CONTENTS

Use of artificial neural network for medical risk assessment analysis

Mariam K Hafshejani[1], Manochehr Sattari Naeini[2], Aboosaleh Mohammadsharifi[3], Ameneh Langari*[4]

[1]Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Department of Biology, Naein Branch, Islamic Azad University, Naein, Iran
[3]Engineering Group, Ramsar Branch, Islamic Azad University, Ramsar, Iran
[4] North Khorasan University of Medical Sciences, Bojnurd, Iran
Corresponding author email: amenehlangari@yahoo.com

Abstract: For new medical products and new drugs, unanticipated side effects that rise after consuming the new product is a dominant factor in decision making. In this project, an artificial neural network (NN) engine is designed and developed by the authors to the aim of a medical risk assessment. Firstly, an appropriate NN system is designed and trained. We mostly concerned with the procedure of how the developed NN construction and training. The designed NN for this case has three layers of neuron. These three layers include an input layer, a hidden layer and finally an output layer, with 25 neurons in the hidden layer. The results from NN models can match the data used for training.

[Hafshejani M K, Sattari Naeini M, Mohammadsharifi A, Langari A. **Use of Artificial Neural Network for Medical Risk Assessment Analysis.** *Life Sci J* 2012;9(4):923-925] (ISSN:1097-8135). http://www.lifesciencesite.com. 143

Keywords: Medical risk assessment, Neural network (NN)

1. Introduction

Medical and health risk assessment is one of the powerful used screening tools in the field of health promotion and is often the first step in multi-component health promotion programs. Moreover, Risk/benefit assessment plays an important role in market of new drugs, medical devices etc. (Al-Awa *et al.*, 2012; Maghrabi, 2012). Risk assessment I a crucial stage in medical risk management. For new products, unanticipated side effects that rise after consuming the new product is a dominant factor. In addition, FDAs focused on ensuring the appropriate use of products in medical practice. Some reports have focused on the human/economic costs of medication errors, as well as serious adverse events that have occurred even when a medical product has been used appropriately (Report to the FDA commissioner, 1999). Risks have different source, hence effective management of each is different (See figure (1)).

In this work we are going to use Neural Network (NN) for medical risk assessment. NN is a system based on the human brain (www.makhfi.com). It is inherently multiprocessor-friendly architecture and without much modification, it goes beyond one or even two processors of the von Neumann architecture. It has ability to account for any functional dependency. No need to postulate a model, to amend it, etc. Neural networks are a powerful technique to solve many real world problems. They typically consist of many simple processing units, which are wired together in a complex communication network (www.nr.no; Zernikow *et al.* 1998). There is no central CPU following a logical sequence of rules - indeed there is no set of rules or program (www.makhfi.com; Shahbaz *et al.,* 2012; Yang *et al.*, 2012). This structure then is close to the physical workings of the brain and leads to a new type of computer that is rather good at a range of complex tasks.

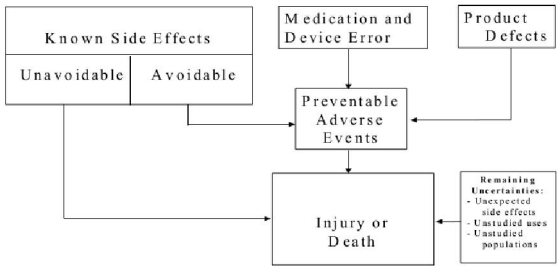


Figure 1. sources of risk from medical products (Report to the FDA commissioner, 1999).

Regardless of the prevention techniques employed, possible threats that could arise the health need to be assessed. Although the exact nature of potential disease or disasters is difficult to determine, it is beneficial to perform a comprehensive medical risk assessment of all threats that can realistically occur to the public health. Quantitative risk assessment employs two fundamental elements; the probability of an event occurring and the likely loss should it occur. Quantitative risk analysis makes use of a single figure produced from these elements (www.nr.no; Zernikow *et al.* 1998).. This is called the 'Annual Loss Expectancy (ALE)' or the

'Estimated Annual Cost (EAC)'. This is calculated for an event by simply multiplying the potential loss by the probability (Balabin & Lomakina, 2009). In this paper, based on an available database, medical risk assessment performed on a typical fault tree. A NN system is designed and trained. This paper is mostly concerned with the procedure of NN construction and training.

2. Fault tree

For this work, in this section a simple fault tree could be considered as has been shown in figure 2. This is a typical fault tree to be applied for designing an artificial Neural Network.

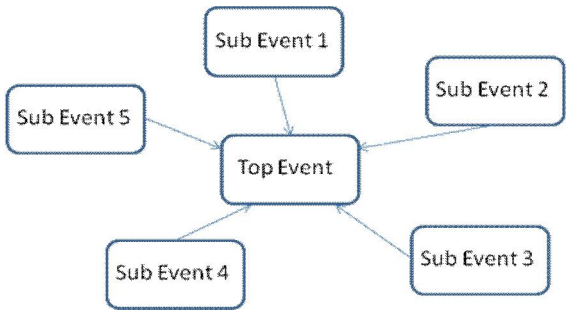


Figure 2. Typical fault tree

3. Available Data Base

Table 1 includes a part of the available data base, performed from our project on risk assessment.

Table 1. Some sets of the fuzzy data base (32768 points)

Frequency of set1	Frequency of set2	Frequency of set 3	Frequency of set 4	Frequency of set 5	Overall frequency
1.25	1.25	1.25	1.25	1.25	1.375
1.25	1.25	1.25	1.25	1.75	1.425
1.25	1.25	1.25	1.25	2.25	1.575
1.25	1.25	1.25	1.75	2.25	1.625
1.25	1.25	1.25	2.75	4.75	2.275
1.25	1.75	1.25	3.25	3.75	2.275
1.25	1.75	3.25	2.75	4.75	2.725
2.25	2.75	3.25	1.75	4.75	2.925
3.25	4.75	1.25	2.75	4.75	3.325
4.75	4.75	4.75	4.75	4.75	4.62

Table 2. Sample of the normalized training data base (32768 points)

Set 1	Set 2	Set 3	Set 4	Set 5	Overall frequency
0.0625	0.0625	0.0625	0.0625	0.0625	0.09375
0.0625	0.0625	0.0625	0.0625	0.1875	0.10625
0.0625	0.0625	0.0625	0.0625	0.3125	0.14375
0.0625	0.0625	0.0625	0.1875	0.3125	0.15625
0.0625	0.0625	0.0625	0.4375	0.9375	0.31875
0.0625	0.1875	0.0625	0.5625	0.6875	0.31875
0.0625	0.1875	0.5625	0.4375	0.9375	0.43125
0.3125	0.4375	0.5625	0.1875	0.9375	0.48125
0.5625	0.9375	0.0625	0.4375	0.9375	0.58125
0.9375	0.9375	0.9375	0.9375	0.9375	0.905

4. Data Normalization

We normalize the data points to be within a specific range. Therefore, the data points are normalized to the range of [0,1] interval. Please note that, the raw data points could be used as they are all in the range of 1 to 5.

5. The Neural Network Constructed

This topology of the constructed neural network is shown in figure 3. The hidden layer includes 25 neurons.

6. The ANN Training Performance

The Levenberg-Marquardt algorithm, as a powerful off-line batch training method for neural networks, is used for training. The learning rate is 0.005. It's run to epoch 300. The final fitness is 0.000187721 (MSE). The performance of training the

assumed neural network is shown in figure 4. The Levenberg-Marquardt algorithm is used for this aim and the learning rate is 0.005.

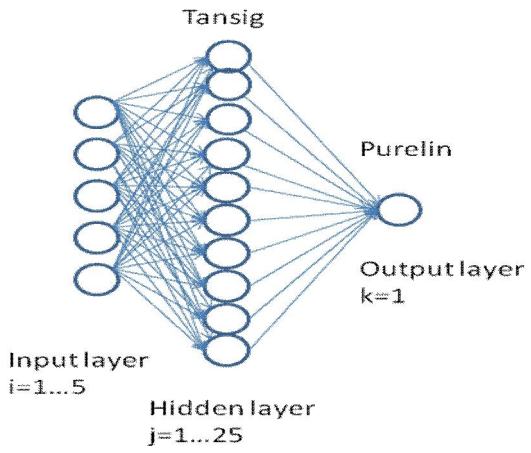


Figure 3. Applied neural network

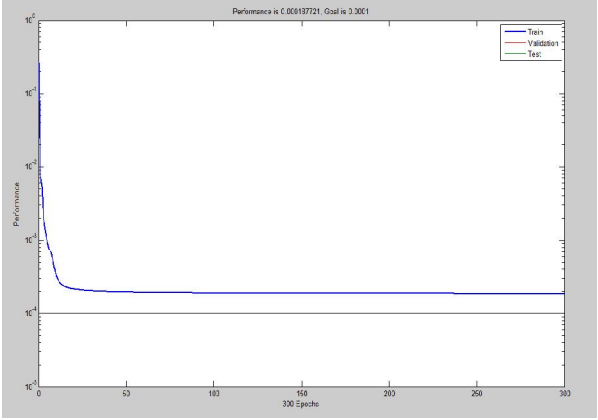


Figure 4. Training performance

7. Conclusions

Medical and health risk assessment is one of the most widely used screening tools in the field of health promotion and is often the first step in multi-component health promotion programs. Moreover, Risk/benefit assessment plays an important role in market of new drugs, medical devices etc.. In this paper, an artificial neural network engine is designed and trained to be applied for a systematic medical risk assessment. The designed neural network for this case has three layers of neuron, input layer, hidden layer and output layer, with 25 neurons in the hidden layer. The results from artificial neural network models can match the data used for training. The Levenberg-Marquardt algorithm is used for this aim and the learning rate is 0.005.

Acknowledgement

The authors of the paper would appreciate to thank all the people contribute in this project.

Corresponding Author:

Ameneh Langari

North Khorasan University of Medical Sciences Bojnurd, Iran

E-mail: amenehlangari@yahoo.com

References

1. Balabin R M, Lomakina E I. Neural network approach to quantum-chemistry data: Accurate prediction of density functional theory energies; Journal of Chemical Physics 2009; 131(7): 074104 (8pp).
2. http://www.makhfi.com/
3. http://www.nr.no
4. Report to the FDA commissioner, managing the risks from medical product use creating a risk management framework, U.S. Department of health and human services, Food and drug administration, 1999.
5. Zernikow B, Holtmannspoetter K, Michel E, Pielemeier W, Hornschuh F, Westermann A, Hennecke K. Artificial neural network for risk assessment; Arch Dis Child Fetal Neonatal Ed. 1998, 79(2):129–134.
6. Al-Awa1 B, Devreux I, Jacquerye A, Alhazmi A, AlBaz H, Habib H, Rayes O. Conceptualization of a Patient Safety Management Model as Practical Approach toward Benchmarking and Improving Healthcare Outcomes; Life Science Journal 2012; 9(3):774-780.
7. Maghrabi K. Impact of Flood Disaster on the Mental Health of Residents in the Eastern Region of Jeddah Governorate, 2010: A Study in Medical geography; Life Science Journal 2012; 9(1):95-110.
8. Shahbaz M, Shaheen M, Aslam M, Ahsan S, Farooq A, Arshad J, Masood S A. Data Mining Methodology in Perspective of Manufacturing Databases; Life Science Journal 2012; 9(3):13-22
9. Yang Y S, Huang C F, Hu B J, Liao T L , Yan J J. Implementation of Real-time Handwriting Recognition System Using Touch Panel Based on Neural Network; Life Science Journal 2012; 9(3):148-15.

9/15/2012

Lichen wealth of Jammu and Kashmir- A promising plant source for Bioprospection

[1]Manzoor Ul Haq, [1]Zafar A Reshi, [2]D. K. Upreti and M.A. Sheikh

[1.]Department of Botany,University of Kashmir, Srinagar-190006, India
[2.]CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India
haqmanzoor@ymail.com

Abstract: So far 279 lichen species belonging to 79 genera and 33 families have been reported from the state of Jammu & Kasmir. The taxa under lichen families Parmeliaceae and Physiaceae dominates the state. Genera such as *Xanthoria, Cladonia, Lecanora and Caloplaca* showed the maximum diversity of species. The paper presents occurrence and probable utilization of lichens for bioprospection in the state.
[Manzoor Ul Haq, Zafar A Reshi, D. K. Upreti and M.A. Sheikh. **Lichen wealth of Jammu and Kashmir- A promising plant source for Bioprospection.** *Life Sci J* 2012;9(4):926-929] (ISSN:1097-8135). http://www.lifesciencesite.com. 144

Keywords: Lichen; Jammu & Kashmir; Diversity; Biomonitoring.

Introduction:

Lichens comprise a unique group of plant that consists of two unrelated organism, a fungus and an alga, growing together in a close symbiotic association. Lichens are extremely biologically diverse (Galloway, 1992; Hawksworth, 2001), and functionally important in terrestrial ecosystems (Knops et al., 1991, 1996; Arseneault et al., 1997). The study of lichen remains quite neglected throughout the world, though they together with mosses form dominant organisms in ecosystem covering over 10% of the earth terrestrial habitats, particularly at higher elevations (Nash and Egan 1988). Given the modern emphasis on biodiversity conservation (Heywood, 1995; Gaston & Spicer, 2004), and the importance of biogeographical research in tackling major conservation issues, it is critical that biogeographical studies include diverse, though often neglected, components of the world's biota (Klironomos 2002). Lichens are one example of a biological group that is often neglected in mainstream biogeographical and ecological study.

Lichens with specific biological structures are known as the best bioindicator organisms of air pollution, due to susceptibility of species to pollutants, especially sulphur dioxide (Saxena et al 2007), and also as biomonitors for trace element and heavy metal accumulation and deposition in their thalli (Garty 2001). However, many lichen species can tolerate extreme environmental conditions, for instance variations in temperature, humidity and light (Hauck et al., 2007). As adaptations for life in marginal habitats, lichens produce a lot of (more than 1,500) unique chemical compounds (Upreti and S. Chatterjee 2007). These organisms produce nalkane, unusual betaine and glycolipids, unsaturated, oxygenated, branched, and halogenated fatty acids (Torres et al 2003). Lichen substances have many medicinal roles, including antibiotic, antibacterial, antiviral, anti-inflammatory, analgesic, antipyretic, anti-proliferative and other activities (Upreti and S. Chatterjee, 2007; Dembitsky, 1992).

The state of Jammu and Kashmir lies between the coordinates 32º 17" and 36º 58" North Latitudes and 73º 26" and 80º 30" East Longitudes and falls in the lichenogeographic zone constituting of mountainous to semi mountainous plains, Shiwalik ranges, mountains of Kashmir valley, Pir Panjal range of Ladakh and Kargil. Including Jammu and Kashmir the Himalaya is often called the "Hot Spot" of lichen diversity in India. The climate in the state varies from tropical, subtropical to alpine. This varied climate together with varied altitudinal range provide different kinds of substrates and niches for colonization and growth of lichens. The lichen collection in the state started during early thirties of last century where Smith (1931) identified and published some lichen species collected by Kashyap. However, more rigorous exploration in the state was started since early 50s and Sheikh *et al* (2006a) provided a historical account of lichen study in Jammu and Kashmir. Recently, workers like Negi and Upreti (2000) collected lichen specimens from Ladakh region of the Kashmir valley and reported 21 species from the area. Sheikh *et al* (2006b) reported 48 species from three districts of the Kashmir valley. Subsequently, Sheikh *et al* (2006a) listed a total of 279 lichen species from whole Jammu & Kashmir. Sheikh *et al.* (2009) reported 30 lichen species from Surinsar-Mansar Wild Life Sanctuary of Jammu district. Though lichens are utilized for different purposes in other states of India but they are scantly utilized in Jammu and Kashmir. The manuscript provides the information as to how lichens can be used in the state.

Methodology:

The study is based on the lichen specimens recently collected from different sites of Jammu & Kashmir and specimens preserved in lichen herbarium of National Botanical Research Institute, Lucknow(LWG); personal lichen collection of D. D. Awasthi (AWAS). The literature available on lichens of Jammu & Kashmir carried out by different workers (Smith 1931; Awasthi & K Singh 1970; Negi & Upreti 2000; Sheikh *et al* 2006 a & b, 2009) from different localities in Jammu & Kashmir were thoroughly consulted. Regarding commercial use of lichens, research work carried out by different workers (Brij Lal 1988, 1990; Brij Lal & Upreti 1995; Saklani & Upreti 1992; Upreti et al 2005; Upreti & Chatterjee 2007 and Nayaka et al 2010) was consulted.

Resuts and Discussion

A total of 279 species belonging to 79 genera and 33 families of lichen species have been reported fom the state of Jammu & Kashmir. Out of different districts of the state the Anantnag district shows the maximum diversity of lichens represented by 105 species. Baramulla and Srinagar district are represented by the occurrence of 70 and 57 species of lichens respectively. In areas of Leh, Pulwama, Jammu, Doda and Budgam districts there are 34, 22, 20, 19 and 12 species of lichens respectively. Gilgit, Kargil and Udhampur districts are poorly explored for lichens as records of only 7, 5, and 1 species are available from these districts respectively. There are absolutely no collection available from frontier distict of Kupwara, Kathwa, Rajouri and Poonch. Lichen families Parmeliaceae and Physciaceae are the dominant families of the state, while *Xanthoria, Cladonia, Lecanora, Caloplaca, Flavoparmelia, Phaeophyscia, Anaptychia, Dermatocarpon, Xanthoparmelia, Heterodermia, Peltigera, parmelina, Chrysothrix, Parmelia and Physconia* are the dominant genera in the state.

Lichens of medicinal importance

In India the ethnomedicinal use of lichens has been carried out since the middle of the last century. Brij Lal(1988,1990); Brij lal & Upreti (1995); Saklani & Upreti (1992); Upreti *et al* (2005); Upreti & Chatterjee (2007) and Nayaka *et al* (2010) provide information regarding the ethnomedicinal use of lichens in India. Nayaka *et al* (2010) listed 137 species of lichens used in antimicrobial, anticancer, antioxidant, anti-inflammatory activities. *Cladonia pyxidata* (L) Hoffm., is used against the symptoms that include difficulty in breathing, anxious and nervousness, dryness of tongue, lips, throat and skin. *Evernia prunastri* (L) Ach., mentioned in Pharmacopia Universalis of 1846 bears antimicrobial and antioxidant activities. *Everniastrum cirrhatum* (Fr.) Hale commonly known as '*Charila*' is the common lichen in temperate Himalaya and is used in Ayurveda and Unani system of medicine for stomach disorders, bronchitis, bleeding piles, scabies, leprosy, spermatorroea, amenorrhea, enlarged spleen, tooth ache, soreness of throat and wound healing. Besides, it has antibacterial and antifungal activities. The intestinal worms are treated by *Flavoparmelia caperata* (L.) Hale, and dried powder of the thallus can be applied on burns. *Heterodermia diademata* (Taylor) D. Awasthi is applied on wounds as plaste to protect from water and infection. The apothecia of *Peltigera canina*(L) Willd., popularly known as 'dog lichen', resembles dog teeth and are widely used in the treatment of dog bites and rabies. *P. polydactyla* (Neck.) Hoffm., is used as antiseptic and applied on cuts to stop bleeding. *Usnea longissima* Ach., along with other ingredients is used in bone fracture. It also benefits urinary tract infection and stop swelling in female genitals. *Xanthopaermelia stenophylla* (Ach.) Ahti & Hawk is used in treating snake bites and is applied for the treatment of venearal diseases such as Syphilis. *Xathoria parietina* (L) Th. Fr. a yellow coloured lichen is used in jaundice.

Dye yielding lichens

A number of lichens, in particular belonging to the genera Xanthoria and Caloplaca are pigmented orange or red due to the presence of hydroxyanthraquinones. One of the widely spread anthraquinone is parietin. The lichen genera as *Evernia, Lobaria, Ochrolecia, Parmelia, Umbilicaria*, *Xanthoria* and *Xanthoparmelia* have different colouring compounds. *Acarospora strigata* (Nyl.) Jatta, *Candellaria vitellina* (Ehrh) Mull. Arh., *Chrysothrix chlorina* (Ach.) Laundon, *Dermatocarpon miniatum* (L) Mann, *Evernia prunastri* (L) Ach. *Lecanora frustolosa* (Dick) Ach., *Lecanora muralis* (Schreber) Rabenh., *Parmelia saxatilis* (L) Ach., *Peltigera canina* (L) Willd., *P. praetextata* (Flork) Zopf., *Rhizoplaca chrysoleuca* (Sm) Leuck. & Poelt, *Xanthoparmelia mexicana* (Gyeln) Hale, and *Xanthoria parietina* (L) Th. Fr. are the lichen species found in the state of Jammu and Kashmir and can be used for dye yielding.

Lichens that can be used as food and fodder

Lichens are extensively used in making spices, ingredients and flavouring agents for meat and vegetables and are a better option than adulterated and carcinogenic counterparts available in the market. Upreti (2005) mentioned a list of lichens exploited for food materials. The common lichen taxa exhaustly collected in other parts of India for their

use as spices are *Canoparmelia texana* (Tuck) Elix & Hale, *Everniastrun cirrhatum* (Fr) Hale, *Flavopunctelia flaventior* (Stirton) Hale, *Heterodermia diademata* (Taylor) D. Awasthi, *Heterodermia lecomela* (L) Poelt, *Parmotrema tinctorum* (Nyl) Hale, *Parmotrema reticulatum* (Taylor) Choisy, *Ramalina sinensis* Jatta, *Usnea orientalis* Mot. and bear a promise in the state of Jammu and Kashmir as well.

Pollution monitoring studies

Due to fast pace of urbanization, the rate of deforestation and vehicular activities have increased tremendously in the state. Lichens have a long history of use as biological indicators of air quality (Rao & LeBlanc, 1967; Vestegaard, 1986). The tolerance of lichens to most of the heavy metals and their slow growth rate, are among the main factors that make them good indicators of both organic and inorganic metal pollution. Among the different lichen species *Phaeophyscia hispidula* (Ach) Essl., has been used for conducting pollution monitoring studies in different parts of India. Being foliose lichen it is easy to collect and can be utilized for pollution monitoring (Shukla & Upreti, 2007). *Phaeophyscia hispidula* together with other members of the lichen family Physciaceae are well known for their toxitolerant nature and are growing frequently in the different region of the state can be used for pollution monitoring.

Ecological studies

Lichens play a major role in balancing the ecosystem and are the pioneers in succession of community. They are proficient in indicating the climate of forest. Lichens usually grow on all kinds of substrata but certain lichens are host specific. Presence of *Lobaria* and *Sticta* species indicate the moist dense undisturbed forest area. *Peltigera, Cladonia, Stereocaulon* and *Xanthoparmelia* indicate the less erosive and animal trampling activities in the forerst. *Usnea* is related to old aged forest with better air quality. Members of family Parmeliaceae indicate the sunlit forest sites, however, members of Physciaceae indicate the dry forest with increasing anthropogenic activities. The coloured nature of *Xanthoria* is related to harsh exposed, open alpine areas with high infra red and UV radiation intervention. Besides this lichens are known to date surfaces near glacier snouts, landslides, old monuments, grave yards and river terrace formation. Only few studies regarding this aspect are accomplished in India. The biomonitoring technique commonly known as lichenometry was undertaken in Gangotri Glacier area.

Conclusion

The state of Jammu & Kashmir sustains an abundantly rich lichen flora. A good number of people live in the remote areas near the forests and have little knowledge about the possible uses of lichens, rather, are bent on cutting the forests and exploiting other forest resources which is a menace and drawback on our conservation policies and strategies. The information provided in the present paper can be used to establish small scale cottage industries for preparation of spices and dyeing material in remote areas. This particular practice may lead to employment generation at village level. This will in turn minimize the pressure on our forests and will act as a strategy for conservation of diversity indirectly. Lichens are promising sources of medicines, dyes, spices and perfumes. They can prove as a best alternative to artificial dyes which are carcinogenic and a lot of capital and human resource can be saved to channelize it in the development of state which otherwise is utilized against these fatal diseases.

To avoid the overexploitation of this gifted resource the conservation measures as suggested by Upreti & Nayaka (2008) should be taken for sustainable use of lichens.

Corresponding Author:
Manzoor Ul Haq
Department of Botany
University of Kashmir
Srinagar 190006, Jammu & Kashmir, India
E-mail: haqmanzoor@ymail.com

References

1. Arseneault, D., Villeneuve, N., Boismenu, C., Leblanc, Y. and Deshaye, J. 1997. Estimating lichen biomass and caribou grazing on the wintering grounds of northern Que´bec: an application of fire history and Landsat data. Journal of Applied Ecology, 34, 65–78.
2. Awasthi, D. D. and Singh K. P. 1970. A note on lichens of Kashmir *Curr. Sci*. **39** : 441-442.
3. Brij Lal and Upreti, D. K. 1995. Ethnomedicinal notes on three Indian lichens. *Lichenologist* **27 (1):** 77-79.
4. Brij Lal. 1988. Traditional remedies for bone-fracture among the tribals of Madhya Pradesh, India, *Aryavaidyan* **1** : 190-1995.
5. Brij Lal. 1990. Ethnomedicinal studies of the Baiga tribe of Madhya Pradesh Ph.D. Thesis Meerut University Meerut.
6. Dembitsky, V. M. 1992. Lipids of Lichens. *Prog. Lipid Res*. **31**, 373-397.

7. Galloway, D.J. 1992. Biodiversity: a lichenological perspective. Biodiversity and Conservation, 1, 312–323.
8. Garty, J. 2001. Biomonitoring atmospheric heavy metals withlichens: Theory and application. Crit. Rev. Plant Sci., 20, 309-371.
9. Gaston, K.J. & Spicer, J.I. 2004. Biodiversity. Blackwell, Oxford.
10. Hauck, M., C. Dulamsuren and M. Mühlenberg 2007. Lichen diversity on steppe slopes in the northern Mongolian mountain taiga and its dependence on microclimate. Flora, 202, 530-546.
11. Hawksworth, D.L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research, 105, 1422–1432.
12. Heywood, V.H. (ed.) 1995 Global biodiversity assessment.Cambridge University Press, Cambridge.
13. Klironomos, J.N. 2002. Another form of bias in conservation research. Science, 298, 749.
14. Knops, J.M.H., Nash, T.H., Boucher, V.L. and Schlesinger, W.H. 1991. Mineral recycling and epiphytic lichens: implications at the ecosystem level. Lichenologist, 23, 309–321.
15. Knops, J.M.H., Nash, T.H. and Schlesinger, W.H. 1996. The influence of epiphytic lichens on the nutrient cycling of an oak woodland. Ecological Monographs, 66, 159–179.
16. Nash, T. H. and Egan, R. S. 1988. The biodiversity of lichens and bryophytes. In: Lichen, Bryophytes and air quality eds. Thomas Nash III & Vilkmar Wirth. Bibl. Carmer in der Gebr. Borntra. Verlag. Berlin, Stuttgart. Lichenol.1988; 30: 11-22.
17. Nayaka, S. Upreti D. K. and Khare R. 2010. Medicinal lichens of india: Drugs from plants.Pp. 1-38. (P.C. Trivedi (ed). Avishkar Publishers, Distributors, Jaipur.
18. Negi, H. R. and Upreti, D. K. 2000. Species diversity and relative abundance in lichens in Rumbuk Catchment of Hemis National Park in Ladakh. *Curr. Sci.* **78(9):**1105-1112.
19. Rao, D.N. and LeBlanc, F. 1967. Influence of an iron sintering planton corticolous epiphytes in Wawa, Ontario. *The Bryologist* **70:** 141-157.
20. Saklani, A and Upreti, D. K. 1992. Folk usres of lichens in Sikkim. *J. Ethnopharmocology* **37;** 229-233.
21. Saxena, Shalini, D.K. Upreti, D. K. and Neeta Sharma 2007 Heavy metal accumulation in lichens growing in north side of Lucknow city, India. J. Environ. Biol., 28, 49-51
22. Sheikh, M. A., Upreti, D. K. and Raina, A. K. 2006a. Lichen diversity in Jammu & Kahmir, India. *Geophytology* **36(1&2):** 69-85.
23. Sheikh, M. A., Upreti, D. K. and Raina, A. K. 2006 b. An enumeration of lichens from three districts of Jammu & Kashmir. *J. Appl.Biosc.***32(2):** 89-191.
24. Sheikh, M. A., Upreti, D. K. and Raina, A. K. 2009. Lichen flora of Surinsar-Mansar Wildlife Sanctuary, Jammu & Kashmir. *J. Appl. Nat. Sci.*b 79-81.
25. Shukla, V. and Upreti, D. K. 2007. Heavy metal accumulation in *Phaeophyscia hispidula* enroute to Badrinath, Uttaranchal, India. *Environ. Monit. Assess.* **141:** 237-243.
26. Smith, A. L. 1931. Lichens from Northern India. *Trans. Brit. Mycol. Soc.* **16:** 128-132
27. Torres, A., Dor I., Rottem, E., Srebnik, M. and Dembitsky, V. M. 2003. n-Alkane and fatty acid variations inthe lichen *Xanthoria parietina*, their photobiont *Trebouxia* sp. and mycobiont, from Jerusalem hills. *Eur.J. Biochem.* **270**, 2120-2125.
28. Upreti, D. K., Divakar, P. K. and Nayaka S. 2005. Commercial and ethnic uses of lichens in India. *Economic Botany* **59(3):** 976-998.
29. Upreti, D. K. and Chatterjee, S. 2007. Significance of lichens and their secondary metabolites: a review.*Fungi* **2**, 169-188.
30. Upreti, D. K. and Nayaka S. 2008. Need for creation of lichen gardens and sanctuaries in India. *Curr. Sci.* **94(8):** 976-978.
31. Vestergaard, N. Stephansen, U., Ramussen, L. and Pilegaard, K. 1986. Airborne heavy metal pollution in the environment of a Danish Steel plant. *Water Air and Soil Pollution* **27:** 363-377.

9/9/2012

Influence of Hesperidin combined with Sinemet on genetical and biochemical abnormalities in rats suffering from Parkinson's disease

Hiam Rushdy A. Salem[1], Amira Abd El-Raouf Mohamed[2]*, Eman M. Saleh[1] and Kamal A.F. Shalaby[1]

[1]Department of Biochemistry, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt, 11566.
[2]Cell Biology Department, National Research Center, El-Dokii, Giza, Egypt.
Corresponding Author: amiraabdelraoof@yahoo.com

Abstract: Parkinson's disease (PD) is a progressive disabling neurodegenerative disorder characterized by severe difficulties with body motions and associated with autonomic dysfunction, depression, and dementia. Oxidative stress is thought to play an important role in the pathogenesis of PD and oxidative damage characterizes proteins, lipids, and DNA in the substantia nigra pars compacta (SNc) of PD patients. To date, L-dopa is the most effective medication for controlling PD symptoms, although long-term treatment can enhance oxidative stress and accelerate the degenerative process of residual cells. Thus, the inhibition of oxidation of L-dopa and the inhibition of reactive oxygen species formation are important strategies for neuro-protective therapy. Therefore, efforts are made not only to improve the effect of L-dopa treatment for PD, but also to investigate new drugs with both antioxidant and neuro-protective effects. Hespiridin (HDN), a naturally occurring flavonoid presents in fruits and vegetables, has been reported to exert a wide range of pharmacological effects including antioxidant, anti-hypercholesterolemic, anti-hyperglycemic, anti-inflammatory, anti-carcinogenic and neuro-protective actions. Chlorpyrifos (CPF) was used in this study as an animal model of PD. Model of CPF-induced Parkinsonism in rats produced neurotoxicity, oxidative stress, hyper-lipidemia, hyperglycemia and DNA damage. Seventy male rats were used in this study and divided into seven equal groups. After 6 weeks, the following groups were studied, control group, CPF group, HDN group, Sinemet group, CPF+HDN group, CPF+Sinemet group, and CPF+HDN+Sinemet group. Here in the present study, the treatment of parkinsonism with HDN alone or combined with sinemet provided a neuroprotection effect when given early in the course of the disease. In conclusion, HDN could be recommended as a disease-modifying therapy when given alone or mixed with L-dopa in course of Parkinson's disease.
[Hiam Rushdy A. Salem, Amira Abd El-Raouf Mohamed, Eman M. Saleh and Kamal A.F. Shalaby. **Influence of Hesperidin combined with Sinemet on genetical and biochemical abnormalities in rats suffering from Parkinson's disease.** *Life Sci J* 2012;9(4):930-945] (ISSN:1097-8135). http://www.lifesciencesite.com. 145

Key words: Hesperidin, Sinemet, Chloropyrifos, Pakinson's disease, Antioxidant enzymes, DNA damage.

1.Introduction

Parkinson's disease (PD) is the second most common neurodegenerativedisease after Alzheimer's disease (AD). According to many epidemiological data, the number of individuals affected by PD in the most populous nations worldwide is expected to rise and will double within the next several decades ***(Dorsey et al., 2007)***. This development constitutes an enormous public health challenge for the future concerning about the medical care and treatment cost ***(Löhle and Reichmann, 2010)***.Pathologically, PD is a neurodegenerative disease characterized, in part, by the death of dopaminergic neurons (DAergic) in the substantia nigra pars compacta (SNc). Clinically, PD has traditionally been defined by the presence of cardinal motor signs such as tremor, rigidity, bradykinesia, akinesia and postural instability. Other symptoms include fatigue, sleep abnormalities, and depression ***(Jankovic, 2008)***. Prominent pathological manifestations associated with degeneration of SNpc-DAergic neurons include mitochondrial abnormalities ***(Dagda and Chu, 2009 ;Nishioka et al., 2010)***, excessive cytosolic dopamine (DA) oxidation, α-synuclein aggregates, autophagolysosome dysfunction, defects in the ubiquitin-proteasome system (UPS), oxidative stress, nitrosative stress, iron released from bound storage and a gradual loss of neuromelanin (NM) ***(Tofaris and Spillantini, 2005; Levy et al., 2009)***.

PD is associated with both genetic and non-genetic contributing factors, with aging as the most prominent risk factor ***(Thomas and Beal, 2007)***. PD brains exhibit a reduction in mitochondrial complex I activity, which is both the rate-limiting step for mitochondrial respiratory chain activity and an important site for generation of reactive oxygen species (ROS). Reduction of complex I activity may lead to accumulation of ROS, which can further induce mitochondrial permeability transition, ATP depletion, and damage of DNA, lipids and proteins ***(Keeney et al., 2006)***.

The oxidation hypothesis suggests that the free radicals damage, resulting from DA's oxidative metabolism through deamination by monoamine oxidase (MAO) enzyme, plays a role in the development or progression of PD. MAO activity

increases with the natural process of aging and can yield toxic products such as hydrogen peroxide, ammonia, and aldehydes ***(Bortolato et al., 2008;Naoi and Maruyama, 2010)***. Hydrogen peroxide normally is cleared rapidly by glutathione (GSH) but if it is not cleared adequately as in PD, it may lead to the formation of highly reactive hydroxyl radicals that can react with lipids of cell membrane to cause lipid peroxidation and cell damage. In PD, levels of glutathione are decreased, suggesting a greater oxidative damage ***(Bharath et al., 2002; Johnson et al., 2005)***.

Environmental factors have been shown to contribute to the incidence of PD by inducing mitochondrial dysfunction ***(Schneider and Zhang, 2010)***. Pesticides represent one of the primary classes of environmental agents that associated with PD ***(Hatcher et al., 2008)***. Organophosphate (OP) compounds are potent neurotoxic chemicals widely used in agriculture, industry ,households and even as chemical weapons ***(Moreno et al., 2008)***. Chlorpyrifos (CPF)[O,O-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothionate] is a crystalline broad spectrum organophosphate insecticide utilized extensively in agriculture and for residential pest control throughout the world under the registered trademarks LORSBAN Insecticide and DURSBAN Insecticide ***(Saulsbury et al., 2009)***. CPF has been reported to exert their primary neurotoxic effects by inhibiting the acetylcholinesterase (AChE), the enzyme responsible for degradation of the neurotransmitter, acetylcholine (ACh), during neurotransmission. It phosphorylates the serine residue at the active site of AChE and thus inhibiting it causing accumulation of acetylcholine at the cholinergic synapses ***(Costa, 2006)***.

In addition to cause neurotoxicity, OPs are also related to a variety of physiological abnormalities including immunotoxicity ***(Galloway and Handy, 2003)***, oxidative stress ***(Kamath et al., 2008;Wu et al., 2011)***, alterations in glucose homeostasis ***(Kamath and Rajini, 2007)*** and hyperglycemia ***(Joshi and Rajini, 2009)***. Also, some studies have shown that these compounds can also induce a disturbance in the lipid status, such as an increase of cholesterol and triglycerides levels that represents a risk factor for premature atherosclerosis ***(Çetin et al., 2010; Lasram et al., 2009)***. However, there is little information in literature concerning the mechanisms involved in hyperlipidemia induced by OPs.

To date, 3,4-dihydroxyphenyl-L-alanine (levodopa/L-dopa) is the most effective medication for controlling PD symptoms, particularly those related to bradykinesia ***(Nagatsua and Sawadab, 2009)***. However, the long-term treatment with L-dopa can generate motor fluctuations, i.e. wearing off and dyskinesia ***(Schapira, 2008)***, since L-dopa and its metabolite dopamine (DA) can enhance oxidative stress and accelerate the degenerative process of residual cell lines ***(Blessing et al., 2003;Hattoria et al., 2009)***. L-dopa is a natural dopamine precursor that can cross the blood–brain barrier (BBB) to reach the brain where it is converted into DA by peripheral decarboxylase and stored in vesicles in order to be progressively released onto postsynaptic receptors. Peripherally, L-dopa is rapidly metabolized to DA by the enzyme aromatic amino acid decarboxylase (AADC) ***(Hardie et al., 1986)***.

Concomitant administration of inhibitors of extra-cerebraldopa decarboxylase (IEDD) e.g. carbidopa (CD) which do not cross the blood–brain barrier, have permitted the peripheral conversion into DA to be blocked and allowed a fourfold reduction in the L-dopa dose requirement as its plasma elimination half-time has been shown to increase from 60 to 90 min ***(Leppert et al., 1988)***. The first marketed product containing a combination of levodopa and carbidopa was an immediate-release (IR) oral dosage form under the trade name of Sinemet and now it is the main treatment for PD ***(Goole and Amighi, 2009)***.

Efforts are made not only to improve the effect of L-dopa treatment for PD, but also to investigate new drugs with both antiparkinsonian and neuroprotective effects ***(Yuan et al., 2010)***. Nowadays, different natural supplements from plant origin are used as a complementary supplement in the treatment of many diseases by improving the efficacy of drug used or by minimizing the toxic side effect and so enhancing the state. Hesperidin (HDN), a flavonone glycoside, is an inexpensive and abundant byproduct of citrus cultivation (***Susana et al., 2008)***. HDN was reported to have many biological effects including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant effects and neuroprotective actions ***(Garg et al., 2001 ;Ebrahimi and Schluesener, 2012)***. It exhibits anti-oxidative properties by several different mechanisms, such as scavenging of free radicals, chelation of metal ions such as iron and copper which are of major importance for the initiation of radical reactions, inhibition of enzymes responsible for free radical generation and facilitation endogenous antioxidative defense system ***(Cai et al., 2006)***.

Hence the present study was aimed to investigate the protective efficacy of HDN as a complementary supplement combined with Sinemet to improve genotoxicity and biochemical abnormalities induced by CPF in the Swiss albino male rats.

2.Materials & Methods

2.1.Materials:

2.1.1.Chemicals

- Chlorpyrifos(CPF) was insecticide purchased from the Egyptian company for pesticides under the trade name "*Drusban"*.
- Hesperidin (HDN) was purchased from Sigma-Aldrich Company-Chemicals Private Ltd., India. It was suspended in distilled water and administered orally to rats.
- Sinemet was obtained as tablets manufactured by Global Napi Pharmaceuticals – Egypt under license from Merck & Corporate Inc., Whitehouse Station, New Jersey, USA. All other chemicals used in the experiment were of analytical grade.

2.1.2 Experimental animals

Seventy adult male Swiss albino rats weighing 150-200 g of the same age were used throughout this study. Animals were obtained from the animal house colony of National Research Center, Dokii, Giza, Egypt. Animals were maintained under standard conditions of ventilation, temperature (25±2°C), humidity (60-70%) and light/dark condition (12/12h). The rats were housed in stainless steel cages and provided with free access to drinking water and food *adlibitum*.

Rats were orally administered their respective doses by gavage every day throughout the study. The local committee approved the design of the experiments, and the protocol confirms the guidelines of the National Institute of Health (NIH).

After two weeks of acclimatization, animals were divided into 7 groups (n=10).

- ***Control group***; rats were fed normally for 6 weeks.
-***Chlorpyrifos group*** *(CPF)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks.
-***Hesperidin group*** *(HDN)*; rats were orally administered with HDN (50mg/kg b.wt./day) according to the method of *Balakrishnan and Menon, (2007)* for 6weeks.
-***Sinemet group;*** rats were orally administered with sinemet (100mg/kg b.wt./day) according to *Lindner et al., (1996)* for 6weeks.
-***Chlorpyrifos plus hesperidin group*** *(CPF+HDN)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then orally administered with HDN (50mg/kg b.wt./day) for another 6 weeks. -***Chlorpyrifos plus sinemet group*** *(CPF+Sinemet)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then administered with sinemet (100mg/kg b.wt./day) for another 6 weeks.
-***Chlorpyrifos plus hesperidin and sinemet group*** *(CPF+HDN+Sinemet)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then administered with HDN (50mg/kg b.wt./day) mixed with sinemet (100mg/kg b.wt./day) for another 6 weeks.

2.2. Methods:

2.2.1.Toxicity studies

The procedure for the determination of LD_{100} and LD_{50} of tested CPF compound was carried out according to ***Reed and Muench, (1938)***.

A total of 60 adult male Swiss Albino rats were used to determine the LD_{100} and LD_{50} of CPF. The rats were divided equally into 6 groups representing doses from 0.25 to 8mg of CPF/ kg b.wt./day for 6weeks with an increasing factor of 2. All animals were administrated by oral gavage with CPF at different doses. Mortality was recorded after 24 hours, and the LD_{50} was calculated as follows:

Log LD_{50}= log LD next below 50% + (log increasing factor x proportionate distance).

Proportionatedistance=

$$\frac{50\% - \%\ \text{mortality next below } 50\%}{\%\ \text{mortality above } 50\% - \%\ \text{mortality below } 50\%}$$

2.2.1.Biochemical analysis

At the end of the experiment, animals were anaesthetized and sacrificed by cervical dislocation after 24 hours fasting period from the final administration. Blood samples were collected in heparinized tubes and centrifuged at 5000 rpm for 10 min to separate plasma samples. Plasma was separated by aspiration, transferred into micro-centrifuge/eppendorf tubes and stored at -80ºC for evaluating of oxidative stress, lipid levels, and glucose. The brain was removed and cleared off blood and transferred immediately to ice cold container containing a sterile 0.9% NaCl and stored at -80ºC for the cholinesterase estimation and comet assay. Bone marrow samples were collected on slides by flushing at least one femur per animal with fetal calf serum for micronucleus assay:

a-Blood analysis

a.1.Estimation of lipid peroxidation:

The quantitative measurement of lipid peroxidation in plasma was performed according to method of ***(Ohkawa et al., 1979)***. The malondialdyhyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS).

a.2.Estimation of antioxidant enzymes:

The activity of reduced glutathione (GSH) was assayed in the plasma according to the method of ***(Beutler et al., 1963)***. Total (Cu–Zn and Mn) superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to the previous described method ***(Nishikimi et al., 1972)***. Catalase enzyme (CAT; EC 1.11.1.6) was measured according to the method of ***(Aebi, 1984)***. Finally, Glutathione S-transferase (GST; EC 2.5.1.18) was measured according to the method of ***(Habig et al., 1974)***.

a.3.Estimation of total cholesterol, triglycerides, and glucose:

Total cholesterol (TC) levels were assayed in the plasma according to the method of ***(Richmond, 1973)***. Triglycerides (TG) levels were determined by enzymatic colorimetric methods according to the previous described method ***(Fassati & Prencipe, 1982)***. Finally, plasma glucose levels were determined by an enzymatic method based on the oxidase/peroxidase system according to the method of ***(Trinder, 1969)***.

b-Brain tissue analysis

b.1.Estimation of acetyl cholinesterase (AchE):

On the day of biochemical estimation, the entire brain was minced and homogenized with (10%w/v) cold phosphate-buffered saline (pH 7.4) by using a glass homogenizer. The homogenates were centrifuged at 5000 rpm for 5 min at 4° C to separate the nuclear debris. AchE (AChE; EC 3.1.1.7) was assayed by the method of ***(Henry, 1974)***.

b.2.Cytogenetic studies:

b.2.1.Bone marrow micronucleus (MN) assay:

To study micronucleus assay, rats were sacrificed 24 h after treatment. Immediately after the animals were sacrificed, both femurs of the rat were removed and freed from the extra muscles. The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum ***(Valette et al., 2002)***.The cell suspension was centrifuged at 1200 rpm for 10 min and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on clean glass slides and left till air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 minutes and stained with Giemsa at pH 6.8 ***(D'Souza et al., 2002)***.The number of micro-nucleated polychromatic erythrocytes (MNPCEs) and the percentage of micro-nucleated cells were scored from the smeared bone marrow slides. The micronucleus frequencies (expressed as percent micro-nucleated cells) were determined by analyzing the number of MNPCEs from at least 3000 polychromatic erythrocytes (PCEs) per animal.

b.2.2.Determination of DNA damage (Comet assay):

The comet assay was performed according to reagent Kit for Single Cell Gel Electrophoresis Assay ***(Angelis et al., 1999)***. A small piece of brain tissue was placed into 1–2 ml of ice cold 1X PBS and20 mM EDTA. Cell suspension was combined with low melting-point agarose (at 37°C) at a ratio of 1:10 (v/v) and immediately 50 µl was pipetted onto Comet Slide. Slides were placed flat at 4°C in the dark for 10 minutes. Slides were immersed in prechilledLysis Solution (2.5 M NaCl, 100 mM EDTA, 10 mMTris, and 1% Triton X-100, pH 10) for 1 h at 4°C in the dark. After lysis, the slides were transferred to a horizontal electrophoresis tank containing freshly prepared pre-cooled (4°C) alkaline electrophoresis buffer pH>13 (200 mMNaOH, 1 mM EDTA) and leaved for 20 min at 4°C in the dark for DNA unwinding. After DNA unwinding process, electrophoresis was carried out in the same buffer for 30min at 0.7V/Cm (20–30 cm between electrodes) at 4˚ C. Excess electrophoresis solution was gently drained and slides were immersed twice in dH_2O for 5 minutes each, then in 70% ethanol for 5 minutes. Samples were dried at ≤ 45°C for 10-15 minutes. 100 µl of diluted SYBR Green I was placed onto each circle of dried agarose and placed in refrigerator for 5minutes. After staining, the slides were dipped in chilled distilled water to remove excess stain and subsequently the covers lips were placed over the slides.The slides were examined under a fluorescent microscope. The length of DNA migration, image length, and DNA damage parameters were calculated.

3.Results

3.1. Single dose acute oral toxicity study of CPF

Table (1):Acute lethal dose (LD_{100}) and median lethal dose (LD_{50}) of CPF in rats

Dose (mg/kg b. wt.)	Number of animals	Survivals (S)	Deaths (D)	Mortality %
0.25	10	10	-	-
0.5	10	8	2	20%
1	10	6	4	40%
2	10	5	5	50%
3	10	3	7	70%
8	10	-	10	100%

Following administration of a single oral gavage dose of CPF, the rats were monitored daily for mortality. The results presented in Table (1) revealed that, the acute lethal dose (LD_{100}) was 8mg/kg body weight, while the median lethal dose (LD_{50}) was 2mg/kg body weight for CPF after oral administration in Swiss Albino rats.

3.2.Clinical signs:

No signs of toxicity were observed in the control, HDN, Sinemet groups. Toxic signs recorded in the CPF group include tremor, weakness, bradykinesia and death in animals Table (2). Animals in the CPF+HDN and CPF+Sinemet groups manifested mild tremor, improvement in the motor coordination and less number in death.

Table (2): Effect of different treatment on the clinical signs in male rats through 6weeks.

Treatment		Week1	Week2	Week3	Week4	Week5	Week6	Total of death	Total number of rats
Control	Clinical signs	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
CPF	*Clinical signs*	*Slow motion*	*Slow motion & Tremor*			*Slow motion & excessive tremor*		6	10
	Number of death	1	-	1	1	1	2		
HDN	*Clinical signs*	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
Sinemet	Clinical signs	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
CPF+HDN	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*			-	-	1	10
	Number of death	1	-	-	-	-	-		
CPF+Sinemet	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*					2	10
	Number of death	-	-	1	1	-	-		
CPF+HDN+ Sinemet	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*				-	1	10
	Number of death	-	1	-	-	-	-		

3.3. Biochemical parameters

Concerning with the drugs used in this study, HDN treated group did not show statistically significant changes in MDA, GSH, TC, TG and glucose levels and SOD, CAT, GST and AchE activities compared to control group. Also, sinemet group showed no significant changes in SOD, CAT activities and TC levels compared to control group, however, GSH, MDA, GST, TG, glucose and AchE showed less significant changes (P<0.05) (Figures 1–9).

3.3.1. Malondialdehyde (MDA) levels:

The data summarized in Figure (1) indicated that, MDA levels were significantly increased (p<0.05) in rats received CPF alone or in combination with HDN and/or sinemet (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet groups) when compared to control group. However, the same parameter of the latter three groups was significantly decreased (p<0.05) when compared to CPF treated group. Also, the positive effect of HDN was detected in CPF+HDN and CPF+HDN+Sinemet groups by decreasing in MDA level while, the sinemet did not show the same effect in CPF+Sinemet group comparing to control.

3.3.2.. Superoxide dismutase (SOD) activity:

Figure (2) showed a significant increase ($p<0.05$) in SOD activity in groups treated with CPF, CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet. Also, the figure clarified the alleviation effect of HDN which exceeded the positive effect of sinemet. On the other hand, SOD activity was significantly sharp decreased in CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet treated groups compared with CPF-treated group. In addition, Figure (2) demonstrated the positive effect of HDN in CPF+HDN and CPF+HDN+Sinemet groups as detected by the significant decreasing (p<0.05) activity of SOD.

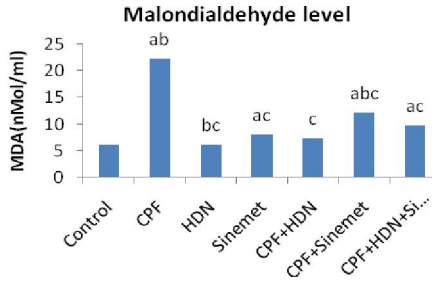


Figure (1): Levels of MDA in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group Significance at p< 0.05. [a] Significant difference from control and other groups.[b] Significant difference from sinemet group and other groups. [c] Significant difference from chlorpyrifos group and other groups. CPF: chlorpyrifos; HDN: hesperidin.

3.3.3. Catalase (CAT) activity:

The present study revealed that, CAT activity was significantly decreased (p<0.05) in rats received CPF alone or combined with sinemet and/or HDN when compared with the control group, while the same parameter was significantly increase (p<0.05) in the mixed groups (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet) compared to CPF group as shown in Figure (3).

3.3.4. Glutathione-S-transferase (GST) activity:

Figure (4) demonstrated the positive effect of HDN in all groups containing it either alone or mixed with CPF and/or sinemet as detected by the significant increase (p<0.05) of GST activity compared to CPF-treated group. On the other hand, a significant decrease (p<0.05) was detected in the

activity of the same enzyme in CPF and Sinemet groups compared to the control group.

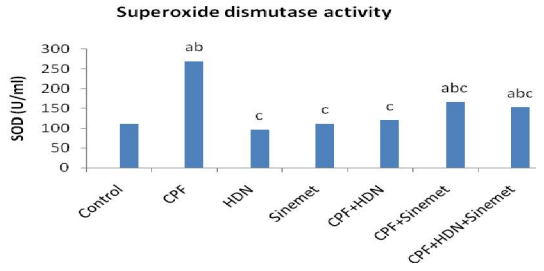


Figure (2): SOD activityin the plasma of male albino ratsin control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference fromchlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

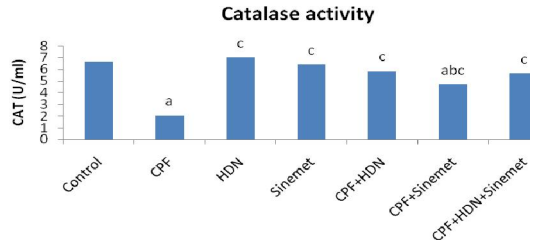


Figure (3): CAT activity in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference fromchlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

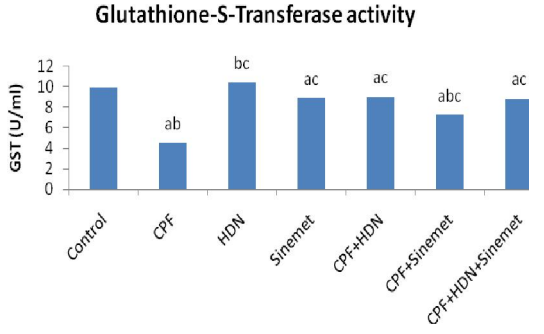


Figure (4): GST activity in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.5. Reduced glutathione (GSH) levels:

Figure (5) indicated that, CPF treatment led to a significant decrease (p<0.05) in GSH levels, while HDN showed significant recovery of this parameter (p<0.05) by enhancing its concentration in plasma. Treatment with HDN alone or mixed with CPF and/or Sinemet significantly alleviated the undesirable decreased levels of GSH comparing with CPF treated animals. It was also obvious that, the effect of HDN surpassed the traditional effect of sinemet.

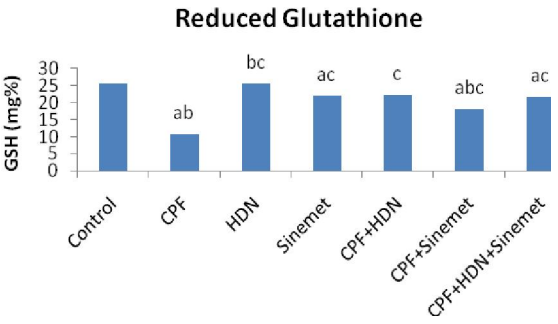


Figure (5): Levels of GSH in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05.
[a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.6. Total cholesterol (TC) levels:

The data summarized in Figure (6) indicated that, TC levels were significantly increased (p<0.05) in rats received CPF alone or in combination with HDN and/or sinemet (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet groups) when compared to control group. However, the same parameter of the latter three groups was significantly decreased (p<0.05) when compared to CPF treated group. Also, the positive effect of HDN was detected in CPF+HDN and CPF+HDN+Sinemet groups by decreasing in TC levels while, the sinemet showed the same effect in CPF+Sinemet group with less degree comparing to control.

3.3.7. Triglycerides (TG) levels:

Figure (7) demonstrated the positive effect of HDN in all groups containing it either alone or mixed with CPF and/or sinemet as detected by the significant decrease (p<0.05) of plasma TG levels compared to CPF-treated group. On the other hand, a significant increase (p<0.05) was detected in the TG level in CPF and Sinemet groups compared to the control group

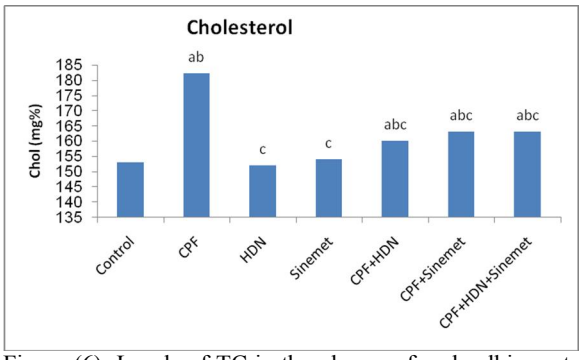


Figure (6): Levels of TC in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05.
[a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

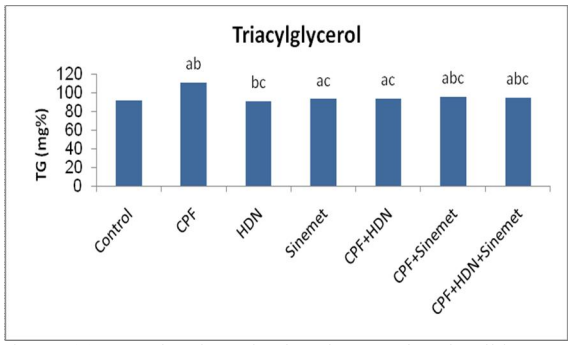


Figure (7): Levels of TG in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups. [b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.8. Glucose levels:

The present study revealed that, glucose level was significantly increased (p<0.05) in rats received CPF alone or combined with sinemet and/or HDN when compared with the control group, while the same parameter was significantly decrease (p<0.05) in the mixed groups (CPF+HDN, CPF+ Sinemet and CPF+HDN+Sinemet) compared to CPF group as shown in Figure (8).

3.4.Brain tissue

3.4.1.Estimation of acetyl cholinesterase (AchE):

The activity of AchEin brain tissues was highly significantly decreased in all CPF groups compared to the control group, while a significant increase (P < 0.05) was detected in all sinemt groups compared with CPF-treated group Figure (9).Simultaneous treatment with HDN (HDN, CPF+HDN and CPF+HDN+sinemt groups) significantly abolished the inductive effect of CPF on AchE activity, this process expressed asenhancing the level of the enzyme in brain tissue (p<0.05).

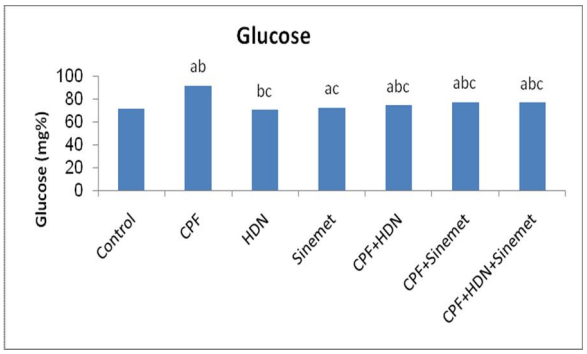


Figure (8): Levels of glucose in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

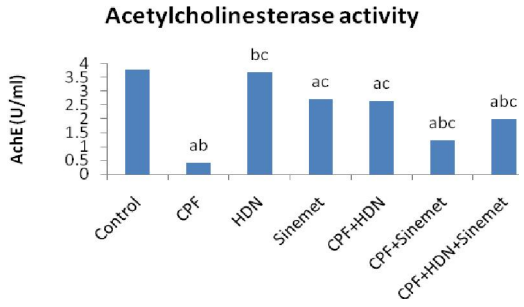


Figure (9): AchE activity in the brain tissue of male albino rats in control and experimental groups.Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.4.2.Cytogenetic studies:

3.4.2.-Bone marrow micronucleus (MN) assay:

At the end of the treatment, there were no statistically significant changes in number of MNPCEs in HDN treated group compared with the control group. While, the comparison between the sinemet treated group and control group showed less significant changes in these parameters. On the other hand, CPF-treated group showed a sharp significant increase in the number of MNPCEs, indicating the occurrence of chromosome damages, compared with the control group Table (3).

Table(3): Results of bone marrow micronucleus assay in control and experimental groups

Groups	Number of MNPCEs per 3000 PCEs
Control	(11±2.5)
CPF	(115±20)[ab]
HDN	(10±2)[c]
Sinemet	(14±2.6)[c]
CPF+HDN	(23±3.4)[abc]
CPF+Sinemet	(46±8.4)[abc]
CPF+HDN+Sinemet	(30±4.3)[abc]

Data are represented as means ± SD; n=10 for each group; Significance at $p< 0.05$.
[a]Significant difference from control and other groups.[b] Significant difference from sinemet group and other groups. [c] Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin; MNPCEs: micronucleated polychromatic erythrocytes

3.4.3.Determination of DNA damage (Comet assay):

Compared to the control group, there was a significant increase in the frequency (percentage) of MNPCEs in CPF-treated groups indicating the occurrence of chromosome damages, while comparing to CPF treated group, there was a significant decreased in the number of MNPCEs in all HDN and sinemet groups even those treated with CPF but the decrease in the CPF+HDN group was significantly sharp supporting that HDN was more effecting in treatment than sinemet.

In cytogenetics study, the DNA damage was assigned in the different groups by the comet assay. Following single-cell electrophoresis, the lengths of the comets (DNA tails) relied on the treatment, where longer tails indicating more DNA damage. Untreated control group and HDN group showed undamaged cell, most of the DNA is located in the head of the comet and no tails are evident,(Fig (10A) and (10B), respectively), which means no DNA damage is observed, while Fig (10C) illustrated the presence of less DNA in the tail (small tail length) in Sinemet.

On the other hand, Fig (10D) showed apoptotic cell, the nucleotide core is not distinguishable and comet tail was sharply longer after treatment of rats with CPF. For mixed groups, Fig (10E) showed a less significant damage of DNA in CPF+HDN treated groups, indicating the positive recovery impact of HDN to counteract the damage effect of CPF. Comet tails were substantially longer in CPF+Sinemet and CPF+HDN+Sinemet treated groups as shown in Fig (10F) and (10G), respectively. These results reflect the less effect of sinemet in ameliorating of the DNA damage.

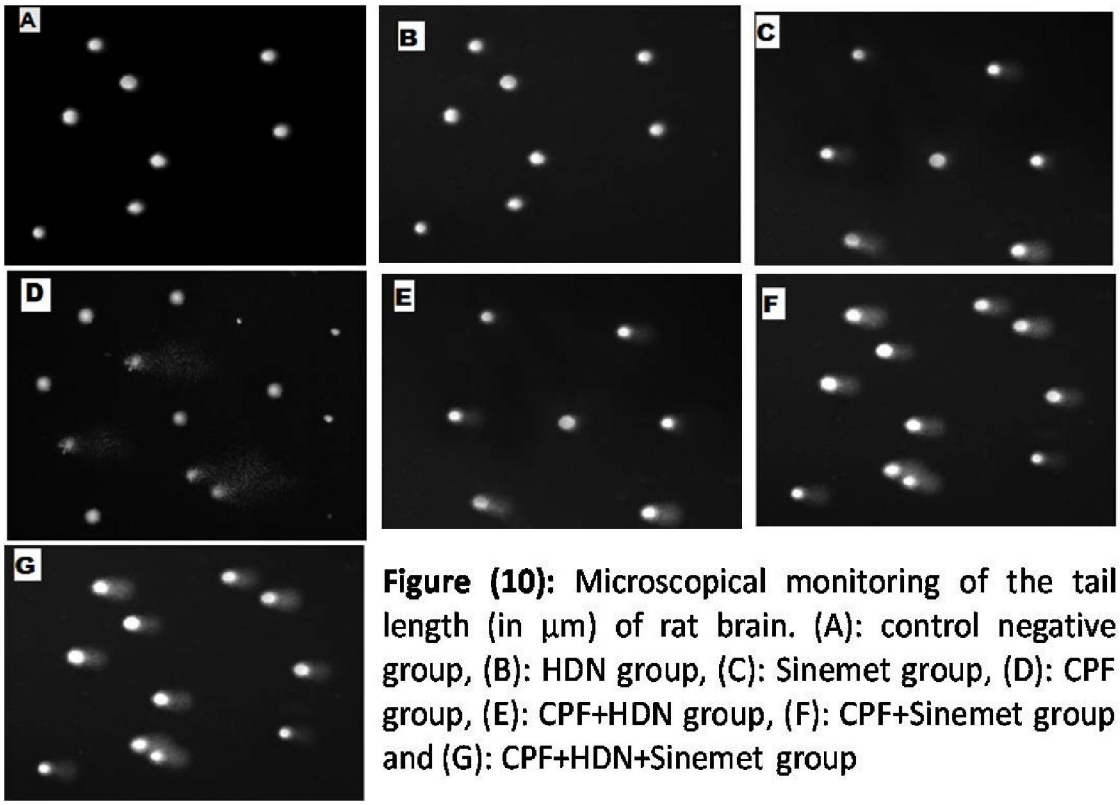


Figure (10): Microscopical monitoring of the tail length (in µm) of rat brain. (A): control negative group, (B): HDN group, (C): Sinemet group, (D): CPF group, (E): CPF+HDN group, (F): CPF+Sinemet group and (G): CPF+HDN+Sinemet group

4. Disscusion

The widespread use of pesticides and herbicides in the environment is of increasing concern because of their effects on the human health, wildlife, and ecosystems. Organophosphate (OP) pesticides are among the leading chemicals widely used for agricultural pest control throughout the world ***(Mehta et al., 2009)***.

Numerous experimental, epidemiological and clinical data showed the connection between both acute and chronic exposure to OP pesticides and damage to the nervous system ***(Lasram et al., 2009;SekarBabu et al., 2010)***. The neuro-toxic action of these compounds has been mainly explained by their ability to inhibit the activities of Ach E which is responsible for hydrolysis of acetylcholine playing a principal role in the transmission of neural impulses in the central and peripheral nervous system ***(Lotti, 2001)***.

An important causative role in the development of diseases of the nervous system has been played by the oxidative /antioxidative imbalance and disorders in lipid metabolism in the nervous tissue, as well as, a decrease in the number of the nervous cells ***(Oliveira and Di Paolo, 2010; Ziv and Melamed, 2010)***.

OP compounds have been reported to cause oxidative stress with consequent lipid per-oxidation and to induce apoptosis and necrosis in the brain under acute and chronic exposure ***(Üner et al., 2006; El-Demerdash, 2011)***. Moreover, it has been reported that OP pesticides can cross the blood–brain barrier (BBB) and cause a loss of neurons in Particular regions of the brain that results in subsequent cognitive decline, impaired memory and attention, and motor function ***(Parran et al., 2005)***. These neuro-behavioral disturbances may eventually lead to Alzhimer's disease, PD and other neuro-degenerative diseases ***(Kamel et al., 2007; Li et al., 2011)***.Epidemiologic studies reported that occupational exposures to OP insecticides are highly associated with increased risk of PD ***(Landrigan et al., 2005;Hancock et al., 2008)***.

One such OP which has spurred renewed interest is chlorpyrifos (CPF). CPF is an organophosphate insecticide known to produce oxidative stress in different human and animal tissues ***(Zama et al., 2007;Uzun et al., 2010)***. In previous studies, it is reported that CPF causes oxidative damage and histopathological changes in various tissues such as brain ***(Giordano et al., 2007)***. Due to the fact that the OPs; particularly CPF, causes oxidative damage in the central nervous system, CPF was used in this current study to be injected in male rats which were used as CPF model of Parkinson's disease. Previous studies showed that exposure of male rats to OP pesticides induced oxidative stress, hyperglycemia, Hypercholesterolemia, hyper-triglyceridemia DNA damage and neurotoxicity in various rat tissues ***(Aly et al., 2010; Acker & Nogueira, 2012).***

Reactive oxygen species (ROS) and its metabolites is the subject of intense research because of their active role in cellular physiology and pathogenesis of number of diseases. In healthy organisms, ROS production is counter balanced by antioxidant defense system to maintain an appropriate redox balance ***(Kruidenier and Verspaget, 2002)***. Organisms possess defense systems to escape the consequences of cell damage caused by ROS including enzymes such as catalase and superoxide dismutase and low molecular compounds, e.g., glutathione. Oxidative stress results when the balance between antioxidant systems and ROS is lost ***(Rao and Chhunchha, 2010)***.

Lipid per-oxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane fluidity and inactivation of a several membrane bound enzymes ***(Goel et al., 2005)***. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids, and thus increased MDA content is an important indicator of lipid per-oxidation ***(Demir et al., 2011)***. In this study, MDA levels were increased in the CPF treated groups. So, in agreement with ***Gultekin et al., (2000)***, it might be due to potent lipid per-oxidation effects of CPF.

SOD and CAT are the most important defense mechanisms against toxic effects of ROS. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, also termed as a primary defence, as it prevents further generation of free radicals. CAT converts hydrogen peroxide into water and molecular oxygen, and hence catalyzes its removal ***(Schneider and de Oliveira, 2004)***. It has been reported that the increased activity of SOD is known to serve as protective responses to eliminate reactive free radicals ***(Celik and Suzek, 2009)***. Other studies reported that increased SOD activity may induce cell death through the accumulation of hydrogen peroxide ***(Saggu et al., 1989)***. The present study demonstrated that CPF intoxication induces an elevation in SOD activity as well as a reduction in CAT activity. Similarly, in the previous studies, it has been reported that SOD activity increased in rat tissues by OP pesticides exposure ***(Sarabia et al., 2009)***. ***(Tuzmen et al., 2007; Shittu et al., 2012)*** showed that chronic administration of CPF causes the decrease in pituitary gland and testicular CAT activities. The decline in CAT activity, observed in this study, could be due to the excess production of superoxide radicals ***(Eraslan et al., 2007)*** since superoxide radicals converts feroxy state of CAT to ferryl state, which is an inactive form of the CAT ***(Freeman and Crapo, 1982)***. The increase in SOD activity and decrease in CAT

activity could have been related to increased oxidative stress caused by CPF exposure.

GSH has been reported to act as free radical scavenger and also to replenish intracellular stores of endogenous antioxidants, or as thiol-reducing agents ***(Valko et al., 2007)***. During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulfide compound, GSSG (oxidized form) ***(Meister and Anderson, 1983)***. In the current study, a significant depletion of GSH was noted in the CPF treated group. The decrease in GSH level could be due to the presence of free radicals produced by CPF. These effects have been previously observed *in vitro* and *in vivo* studies ***(Maran et al., 2009)***. ***Verma et al., (2007)*** proved that rats exposed to CPF exhibited reductions in GSH levels and increases in lipid peroxidation in the brains and livers. Glutathione-S-transferases (GST) are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to thiol group of GSH, producing less toxic forms ***(Mansour and Mossa, 2009)***. OP pesticides have been reported to significantly inhibit GST activity in various rat tissues ***(Khan and Kour, 2007)***. The results of the present study show a significant decrease in GST activity in CPF group which is in line with previous reported studies (***Kalender et al., 2012)***. The decrease in GST activity can be seen as a result of decreasing levels of GSH following CPF exposure ***(Rai and Sharma, 2007)***. In the present study, the decreased GST activities and GSH level might reflect cellular oxidative stress due to CPF exposure.

In the current study, we reported the hyperglycemic and hyperlipidemic effects of CPF after an administration in rats. Evidence has been found to suggest hyperglycemia as a characteristic outcome of CPF poisoning ***(Rahimi and Abdollahi, 2007)***. Hyperglycemia in experimental animals following acute exposure to OPs appears to be rapid in onset and transient in nature ***(Lasram et al., 2008;Joshi and Rajini, 2012)***. Hypercholesterolemic and hypertriglyceridemic effects indicate the toxicity of CPF. Accordingly, (***Acker & Nogueira, 2012)*** demonstrated that a single acute CPF administration in rats caused hyperglycemic and hyperlipidemic effects in rats.This increased level of cholesterol is attributed to the increased activity of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase) and increased incorporation of labelled acetate into cholesterol ***(Brunzell et al., 1983)***.

Neurotoxicity of OP compounds has been mainly explained by accumulation of acetylcholine (Ach), due to the inhibition of AChE activities, that produces cholinergic effects ***(Celik and Isik, 2009)***. AChE activity is known as biomarker of chronic toxicity in human ***(Singh et al., 2011)*** and experimental animals ***(Dwivedi and Flora, 2011)*** following pesticide exposure. The present study reported a significant decrease in brain AChE activity in the CPF group as compared to control. This result is in agreement with the previous studies that reported the inhibition of AChE activity in animals intoxicated with OP insecticides ***(Jintana et al., 2009; Catano et al., 2008)***. ***Darvesh et al., (2003)*** reported that the decreased AChE might be referred to the increase in lipid per-oxidation inducing deformities of cellular membranes, and disturbing metabolic and nervous activity.

CPF-exposed animals were investigated in the current study for genotoxic effects using the comet assay and micronucleus assay. CPF-induced DNA damage was observed in brain tissues, as evidenced by a significant increase in the Comet tail length (µm) in the exposed rats, which is consistent with other reports (***Shadnia et al., 2005;Atherton et al., 2009)***. In this context, a positive correlation was drawn between ROS generation and tail length in the exposed animals of this study. As shown the increase in the comet tail length due to DNA damage in CPF intoxicated rats may be due to the increase in the oxidative stress. ***Bagchi et al., (1995)*** examined that *in vitro* and*in vivo* administration of CPFresulted in two fold increases in DNA single strand breaks which may be due to the generation of ROS. CPF is known to inhibit P_{450} enzyme system and results in free radical production, which causes DNA damage ***(Dinsdale and Verschoyle, 2001)***. The current result was in agreement with ***Ding et al., (2012)*** who reported that the exposure of children to OP was associated with the increased generation of 8-hydroxydeoxyguanosine (8-OHdG), suggesting that exposure to OP may play an important role in DNA damage in children. In the present study, the results of CPF proved its toxic effect by causing of several changes like brain DNA damage, neurotoxicity, oxidative stress, hyperlipidemia, and hyperglycemia.

In the present study, some clinical signs were observed in the CPF group. The deficit in the motor coordination and appearance of tremor were occured in the rats of CPF group as signs of toxicity. Also, a number of rats were dead by administration of this insecticide. This finding is similar to those recorded by ***Ambali et al., (2010)***. Recently, these results were confirmed by ***Ambali and Aliyu, (2012)***. This may be due to damage to the portion of the brain responsible for motor coordination, probably due to oxidative stress as exemplified by increased oxidative stress, hyperlipidemia, hyperglycemia and DNA damage in the group. Also, the locomotor deficits recorded in the CPF group may be partly due to either oxidative damage to the muscle or impairment of neuronal transmission as a result of paralysis of the neuromuscular junction apparently resulting from prolonged AChE inhibition, leading to accumulation of

ACh in the cholinergic receptors in the peripheral and central nervous system. The accumulation of ACh in the cholinergic receptors leads to muscarinic, nicotinic and central nervous cholinergic syndromes ***(Eaton et al., 2008)***.

Although L-dopa remains as the gold standard replacement symptomatic therapy, it could contribute to bad prognosis of PD. A number of papers have suggested that L-dopa promotes neurodegeneration and accelerates progression of PD by way of oxidative metabolism ***(Du et al., 2009; Muller et al., 2004)***. In the present study, sinemet treated group showed a less significant increase in DNA damage with slightly increase in the lipid peroxidation and decrease in the levels of GSH, GST and AchE, compared to control group. It also showed a less significant increase in the levels of glucose and TG with slightly increase in TC levels. Numerous studies reported exogenous L-dopa could contribute to neurotoxicity via either oxidative stress as it has the potential to auto-oxidize to quinone and semiquinone, generating ROS and depleting striatal GSH ***(Hattoria et al., 2009)***, or by induction of apoptosis, as evidenced by accelerated activity of caspase-3 and DNA damage by a mechanism independent of oxidative stress ***(Maharaj et al., 2005; Pedrosa and Soaresda-Silva, 2002)***. The quinone products of L-Dopa auto-oxidation could also bind to and deplete GSH levels, further reducing antioxidant defenses available to dopamine neurons. But, there were no changes in the activities of SOD and CAT in sinemet treated group compared to control group. Moreover, L-Dopa treatment showed a hyperglycemic effect in the plasma of fasted wister rats ***(Furman and Wilson, 1979)***. Also, in this study, the treatment of CPF-intoxicated rats with sinemet showed a less significant amelioration in the antioxidant activities, MDA and GSH levels compared to the CPF treated group.

Dopaminergic neurons oxidize dopamine by monoamine oxidase, a reaction known to cause production of superoxide and hydrogen peroxide. Consequently, dopaminergic neurons are in a perpetual state of oxidative stress, and this imbalance may lead to reduced levels of endogenous antioxidants. The brain is only 2–3% of the total body mass, but it consumes 20% of body oxygen. Cells in the brain are particularly susceptible to oxidative damage due to high levels of polyunsaturated fatty acids in their membranes and relatively low activity of endogenous antioxidant enzymes ***(Miller et al., 2009)***. Recent studies demonstrated that adding L-dopa to the mesencephalic cultures showed a decrease in the number of viable cells, reduction in neurite length, DNA damage, and distinct signs of oxidative stress ***(Mosharov et al., 2009;Sabens et al., 2010)***. L-dopa toxicity was reduced in mesencephalic cultures obtained from parkin knockout mice that have increased content of glutathione ***(Casarejos et al., 2005)***. A reduction in the number of DAergic neurons was reported after direct intranigral infusion of L-dopa in rats ***(Reksidler et al., 2009)***. Brain infusion or *i.p.* injection of the drug increased the levels of hydroxyl radical ($^{\cdot}OH$) and nitric oxide (NO) in substantianigra and the striatum ***(Itokawa et al., 2006;Golembiowska et al., 2008)***. There were signs of lipid peroxidation in the cortex in monkeys treated with L-dopa (80 mg/kg with carbidopa or entacapone) for 13 weeks ***(Zeng et al., 2001; Lyras et al., 2002)***. ***Isobe et al., (2006)*** observed higher levels of 3- nitro-tyrosine (a marker of oxidative stress caused by $ONOO^{-}$) in the cerebrospinal fluid (CSF) of patients treated with L-dopa (450 mg, 8 weeks). Another effect of L-dopa therapy is an increase of homocysteine in the plasma level. Homocysteine levels correlate with the plasma levels of β-amyloid and α-synuclein, considered the systemic markers of neuro-degeneration ***(Obeid et al., 2009; Zoccolella et al., 2010)***.

Numerous neurodegenerative diseases such as PD are associated with neurodegeneration; describes the loss of neuronal structure and function. Nowadays, consumption of dietary flavonoids has been associated with reduced risk of neurodegenerative diseases in human ***(Weinreb et al., 2004; Sutherland et al., 2005)*** and considered as a neuroprotective agent against many neurodegenerative diseases in mice and rats ***(Zbarsky et al., 2005***; ***Kumar and Kumar, 2010)***. The antioxidant properties of flavonoids depend on both metal-chelating properties and free radical scavenging of ROS ***(Haller and Hizoh, 2004)***. HDN, a naturally occurring methoxylated citrus flavonoid presents in fruits and vegetables and hence lipophilic in nature, has been reported to exert a wide range of pharmacological effects including antioxidant, antihypercholesterolemic, anti-inflammatory, anticarcinogenic and neuroprotective actions ***(Tommasini et al., 2005; Ebrahimi and Schluesener 2012)***. It is able to penetrate BBB and used as a potential antioxidant neuroprotective agent ***(Youdim et al., 2003;Hwang and Yen, 2008)***. Over all above data suggests that HDN can be added to the growing list of dietary-derived polyphenolic compounds that exert beneficial actions in the central nervous system.In this study HDN was used as a neuroprotective drug alone and with sinemet in a CPF rat model of Parkinson's disease. The direct protective effect of HDN has been described in the various cell types of the central nervous system, increasing neuronal progenitors survival, improving neurobehavioral alterations, attenuating oxidative damage and restoring antioxidant and mitochondrial complex enzyme activities ***(Nones et al., 2010;Gaur et al., 2011)***.

In the present study, administration of HDN to CPF-treated group showed an improvement in the levels of endogenous antioxidant enzymes (SOD, CAT and GST), GSH and AchE. Also, HDN significantly attenuated the oxidative stress along with lipid peroxidation (MDA) compared with CPF-treated group. HDN supplementation lowered the levels of plasma total cholesterol and triglycerides. Antihypercholesterolamic activities of HDN in CCl4 induced hypercholesterolamic rats have been reported ***(Naveen et al., 2005)***. This observation was in line with previous studies that supplementation of HDN has been found to significantly lower the cholesterol level and triglyceride levels in nicotine-treated rats ***(Balakrishnan and Menon, 2007)***.

Hypocholesterolemic effect of HDN may be mediated via reduction in the HMG-CoA reductase activity ***(Lee et al., 1999)***. Moreover, HDN was recorded to ameliorate the hyperglycemic effect of CPF. This is in line with studies that reported the antihyperglycemic properties of HDN against diabetic complications in a rat model of type 2 diabetes mellitus (T2DM) by potentiating the antioxidant defense system ***(Abdel-moneim et al., 2011;Mahmoud et al.,2012)***.

Regarding to cytogenetics studies, HDN significantly decreased the genetic damage which visualized by the decrease in comet tail length in brain tissues and the number of MNPCEs in bone marrow compared with CPF-treated group. Moreover, HDN has a positive effect when combined with sinemet in CPF+HDN+Sinemet group by ameliorating the toxicity of CPF and the side effects of sinemet drug. On the other hand, HDN alone does not show any biochemical alterations or DNA damage which in turn indicating the protective nature of the product. Improvement of these results in CPF+HDN-treated rats in comparison to CPF intoxicated rats demonstrated the effectivness of HDN in the treatment of oxidative stress, DNA damage and neurotoxicity. This observation was in line with previous studies ***(Wilmsen et al., 2005;Balakrishnan and Menon, 2007)***. HDN treatment has been demonstrated to improve GSH levels in liver and kidneys and decrease in levels of 8-hydroxydeoxyguanosine (8-OHdG), a product of DNA damage, in the urine of diabetic rats ***(Miyake et al., 1998)***. A report by ***(Chen et al., 2010)*** showed that HDN was able to prevent tert-butyl hydroperoxide (t-BuOOH)-induced cell damage by augmenting cellular antioxidant defense. HDN has also been reported to act as a powerful consumer of superoxide, singlet oxygen and hydroxyl radicals ***(Pradeep et al., 2008)***, contributing significantly to the intracellular antioxidant defense system. Recently studies demonstrated that HDN has powerful protective effects against DNA damage induced by gamma irradiation in mice by effectively scavenging the free radicals that produces damage to DNA ***(Hosseinimehr et al., 2009; Kalpana et al., 2011)***. Further, ***Hosseinimehr and Nemati (2006)*** have also reported that HDN possesses strong anti-clastogenic activity against γ-irradiation induced bone marrow damage in mice by reducing the micronuclei frequency. ROS has the ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid per-oxidation and hence threat neuronal survival. The neurons are highly vulnerable to oxidative insults due to low level of GSH ***(Dringen, 2000)***. The present study recorded an increase in the content of GSH and decrease in the extent of lipid per-oxidation with the treatment of HDN. These results were in concordance with earlier reports, where flavonoids had been used for the treatment of different type of brain diseases ***(Jungsook, 2006;El-Sayed et al., 2008)***. It was also reported that HDN offers protection by terminating the lipid per-oxidation side chain rather than scavenging extracellular non-lipid radicals that initiate lipid per-oxidation ***(Heffner and Repine, 1989)***.

Treatment with HDN was shown to have Uimpaired by CPF. This reaffirms the fact that oxidative stress is partly involved in the short-term motor coordination deficit induced by acute CPF exposure. HDN was able to improve the efficiency of locomotion partly due to its antioxidant and AChE restoration properties which ultimately improve neuronal activity. Moreover, HDN is a good hypo-triglycerolemic and hypo-cholesterolemic agent ***(Steinberg and Khoo, 1977)*** as recorded in the present study.

Long term treatment with a flavonoid (epigallocatechingallate) increased the life span and enhanced movement abilities in a transgenic Drosophila melanogaster model of PD ***(Ortega-Arellano et al., 2011)***. Recently with respect to the brain, flavonoids, such as those found in berries and citrus, have been shown to be highly effective in preventing neurodegeneration in both animals and humans. Despite the need for these additional studies, the evidence to date strongly suggests that flavonoids represent precursor molecules in the quest to develop of a new generation of drugs capable of counteracting neuroinflammation and associated neurodegenerative disease ***(Spencer et al., 2012)***.

Conclusion:

In conclusion, Overall data suggested that HDN can be used as a therapeutical drug in neurodegenerative diseases by adding it to dietary-derived poly-phenolic compounds that exert beneficial actions in the central nervous system. Also, the results of present investigation suggest that HDN acts an efficient neuro-protector against the neuro-degeneration induced by CPF of Swiss albino rats and it could be recommended

as a disease-modifying therapy when given with sinemet early in course of Parkinson's disease.

5. Reference

1. ***Abdel-Moneim A., Ashour M.B., Mahmoud A.M., and Ahmed O.M., (2011):*** Insulin sensitizing effects of hesperidin and naringin in experimental model of induced type 2 diabetes in rats: focus on tumor necrosis factor-alpha and resistin. Nature and Science; 7(10): 134–141.
2. ***Acker C.I., and Nogueira C.W., (2012):*** Chlorpyrifos acute exposure induces hyperglycemia and hyper-lipidemia in rats. Chemosphere; 89: 602–608.
3. ***Aebi H., (1984):*** Catalase *in vitro*. Methods in Enzymology; 105:121-126.
4. ***Aly N., El-Gendy K., Mahmoud F., and El-Sebae A.K., (2010):*** Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pesticide and Biochemical Physiology; 97:7-12.
5. ***Ambali S.F., Idris S.B., Onukak C., Shittu M., and Ayo J.O., (2010):*** Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats. Toxicol. Ind. Health; 26: 547-558.
6. ***Angelis K.J., Dusinska M., and Collins A.R., (1999):*** Single cell gel electrophoresis: Detection of DNA damage at different levels of sensitivity. *Electrophoresis*; 20:2133-38.
7. ***Atherton K.M., Williams F.M., Gonzez F.J., Glass R., Rushton S., Blain P.G., and Mutch E., (2009):*** DNA damage in horticultural farmers: a pilot study showing an association with organophosphate pesticide exposure. Biomarkers; 14(7):443-451.
8. ***Bagchi D., Bagchi M., Hassoun E.A., and Stohs S.J., (1995):*** In vitro and in vivo generation of reactive oxygen species. DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology; 104:129-140.
9. ***Balakrishnan A., and Menon V.P., (2007):*** Protective effect of hesperidin on nicotine induced toxicity in rats. Indian Journal of Experimental Biology; 45:194-202
10. ***Beutler E., Duron O., and Kelly B.M., (1963):*** Improved method for the determination of blood glutathione. J Lab Clin Med.*; 61:882–888.*
11. ***Bharath S., Hsu M.,Kaur D.,Rajagopalan S.,and Andersen J.K., (2002):*** Glutathione, iron and Parkinson's disease. Biochem.Pharmacol.;64:1037-1048.
12. ***Blessing H., Bareiss M., Zettlmeisl H., Schwarz J., and Storch A., (2003):*** Catechol-O-methyltransferase inhibition protects against 3,4-dihydroxyphenylalanine (DOPA) toxicity in primary mesencephalic cultures: new insights into levodopa toxicity. Neurochem. Int.; 42(2): 139-151.
13. ***Bortolato M., Chen K.,and Shih J.C., (2008):*** Monoamine oxidase inactivation: from pathophysiology to therapeutics. Adv. Drug Deliv. Rev.; 60: 1527-1533.
14. ***Brunzell J.D., Miller N.E., Alaupovic P., St. Hilaire R.J., and Wang C.S., (1983):*** Familial chylomicronemia due to a circulation inhibitor of lipoprotein lipase activity. *J. Lipid Res*.; 24: 12.
15. ***Cai Y.Z., Sun M., Xing J., Luo Q., and Corke H., (2006):*** Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci.;78:2872-2888.
16. ***Casarejos M.J., Solano R.M., Mene´ ndez J., Rodrı´guez-Navarro J.A., Correa C., Garcı´a de Ye´ benes J., and Mena M.A., (2005):*** Differential effects of L-DOPA on monoamine metabolism, cell survival and glutathione production in midbrain neuronal enriched cultures from parkin knockout and wild-type mice. J. Neurochem.; 94(4):1005-1014.
17. ***Catano H.C., Carranza E., Huamani C., and Hernandez A.F., (2008):*** Plasma cholinesterase levels and health symptoms in Peruvian farm workers exposed to organophosphate pesticides. Arch. Environ. Contam.Toxicol.; 55(1):153-159.
18. ***Celik I., and Isik I., (2009):*** Neurotoxic effects of subacute exposure of dichlorvos and methyl parathion at sublethal dosages in rats. Pest Biochem. Physiol.; 94:1-4.
19. ***Celik I., and Suzek H., (2009):*** Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid per-oxidation in rats. Ecotoxicology and Environmental Safety; 72:905-908.
20. ***Çetin E., Kanbur M., Silici S., and Eraslan G., (2010):*** Propetamphos-induced changes in haematological and biochemical parameters of female rats: protective role of propolis. Food Chem. Toxicol.; 48: 1806–11810.
21. ***Chen M., Gu H., Ye Y., Lin B., Sun L., Deng W., Zhang J., and Liu J., (2010):*** Protective effects of hesperidin against oxidative stress of tert-butyl hydroperoxide in human hepatocytes. Food and Chemical Toxicology; 48:2980-2987.
22. ***Costa L.G., (2006):*** Current issues in organophosphate toxicology. Clin.Chim.Acta; 366:1-13.
23. ***D'Souza U.J.A., Zain A., and Raju S., (2002):***Genotoxic and cytotoxic effects bone marrow of rats exposed to low dose of paquat via the dermal route. *Mutation Research*; 581:187-190.
24. ***Dagda R.K., and Chu C.T., (2009):*** Mitochondrial quality control: insights on how Parkinson's disease related genes PINK1, parkin, and Omi/HtrA2 interact to maintain mitochondrial homeostasis. J. Bioenerg. Biomembr.; 41: 473-479.
25. ***Darvesh S., Hopkins D.A., and Geula C., (2003):*** Neurobiology of butyryl cholinesterase. Nat Rev Neurosci.; 4:131-8.
26. ***Demir F., Uzun F.G., Durak D., and Kalender Y., (2011):*** Sub-acute-chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. Pesticide Biochemistry and Physiology; 99:77-81.
27. ***Ding D., Han S., Wang P., Gao Y., Shi R., Wang G., and Tian Y., (2012):*** Increased levels of 8-hydroxy-2'-deoxyguanosine are attributable to organophosphate pesticide exposure among young children. Environmental Pollution; 167:110-114.
28. ***Dinsdale D., and Verschoyle R.D., (2001):*** Cell-specific loss of cytochrome Microorganisms450 2B1 in rat lung following treatment with pneumotoxic and non-pneumotoxic trialkyl phosphorothioates. Biochem Pharmacol.; 61:493-501.
29. ***Dorsey E.R., Constantinescu R., Thompson J.P., Biglan K.M., Holloway R.G., Kieburtz K., (2007):*** Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology; 68:384-386.
30. ***Dringen R., (2000):*** Metabolism and functions of glutathione in brain. Prog. Neurobiol.; 62:649-671.
31. ***Du F., Qian Z.M., Zhu L., Wu X.M., Yung W.H., Tsim T.Y., and Ke Y., (2009):*** l-DOPA neurotoxicity is mediated by up-regulation of DMT1-IRE expression. PLoS One 2, e4593.
32. ***Dwivedi N., and Flora S.J.S., (2011):*** Concomitant exposure to arsenic and organophosphates on tissue oxidative stress in rats. Food Chem.Toxicol.; 49:1152-9.
33. **Eaton D.L., Daroff R.B., Autrup H., Bridge J., P. Buffler P., Costa L.G., Coyle J., Mckhann G., Mobley W.C., Nadel L., Neubert D., Schulte-Hermann R., Spencer P.S., (2008):** Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol.; 38: 1-125.
34. ***Ebrahimi A., and Schluesener H., (2012):*** Natural polyphenols against neurodegenerative disorders: Potentials and pitfalls. Ageing Research Reviews; 11: 329-345.
35. ***El-Demerdash F.M., (2011):*** Lipid peroxidation, oxidative stress and acetyl cholinesterase in rat brain exposed to organophosphate and pyretroid insecticides. Food Chem Toxicol; 49:1346–52.
36. ***El-Sayed S.M., Abo-Salem O.M., Abd-Ellah M.F., and Abd-Alla G.M., (2008):*** Hesperidin, an antioxidant flavonoid, prevents acrylonitrile-induced oxidative stress in rat brain. J. Biochem. Mol. Toxicol.; 22:268-273.
37. ***Eraslan G., Saygi S., Essiz D., Aksoy A., Gul H., and Macit E., (2007):*** Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocyanidin and Nacetylcysteine against exposure to cyfluthrin in mice, Pestic. Biochem. Phys.; 88:43-49.
38. ***Fassati P., and Prencipe L., (1982):*** Triglycerides enzymatic colormetric method. Clin. Chem., 20: 2077-2083.
39. ***Freeman B.A., and Crapo J.D., (1982):*** Biology of disease: free radicals and tissue injury, Lab. Invest.; 47:412-426.
40. ***Furman B.L., Wilson G.A., (1979):*** The effects of levodopa on plasma glucose in two strains of rat**.** European Journal of Pharmacology; 55(3): 241–246.
41. ***Galloway T., and Handy R., (2003):*** Immunotoxicities of organo-phosphorus pesticides. Ecotoxicology; 12: 345–363.
42. ***Garg A., Garg S., Zaneveld L.J., and Singla A.K., (2001):*** Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. Phyto. ther. Res.; 15:655-669.
43. ***Gaur V., Aggarwal A., and Kumar A., (2011):*** Possible nitric oxide mechanism in the protective effect of hesperidin against

ischemic reperfusion cerebral injury in rats. Indian J. Exp. Biol.; 49:609–618.

44. ***Giordano G., Afsharinejad Z., Guizetti M., Vtalone A., Kavanagh T.J., and Costa L.G., (2007):*** Organo phosphorus insecticides chlorpyrifos and diazinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. Toxicology and Applied Pharmacology; 219:181–189.
45. ***Goel A., Dani V., and Dhawan D.K., (2005):*** Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chemico-Biological Interactions; 156:131-140.
46. ***Golembiowska K., Dziubina A., Kowalska M., and Kamin´ ska K., (2008):*** Paradoxical effects of adenosine receptor ligands on hydroxyl radical generation by L-DOPA in the rat striatum. Pharmacol. Rep.; 60(3):319-330.
47. ***Goole J., and Amighi K., (2009):*** Levodopa delivery systems for the treatment of Parkinson's disease: An overview. International Journal of Pharmaceutics; 380: 1-15.
48. ***Gultekin F., Ozturk M., and Akdogan M., (2000):*** The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid per-oxidation and antioxidant enzymes (in vitro), Arch. Toxicol.; 74:533-538.
49. ***Habig W.H., Pabst M.J., and Jacob W.B., (1974):*** Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
50. ***Haller C., and Hizoh I., (2004):*** The cyto-toxicity of iodinated radiocontrast agents on renal cells in vitro. Invest. Radiol.; 39:149-154.
51. Hancock D.B., Martin E.R., Mayhew G.M., StajichJ.M., Jewett R., Stacy M.A., Scott B.L., Vance J.M., and Scott W.K., (2008): Pesticide exposure and risk of Parkinson's disease: a family-based case–control study, BMC Neurol;**8**:6.
52. ***Hardie R.J., Malcom S.L., and Lees A.J., (1986):*** The pharmacokinetics of intravenous and oral levodopa in patients with Parkinson's disease who exhibit on-off fluctuations. Br. J. Pharmacol.; 22:429-436.
53. ***Hatcher J.M., Pennell K.D., and Miller G.W., (2008):*** Parkinson's disease and pesticides: a toxicological perspective. Cell Press; 322-329.
54. ***Hattoria N., Wanga M., Taka H., Fujimura T., Yoritaka A., Kubo S., and Mochizuki H., (2009):*** Toxic effects of dopamine metabolism in Parkinson's disease. Parkinsonism Relat. Disord.; 15(Suppl. 1): S35-S38.
55. ***Heffner J.E., and Repine J.E., (1989):*** Pulmonary strategies of antioxidant defense. Am. Rev. Respir. Dis.; 140:531-554.
56. ***Henry R.J., (1974):*** Clinical Chemistry: Principles and Techniques, Second Edition, Harper and Row, Publishers, Inc., New York, p. 917.
57. ***Hosseinimehr S.J., and Nemati A., (2006):*** Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. Br. J.Radiol.; 79:415-418.
58. ***HosseinimehrS.J., Ahmadi A., Beiki D., Habibi E., and Mahmoudzadeh A., (2009):*** Protective effects of hesperidin against genotoxicity induced by 99mTc-MIBI in human cultured lymphocyte cells. Nuclear Medicine and Biology; 36:863-867.
59. ***Hwang S.L., and Yen G.C., (2008):*** Neuroprotective effects of the citrus Flavanones against H2O2-induced cytotoxicity in PC12 cells. J. Agric. Food Chem.; 56:859-864.
60. ***Isobe C., Abe T., Kikuchi T., Murata T., Sato C., and Terayama Y., (2006):*** Cabergoline scavenges peroxynitrite enhanced by L-DOPA therapy in patients with Parkinson's disease. Eur. J. Neurol.; 13(4):346-350.
61. ***Itokawa K., Ohkuma A., Araki N., Tamura N., and Shimazu K., (2006):*** Effect of L-DOPA on nitric oxide production in striatum of freely mobile mice. Neurosci.Lett.; 402(1–2):142-144.
62. ***Jankovic J., (2008):*** Parkinson's disease: clinical features and diagnosis. J. Neurol. Neurosurg. Psychiatr.; 79(4): 368-376.
63. ***Jintana S., Sming K., Krongtong Y., and Thanyachai S., (2009):*** Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. Int. Arch. Occup. Environ. Health; 82(7):833-842.
64. **Johnson M.D., Yu L.R.,Conrads T.P., Kinoshita Y.,Uo T., McBee J.K.,Veenstra T.D., and Morrison R.S.,(2005):**The proteomics of neurodegeneration. Am. J. Pharmacogenomics; 5: 259-270.
65. ***Joshi A.K.R., and Rajini P.S., (2009):*** Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: role of gluconeogenesis. Toxicology; 257: 40–45.
66. ***Joshi A.K.R., and Rajini P.S., (2012):*** Hyperglycemic and stressogenic effects of monocrotophos in rats: evidence for the involvement of acetyl cholinesterase inhibition. Exp. Toxicol. Pathol.; 64: 115–120.
67. ***Jungsook C., (2006):*** Antioxidant and neuroprotective effects of hesperidin and its aglyconehesperetin. Arch. Pharm. Res.; 29:699-706.
68. ***Kalender Y., Kaya S., Durak D., Uzun F.G., andDemir F., (2012):*** Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histo-architecture induced by chlorpyrifos in male rats. Environmental toxicology and pharmacology; 33:141-148.
69. ***Kalpana K.B., Devipriya N., Srinivasan M., Vishwanathan P., Thayalan K., and Menon V.P., (2011):*** Evaluating the radioprotective effect of hesperidin in the liver of Swiss albino mice. European Journal of Pharmacology; 658:206-212.
70. ***Kamath V., and Rajini P.S., (2007):*** Altered glucose homeostasis and oxidative impairment in pancreas of rat subjected to dimethoate intoxication. Toxicology; 231: 137–146.
71. ***Kamath V., Joshi A.K.R., and Rajini P.S., (2008):*** Dimethoate induced biochemical perturbations in rat pancreas and its attenuation by cashew nut skin extract. Pest. Biochem. Physiol.; 90: 58–65.
72. **Kamel F., Tanner C., Umbach D., Hoppin J., Alavanja M., Blair A., Comyns K., Goldman S., Korell M., Langston J., Ross G., and Sandler D., (2007):** Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am. J. Epidemiol; 165: 364–374.
73. ***Keeney P.M., Xie J., Capaldi R.A., and Bennett J.P. Jr., (2006):*** Parkinson's disease brain mitochondrial complex I hasoxidatively damaged subunits and is functionally impaired and misassembled. J.Neurosci.; 26:5256-5264.
74. ***Khan S.M., and Kour G., (2007):*** Subacute oral toxicity of chlorpyrifos and protective effect of green tea extract. Pesticide and Biochemical Physiology; 89:118-123.
75. ***Kruidenier L., and Verspaget H.W., (2002):*** Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease – radicals or ridiculous. Aliment Pharm. Therap.; 16:1997-2015.
76. ***Kumar P., and Kumar A., (2010):*** Protective effect of hesperidin and naringin against 3-nitropropionic acid induced Huntington's like symptoms in rats, Possible role of nitric oxide. Behav. Brain Res.; 206:38-46.
77. ***Landrigan P.J., SonawaneB., Butler R.N., TrasandeL., CallanR., and Droller D., (2005):*** Early environmental origins of neurodegenerative disease in later life, Environ. Health Perspect; 113: 1230–1233.
78. ***Lasram M.M., Annabi A.B., Elj N.E., Selmi S., Kamoun A., El-Fazaa S., and Gharbi N., (2009):*** Metabolic disorders of acute exposure to malathion in adult Wistar rats. J. Hazard. Mater.; 163: 1052–1055.
79. ***Lasram M.M., Annabi A.B., Rezg R., Elj N., Slimen S., Kamoun A., El-Fazaa S., and Gharbi, N., (2008):*** Effect of short-time malathion administration on glucose homeostasis in Wistar rat. Pestic. Biochem. Physiol.; 92: 114–119.
80. ***Lee S.H., Jeong T.S., Park Y.B., Kwon Y.K., Choi M.S., and Bok S.H., (1999):*** Hypo-cholesterolemic effect of hesperetin mediated by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyl transferase in rats fed high cholesterol diet. Nutrition Research; 19: 1245–58.
81. ***Leppert P.S., Cortese M., and Fix J.A., (1988):*** The effects of carbidopa dose and time and route of administration on systemic l-dopa levels in rats. Pharm. Res.; 5: 587-591.
82. ***Levy O.A., Malagelada C., Greene L.A., (2009):*** Cell death pathways in Parkinson's disease: Proximal triggers, distal effectors, and final steps. Apoptosis; 14:478-500.
83. ***Li Y., Lein P.J., Liu C., Bruun D.A., Tewolde T., and Ford G., (2011):*** Spatiotemporal pattern of neuronal injury by DFP in rats: a model for delayed neuronal cell death following acute OP intoxication. Toxicol Appl Pharmacol; 253:261–9.

84. ***Löhle M., and Reichmann H., (2010):*** Clinical neuro-protection in Parkinson's disease–Still waiting for the breakthrough. Journal of the Neurological Sciences; 289:104-114.
85. ***Lotti M., (2001):*** Clinical toxicology of anti-cholinesterase agents in humans. In: Krieger R, editor. Handbook of pesticide toxicology, vol. 2. USA: Academic Press; P. 1043–86.
86. ***Lyras L., Zeng B.Y., McKenzie G., Pearce R.K., Halliwell B., and Jenner P., (2002):*** Chronic high dose L-DOPA alone or in combination with the COMT inhibitor entacapone does not increase oxidative damage or impair the function of the nigro-striatal pathway in normal cynomologus monkeys. J. Neural Transm.; 109(1):53-67.
87. ***Maharaj H., Maharaj D.S., Scheepers M., Mokokong R., and Daya S., (2005):***L-dopa administration enhances 6-hydroxydopamine generation. Brain Res.; 1063:180-186.
88. ***Mahmoud A.M., Ashour M.B., Abdel-Moneim A., and Ahmed O.M., (2012):*** Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and pro-inflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats. Journal of Diabetes and its Complications. doi:10.1016/j.jdiacomp.2012.06.001. In press.
89. ***Mansour S.A., and Mossa A.H., (2009):*** Lipid per-oxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pesticide and Biochemical Physiology; 93:34-39.
90. ***Maran E., Fernandez M., Barbieri P., Font G., and Ruiz M.J., (2009):*** Effects of four carbamate compounds on antioxidant parameters. Ecotoxicol. Environ. Saf.; 72:922-930.
91. ***Mehta A., VermaR.S.,and Srivastava N., (2009):***Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver. Pesticide and Biochemical Physiology 94, 55–59.
92. ***MeisterA., and Anderson M.E., (1983):*** Glutathione. Ann. Rev. Biochem.; 52:711-760.
93. ***Miller R.L., James-Kracke M., Sun G.Y., and Sun A.Y., (2009):*** Oxidative and inflammatory pathways in Parkinson's disease. Neurochem. Res.; 34 (1):55-65.
94. ***Miyake Y., Yamamoto K., Tsujihara N., and Osawa T., (1998):*** Protective effects of lemon flavonoids on oxidative stress in diabetic rats. Lipids; 33:689-695.
95. ***Moreno M., Ca˜nadas F., Cardona D., Su˜nol C., Campa L., S´anchez-Amate M.C., Flores P., and Sanchez-Santed F., (2008):*** Long-term monoamine changes in the striatum and nucleus accumbens after acute chlorpyrifos exposure. Toxicology Letters; 176:162-167.
96. **Mosharov E.V., Larsen K.E., Kanter E., Phillips K.A., Wilson K., Schmitz Y., Krantz D.E., Kobayashi K., Edwards R.H., and Sulzer D., (2009):** Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantianigra neurons. Neuron; 62(2):218-229.
97. **Muller T., Hefter H., Hueber R., Jost W.H., Leenders K.L., Odin P., and Schwarz J., (2004):** Is levodopa toxic? J. Neurol.; (Suppl. 6):44-46.
98. ***Nagatsua T., and Sawadab M., (2009):*** L-dopa therapy for Parkinson's disease: past, present, and future. Parkinsonism Relat. Disord.; 15(Suppl 1):S3–S8.
99. ***Naoi M., and Maruyama W., (2010):*** Monoamine oxidase inhibitors as neuroprotective agents in age-dependent neurodegenerative disorders. Curr. Pharm. Des.; 16: 2799-2817.
100. ***Naveen T., Sangeeta P., Anurag K., and Kanwaljit C., (2005):*** Hesperidin, A citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacology; 5: 1.
101. ***Nishikimi M., Roa N.A., and Yogi K., (1972):*** Measurement of superoxide dismutase. Biochem. Biophys. Res. Common., 46: 849-854.
102. ***Nishioka K.,Vilariño-Güell C., Cobb S.A.,Kachergus J.M., Ross O.A.,Hentati E.,Hentati F., andFarrer M.J., (2010):***Genetic variation of the mitochondrial complex I subunit NDUFV2 and Parkinson's disease. Parkinsonism Relat. Disord.; 10: 686-687.
103. ***Nones J., Stipursky J., CostaL.C., and Gomes F.C.A., (2010):*** Flavonoids and astrocytes crosstalking: implications for brain development and pathology. Neurochem. Res.; 35:955-966.
104. ***Obeid R., Schadt A., Dillmann U., Kostopoulos P., Fassbender K., and Herrmann W., (2009):*** Methylation status and neurodegenerative markers in Parkinson disease. Clin. Chem.; 55(10):1852-1860.
105. ***Ohkawa, H, Ohishi, N and Yagi, K. (1979):*** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351–358.
106. ***Oliveira T.G., and Di Paolo G., (2010):*** Phospholipase D in brain function and Alzheimer's disease. Biochem Biophys Acta; 1801:799–805.
107. ***Ortega-Arellano H.F., Jimenez-Del-Rio M., and Velez-Pardo C., (2011):*** Life span and locomotor activity modification by glucose and polyphenols in Drosophila melanogaster chronically exposed to oxidative stress-stimuli: implications in Parkinson's disease. Neurochem. Res.; 36:1073-1086.
108. ***Parran D.K., Magnin G., li W., Jortner B.S., and Ehrich M., (2005):*** Chlorpyrifos alters functional integrity and structure of an in vitro bbb model: co-cultures of bovine endothelial cells and neonatal rat astrocytes, Neuro-toxicology; 26: 77–88.
109. ***Pedrosa R., and Soares-da-Silva P., (2002):*** Oxidative and non-oxidative mechanisms of neuronal cell death and apoptosis by l-3,4dihydroxyphenylalanine (l-DOPA) and dopamine. Br. J. Pharmacol.; 137(8):1305-1313.
110. ***Pradeep K., Park S.H., and Ko K.C., (2008):*** Hesperidin a flavano-glycone protects against γ-irradiation induced hepato-cellular damage and oxidative stress in Sprague–Dawley rats. Eur. J. Pharmacol.; 587:273-280.
111. ***Rahimi R., and Abdollahi M., (2007):*** A review on mechanisms involved in hyperglycemia induced by organo-phosphorus insecticides. Pest. Biochem. Physiol.; 88: 115–121.
112. ***Rai D.K., and Sharma B., (2007):*** Carbofuran-induced oxidative stress in mammalian brain, Mol. Biotechnol.; 37:66-71.
113. ***Rao M.V., and Chhunchha B., (2010):*** Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid, Food Chem. Toxicol.; 48:7-10.
114. ***Reed L.H., and Muench H., (1938):*** A simple method of estimating fifty percent point. AM. J. Hyg.; 37: 493-501.
115. ***Reksidler A.B., Lima M.M., Dombrowski P.A., Barnabe G.F., Andersen M.L., Tufik S., and Vital M.A., (2009):*** Distinct effects of intranigral L-DOPA infusion in the MPTP rat model of Parkinson's disease. J. Neural Transm. Suppl.; (73):259-268.
116. ***Richmond W., (1973):*** Determination of cholesterol by enzymatic colormetric method. Clin. Chem.; 19: 1350-1361.
117. ***Sabens E.A., Distler A.M., and Mieyal J.J., (2010):*** Levodopa deactivates enzymes that regulate thiol-disulfide homeostasis and promotes neuronal cell death: implications for therapy of Parkinson's disease. Biochemistry; 49(12):2715-2724.
118. ***Saggu H., Cooksey J., Dexter D., Wells F.R., Lees A., Jenner P., and Marsden C.D., (1989):*** A selective increase in particulate superoxide dismutase activity in parkinsonian substantianigra. J Neurochem.; 53(3):692-7.
119. ***Sarabia L., Mauer I., and Bustos-Obregon, E., (2009):*** Melatonin prevents damage elicited by the organo-phosphorous pesticide diazinon on mouse sperm DNA. Eco-toxicology and Environmental Safety; 72:663-668.
120. ***Saulsbury M.D., Heyliger S.O., KaiyuW., and Johnson D.J., (2009):*** Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. Toxicology; 1-9.
121. ***Schapira A.H., (2008):*** The clinical relevance of levodopa toxicity in the treatment of Parkinson's disease. Mov. Disord; 23 (Suppl 3): S515–S520.
122. ***Schneider C.D., and de Oliveira A.R., (2004):***Oxygen free radicals and exercise: mechanisms of synthesis and adaptation to the physical training, Rev. Bras. Med.; 10:314-318.
123. ***Schneider L., and Zhang J., (2010):*** Lysosomal function in macromolecular homeostasis and bioenergetics in Parkinson's disease. Molecular Neuro-degeneration; 5:14.
124. ***SekarBabu H., Jayaraman P., and Aarthy P., (2010):*** Screening of Ach Ease inhibition in blood plasma and brain of Wistar rats by Neurella D [combination pesticide]. IJPBS; 1:574–8.
125. ***Shadnia S., Azizi E., and Hosseini R.,(2005):*** Evaluation of oxidative stress and geno-toxicity in organo-phosphorous insecticide formulators. Hum. Exp. Toxicol.; 24:439-445.
126. ***Shittu M., Ayo J.O., Ambali S.F., Fatihu M.Y., Onyeanusi B.I., and Kawu M.U., (2012):*** Chronic chlorpyrifos-induced oxidative changes in the testes and pituitary gland of Wistar rats:

Ameliorative effects of vitamin C. Pesticide Biochemistry and Physiology, 102:79-85.

127. ***Singh S., Kumar V., Thakur S., Dev Banerjee B., Chandna S., and Rautela R.S., (2011):*** DNA damage and cholinesterase activity in occupational workers exposed to pesticides. Environ Toxicol Pharmacol.; 31:278–85.
128. ***Spencer J.P.E., Vafeiadou K., Williams R.J., and Vauzour D., (2012):*** Neuroinflammation: Modulation by flavonoids and mechanisms of action. Molecular Aspects of Medicine; 33:83–97.
129. ***Steinberg D., and Khoo J.C., (1977):*** Hormone sensitive lipase of adipose tissue. Fed Proc.; 36: 1986.
130. ***Susana B.E., Evelina G.F., Luciana N., Josefina R., Victoria S., and Patricia A.M.W., (2008):*** Antioxidant effects of the VO (IV) hesperidin complex and its role in cancer chemoprevention. J. Biol. Inorg. Chem.; 13:435-447.
131. ***Sutherland B.A., Rahman R.M., and Appleton I., (2005):*** Mechanisms of action of green tea catechins, with a focus on ischemia-induced neuro-degeneration, J. Nutr. Biochem.; 17:291–306.
132. ***Thomas B., and Beal M.F., (2007):*** Parkinson's disease. Hum. Mol. Genet.; 16(No 2):R183-R194.
133. ***Tofaris G.K.,and Spillantini M.G., (2005):*** Alpha-synuclein dysfunction in Lewy body diseases. Mov. Disord.;20:S37-S44.
134. ***Tommasini S., Calabro M.L., Stancanelli R., Donato P., CostaC., Catania S., Villari V., Ficarra P., and Ficarra R., (2005):*** The inclusion complexes of hesperetin and its 7- rhamnoglucoside with (2-hydroxypropyl)-b-cyclodextrin. J. Pharmaceut. Biomed.; 39:572-580.
135. ***Trinder P., (1969):*** Determination of glucose by colorimetric method. Ann. Clin. Biochem.; 6: 24-33.
136. ***Tuzmen N., Candan N., and Kaya E., (2007):*** The evaluation of altered anti-oxidative defense mechanism and acetyl cholinesterase activity in rat brain exposed to chlorpyrifos, delta-methrin, and their combination, Toxicol. Mech. Methods; 17:535-540.
137. ***Üner N., Oruc E., Sevgiler Y.S.,Ahin N., Durmaz H., and Usta D., (2006):*** Effects of diazinon on acetyl cholinesterase activity and lipid per-oxidation in the brain of Oreochromisniloticus. Environ ToxicolPharmacol;21:241–5.
138. ***Uzun F.G., Demir F., Kalender S., Bas H., and Kalender Y., (2010):*** Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food and Chemical Toxicology; 48:1714–1720.
139. ***Valette H., Dolle F., Bottlaender M., Hinnen F., and Marzin D., (2002):*** Fluro-A-85380 demonstrated no mutagenic properties in in vivo rat micronucleus and Ames tests. Nuclear Medicine and Biology; 29:849-853.
140. ***Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., and Telser J., (2007):*** Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol.; 39:44-84.
141. ***Verma R.S., Mehta A., and Srivastava N., (2007):*** In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pesticide and Biochemical Physiology; 88:191-196.
142. ***Weinreb O., Mandel S., Amit T., and Youdim M.B., (2004):*** Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases, J. Nutr. Biochem.; 15:506–516.
143. ***Wilmsen P.K., Spada D.S., and Salvador M., (2005):*** Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. J. Agric. Food Chem.; 53:4757-4761.
144. ***Wu H., Zhang R., Liu J., Guo Y., and Ma E., (2011):*** Effects of malathion and chlorpyrifos on acetyl cholinesterase and antioxidant defense system in Oxya chinensis (Thunberg) (Orthoptera: Acrididae). Chemosphere; 83: 599–604.
145. ***Youdim K.A., Dobbie M.S., Kuhnle G., Proteggente A.R., Abbott N.J., and Rice-Evans C., (2003):*** Interaction between flavonoids and the blood-brain barrier: in vitro studies. J. Neurochem.; 85:180-192.
146. Yuan H., Zhang Z.W., Liang L.W., Shen Q., Wang X.D., Ren S.M., Ma H.J., Jiao S.J., and Liu P., (2010): Treatment strategies for Parkinson's disease. Neurosci. Bull.; 26(1): 66-76.
147. ***Zama D., Meraihi Z., Tebibel S., Benayssa W., Benayache F., Benayache S., and Vlietinck A.J., (2007):*** Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: the protective role of the butanolic extract of Paronychia argentea L. Indian Journal of Pharmacology; 39:145–150.
148. ***Zbarsky V., Datla K.P., Parkar S., Rai D.K., Aruoma O.I., and Dexter D.T., (2005):*** Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease, Free Radic. Res.; 39:1119–1125.
149. ***Zeng B.Y., Pearce R.K., MacKenzie G.M., and Jenner P., (2001):*** Chronic high dose L-dopa treatment does not alter the levels of dopamine D-1, D-2 or D-3 receptor in the striatum of normal monkeys: an autoradiographic study. J. Neural Transm.; 108(8–9):925-941.
150. ***Ziv I., and Melamed E., (2010):*** Editorial: apoptosis in the aging brain. Apoptosis; 15:1285–91.
151. ***Zoccolella S., LambertiS.V., Iliceto G., Santamato A., Lamberti P., and Logroscino G., (2010):*** Hyper-homocysteinemia in L-dopa treated patients with Parkinson's disease: potential implications in cognitive dysfunction and dementia? Curr. Med. Chem.; 17(28):3253-3261.

9/26/2012

Identifying and Prioritization Effective Factors in MRP implementation Using FAHP Approach

[1]Reza Kiani mavi, [2]Kiamars Fathi Hafshejani, [3]Hamid Bahrami, [4*]Davood Gharakhani

[1]Assistant Professor, Department of Industrial Management, Qazvin branch, Islamic Azad University (IAU), Qazvin, Iran
[2]Assistant Professor, Department of Management, South Tehran Branch Islamic Azad University (IAU), Tehran, Iran
[3]Department of Industrial Management, Qazvin branch, Islamic Azad University (IAU), Qazvin, Iran
[4*]Department of Industrial Management, Qazvin branch, Islamic Azad University (IAU), Qazvin, Iran
Davood Gharakhani (Corresponding author) E-mail: Davoodgharakhany@yahoo.com

Abstract: MRP is a plan for the production and purchase of the components used in making items in the master production schedule. It shows the quantities needed and when manufacturing intends to make or use them.MRP is a commonly accepted approach for replenishment planning in major companies (Gharakhani et al., 2011). Implementation of any production system requires that all of the factors identified in its implementation and also determine the importance of each. In this study the researcher to identify and prioritize the Effective Factors in MRP implementation. The survey results show that the most important Effective Factors in MRP implementation are Top management support and Formal project planning. Moreover, the less important factor is software / hardware Characteristics.

[Reza Kiani mavi, Kiamars Fathi Hafshejani, Hamid Bahrami, Davood Gharakhani. **Identifying and Prioritization Effective Factors in MRP implementation Using FAHP Approach.** *Life Sci J* 2012;9(4):946-951] (ISSN:1097-8135). http://www.lifesciencesite.com. 146

Keywords: material requirements planning, project planning, Fuzzy set, AHP

1. Introduction

MRP was first introduced in 1970's; thereafter, many researchers, papers, books, industries, companies and even different sciences have applied it (Dolgui et al., 2007). In manufacturing environments with complex product structures and multiple production stages, material requirements planning (MRP) systems are the most commonly used for production planning and material supply decision making. In some researches, the advantages and disadvantages of MRPs have been explained. (see Safizadeh et al., 1986; Harhen, 1988 ; Browne et al., 1996). Material requirement planning (MRP) is deployed in order to model integrated business systems. MRP is a computer-based information system designed to handle ordering and scheduling of inventories (e.g. raw materials, component parts, and subassemblies). It has been designed primarily for complex production planning environments. Significant benefits such as improved customer service, better production scheduling, and reduced manufacturing costs are some of the key benefits. MRP has evolved from a simplistic representation in the 1980s to today's powerful and comprehensive versions, i.e. manufacturing resource planning (MRPII) and enterprise resource planning (ERP).

In MRP systems, the master production schedule (MPS) represents a plan for the production of all end-items over a given planning horizon. It specifies how much of each end-item will be produced in each planning period, so that future component production requirements and materials purchases can be calculated using MRP component- explosion logic. As such, the MPS has to be feasible so that components can be produced within the capacityavail able in each time period. It is clear that there is a role here for a planning tool that efficientlytakes capacityand the MRP explosion into account at the same time, a point made by Shapiro (1993).

MRP determines the quantityand timing of the acquisition of dependent demand items needed to satisfy master schedule requirements. One of its main objectives is to keep the due date equal to the need date, eliminating material shortages and excess stocks. MRP breaks a component into parts and subassemblies, and plans for those parts to come into stock when needed. MRP relates each component or subassemblyto every other part and to the component as a whole. With computer technologyadvances, maintenance and repairs have become integrated within the system. MRP is sold for manufacturing applications, but it could potentiallybe useful in aircraft parts inventory. Material Requirements Planning (MRP) has fallen into disfavor during the last ten years, as demonstrated by the extensive literature and conference material coming out of organizations like the American Production and Inventory Control Society (APICS) which discuss its shortcomings (Berger, 1987). MRP has received intense challenges of its e€ectiveness from Japan. In

batch manufacturing environment, material requirements planning (MRP), manufacturing resource planning (MRPII) or enterprise resource planning (ERP) has been recommended as the ideal system. Since the MRP release logic is deployed in MRPII and ERP systems, when they are used for production planning and scheduling, the scheduled outputs are identical. This research focuses on their roles in production planning and scheduling, therefore the use of these systems in batch manufacturing enterprises is referred as MRP-controlled manufacturing environment. The Materials Requirements Planning (MRP) approach, applied in production planning and management has some weaknesses. Despite several shortcomings, the MRP concept is still widely used in practice for materials planning and control. One of the major drawbacks is that MRP does not explicitly take into consideration any uncertainty inherent in the planning data (see Vollmann et al., 2005).

Decision makers usually are more confident making linguistic judgments than crisp value judgments. This phenomenon results from inability to explicitly state their preferences owing to the fuzzy nature of the comparison process. Many studies have continually introduced the fuzzy concept to improve MCDM and solve linguistic and cognitive fuzziness problems. For example, fuzzy theory and AHP are combined to become the Fuzzy AHP (FAHP) method (Cheng, 1997; Cheng et al., 1999), which is a fuzzy extension of AHP, and was developed to solve hierarchical fuzzy problems. FAHPs are systematic approaches to the alternative selection and justification problem that use the concepts of fuzzy set theory and hierarchical structure analysis. FAHP can be applied to measure fuzzy linguistic cognition, and suffers form the disadvantage of unstable (i.e., non-unique) results being obtained by different defuzzification methods, and the ordering of alternatives will arise ranking reversion (Gharakhani،2012).

The remainder of this paper is organized as follows: Section 2 discusses the Effective Factors in MRP implementation. Section 3 discusses the fuzzy AHP method. Section 4 outlines an empirical study to show the process of AHP method to priorities the Effective Factors in MRP implementation. Section 5 carries our conclusions and suggestions.

2. Effective Factors in MRP implementation

Material requirement planning (MRP) is a plan for the production and purchase of the components used in making items in the master production schedule. It shows the quantities needed and when manufacturing intends to make or use them (Arnold، 1998). The application of this popular tool in materials management has greatly reduced inventory levels and improved productivity. Based on the previous literatures, we focus on eight Effective Factors in MRP implementation. The factors used in relevant literatures are as follows:

Top management support, Formal project planning, Data Accuracy, Organizational arrangement, Education, Control policies and procedures, software / hardware Characteristics and employees' individual Characteristics (Alberto petroni, 2002).

3. The fuzzy AHP method

3.1. Analytic hierarchy process (AHP)

The AHP methodology, which was developed by Saaty (1980), is a powerful tool in solving complex decision problems.AHP integrates experts' opinions and evaluation scores, and devises the complex decision-making system into a simple elementary hierarchy system. The evaluation method in terms of ratio scale is then employed to perform relative importance pair-wise comparison among every criterion. This method decomposes complicated problems from higher hierarchies to lower ones. In the AHP approach, the decision problem is structured hierarchically at different levels with each level consisting of a finite number of decision elements. The upper level of the hierarchy represents the overall goal, while the lower level consists of all possible alternatives. One or more intermediate levels embody the decision criteria and sub-criteria (Partovi, 1994).Through AHP, the importance of several attributes is obtained from a process of paired comparison, in which the relevance of the attributes or categories of drivers of intangible assets are matched two-on-two in a hierarchic structure.

3.2. Determining the evaluation dimensions weights

This research employs fuzzy AHP to fuzzify hierarchical analysis by allowing fuzzy numbers for the pairwise comparisons and find the fuzzy preference weights. In this section, we briefly review concepts for fuzzy hierarchical evaluation. Then, the following sections will introduce the computational process about fuzzy AHP in detail.

3.2.1. Establishing fuzzy number

To deal with vagueness of human thought, Zadeh (1965) first introduced the fuzzy set theory, which was oriented to the rationality of uncertainty due to imprecision or vagueness. A major contribution of fuzzy set theory is its capability of representing vague data. The theory also allows mathematical operators and programming to apply to the fuzzy domain. A fuzzy set is a class of objects with a continuum of grades of membership.

The mathematics concept borrowed from Hsieh, Lu, and Tzeng (2004) and Liou et al. (2007).

A fuzzy number Ã on R to be a TFN if its membership function is $\mu_{\tilde{A}}(x)$: R [0, 1] is equal to following Eq. (1):

$$\mu_{\tilde{A}}(x) = \begin{cases} \dfrac{(x-l)}{(m-l)} & l \le x \le m \\ \dfrac{(u-x)}{(u-m)} & m \le y \le u \\ 0 & otherwise \end{cases} \quad (1)$$

From Eq. (1), l and u mean the lower and upper bounds of the fuzzy number Ã, and m is the modal value for Ã (as Fig. 1). The TFN can be denoted by Ã= (l, m, u).

3.2.2. Determining the linguistic variables

Generally, the decision-making problem is made under uncertainties, vagueness, fuzziness, risk, time pressure and some information is incomplete or missing. For example, it is difficult for decision makers to give an exact value to express their opinion on a company's capability. They prefer to describe their feeling in the fuzzy term. The triangular fuzzy number is the simplest fuzzy number and is used most frequently for expressing linguistic terms in research (Chen, 2000; Deng, 2006).

An appropriate linguistic variable set can help decision makers to give right judgments on decisions.

Here, we use this kind of expression to evaluation dimension by nine basic linguistic terms, as ''Perfect,'' ''Absolute,'' ''Very good,'' ''Fairly good,'' ''Good,'' ''Preferable,'' ''Not Bad,'' ''Weak advantage'' and ''Equal'' with respect to a fuzzy nine level scale. In this paper, the computational technique is based on the following fuzzy numbers defined by Gumus (2009) in Table 1. Here, each membership function (scale of fuzzy number) is defined by three parameters of the symmetric triangular fuzzy number, the left point, middle point, and right point of the range over which the function is defined. (Note 1).

3.2.3. Fuzzy AHP

Fuzzy theory has been widely used for assisting in decision making where fuzziness exists in defining variables (Seo et al., 2004).

AHP involves the principles of decomposition, pairwise comparisons, and priority vector generation and synthesis. Though the purpose of AHP is to capture the expert's knowledge, the conventional AHP still cannot reflect the human thinking style. Therefore, fuzzy AHP, a fuzzy extension of AHP, was developed to solve the hierarchical fuzzy problems. In the fuzzy AHP procedure; the pairwise comparisons in the judgment matrix are fuzzy numbers that are modified by the designer's emphasis.

Then, we will briefly introduce that how to carry out the fuzzy AHP in the following sections.

Step1: Construct pairwise comparison matrices among all the elements/criteria in the dimensions of the hierarchy system. Assign linguistic terms to the pairwise comparisons by asking which is the more important of each two dimensions, as following matrix Ã.

$$\tilde{A} = \begin{bmatrix} 1 & \tilde{\alpha}_{12} & \cdots & \tilde{\alpha}_{1n} \\ \tilde{\alpha}_{21} & 1 & \cdots & \tilde{\alpha}_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \tilde{\alpha}_{n1} & \tilde{\alpha}_{n2} & \cdots & 1 \end{bmatrix} = \begin{bmatrix} 1 & \tilde{\alpha}_{12} & \cdots & \tilde{\alpha}_{1n} \\ 1/_{\tilde{\alpha}_{12}} & 1 & \cdots & \tilde{\alpha}_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ 1/_{\tilde{\alpha}_{1n}} & 1/_{\tilde{\alpha}_{2n}} & \cdots & 1 \end{bmatrix}$$

Where

$$\tilde{\alpha}_{ij} = \begin{cases} \tilde{9}^{-1}, \tilde{8}^{-1}, \tilde{7}^{-1}, \tilde{6}^{-1}, \tilde{5}^{-1}, \tilde{4}^{-1}, \tilde{3}^{-1}, \tilde{2}^{-1}, \tilde{1}^{-1}, \tilde{8}^{-1}, \tilde{1}, \tilde{2}, \tilde{3}, \tilde{4}, \tilde{5}, \tilde{6}, \tilde{7}, \tilde{8}, \tilde{9}, & 1, i \neq j \\ 1 & i = j \end{cases}$$

Step 2: To use geometric mean technique to define the fuzzy geometric mean and fuzzy weights of each criterion by Hsieh et al. (2004)

$$\tilde{r}_i = \left(\tilde{\alpha}_{i1} \otimes \ldots \otimes \tilde{\alpha}_{ij} \otimes \ldots \otimes \tilde{\alpha}_{in}\right)^{1/n}$$

$$\tilde{w}_i = \tilde{r}_i \otimes [\tilde{r}_1 \oplus \ldots \otimes \tilde{r}_i \oplus \ldots \oplus \tilde{r}_n]^{-1}$$

Where $\tilde{\alpha}_{ij}$ is fuzzy comparison value of dimension i to criterion j, thus, $\tilde{r}_i$ is a geometric mean of fuzzy comparison value of criterion i to each criterion, $\tilde{w}_i$ is the fuzzy weight of the ith criterion, can be indicated by a TFN, $\tilde{w}_i =$

(lw_i, mw_i, uw_i). The lw_i, mw_i and uw_i stand for the lower, middle, and upper values of the fuzzy weight of the ith dimension.

4. Data analysis

Data analysis is divided into three sub-sections: (1) Fuzzy AHP questionnaire design, (2) The calculation process of fuzzy AHP method, (3) analyzing the evaluation criteria of significance.

4.1. Fuzzy AHP questionnaire design

This research designed questionnaire for fuzzy AHP. This questionnaire composed of two parts; the first part outlines each criteria definition for easy understanding and response. The second part is a pairwise comparison to evaluate the importance of each criterion using Scale of fuzzy number that displayed in table 1.

4.2. The calculation process of fuzzy AHP method

This study uses an expert interview method. The objects were professional experts of the Iran khodro Company in Iran (15 experts). The evaluation factors symbols in this study are as follows: Top management support (A), Formal project planning (B), Data Accuracy (C), Organizational arrangement (D), Education (E), Control policies and procedures (F), software / hardware Characteristics (G) and employees' individual Characteristics (H).

Data collected from the experts was analyzed with the fuzzy AHP method. The steps were conducted as the following.

We adopt fuzzy AHP method to evaluate the weights of different dimensions for the Effective Factors in MRP implementation. Following the construction of fuzzy AHP model, it is extremely important that experts fill the judgment matrix. The following section demonstrates the computational procedure of the weights of factors.

(1) According to the committee with fifteen representatives about the relative important of factor, then the pairwise comparison matrices of factors will be obtained. We apply the fuzzy numbers defined in Table 1. We transfer the linguistic scales to the corresponding fuzzy numbers.

(2) Computing the elements of synthetic pairwise comparison matrix by using the geometric mean method suggested by Buckley (1985) that is:

$\tilde{\alpha}_{ij} = \left(\tilde{\alpha}_{ij}^{1} \otimes \tilde{\alpha}_{ij}^{2} \otimes \ldots \otimes \tilde{\alpha}_{ij}^{12}\right)$, for $\tilde{\alpha}_{12}$ as the example:

$\tilde{\alpha}_{12} = (1/6,1/7,1/8) \otimes (1/3,1/4,1/5) \otimes \cdots \otimes (1/2,1/3,1/4)^{1/15} = (1/6 \times 1/3 \times \cdots \times 1/2)^{1/15}$, $(1/7 \times 1/4 \times \cdots \times 1/3)^{1/15}$, $(1/8 \times 1/5 \times \cdots \times 1/4)^{1/15}$

= (0.680,0.591,0.534)

It can be obtained the other matrix elements by the same computational procedure, therefore, the synthetic pairwise comparison matrices of the nine representatives will be constructed as follows:

	A	B	C	D	E	F	G	H
A	1	(0.680,0.591, 0.534)	(1.258,1.350, 1.419)	(3.543,4.699, 5.773)	(2.632,3.369, 4.181)	(4.047,5.316, 6.193)	(3.007,3.896, 4.714)	(0.625,0.462, 0.289)
B	(1.469,1.692, 1.873)	1	(1.239,1.140, 1.079)	(2.971,3.856, 4.712)	(0.714,0.556, 0.409)	(1.176,2.103, 2.984)	(1.003,1.053, 1.218)	(5.413,6.389, 7.143)
C	(0.803,0.748, 0.714)	(0.807,0.911, 0.937)	1	(5.487,6.242, 7.121)	(0.269,0.217, 0.152)	(1.905,2.863, 3.614)	(0.723,0.539, 0.381)	(1.922,2.783, 3.568)
D	(0.282,0.211, 0.167)	(0.336,0.261, 0.214)	(0.182,0.158, 0.141)	1	(3.420,4.286, 5.121)	(0.834,0.691, 0.476)	(1.148,0.861, 0.612)	(3.274,4.161, 5.081)
E	(0.368,0.287, 0.226)	(1.402,1.798, 2.444)	(3.717,4.608, 6.578)	(0.292,0.228, 0.192)	1	(1.259,1.592, 2.127)	(4.685,5.514, 6.704)	(0.980,1.115, 1.362)
F	(0.245,0.182, 0.161)	(0.850,0.475, 0.335)	(0.525,0.349, 0.276)	(1.187,1.443, 2.101)	(0.794,0.628, 0.470)	1	(1.333,1.813, 2.341)	(1.239,1.674, 2.114)
G	(0.329,0.243, 0.210)	(0.997,0.949, 0.821)	(1.383,1.855, 2.624)	(0.871,1.159, 1.631)	(0.213,0.180, 0.147)	(0.747,0.523, 0.364)	1	(0.514,0.394, 0.342)
H	(1.621,2.158, 3.452)	(0.184,0.156, 0.139)	(0.520,0.359, 0.281)	(0.305,0.239, 0.194)	(1.020,0.897, 0.732)	(0.807,0.597, 0.473)	(1.923,2.532, 2.912)	1

(3) To calculate the fuzzy weights of factors, the computational procedures are displayed as following parts

$\tilde{r}_1 = \left(\tilde{\alpha}_{11} \otimes \tilde{\alpha}_{12} \otimes \tilde{\alpha}_{13} \otimes \ldots \otimes \tilde{\alpha}_{1,12}\right)^{1/8}$

$= ((1 \times 0.680 \times \cdots \times 0.625)^{1/8}, (1 \times 0.591 \times \cdots \times 0.462)^{1/8}, (1 \times 0.534 \times \cdots \times 0.289)^{1/8})$

= (1.669, 1.821, 1.877)

Similarly, we can obtain the remaining $\tilde{r}_i$, there are:
$\tilde{r}_2 = (1.493, 1.663, 1.780)$
$\tilde{r}_3 = (1.123, 1.157, 1.172)$
$\tilde{r}_4 = (0.809, 0.742, 0.664)$
$\tilde{r}_5 = (1.158, 1.232, 1.385)$
$\tilde{r}_6 = (0.801, 0.732, 0.645)$
$\tilde{r}_7 = (0.651, 0.606, 0.583)$
$\tilde{r}_8 = (0.723, 0.667, 0.634)$

For the weight of each factor, they can be done as follows:

$$\tilde{w}_1 = \tilde{r}_1 \otimes (\tilde{r}_1 \oplus \tilde{r}_2 \oplus \tilde{r}_3 \oplus \tilde{r}_4 \oplus \tilde{r}_5 \oplus \tilde{r}_6 \oplus \tilde{r}_7 \oplus \tilde{r}_8)^{-1}$$
$$= (1.669, 1.821, 1.877) \otimes (1/(1.877 + \cdots + 0.634)\ ,1/(1.821 + \cdots + 0.667), 1/(1.669 + \cdots + 0.723))$$
= (0.190, 0.211, 0.223)

We also can calculate the remaining $\tilde{w}_i$, there are:

$\tilde{w}_2 = (0.170, 0.193, 0.212)$
$\tilde{w}_3 = (0.128, 0.134, 0.139)$
$\tilde{w}_4 = (0.092, 0.086, 0.079)$
$\tilde{w}_5 = (0.132, 0.149, 0.165)$
$\tilde{w}_6 = (0.091, 0.085, 0.076)$
$\tilde{w}_7 = (0.074, 0.070, 0.069)$
$\tilde{w}_8 = (0.082, 0.077, 0.075)$

(4). to apply the COA method to compute the BNP value of the fuzzy weights of each factor: To take the BNP value of the weight of A1 (Inadequate selection) as an example, the calculation process is as follows

$$BNP_{w1} = \frac{[(U_{w1} - L_{w1}) + (M_{w1} - L_{w1})]}{3 + L_{w1}}$$

$$= \frac{[(0.223 - 0.190) + (0.211 - 0.190)]}{3 + 0.190} = 0.208$$

Then, the weights for the remaining factors can be found as shown in Table 2. Table 2 shows the relative weight of eight Effective Factors in MRP implementation, which obtained by FAHP method.

***4.3.* Analyzing the evaluation factors of significance**

This study integrates fifteen questionnaires from expert interviews to find out the evaluation factors of significant, and then calculates the Weights of factors as shown in Table 2.The weights for each factor are: Top management support (0. 208), Formal project planning (0. 191), Data Accuracy (0. 133), Organizational arrangement (0. 086), Education (0. 149), Control policies and procedures (0. 084), software / hardware Characteristics (0. 071), and employees' individual Characteristics (0. 078) . From the fuzzy AHP results, we can understand the most important Effective Factors in MRP implementation are Top management support (0. 208) and Formal project planning (0. 191).Moreover, the less important factor is software / hardware Characteristics (0. 071).

Table 1. Membership functions of linguistic scale (example)

Fuzzy number	Linguistic	Scale of fuzzy number
9	Perfect	(8, 9, 10)
8	Absolute	(7, 8,9)
7	Very good	(6, 7,8)
6	Fairly good	(5, 6,7)
5	Good	(4, 5,6)
4	Preferable	(3, 4,5)
3	Not bad	(2, 3,4)
2	Weak advantage	(1, 2,3)
1	Equal	(1, 1,1)

Table 2. The weights and rank of factors**.**

Dimensions	Weights	BNP	Rank
(A) Top management support	(0.190, 0.211, 0.223)	0.208	1
(B) Formal project planning	(0.170, 0.193, 0.212)	0.191	2
(C) Data Accuracy	(0.128, 0.134, 0.139)	0.133	4
(D) Organizational arrangement	(0.092, 0.086, 0.079)	0.086	5
(E) Education	(0.132, 0.149, 0.165)	0.149	3
(F) Control policies and procedures	(0.091, 0.085, 0.076)	0.084	6
(G) software / hardware Characteristics	(0.074, 0.070, 0.069)	0.071	8
(H) employees' individual Characteristics	(0.082, 0.077, 0.075)	0.078	7

5. Conclusion

MRP is a commonly accepted approach for replenishment planning in major companies. The MRP-based software tools are accepted readily. Most industrial decision makers are familiar with their use. The practical aspect of MRP lies in the fact that this is based on comprehensible rules, and provides cognitive support, as well as a powerful information system for decision making (Gharakhani, 2011).The purpose of this study is Identifying and Prioritization Effective Factors in MRP implementation Using FAHP Approach. The survey results show that the

most important Effective Factors in MRP implementation are Top management support and Formal project planning.Moreover, the less important factor is software / hardware Characteristics. If Iran Khodro Company will implement Material requirements planning system the best way, it should much attention focused on the Top management support, Formal project planning and Education.

In this paper, one major limitation is the evaluation effort required with this technique. In a decision-making process, the use of linguistic variables in decision problems is highly beneficial when performance values cannot be expressed by means of crisp values. The next limitation of this study it can be noted that this research has been done only in Iran Khodro Company and its results can not be fully extended to all companiesLack of necessary resources and time constraints of this project are another limitation.In this paper, we present AHP as a generalized method to ranking risk factors under a fuzzy environment. Future study can identify and ranking Effective Factors in MRP implementation by different methods such as ELECTRE, TOPSIS and VIKOUR. Further research can survey direct and indirect effects of each factor through dematel method. Researchers can identify and rank factors in implementing material requirements planning systems in other manufacturing companies.

References

1. Arnold JR. Introduction to material management. 3rd ed. New Jersey: Prentice-Hall International, 1998.
2. Berger, G., 1987. Ten ways MRP can defeat you. APICS Conference Proceedings, APICS.
3. Browne J., Harben J. and Shivnan J., Production Management Systems: an integrated perspective, Second Edition, Addison-Wesley Press, (1996).
4. Buckley, J.-J. (1985). Fuzzy hierarchical analysis. Fuzzy Sets and Systems, 17(1), 233–247.
5. Chen, C.T. (2000), "Extensions of the TOPSIS for group decision-making under fuzzy environment", Fuzzy Sets and Systems, Vol. 114 No. 1, pp. 1-9.
6. Cheng, C.H. (1997).Evaluating naval tactical missile system by fuzzy AHP based on the grade value of membership function, Eur. J. Operational Res. 96 pp.343– 350.
7. Cheng, C.H, Yang, L.L. and Hwang, C.L. (1999). Evaluating attack helicopter by AHP based on linguistic variable weight, Eur. J. Operational Res. 116 pp. 423–435.
8. Dolgui A. and Prodhon C., Supply planning under uncertainties in MRP environments: A state of the art, Annual Reviews in Control, (2007), 31, 269–279.
9. Deng, Y. (2006), "Plant location selection based on fuzzy TOPSIS", International Journal of Advanced Manufacturing Technology, Vol. 28 Nos 7-8, pp. 839-44.
10. Gharakhani, D., (2011)، Optimization of material requirement planning by Goal programming model، Asian Journal of Management Research، Vol. 2, No. 1 , pp. 297-311.
11. Gharakhani, D., (2012)، Identifying and ranking Risk factors in New Product Development Projects by Fuzzy AHP technique ، American Journal of Scientific Research، Vol. 4, No. 1 , pp. 106-116.
12. Gharakhani, D., Kiani mavi, R., Fathi Hafshejani. K., (2011)، A Goal Programming Model for Optimization of MRP with Fuzzy Demand، American Journal of Scientific Research، Issue 28(2011), pp. 153-168.
13. Gumus, A.-T. (2009). Evaluation of hazardous waste transportation firms by using a two step fuzzy-AHP and TOPSIS methodology. Expert Systems with Applications, 36(2), pp.4067–4074
14. Harhen J., The state of the art of MRP/MRP II, Computer Aided Production Management: The State of the Art, Springer-verlag, Germany, (1988).
15. Hsieh, T.-Y., Lu, S.-T., & Tzeng, G.-H. (2004). Fuzzy MCDM approach for planning and 751 design tenders selection in public office buildings. International Journal of Project Management, 22(7), pp.573–584.
16. Liou, J.-J.-H., Yen, L., & Tzeng, G.-H. (2007). Building an effective safety management system for airlines. Journal of Air Transport Management, 14(1), pp. 20–26.
17. Partovi, F.Y. (1994), "Determining what to benchmark: an analytic hierarchy process approach", International Journal of Operations & Production Management., Vol. 14 No. 6, pp. 25-39.
18. petroni,.A, (2002), Critical factors of MRP implementation in small and medium sized firms, International Journal of Operations & Production Management, Vol. 22 No. 3, pp.329-348.
19. Saaty, T.L. (1980), the Analytical Hierarchy Process, McGraw-Hill, New York, NY.
20. Safizadeh M. and Raafat F., Formal/informal systems and MRP implementation, Production and Inventory Management, (1986), 27 (1)
21. Seo, S., Aramaki, T., Hwang, Y.W. and Hanaki, K. (2004), "Fuzzy decision-making tool for environmental sustainable buildings", Journal of Construction Engineering and Management, Vol. 130 No. 3, pp. 415-23.
22. Shapiro, J.F., 1993. Mathematical programming models and methods for production planning and scheduling. In: Graves, S.C. (Ed.), Handbooks in Operations Research and Management Science, Vol. 4. Elsevier Science Publishers, New York, pp. 371–443.
23. Vollmann, T.E., Berry, W.L., Whybark, D.C., Jacobs, F.R., 2005. Manufacturing planning and control systems for supply chain management, fifth ed. McGraw-Hill, New York.
24. Zadeh, L. A. (1965). Fuzzy sets. Information and Control, 8(3), pp.338–353.

10/4/2012

Policy Analysis: investigating the critical success factors toward financial sector of Iran

Dr. Lotfollah Forouzandeh [1], Mohammad Aidi [2]

[1.] Assistant Professor at Tarbiat Modaress University
[2.] Faculty Member of Ilam University

Abstract: Policy analysis is determining which of various alternative policies will most achieve a given set of goals in light of the relations between the policies and the goals. For an organization or any institution it is vital to determine which on strategies, plans or policies are better performed than others and finally policy makers can make better decisions towards organizational objectives or vision. The main purpose of this applied research is to investigate the critical success factors of policy analysis in the financial sector of Iran. To achieve this purpose, after detailed review of literature by using a questionnaire essential critical success factors of policy analysis in the financial sector have been identified and presented. Indeed, Critical success factors (CSFs) have been used significantly to present or identify a few key factors that organisations should focus on to be successful. Required data has been gathered by using viewpoints of study's experts. Basic tool for data analysis are SPSS18.0. Results of this study can be very useful both in theoretical and applied viewpoints especially based on the research conceptual framework.

[Lotfollah Forouzandeh, Mohammad Aidi. **Policy Analysis: investigating the critical success factors toward financial sector of Iran.** *Life Sci J* 2012;9(4):952-956] (ISSN:1097-8135). http://www.lifesciencesite.com. 147

Keywords: policy analysis, financial sector, CSFs, strategy, applied research.

1. Introduction

Human societies seem complex. People who study them spend a lot of time comparing differences, and trying to figure out why people do what they do. This is the task of the social sciences, including psychology, sociology, political science, economics, and history. Even though the social sciences, in their current form, have only existed for about a century, we have made lots of progress toward understanding many pieces of the puzzle of human behaviour (Munger, 2007).

Policy analysis and policy analysts have an important role in the literature on public policy making. Usually when we talk about public policy making, policy analysis too attracts much attention as a complimentary part of it. From this point of view, policy analysis gets related to the policy making process. Many authors in public policy making field not only consider the "issues" related to this field but also drag "selections", "options" and "solutions" into the realm of analysis test. Hence, the policy analysis is accompanied by some actions whose axis is comprised of knowledge and awareness. This knowledge includes those inside the policy making process, as well as those for this process and those about this process.

In this research the critical success factors of the policy analysis have been investigated. Critical success factors (CSFs) have been used significantly to present or identify a few key factors that organisations should focus on to be successful. As a definition, critical success factors refer to "the limited number of areas in which satisfactory results will ensure successful competitive performance for the individual, department, or organisation" (Rockart and Bullen, 1981). Identifying CSFs is important as it allows firms to focus their efforts on building their capabilities to meet the CSFs, or even allow firms to decide if they have the capability to build the requirements necessary to meet critical success factors (CSFs). So, basic research question of this study is: what are the critical success factors of policy analysis in the financial sector?

2. Policy Analysis

Policy analysis is determining which of various alternative policies will most achieve a given set of goals in light of the relations between the policies and the goals. However, policy analysis can be divided into two major fields. Analysis of policy is analytical and descriptive; it attempts to explain policies and their development. Analysis for policy is prescriptive; it is involved with formulating policies and proposals (Munger, 2007).

Policy analysis is methodologically diverse using both qualitative methods and quantitative methods, including case studies, survey research, statistical analysis, and model building among others. One common methodology is to define the problem and evaluation criteria; identify all alternatives; evaluate them; and recommend the best policy agenda per favor.

Policy analysis is a process. More specifically, policy analysis is the process of assessing, and deciding among, alternatives based on their usefulness in satisfying one or more goals or values. Generally, the policy recommendation is made by one person, a decision is made by another entity, and enforcement of

the policy actually chosen is left to yet a third person or group (Munger, 2007).

Policy analysts have some common concerns no matter where they are and what policy making area they are attracted to. These concerns include:

- They have concerns about the social issues and the relationship between public policies and these issues.
- They pay attention to the content of public policies.
- They pay attention to whatever policy makers are doing or not doing and they like to ponder upon the inputs and the processes of policymaking field.
- They pay attention to the results of a policy as outputs and outcomes.

Besides the things said about the common characteristics of policy analyzers, it should be noted that the policy analyzer is a kind of experts in policies. She/he is the person who evaluates, investigates and interprets the policies whether based on the framework for policy making process or any other framework. From this point of view his job is a cognitive one. But the recognition of this cognitive job can be evaluated on two levels: Firstly, the level of personal characteristics; and secondly the level of methodological characteristics. In the personal characteristics level, regarding the special status of the public policies, particularly because it's a cross sectional field, the policy analyzer should be equipped with a variety of knowledge tools so that not only he can mingle them with the other needed characteristics of the analysis field, but also be able to earn the standards regarding personal characteristics. Regarding the level of methodological characteristics, the policy analyzer should be an expert in questioning. This expertness is the product of the faith of the questioner in the necessity of the four aforementioned cognitive questions and using them skillfully to reach unbiased answers. The method which the policy analyzer chooses should be an all scientific method. However in policy determination and the adjustment of approaches and tactics based on the time and place there might be so much different factors that some of their scientific aspects may be daintier than unscientific aspects. In such a case, the analysis method should be based on the scientific foundation so that the resulted answers of policy analysis would have enough scientific accuracy.

Personal and methodological characteristics are counted as complementary characteristics in the cognition of policy analyzer so that without a proper balance between these two it is likely that we end up with a person which is capable enough but without tools and methods or a person without enough capability with more than ten possible approaches available to him (Malek Mohammadi, Hamidreza, 2010).

3. Critical Success Factors (CSFs)

Critical success factors (CSFs) have been used significantly to present or identify a few key factors that organisations should focus on to be successful. As a definition, critical success factors refer to "the limited number of areas in which satisfactory results will ensure successful competitive performance for the individual, department, or organisation" (Rockart and Bullen, 1981). Critical success factors were introduced by John F. Rockart and the MIT Sloan School of Management in 1979 as a way to help senior executives define their information needs for the purpose of managing their organizations (Gates, 2010).

Identifying CSFs is important as it allows firms to focus their efforts on building their capabilities to meet the CSFs, or even allow firms to decide if they have the capability to build the requirements necessary to meet critical success factors (CSFs).

Success factors were already being used as a term in management when Rockart and Bullen reintroduced the concept to provide greater understanding of the concept and, at the same time, give greater clarity of how CSFs can be identified.

CSFs are tailored to a firm's or manager's particular situation as different situations (e.g. industry, division, individual) lead to different critical success factors. Rockart and Bullen presented five key sources of CSFs: **the industry, competitive strategy and industry position, environmental factors, temporal factors,** and **managerial position.** Each of these factors is explained in greater detail below.

3.1. The Industry

An industry's set of characteristics define its own CSFs. Different industries will thus have different CSFs, for example research into the CSFs for the business services, health care and education sectors showed each to be different after starting with a hypothesis of all sectors having their CSFs as market orientation, learning orientation, entrepreneurial management style and organisational flexibility (Barrett, Balloun and Weinstein, 2005).

3.2. Competitive Strategy and Industry Position

Not all firms in an industry will have the same CSFs in a particular industry. A firm's current position in the industry (where it is relative to other competitors in the industry and also the market leader), its strategy, and its resources and capabilities will define its CSFs. For example, in 2005 Caterpillar defined a new strategy to aggressively grow revenues over the long term. As part of that new strategy,

Caterpillar defined several CSFs specific to the firm which were (Gordon, 2005):

Organisational culture: "creating a culture that engaged employees, while focusing on safety and diversity"

- Quality control: "accelerating the pace of quality improvement for its products, while focusing on improving new product introduction and continuous product improvement processes"
- Cost focus: "implementing processes to become the highest-quality, lowest cost producer of our high-volume products in each hemispheric currency zone"

Other firms in Caterpillar's industry may or may not have the same CSFs, and are unlikely to have the same complete set.

3.3. Environmental Factors

These relate to environmental factors that are not in the control of the organisation but which an organisation must consider in developing CSFs. Examples for these are the industry regulation, political development and economic performance of a country, and population trends. For example, Ladbrokes, a UK bookmaker, will be establishing an international business in Italy where it has just acquired a business license, a requirement for foreign sports betting firms prior to establishing a business in the country.

3.4. Temporal Factors

Temporal factors are temporary or one-off CSFs resulting from a specific event necessitating their inclusion. Rockart and Bullen (1981) state that typically, a temporal CSF would not exist and they give as an example of a firm which "lost executives as a result of a plane crash requiring a critical success factor of rebuilding the executive group". However, with the evolution and integration of markets globally, one could argue that temporal factors are not temporal anymore as they could exist regularly in organisations. For example, a firm aggressively building its business internationally would have a need for a core group of executives in its new markets. Thus, it would have the CSF of "building the executive group in a specific market" and it could have this every year for different markets.

3.5. Managerial Position

A final primary source of CSF is managerial position. This is important if CSFs are considered from an individual's point of view. Rockart and Bullen (1981) give an example of manufacturing managers who would typically have the following CSFs: product quality, inventory control and cash control. In organisations with departments focused on customer relationships, a CSF for managers in these departments is customer relationship management (Mendoza et al., 2007).

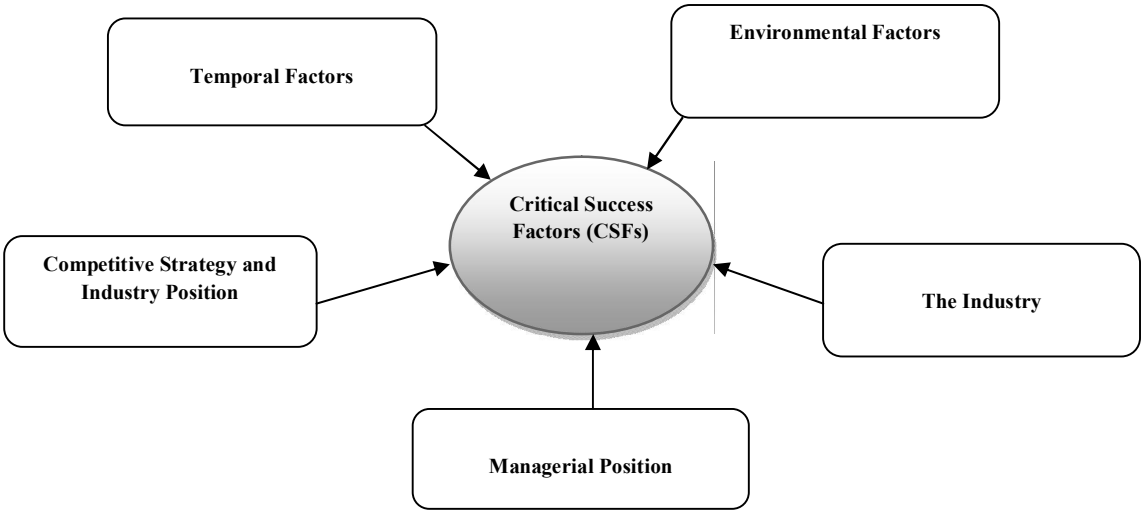


Figure 1. A general view and perspective to the Critical Success Factors (CSFs) (Amiri et al, 2011)

4. Research Methodology

The method of this research are Applied Research as the objective, and in the view point of data gathering is Descriptive-Analytic (non-experimental) that is implemented with the case study format. In this study, after appropriate literature review by using a standard questionnaire critical success factors for policy analysis in the area of financial sector have been identified and presented. The questionnaire has been extracted from the literature review and detailed focus on the research background.

The area of study in this research is financial sector. For identify the critical success factors for financial sector, the number of 8 factors have been identified. The research population of this study was experts of the field that participated in the survey. 10 experts have been identified and then the questionnaires have been distributed among these experts and finally required data has been and gathered.

Validity and Reliability

Validity and reliability are two necessary features for every measuring material such as questionnaire because these materials should analyze data and provide final conclusions for researchers. To sum up, validity means that a measuring material is used to measure the characteristics.

In this study to measure the reliability the Cronbach's Alpha has been used. The Cronbach's Alpha the calculated for this research was 0.77 that is more than the mean acceptable alpha. Hence, the questionnaire is reliable (a> 0.7). Also the validity of the tool of this study has been confirmed by using the viewpoints of the research's experts. Using valuable comments of these experts valid questionnaire has been used in order to gather the required data.

5. Findings

First by literature review and some interviews with the experts initial CSFs was identified. Then by using a questionnaire that provided for gathering the experts viewpoint about the indentified CSFs and also increases the CSFs was distributed among the research population. Data that gathered from the statistical population by using statistical software including SPSS was analysed and finally number of 8 CSFs for policy analysis based on the expert's response was identified. The importance of these CSFs was different as the view point of the respondents of the research was cited. Table 1 shows the critical success factors.

Table 1. The final CSFs for SKM implementation for the service sector

No.	CSFs
1	Organizational strategic planning
2	Inspirational leadership
3	Communication
4	Organizational readiness for change
5	Employees volunteering
6	Organizational commitment
7	Cooperation
8	Knowledge sharing

Hypothesis: "The identified CSFs (including 8 CSFs) in this research are affecting on the policy analysis for the financial sector".

In this section of this research, the affectability of the identified critical success factors for the policy analysis for the financial sector based on the experts viewpoints is investigated. For this purpose the T-Test is runes and results of this statistical test in the table 2 are illustrated.

Table 2. The results of affectability of CSFs for the policy analysis for the financial sector by using t-test analysis

CSFs for CSR implementation	T- Statistic	df	Sig*	mean	Test-Value	Confirm / Reject
Organizational strategic planning	17.355	9	0.000	5.345	3	Confirm
Inspirational leadership	14.125	9	0.000	4.609	3	Confirm
Communication	22.435	9	0.000	5.038	3	Confirm
Organizational readiness for change	17.163	9	0.000	4.569	3	Confirm
Employees volunteering	12.760	9	0.000	5.112	3	Confirm
Organizational commitment	20.370	9	0.000	5.021	3	Confirm
Cooperation	11.879	9	0.000	4.886	3	Confirm
Knowledge sharing	14.901	9	0.000	5.033	3	Confirm

***α=0.05**

7. Conclusion

Analysis the stated policies, strategies, plans, programs and etc. is a vital and key elements in the progressiveness of any complex or organization. To achieve a best way to analysis the policies determining the key effected factors is very useful and important. On the other hand Policy analysts are a set of people working in a variety of different fields. Since public policy making includes different areas such as health, education, environment, transportation, real estate and the like, policy analysts based on any one of the above-mentioned areas are performing their activities in different levels. On the other hand, any one of the different special fields, it is possible that the policy analyzer focuses on a special phase of the policy making circle.

This paper aimed to investigate the critical success factors toward policy analysis with focus on the financial sector in Iran. To achieve this purpose, a framework consisted on the key factors have been extracted from the literature review. Then by using the expert's viewpoints the confirmations of these basic factors has been applied. These essential factors or CSFs were including: Organizational strategic planning, Inspirational leadership, Communication, Organizational readiness for change, Employees volunteering, Organizational commitment, Cooperation and Knowledge sharing. It is recommended to the both researchers and policy makers in the studies field that attention to this conceptual model identified in this research is very useful to get better and efficient activities. So, analysts in the studied filed recommended to have comprehensive approach to analysis main policies in the organizations. Also, future researchers recommended to investigate the critical success factors in the other field especially in the public sector organizations and firms.

10/6/2012

References

1. Amiri, Mojtaba; Sarfi, Alireza; Safari Kahreh, Mohammad and Maleki, Mohammad Hasan. (2010). Investigation the Critical Success Factors of CRM Implementation in the Urban Management; Case Study: Tehran Municipality. International Bulletin of Business Administration, Issue 9.
2. Barrett, H., Balloun, J. and Weinstein, A. (2005). Success Factors for Organizational Performance: Comparing Business Services, Health Care, and Education. SAM Advanced Management Journal, 70 (4).
3. Gates, Linda Parker. (2010). Strategic Planning with Critical Success Factors and Future Scenarios: An Integrated Strategic Planning Framework. Technical report, available at: http://www.sei.cmu.edu.
4. Malek mohammadi, Hamidreza. (2010). Study on the Policy Analysis and Analyzers. Humanities and Cultural Research Center.
5. Mendoza, L., Marius, A., Pérez, M. and Grimán, A. (2007). Critical success factors for a customer relationship management strategy. Information & Software Technology, 49 (8).
6. Munger, Michael C. (2007). Analyzing Policy: Introduction to the Analysis of Choice and Management. Duke University.
7. Rockart, J. and Bullen, C., (1981). A primer on critical success factors. Center for Information Systems Research Working Paper No 69. Sloan School of Management, MIT, Cambridge, Massachuset ts.

Effect of Ultra Short Pulse Laser on dentin structural changes and surface roughness

Ola. M. Sakr

Departments of Operative Dentistry, College of Dentistry, Qassim University, Kingdom of Saudi Arabia & Misr University for Science and Technology\, Egypt
olasakr2004@yahoo.com

Abstract: In this study, the structure, and surface morphology of dentin after ablation with ultra-short pulses (USPL) were evaluated using environmental scanning electron microscopy (ESEM) with EDAX analysis and scanning electron microscope micrographs. The dentin specimens examined were irradiated by (100, 300 and 400 mJ) of ultra short pulse laser . Based on EDAX results, it was possible to identify the suitable energy density as the ablation threshold for dentin. The results demonstrate that by selecting suitable parameters one can obtain efficient dentin surface preparation without evidence of structural changes and thermal damage, i.e., with minimized heat affected zones and reduced collateral damage, which characterized by formation of microcracks , grain growth and recrystallization in the heat affected zones.
[Ola. M. Sakr. **Effect of Ulrra Short Pulse Laser on dentin structural changes and surface roughness.** *Life Sci J* 2012;9(4):957-962] (ISSN:1097-8135). http://www.lifesciencesite.com. 148

Keywords: Dentin, USPL ablation, Surface structure modification, EDAX, ESEM

1. Introduction

Several approaches for localized, painless, rapid laser treatment of dental hard tissue have been the aim of different continuing researches [1-3]. The hard dental structure comprises several layers, enamel, dentin, cementium and pulp. The dentin is the major constituent of teeth and consists of 70% hydroxyaptite crystals [$Ca10(PO_4)6(OH)_2$], 20% organic matter (collagen fibers) and 10% water. Its internal structure consists of dentinal tubules, which are essential for the sensation and growth of the tooth [4,5] . Dentin hypersensitivity is characterized by short sharp pain arising from exposed dentin in response to stimuli . The stimuli may be thermal, evaporative, tactile, osmotic or chemical. Most commonly, cases of dentine hypersensitivity are associated with erosion or gingival recession. Dentin hypersensitivity is one of the most common complications that affect patients after periodontal therapy and is reduced when the dentinal tubules are occluded. Several attempts have been performed previously to obtain closure of dentinal tubules using Nd:YAG laser treatment by inducing melting and resolidification of the dentin [5,6]. Studies of dentin ablation with Er:YAG and Ho:YAG lasers have indicated several weaknesses in the surface preparation process expressed in heat accumulation, fissuring and slow material removal rates. The attempts to apply these lasers to certain procedures in dentistry, especially for caries therapy in order to replace conventional drilling, have not been fully successful. Due to the high absorption of Er:YAG radiation by water molecules in the dentin tissue, the preparation process results in micro explosions breaking the hydroxyapatite structure and leading to the appearance of microcracks [7].

These microcracks could become initial points for the development of new carious lesions. Modifications of these systems (Er, Cr:YSGG) have been introduced in the dental practice in combination with water spray in order to improve the surface preparation by cooling the ablated region and increasing the ablation rate[8-10] . In recent studies, new ultra-short laser techniques for modification and structuring of dental hard tissue have emerged as a promising tool and an alternative to Er:YAG lasers [11-13] due to the advantages expressed in negligible heat and shock wave impact. The interaction of high-intensity ultra-short laser pulses with materials is characterized by fewer thermal side effects. Studies of the surface topography evolution in dentin processed by femtosecond lasers revealed surface effects, such as sealing of dentinal tubules, microdrilling of human enamel and dentin for caries treatment. Depending on the dental treatment procedure to be performed, one could utilize different types of laser-induced physical effects to obtain maximum benefits. For example, the effect of melting is used for sealing dentinal tubules in order to reduce dentin hypersensitivity and pathogenic agents' penetration. This procedure has so far been carried out by CO2, Ho:YAG and Er:YAG lasers; however, the thermal side-effect associated with the use of these conventional lasers like appearance of a heat affected zone in the order of micrometers, results in rough surface structuring with deep cracks [14,15]. The present study was motivated to detect the microstructure of dentin surfaces after different energy levels of ultra-short pulse laser ablation.

The review of literature revealed that the application ultra short pulse laser with duration <50 fs

for ablation of dental tissue, followed by examination with ESEM , EDAX analysis and different energies and their effect on dentin, has been rarely attempted. Few studies have been performed to compare the use of different number of femto second laser pulses [14,16]. The purpose of our study was to obtain and understanding on the structure and morphological changes of the dentin surface after three different energies of ultra-short laser ablation of dentin.

2. Materials and Methods:

I- Preparation of the samples:

A total of 30 of human molar teeth were extracted for periodontal diseases. The teeth were free from visible caries and other surface defects. Teeth were cleaned with a rotary brush and pumice and stored in saline and used within 6 months from time of extraction to inclusion in the study. The roots were sectioned 2 mm beyond the cemento-enamel junction .The occlusal enamel was removed by horizontal sectioning till reaching the dentin just below the dentinoenamel junction using the Isomet slow-speed diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). The dentin surface was abraded with decreasing grits of silicon carbide (SiC) paper (from #800 to # 1200) under water-cooling for 30 s/ paper. A standard superficial dentin surface of about 0.5 mm from dentinoenamel with standard smear layer was produced.

II- Sample classification:

The total 30 samples were classified into three main groups:

Group I: 10 samples for ablation with100 mJ ultra short pulse laser

Group II: 10 samples for ablation with 300 mJ ultra short pulse laser

Group III: 10 samples for ablation with 400 mJ ultra short pulse laser

III- Laser ablation condition:

Dentin surfaces were ablated by Ultra short pulsed laser (USPL). Its wavelength range was around 2940 nm in infra red region. Spot size was 3 mm. The beam was applied perpendicularly to the specimens, with the tested different energies of (100, 300, 400 mJ).

IV-Topographic evaluation:

The Scanning Electron Microscopic (SEM) examination and microanalysis were carried out using QUANTA 200 scanning electron microscope attached with EDAX unit, with accelerating voltage 30 K.V. magnification 10x up to 400.00x

Results:

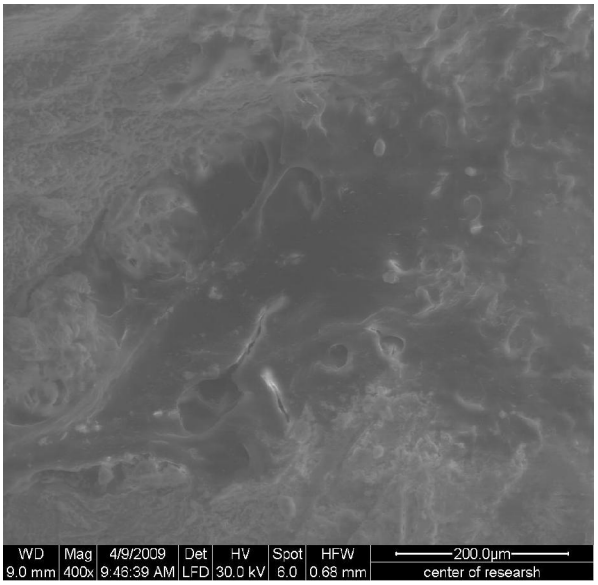
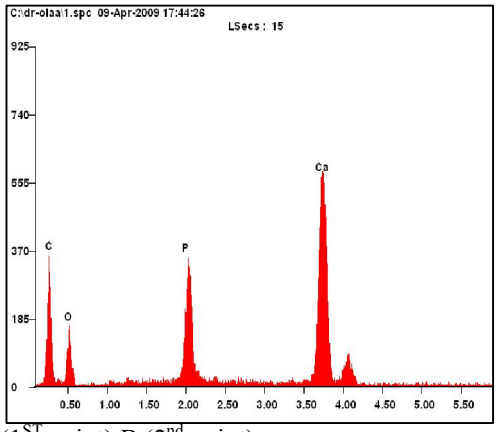
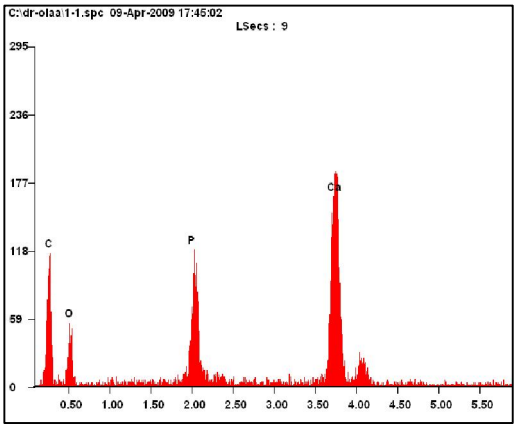
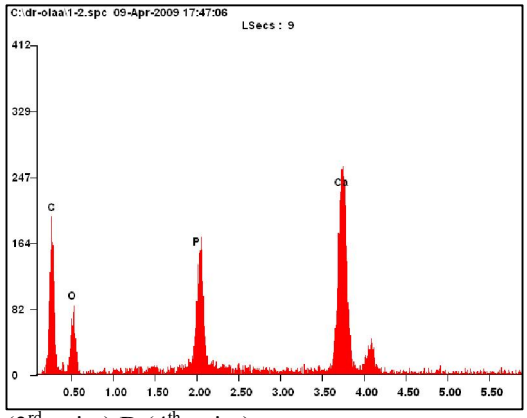


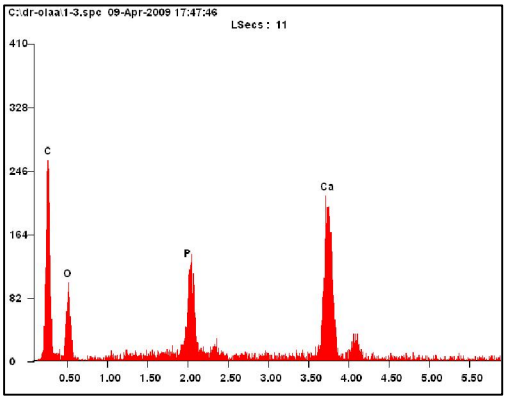
Fig.(1): Scanning electron micrograph showing dentin surface ablated with 100 mJ of USPL showing recrystalization and solidification





A(1ST point) B (2nd point)





C (3rd point) D (4th point)

Fig.(2): Four points EDAX microanalysis of first group showing highest carbon level and lowest calcium level

Element	*Wt %*	*At %*
C K	60.43	72.32
O K	23.90	21.47
P K	05.67	02.63
CaK	10.00	03.59

Table (1) :Mean of surface dentin elements , BY EDAX ZAF QUANTIFICATION , ablated with 100 mJ of USPL

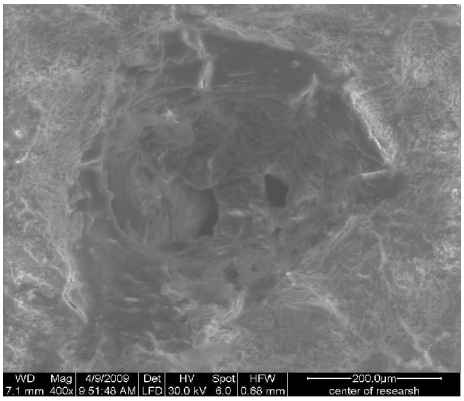
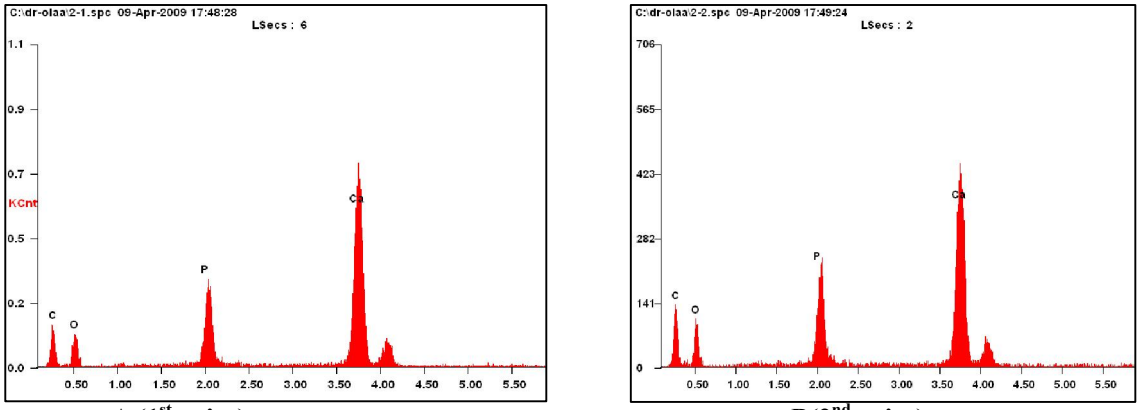


Fig.(3) : Scanning electron micrograph showing dentin surface ablated with 300 mJ of USPL showing crater like cavity



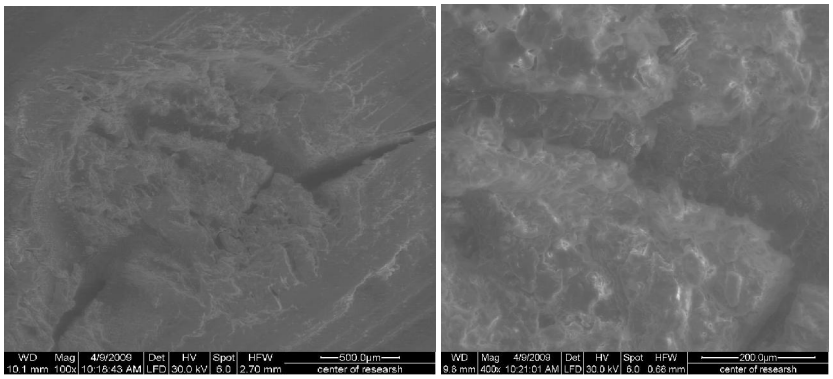
A (1st point) B(2nd point)

C (3rd point) D (4th point)

Fig.(4): Four points EDAX microanalysis of 2nd group showing moderate carbon and calcium level

Table (2): Mean of surface dentin elements , BY EDAX ZAF QUANTIFICATION , ablated with 300 mJ of USPL

Element	Wt %	At %
C K	48.92	62.79
O K	28.83	27.78
P K	07.73	03.85
CaK	14.52	05.58



A B

Fig.(5) : Scanning electron micrograph showing dentin surface ablated with 400 mJ of USPL showing increasing of surface roughness and cracking. A at 100 magnification , B at 400 magnification

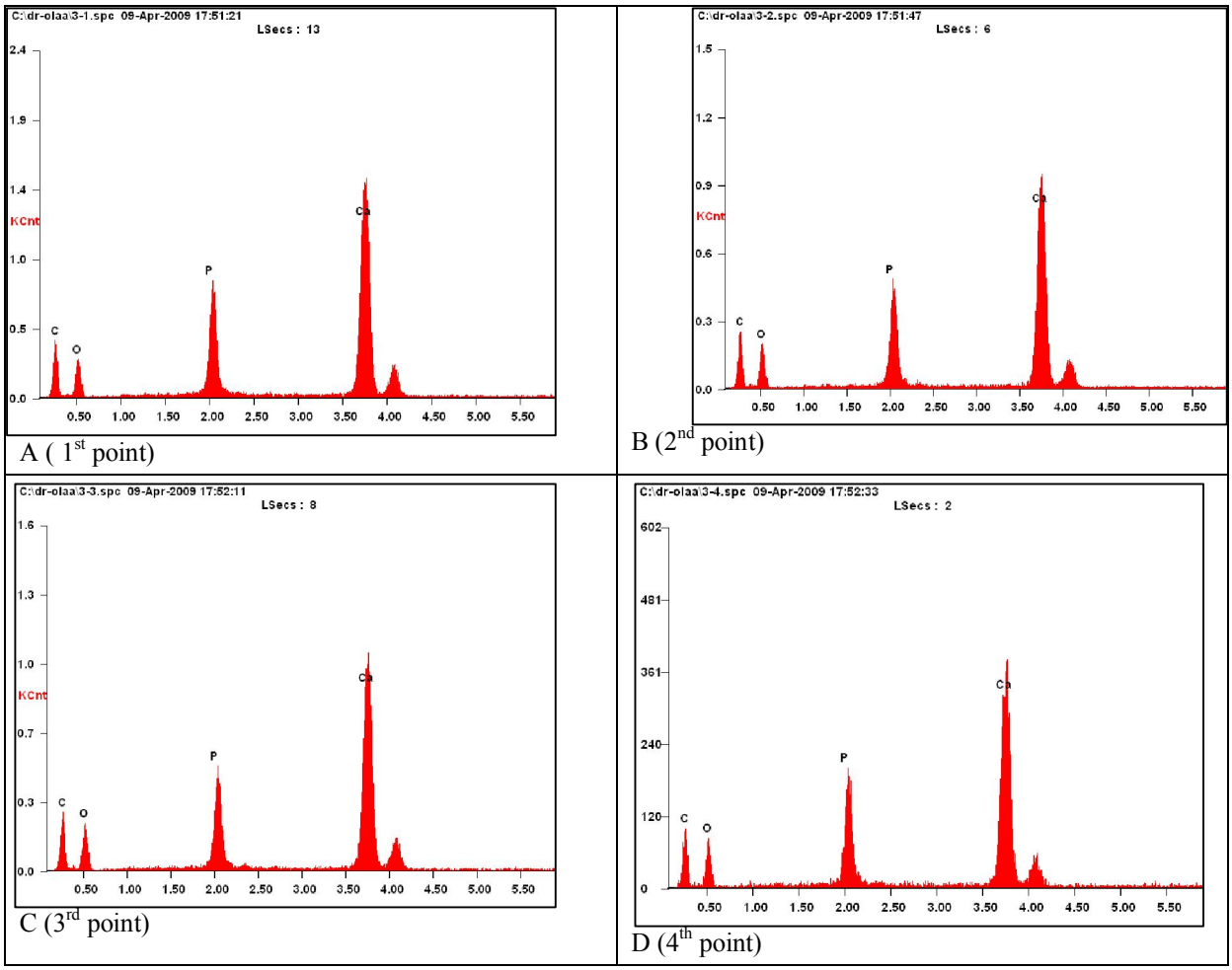


Fig.(6): Four points EDAX microanalysis of 3rd group showing lowest carbon level and highest calcium level

Table (3) :Mean of surface dentin elements , BY EDAX ZAF QUANTIFICATION , ablated with 400 mJ of USPL

Element	Wt %	At %
C K	47.01	61.43
O K	28.64	28.09
P K	08.18	04.15
CaK	16.17	06.33

4. Discussion :

The SEM micrographs of (first group)100mJ of USPL ablated dentin surface revealed a flat smooth without cracks surface with recrystalization and solidification of dentin (Fig. 1) . EDAX microanalysis(Fig.2 and Table 1) show highest carbon level and lowest calcium level .this evidence may be explained as lower USPL thermal effect on organic component of dentin tissue .The SEM micrographs of (2nd group) 300 mJ showed a development of a crater (Fig. 3) without cracking . EDAX microanalysis (Fig.4 and Table 2) show lower carbon level and higher calcium level than first group. This evidence may be attributed to increase of USPL thermal effect on organic component of dentin and melting of non organic component. The SEM micrographs of (3rd group) 400 mJ showed a development of a crater with cracking (Fig. 5). EDAX microanalysis (Fig.6 and Table 3) show lowest carbon level and highest calcium level. In this study, this evidence may be attributed to high energy density of USPL show thermal side effect on organic

component of dentin. We can explain that thermomechanical ablation of dentin tissue, as Li *et al.,* ([17]) mentioned in case of Er:YAG dentin ablation , by causing micro-explosions within inorganic structures in teeth. Then laser vaporizes water and other hydrated organic components until internal pressure causes the destructive explosion of the inorganic component before the melting point is reached. So increase of laser density may cause decrease in organic component of dentin and melting with rational increase to inorganic component of dentin. This results coincide with Daskalova *et al.,* ([16]) who stated that As the number of pulses was raised , the presence of cracks at the bottom of the ablated crater was observed, heat is generated in a volumetric region in depth. After a certain number of pulses, a sufficient amount of heat per pulse is deposited in a cumulative manner leading to an increase in the local temperature and initiating a process of thermal ablation followed by formation of cracks at the cavity bottom.

Conclusion:

-Dentin ablation with USPL in lower energies led to development of a characteristic molten morphological layer. These findings can be applied to achieve sealing of the dentinal tubules necessary for the prevention and treatment of an early-stage carious lesions and to make the tissue more resistant to development of new caries.

-There are a direct correlation between USPL energy level and thermomechanical ablation of dentin surface . So high energy levels of USPL should have restricted role clinically.

Corresponding author
Ola. M. Sakr
Departments of Operative Dentistry, College of Dentistry, Qassim University, Kingdom of Saudi Arabia & Misr University for Science and Technology, Egypt
olasakr2004@yahoo.com

References

1. Walsh L.J. (2003): The current status of laser applications in dentistry, Dent. J. 48; 146–155
2. Neev, J., L.B. Siiva, M.D. Feit, M.D. Perry, A.M. Rubenchik, B.C. Stuart, (1996): Ultra short pulse lasers for hard tissue ablation. IEEE Journal of Selected Topics in Quantum Electronics: 2: 790-799.
3. Yip H.K., L.P. Samaranayake (1998):Caries removal techniques and instrumentation ,:a review, Clin. Oral Investing. 2 148–154.
4. James KA (2000).: Essentials of oral histology and embryology. 2nd ed , Mosby Co, Inc.;P.: 94 - 106 ;
5. Giachetti, L., D. Scaminaci Russo, F. Scarpelli and M. Vitale, (2004): SEM analysis of dentin treated with the Er:YAG laser:A pilot study of consequences resulting from laser use on adhesion mechanisms. Clin Laser Surg Med., 22: 35.
6. Liu H.C., C.P. Lin, W.H. Lan (1997): Sealing depth of Nd:YAG laser on human dentinal tubules, J. Endod., 23 691–693.
7. Attril DC , Davies RM , King TA , Dickinson MR and Blinkhorn AS (2004): Thermal effect of Er:YAG lasers on simulated dental pulp : a quantitative evaluation of the effect of water spray . J Dent Res , 32: 35-40.
8. Visuri S.R., J.T. Walsh Jr., H.A. Wigdor, (1996) : Shear strength of composite bonded to Er:YAG laser-prepared dentin, Lasers Surg. Med. 18 294–300.
9. Apel C., J. Meister, R.S. Ioana, R. Franzen, P. Hering, N. Gutknecht (2002): The ablation threshold of Er:YAG and Er:YSGG laser radiation in dental enamel, Lasers Med. Sci., 17 ;246–252.
10. Ebihara A., B. Majaron, L-H.L. Liaw, T.B. Krasieva, P. Wilder-Smith (2002) : Er:YAG laser modification of root canal dentine: influence of pulse duration, repetitive irradiation and water spray, Lasers Med. Sci. 17: 198–207.
11. Kraft J.F., K. Vestentoft, B.H. Christensen, H. Lovschall, P. Balling (2008): Calculus removal on a root cement surface by ultrashort laser pulses, Appl. Surf. Sci., 254 :1895–1899.
12. Ozono K., M. Obara(2003): Tailored ablation processing of advanced biomedical hydroxyapatite by femtosecond laser pulses, Appl. Phys. A 77; 303–306.
13. Kamata M., T. Imahoko, K. Ozono, M. Obara(2004): Materials processing by use of a Ti:sapphire laser with automatically-adjustable pulse duration, Appl. Phys., A79:1679–1685.
14. Chung S., E. Mazur (2009): Femtosecond laser ablation of neurons in C. elegans for behavioral studies, Appl. Phys., A; 96 335–341.
15. Lin C.P., B.S. Lee, S.H. Kok, W.H. Lan (2000): Treatment of tooth fracture by medium energy CO2 laser and DP-bioactive glass paste: thermal behavior and phase transformation of human tooth enamel and dentin after irradiation by CO_2 laser, J. Mater. Sci.: Mater. Med. 11:373–381.
16. Daskalova A., S. Bashirb, and W. Husinsky (2010): Morphology of ablation craters generated by ultra-short laser pulses in dentin surfaces: AFM and ESEM evaluation. Applied Surface Science 257; 1119–1124.
17. Li ZZ, Code JE and Van de Merwe WP(1992): Er:YAG laser ablation of enamel and dentin of human teeth: determination of ablation rates at various fluencies and pulse repetition rates. Laser Surg Med., 12:625–30.

5/21/2012

Study of extremely low frequency electromagnetic wave effects on the acetylcholine and, achievements on the Alzheimer disease

A.Mollai[1], Z.Emami[1*], H.Damsaz[1], A.Haghpeima[1], B.Haghighi[1]
[1]Department of physics, Faculty of Sciences, Mashhad Branch , Islamic Azad University Mashhad Branch, Iran
***Corresponding author:** Zahra emami, Department of physics, Faculty of Sciences,
Mashhad Branch , Islamic Azad University Mashhad Branch, Iran
E-mail: zahra_sh_emami@yahoo.com
Phone# +985118435000, Fax# +985118424020

Abstract: There are different achievements about the Electromagnetic waves interaction with acetylcholine and the resultant studies on the Alzheimer patients. Because the acetylcholine intermediate enzyme is reduced in the brain of these patients, based upon some research works it is believed that the absorption of electromagnetic field in acetylcholine may tend to increased this enzyme amounts in the brain of these patients, then in this research we studied the excitation and the radiation absorption process effects of the extremely low frequency (ELF) electromagnetic waves in the acetylcholine from this magnetic field. The absorption process calculated with the aid of IR (Infra Red), UV (Ultra violet), and the Visible spectroscope devices.

[A.Mollai, Z.Emami, H.Damsaz,A.Haghpeima and B.Haghighi. **Study of extremely low frequency electromagnetic wave effects on the acetylcholine and, achievements on the Alzheimer disease.** *Life Sci J* 2012;9(4):963-973] (ISSN:1097-8135). http://www.lifesciencesite.com. 149
Key words:: Aging disease, Alzheimer disease, Acetylcholine, UV-visible spectroscopy
Pacs: 81.40Cd, 87.19.Xr

Introduction

The fast progress in technology of mankind life's is not together with his physical & psychological healthfulness[1-2].One problem of industrialized and developing societies is the neuropathic disorders and anomaly of brain, that is together with unknown causalities[3].One of this anomaly& brain disorder is Alzheimer disease. This disease is one of the most complicated mentally disease that only after death of the patient it's crucial diagnostics through the brain autopsy is rather possible [4].The progress of the disease in the patients is slow and at last steps it reached to the end stage with patients death, sometime also the symptom of disease, mistakes with the patients senility because boat are the aged diseases.

It is known that this disease, involved 2- 5% of the aged people and is because of the hypo camp region cells destruction and prevent to produced acetylcholine more. The electromagnetic radiation absorption effects on the human body first discovered in the1960s decade. This wave in long terms tend to vision disordered like cataract, blood changeness and the brain systems disturbances [5].High does electromagnetic radiation exposure effects on mankind are included as follows:

- Spontaneous misabortion
- Menstrual irregularity
- Sudden epilepsy
- Speech inability
- Some cancers involving
- Even the possibility of occurrences of brain disturbances from long term exposure of radiation could be schizophrenia, M.S etc. Early at the beginning of the 20th century the first Alzheimer disease symptoms were known from those one can mentioned weakness memory and amnesia that are caused from the lack of acetylcholine intermediate enzyme in brain cells that transfer the messages .Then reduction of acetylcholine in brain cells is a symptom of the progress of Alzheimer disease [6].This is why we studied the short term electromagnetic radiation absorption as a symptom of increasing this enzyme in brain. The exposure of radiation in this research studiedI- by a homemade device and the radiation absorption studied by2- an spectroscope in the IR, UV, and visible range of electromagnetic radiation.

Material method

1- With pure water as buffer avail of acetylcholine transform to liquid.
2- We used our homemade device for producing electromagnetic waves and magnetic field(Fig-1)
3- This device also equipped with a unite for temperature control.
4- Spectrophotometer in the IR UV and Visible Rang for absorption calculation.
5- A 2cc sampler with 1000 ppm.
6- The electromagnetic waves ranges from 1Hz-1KHz

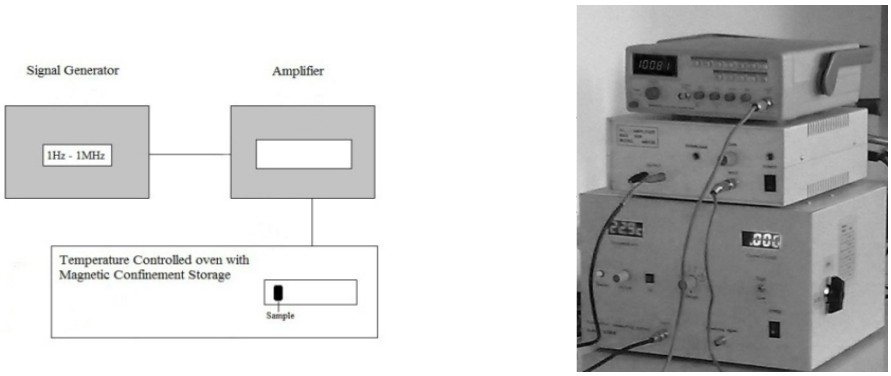


Fig-1 Home made device for electromagnetic radiation production and temperature unite control

To fix the temperature of the acetylcholine we put it into refrigerator for a time period about 24hours before the exposure, at the time of experiment we put it into the temperature unite control oven to reach the 37ċ. This process is for fixing the enzyme temperatureabout37ċ, then we selected 2mm sampler, to expose to (ELF) electromagnetic radiation for time ranges about 5,15,30,45 and60minutes respectively. the spectrophotometer are working in IR, visible and UV range of electromagnetic and the calculated specter taken during time divisions as mentioned above, while the frequency of exposure also are selected in ELF ranges. It should be mentioned that one sample also put into the spectroscope instrument without electromagnetic exposure that could be act as an evidence in the spectroscopic measurements .All achievements are seen in the table-1.All experimental resultants are seen in Figs.1

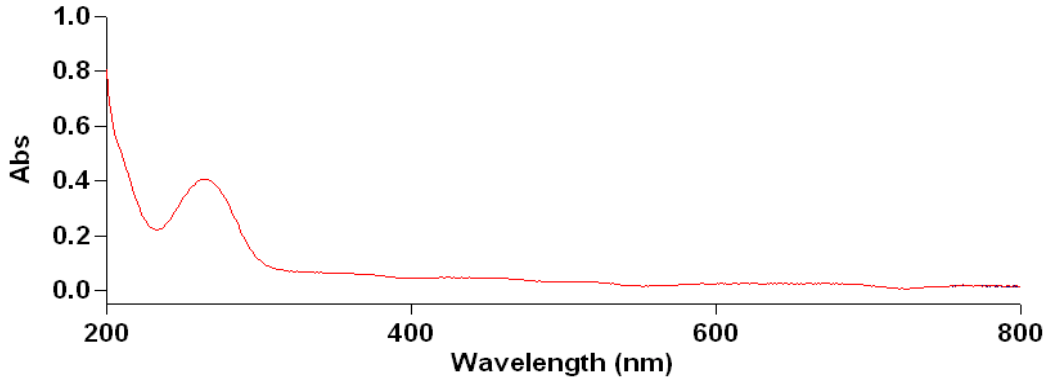


Fig-2. Far-UV-visible Acetylcholine absorption in the absent of electromagnetic field

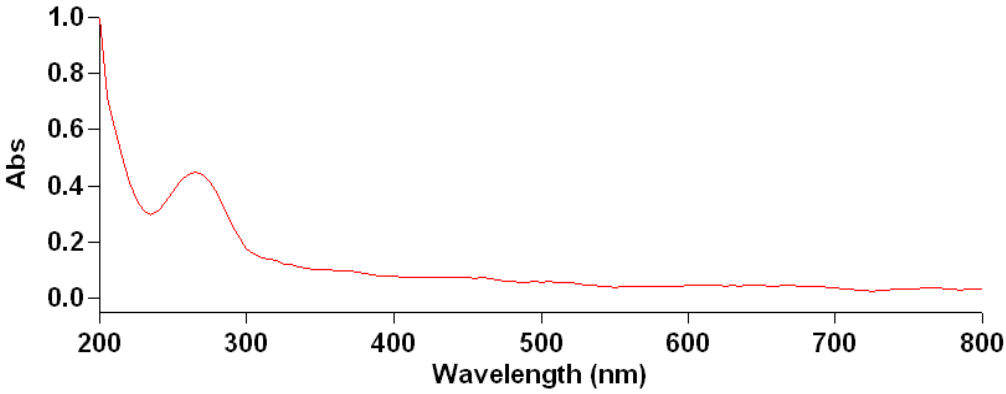


Fig-3.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 50HZ, frequency, 7.65µT magnetic field. The maximum absorption wave length is265nm,the time of exposure is 5 min and the enzyme temperature 37ċ

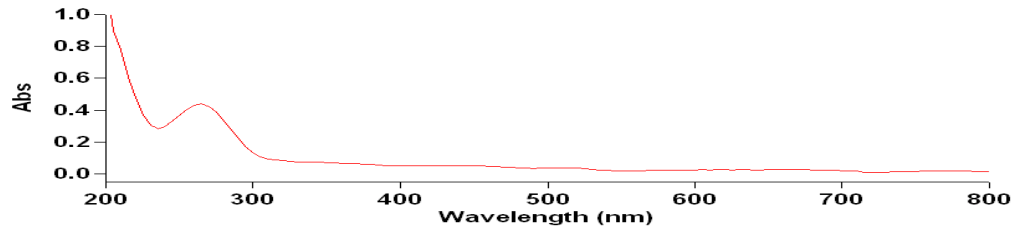


Fig-4.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 50HZ, frequency, 7.65µT magnetic field. The maximum absorption wave length is265nm.the time of exposure is 15 min and the enzyme temperature37ċ

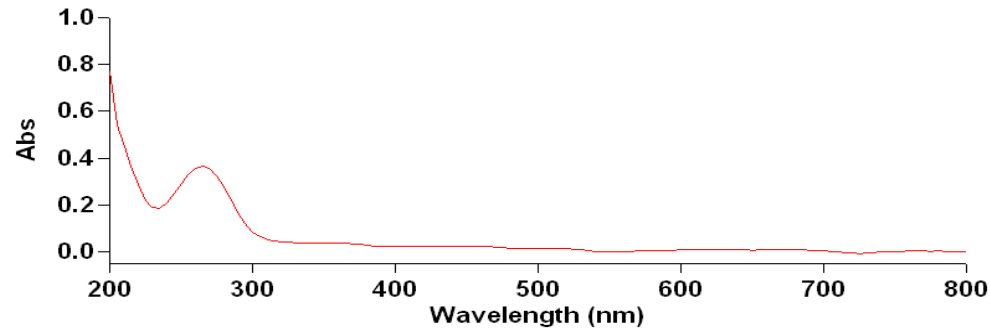


Fig-5.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 50HZ, frequency 7.65µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is30 min and enzyme temperature37ċ.

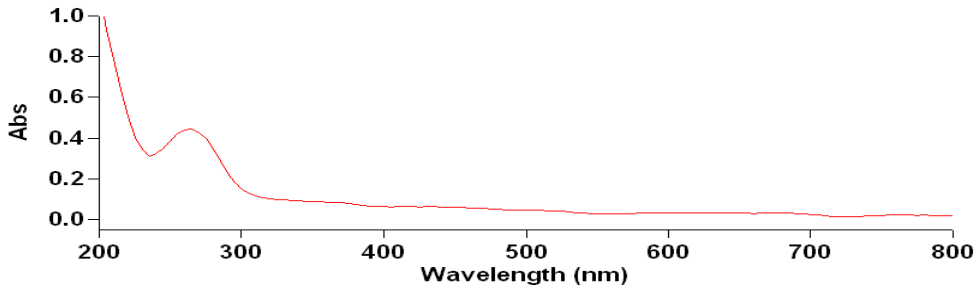


Fig-6.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 50HZ, frequency 7.65µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is45 min and the enzyme temperature37ċ.

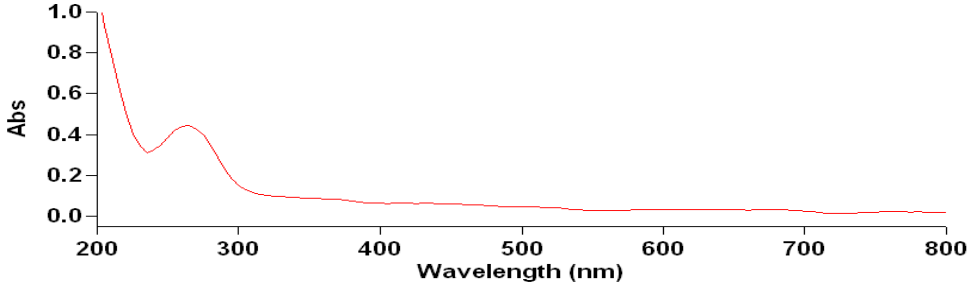


Fig-7.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 50HZ, frequency 7.65µT magnetic field. The maximum absorption wave length is265nm, the time of exposureis60 min and the enzyme temperature37ċ.

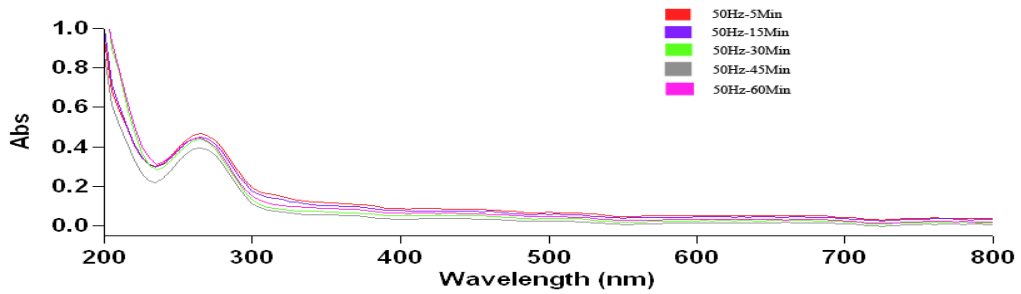


Fig-8.UV-visible. Absorption specters of Acetylcholine in the present of electromagnetic wave 50HZ frequency and 7.65µT magnetic field. With the time ranges 5-60 min for comparison, the enzyme temperature again is 37ċ

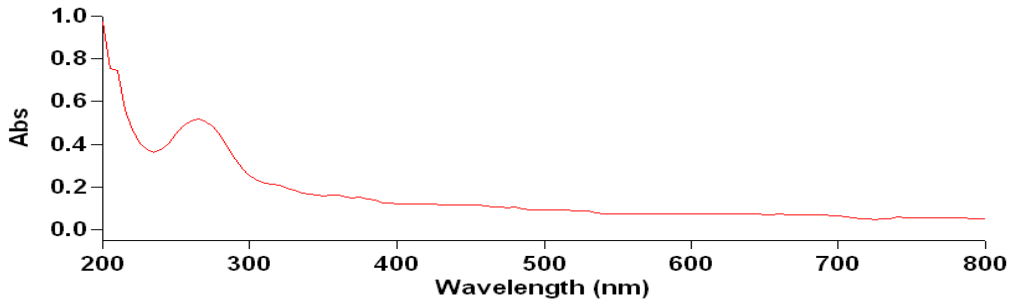


Fig-9.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 500HZ, frequency 9.114µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is5 min and the enzyme temperature37ċ.

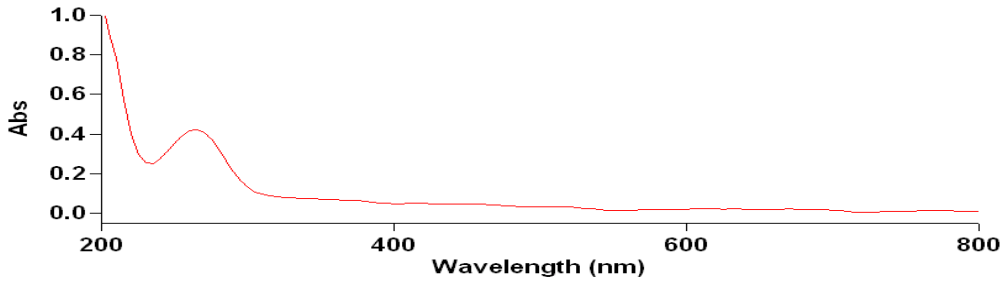


Fig-10.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 500HZ, frequency 9.114µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is15min and the enzyme temperature 37ċ .

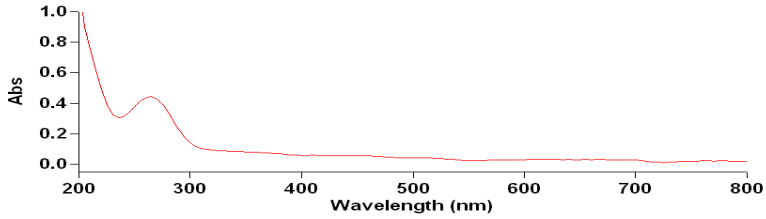


Fig-11.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 500HZ, frequency 9.114µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is30min and the enzyme temperature 37ċ

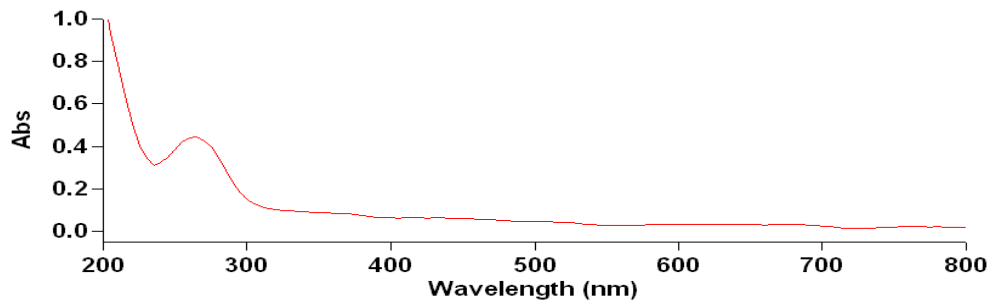


Fig-12.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 500HZ, frequency 9.114µT magnetic field. The maximum absorption wave length is265nm, the time of exposureis45min and the enzyme temperature37ċ.

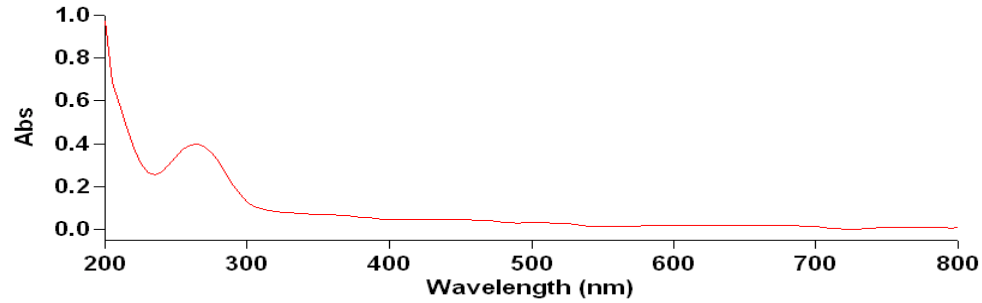


Fig-13.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 500HZ, frequency 9.114µT magnetic field. The maximum absorption wave length is265nm, the time of exposureis60min and the enzyme temperature37ċ.

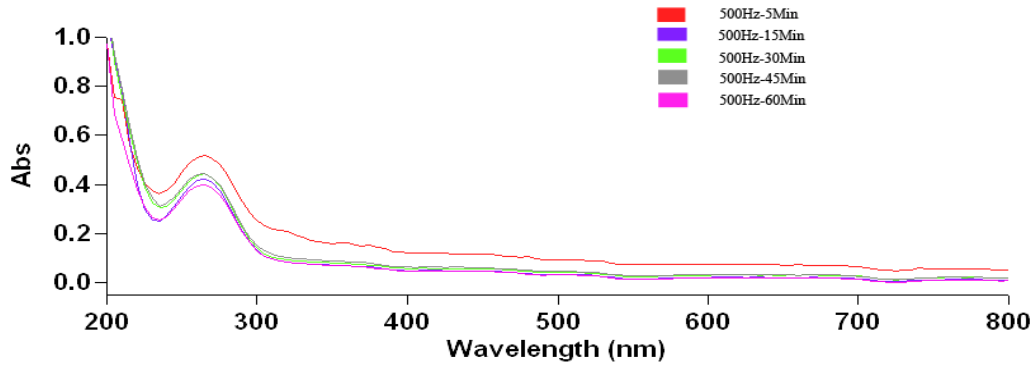


Fig-14.UV-visible. Absorption specters of Acetylcholine in the present of electromagnetic wave 500HZ frequency and 9.114µT magnetic field. With the time of exposures ranges 5-60 min for comparison, the enzyme temperature again 37ċ.

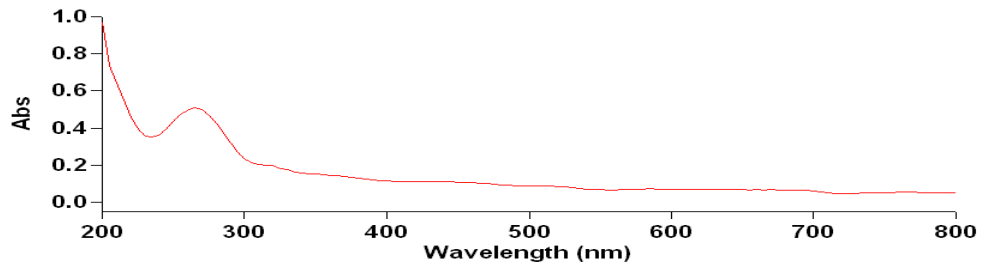


Fig-15.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 1KHZ, frequency 9.268µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is 5min and the enzyme temperature37ċ.

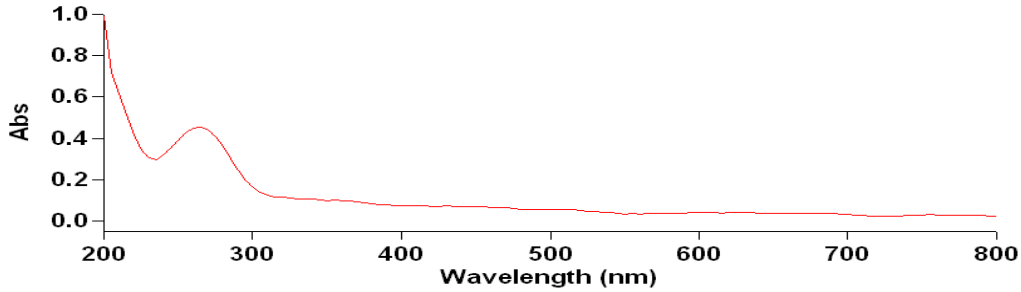


Fig-16.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 1KHZ, frequency 9.268µT magnetic field. The maximum absorption wave length is265nm, the time of exposureis15min and the enzyme temperature37ċ.

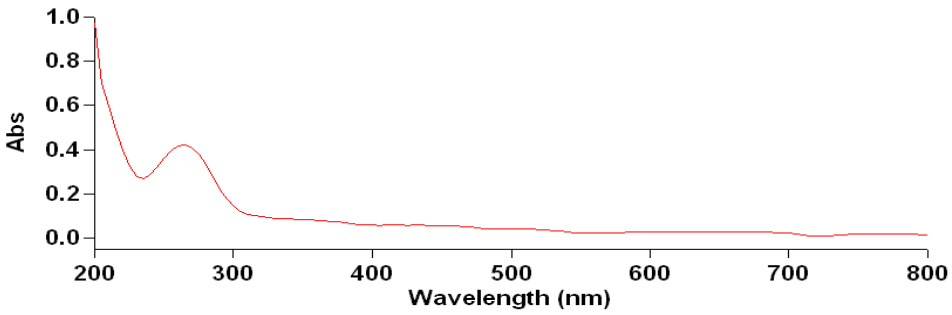


Fig17.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 1KHZ, frequency 9.268µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is30 min and the enzyme temperature37ċ.

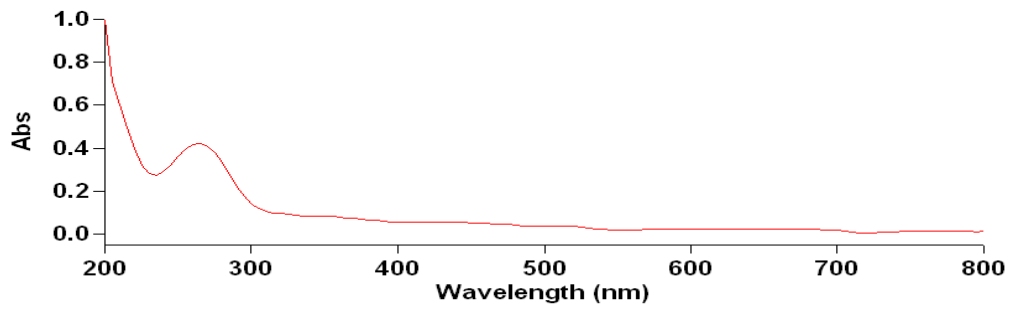


Fig-18.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 1KHZ, frequency 9.268µT magnetic field. The maximum absorption wave length is265nm, the time of exposureis45min and the enzyme temperature37ċ.

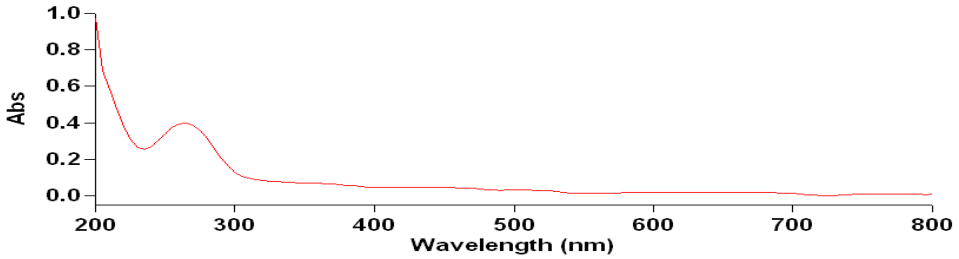


Fig-19.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 1KHZ, frequency 9.268µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is60min and the enzyme temperature37ċ

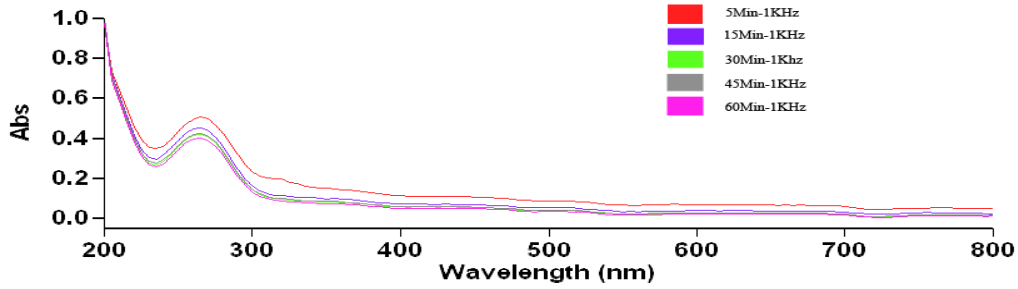


Fig-20.UV-visible. Absorption specters of Acetylcholine in the present of electromagnetic wave 1KHZ frequency and 9.268µT magnetic field. With the time ranges 5-60 min for comparison, the enzyme temperature again 37ċ.

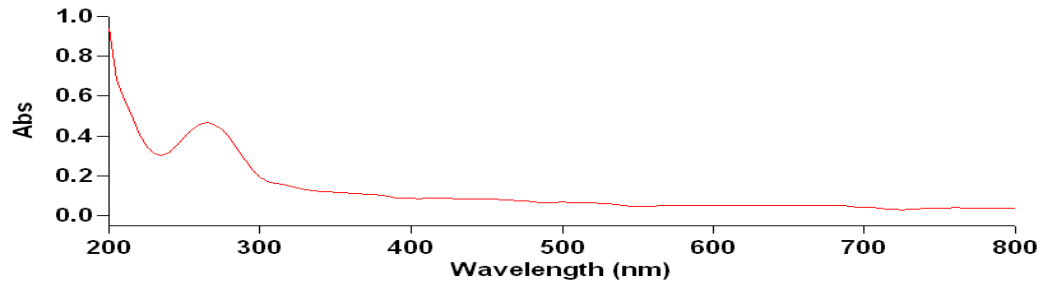


Fig-21.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 5KHZ, frequency 9.173µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is5min and the enzyme temperature 37ċ

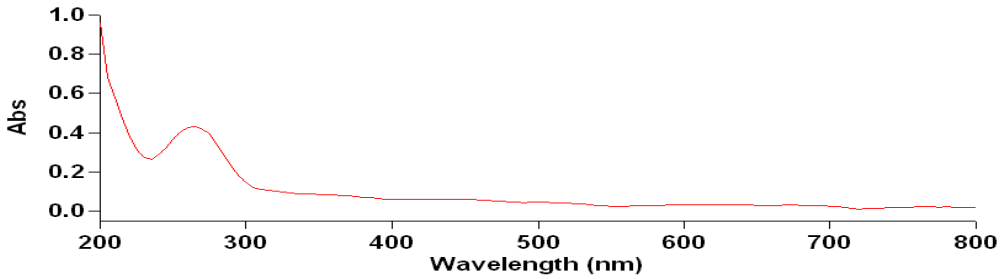


Fig-22.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 5KHZ, frequency 9.173µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is15min and the enzyme temperature37ċ

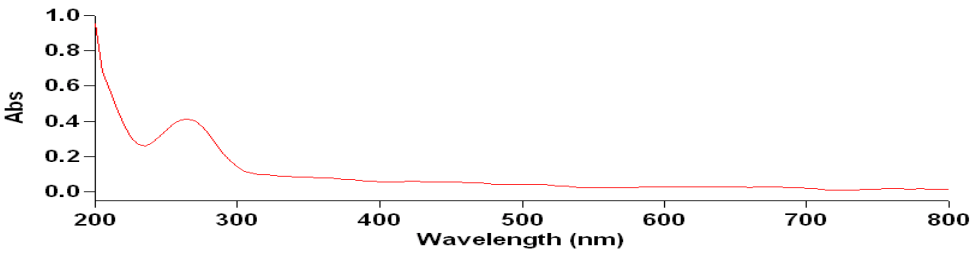


Fig-23.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 5KHZ, frequency 9.173µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is30min and the enzyme temperature37ċ.

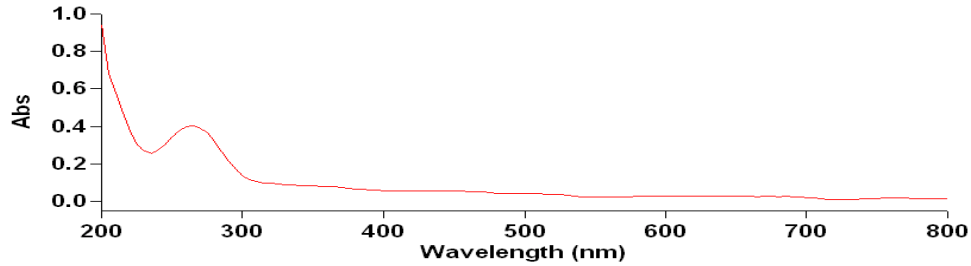


Figure24.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 5KHZ, frequency 9.173µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is45min and the enzyme temperature37ċ.

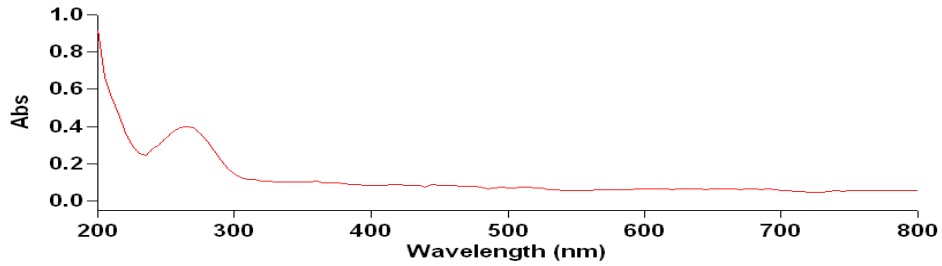


Fig-25.UV-visible. Absorption spectra of Acetylcholine in the present of electromagnetic wave 5KHZ, frequency 9.173µT magnetic field. The maximum absorption wave length is265nm, the time of exposure is60min and the enzyme temperature 37ċ

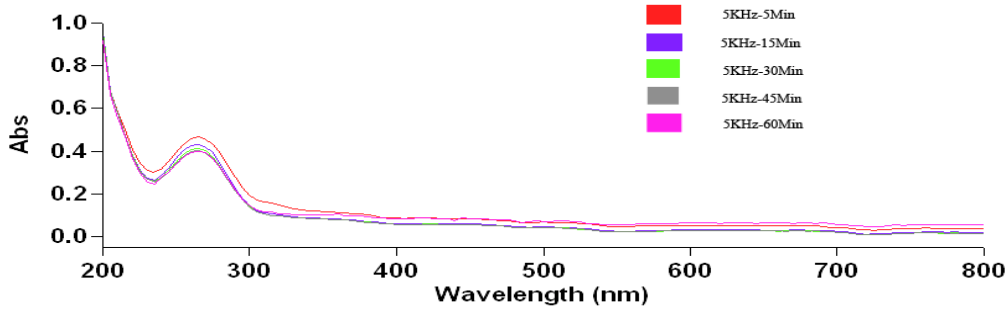


Fig-26.UV-visible. Absorption specters of Acetylcholine in the present of electromagnetic wave 5KHZ frequency and 9.173µT magnetic field. With the time ranges 5-60 min for comparison, the enzyme temperature again 37ċ.

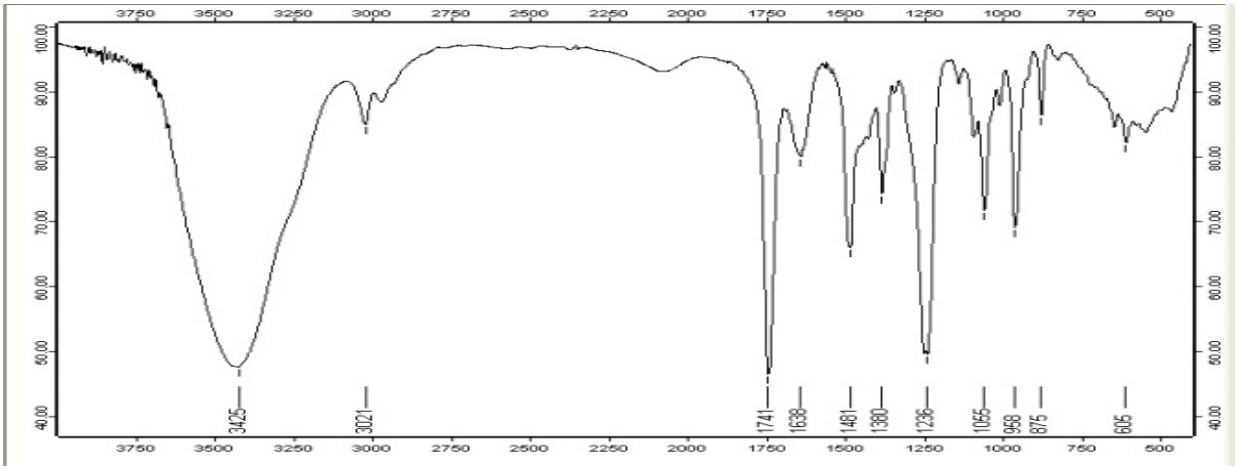


Fig-27.IRabsorption specter for Acetylcholine in the absent of Electromagnetic field at the temperature 37ċ.

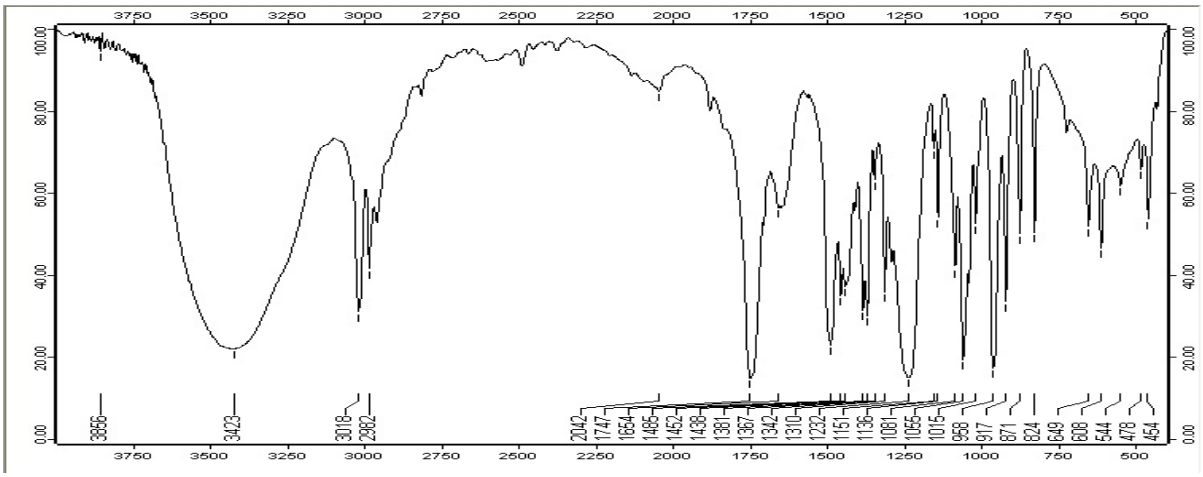


Fig-28.IR Relative absorption of the Acetylcholine in the present of Electromagnetic field, frequency 50 Hz, and Magnetic field 7.65µT, at the temperature37ċ

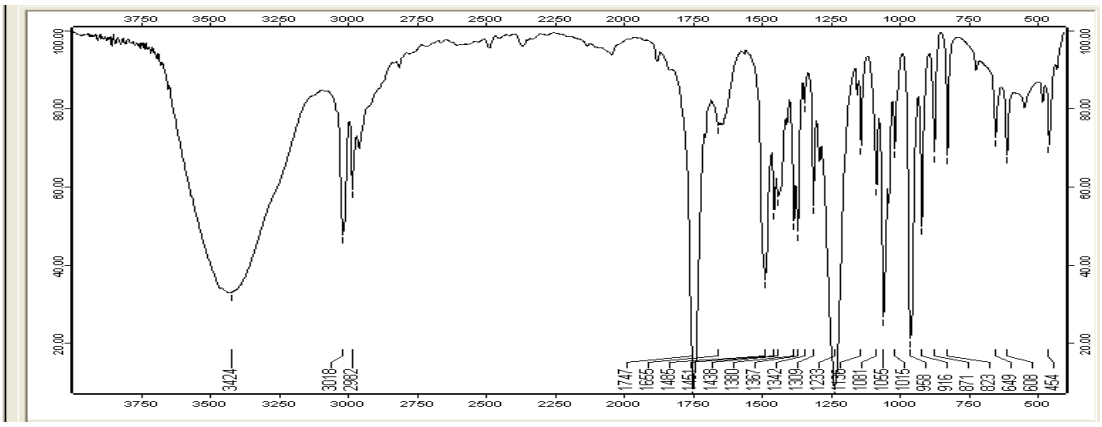


Fig29.IR Relative absorption of the Acetylcholine in the present of Electromagnetic field, frequency 500 Hz, and Magnetic field 9.114µT, at the temperature37ċ

Conclusion Remarks

Extremely Low Frequency (ELF)electromagnetic field induced currents& electromagnetic field in biological systems depending to the waves intensity that may be very small and sometimes regardless.[7] in a special range of the waves .If frequency in the ELF range increased more the noticeably absorption of waves presented[8]. Molecular excitation during high amounts absorption of radiation in physical words is a proof of increased of entropy and the increasing of molecular impacts [9]. Although high amount of radiation absorption may tend to stress, tiredness in one hand, it may in turn tend to increased acetylcholine amount in the brain of patients in other hand, increasing Acetylcholine in the brain cells of Alzheimer patients could tend to relative relief of the Alzheimer disease. Our studies shows that the best absorption occurred in the wave length(see please Figs2-25)of265nm-275nm on the spectroscopic studies that belongs to the wave frequency 1KHz ,with 9.268μT magnetic field and the 5 minute time of exposure . We agreed that some biological action-reaction may be because of electromagnetic wave absorption at the time of exposure which tends to increased of the acetylcholine in the brain cells of the patients that are unknown yet. The lower limit of ELF waves and exceeding time of exposure did not tend to increase noticeable amounts of the acetylcholine .Also our research showed that the waves absorption in the visible range is not so many important (as all Figs showed) because in all ELF wave length very low amounts of the absorption of radiation in the wavelength greater than the UV occurred.

References

[1]. Price, D.L., Sisodia, S.S., and Borchelt, D.R. (1998). Genetic Neurodegenerative Diseases: The Human Illness and Transgenic Models. Science 282, 1079-1093

10/15/2012

[2]. Vassar, R., and Bennet, B.D. (1998) Beta-secretase cleavage of alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE.Science 286, 5440-5464

[3]. LaFerla, F.M. (2002) Calcium dyshomeostasis and intracellular signalling in alzheimer's disease. Nature Reviews Neuroscience 3, 862-872

[4]. Gregersen, N., Bross, P., and Andresen, B.S. (2001) the role of chaperone-assisted folding and quality control in inborn errors of metabolism: Protein folding disorders. Journal of Inherited Metabolic Disorders 24, 189-212

[5]- MacCalman C, Blaschuk OW (1994) effect electromagnetic wave on human. Endocrinology 134:630-639.

[6]. Price, D.L., Sisodia, S.S., and Borchelt, D.R. (1998). Genetic Neurodegenerative Diseases: The Human Illness and Transgenic Models. Science 282, 1079-1093

[7]- Vertessy G, Szollosy J (1994) High sensitivity magnetic field meter. In Simunic D ed., Mobile communication and extremely low frequencyfields& instrumentation and measurements in bioelectromagnetic research

[8] R.P. Liburdy, D.E. Callahan, J. Harland, E. Dunham, T.R. Sloma, P.Yaswen, Experimental evidence for 60 Hz magnetic fields operating through the signal transduction cascade. Effects on APOELett. 334 (1993) 301–308.

[9]- De Seze R, Lahitte A, Moreau JM, Veyret B (1994) Generation of extremely low frequency magnetic fields with standard available commercial equipment: Implications for experimental bioelectromagneticwork.BioelectrochemBioenerg 35:127-131.

Prediction of PEF and LITH logs using MRGC approach

Mahdi Pabakhsh[1], Kamyar Ahmadi[2]*, Mohammad Ali Riahi[3] and Abbas Abbaszadeh Shahri[4]

[1]M.Sc. Student of Exploration Petroleum Engineering, Islamic Azad University, Science & Research Branch, Tehran, Iran; m.pabakhsh.oil.eng@gmail.com
[2]Academic staff and Faculty member, Department of Petroleum Engineering, Islamic Azad University, South Tehran Branch, Tehran, Iran
[3]Associate professor, Institute of Geophysics, University of Tehran, Tehran, Iran; mariachi@ut.ac.ir
[4]Assistant professor, Department of Geophysics, Islamic Azad University, Hamedan branch, Hamedan, Iran; a_abbaszadeh@iauh.ac.ir
***Corresponding Author**: k.ahmadi@srbiau.ac.ir

Abstract: The Fuzzy logic method offers superior log estimation properties for a large class of well log functions and has been employed as a standard tool in formation evaluation of Oil production zones. However it suffers from spurious behavior in the vicinity of edge trends in log signals. In this article, we used The MULTI-RESOLUTION GRAPH-BASED CLUSTERIN (MRGC) Supervised framework for obtaining lithology properties from PEF & LITH logs that estimated in one of the Well (D) in the field since there is no core data in most wells. Estimations are performed from basic information and model logs of another well (A), including RHOB, NPHI, DT, PHIE, and NDS. Taking advantage of this framework, we show that it is feasible to recover log data from a relatively accurate method especially in inhomogeneous formation than the Fuzzy logic method.

[Mahdi Pabakhsh, Kamyar Ahmadi, Mohammad Ali Riahi and Abbas Abbaszadeh Shahri. **Prediction of PEF and LITH logs using MRGC approach.** *Life Sci J* 2012;9(4):974-982] (ISSN:1097-8135). http://www.lifesciencesite.com. 150

Keywords: Pef log, Lith log, Estimate, MRGC, Fuzzy Logic

Introduction

In recent years, fuzzy logic and artificial neural networks have been widely used for reservoir studies. For example, several researchers (Saggaf & Nebrijs, 2003; Cuddy, 1997; Nordlund, 1996; Bois, 1984) have applied neural networks and fuzzy logic in reservoir studies of several fields.

Fuzzy logic was introduced in 1956 by Lotfi zadeh in a paper entitled Fuzzy set. In the fuzzy logic, a membership function is described which allows the membership of more than one class with different membership degrees.

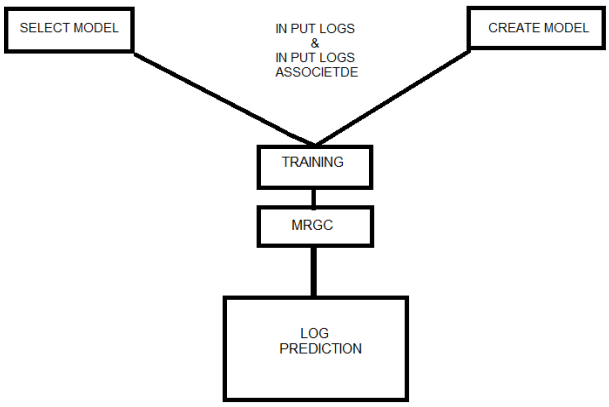
Most previous researchers (Olson and Brill, 1997; Bvbyk et al. 1999; Conte A. et al, 2006; Svtady Vieira, 2008) have attempted to determine PEF and LITH logs but none of them have used MULTI-RESOLUTION GRAPH-BASED CLUSTERIN (MRGC) method.

MRGC is one of the most important hierarchical methods. In this research, MRGC approach is presented to determine petrophysical parameters from well logs in order to create different resolution of the data. In this study, two logs from an oil field are used. In well A, the PEF log is available and the LITH log is derived from core data. These information are used to predict the same logs in well D where PEF and LITH logs are not available.

Methodology

MRGC method uses model logs and associated logs to find a relationship between them to predict PEF and LITH logs, so the estimated logs can be propagated to the rest of the wells.

The MRGS method is, in fact, a combination of artificial intelligence techniques and a hierarchical clustering method. This method uses KRI and NI index parameters which discern it from conventional methods.



WORKFLOW

Suppose there are 6 members, 3 clusters in a resolution and 2 clusters in a higher resolution. In this case, the MRGC method uses a parameter called KRI. In cross-plots, especially in Neutron- Sonic where two points are close to each other and there is no resemblance between them, this method successfully distinguishes them. In this approach, the following indices are added to make the MRGC method more robust than other hierarchical methods:

Neighborhood Index (Neighboring Index): This parameter substitutes the distance parameter. As mentioned before, when two points are close to each other, they can be easily separated if they have high NI. Unlike other hierarchical methods, depending on the facies' behavior, the user can specify the number of facies.

KRI Index: It is a combination of NI, distance and weighted distance function M(x, y) which specifies the Neighborhood or the degree of membership for M. If it is low, it is affected by M; otherwise it has a high membership degree and is not affected by M.

$$NI(X) = \sum_{N=1}^{n-1} \exp(-m_{n.a}) \qquad (1)$$

Where m the neighbor ranking, a is the resolution parameter.

$$KRI = NI(x)M(x,y)D(x,y) \qquad (2)$$

In which M, is the weighting distance, D is the distance between x and y.
First kernel or the center point which influences all of its neighboring members" is specified", and then all the members will be compared. The members that are influenced by the kernel affect other members as well. The boundaries are, therefore, specified where a member is affected by its previous member but cannot affect other members. So, the boundaries determine the Split point and distinguish different groups based on the parameters. According to this method, the model logs (NDS, RHOB, NPHI, PHIE, and DT) are introduced into the facimage section of the software to get the clusters. The LITH log is then inputted as an associated log and the data are trained.

Figure 1, at first, each log is divided into 2 clusters. Next, clustering is done based on the MRGC method with minimum of 6 and maximum of 15 clusters.

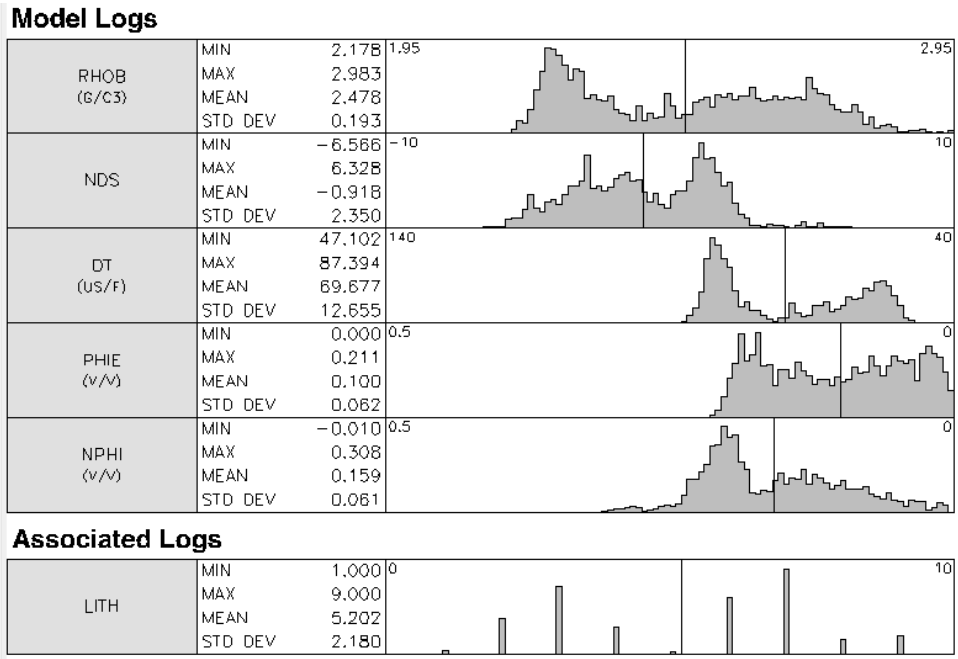


Figure 1: Training the data's model logs as follow:
Model log:"RHOB, NDS, DT, PHIE, NPHI
Associated log: LITH log

Figure 2, 15 clusters have been selected to estimate the PEF log in Well A as a model, where the PEF log is available. The estimated PEF log showed correlation with the actual PEF log in well A. figure 3, the PEF log is propagated to the well D and the derived lithology is drawn.

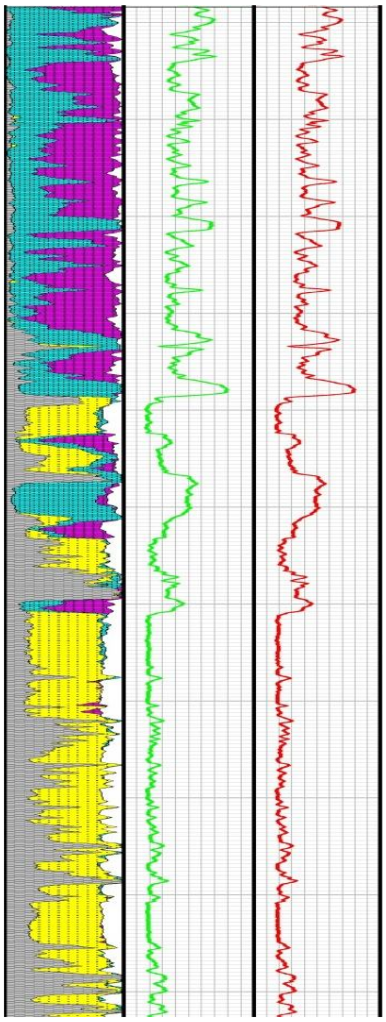


Figure2.Estimated PEF LOG in A well in red Color which has been drawn 0 to 10 and it is having correlation with PEF log in A well

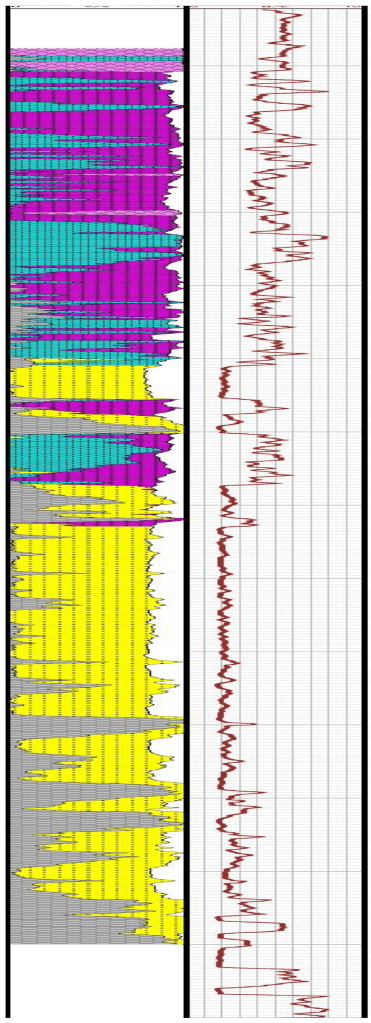


Figure 3.Estimated PEF log in D well IN brown color which has been drawn 0 to 10 and it is having correlation with formation of lithology.

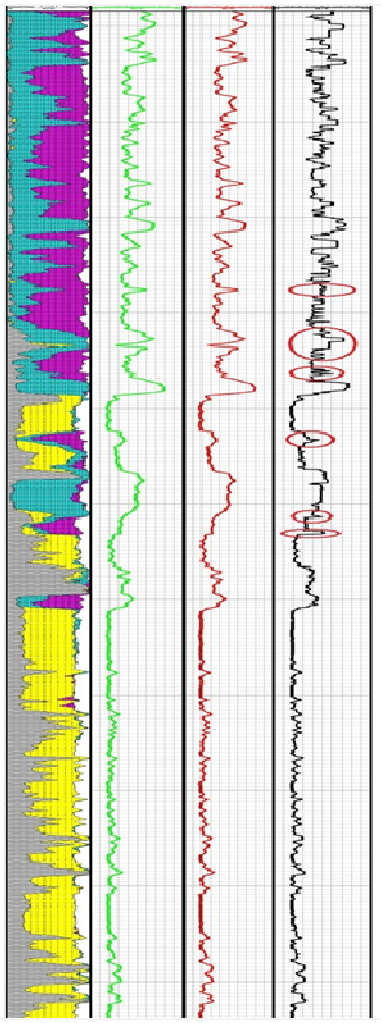


Figure 4.Estimated PEF log by Fuzzy method in black color which has been drawn from 0 t0 10 compared with estimated PEF log by MRGC method in red color and available PEF log in A well. In pointed circle, Fuzzy method has not been operated perfectly and MRGC method has been correlated completely with available PEF log in A well.

Figure 4, the results of FUZZY method is shown in which the RHOB, PHIE, NPHI, DT, and NDS logs are entered and the PEF log is estimated. Clearly, the where it shows a perfect correlation with the original log.

predicted PEF log in well A with the Fuzzy method less accurate than the one predicted with the MRGC method

Results and Discussion:

The Lithology of formation is heterogeneous and is divided into dolomitic limestone, sandstone with Anhydrate and shale which is accurately estimated from LITH and PEF logs using MRGC method. The Formation is divided into seven zones A1, A2, A3, A4, A5, A6, and A7.

Shown in Figure 5 from left to right, the first track is the derived lithology, the second track indicates LITH log which is computed from core data and the last track is the estimated LITH log from MRGC method which shows a very good correlation.

Figure 3 shows well D where LITH and PEF logs are not available. The obtained lithology from predicted PEF log shows a good correlation with the obtained lithology from petrophysical logs in Well D. As it can be seen, the result of predicted PEF log is very reliable, i.e., PEF number is 2 in the sandstone, 3 in shale, nearly 3 in limestone and 5 in anhydrite.

The estimated LITH log at well D in Figure 6 shows a high correlation with PEF log and the derived lithology.

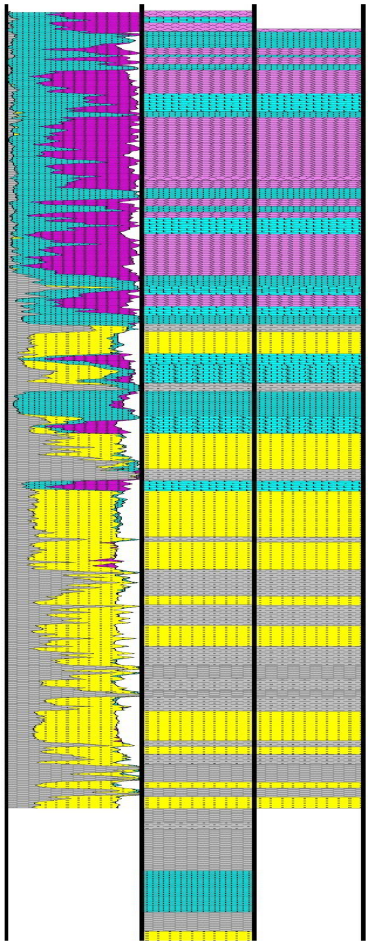


Figure 5. estimated LITH log by MRGC method in left track compared with LITH log obtained from Core in right track in A well.

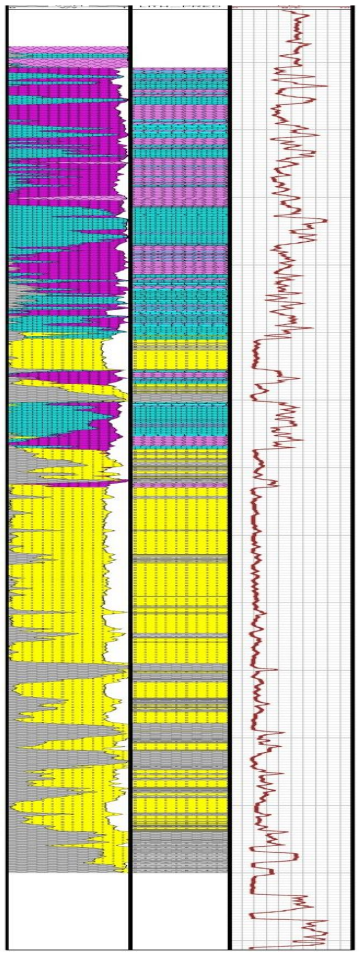


Figure 6.Estimated LITH and PEF log by MRGC method in D well

Figure 7, well A and D are shown in a section which demonstrates a good correlation between them and the zone A6 is the sandstone reservoir zone.

Figure 8, Cross plot of the PEF log and the sonic logs in well A is drawn, in which sonic log data are plotted in the horizontal axis from 40 to 140 microseconds. The colored data, green and yellow points, are the gamma ray log from 0 to 200 API, representing clean sands, shales and shaly sands respectively.

A linear relationship between DT and PEF logs is established as PEF= 9.70276-0.0906589DT and CC= 0.82 which is a high correlation between these logs. As the transit time in the formation is decreased, the corresponding PEF log value is increased.

In this case, the rocks are more condensate and porosity is decreased. The PEF log value approaches 5 to 5.5 which is an indicative of limestone or anhydrate. However, when the sonic log transit time is increased, the PEF log reads a lower value and the formation is more porous.

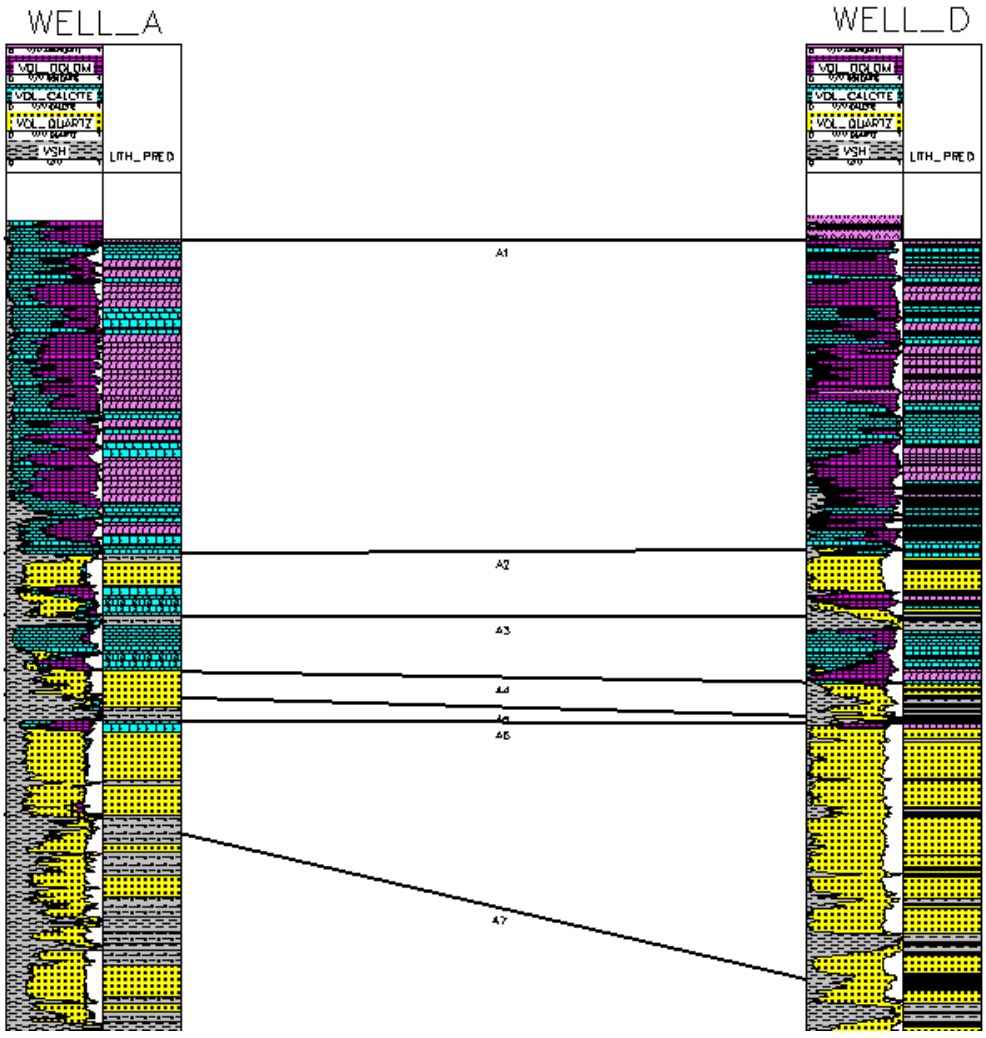


Figure 7. The section of A and B wells and the pointed correlation 7 Zones .

The range 2 to 3 of PEF log refers to sandstone to shale and dolomite (Figure 8). The negative slope of the regression line indicates an inverse relationship between Sonic and PEF log.

Figure 9, the Cross-plot of PEF and neutron logs in well A is shown. Neutron log data are plotted from -0.1 to 0.4 in the horizontal axis and the colored data refer to gamma ray from 0 to 200 API in which the green and yellow points are clean *sands, shaly sands and shales respectively. The linear relationship* between NPHI and

PEF logs is PEF= 5.76276-15.0999NPHI in which CC= 0.69 shows a high correlation.

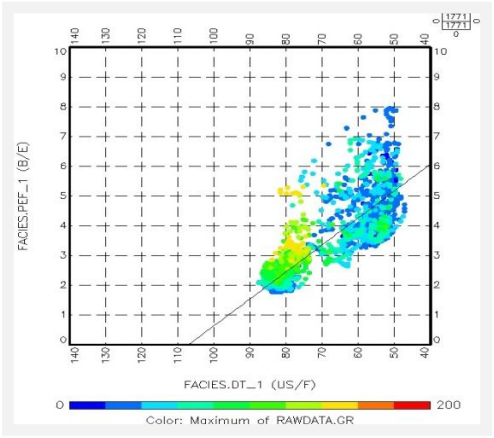


Figure 8. PEF and DT logs cross plot in well A

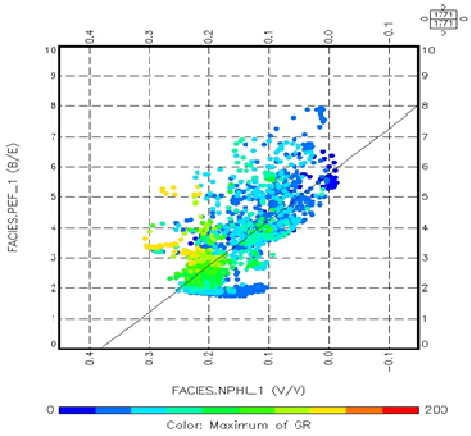


Figure 9. PEF and NPHI logs Cross plot in well

As neutron log reads a lower value, the PEF value increases and the rock tends to be more condensate, with PEF reading 5 belongs to anhydrate and limestone. On the other hand, as neutron log increases, the rocks is more porous and the PEF value decreases which is between 2 and 3 for porous sandstones and shale. The negative coefficient of the regression line indicates an inverse relationship between neutron and PEF logs.

In Figure 10, the Cross-plot of PEF and density logs in well A is shown. Density log data are plotted from 2 to 2.9 g/cm^2 in the horizontal axis and the colored data refer to gamma ray from 0 to 200 API in which the green and yellow points are clean sands, shaly sands and shales respectively.

The linear relationship between RHOB and PEF logs is PEF=-10.4896+5.59337RHOB in which CC= 0.78 shows a high correlation. AS the density log increases, the rocks become more condensate and the PEF value increases too, which refers to limestone and anhydrate. As the density log decreases, the porosity of formation increases.

The PEF value decreases to 2 and 3 for porous sandstones and shale. The positive coefficient of the regression line indicates a direct relationship between neutron and PEF logs.

Figure 11 is the Cross-plot of PEF and NDS logs in well A. The horizontal axis is the NDS log from -10 to 10 and the PEF log is plotted on the vertical axis from 0 to 10 and the colored data are gamma ray log. The NDS log value is negative in sandstones.

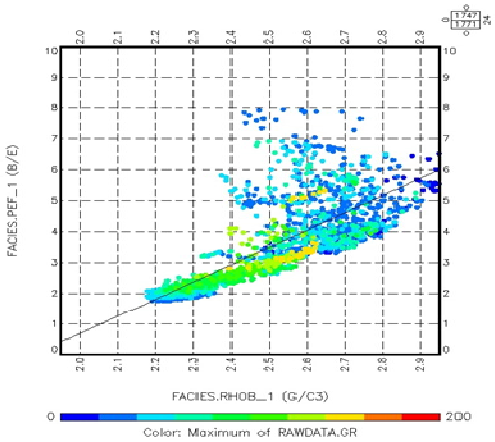


Figure 10. PEF and RHOB logs Cross plot in well A

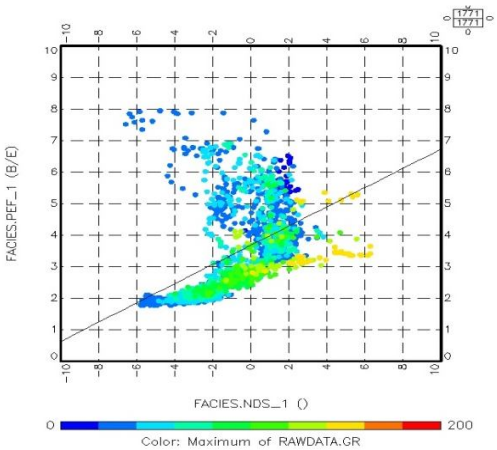


Figure 11. PEF and NDS logs Cross plot in well A

The NDS log value is positive in Anhydrite, dolomite and shale and near 0 where pure lime and the PEF log reads 2 in sandstones. The linear relationship is as PEF= 3.681140+3046690 * NDS and CC= 0.50, which shows a good correlation.
The NDS log is the separation of the neutron – density. In order to be separated, they should be normalized and shown with the same scale. The equation below is used for this purpose (Liu, 2012):

NDS=(RHOB-1.95)/0.06-(0.45-NPHI)/0.03 (3)

Figure 12, the Cross-plot of the estimated PEF log and the sonic log in well D is drawn with sonic log data plotted in the horizontal axis from 40 to 140 microseconds. The PEF log data are drawn from 0 to 10 in the vertical axis. Points where the transit time decreases show a more condensate rock such as limestone and anhydrate.

At points where the transit time increases, the formation is more porous which tends to be sandstone and shale with green to yellow colors. The estimated PEF log in condensate formations reads 5 for porous rocks.

Additionally, where Sonic log transit time increases, the estimated PEF log reads between 2 and 3 which is related to sandstone and shale. This is an indicative of the correctness of the estimated PEF log by the MRGC method. The linear relationship between sonic log and the PEF log is as: PEF =9.70276-0.0906589 * DT and CC =0.82, which is a high correlation between DT and PEF logs.

Figure 13 is the Cross-plot of the estimated PEF and the neutron logs in well D in which the neutron log is plotted in horizontal axis from -0.1 to 0.4 mV and the estimated PEF log from 0 to 10. As the neutron log increases, the porosity increases as well and the estimated PEF log reads a low value between 2 and 3 which is related to sandstone and shale. As the neutron log decreases, the PEF value reads between 5 and 6.
The range between 5 and 6 shows a dense and less porous formation which is related to limestone and Anhydrite. This indicates that the estimated PEF log by the MRGC method is acceptable. The linear relationship between NPHI and PEF logs is as: PEF= 5.80432-14.7678 * NPHI in which CC= 0.69 .

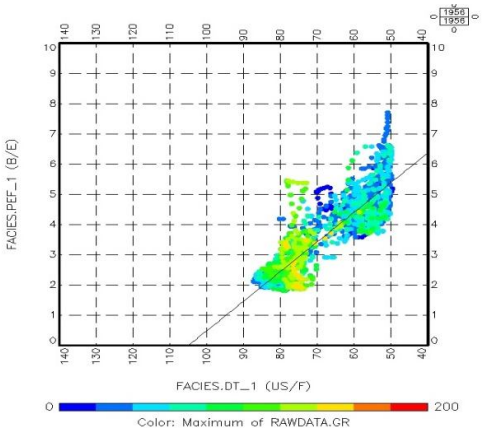


Figure 12: PEF and NPHI logs Cross plot in D well

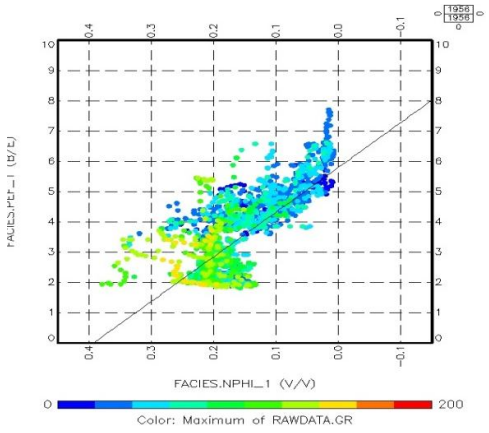


Figure 13. PEF and NPHI logs Cross plot in well D

Figure 14 the Cross-plot of the estimated PEF and the density logs in well D is drawn with the density log data plotted in the horizontal axis from 2 to 2.9 g/cm^2 and the PEF log data are drawn from 0 to 10 in the vertical axis. As the density is increased, the formation becomes less porous and more condense and the estimated PEF log reads between 5 and 5.5 which relates to limestone and anhydrate.
As the Density log reading decreases, the PEF reading is between 2 to 3 which shows porous formations like sandstone and shale rocks .This shows that the estimated PEF log by the MRGC method is excellent. The linear relationship between RHOB and PEF logs is

PEF= -11.53916.07501 with a high correlation of CC = 0.80.

Figure 15 is the Cross-plot of the estimated PEF and NDS in well D. The NDS log is plotted in horizontal axis from -10 to 10 and the estimated PEF log from 0 to 10. In NDS log, the negative points show sandstones and the estimated PEF log reads 2. The linear relationship between NDS and PEF logs is PEF 3.669210.235632 with CC= 0.41.

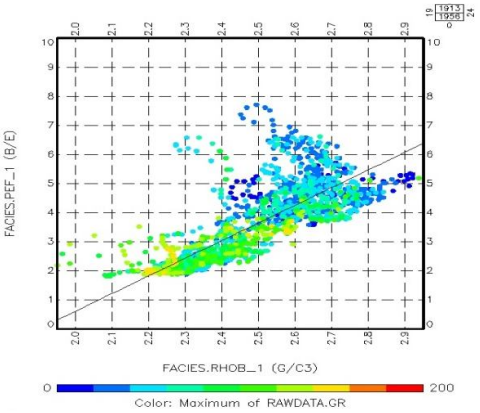


Figure 14. PEF and NPHI logs Cross plot in well D

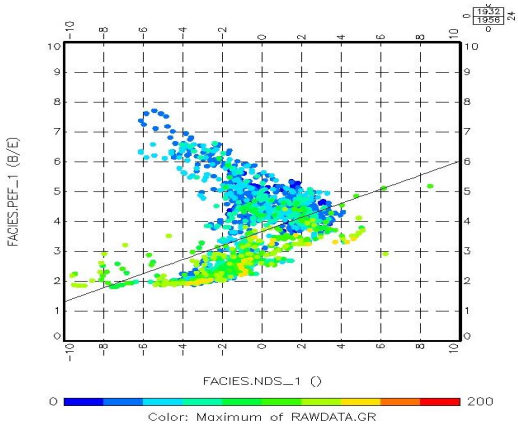


Figure 15. PEF and NDS logs Cross plot in well D

CONCLUSION

To identify formation lithology, it is required to have PEF and LITH logs. However, these logs are not available in old wells. Additionally, there is a limitation of core data and lack of coring in all the wells for lithology identification purposes.

In this study, the MRGC method is performed to estimate well logs. In this method, petrophysical data are grouped into a number of clusters based on NI and KRI indices. Compared to conventional methods, the introduced procedure is robust and shows superior results.

This type of clustering is the advantage of the MRGC method in respect to other clustering techniques. In each obtained cluster from the MRGC method, a relationship between PEF log and the model logs and also, between LITH log and the model logs is derived.

In this study, the formation is divided into 15 different clusters using MRGC method, in which, for each cluster the majority of PEF and LITH logs are derived and the average value of each cluster is related to PEF and LITH logs. For each average value, a number is defined which is the estimated PEF and LITH, so the MRGC method is more accurate. As the number of clusters are increased, the estimated PEF and LITH logs are smoother.

References

1. Bobick, A., Intille, S., Davis, J., Baird, F., Pinhanez, C., Campbell, L., Ivanov, Y., Schtte, A., and Wilson, A., 1999, The KidsRoom: a perceptually based interactive and immersive story environment, RESENCE: Teleoperators Virtual Environ. 8, 367–391.
2. Contea, D., Foggiab, P., Jolionc, J.-M., and Ventoa, M., 2006, AGRaph-based, multi-resolution algorithm for tracking objects in presence of oCClusions, Pattern Recognition 39, 562 – 572.
3. Liu Chengbing, 2012, SYSTEM AND METHOD FOR SWEET ZONE IDENTIFICATION IN SHALE GAS RESERVOIRS, Earth science well logging or borehole study by induction or resistivity logging tool, Patent application number: 20120065887.
4. Olson, T.J., and Brill, F.Z., 1997, Moving object detection and event recognition algorithm for smart cameras, Proceedings of DARPA Image Understanding Workshop, 159–175.
5. Sutadiwirya, Y., Abrar, B., Henardi, D., NuGRoho, B. H., and Wibowo, R. A., 2008, Using MRGC (Multi Resolution GRaph-Based Clustering) Method to InteGRate Log Data Analysis and Core Facies to Define Electrofacies, in the Benua Field, Central Sumatera Basin,

Indonesia, International Gas Union Research Conference, IGRC, Paris.

6. Ye, S.J., and Rabiller, Ph., 2000, A New Tool For ElectroFacies Analysis: Multi-Resolution GRaph-Based Clustering, paper, SPWLA 41st Annual Logging Symposium, June 4-7.

10/20/2012

Perceived risk of security and privacy in online shopping: A study of Malaysia context

Marzieh Zendehdel[1], Laily Hj Paim (Corresponding author)[2]

1. Department of Resource Management and Consumer Studies, Faculty of Human Ecology, University Putra Malaysia, 43400 UPM Serdang Selangor
2. Department of Resource Management and Consumer Studies, Faculty of Human Ecology, University Putra Malaysia, 43400 UPM Serdang Selangor Tel: 006-03-89467051

niaz_z7@yahoo.com Laily@putra.upm.edu.my

Abstract: Online shopping is a new phenomenon in the field of E-Business and is certainly going to be the future of shopping in the world. Though online shopping is very common outside Malaysia, its growth in Malaysian Market, which is a large and strategic consumer market, is still not in line with the global market. The present research paper has used exploratory study to highlight the various factors and variables impacting the behavior of consumers towards on-line shopping in Malaysia. Data was collected from students' samples in Malaysia. SEM (Structural Equation Model) was used to test the hypotheses and confirmed the fit of the model. The researcher found that there are three factors for explaining attitude and intention towards online shopping, which are privacy, security and subjective norm. The results show that if students worry about whether their information due to the process of online shopping will be used for other purposes, it will reduce their purchase attitude.
[M. Zendehdel, L. Paim. **Perceived risk of security and privacy in online shopping: A study of Malaysia context.** *Life Sci J* 2012;9(4):983-987] (ISSN:1097-8135). http://www.lifesciencesite.com. 151

Key words: Online shopping, Perceived risk, privacy, security, attitude, intention.

1. Introduction

With the rapid growth of the Internet, online shopping has become one of the most profitable e-commerce on the Internet over the last decade .Electronic commerce has created significant benefits, but has also increased the likelihood or negative consequences of some risks that already exist in the offline environment and created some risks that are completely new. Understanding people's perceptions of attitudes towards online risks is therefore, important for ecommerce(Gabriel & Nyshadham, 2008).This study addresses perceived risk of security and privacy with respect to online shopping services as trading platforms, which can remove any middlemen between the vendors and consumers and facilitate a safe and legal authentication mechanism as well as a secure transaction platform (Kayworth & Whitten, 2010; Martin & Camarero, 2008). Prior research suggests that perceived risk is an important factor influencing online consumer behaviour (Cunningham, Gerlach, & Harper, 2005; Pavlou, 2003; Salam, Iyer, Palvia, & Singh, 2005; Schlosser, White, & Lloyd, 2006). Previous studies have shown that perceived risk is derived from consumer uncertainty, especially in the case of Internet shopping (Bakos, 1997; Martin & Camarero, 2008).

Similarly, Miyazaki and Fernandez (2001), found that perceived risk had a significant impact on online purchasing behaviour. Behaviour researchers, for the most part, defined perceived risk in terms of the consumer's perceptions of the uncertainty and potential adverse consequences of buying a product or service (Littler & Melanthiou, 2006), and is frequently defined as the possibility of negative results (Das & Teng, 2004).According to Lallmahamood (2007), discussed perceived security and privacy as the user's perception of protection against security threats and control of their personal data information in an online environment. In this study, perceived risk refers to the uncertainty that consumers face when they cannot predict the consequences of their online transaction behavior (Samadi & Yaghoob-Nejadi, 2009). Security and privacy is the negative result in online buying that is not found in traditional commerce (Corner & Thompson, 2005; Salo & Karjaluoto, 2007; Zhou, Dresner, & Windle, 2008). Miyazaki and Fernandez (2001) pointed out that the Internet security of Intimate details is the main barrier to consumer online shopping. Personal data being transferred to others without authorization, insecure transactions, and personal information being stolen by others are the aspects associated with the security and privacy of the Internet (Aldás-Manzano, Lassala-Navarré, Ruiz-Mafé, & Sanz-Blas, 2009; Polasik & Wisniewski, 2009). In present study, perceived risk focuses on users' concerns about possible losses due to fraud, personal information being intercepted by others, the unauthorized use of personal information, and unprotected transactions as well as consumer behaviour researchers. In a number of studies, a significant negative impact of risk perception on the attitude towards online shopping or likelihood to

purchase online was found (Grabner-Kruter & Faullant, 2008; Jarvenpaa, Tractinsky, & Michael 2000; Teo & Liu 2007). The influence of risk perception on consumer attitudes and intention may be different in situations that are dominated by different types of risk (Mandrik & Bao, 2005). The impact of risk factors on attitudes, intentions or actual usage of online-shopping has been investigated in several studies (Chang , Cheung , & Lai, 2005). Pavlou (2003) found that perceived risk has a negative effect on the intention to do e-commerce transactions. Kim (2008) also demonstrates that perceived risk has a strong negative impact on the intention to purchase via the Internet. As a result, in the online channel, perceived risk reduces consumers' intention to conduct transactions. Consumers hesitate to utilize a retail store when they feel insecure and perceive risk related to purchase (Korgaonkar & Karson, 2007). Consumer's fears their private information may be misused by some unauthorized person, and, thus, are reluctant to provide information on the Internet. Jarvenpaa and Tractinsky (1999) argued that a customer might be willing to buy from an online store if it is perceived to be of low risk even if they do not have a highly positive attitude towards the store.

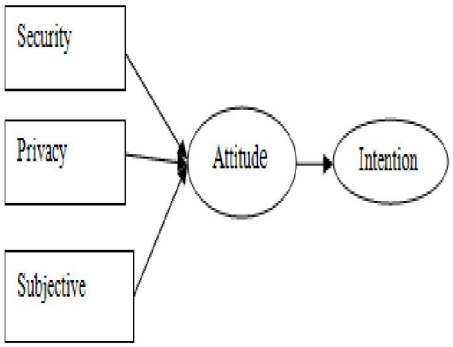


Figure 1. Research Model

Beside attitude refers to ''the degree of a person's favorable or unfavorable evaluation or appraisal of the behavior in question'' (Fishbein and Ajzen, 1975). According to the TPB, attitude impacts users' behavioral intention, which in turn influences their actual behavior. When individuals form positive attitude towards online shopping, they will have a stronger intention toward adopting it, and thus they are more likely to use it. Subjective norm refers to ''the perceived social pressure to perform or not to perform the behavior'' (Ajzen, 1991). In other words, subjective norm is related to the normative beliefs about the expectation from other people. Many Internet users choose to shop online because their friends shop online too, and they recommend it to Them. Research model (Figure 1) was adhered to observe the factors affecting online shopping which have demonstrated literature support.

Therefore, based on our earlier discussion, we propose the following hypotheses:

H1: There is a negative relationship between perceived risk (privacy, security) and intention toward online shopping.

H2: There is a positive relationship between subjective norm and intention online shopping.

2. Materials and Method

Present study used a questionnaire survey with two parts to test our theoretical model. The first part had questions measuring the constructs in the research model, while the second part had demographic questions about the participants. Each item corresponding to the constructs was measured using a seven-point Likert scale, with answer choices ranging from "disagree strongly" (1) to "agree strongly" (7), and most of these items were adapted from the existing literature while the scale items for subjective norm, , and attitude were adapted from Taylor and Todd (1995). In addition, the items measuring perceived risk were adapted from Lee (2009).

Survey approach was chosen to gather information directly from students in universities located in the Klang valley .students are potentially able of utilizing internet services and are believed to be the most frequent and active internet users (Zendehdel, Paim, Bojei, & Osman, 2011). Thus, it is significant for retailers and consumers' behavior researcher to recognize Malaysian students' population intention toward online shopping due to student's role in online marketing in Malaysia(Sabri et al., 2008). Thus selecting students as our sample was appropriate. And cluster sampling method was used. Firstly, whole population (population of universities in klang valley) was separated into two clusters (public and private). Then, from the selected clusters the researcher chose four universities from each cluster through simple random sampling. Hence, students who enrolled in a broad range of courses in each faculty and institute were chosen.

Among 400 questionnaires that were dispersed, about 380 were returned, but only 375 completely answered. Frequency distribution profile of respondents showed that 60 percent of the respondents were female while 40 percent were male. The majority of the respondents (55.2 %) fall in the age range between 20 to 25 years of age. Respondents having a monthly income ranging from less than RM 2400 were the majority income group (72.3 %). From the ethnic point of view, Malays comprised 52.4%, followed by Chinese and Indians

that composed 32.8% and 14. 4% of the study sample respectively.

3. Result

In order to achieve the research aim and test the hypotheses, AMOS 16 was used to perform these analyses. Comprehensive instrument validation and reliability procedures were followed in order to validate the instrument empirically. For the constructs of perceived risk (privacy, Security) and attitude, intention, subjective norm), this study used CFA as a confirmatory test to measure validity and reliability, while other constructs were examined with SEM was used to test the model fit. "Structural equation modeling seeks to explain the relationships among multiple variables; it examines the structure of interrelationships.

3.1 Analysis of the structural model

We assessed the overall goodness of fit using the chi-square test. The chi-square test assesses the adequacy of a hypothesized model in terms of its ability to reflect the variance and covariance of the data. The results of structural equation modeling obtained for the proposed conceptual model (Table 1). Approximation (RMSEA) of 0.055. Generally, fit statistics greater than or equal to 0.9 for GFI, NFI, RFI, and CFI indicate a good model fit (Hairs et al., 1998). In addition, RMSEA Values ranging from 0.05 to 0.08 are acceptable (Hairs et al., 1998), indicating that our model fit was acceptable. The other fit indices, except AGFI, indicated that our proposed model obtained an adequate model fit.

Table 1.Overall Fits of the Research Model

Fit indices	_²/df	CFI	NFI	RMSEA	AGFI	GFI
Observed value	2.645	.942	.911	.066	0.85	0.90
Recommended value	<3	>.9	>.9	<.08	>.9	>.9

Table2. Results of Hypotheses Testing of this Study

Hypothesis	IV	DV	Coefficient	(P-value)	Remark
H1	Privacy	Attiude	.-.413	***	Supported
H2	Security	Attitude	-.327	***	Supported
H3	Subjective norm	Attitude	.470	***	Supported

4. Discussion

The results, as displayed in (**Error! Reference source not found.**), show that there is a significant negative relationship between perceived risk and attitude towards online shopping Therefore, H1-2 was supported by the data in this study. This is consistent with previous studies in which risk perception was found to have a significant negative impact on the attitude towards online shopping or likelihood to purchase online (Grabner-Kruter & Faullant, 2008; Jarvenpaa et al., 2000; Teo & Liu 2007).The results show that if students worry about whether their information due to the process of online shopping will be used for other purposes, it will reduce their purchase attitude. Furthermore, this study found that if information security is not guaranteed, students will actually reduce consumption. Thus, online shopping managers must pay attention. The government must also protect the Privacy and security of consumers as one of the main roles it can contribute in growing internet shopping.

The results, as depicted in (table 2), show that the exogenous variable of subjective norm made a significant contribution in the prediction of attitude towards online shopping. Therefore, the significant relationship between normative and attitude H3 was supported. This finding is consistent with Miniard and Cohen (1983),and Ryan (1982) who proposed that normative beliefs influence attitude formation, as attitude may be formed on the basis of information provided by an expert referent or expectations of other significant people. In addition, a few studies have examined potential relationships between attitude and subjective norms (Quintal et al., 2010), that is, students are likely to believe the opinion of others who are important to them (e.g., peers, family) when they decide whether or not to buy a product. The results also lend support to previous findings, indicating that purchase intention is positively influenced by subjective norm. This finding implies that when students find that people around them have adopted online shopping, they will be more willing to use it.

5. Implications

In terms of subjective norm they should build positive word of mouth to enhance the perception of friends and family members of current customers. In this respect, online shopping providers may use a positive word-of-mouth strategy to enhance the awareness of their services and promote their benefits. Further, from a managerial standpoint, identifying consumer risks is important and how to reduce these is of great importance to e-marketers, who need to ensure that security and privacy policies are appropriately defined, implemented, and communicated to the relevant stakeholder. Online shopping marketers could also look into the possibility of spreading and promoting security and privacy related information on the Internet. First, e-commerce computer network security technologies, such as firewalls, virtual private networks, intrusion detection systems, and virus prevention techniques, should be used to strengthen online security. Second, e-commerce security management systems should be developed, including personnel management, security systems, tracking audit systems, maintenance systems, data backup systems, and regular cleaning of virus systems.

Finally, the managerial implications of this study are to provide online shopping companies with information security policy considerations, and to help companies to develop online marketing strategies using information technology regarding consumer behaviour and psychological impact. Future research can create an in-depth understanding of security perceived risk and consumer behaviour. This implies that controlling the risk of online shopping is particularly important for managers as they decide how to assign resources to retain and expand their current customer base. However, building a risk-free online shopping transaction environment is a difficult thing. Therefore, online stores need to search for risk-reducing strategies that might assist in inspiring high confidence in potential customers. This study suggests that they should consider focusing on the prevention of fraud, identity theft, and financial loss. For example, building secure firewalls to avoid intrusion, developing methods for strengthening encryption and authenticating websites are all measures that should be undertaken.

Acknowledgement:

I would like to take this opportunity to thank my supervisor, Prof Dr Laily Hj Paim. I would also like to thank my friends for their support and valuable assistance with my project.

References

1. Aldás-Manzano J, Lassala-Navarré C, Ruiz-Mafé C Sanz-Blas S. The role of consumer innovativeness and perceived risk in online banking usage. International Journal of Bank Marketing2009;27(1): 53-75.
2. Bakos J Y. Reducing buyer search costs: Implications for electronic marketplaces. Management science1997; 43(12): 1676-1692.
3. Chang M K, Cheung W, Lai V S. Literature derived reference models for the adoption of online shopping. Information & Management2005; 42(4):543-559.
4. Corner J L Thompson F. Perceived risk, the internet shopping experience and online purchasing behavior: a New Zealand perspective. Journal of Electronic Commerce in Organizations2005; 13(2): 324-326.
5. Cunningham L F Gerlach J Harper M D Perceived risk and e-banking services: An analysis from the perspective of the consumer. Journal of Financial Services Marketing2005; 10(2):165-178.
6. Das T K Teng B S. The risk-based view of trust: A conceptual framework. journal of Business and Psychology2004; 19(1): 85-116.
7. Gabriel I J, Nyshadham E. A Cognitive Map of People's Online Risk Perceptions and Attitudes: An Empirical Study. Paper presented at the International Conference on System Sciences, Hawaii 2008.
8. Grabner-Kruter S, Faullant R. Consumer acceptance of internet banking: the influence of internet trust. International Journal of Bank Marketing2008; 26(7): 483-504.
9. Jarvenpaa S L Tractinsky N Michael V. Consumer Trust in an Internet StoreInformation Technology and Management2000; 1(1-2): 45-75.
10. Kayworth T Whitten D .Effective information security requires a balance of social and technology factors. MIS Q. Ex2010; 9(3): 163-175.
11. Korgaonkar P K, Karson E G. The influence of perceived product risk on consumers' e-trailer shopping preference. journal of Business and Psychology2007; 22(1):55-64.
12. Lallmahamood M. An examination of individual's perceived security and privacy of the internet in Malaysia and the influence of this on their intention to use e-commerce: Using an extension of the technology

acceptance model. Journal of Internet Banking and Commerce 2007; 12(3): 1-26.
13. Littler D, Melanthiou D. Consumer perceptions of risk and uncertainty and the implications for behaviour towards innovative retail services: The case of Internet Banking. Journal of Retailing and Consumer Services2006; 13(6): 431-443.
14. Mandrik C A Bao Y. Exploring the concept and measurement of general risk aversion. Advances in Consumer Research2005; 32(4):531-539.
15. Martin S S Camarero C. Consumer trust to a web site: moderating effect of attitudes toward online shopping. CyberPsychology & Behavior2008; 11(5): 549-554.
16. Miyazaki A D Fernandez A. Consumer perceptions of privacy and security risks for online shopping. Journal of Consumer Affairs2001; 35(1): 27-44.
17. Pavlou P A. Consumer acceptance of electronic commerce: Integrating trust and risk with the technology acceptance model. International Journal of electronic commerce2003; 7(3):101-134.
18. Polasik M, Wisniewski T P. Empirical analysis of internet banking adoption in Poland. International Journal of Bank Marketing2009; 27(1): 32-52.
19. Sabri MF, MacDonald M, Masud J, Paim L, Hira T K ,Othman M A .Financial Behavior and Problems among College Students in Malaysia: Research and Education Implication. Consumer Interests Annual2008;54(3):166-170.
20. Salam A F, Iyer L, Palvia P, Singh R. Trust in e-commerce. Communications of the ACM 2005; 48(2): 72-77.
21. Salo J, Karjaluoto H. A conceptual model of trust in the online environment. Online Information Review 2007; 31(5):604-621.
22. Samadi M , Yaghoob-Nejadi, A. A survey of the effect of consumers' perceived on purchase intention in e-shopping. Business Intelligence Journal 2009; 2(2): 261-275.
23. Schlosser A E, White T B, Lloyd S M.Converting web site visitors into buyers: how web site investment increases consumer trusting beliefs and online purchase intentions. Journal of Marketing 2006;70(2): 133-148.
24. Taylor S, Todd P A. Understanding information technology usage: A test of competing models. Information systems research 1995; 6(2): 144-176.
25. Teo T S H, Liu J. Consumer trust in e-commerce in the United States, Singapore and China. Omega 2007; 35(1): 22-38.
26. Zendehdel M, Paim L B H, Bojei J B, Osman S B. The Effects of Trust on Online Malaysian Students Buying Behavior. Australian Journal of Basic and Applied Sciences2011; 5(12): 1125-1132.
27. Zhou L, Dresner M, Windle R J. (2008). Online reputation systems: Design and strategic practices. Decision support systems 2008; 44(4):785-797.

9/13/2012

Formal Modeling towards the Context Free Grammar

Nazir Ahmad Zafar[1], Sher Afzal Khan[2], Fahad Alhumaidan[1], Bushra Kamran[3]

[1]Department of Computer Science, King Faisal University, Hofuf, Saudi Arabia
[2]Department of Computer Sciences, Abdul Wali Khan University, Mardan, Pakistan
[3]Faculty of Information Technology, University of Central Punjab, Lahore, Pakistan
nazafar@kfu.edu.sa;sher.afzal@awkum.edu.pk;falhumaidan@kfu.edu.sa;bushra.kamran@ucp.edu.pk

Abstract: The language to control objects is a primary requirement in design of a complex system. Context free grammar plays an important role in modeling control functionalities of a system by grammatical rules. This generates naturally the operation of a system by the language which having commands in the form of strings generated by variables which are nested inside variables arbitrarily deeply. The formal method Z is an ideal notation which is used for describing state space of a system and then defining operations over it. Consequently, an integration of context free grammar and Z will be an effective tool for increasing modeling power for a complex system. In this paper, we have given a procedure for integrating CFG and Z. Formal definition of a CFG is defined. Then derivation of a string and further development of formal language is formalized. The specification of this relationship is analyzed and validated using Z/EVES tool.
[Zafar NA, Khan SA, Alhumaidan F, Kamran B. **Formal Modeling towards the Context Free Grammar**. *Life Sci J* 2012;9(4):988-993] (ISSN:1097-8135). http://www.lifesciencesite.com. 152

Keywords: Integration of approaches; Context free languages; Formal specification Z; Model checking.

1. Introduction

Machines are controlled by computer based systems and, of course, computers are controlled by software systems. When software is used in controlling a complex system, for example, safety critical system; its failure may cause a big loss in terms of wealth, deaths, injuries or environmental damages. Consequently, constructing correct software is as important as its other counterparts, i.e., hardware or electro-mechanical systems (Hall, 2002). Formal methods are mathematical based techniques used for specification of properties of software and hardware systems for insuring their correctness (Burgess, 1995). We can describe a mathematical model of a system and then it can be analyzed and validated increasing confidence of development (Gwandu et al., 1994).

At the current stage of development in formal methods, it is not possible to develop a system using a single formal technique and as a result integration of approaches is required. That is why integration of approaches has become a well-researched area. Further, it is an open research area in computer science and engineering leading to development of automated computer tools and techniques.

Design of a complex system, not only requires functionality but it also needs to model its control behavior. There are a large variety of techniques for software specification which are suitable for specific aspects in the process of the software development. For example, Z notation, Vienna Development Methods, B Method and algebraic techniques are usually used for defining data type while Petri nets, process algebras, automata and state-charts are best suited for capturing dynamic aspects 4. Therefore it is required to identify a relationship between static and dynamic modeling techniques for complete development of a system.

Although integration of approaches is a well researched are (Beek et al., 2004; Hasan et al., 2007; Gervais et al., 2005; Araki et al., 1999; Akbarpour et al., 2002; Raymond, 2004) but there does not exist much work on formalization of structures which generates formal languages. Dong et al (2004, 2005) described the integration of Object Z and timed automata. Another piece of good work is reported by Constable (1997, 2000) has proposed a constructive formalization of some important concepts of automata using Nuprl. A relationship is investigated between Petri-nets and Z notation in (Heiner et al., 1999; He, 2001). An integration of B method and UML is presented in (Leadinng et al., 2002,2002a). Wechler, W. has introduced some important algebraic structures in fuzzy automata (Wechler, 1978). In (John et al., 2002), a treatment of fuzzy automata and fuzzy language theory is discussed when the set of possible values is a closed interval [0, 1]. Ito, M., has described automata and formal languages from the algebraic point of view. (Mansoor et al.,2007). Proposed, an algorithm to eliminate the useless productions of CFG.

In this paper, a relationship between Z notation and CFG is checked and verified after removing inconsistencies. Formal construction is given using Z notation, and it is analyzed and validated using Z/EVES tool. The major objectives of this research

are: (i) identifying and proposing an integration of context free grammar and formal methods enhancing modeling power of complex systems and (ii) providing a syntactic and semantic relationship between Z and CFG. In section 2, an introduction to formal methods is given. In section 3, an overview of context free grammar is provided. Formal specification of context free grammar is described in section 4. Section 5 describes the modeling analysis. Finally, conclusion and future work are discussed in section 6.

2 Formal Methods

Formal methods are mathematical approaches used for describing and analyzing properties of software systems (Khan et al., 2011, 2011(a), 2011(b); Zafar et al., 2012). These techniques are based on discrete mathematics such as logic, set theory and graph theory. Formal methods may be classified in several ways. Property and model oriented methods are two main classification of it (Brendan, 1998; Khan et al., 2007). Property oriented methods are used to describe software in terms of properties or constraints that must be satisfied. Model oriented methods are used to construct a model of a system's behavior (Spivey, 1989; Ahmad et al., 2011,; Ali et al., 2012, 2012(a)). Formal methods are used to improve quality of software systems by means of documenting and specifying in a precise and structured manner. Although formal methods are successfully applied in many research areas of computer science but at the current stage of their development, it requires an integration of formal and traditional approaches.

Z notation is one of the most popular specification languages in formal method. The Z (Spivey, 1989) is a model oriented approach, which is based on set theory and first order predicate logic. It is also used for specifying the behavior of systems as an abstract data types and sequential programs can also be modeled using it. In this paper, Z is selected to be integrated with algebraic automata because a natural relationship exists between these approaches. The Z is based upon set theory including standard set operators, set comprehensions, Cartesian products, and power sets. On the other hand the logic of Z is formulated using first order predicate calculus. The Z notation is used in our research because it allows organizing a system in smaller pieces known as schemas. The schema defines the way in which state of the system can be modified. A promising aspect of Z is the mathematical refinement which is a stepwise verifiable transformation of an abstract specification into a concrete executable program. Once formal specifications in Z are written it can be refined into actually implemented system by the process of stepwise mathematical refinements.

3 Context free grammar

A context-free grammar (CFG) generates a formal language where a clause is nested inside another clause making a best use of recursion. Every production of a context free grammar is of the form: $S \rightarrow t$, where S is a non-terminal consisting of a single character/symbol and t is a string which may contain only terminals or non-terminals on combination of both. Further, t might me an empty string. The notation: $S \rightarrow t$ is called a production or a rule. Context free grammar consists of such kind of rules which are applied one after other producing a parse tree. The tree ends with terminals which are leaves of the tree and each internal node is a non-terminal which produces one or more further nodes. The left hand side of a production rule of a context free grammar is always a single non-terminal. Because all rules only have non-terminals on the left hand side and it can easily be replaced with the string on the right hand side of this rule. Further the context in which the symbol occurs is therefore not important and hence the grammar is called context free grammar. It is to be noted that the context free grammar(s) are always recognized by finite state machines having a single infinite taps. For keeping track of nested units, the current parsing state is pushed at the start of the unit and it is recovered at the end. The context free grammars are very important in designing and description of the programming languages and their compilers. The syntax of natural languages can also be analyzed by using it.

The formalism of context-free grammar was developed by Noam Chomsky who described the linguistics in a grammatical form and finally converted into mathematical models providing a precise and simple mechanism for describing of languages. This way of description of languages makes the formalism producing rigorous mathematical studies. The context free grammars allow an efficient parsing of algorithms and their constructions in a simple way. Using the grammar, it can be determined whether a string can be generated or not. Further, the way of generation is also determined. On the other hand, context free languages have their own limitations. For example, some of the operators, which are well-defined in many models of automata theory, do not behave well in case of context free grammar. The intersection of two context free grammars, in general, is not context free, is an example of those operators. The complement of a context free language is not context free, is another example of it. However, union, concatenation and Kleene star operators produce context free language when applied to context-free language or languages.

4 Formal Specification of Context Free Grammar

In this section, an integration of some important concepts of CFG and Z notation is given. It is mentioned that the definitions used are based on the book with title "Algebraic Theory of Automata and Languages" (Brendan et al., 1998). The set of structures used are: (i) CFG (ii) *Derivation* (iii) *Derivations* (iv) *WorldOfCFG* and (v) *LanguageOfCFG*.

We start with the definition of context free grammar which is a 4-tuple $(V,\sum, R, S)$, where, V is a finite set of non-terminals, $\sum$ is the set of terminals, it is disjoint from V, this make the words for a language. S is the start non-terminal. R is the relation from V to $(V \cup \sum)^*$such that $\exists w \in (V \cup \sum)^*: (S,w) \in R$. In the specification denoted by CFG, we define the sets of non-terminal by V, set of terminal by Sigma, the set X denotes both the set of terminals and non-terminals. The notation rules define the relation between V and *seqX*. The *seqX*, denotes the set of all sequences containing terminals and non-terminals. The predicate part defines, *Sigma* is a finite set of terminals, it is disjoint from the set of non-terminals. The production rules are defined by the relation denoted by *rules* such that if ∃ a stringof type *seqX* then $(s0, w) \in rules$. Where *s0* is the start non-terminal used to represent the whole sentence or program.

$[X]$

$V == X$

$Sigma == X$

CFG

$variables: \mathbb{F}\ V$

$terminals: \mathbb{F}\ Sigma$

$alphabets: \mathbb{F}\ X$

$rules: V \leftrightarrow \text{seq}\ X$

$s0: V$

$s0 \in variables$

$\text{dom}\ rules \subseteq variables$

$variables \cap terminals = \{\}$

$alphabets = variables \cup terminals$

$\exists w: \text{seq}\ X \mid w \in \text{ran}\ rules \bullet (s0, w) \in rules$

$\forall st: \text{seq}\ X \mid st \in \text{ran}\ rules \bullet \text{ran}\ st \subseteq alphabets$

Invariants:

1. The variable *s0* must be an element of *variables*.
2. The domain of *rules* relation is subset of variables.
3. The terminals and non-terminals are disjoint sets.
4. The entire set of alphabets is union of terminals and non-terminals.
5. There exists at least one rule which contains start variable on the left hand side.
6. Elements of all the rules are members of alphabets.

Formal Construction of Productions

In this section, we describe the formal specification of production rules. Production rule is substitution rule perform recursively to derive new string of terminal and non-terminal from the string of terminal and non-terminal.

In the specification denoted by the *Derivation*, we specify the process of production of one string from another string of terminal and non-terminal. In the specification *st1* and *st2* are two strings of type *seqX*. We say *st1* yields *st2* if $\exists a \in V, b, st3$ and $st4 \in seqX$ such that:

$st1 = st3 \frown \langle a \rangle \frown st4 \wedge st2 = st3 \frown b \frown st4.$

Thus, *st2* is the result of obtained by the rule *(a, b)* to *st2*.

Derivation

ΞCFG

$drives: \text{seq}\ X \leftrightarrow \text{seq}\ X$

$\forall st1, st2: \text{seq}\ X \mid \text{ran}\ st1 \subseteq alphabets \wedge \text{ran}\ st2 \subseteq alphabets$

$\bullet\ (st1, st2) \in drives$

$\Rightarrow (\exists a: V; b: \text{seq}\ X; st3, st4: \text{seq}\ X$

$st3 \in \text{ran}\ rules \wedge st4 \in \text{ran}\ rules \wedge (a, b) \in rules$

$\bullet\ st1 = st3 \frown \langle a \rangle \frown st4 \wedge st2 = st3 \frown b \frown st4)$

Formal Construction of further Derivations

The specification schema denoted by Derivations is the extension of schema Derivation. This specify the generation of one string of non-terminals or terminals to the string of non-terminals or terminals. In the specification we consider two strings of non-terminals or terminals denoted by st1 and st2. The schema Derivations call the schema Derivation in successive manner and develop the productions: st1⇒st3∧ st3⇒st4 ∧ s4⇒st5∧ st5⇒st2 as specified.

In many fields of computer sciences words generated from grammar to code certain programs used to functionalize a system. The schema denoted by *WorldOfCFG* is used to generate strings of terminals from the strings of non-terminals and terminals. Word of CFG is define to be a string of terminals of type *seq Sigma* generated by successive production from the string of non-terminals or

terminals of type seqX. For generating word *WordOfCFG* uses operation of schema *Derivations* to perform the desire production.

Derivations

$$\Xi Derivation$$
$$drivess: \text{seq}\, X \leftrightarrow \text{seq}\, X$$

$$\forall st1, st2: \text{seq}\, X \mid \text{ran}\, st1 \subseteq alphabets \land \text{ran}\, st2 \subseteq alphabets$$
$$\bullet\ (st1, st2) \in drivess$$
$$\Rightarrow (\exists st3: \text{seq}\,(\text{seq}\, X) \mid 1 \leqslant \#st3 \land \text{ran}\, st3 \subseteq \text{ran}\, rules$$
$$\bullet\ ((st1, st3\ 1) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ st1 = st4 \frown \langle a \rangle \frown st5 \land st3\ 1 = st4 \frown b \frown st5))$$
$$\land (\forall i: \mathbb{N} \mid i \in 2 \mathinner{..} \#st3$$
$$\bullet\ ((st3\ (i - 1), st3\ i) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules$$
$$\land st5 \in \text{ran}\, rules$$
$$\land (a, b) \in rules$$
$$\bullet\ st3\ (i - 1) = st4 \frown \langle a \rangle \frown st5$$
$$\land st3\ i = st4 \frown b \frown st5)))$$
$$\land ((st3\ (\#st3), st2) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ st3\ (\#st3) = st4 \frown \langle a \rangle \frown st5 \land st2 = st4 \frown b \frown st5)))$$

Worlds generated by CFG

WordOfCFG

$$\Xi Derivations$$
$$word?: \text{seq}\, Sigma$$

$$\text{ran}\, word? \subseteq terminals$$
$$(\langle s0 \rangle, word?) \in drivess$$
$$\Rightarrow (\exists st3: \text{seq}\,(\text{seq}\, X) \mid 1 \leqslant \#st3 \land \text{ran}\, st3 \subseteq \text{ran}\, rules$$
$$\bullet\ ((\langle s0 \rangle, st3\ 1) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ \langle s0 \rangle = st4 \frown \langle a \rangle \frown st5 \land st3\ 1 = st4 \frown b \frown st5))$$
$$\land (\forall i: \mathbb{N} \mid i \in 2 \mathinner{..} \#st3$$
$$\bullet\ ((st3\ (i - 1), st3\ i) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ st3\ (i - 1) = st4 \frown \langle a \rangle \frown st5$$
$$\land st3\ i = st4 \frown b \frown st5)))$$
$$\land ((st3\ (\#st3), word?) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ st3\ (\#st3) = st4 \frown \langle a \rangle \frown st5 \land word?$$
$$= st4 \frown b \frown st5)))$$

Language generated by CFG.

The set of words generated by a schema *WordOfCFG* is called a context-free language. In the specification *LanguageOfCFG* a sequence of terminal is denoted by *w* is of type *seq Sigma* belongs to the set *language* if there exist a set of derivations from s0 to w operated by the schema *Derivations* as specified in the following schema.

LanguageOfCFG

$$\Xi Derivations$$
$$language?: \mathbb{P}\,(\text{seq}\, Sigma)$$

$$\forall w: \text{seq}\, Sigma \mid w \in language? \bullet \text{ran}\, w \subseteq terminals$$
$$\forall w: \text{seq}\, Sigma \mid w \in language?$$
$$\bullet\ (\langle s0 \rangle, w) \in drivess \Rightarrow$$
$$(\exists st3: \text{seq}\,(\text{seq}\, X) \mid 1 \leqslant \#st3 \land \text{ran}\, st3 \subseteq \text{ran}\, rules$$
$$\bullet\ ((\langle s0 \rangle, st3\ 1) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ \langle s0 \rangle = st4 \frown \langle a \rangle \frown st5 \land st3\ 1 = st4 \frown b \frown st5))$$
$$\land (\forall i: \mathbb{N} \mid i \in 2 \mathinner{..} \#st3 \bullet ((st3\ (i - 1), st3\ i) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules$$
$$\land st5 \in \text{ran}\, rules$$
$$\land (a, b) \in rules$$
$$\bullet\ st3\ (i - 1) = st4 \frown \langle a \rangle \frown st5$$
$$\land st3\ i = st4 \frown b \frown st5)))$$
$$\land ((st3\ (\#st3), w) \in drives$$
$$\Rightarrow (\exists a: V; b: \text{seq}\, X; st4, st5: \text{seq}\, X$$
$$st4 \in \text{ran}\, rules \land st5 \in \text{ran}\, rules \land (a, b) \in rules$$
$$\bullet\ st3\ (\#st3) = st4 \frown \langle a \rangle \frown st5 \land w = st4 \frown b \frown st5)))$$

---

5 Model Analysis

As we know that there does not exist any computer tool that may guarantee about the complete correctness of a computer model. That is why we can believe that even the specification is written, in any of the formal languages, it may contain potential errors. That means the art of writing formal specification never assures the consistencies, correctness and completeness of the system to be developed. But, on the other hand, if the formal specification is checked and analyzed with computer tools it certainly identifies, if exist, the potential errors in syntax and semantics of the formal description of a system. The Z/EVES is one of the most powerful tools which we have used for writing validating and analyzing the formal specification written in Z.

Conclusion

In this research, the approach of context free grammar is combined with Z notation which defining a relationship between fundamentals of these approaches. At first, we have described formally the structures of context free grammar then formal models of derivation process from a string to a string of non-terminals or terminals is presented. Further we specified the process of production in sequence by using the schema Derivations. At the end we presented the formal model to generate the words from CFG and further its language. Formal proofs of the above models are presented under the certain assumptions. Formal models of few interesting algebraic structures and its variants are proposed by reusing the definitions of the abstract structures. The specification of this integration is verified and validated using Z/Eves tool. An extensive survey of existing work was done before initiating this research. Some interesting work (Wechler, 1978; Tuan, 2000, Bowen, 1996, Vilkomir, 2001) was found but our work and approach are different because of conceptual and abstract level integration of Z and CFG. Why and what kind of integration is required, were two basic questions in our mind before initiating this research. Since, CFG is best suited for modeling system's behavior by using proper sequence of strings, while Z is an ideal one used for describing state of a system. This distinct in nature but supporting behavior of Z encouraged us to integrate Z with CFG.

Most of the researchers have either taken some examples in defining integration of approaches or have addressed only some aspects of it. Further, there is a lack of formal analysis supported by computer tools. Our work is different from others because we have given a generic approach to link Z and CFG. A computer tool support is provided for analysis and validation of this relationship as well.

Few benefits of using Z are: (i) Every object is assigned a unique type providing useful programming practice. (ii) Several type-checking tools exist to support the specification. (iii) The Z/Eves is a powerful free tool to prove and analyze the specification. (iv) the rich mathematical notations make it possible to reason about behavior of a specified system effectively.

References

1. A. Hall, Correctness by Construction: Integrating Formality into a Commercial Development Process, Praxis Critical Systems Limited, Springer, vol. 2391, pp. 139-157, 2002.
2. C. J. Burgess, The Role of Formal Methods in Software Engineering Education and Industry, University of Bristol, UK, 1995.
3. B. A. L. Gwandu and D. J. Creasey, The Importance of Formal Specification in the Design of Hardware Systems, School of Electron. & Electr. Eng., Birmingham University, 1994.
4. H. A. Gabbar, Fundamentals of Formal Methods, Modern Formal Methods and Applications, Springer, 2006.
5. H. Beek, A. Fantechi, S. Gnesi and F. Mazzanti, State/Event-Based Software Model Checking, Integrated Formal Methods, Springer, vol. 2999, pp. 128-147, 2004.
6. O. Hasan and S. Tahar, Verification of Probabilistic Properties in the HOL Theorem Prover, Integrated Formal Methods, Springer, vol. 4591, pp. 333-352, 2007.
7. F. Gervais, M. Frappier and R. Laleau, Synthesizing B specifications from EB3 Attribute Definitions, Integrated Formal Methods, Springer, vol. 3771, pp. 207-226, 2005.
8. K. Araki, A. Galloway and K. Taguchi, Integrated Formal Methods, Proceedings of the 1st International Conference on Integrated Formal Methods, Springer 1999.
9. B. Akbarpour and S. Tahar and A. Dekdouk, Formalization of Cadence SPW Fixed-Point Arithmetic in HOL, Integrated Formal Methods, Springer, vol. 2335, pp. 185-204, 2002.
10. J. Derrick and G. Smith, Structural Refinement of Object-Z/CSP Specifications, Integrated Formal Methods, Springer, vol. 1945, 2000.
11. T. B. Raymond, Integrating Formal Methods by Unifying Abstractions, Springer, vol. 2999, 2004.
12. J. S. Dong, R. Duke and P. Hao, Integrating Object-Z with Timed Automata, pp 488-497, 2005.
13. J. S. Dong, et al., Timed Patterns: TCOZ to Timed Automata, The 6th International Conference on Formal Engineering Methods, pp 483-498, 2004.
14. R. L. Constable, P. B. Jackson, P. Naumov and J. Uribe, Formalizing Automata II: Decidable Properties, Cornell University, 1997.
15. R. L. Constable, P. B. Jackson, P. Naumov and J. Uribe, Constructively Formalizing Automata Theory, Foundations of Computing Series, MIT Press, 2000.
16. M. Heiner and M. Heisel, Modeling Safety Critical Systems with Z and Petri nets, International Conference on Computer Safety, Reliability and Security, Springer, pp. 361–374, 1999.

17. X. He, Pz nets a Formal Method Integrating Petri nets with Z, Information & Software Technology, vol. 43(1), pp.1–18, 2001.
18. H. Leading and J. Souquieres, Integration of UML and B Specification Techniques: Systematic Transformation from OCL Expressions into B, Asia-Pacific Software Engineering Conference, 2002.
19. H. Leading and J. Souquieres, Integration of UML Views using B Notation, Proceedings of Workshop on Integration and Transformation of UML Models, 2002a.
20. W. Wechler. The Concept of Fuzziness in Automata and Language Theory, Akademic-Verlag, Berlin, 1978.
21. N. M. John and S. M. Davender, Fuzzy Automata and Languages: Theory and Applications, Chapman & HALL, CRC, 2002.
22. D. Conrad and B. Hotzer, Selective Integration of Formal Methods in the Development of Electronic Control Units, Research Institute for Automotive Engineering and Vehicle Engines, 1998.
23. M. Brendan and J. S. Dong, Blending Object-Z and Timed CSP: An Introduction to TCOZ, Proceedings of International Conference on Software Engineering, 1998.
24. J. M. Spivey, The Z notation: A Reference Manual, Englewood Cliffs, NJ, Printice-Hall, 1989.
25. D. P. Tuan, Computing with Words in Formal Methods, University of Canberra, Australia, 2000.
26. J. P. Bowen, Formal Specification and Documentation Using Z: A Case Study Approach, International Thomson Computer Press, 1996.
27. S. A. Vilkomir and J.P. Bowen, Formalization of Software Testing Criterion, South Bank University, London, 2001.
28. Mansoor, A. A., Khan, A. A.: Removing Useless Productions of a Context Free Grammar through Petri Net, Journal of Computer Science, vol. 3 (7), pp. 494-498, 2007.
29. S.A. Khan and NA Zafar, Improving Moving Block Railway System using Fuzzy Multi-Agent Specification Language, Int. J. Innov. Computing, Inform. Control, 7(7(B)):4517-34, 2011.
30. S.A. Khan and NA Zafar and F Ahmad, Petri Net Modeling of Railway Crossing System using Fuzzy Brakes, International J. Phy. Sci, 6(14): 3389-3397, 2011(a).
31. Zafar, N.A, Khan, S.A and Araki, K, Towards the Safety Properties of Moving Block Railway Interlocking System, Int. J. Innov. Computing, Inform. Control, 8(8): 2012 .
32. S.A. Khan and NA Zafar, Extending promotion for the management of moving block interlocking components., International J. Phy. Sci, 6(31), 7262-70. 2011(b).
33. S.A. Khan and NA Zafar, Promotion of Local to Global Operation in Train Control System, Journal of Digital Information Management (page 228-233)., 2007.
34. F. Ahmad, SA Khan. Module-based Architecture for Periodic job-Shop Scheduling problem, Computers & Mathematics with Applications, 64(1), 1-10, 2012.
35. G Ali, SA Khan, F Ahmad and Zafar, N.A, Visualized and Abstract Formal Modeling towards the Multi-Agent Systems, International Journal of basic and Applied Sciences 2(8)8272-8284, 2012
36. G Ali, SA Khan, F Ahmad and NA Zafar, Formal Modeling towards a Dynamic Organization of Multi-Agent Systems Using Communicating X-Machine and Z-Notation, Indian Journal of Science and Technology, Vol. 5 No. 7, 2012(a).

9/19/2012

Semantic Web Specification using Z-Notation

Sher Afzal Khan[1], Aamir Aziz Hashmi [2], Fahad Alhumaidan[3] and Nazir Ahmad Zafar[3]

[1]Department of Computer Sciences, Abdul Wali Khan University, Mardan, Pakistan
[2]Department of Networking, Virtual University of Pakistan, Islamabad, Pakistan
[3]Department of Computer Science, King Faisal University, Hofuf, Saudi Arabia
sher.afzal@awkum.edu.pk; ana1-isb@vu.edu.pk; {falhumaidan ,nazafar}@kfu.edu.sa

Abstract: Current World Wide Web means to display pages to end user, while the Semantic Web is a vision of a next-generation network focuses on "Meaning" instead of merely pasting arbitrary text on a page. An intelligent software agents use information to organize and filter data to meet the user's needs. DAML+OIL and Web Ontology Language OWL are the current environments to create Ontology over RDF and XML structures which are used to represent data intelligently among different Ontologies. To assure quality and accurateness in Ontologies in the early design stage, we used the Z-specification which is a formal language based on discrete mathematics such as predicate logic, sets, relations and functions to specify the behavior of Semantic Web. Further, we applied a transformation from schemas written in Z-specification to OWL. The formal specification is described and validated using Z/EVES tool. A fundamental goal of this research is to transform a verified and validated specification to OWL to design Ontologies.
[Khan SA, Hashmi AA, Alhumaidan F, Zafar NA. **Semantic Web Specification using Z-Notation.** *Life Sci J* 2012;9(4):994-1000] (ISSN:1097-8135). http://www.lifesciencesite.com. 153

Keywords: Semantic Web; Ontology; Formal Methods; Z-specification

1. Introduction

The Semantic Web (Berners, et al, 2003) is an intelligent extension of current World Wide Web. It describes Web services in such a way that computers or intelligent software agents can understand the meaning of Web pages. It is still in its evolving period, challenges are still there to meet the goals. Semantic Web's developers are looking to XML and RDF to meet these challenges. DAML+OIL (Harmelen et al., 2001) and OWL (Dean et al., 2004) are the current environments over the top of XML and RDF. They are playing remarkable role in designing Ontologies and interacting among different Ontologies over the Internet. These languages are based on descriptive logic and they are designed to be decidable (World Wide Web, 2003). The Z-notation (Khan et al., 2008, 2009, 2011) is a formal specification language based on descriptive logic, sets, relations and functions. Descriptive logic can be regarded as the subset of predicate logic. Therefore, Z is more expressive than other Ontology languages. Z/EVES (Meisels, 1997) is a proof tool for reasoning and checking Z-specifications. In this paper we use Z-specification to specify and verify the requirements of the Semantic Web. In the idea we first specify system in Z-specification then check its proof and syntax by Z/EVES tool. Further convert Z-model into OWL to design Ontology. The use of Z-specification and Z/EVES tool removes inconsistencies and ambiguities in Ontology. The transformation between Z-specification and OWL can confine the properties of Ontology that the OWL can not. As (Dong et al., 2004) describes that the intrinsic homogeneity between semantic bases of ontology languages and Z implies that Z can be regarded as an ontology meta-language and it can even capture properties that ontology languages cannot. Further, (Dong et al., 2002) expresses that Z-specification can capture various requirements of Semantic Web services including ontology and service functionalities. The research of using the transformation of Z-specification and Semantic Web has already been used. As (Dong et al., 2004(a)) use Z-specification on DAML+OIL to design and reuse ontology. As a forward approach proposed by J.S. Dong et al. (2002), they describe that the use of Z semantics to design Ontology would be easy to reduce Ontology flaws. In his technique they simply introduced a transformation of Z model into DAML+OIL to design Ontology and also provide some rules of transformation. The idea was simple but he left a reverse technique that is to transform the DAML+OIL to Z-specification for their future work. Further J.S Dong and his team propose the reverse technique in which they use Racer with Z-specification and introduced that DAML+OIL can be re transformed into Z Model to check inconsistencies of Ontologies. The idea became complete here with forward and reverse transformations together to remove Ontology related flaws by the description logic. Dong et al. (2002) describe the combined approach of DAML+OIL, Z-specification language, RACER and Alloy, in the research they first apply Z-

specification to design Ontology, next RACER is used to identify any inconsistency in Ontology, after that Alloy is used to trace origin of an error(s) and lastly Z-specification is used to express complex Ontology properties. The only problem seen in this approach is a long procedure. Whereas, this can be possible more efficiently with the integration of Ontology Web Language OWL and Z-specification. The OWL has more and extra efficient features over other ontology's based languages, W3C recently introduced OWL for Semantic Web which is on top of XML/RDF.

In the paper we use OWL as Ontology language. The language OWL is derived from DAML+OIL which does not include qualified number of restrictions. The OWL can define symmetric properties and does not rename RDF-S primitives which increase the power of Semantic Web. The mentioned importance forced us to map Z-specifications to OWL with the motivation from basic idea described by (Dong et al., 2002, 2004).

The rest of the paper is organized as follows. In section 2, an introduction to formal method is given. In section 3 we describe an overview of Semantic Web, OWL, DAML+OIL, XML and RDF and further describe the conceptual model for Z approach to OWL. Section 4 consists of implementation and verification of model with Z/EVES tools and section 5 contains the conclusion of the study and a future work

2. Formal Methods

Formal methods are based on mathematical techniques and notations uses for describing and analyzing properties of software systems (Clarke et al., 1996; Khan et al., 2011(a); Zafar et al., 2012; Ahmad et al 2012; Ali et al., 2012). These mathematical techniques are based on discrete mathematics such as predicate logic, set theory, relations, functions, and graph theory. The process, to develop software systems using formal methods is shown in the Figure 1 (Liu et al.,1995).

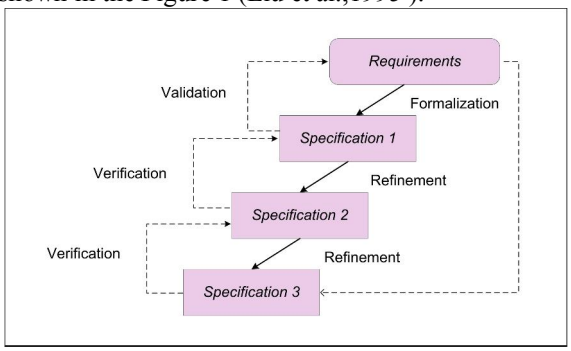


Figure 1. The Process of Software Development using Formal Methods

The "Requirements" are the result of requirements analysis and are normally described in informal language. 'Specification1' represents the stage of transformation from requirements to formal methods. Further, the process from 'Specification 2' to 'Specification n' corresponds to the stage of design. The process from 'Specification n' to 'Program' corresponds to the stage of implementation or coding. Validation and verification are the two basic principles that arise in system development. Validation addresses whether the produced system fulfills the requirements and verification check whether the software meets the requirements established in the previous phases. The aim of this approach is to demonstrate the process of development of a system from requirement to coding using formal specification. The use of this approach identifies errors and oversights early in the design life-cycle which are then easy to remove, with consequent high quality and cost saving software development. The Z-notation (woodcock et al., 1996), is model-oriented approach, it is used for specifying the behavior of abstract data type and sequential programs. The Z-specification divides the specification of complex system in different states called schemas. The schema consists of three parts; schema name, schema signature and schema predicate. These schemas can be combined to produce the overall description of the system. The paper addresses schemas in the specification part of the paper. Z-specification cannot typically be executed by computers, but the standard tools are available which are used for checking syntax and proof of the specification, leads to quality of specification and this allows mistakes to be detected and corrected sooner in the design life cycle.

3. The Semantic Web

Semantic Web is a future of current Web in which information is given with well-defined meaning in such a way that computers or intelligent software agents can understand the meaning of Web pages (Berners et al., 2002. Allwood et al., (2008) describe that the Semantic Web is an evolving extension of the World Wide Web in which Web content can be expressed not only in natural language, but also in a form that can be understand, interpret and use by software agents, thus permitting them to find, share and integrate information more easily. Semantic Web is a combination of different Ontology over Internet in such a manner that they can understand, interpret data intelligently without human involvement. Where ontology is a data model represents a set of concepts within a domain and the relationships between those concepts (Yang et al, 2008). Ontologies are represented by Ontology

languages such as DAML+OIL and OWL, which are based on top of XML and RDF. XML is a set of rules for defining and representing information as structured documents for applications on the Internet. RDF is a model for describing Web resources. Differentiating from HTML, HTML is aimed to deliver data to end user while XML is an extensible language: a language to describe other languages. XML is focused on syntax of the document rather than text. RDF defines resources on the Internet and provides interoperability between applications to exchange data. So that RDF uses XML to exchange description of Web resources. RDF Schema provides the built in vocabularies for RDF library. It is used to define properties of Web resources. DAML is a semantic markup language based on XML/RDF for Web services. DAML combined with Ontology-Interface Layer is referred as DAML+OIL. By using existing classes and properties, new concept can be added. This enables the DAML+OIL to reuse the existing technology. In 2003, W3C proposed a new markup language for Semantic Web known as OWL. It is based on top of DAML+OIL. Main differences are, OWL does not include qualified number restrictions, further, it can define symmetric properties and does not rename RDF-S primitives. In other words, the power of Semantic Web is increased with OWL. It has three flavors: Lite, DL and Full with enhanced capabilities used according to demand.

The semantic Web and Z-Specification

While communicating over the Web, these Ontologies need to be proper functioning. If Ontologies are not properly defined then obviously wrong results will cause problems. Z-specification is a descriptive logic which can perform well for Semantic Web. We are providing the power of Z-specification to built Ontology to meet the challenges in designing, testing and verification stages.

3 Z-Specification towards OWL

In the paper initially we specify the system using Z-specification. Further check their proof and syntax by using Z/EVES. After verifying we transform Z-model (encoded in ZML (Sun et al., 2001) into OWL to design Ontology and after checking and testing, retransformation of OWL into Z-model (again encoded in ZML) to remove inconsistencies in Ontology. The above process is shown in Figure 2.

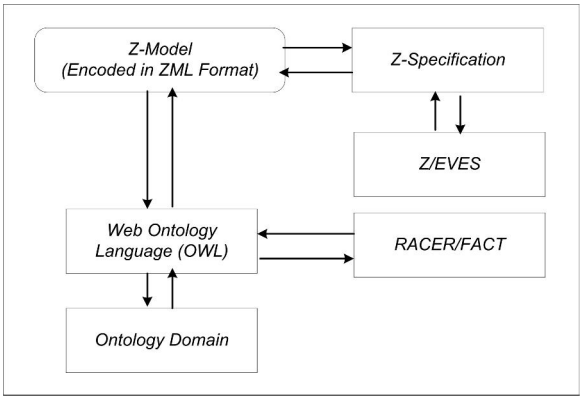


Figure 2. The Process of Z Approach to OWL

Transformation of Z-Specification to OWL

In order to transform Z semantics in OWL, we go through an example of IT talk discovery system TDS. The TDS is an on line Web portal offering the service of information about seminars. It also offers IT related upcoming talks that may appeal a register user according to his personal interests or schedules. It can be categorize in four agents.

- User's Calendar Agent
- Distance Map Agent
- User's Personal Agent
- Talk Discovery Agent

All these agents work collaboratively on behalf of human to extract the information about user's travel guide. In the next subsections we describe Z-specification in Z/EVES and then there corresponding OWL semantics to highlight the idea of transformation.

Z Model for IT Discovery System and OWL code

User's personal agent needs to consult with User's Calendar Agent to determine whether user is available or not. The calendar agent in IT Talk Discovery System can be defined in Z-specification as:

[TIME, DATE]

Where TIME and DTAE are of set types. The date and time can be defined as the schema DateTime.

Date_Time

d: DATE
t: TIME

The Status defined by Z free type definition shows that either user is busy or not.

Status ::= Free/Busy

The Calendar schema is as:

Calender

Schedule: Date_Time → Status

Transformation of Date and Time type definition into OWL can be written as:

```
<?xml version="1.0"?>
<rdf:Description rdf:about="d:DATE">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
    <rdf:Description rdf:about="t:TIME">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
</rdf:RDF>
```

Note: Code is simplified. Name spaces are omitted.

Distance Map Agent

After checking the availability of user, the user's personal agent needs to determine the distance between user's office and talk place. The Distance Map Agent outputs the distance for the user's personal agent.

We define [PLACE] is of a set type.

Map

$places : \mathbb{P}\ PLACE$

$dist. : places \times places \rightarrow \mathbb{R}^{+}$

The OWL code can be written as:

```
<rdf:Description rdf:about="d:dist">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#ObjectP
roperty"/>
        </rdf:type>
        <rdfs:domain>
            <rdf:Description
rdf:about="place:places"/>
        </rdfs:domain>
        <rdfs:range>
            <rdf:Description
rdf:about="r:R"/>
        </rdfs:range>
        <rdfs:domain>
            <rdf:Description
rdf:about="place:places"/>
        </rdfs:domain>
    </rdf:Description>
    <rdf:Description rdf:about="r:R">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
```

Note: For simplicity, Namespaces and classes are omitted.

User's Personal Agent

User's personal agent keeps user's personal information i.e. user's profile, user's office location, interests etc.

Z specification can be written as:

[NAME, TOPICS]

where NAME and TOPICS are of set type.

Personal

Name: NAME

Office: PLACE

Interest: $\mathbb{P}$ *TOPICS*

The OWL code can be written as:

```
<rdf:Description rdf:about="nam:NAME">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
    <rdf:Description
rdf:about="office:PLACE">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
    <rdf:Description
rdf:about="interest:TOPICS">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
    </rdf:Description>
    <rdf:Description rdf:about="int:Interest">
        <rdf:type>
            <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#Class"/>
        </rdf:type>
        <rdfs:subClassOf>
            <rdf:Description
rdf:about="interest:TOPICS"/>
        </rdfs:subClassOf>
    </rdf:Description>
```

Talk Discovery Agent

Finally, Talk discovery agent outputs various results based on users interests. The Talk

schema is defined for a general talk type. Interested talks are recorded in interestedtalk for the user.

Talk

place: PLACE
dt: Date_Time
subject: ℙ SUBJECT

The OWL code for Talk schema can be written as in user's personal agent

```
<rdf:Description rdf:about="Int:Interested_Talk">
        <rdf:type>
                <rdf:Description
rdf:about="http://www.w3.org/2002/07/owl#ObjectP
roperty"/>
                </rdf:type>
                <rdfs:domain>
                        <rdf:Description
rdf:about="p:Personal"/>
                </rdfs:domain>
                <rdfs:range>
                        <rdf:Description
rdf:about="T:Talk"/>
                </rdfs:range>
        </rdf:Description>
```

Implementation and verification

We checked the schemas's proof and syntax by the tool Z/EVES, the snapshot of the proof is shown in Figure 3.

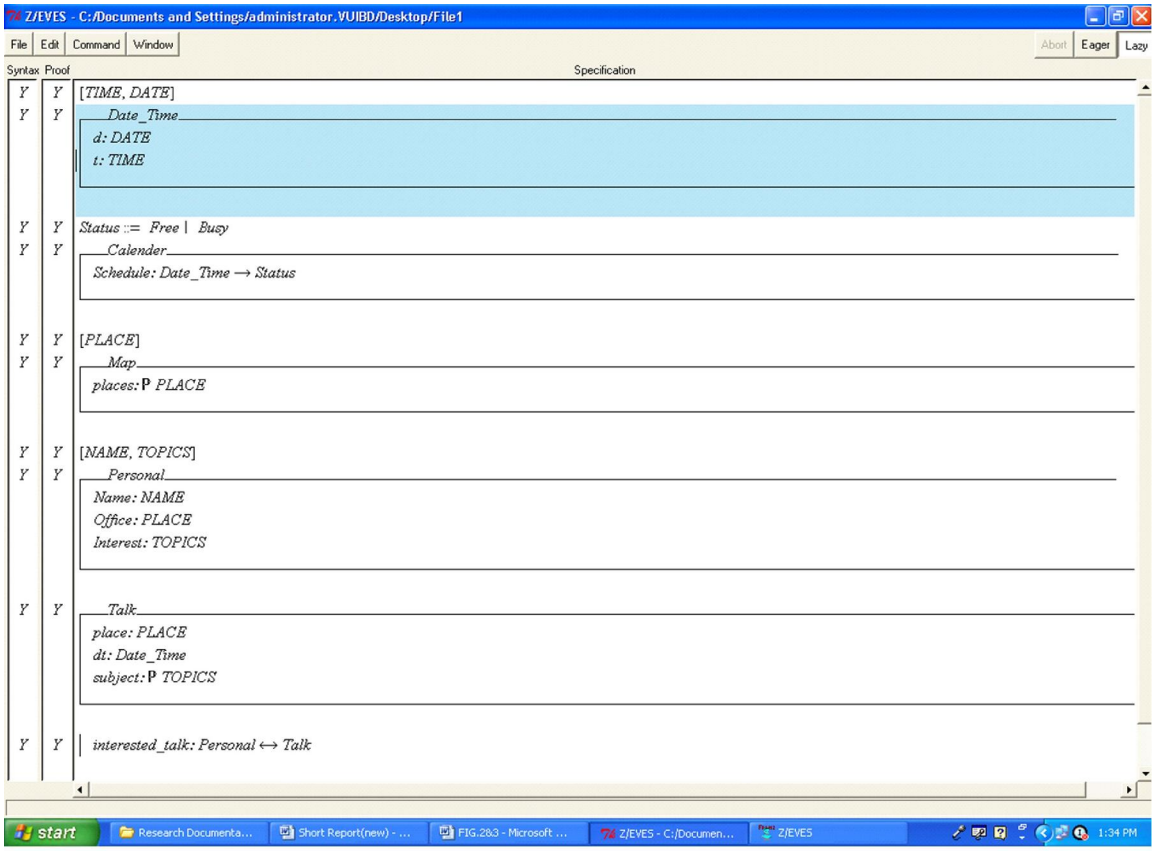


Figure 3. Z/EVES Schemas for IT Talk Discovery

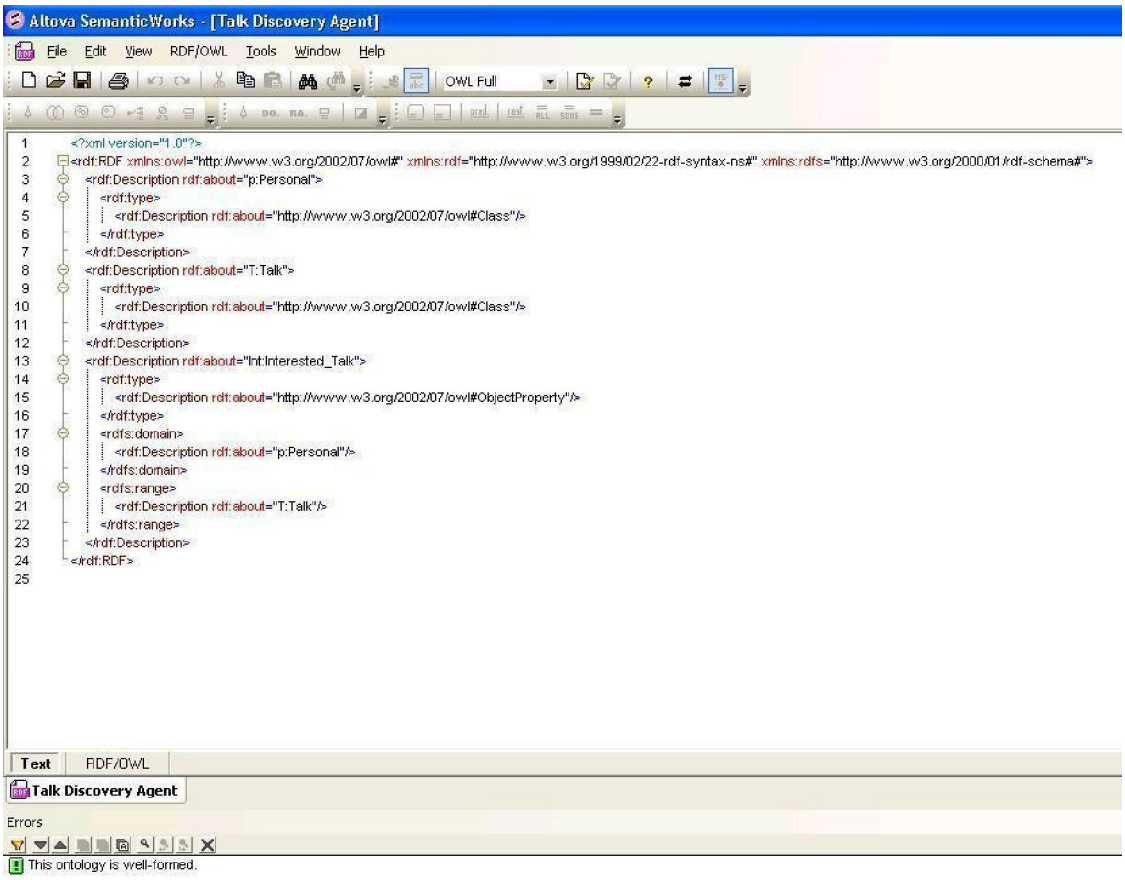


Figure 4. OWL code for IT Talk Discovery

Further we transform schemas to their corresponding OWL code in Altova Semantic Works 2008 which is shown in Figure 4.

Conclusion

In this paper, we have discussed an idea of designing Ontology, using Z-specification schemas. The verification is done by Z/EVES and further transformation is done into OWL code. To observe the approach, we have used an example of IT Talk Discovery System which is an on line Web portal offering the service of information about seminars and IT related upcoming talks that may appeal a register user according to his personal interests or schedules. We have shown, via an example, a one to one correspondence between Z-schemas and OWL code which provides an environment to transform Z-schemas to OWL after removing an inconsistencies and ambiguities from a specification.

References

1. Allwood, J.: Some remarks on the relationship between the semantic and the pragmatic Web. In: Proceedings of the 3rd international Conference on the Pragmatic Web: innovating the interactive Society, (2008)
2. Berners, L.T., Miller, E.: The semantic Web lifts off. Ercim News Online Edition, No. 51, (2002)
3. Berners, L.T., Hendler, J., Lassila, O.: The Semantic Web. Scientific American, (2001)
4. Clarke, E., Wing, J.: Formal methods: State of the art and future directions. ACM Computing Surveys 28, 4, pp. 626-646 (1996)
5. Dean, M., Schreiber, G. (editors).: OWL Web Ontology Language Reference. W3C Recommendation, http://www.w3.org/TR/owl-ref/, (2004)
6. Dong, J.S., Lee, C.H., Li, Y.F., Wang, H.: Verifying DAML+OIL and Beyond in Z/EVES. In: Proceedings of the 26th International Conference on Software Engineering. pp. 201-210 (2004)
7. Dong, J.S., Sun, J., Wang. H.: Z Approach to Semantic Web. In: International Conference on Formal Engineering Methods. LNCS, Springer-Verlag, pp. 156-167 (2002)
8. Dong, J.S., Lee, C.H., Li, Y.F., Wang, H.: A Combined Approach to Checking Web Ontologies. In: Proceedings of 13th World Wide

Web Conference, New York, USA, pp. 714-722 (2004(a))
9. Harmelen, F.V., Peter, F.P.S., Horrocks, I. (editors).: Reference description of the daml+oil ontology markup language. http://www.daml.org/2001/03/reference.html, (2001).
10. Khan, S.A and Zafar, N.A, Promotion of Local to Global Operation in Train Control System, Journal of Digital Information Management (page 228-233).,2007.
11. Khan, S.A., Zafar, N.A.: Towards the formalization of railway interlocking system using z-notations. In: Proceedings of the 2nd IEEE International Conference on Computer, Control and Communication, pages 750-755 (2009)
12. Liu, S., Adams, R.: Limitations of Formal Methods and an Approach to Implement. Technical Report ,Hiroshima City University, (1995)
13. Meisels, I. and Saaltink, M.: The Z/EVES reference manual. TR-975493-03, ORA, Canada, (1997)
14. Sun, J. Dong, J.S. Liu, J. and Wang, H. Object-Z Web Environment and Projections to UML. In: 10th International World Wide Web Conference, pp. 725-734 (2001)
15. World Wide Web Consortium (W3C). Web Ontology Language (OWL) Use Cases and Requirements. http://www.w3.org/TR/webont-req/, (2003)
16. Woodcock, J. and Davies, J.: Using Z: Specification, Refinement, and Proof. Prentice-Hall International, (1996)
17. Yang, H. and Callan, J.: Human-Guided Ontology Learning. Second Workshop on Human-Computer Interaction and Information Retrieval. Microsoft Research, Redmond, WA, USA (2008)
18. Khan, S.A and Zafar, N.A, Improving Moving Block Railway System using Fuzzy Multi-Agent Specification Language, Int. J. Innov. Computing, Inform. Control, 7(7(B)):4517-34, 2011.
19. Khan, S.A, Zafar, N.A and Ahmad, F, Petri Net Modeling of Railway Crossing System using Fuzzy Brakes, International J. Phy. Sci, 6(14): 3389-3397, 2011(a).
20. Zafar, N.A, Khan, S.A and Araki, K, Towards the Safety Properties of Moving Block Railway Interlocking System, Int. J. Innov. Computing, Inform. Control, 8(8): 2012 .
21. Khan, S.A, Zafar, N.A, Extending promotion for the management of moving block interlocking components., International J. Phy. Sci, 6(31), 7262-70. 2011(b).
22. Ahmad, F. Khan, S.A, Module-based Architecture for Periodic job-Shop Scheduling problem, Computers & Mathematics with Applications, 64(1), 1-10, 2012.
23. Ali, G., Khan, S.A., Ahmad, M.F. and Zafar, N.A, Visualized and Abstract Formal Modeling towards the Multi-Agent Systems, International Journal of basic and Applied Sciences 2(8)8272-8284, 2012
24. Ali, G., Khan, S.A., Ahmad, M.F. and Zafar, N.A, Formal Modeling towards a Dynamic Organization of Multi-Agent Systems Using Communicating X-Machine and Z-Notation, Indian Journal of Science and Technology, Vol. 5 No. 7, 2012(a)

9/23/2012

Anatomical and Morphometrical Study of the Alimentary Canal of the Lizard *Scincus scincus* and the snake *Natrix tessellata*

[1]Ahlam M. El- Bakry, [2]Ahmed M. Abdeen and [1]Rasha E. Abo- Eleneen

[1]Department of Zoology- Faculty of Science, Beni-Suef University
[2]Department of Zoology- Faculty of Science- Mansoura University
amalbakry2@yahoo.com

Abstract: The gastrointestinal tract (GIT) is one of the most interesting systems of reptiles. The present study aimed to study the anatomical, histological and morphometrical features of the gastrointestinal tract (GIT) of the lizard *Scincus scincus* and the snake *Natrix tessellata*. The wall of the different regions of the alimentary tract is built up of four layers, from outside inwards: serosa, muscularis, submucosa and mucosa. The oesophageal mucosa is formed of simple columnar epithelium in *Scincus scincus*, while in *Natrix tessellata* it consists of simple columnar ciliated epithelium with goblet cells. In the studied species, the mucosa of the stomach displayed numerous gastric glands which opened with gastric pits while, the mucosa of the intestine is consisted of simple columnar epithelium containing goblet cells. The intestinal glands are completely absent in the two species. There are great differences between the oesophagus, stomach and intestine histochemically in both species. Generally, the length of the whole gastrointestinal organs is sex-dependent. No significant difference was found in between the organ lengths in both sexes of the studied species. In conclusion, variation in the structure of the GIT appears to be related to the difference in their habitats or their vital activities.

[Ahlam M. El- Bakry, Ahmed M. Abdeen and Rasha E. Abo- Eleneen. **Anatomical and Morphometrical Study of the Alimentary Canal of the Lizard *Scincus scincus* and the snake *Natrix tessellate.*** *Life Sci J* 2012;9(4):1010-1022] (ISSN:1097-8135). http://www.lifesciencesite.com. 155

Key words: Anatomy- Histology- Morphometry- Alimentary canal- Reptiles

1. Introduction

Reptiles are always a matter of great interest in many fields of biological sciences. Recently, efforts of the present authors have been directed towards the reptilian gastrointestinal tract (GIT) in order to merge specific morphological merits that would be utilized as an identification key. The gut of various reptiles has been the subject for anatomical, morphometrical, histological and histochemical studies **(Zaher *et al*., 1987 a, b, c & 1989 a; Abo-Taira *et al*., 1989; Afifi *et al*., 1989 and Abdeen *et al*., 1992).** Among lower vertebrates, reptiles always stimulate much interest when compared with the higher ones.

The gastrointestinal tract (GIT) is one of the most interesting systems of reptiles and its morphology, anatomy and histology have been studied extensively on lizards; *Scincus officinalis* and *Scincus scincus* (Family: Scincidae), **El- Toubi, 1936 and Biomy, 2010** respectively, *Uromastyx aegyptia* (family: Agamidae, **El- Toubi and Bishai, 1958**) and *Chamaeleon vulgaris* (family: Chamaeleontidae, **Bishai, 1960**). The previous authors showed how the morphological, anatomical and histological patterns of the alimentary tract organs are well adapted to perform their vital functions. Valuable informations were later on added to the subject through the study of other reptilian species; the amphisbaenian *Diplometopon zarudnyi* (**Al- Nassar, 1976**), the gecko *Gehyra mutilate* (**Chou, 1977**), the scincid lizard *Chalcides levitoni* (**El- Taib *et al*., 1982**) and the lacertid lizard *Acanthodactylus ophedureus* (**Zaher *et al*., 1987 a**).

Furthermore, combined anatomical and histological studies were carried out on the different species of lizards and snakes, as well as morphometrical investigations were done on the reptilian tract organs of some species in order to cast more light on the importance of the morphometric analysis as a valuable parameter for the identification of different species. In this respect different species of lizard were studied family Lacertidae as *Acanthodactylus boskianus* (**Abo- Taira *et al*., 1988**), family Geckonidae as *Tarentola annularis* (**Abo-Taira *et al*., 1990**) and *Chalcides sepsoides* **(Zaher *et al*., 1990 b & c).** Stimultaneously, great attention was directed to the anatomy and histology of snakes, family Colubridae as *Natrix natrix* **(Przystalski, 1980, Kozaric *et al*. 2011).**

Abdeen *et al*. (1992) studied the distribution of carbohydrates, proteins and nucleic acids in the oesophageal and gastric mucosal epithelium of three colubrid snakes; *Malpolon monspessulanus*, *Coluber florulentus* and *Tarbophis obtusus* (Family: Colubridae). Several studies are carried out on the histochemical configurations of the reptilian gut mucosa **(Zaher *et al*., 1991 a & b). Mahmoud (1975)** resolved the distribution of carbohydrates and proteins in the mucosa of the ileum and rectum of

Mabuya quinquetaeniata and *Chalcides ocellatus*. Recently, in an attempt to creat a novel criterion for classification of reptiles, a regression analysis was applied relating to the snout vent with that of the alimentary canal in the gecko *Tarentola annularis* **(Abo- Taira *et al*., 1990)** and *Mabuya quinquetaeniata* **(Zaher *et al*., 1990 a).** These previous works have attracted our attention to design the present investigation which deals with the study of the anatomical, histological and morphometrical aspects of the alimentary tract of the two studied species; the insectivore lizard *Scincus scincus* (Family: Scincidae) and the water snake *Natrix tessellata* (Family: Colubridae). This investigation was done to cast more light on the correlation between the anatomical, histological as well as morphometrical configurations of the alimentary canal of *Scincus scincus* and *Natrix tessellata* in relation to their habitats. Also, to elucidate if such parameters could be considered with great importance in the field of taxonomy of reptiles.

2. Material and Methods

1- Animals:-

Animal	Family	Habitat
Scincus scincus (Fig. 1)	Scincidae	Restricted to sandy desert areas where it lives mostly under the sand but surfaces occasionally to run on the sand surface at night. It feeds on a variety of insects and captured from Sinai.
Natrix tessellata (Fig. 4)	Colubridae	Never found far from water, often resting or crawling on the bottom of streams or irrigation canals. When distributed it invariably tends to escape by diving into the water. In suitable habitats, large populations can be found in a relatively small area. It feeds on fish and amphibian and collected from Faiyum.

- The animals used were 10 adults of each sex of *Scincus scincus* and *Natrix tessellata*.

2- Gross Anatomy:-

All animals were sacrificed to remove the gut portions (oesophagus, stomach and intestine). These organs were anatomically examined and measured.

3- Morphometry:-

All animals were measured from the snout to the cloacal opening (snout-vent). Lengths of the gastrointestinal tract (GIT) of males and females were expressed as indices and percentages of the total tract length.

4- Statistic Analysis

Statistical significance of female values versus male ones was determined by the application of student test **(Van der Waerden and Nievergelt, 1956).** T. test was also applied between the whole gut length and its organs as well as the snout-vent of both sexes of the two species studied.

5- Histological and Histochemical Studies

For histological study of the alimentary tract, parts from different regions of the gut were taken, cleaned with saline and fixed in 10% neutral formalin, then washed and dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were then cut serially at 5 µm thickness and stained with Ehrich's haematoxylin and eosin **(Mallory, 1944)** and Masson trichrome stain **(Drury *et al*., 1976).** For histochemical study, the identification of polysaccharides (McManus, 1946), acid and neutral mucins **(Mowry, 1956)** and total proteins **(Mazia *et al*., 1953)** were adopted. The stained sections were examined and photographed using Leitz microscope (Germany).

3. Results

Anatomical Features (Figs. 2, 3, 5 &6):

In *Scincus scincus* (Figs. 2 & 3), the alimentary tract starts with a transverse terminal mouth which surrounds a buccal cavity leading to a short pharynx. The oesophagus is a narrow elongated funnel tube opening directed into the stomach without any external constriction. The stomach is left- curved and slightly wider and longer than the oesophagus. The stomach is terminated by a narrow portion which opens into the intestine.

In *Natrix tessellata* (Figs. 5 & 6), the oesophagus is subdivided into an anterior wide, funnel- shaped portion and a posterior narrower and extremely longer cylindrical one. The latter enters the visceral cavity dorsal to the pericardioperitoneal septum. The border between the oesophagus and the stomach is only indicated by the nature of the mucous membrane, i.e., there is no anatomical distinction between the oesophagus and the stomach. The stomach has a larger left convex and a smaller right concave curvatures. The stomach leads to the intestine. The intestine is distinguished into the small and large intestine. The large intestine is nearly straight and can be subdivided into two sections: The colon and the rectum. The latter opens into the cloaca.

Morphometric observations (Tables 1 – 6 and Figs. 49 – 52):

The application of t- test on the morphometrical data collected from the alimentary canal of the two studied species *Scincus scincus* and *Natrix tessellata* showed that in *Scincus scincus*, there is a significant difference observed between the snout-vent of female (8.4 ± 0.28) and males (9.4 ± 0.15). The gastrointestinal tract (GIT) of females (12.7 ± 1.72) is always longer than that of males (11.7 ± 0.79) and there is no significant difference is detected in

between them (P > 0.05 level, student t- test) Table (1). In the studied lizard, the female oesophagus length (2.1 ± 0.29) is always longer than the male's oesophagus (1.6 ± 0.37). No significant difference is detected in between the stomach and intestinal lengths in both species. In both sexes, the longest and shortest organs are the intestine and the oesophagus, respectively. The length of the GIT and its organs, in females and males are diagrammatically represented in (Fig. 49).

There is no significant difference is detected among the index values of the GIT organs in females and males except for the oesophagus (Table 2).The percentage ratios of the GIT organs of female do not significantly differ from those of males in stomach and intestine. However, there is a significant difference in the oesophagus (Table 3). These ratios increase progressively in both sexes through the oesophagus, stomach and intestine. Index and percentage values of the GIT organs in both sexes of *Scincus scincus* are diagrammatically represented in (Fig. 50), these values are resulted in two closely related curves for the index values, for females and males, and other opposing two for the percentage ratios. These two couples of curves enclose a single genometrically tetragonal area.

The morphometric finding of the alimentary tract organs in both sexes of *Natrix tessellata* disclosed that there is a significant difference between the snout-vent length of females (85.3 ± 3.34) and males (74.8 ± 9.75). The GIT length of the females (80.3 ± 3.34) is always longer than that of the males (69.8 ± 9.75), and there is a significant difference is detected between them (P < 0.005 level, student t-test) Table (4). In both sexes, the longest and shortest organs are the intestine and the stomach respectively. There is a high significant difference between the length of the females and males stomach (P < 0.001 level) and a significant difference appears also between females and males intestine (P < 0.05 level). No significant difference is detected between the length of the oesophagus in the two sexes (Table 4 and Fig. 51).

The morphometric data of the alimentary tract organs in both sexes of *Natrix tessellata* showed that there is no significant difference is detected between the index values of stomach and small intestine in females and males but there is a significant difference appears between the oesophagus and large intestine (Table 5).

The percentage ratios increase progressively in both sexes of *Natrix tessellata*, through the stomach, oesophagus and intestine. There is a significant difference between the oesophagus and small intestine (P < 0.005 level, student t- test), but there is no significant difference appears between the stomach and large intestine in both sexes (P > 0.05 level, student t- test) Table (6).

Index and percentage values of GIT organs in both sexes of *Natrix tessellata* are diagrammatically represent in (Fig. 52). The diagrammatical designation of the index and percentage values of the GIT organs in both sexes of the *Natrix tessellta* disclosed two closely related curves inclosing between the two tetragons of unequal areas.

Histological and histochemical studies (Figs. 7-48):

The alimentary canal of *Scincus scincus* and *Natrix tessellata* consists of the usual known regions which are the oesophagus, stomach and intestine. The oesophagus, stomach and intestine of the two studied species are formed of the mucosa, submucosa, muscularis and serosa. **The serosa** represents the outermost layer of the wall in all GIT organs and consists of simple squamous epithelium. This layer is followed by **the muscularis** which consists of smooth muscles and is differentiated into an outer longitudinal and an inner circular muscle layer. In the oesophagus, both layers of muscularis appear, nearly, in the same thickness. However, in all other GIT organs, the outer longitudinal muscle layer is always less developed than the inner circular one.

The muscularis mucosa is well represented in the oesophagus, as well as in the stomach. The oesophageal muscularis mucosa is built up of an outer longitudinal muscle layer which is arranged in several rows and an inner circular muscle layer which appears as a thin sheet. The muscularis mucosa is arranged in the form of a well-developed outer longitudinal muscle layer and well-developed inner circular one.

The submucosa, is well developed throughout all the alimentary canal organs. It consists of an areolar connective tissue and is involved in the structure of the oesophageal, gastric and intestinal folds. The submucosa contains also blood vessels and capillaries in all gut organs. Although the previous layers are nearly similar, some differences are found in the mucosal coat of the oesophagus, the stomach and the intestine of *Scincus scincus* and *Natrix tessellata* which may be referred to their mode of feeding.

1- The oesophagus

Histologically, the oesophageal mucosa of *Scincus scincus* (Figs. 7 & 9), consists of a thin simple columnar epithelium and contains oesophageal glands, while it is thrown into many folds with variable lengths and wavy appearance in *Natrix tessellata* (Figs. 8 & 10). The mucosal epithelium of *Natrix tessellata*, is simple and formed of two types of cells, the elongated ciliated epithelial cells and the goblet cells. The goblet cells are a unicellular glands

and markedly numerous at the bottom of the oesophageal folds. The ciliated epithelial cells of the oesophageal mucosa scattered between the goblet cells.

In *Scincus scincus* (Figs. 11 & 13), the collagenous fibres are scarcely distributed through the mucosa, while in *Natrix tessellata* (Figs. 12 & 14), the collagenous fibres are widely distributed through the mucosa layer.

Histochemically, high contents of polysaccharides which represented by PAS- positive materials are found in the mucosa of both species. The submucosa is moderately stained in *Scincus scincus* (Fig. 15) while, in *Natrix tessellata* (Fig. 16), the submucosa is weakly stained. A mixture of neutral polysaccharides (PAS- positive) and acidic ones (Alcian blue positive) are strongly stained in the mucosa of both species in magenta colour (Figs. 17 & 18). Using bromophenol blue stain the oesophageal mucosa of the two studied species shows high content of protein (Figs. 19 & 20). However, low protein content is found in the oesophageal glands of *S. scincus* (Fig. 19).

2- The stomach

Histologically, in both species, the mucosa of the stomach is mostly consisted of simple columnar cells with oval basally located nuclei. The gastric mucosal coat appeared thick due to the presence of the gastric glands. These gastric glands are embedded in the lamina propria and open into the gastric folds through the gastric pits. The glands consist mainly of a secretory portion, gland neck and gastric pit which open into the lumen (Figs. 21 – 28).

Histochemically, the stomach mucosa of *Scincus scincus* is strongly stained with PAS indicating high content of neutral polysaccharides (Fig. 29), while the stomach mucosa of *Natrix tessellata* is moderately stained with PAS indicating the presence of moderate content of neutral polysaccharides (Fig. 30). On the other hand, the muscularis and the submucosa of the two species are moderately loaded with neutral polysaccharides. PAS- Alcian blue (pH 2.5), showed weak reaction in the mucosa, the muscularis and the gastric glands of *Scincus scincus* (Fig. 31), while PAS- Alcian blue (pH 2.5), showed strong reaction in the mucosa and the gastric glands of *Natrix tessellata* (Fig. 32). This reaction appeared magenta indicating high content of mixed neutral (purple) and acidic (blue) polysaccharides present in the mucosa. In addition, moderate magenta colour (mixed polysaccharides) was observed in the muscularis layer. Bromophenol blue stained materials indicated high protein contents in the mucosa of both species, while the submucosa appeared moderately stained. However, the low protein contents in gastric glands are weakly stained (Figs. 33 & 34).

3- The intestine

Histologically, the intestinal mucosae of both studied species are thrown up into numerous elongated and deep folds lined with simple epithelia containing scattered goblet cells (Figs. 35 – 38). The intestinal glands are absent in the two species. The collagenous fibres are scarcely distributed through the muscularis layer of *Scincus scincus* and *Natrix tessellata* (Figs. 39 & 40), while these fibres are greatly distributed in the mucosa of both species (Figs. 41 & 42).

Histochemically, PAS positive materials are represented in the muscularis and mucosa of *Scincus scincus* indicating moderate content of neutral polysaccharides (Fig. 43). However, strong PAS reaction (high content of neutral mucins) is indicated in the mucosa of *Natrix tessellata* (Fig. 44), while the muscularis is moderately stained with PAS.

PAS- Alcian blue (pH 2.5), showed moderate magenta colour (mixed neutral and acidic mucins) in the mucosa of both species. The goblet cells are stained magenta for mixed mucins and blue for acid mucins in both species (Figs. 45 & 46), while in *Natrix tessellata* acid mucins predominate in the goblet cells (Fig. 46). Application of bromophenol blue, showed high protein contents in the mucosa and the muscularis layer of both species (Figs. 47 & 48). On the other hand, the goblet cells appeared weakly stained for protein in *Scincus scincus* and devoiding proteins in *Natrix tessellata*.

Table (1): Lengths (cm) of the snout vent and alimentary tract organs in both sexes of *Scincus scincus*

GIT organ	Females	Males	P
Snout-vent	8.4 ± 0.28	9.4 ± 0.15	**
Alimentary tract	12.7 ± 1.72	11.7 ± 0.79	ns
Oesophagus	2.1 ± 0. 29	1.6 ± 0. 37	**
Stomach	2.7 ± 0.52	2.4 ± 0. 28	ns
Small intestin	0.3 ± 1.2	5.2 ± 1. 36	ns
Large intestine	2.6 ± 0.6	2.5 ± 0.6	ns

The values are means ± standard deviation (n = 10 0f each sex); ns: Non- Significant (student t-test, P > 0.05 level); **: Significant values (student t- test, P < 0.001 level).

Table (2): Index values of gastrointestinal tract (GIT) organs in both sexes of *S. scincus*

GIT organ	Females	Males	P
Oesophagus	6.6 ± 1.17	7.3 ± 1.16	*
Stomach	4.4 ± 0.5	4.3 ± 0. 61	ns
Small intestine	2.4 ± 0.25	2.5 ± 0. 41	ns
Large intestine	5.4 ± 0.96	4.7 ± 0.78	ns

Index is the gastrointestinal tract length / organ length; Values are means ± standard deviation (n = 10 0f each sex); ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t- test, P < 0.05 level).

Table (3): Percentage ratios of the gastrointestinal tract (GIT) organs in both sexes of *Scincus scincus*

GIT organ	Females	Males	P
Oesophagus	16.7 ± 2.35	13.6 ± 2.9	*
Stomach	21.4 ± 3.16	20.8 ± 2. 54	ns
Small intestine	42 ± 4.42	44.4 ± 5.3	ns
Large intestine	20.4 ± 2.3	21.3 ± 3.2	ns

The values are means ± standard deviation (n = 10 0f each sex) Percentage ratio is the organ length × 100 / gastro-intestinal length; ns: Non- Significant (student t- test, p > 0.05 level); *: Significant values (student t-test, p < 0.05 level).

Table (4): Lengths (cm) of the snout vent and alimentary tract organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	P
Snout-vent	85.3 ± 3. 34	74.8 ± 9.75	*
Alimentary tract	80.3 ± 3. 34	69.8 ± 9.75	*
Oesophagus	21.6 ± 1.58	20.5 ± 1.96	ns
Stomach	10.4 ± 1.19	8.1 ± 0.74	**
Small intestine	36.6 ± 11.8	30.2 ± 8. 31	*
Large intestine	11.7 ± 3.4	11 ± 2.8	*

The values are means ± standard deviation (n = 10 0f each sex); ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t- test, P < 0.05 level); **: Significant values (student t- test, P < 0.001 level).

Table (5): Index values of gastrointestinal tract (GIT) organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	P
Oesophagus	3.5 ± 0.69	3.2 ± 0.23	*
Stomach	7.7 ± 1.39	8.6 ± 1.25	ns
Small intestine	2.3 ± 0.4	2.5± 0. 19	ns
Large intestine	6.8 ± 1.45	6.6 ± 1.3	*

Index is the gastrointestinal tract length / organ length. Values are means ± standard deviation (n = 10 0f each sex); ns: Non- Significant(student t- test, p > 0.05 level); *: Significant values (student t- test, p < 0.05 level).

Table (6): Percentage ratios of the gastrointestinal tract (GIT) organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	P
Oesophagus	26.9 ± 2.1	29.6 ± 2.84	*
Stomach	12.9 ± 1.63	11.6 ± 1.13	ns
Small intestine	45.5 ± 7.2	43.2 ± 3.8	*
Large intestine	14.5 ± 2.	16 ± 2.6	ns

The values are means ± standard deviation (n = 10 0f each sex); Percentage ratio is the organ length × 100 / gastro-intestinal length; ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t-test, P < 0.05 level).

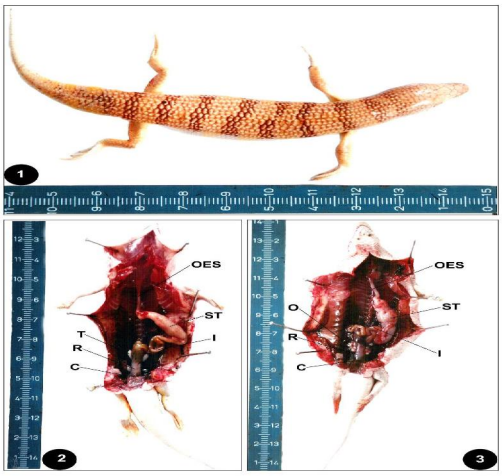


Fig. 1: Photograph of the lizard (*Scincus scincus*) in a lateral view.

Fig. 2: Dissected specimen of male *Scincus scincus* exposing the general viscera.Oesophagus (OES), Stomach (ST), Intestine (I), Testis(T), Rectum (R), Coloaca (C).

Fig. 3: Dissected specimen of female *Scincus scincus* exposing the general viscera. Oesophagus (OES), Stomach (ST), Intestine (I), Ovary(O), Rectum (R), Coloaca (C).

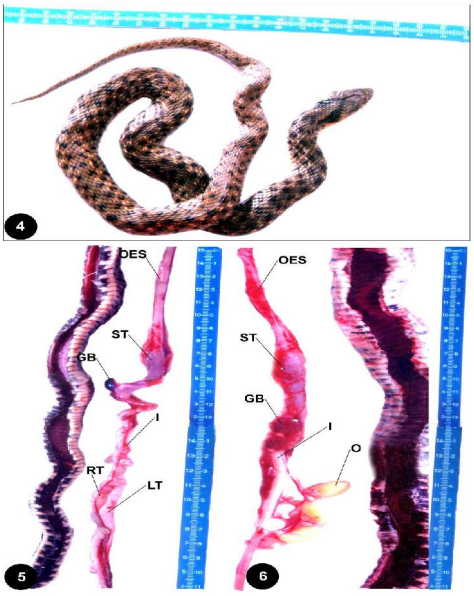


Fig. 4: Photograph of the snake (*Natrix tessellata*) in a lateral view.

Fig. 5: Dissected specimen of male *Natrix tessellata* exposing the general viscera. Oesophagus (OES), Stomach (ST), Gall Bladder (GB), Intestine (I), Right Testis(RT), Left Testis(LT).

Fig. 6: Dissected specimen of female *Natrix tessellata* exposing the general viscera. Oesophagus (OES), Stomach (ST), Gall Bladder (GB), Intestine (I), Ovary (O).

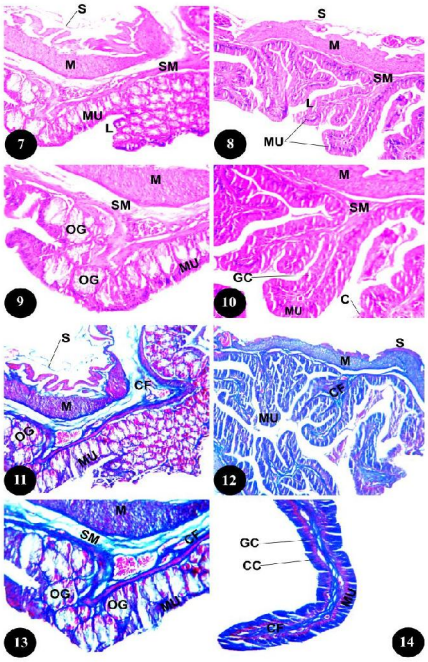


Fig. 7: T.S. of the oesophagus of *Scincus scincus* showing the structure of the mucosa (MU) and the submucosa (SM) of loose connective tissue. H.E., X 100.

Fig. 8: T.S. of the oesophagus of *Natrix tessellata* displaying the columnar epithelial structure of the mucosa (MU). The submucosa (SM) is formed of loose connective tissue and thick muscularis layer (M) of striated muscles covered with serosa (S). H.E., X 100.

Fig. 9: T.S. of the oesophagus of *Scincus scincus* showing the structure of the mucosa (MU), the esophageal glands (OG) and the submucosa (SM). H.E., X 200.

Fig. 10: T.S. of the oesophagus of *Natrix tessellata* displaying the ciliated epithelial cell (C) and the goblet cell (GC). H.E., X 200.

Fig. 11: T.S. of the oesophagus of *Scincus scincus* showing the columnar epithelial structure of the mucosa (MU) and the collagen fibres (CF) scarcely distributed in the submucosa (SM). Masson's trichrome stain., X 100.

Fig. 12: T.S. of the oesophagus of *Natrix tessellata* showing the condensation of the collagen fibres (CF) in the muscularis (M) and the mucosa (MU). Masson's trichrome stain., X 100.

Fig. 13: T.S. of the oesophagus of *Scincus scincus* showing the collagen fibres (CF) in the muscularis (M), the submucosa (SM) and the mucosa (MU). Masson's trichrome stain., X 200.

Fig. 14: T.S. of the oesophagus of *Natrix tessellata* showing the collagen fibres (CF) are distributed in the mucosa (MU). Masson's trichrome stain., X 200.

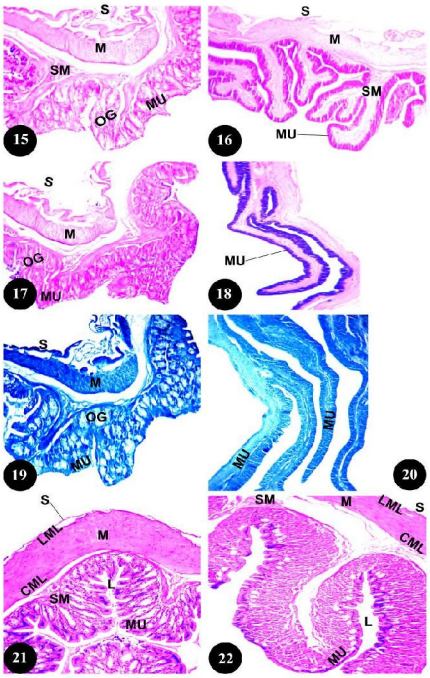


Fig. 15: T.S. of the oesophagus of *Scincus scincus* denoting high polysaccharide content in the mucosa (MU) and the esophageal glands (OG). PAS, X 100.

Fig. 16: T.S. of the oesophagus of *Natrix tessellata* showing accumulation of high polysaccharide content in the mucosa (MU). PAS., X 100.

Fig. 17: T.S. of the oesophagus of *Scincus scincus* displaying high content of neutral and acidic mucins in the mucosa (MU) and showing high content of acidic mucins in the esophageal glands (OG) (in blue). PAS-Alcian., X 100.

Fig. 18: T.S of the oesophagus of *Natrix tessellata* denoting high content of acidic mucins in the mucosa (MU). The muscularis (M) are moderately stained magenta. PAS-Alcian., X 100.

Fig. 19: T.S. of the oesophagus of *Scincus scincus* showing high protein content in the mucosa (MU), the muscularis (M) and the serosa (S). The esophageal glands (OG) are moderately stained. Bromophenol blue., X 100.

Fig. 20: T.S. of the oesophagus of *Natrix tessellata* displaying high protein content in the mucosa (MU). Bromophenol blue., X 100.

Fig. 21: T.S. of the stomach of *Scincus scincus* disclosing the serosa (S), the muscularis (M), the submucosa (SM) and the mucosa (MU). H.E., X 100.

Fig. 22: T.S. of the stomach of *Natrix tessellata* displaying the serosa (S), the muscularis (M), the submucosa (SM) and the mucosa (MU). H.E., X 100.

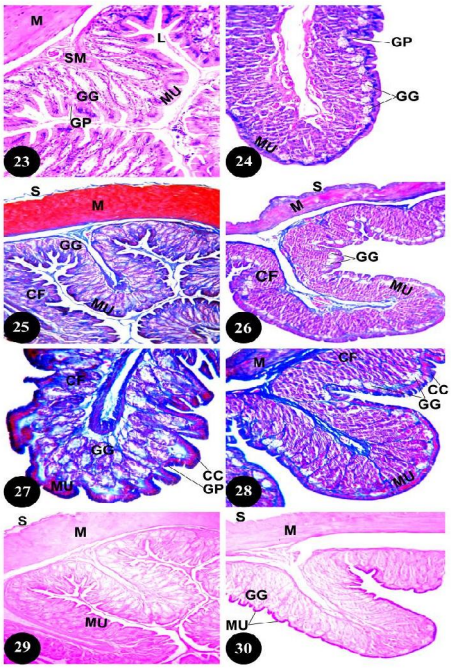


Fig. 23: T.S. of the stomach of *Scincus scincus* showing the mucosa (MU), the gastric pits (GP) and the gastric glands (GG). H.E., X 200.

Fig. 24: T.S. of the stomach of *Natrix tessellata* displaying the mucosa (MU), the gastric pits (GP) and the gastric glands (GG). H.E., X 200.

Fig. 25: T.S. of the stomach of *Scincus scincus* showing the collagen fibres (CF) in the serosa (S) and the mucosa (MU). Masson's trichrome stain., X 100.

Fig. 26: T.S. of the stomach of *Natrix tessellata* showing the collagen fibres (CF) in the serosa (S) and the mucosa (MU). Masson's trichrome stain., X 100.

Fig. 27: T.S. of the stomach of *Scincus scincus* showing the collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.

Fig. 28: T.S. of the stomach of *Natrix tessellata* showing the collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.

Fig. 29: T.S. of the stomach of *Scincus scincus* showing high polysaccharide contents in the mucosa (MU). The muscularis (M) is weakly stained. PAS., X 100.

Fig. 30: T.S. of the stomach of *Natrix tessellata* showing moderate content of polysaccharides in the mucosa (MU). The muscularis (M) is lightly stained. PAS., X 100.

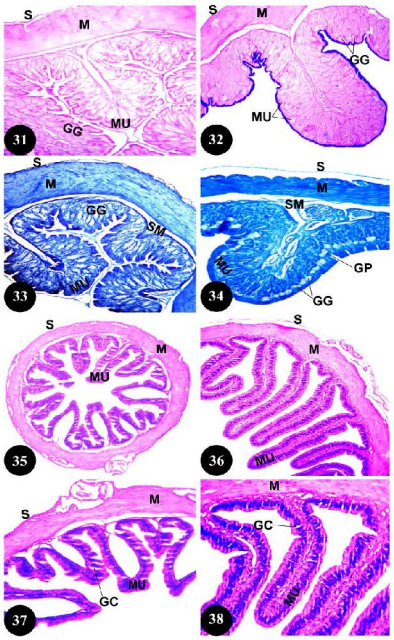


Fig. 31: T.S. of the stomach of *Scincus scincus* displaying weak reaction of PAS-Alcian blue in the mucosa (MU) and the muscularis (M). PAS-Alcian blue., X 100.

Fig. 32: T.S. of the stomach of *Natrix tessellata* showing high content of neutral and acidic-mucins in the mucosa (MU). The muscularis (M) appear moderately stained magenta. PAS-Alcian., X 100.

Fig. 33: T.S. of the stomach of *Scincus scincus* displaying high protein content in the mucosa (MU). The muscularis (M) is moderately stained while the gastric glands (GG) are weakly stained. Bromophenol blue., X 100.

Fig. 34: T.S. of the stomach of *Natrix tessellata* displaying high protein content in the mucosa (MU) and the muscularis (M). The gastric glands (GG) are weakly stained. Bromophenol blue., X 100.

Fig. 35: T.S. of the intestine of *Scincus scincus* showing the serosa (S), the muscularis (M) and the mucosa (MU). H.E., X 100.

Fig. 36: T.S. of the intestine of *Natrix tessellata* displaying the serosa (S), the muscularis (M) and the mucosa (MU). H.E., X 100.

Fig. 37: T.S. of the intestine of *Scincus scincus* showing the mucosa (MU) and numerous goblet cells (GC). H.E., X 200.

Fig. 38: T.S. of the intestine of *Natrix tessellata* showing the mucosa (MU) and the goblet cells (GC). H.E., X 200.

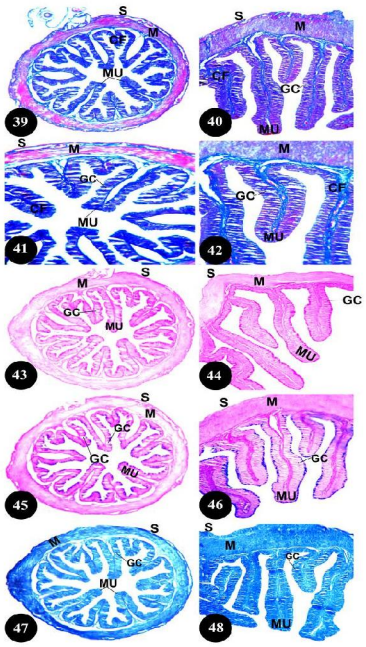


Fig. 39: T.S. of the intestine of *Scincus scincus* showing the collagen fibres (CF) scarcely distributed in the muscularis (M). Masson's trichrome stain., X 100.

Fig. 40: T.S. of the intestine of *Natrix tessellata* disclosing the collagen fibres (CF) in the muscularis (M). Masson's trichrome stain., X 100.

Fig. 41: T.S. of the intestine of *Scincus scincus* showing the condensation of collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.

Fig. 42: T.S. of the intestine of *Natrix tessellata* displaying the condensation of collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.

Fig. 43: T.S. of the intestine of *Scincus scincus* disclosing moderate polysaccharide content in the muscularis (M) and the mucosa (MU). The goblet cells (GC) appear unstained. PAS., X 100.

Fig. 44: T.S. of the intestine of *Natrix tessellata* displaying high content of polysaccharide in the mucosa (MU). The muscularis (M) is moderately unstained. PAS., X 100.

Fig. 45: T.S. of the intestine of *Scincus scincus* showing moderate PAS-Alcian blue positive materials in magenta colour (neutral and acidic mucins) in the mucosa (MU) and the muscularis (M). The goblet cells (GC) are strongly stained with a mixture of Alcian blue (acid mucins) and PAS (neutral mucins). PAS-Alcian blue., X 100.

Fig. 46: T.S. of the intestine of *Natrix tessellata* showing mixed neutral (red) and acidic (blue) polysaccharides (magenta) in the mucosa (MU). Acid mucins predominate in the goblet cells (GC). PAS-Alcian blue., X 100.

Fig. 47: T.S. of the intestine of *Scincus scincus* showing high content of protein in the muscularis (M) and the mucosa (MU). The goblet cells (GC) are devoid of protein. Bromophenol blue., X 100.

Fig. 48: T.S. of the intestine of *Natrix tessellata* disclosing high protein content in the muscularis (M) and the mucosa (MU). The goblet cells (GC) are negatively stained for protein. Bromophenol blue., X 100.

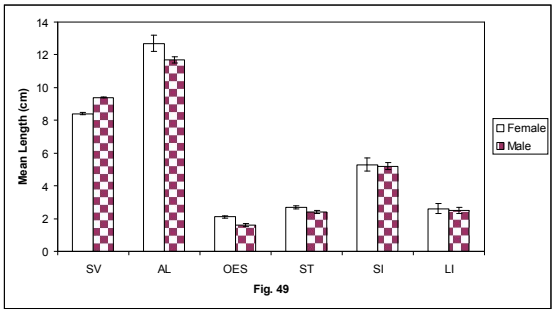


Fig. 49: Histogram illustrating the snout-vent, mean length of the alimentary tract and its organs in both sexes of *Scincus scincus*.

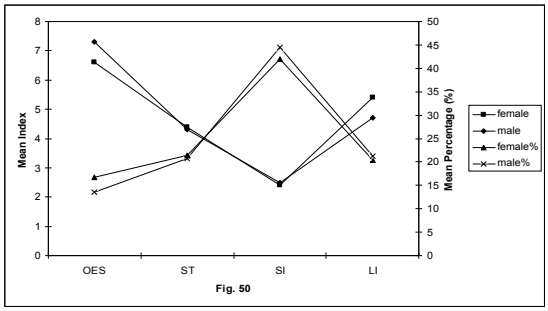


Fig. 50: Indices and percentage ratios of organ length relative to the length of the whole alimentary tract in both sexes of *Scincus scincus*. Note the tetragonal areas in between the two curves.

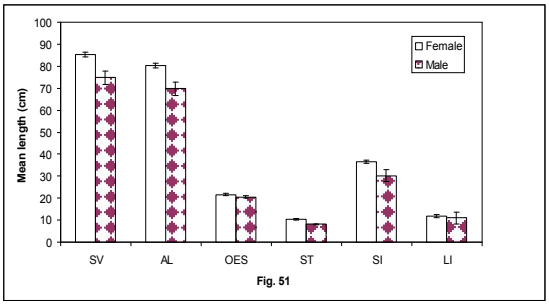


Fig. 51: Histogram representing the mean length of the snout-vent, alimentary tract and its organs in both sexes of *Natrix tessellata*.

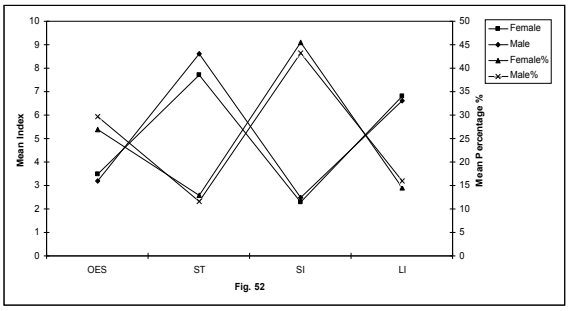


Fig. 52: Indices and percentage ratios of organ lengths in relation to the total tract length in both sexes of *Natrix tessellata*. Note the two, more or less, similar tetragonal areas in between the curves.

4. Discussion

Anatomically, in the two examined species, there is a relatively long and narrow oesophagus. It becomes gradually wider towards the stomach. The stomach is formed of an elongated straight sac. Stated previously, the insectivorous species *Scincus scincus* has no delimitation between the oesophagus and the stomach. This is a relevant adaptation to permit the passing of insect prey without any obstruction, similar to that found in *Scincus officinalis* **(El- Toubi, 1936),** *Mabuya brevicollis* **(Zaher *et al*., 1989 b)** and *Scincus scincus* **(Biomy, 2010)** displayed also the absence of such a constriction. The stomach is highly elongated and extremely narrow in the present lizard. The differentiation of the intestine of *Scincus scincus* is closely similar to that described in other members of scincidae **(El-Toubi, 1936; Zaher *et al*., 1989 b and Biomy, 2010,).** Also, the present finding indicates the absence of the oesophageal stomach demarcation in *Natrix tessellata*. This is also observed in other snakes, *Coluber florulentus* **(Dehlawi and Zaher, 1989 and Abdeen *et al*., 1992).** The disappearance of such anatomical limit in snakes is believed to be the major reason for facilitating the passage of prey which is swallowed occasionly from the oesophagus to stomach. As observed in several lizards and snakes, the stomach of both studied species displays a large left convex curvature and a small right curvature. Therefore, this gastric appearance is most likely a characteristic feature of order squamata. A similar observation was recorded in lacertilian and ophidian species **(Bishai, 1959; Zaher *et al*., 1990 a & b and Afifi *et al*., 1990, Kozaric *et al*. 2011).**

The morphometric analysis:

The statistical analysis of the morphometric variabilities has got a special attention in the last few years. Good results were obtained for evaluation of sexual dimorphism in reptiles **(Kminiak and Kaluz, 1983)** and for determination of some indices **(Estrada and Rodriguez, 1984).** Moreover, extensive studies were carried out by icthyologists using regression analysis and morphometric indices in the field of fish taxonomy **(Botros *et al*., 1970; Lachner and Jenkins, 1971; Ezzat *et al*., 1979; Mahmoud, 1980, Kozaric *et al*. 2011).**

As demonstrated morphometrically, there is no statistical significant difference in between the gastrointestinal organs of the males and females of the two studied species. However, in *Natrix tessellata*, the small intestine of females was found to be longer than that of males.

The present morphometric demonstration divulged that no differences were observed between males and females of *S. scincus* in all studied measurements. Except for the stomach of *M. brevicollis* which is longer in males than females all other morphometric aspects have no sex differences **(Zaher *et al*., 1989 b).** This may suggest that the sex relationships which inferred from the present data are not correlated with sex dimorphism in family scincidae. It is not surprising that the small intestine is the longest gut organ in *S. scincus*. This elongation is most probably to compensate the limited number of intestinal villi observed microscopically and consequently to increase the surface of absorption. In contrast, which in the present *Scincus scincus*, the oesophagus appears as the shortest organ. Since the mucosal folds of the oesophagus are highly convoluted, the shortening of this organ does not interrupt the oesophageal function. The present morphometric data disclose that in *S. scincus* the snout- vent represents 70 % of the alimentary tract length. This means that, when the snout-vent as an external element of an animal is measured, the length of the GIT and its organs, as an internal system can be estimated without the need for animal sacrifice. Furthermore, the illustrations of both index and percentage ratio of all tract regions of an animal always gives rise to a specific geometrical outline. This specific illustration may support the predicted species identification. In the present species (*S. scincus*), the resulted geometrical outline is a tetragonal area which is dissimilar to the hexagonal one obtained in the skink lizard *M. brevicollis* **(Zaher *et al*., 1989 b).** The present tetragonal outline is similar to that described in the lizards (*Chalcides sepoides*; **Zaher *et al*., 1990 b & c** and *Acanthodactylus boskianus*; Abo- Taira *et al*., 1988) and in the snake (*Echis carinatus*; **Afifi *et al*., 1990).**

The morphometric analysis of *N. tessellata* revealed that the female GIT is more elongated than that of the male. In addition, the length of each alimentary canal organ except the large intestine is significantly higher in females than in males. Similar observations have been reported in other reptilian species **(Przystalski, 1980 and Abo- Taira *et al*., 1988).** However, it seems likely that the correlation between the female sex and the length of GIT is a remarkable character of snakes. This sex- dependency is perhaps connected with the production of eggs in females **(Przystalski, 1980).**

In the present species, both the stomach and oesophagus are shortest and longest GIT organs, respectively. Similar results were obtained by **Zaher *et al*. (1991a)** in *Echis carinatus*.

By considering the index and percentage of all GIT regions of *N. tessellata* none of the organs exhibits any significant difference between both sexes except for the index of the female large intestine which is slightly higher than that in males. Therefore,

both parameters (index and percentage) cannot be accepted as a base for differentiating the sex. In addition, these values are different from those reported in *Chalcides sepoides* **(Zaher *et al*., 1990 b & c)**. However, such measurements are most probably related to the zone where the animal lives and where the nature and amount of food as well as ecological conditions differ from one zone to another.

The combined representation of both index and percentage values in *N. tessellata* discloses a double geometrical design. Each of the resulting two areas is a tetragon and most likely a specific rule for snakes, since the geometrical description reported previously was a single hexagon in *Mabuya brevicollis* **(Zaher *et al*., 1989 b)** and also a single tetragon in *Stenodactylus slevini* **(Zaher *et al*., 1989 a).**

Histologically, the oesophageal mucosa of *Scincus scincus* is formed of simple columnar epithelium and consists mostly of oesophageal glands, while the oesophageal mucosa of *Natrix tessellata* is simple and formed of two types of cells, the elongated thin ciliated epithelial cells and the goblet cells. Such a condition was also recorded in other reptilian species such as *Chalcides levitoni* and *Mauremyes caspica* **(El- Taib *et al*., 1982 and El-Taib and Jarrar, 1983, Kozaric *et al*. 2011)**. The oesophageal mucosa of *Scincus scincus*, is nearly similar to that of *Uromastyx aegyptius* **(Abo-Eleneen, 2010)**. In *Natrix tessellata*, such a structure is greatly similar to that described in *Varanus niloticus* **(Abo- Eleneen, 2010)** and in *Acanthodactylus boskianus* **(Dehlawi and Zaher, 1985a)**. The presence of the oesophageal glands in *Scincus scincus* is nearly similar to that of several reptiles such as *C. vulgaris* **(Bishai, 1960)** and turtles **(Gabe, 1973).** The oesophageal glands are mucous in nature and secrete mucin to help the oesophagus to convey food from the buccal cavity to the stomach.

On the other hand, in *Natrix tessellata*, the oesophageal glands are completely absent. This observation was also recorded in *C. levitoni* **(El- Taib *et al*., 1982)**, *T. vermicularis* **(Heyder, 1974)** and *A. boskianus* and *P. rupestris* **(Dehlawy and Zaher, 1985a & b).** The presence of the goblet cells in the oesophageal mucosa of *Natrix tessellata* as well as of other reptiles, such as *Ablephorous pannonicus* **(Greschik, 1917),** *Chamaeleon vulgaris* **(Bishai, 1960)** and *U. philbyi* **(Farag, 1982)**, agrees with the basic task of the oesophagus which conveys food from the buccal cavity to the stomach. This function is facilitated by the mucous secretion of the goblet cells, which causes the inner surface of the oesophageal lumen to be viscous and thus facilitates the swallowing of the prey and its gliding through the oesophageal lumen to reach the stomach.

The collagenous fibres of *Scincus scincus* are scarcely distributed through the mucosa while, the collagenous fibres of *Natrix tessellata* are widely distributed through the mucosa. This collagenous fibre gives the oesophagus its strength.

Histochemically, the present study revealed the presence of exaggerated amounts of carbohydrates in the mucosal epithelium of both species. This finding is in agreement with that observed by **Abo- Eleneen (2010)** in *Uromastyx aegyptius* and *Varanus niloticus*. The oesophageal mucosa of *Scincus scincus* and *Natrix tessellata* showed accumulation of PAS and Alcian blue positive materials indicating the presence of mixed neutral and acidic mucins which help in producing mucous necessary for lubricating food. In *S. scincus* and *N. tessellata*, the oesophagus is richly supplied with protein particularly in the mucosa and muscularis. This indicates the importance of protein materials in constituting the contracting muscles. However, low protein content is found in the oesophageal glands of *S. scincus* showing that these glands are more responsible for the production of mucus which is mostly acidic in nature.

The stomch mucosa of *Scincus scincus* and *Natrix tessellata* is consisted of gastric glands and gastric pits. In *S. scincus* (insectivorous), the gastric glands are represented with the oxyntic type while, these glands are represented with peptic cells in *Natrix tessellata* (carnivorous). A review to the published works of the history of the alimentary tract of reptiles shows a considerable controversy on the nature of the granular cells forming the glandular bodies of the gastric glands of the reptilian stomach**. Smit (1962), Gabe and Saint Girons (1964) and Skoczylas (1970)** reported that these cells play a role in the secretion of pepsin and hydrochloric acid. They termed them oxyntico-peptic cells. However, in *U. aegyptia*, which is purely herbivorous **(El- Tobui and Bishai, 1958),** termed these cells oxyntic cells. On the other hand, **Bishai (1959)** in *Varanus griseus*, which is purely carnivorous, termed these cells peptic cells. The same author identified such in the insectivorous *C. vulgaris* as oxyntic cells. In both species studied, the collagenous fibres are scarcely distributed through the serosa and the mucosa.

Histochemically, the gastric mucosa is heavily loaded with mixed neutral and acidic mucins in *Natrix tessellata* while, the gastric mucosa is weak reaction with PAS- Alcian blue. The positive response of the gastric secretory portion cells of PAS-Alcian blue stain denotes the mucous nature of their secretions that facilitate the passage of digested food from the stomach to the intestine. Meanwhile, the presences of polysaccharides which play an important role in the production of energy are greatly represented in the mucosa. Moreover, the gastric

mucosa of both species shows heavy protein contents. The same results were recorded in *Uromastyx aegyptius* and *Varanus niloticus* **(Abo- Eleneen, 2010, Zaher *et al*., 1991c).**

The absence of the intestinal glands observed in the present work was similar to that found in *Mabuya quinquetaentiata* **(Amer and Ismail, 1975),** *Acanthodactylus boskianus* **(Dehlawi and Zaher, 1985 a)**, in *Typhlops vermicularis* and *Echis carinatus* **(Heyder, 1974 and Afifi *et al*., 1990)** respectively and in *Uromastyx aegyptius* and *Varanus niloticus* **(Abo- Eleneen, 2010).**

As outlined microscopically, the intestinal mucosa of *Scincus scincus* and *Natrix tessellata* lacks granular crypts. This outcome is closely similar to that recorded in both lacertilian and ophidian species **(Greschik, 1917).** Moreover, the response of the mucous membrane cells and goblet cells in the intestinal wall indicates the abundance of mucous secretion. This may be considered as an adaptation for the intestine to perform well its role in water absorption **(Bishai, 1960).** The collagenous fibres are greatly distributed in the mucosa of both species studied. These fibres give the intestinal mucosa its strength and compactness which are associated with the mechanical properties.

Histochemically, the intestinal goblet cells of the two species studied are strongly stained with a mixture of neutral and acidic mucins. The secretion of these cells is probably responsible for mucus secretion. However, acidic mucins are predominated in the goblet cells of *Natrix tessellata*. Meanwhile, the goblet cells of both species are weakly loaded with proteins, whereas high content of protein is found in the mucosa. Similar observations were reported in reptiles **(Abo- Eleneen, 2010)** and in mammals **(Abdeen *et al*., 2008).**

In conclusion, it is obvious that the anatomy and morphometry as well as the histology of the alimentary tract in *S. scincus* and *N. tessellata* demonstrate certain specific characteristics of functional adaptation.

Corresponding author
Ahlam M. El- Bakry
Department of Zoology- Faculty of Science- Beni-Suef University
amalbakry2@yahoo.com

References

1. Abdeen, A.M., Zaher, M.M. and Abdel- Rahman, A.A. (1992): Comparative histochemical studies on the gut mucosa of the colubrid snakes, *Malpolon monspessulanus*, *Coluber florulentus* and *Tarbophis obtusus*.I. Esophagus and stomach. J. Egypt. Ger. Soc. Zool., 7: 75- 86.
2. Abdeen, A.M.; Mohammed, M.A. and Moustafa, N.A. (2008): Histological and histochemical study of the alimentary canal of the insectivore hedgehog *Hemiechinus auritus* and the carnivore weasel *Mustela nivalis*. J. Egypt. Ger. Soc. Zool., 55c: 161- 205.
3. Abo- Eleneen, R.E. (2010): Comparative histological and histochemical studies on the mucosa of the digestive tract of the herbivore *Uromasatyx aegyptius* and the carnivore *Varanus niloticus*. J. Egypt. Ger. Soc. Zool., 60 B: 1- 35.
4. Abo- Taira, A.M.; Mansour, A.B.; Amer, M.A. and Zaher, M.M. (1988): Anatomical, morphological and histological studies on the alimentary tract of the lacertid lizard *Acanthodactylus boskianus* (Family: Lacertidae). Proc. Egypt. Acad. Sci., 38: 87- 101.
5. Abo- Taira, A.M.; Afifi, A.M.F.; Zaher.; M.M.; Badr El- Din, N.K. and Moharram, N.Z. (1989): Gatrointestinal tract of snakes: II. Histochemical phenotype of mucosal coat in *Cerastes cerastes* (Viperidae). Zool. Soc. Egypt. Bull., 39: 87- 101.
6. Abo- Taira, A.M.; Zaher, M.M. and Afifi, A.M.F. (1990): Anatomical manifestation of the alimentary tract of the gecko *Tarentola annularis* (Reptilia: geckonidae). Proc. Zool. Soc. A.R. Egypt, 17: 361- 388.
7. Afifi, A.M.F.; Abo- Taira, A.M.; Zaher.; M.M.; Badr El- Din, N.K. and Moharram, N.Z. (1989): Further aspects of lacertilian alimentary tract: I. Histochemical approach of the mucosal membrane in *Mabuya brevicollis* (Family: Scincidae). Zool. Soc. Egypt. Bull. 11 (2): 221- 232.
8. Afifi, A.M.F.; Zaher, M.M.; Abo-Taira, A.M.; Abdeen, A.M. and Badr El- Din, N.K. (1990): Gastrointestinal tract of snakes: some contributions to gross anatomy, morphometry and optic microscopy of the alimentary tract of *Echis carinatus* (Viperidae). Bull. Zool. Soc. Egypt., 11 (2): 221- 232.
9. Al- Nassar, N.A. (1976): Anatomical studies osteology and gut histology of the amphisbaenian *Diplometopon zarudnyi* inhibiting Kuwait. M.Sc. Thesis, Kuwait Univ.
10. Amer, F. and Ismail, M.H. (1975): The microscopic structure of the digestive tract of the lizard *Mabuya quinquetaeniata*, Bull. Fac. Sci., Ain Shams Univ. 18: 25- 40.
11. Biomy, A.A. (2010): Ultrastructural and histochemical characterization of the alimentary tract of the insectivorous *Scincus scincus* (Scincidae). J. Environ. Sci. Mans. Univ., 39 (4): 525- 545.
12. Bishai, H.M. (1959): The anatomy and histology of the alimentary tract of the lizard *Varanus griseus*, Daud., Bull. Fac. Sci., Cairo Univ., 35: 53- 73.

13. Bishai, H.M. (1960): The anatomy and histology of the alimentary tract of *Chamaeleon vulgaris* Daud. Bull. Fac. Sci. Cairo Univ., 36: 44- 61.
14. Chou, L.M. (1977): Anatomy, histology and histochemistry of the alimentary canal of *Gehyra mutilata* (Reptilia, Lacertilia, Gekkonidae). J. Herpetol., 11 (3): 349- 357.
15. Dehlawi, G.Y. and Zaher, M.M. (1985a): Histological studies on the mucosal epithelium of the alimentary canal of the lizard *Acanthodactylus boskianus* (Family: Lacertidae). Proc. Zool. Soc. A. R. Egypt. 9: 67- 90.
16. Dehlawi, G.Y. and Zaher, M.M. (1985b): Histological studies on the mucosal epithelium of the alimentary canal of the gecko *Pristurus rupestris* (Family: Geckonidae). Proc. Zool. Soc. A. R. Egypt. 9: 91- 112.
17. Dehlawi, G.Y. and Zaher, M.M. (1989): Histological studies on the alimentary tract of the colubrid snake *Coluber florulentus* (Family: Colubridae). J. K. A. U. Sci., (1): 95- 112.
18. Drury, R.A.; Wallington, E.A. and Cancerson, R. (1976): In: Carlton's Histopathological Techniques. 4th Edn. Oxford University press, Oxford, London, New York.
19. EL- Taib, N.T. and Jarrar, B.; El- Ghandour, M.H. (1982): Morphology and histology of the alimentary tract of *Chalcides levidtoni* (Reptilia: Scincidae). Bangladesh J. Zool., 10 (1): 1- 14.
20. EL- Taib, N.T. and Jarrar, B. (1983): Morphology and histology of the alimentary canal of *Mauremys caspica* (Reptilia: Emydidae), Ind. J. Zool., 11: 1- 12.
21. El- Toubi, M.R. (1936): Macroscopic and microscopic anatomy of *Scincus officinalis*. M.Sc. Thesis, Fac. Sci., Cairo Univ.
22. El- Toubi, M.R. and Bishai, H.M. (1958): The anatomy and histology of the alimentary tract of the lizard *Uromastyx aegyptia*. Forskal, Bull. Fac. Sci. 34: 13- 25.
23. Estrada, A.R. and Rodriguez, A.S. (1984): Analisis de la ecomorfologia de 23 species de lagartos Cubanos del genero Anolis. Ciencias Biologicas, Acad. Cienc. Cuba, 12: 91- 104.
24. Ezzat, A.A.; Hashem, M.T.; El- Garabawy, M.M. (1979): Biometric variations in *Solea vulgaris* acclimatized in Lake Qarun, Upper Egypt. J. Fish. Biol., 14 (1): 39- 46.
25. Farag, A.A. (1982): Histological studies on the mucosal epithelium of the alimentary tract of the Agamid lizard *Uromastyx philibyi* Parker. Ann. Zool., XIX (1): 1- 23.
26. Gabe, M. (1973): Contribution a I'histologie des cellules endocrines duodenales des sausopsides. Acta. Anat., 85: 434- 449.
27. Gabe, M. and Saint Girons, H. (1964): Contribution a I'histologie de *Sphenodon punctatus* Gray., Centre National de la Recherch Scientifique, Pairs.
28. Greschik, E. (1917): Über den dannkanal von Ablephrus pannonicus Fritz., und Anguis fragilis L. Anat. Anz., 50: 70- 80.
29. Heyder, G. (1974): Das Verdaungs System von *Typhlops vermicularis* Merrem 1820. Morph. Jb. 120: 185- 197.
30. Kminiak, M. and Kaluz, S. (1983): Evaluation of Sexual dimorphism in snakes (Ophidia, Squamata) based on external morphological characters. Folia Zool., 32 (3): 259- 270.
31. Kozaric,*Z.,* Petrinec,*Z.,* Kuzir, *S.,* Gjurc˘evic,*E. and* Baz˘daric, B., (2011): Histochemical Analyses of Digestive Enzymes in the Intestine of Adult Large-Scaled Gurnard (Lepidotrigla cavillone, Lacepe` de, 1801). Anat. Histol. Embryol. 40 (2011) 314–320
32. Lachner, E.A.; Jenkins, R.E. (1971): Systematics, distribution and evolution of the *Nocomis biguttatus* species group (family: Cyprinidae, Pisces) with a description of a new species from the Ozark Upland. Smitheonian Contributions to Zoology, 91: 1- 28.
33. Mahmoud, M.M. (1975): Distribution of carbohydrate and proteins in the mucosa of the ileum and rectum of *Mabuya quinquetaeniata* and *Chalcides ocellatus*. Egypt. J. Histol., 6: 27- 42.
34. Mahmoud, M.M. (1980): Taxonomic studies of the Nile fishes, family Cyprinidae. M. Sc. Thesis, Assiut University, Egypt.
35. Mallory, F.B. (1944): Pathological Technique. Philadephia. W.B. Saunders. UK.
36. Mazia, D.; Brewer, P.A. and Alfert, M. (1953): The cytochemical staining and measurement of protein and mercuric bromophenol blue. Biol. Bull., 104: 57- 67.
37. McManus, J.F.A. (1946): The histological demonstration of mucin after periodic acid. Nature, 158: 202.
38. Mowry, R.W. (1956): Alcian blue technique for histochemical study of acidic carbohydrate. J. Histochem. Cytochem. 4: 407- 412.
39. Przystalski, A. (1980): The dimension of the mucosa and the structure of the alimentary canal in some reptiles, Acta Biol. Cracov, Series Zoology, Vol. XXIII. 325- 341.
40. Skoczylas, R. (1970): Salivary and gastric juice secretion in the grass snake *Natrix natrix*. Comp. Biochem. Physiol., 53: 885- 903.
41. Smit, H. (1962): Gsatric secretion in the lower vertebrates and birds. In "Handbook of physiology". Alimentary canal, Vol. 5, pp. 2791- 2805. American Physiological Society, Washington, D.C.
42. Van der Waerden, B.L. and Nieverglt, E. (1956): Tafeln zum vergleich Zweier stichproben Mitteles X Test und zeichentest. Springer verlag, Berlin, Goettingen und Heidelberg. Germany.
43. Zaher, M.M.; Jamaal El- Lail, S. and Dehlawi, G.Y. (1987a): Anatomical and histological studies on the alimentary tract of the lacertid lizard

Acanthodactylus ophedureus (Family: Lacertidae). Egypt. J. Histol., 10 (2): 207- 221.
44. Zaher.; M.M.; Dehlawy, G.Y.; Amer, M.A. and Abo- Taira, A.M. (1987b): Histochemical studies of lipids, proteins and nucleic acids in the mucosal epithelium of the alimentary of the gecko *Pristurus rupestris*. Egypt. J. Histol., 10 (2): 323- 329.
45. Zaher.; M.M.; Amer, M.A.; Dehlawy, G.Y. and Abo- Taira, A.M. (1987c): Histochemical localization of lipids, proteins and nucleic acids in the alimentary canal mucosa of the lizard *Acanthodactylus boskianus*. Egypt. J. Histol., 10 (2): 309- 315.
46. Zaher.; M.M.; Abo-Taira, A.M.; Afifi, A.M.F. and Dehlawy, G.Y. (1989a): Apparent merits of anatomy, morphometry and histology of the alimentary tract in the insectivorous gecko *Stenodactylus slevini* (Family: Geckonidae). Proc. Zool. Soc. A.R. Egypt., 17: 317- 338.
47. Zaher, M.M.; Abo- Taira, A.M.; Afifi, A.M.F.; Dehlawi, G.Y. (1989b): High lights of anatomy, morphometry and histology of the gastrointestinal tract of the insectivorous skink *Mabuya brevicollis* (Family: Scincidae). Zool. Soc. A. R. Egypt., 17: 339 - 360.
48. Zaher, M.M.; Abo-Taira, A.M. and Abdeen, A.M. (1990a): A morphological study on the alimentary tract of *Mabuya quinquetaeniata*. Egypt. J. Anat., 13 (2): 27- 42.
49. Zaher, M.M.; Abo- Taira, A.M.; Afifi, A.M.; Abdeen, A.M. and Badr El- Din, N.K. (1990b): Morphological characterization of the alimentary canal of *Chalcides sepoides* (Scincidae): Some anatomical, morphometrical and histological aspects. Egypt. J. Anat., 13 (2): 43 - 57.
50. Zaher, M.M.; Abdeen, A.M.; Afifi, A.M.F.; Abo-Taira, A.M. and Kenawy, M.A. (1990c): Histochemical appearance of gastrointestinal mucosa in Scincidae: II. Localization of carbohydrates, proteins, nucleic acids and lipids in *Chalcides sepoides* (Lacertilia: Scincidae). Proc. Zool. Soc. A.R. Egypt., 18: 135- 145.
51. Zaher, M.M.; Abo- Taira, A.M.; Abdeen, A.M.; Badr El- Din, N.K. and Afifi, A.M. (1991a): Gastrointestinal tract of snakes: Contributions to gross anatomy, morphometry and microscopic structure of the alimentary tract in *Echis carinatus* (Viperidae). J. Egypt. Ger. Soc. Zool., 5: 469- 488.
52. Zaher, M.M.; Badr EL- Din, N.K.; Abdeen, A.M. and Bassiouni, W.M. (1991b): Comparative histochemical studies on the gut mucosa of the carnivorous *Varanus griseus* and the insectivorous *Chameleon vulgaris*. II. Small and large intestines. J. Egypt. Ger. Soc. Zool., 6: 75- 86.
53. Zaher, M.M.; Bassiouni, W.M.; Badr EL- Din, N.K. and Abdeen, A.M. (1991c): Comparative histochemical studies on the gut mucosa of the carnivorous *Varanus griseus* and the insectivorous *Chameleon vulgaris*. I. Oesophagus and stomach. J. Egypt. Ger. Soc. Zool., 6: 211- 227.

9/28/2012

A Review of different Approaches of Land Cover Mapping

Gul Afzal Khan[1], Sher Afzal Khan[2], Nazir Ahmad Zafar[3], Saeed Islam[4] and Farooq Ahmad[5]

[1]College of Aeronautical Engineering, National University of Sciences and Technology, Islamabad, Pakistan
[2]Department of Computer sciences, Abdul Wali Khan University, Mardan, Pakistan
[3]Department of Computer Science, King Faisal University, Hofuf, Saudi Arabia
[4]Department of Mathematics, Abdul Wali Khan University, Mardan, Pakistan
[5]Department of Information Technology, University of Central Punjab, Lahore, Pakistan
gafzal@cae.nust.edu.pk

Abstract: In this study, a survey of land cover mapping and their classification techniques is done. Land cover mapping plays a very important role in making land policy, land management and land analysis. In this survey different approaches are studied that were applied for land cover mapping such as an Artificial Neural Network (ANNs), Fuzzy Logic, Supervised, Unsupervised and Maximum Likelihood. The objective of this research is to analyze, evaluate and compare different algorithms for the classification of land cover and also evaluate and compare the methods to overcome the problems which are faced during classifications
[Khan GA, Khan SA, Zafar NA and Islam S. **A Review of different Approaches of Land Cover Mapping.** *Life Sci J* 2012;9(4):1023-1032] (ISSN:1097-8135). http://www.lifesciencesite.com. 156

.

Keywords: Artificial Neural Network (ANN), Fuzzy Logic and Maximum Likelihood.

1. Introduction

The land cover mapping is very valuable for planning, resources management, identification of environmental changes, identifying deforestation and forecasting. Many organizations require accurate land cover and land use information for a variety of applications. Several researchers point out the importance of land cover information such as [3] presents that the land cover information is required for different purposes e.g. Scientific research (e.g. Climate change modeling, flood prediction) and management (e.g. City planning, disaster mitigation). The approach of remote sensing is the biggest source of acquiring information on land cover and land use [15]. It is too hard to classify the remote sensing data manually [14]. Therefore computer aided techniques are used to extract information from remotely sensed data by means of classification. The land cover and land use classification of satellite images are vital activities for extracting geospatial data for military and civil purposes like crop disease monitoring, flood disaster analysis and unreachable areas etc. Therefore [14] proposed the soft computing techniques used for image classification because these techniques are based on uncertainty e.g. ANN, fuzzy set theory and rough set theory. Classification of land cover and land use are important to the modeling of global changes and management of ecosystem [13]. According to the importance of classify land cover and land use which is related to modeling of global change and the management of other natural resources for this purpose there are some local and international organizations which are working for the monitoring and prediction of land cover and land use changes and promoting awareness among the people about the management of land resources, one of these is ICIMOD (International Centre for Integrated Mountain Development) ICIMOD is working for development of HKH (Hindu Kush-Himalayas) and monitoring the changes in mountain regions. The objective of this research is to analyze and evaluate and compare different algorithms for the classification of land cover and also evaluate and compare methods to overcome the problems which are faced during classifications. Constructing a mathematical modeling approach in which spatial operations of multi-dimensional spaces are integrated through incorporation of formal methods in [26]. The formal methods are the languages based on predicate logic, set theory and functions use for specification and verification of software and hardware systems [25-32].

The organization of the paper is as follows. We first discuss the background of the paper in section 2, examine the different approaches for the land cover and land use classification. Section 3, Discus the problems with remote sensing image classification and the approaches which are used to solve mix pixel problems and summarize our findings in Section 4.

2 Background

This research survey is about the land cover map classification. The different algorithms of land cover classification such as artificial neural network, fuzzy logic, maximum likelihood and linear mixing have been proposed. Land cover map can be generated by different ways but remote sensing has the strong ability to generate the land cover map. There are

many classification techniques exist such as the statistical algorithms and soft computing techniques. In statistical algorithm included discriminate analysis and the maximum likelihood classification techniques. The maximum likelihood, which assign each image pixel to the land cover class based on highest probability of membership [21]. The soft classification techniques such as an Artificial Neural Network (ANNs) and Fuzzy Logic are used for classification. ANN may be more robust when distribution is powerfully non-Gaussian because Neural-network classifiers are nonparametric. The ANNs forming arbitrary decision boundaries in the feature space during training. The authors proposed ANNs and GIS for predicting the changes of Land use [1]. GIS can be defined as a powerful set of tools used to capture, store, and process, analyze and interpret Spatial data. GIS used in paper [1] to create a GIS layer which includes Digital Elevation Model (DEM) and resampling resolution to 90 m. The DEM is a digital representation of land surface geography. The land images acquired by different satellite in [11] authors used MODIS-MYD3Q1 satellite images for classification. The Moderate Resolution Imaging Spectrora diameter (MODIS) launched in December 1999. MODIS provides continuous global coverage every one to two days. ANN based five models was presented to classify the MODIS image. The models used NDVI (Normalized Difference Vegetation index), EVI (Enhanced Vegetation Index), red (RED) and NIR reflectance as input. The NDVI is a simple graphical sign that used to analyze remote sensing measurements. The EVI was developed to improve the sensitivity of vegetation signal in high biomass areas and enhanced vegetation monitoring. New Supervised learning of ANNs [12] is proposed: the data were acquired by two different ways one was used optical detector and a second was used microwave radar. The optical detector is a device that measures a physical quantity of the light and the becomes a signal which can be read by an observer or by an instrument using the radar to microwave high frequency radiation. Its accuracy and ability to penetrate the clouds give it great navigation and to the achievement of images used. ANNs approach to classify land use land cover using ancillary data [10]. The ancillary data is the data from other sources than remote sensing that can be used to aid in the classification. Three approaches were presented [21] which used the data of IKNOS multispectral Satellite. The IKONOS satellite is publicly available with high resolution imagery up to 1 to 4 meter resolution.

The common problem with remote sensing is the mixed pixel. The mixed pixel represents the area which occupies more than one feature of land cover. To solve the problems of mixed pixels in paper [18] authors proposed two methods maximum likelihood and Fuzzy Classifier the data were acquired from global land cover network. The classifications were performing in forestry, urban planning and Swana woodland (grassland). Similarly in [19] [18] the authors present the methods to estimate the components of land cover features to solve the mix pixels problems. In [23] the authors present Maximum a Posteriori (MAP) model to provide the solutions to the mix pixel problem.

3 Approaches for land cover classification

Several ways [12] in which classifies the image of land cover and land use such as statistical techniques and soft computing. The statistical technique such as the maximum likelihood method was used for the classification of land cover and land use based on multi-band data which acquired by the satellite. These techniques need distribution assumption of observed data, but generally the observed data do not satisfy the assumption so the using of statistical techniques failed in classification. However, the ANN BP recently has been applied for image classification, BP method as learning technique which does not need the distribution assumptions.

In paper [14] presents the importance and advantages of ANNs for image classification, ANN is tool for pattern classification. ANN are may be more robust when distribution is powerfully non-Gaussian because ANN classifiers are nonparametric. ANNs is able of drawing arbitrary decision boundaries in the feature space, during training. The advantages of ANNs can thus be summarized as:

- Ability of noise resistance
- Tolerance to distorted patterns/image
- Greater ability to recognize overlapping pattern classes with high nonlinear boundaries or Partially or Degraded Image
- Potential for parallel processing
- Non parametric

In consequence of above advantages the ANNs method generally can acquire more high precision of the output and this approach widely used in land cover and land use classification.

a. ANNs and GIS applied to forecast land use changes [1].

In this paper author applied a spatial model for prediction of land cover changes. Combining ANNs and GIS, the model applied on the Lesvos NE Greece Island for the time period between 1975 and 1999 for predicting the pattern of growth of the island's urban areas and olive grove.

The Feed Forward ANNs has been developed with one hidden layer. In the first step the data that

were collected imported into GIS and make different GIS layers, referencing to cartographic projection. The data included the transportation network, coastline vector, geological and soil data and DEM, classification information on land cover and population data for the years 1975, 1990 and 1999. The Hellenic Geodetic Reference system used and resample resolution of 90 m. In the second step, the ANNs trained using an independent data and applied for the years 1975 to 1990. The model used the year 1975 data as input and the year 1990 data as output. The model is applied to forecast the changes in urban growth and pattern of olive cultivation in for the year 1999.

The accuracies of classification of urban and olive grove classes were 96% and 94% respectively for year 1975 and 96% and 93% for the year 1990. The back propagation algorithm used for training process the network trained after 500 iterations. The model was then applied to predict changes in urban land cover. The model produces two numbers within the range 0 and 1 the predicting urban growth class cells for the year 1999 as shown in Figure. 1. The cell's value greater than 0.5 is representing the likelihood greater than 50% which shows belonging to the urban class. Similarly the model applied for the year 1999 to predict the olive grave patterns as a result the value generates a very low (between 0 and 0.17).

Figure 1. Result of the model [1]

Thus the result shows that the model performs well in forecasting urban growth as compared to predict the changes in the pattern of olive cultivation.

b. ANNs models for land use classification from satellite images [11].

In this paper author proposed ANNs Models to classify land cover images. The satellite image of MODIS-MYD13Q1 and data of 85 plots in Cordoba, Argentina were using the data that comprised 13% of the plots covered bare soil, 63.5 % were grown with soybean and corn with 23.5%. Five different ANN model of multi-layer feed-forward perceptron were developed shown in Figure.2. The input used by the four models were NDVI (Normalized Difference Vegetation index), EVI (Enhanced Vegetation Index), red (RED) and near infrared (NIR), the numbers of input neurons used by the fifth model were RED and NIR reflectance. The model one to four built with 3 neurons in the input layer and 6 numbers of input neurons in the hidden layer. For all models the numbers of neurons in hidden layer were same. The results of all models were good for land use estimation; all models correctly classify the Soil, Corn and Bare Soil. The performance was quantified. All models evaluated the accuracy and Kappa statistic as shown in Table 1.

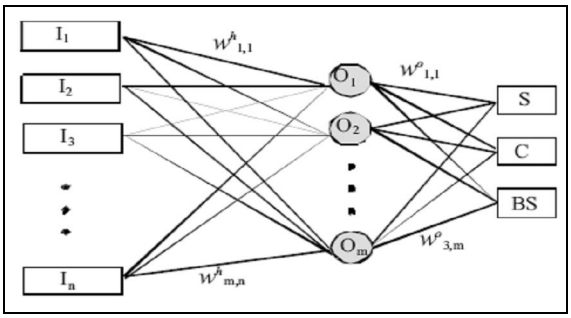


Figure 2. Scheme of an ANN of the multilayer perceptron type [11].

Ii. Input neurons,
Oj Hidden neurons wh
i, j Connection weight between Ii and Oj, wo
k, j weight between Oj and the output layer
Soybean(S), Corn(C) and Bare Soil(BS)
(For $1 \leq i \leq n$, $1 \leq j \leq m$).

c. New supervised learning of ANNs for satellite image classification [12].

A new technique proposed consists of three layered ANNs using the concept of domain of recognition in the input space. The multi band data were acquired by two different ways, one is by an optical sensor and second used microwave radar. The model has been used to classify the multiband data and assigned some categories. Recently the author proposed the same technique for the 7 band observation data which obtained by an optical sensor. In proposed work to improve the classification accuracy by using not only multiband data of an optical sensor but also used to process the data which obtained by microwave radar. For leaning designed two ANNs models, one model was learnt using seven bands TM data, and the other was learnt using eight band data by adding an Active microwave instrument (AMI) data to the 7 bands. However the result shows the classification of 8 band data was good and decrease unclassified pixel in the map as compared to those which construct using 7 bands data thus shows

that the classification ability of ANN depends on the choice of training data.

Table 1. The Accuracy and kappa statistic of all models with different combinations of input data [11].

Models	Overall Accuracy%	Accuracy Procedure/user (%)			Kappa statistics(%)
Model1	85.70	89.30/89.30	80.00/80.00	75.00/75.0	70.80
Model2	83.30	89.30/86.20	60.00/66.70	100/100	65.30
Model3	76.20	85.70/80.00	70.00/70.00	25.00/50.00	48.50
Model4	81.00	92..90/81.30	40.00/80.00	00/80.00	7.90
Model5	92.90	92.90/96.30	90.00/81.80	00/100	85.70

d. An ANNs approach to map land use/cover using Land sat imagery and ancillary data [10].

In this paper author discussed the errors with remote sensing image classification. The accuracy of land cover feature classification depends on the spectral signature and on the methodology of classification procedure. Different land cover class has a many similar spectral features and unique signature. This confusion of interclass introduced spectral classification errors. In [8] [9] Applied ancillary data to improve the spectral classification accuracy. The ancillary data cannot use directly because statistical assumption of the statistical algorithm e.g. maximum likelihood. Therefore in [2] proposed a model to provide a new approach to classify spectral and ancillary information using ANNs.

In the paper [10] the multilayer perceptron (MLP) applied for land cover mapping. In this research, the multilayer perceptron (MLP) was trained by back propagation (BP) algorithm. In the proposed work the data were acquired from the National Institute of Geography the data include a Landsat ETM+ image dated April 3, 2000, a digital model of elevation along with soil, land cover and land use, and digital maps of road network with a scale of 1:250,000. There are two classification models were designed. The first model trained the division of land cover and land use class for making spatial relationships between the land use, land cover map and the ancillary information. The first model creates a digital fuzzy map which shows the likelihood of the presence of each land cover and land use type through by each pixel. Similarly the second classifier used remotely sensed images and produced a fuzzy map using a spectral classification. Thus there were two fuzzy maps for each pixel which indicated a membership value. These maps were describing the likelihood of the occurrence of a land cover class from its spectral features, respectively. In [6] presents a model used AND operator to combine the two fuzzy map, the model calculates the minimum of two values of belonging. A final no fuzzy map has been generated and marks each pixel with the greater value of belonging. The MLP training process used the BP training algorithm. The data including the training set, the verification set, and the test set. The verification set shall apply to determine the best network and to stop the process of training in the case of over learning occurred. The test set was given the independent judgment of network performance when the network design process was completed.

In the proposed work the image was divided into 6 land cover and land use classes: 1) tropical forest, 2) mangroves, 3) wetlands, 4) agriculture (grasslands, pasturelands and croplands), 5) water and, 6) urban areas. There were nine inputs: six soils type, elevation distance to the road and distance to the coast to MLP for classification and 6 hidden nodes. The result of classification of the test set is 73% correctly classified. The spectral classification the MLP with 5 inputs bands 2, 3, 4, 5 and 7 and two hidden layers with 3 and 4 nodes respectively. The classification results show good performance 82% accurate classification of the test set. The first classification result obtained using spectral information, classifying each pixel into the class which have the higher membership value. Table 2 shows the errors for the classification of crop, water, Mangrove, Forest, Wetland and Urban areas

As a summarize step, the ancillary maps combined with spectral fuzzy maps. The accuracy of the resulting map was verified with same verification data. Overall accuracy improved to 79%. The accuracy of different land cover classes' improves significantly by using the ancillary. Both commission and omission errors reduce 25% but for the error of commission of the crop/pasture land and both commission and omission errors of urban area which remained the same as shown in Table 2.

e. Land Cover Classification of IKONOS Multispectral Satellite Data [21].

The paper presents the maximum likelihood classifier. In the maximum likelihood classification each image pixel is assigned to land cover class based on highest probability of membership. The IKNOS satellite data were used. It is imaged over the part of Daejeon city, Korea. The image consisted of 399 lines, with 550 cells per line, a cell's size of about 4x4m and one near-infrared and three visible bands. In this research channel 1, 2, 3 and 4 of the images were used. Nine classes of the land cover features were acquired in the training sites for getting training datasets.

Table 2. Omission and Commission Errors of Land Class Classifications [10]

Land use class	Spectral	Spectral	Spectral + Ancillary	Spectral + Ancillary
Map	Error of omission	Err of commission	Error of omission	Err of commission
Crop/pasture	0.53	0.29	0.20	0.31
Water	0.02	0.02	0.06	0.00
Mangrove	0.30	0.14	0.22	0.11
Tropical forest	0.31	0.24	0.22	0.22
Wetlands	0.23	0.52	0.23	0.23
Urban AREAS	0.43	0.50	0.43	0.43

The topographic map was used for calculation of classification accuracy.

The steps for land cover classification are as follows.

Step1. Identify the land cover classes within an image which is to be classified, for example water, grass, building, etc.

Step2. Select pixel which is the representation of the desired set of classes.

Step3. Estimate the parameters of the algorithm by using training data. These parameters will be used as properties of the probability model.

Step4. Label every pixel in the image into their corresponding land cover type.

Step5. Generate thematic maps.

The maximum likelihood classification was applied to the IKNOS image. The overall accuracy of maximum likelihood classifier is 76.20% and kappa coefficient is 0.73 as shows in Figure 8. The same data is classified by using neural network and the overall accuracy is 79.00% and the kappa coefficient is 0.76.

The same data are also classified by neuro fuzzy model. The overall accuracy of the model is 85.6 and the kappa coefficient is 0.83. The results of the three model shows that the performance of the neuro fuzzy model is better than neural network and maximum likelihood as shown in Table 3.

Table 3. Performance of Maximum Likelihood, Neural Network AND NEURO-fuzzy methods [22].

	Classification Performance	
Method	Overall Accuracy	Kappa Coefficient
Maximum Likelihood	76.2	0.73
Neural Network	79.0	0.76
Neuro-Fuzzy	85.6	0.83

f. Comparison of maximum likelihood, Neural Network and Neur-fuzzy classifier.

The approaches which are used for classification of land cover and land use are summarized in (Table 4). The comparison shows that the performance of all models of ANNs is good as compared to the statistical technique such as Maximum likelihood. There are some problems with statistical technique such as the integration of ancillary data and assumed normal distribution.

g. Fuzzy logic applied in the remote sensing image classification [16]

Fuzzy logic can be applied in remote sensing for the classification of images. The benefits of this method are that it does not need assumption about the statistical distribution of the data and provide more accurate results for image classification. This paper contains a hierarchical expert system for the classification of remote sensing. The model was tested for land cover classification. The data were obtained by Lansat 7 ETM+ over the Rio Rancho area. 8 spectral bands, DEM and NDVI used as input. If the normal type rules are used then the number of rules would be very large for classification, so requires a long time for classification. To overcome this problem the Hierarchical structure is presented. All the classes were grouped together and each group would be further divided into subgroups. It was important to generate appropriate fuzzy rules by using the fuzzy expert knowledge The main problem of using the fuzzy expert system is the chance to lose some information.

In the proposed work authors used an Adaptive-Neural-Network Based fuzzy Inference to generate fuzzy rules. The system is tested on the images which are taken by Land sat 7 ETM+. There are 9 different land cover classes: water, vegetation, urban irrigated vegetation, barren, calichebarren, Basque, shrub land, natural grassland and juniper. The urban area is blocked by street and mixed with vegetation which creates a problem in classification, similarly natural grassland; shrub land and juniper are highly mixed so its classification is difficult, the result of the proposed model compared with that of maximum likelihood classifier and ANN algorithm

The comparison shows that the result of the proposed work betters than the results of Maximum likelihood classifier and back propagation. The overall accuracy fuzzy system is 91.55% which is higher than maximum likelihood and back propagation algorithms.

Table 4. Comparison of different classification approaches.

Technique	ANN Supervised	ANNs Multilayer Perceptron	Maximum Likelihood	ANNs Back propagation	Nuero-Fuzzy
Approach	ANNs	ANNs	Probabilistic	ANNs	ANNs
Classification	Satellite Image	Satellite Image	Satellite Image	Satellite Image	Satellite Image
Data Source	Optical Sensor and microwave radar	Landsat ETM	IKNOS Satellite	IKNOS Satellite	IKNOS Satellite
Model	7 band and 8 band	M1=Ancillary Data, M2=Spectral Data, M3= Combination of M1 and M2	MLC	BP	Multi-layer Neuro-fuzzy
Accuracy	accuracy of 8 band data better than 7 band data	M1=73%, M2=82% M3=79%	MLC=76.2 Kappa=0.73	BP=79.2 Kappa =0.76	The overall accuracy is 85.2 the Kappa is 0.83

Table 5. Classification matrix for the study area by using MLC [16]

Actual class	Predicted classes									Accuracy
	wt	ui	ziv	br	cb	bq	sb	ng	jp	
WT	0	225	1	0	0	0	0	0	0	0
UI	0	863	0	22	16	0	50	0	8	89.99
IV	0	20	506	0	0	0	0	0	0	96.20
BR	0	53	0	786	384	0	10	31	85	58.27
CB	0	0	0	18	74	0	0	0	0	80.43
BQ	0	81	0	0	0	0	0	0	0	0
SB	0	17	0	0	0	0	296	36	29	78.31
NG	0	4	0	0	0	0	145	44	6	21.89
JP	0	12	0	0	0	0	64	2	89	50.86
	Average accuracy (%)=52.88 overall accuracy (%)=66.67									

Table 6. Classification matrix for the study area by using BP [16]

Actual class	Predicted classes									Accuracy
	wt	ui	iv	br	cb	bq	sb	ng	jp	
WT	223	0	1	0	0	2	0	0	0	98.67
UI	0	852	1	55	0	0	43	5	5	88.84
IV	0	1	522	2	0	1	0	0	0	99.24
BR	0	12	0	1310	0	0	3	3	21	97.11
CB	0	0	0	92	0	0	0	0	0	0
BQ	0	0	0	0	0	81	0	0	0	100
SB	0	19	0	2	0	0	327	25	5	86.51
NG	0	4	0	36	0	0	99	57	5	28.36
JP	0	8	0	45	0	0	47	12	63	36.00
	Average accuracy (%)=70.53 overall accuracy (%)=86.53									

Table 7. Classification matrix for the study area by using Fuzzy System [16]

Actual class	Predicted classes									Accuracy
	wt	ui	iv	br	cb	bq	sb	ng	jp	
WT	215	0	1	0	0	10	0	0	0	95.13
UI	3	933	0	17	0	0	23	1	3	97.29
IV	1	4	510	0	0	11	0	0	0	96.96
BR	4	20	0	1271	35	0	5	6	8	94.22
CB	0	0	0	10	82	0	0	0	0	89.13
BQ	0	0	0	0	0	81	0	0	0	100
SB	0	0	0	1	0	0	356	18	3	94.18
NG	0	0	0	27	0	0	92	68	14	33.83
JP	0	2	0	29	4	0	6	0	134	76.57
	Average accuracy (%)=86.37 overall accuracy (%)=91.55									

4. Methods to solve the mix pixel problems.

The mix pixel is the common problem of remote sensed images [12]. A mixed pixel represents the areas which occupy more than one variable. There are two situations in which the mixed pixel problem occurs. The first case is when the pixel attached with large area objects such as agriculture field. The second case is that when the object is relatively small compared to the spatial resolution of the scanner. Therefore, to accurately estimate the land cover sub pixel analysis is important there are different techniques are used to solve the mixed pixel problem supervised fuzzy c means classification, the spectral mixture model, the

nearest neighbor classifier and multilayer perceptron. In different papers presented different techniques to solve the mix pixels problem.

a. Maximum Likelihood and Fuzzy Classifier [18].

The spatial pattern of ground cover features information can obtained by Remote Sensing, but the imagery which is obtained by Remote Sensing have problems of class mixing within pixels. This paper presents the maximum likelihood and fuzzy classifier, which are used in urban planning, forestry, urban and savanna woodlands. The data for features extraction got from global land cover network. Eight detail land cover classes were mapped for the shire River catchment. First used maximum likelihood classifier this method involves the selection of training area which represent the eight land cover classes, Then signature of training area used to determine to which class were assigned to image pixels. Second use Fuzzy Convolution filter Classification. This method process completes in two steps the first step involves filtering in which create a single classification layer into a window of pixel and total weighted inverse distance of all the classes calculated then assigning the center pixel to the class which have largest total inverse distance. The pixel based classification approach maximum likelihood used in this paper provides results with 87% accuracy while fuzzy convolution provides 77% accurate results. This shows that the maximum likelihood classification performance is good when extracting land covers information from satellite imagery. The comparison of both classification methods is shown in Table 5.

The comparisons of maximum likelihood and fuzzy classifier are summarized in (Table 8). The pixel based classification method maximum likelihood was used in this paper for the classification of forestry, urban planning and savanna woodlands. The maximum likelihood provides results with 87% accuracy while fuzzy convolution provides 77%. This shows that the maximum likelihood analysis has the great ability of classification in the land cover with heterogeneous and higher resolution imagery.

The accuracy of maximum likelihood is better than fuzzy convolution filter. The maximum likelihood classifier is useful in discriminating environments, which is well suited for application such as hydrological modeling.

b. Sub pixel Estimation of land cover in remotely sensed image [19].

There were different approaches proposed to correctly estimate the components of land cover such as Spectral un-mixing, Unsupervised and Supervised Methods [18]. Spectral un-mixing technique was used to estimate the fraction of each component in a pixel using multispectral data. The linear mixing models were used to estimate the proportion of component spectra from training data. However, when the spectral characteristic of the categories within objective areas does not satisfy by the training data, large errors may be occurred with the result. The unsupervised estimation was used to overcome the problems of linear mixing model [20].

This method is good to solve the problem when the variation of pure pixel is small otherwise large error may be appeared in the results.

In a paper [19] authors proposed Semi-supervised estimation this method overcomes the problems of unsupervised estimation in which the variation of pure pixels is large, Semi-supervised estimate the component spectra depends on the surrounding information of pixels, it used the small size of initial training data and first step identifies pure pixel in the image as shown in Figure 3.

Pure pixels which consist of a single class of features exist around a certain point in a feature space. If two classes are mixed within a pixel, It will show that the observed data will exist between the two classes and represent the intermediate characteristic of spectra. The pixel can consider is pure if it exists within a small distance from each features class center. The mean vector and covariance matrix are used to calculate the distance between pixel and category. Next use the pure pixels to predict adaptively the feature spectra in the surrounding areas of each mix pixels. When pure pixels exist in the surrounding area of only one class as shown in Figure 4, and then classify the cells into the same class with neighbors. When there are more than two categories of pure cells in the surrounding areas, then component spectra for each class and use for sub cells estimation.

The result shows that the proposed method works well when the variations of pure pixels are large. The comparison of Supervised, Unsupervised and Semi Supervised methods based on sub pixel mapping as summarized in Table. 9.

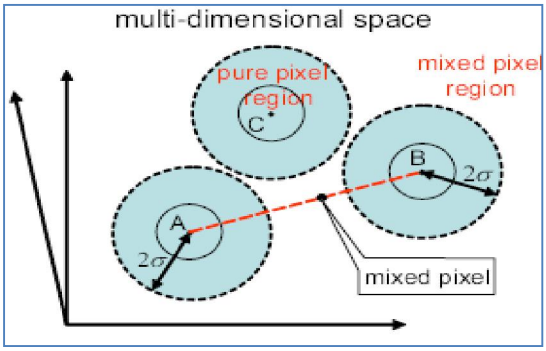


Figure 3. Determination of pure pixels [19].

Table 8. Comparison of maximum likelihood and fuzzy classifier.

Technique	Maximum Likelihood	Fuzzy Classifier
Approach	Statistical	Fuzzy Logic
Classification	Satellite images with mix pixels.	Satellite images with mix pixels.
Data	Forestry, Urban, Swana Woodland	Forestry, Urban, Swana Woodland
Model	Maximum Likelihood classifier	Fuzzy Convolution Filter
Accuracy	1. Overall accuracy 87% 2. Accurately mapped individual classes in more details. 3. Successful in heterogeneous environment.	1. Overall accuracy 77% 2. Not good to map individual classes in more detail. 3. Misclassify pixels
Advantages	High Potential to classify higher resolution imagery.	Good in homogeneous environment.

c. Sub-pixel Mapping of Remote Sensing Image Based on MAP model [23]

In this paper author proposed a new sub pixel mapping approach Maximum a Posteriori (MAP). The MAP model can be used to predict the location of class proportion within each pixel. The locations of the sub pixel in the central pixel identified by using the spatial arrangement of the different class fraction in surrounding pixels. The sub-pixel mapping algorithm is to be applied to high spatial resolution fraction images. The experiment is performed on artificial imagery and real imagery. The result shows that the model performs well against artificial imagery (Table.10)

Similarly the performance of the model is good for real imagery and the result is compared with other classification method which shown in (Table.11).

The confusion matrix (Table.12) shows that the MAP model produces good result as compared to the maximum likelihood classifier.

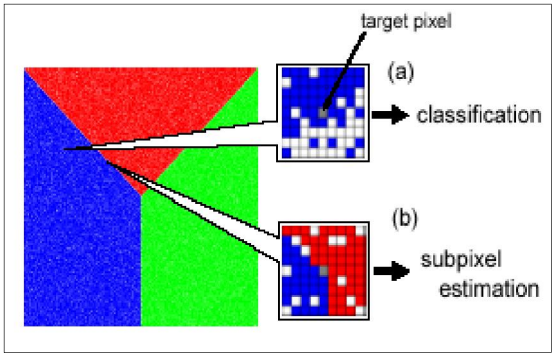


Figure 4. Sub pixel estimation for mixed pixel [19]

Table 9. Comparison of Supervised, Unsupervised and Semi Supervised

Technique	Linear Mixing Model	Unsupervised Analysis	Semi Supervised Analysis
Approach	Supervised	Unsupervised	Semi-Supervised
Performance	Depends on the training data	The result is good when the variation of pure pixels is small	Depends on the initial training data and the pure determination
Limitation	When the training does not represent the spectral characteristic of the categories within objective area	When the variation of pure pixel is large	When the class composition of every pixel is estimated , the spatial distribution of these class components within each pixel remains unknown

Table 10. The accuracy static of the classification result of two methods [23]

Method	PCC	Kappa coefficient
Hard Classification	0.906	0.752
MAP	0.987	0.861

Table 11. The accuracy static of the classification result with MLC and MAP [23]

Method	PCC	Kappa coefficient
Hard Classification	0.813	0.832
MAP	0.907	0.961

Table.12 The classification result by Confusion Matrix (R, L, V, U: River, Lake, Vegetation, Urban) [23]

Method		r	l	v	u
MLC	R	89	89	7	2
	L	8	8	8	6
	V	3	3	66	21
	U	0	0	19	71
MAP	R	95	95	0	2
	L	2	2	6	8
	V	2	2	82	10
	U	1	1	12	79

Conclusion and Recommendations

The increasing demand of land cover and land use information has raised the importance of classification and forecasting of land cover and land use. In this study a survey of current approaches of land cover mapping such as ANN, Fuzzy logic and Statistical techniques is done. The multilayer Feed Forward ANN used for the classification the overall results of this approach is good as compared to the statistical approach. ANN has the very strong ability to recognize mix classes. The performance of statistical approach is good as compared to the fuzzy classifier although Maximum Likelihood classification assumes a normal distribution. This approach can be used to produce a land cover map. Moreover, the survey included the study of different approaches such as ANNs, Maximum Likelihood, Supervised, Unsupervised and Semi Supervised to solve the mix pixel problems. The semi-supervised has the ability to solve the problems of mix pixels. The semi-supervised technique adaptively estimates the proportion of components where the object characteristics changes with the location of the mixed pixels. The majority of approaches discussed in this study are based on ANNs, Fuzzy logic, and probabilistic techniques. The ANNs and fuzzy set theory has the potential to overcome the limitation of probabilistic techniques such as maximum likelihood classifier and parallelepiped etc. In some cases e.g. when the spectral signature of the area is same the Maximum Likelihood produce good result as compared to ANNs and Fuzzy logic, however when the spectral signatures of the area are not same than ANNs and Fuzzy logic are better than Maximum Likelihood.

References

1. Gatsis-K. Gkoltsiou. A.T. Vafeidis, S. Koukoulas, “Forecasting land-use changes with the use of ANNs and GIS,” IEEE International Geoscience and Remote Sensing Symposium, vol. pp. 5068 – 5071, 2007.
2. P.M. Atkinson and A.R.L. Tatnall, “ANNs in remote sensing,” International Journal of Remote Sensing, vol. 18(4), pp. 699-709, 1997.
3. J. Zhang, Chang Yi, Y. Pan, "Multiple-class land use mapping at the sub-pixel scale using an innovated CA model,“ 2006.
4. URL:ieee.org/Xplore/login.jsp?url=http%3A%2F%2Fieeexplore.ieee.org%2Fiel5%2F4087812%2F4087813%2F04088062.pdf&authDecision=-203 Accessed on: 05.02.2012
5. D.L. Civco, “ANN for land use classification and mapping,” International Journal of Geographical Information Systems, vol. 7, pp.173-186, 1993.
6. P. Fisher, "The pixel: a snare and a delusion," International Journal of Remote Sensing, vol. 18, pp.679-685, 1997.
7. G.M. Foody, G. Palubinskas, R.M. Lucas. and P.J. Curran, “An evaluation of fuzzy and texture-based classification approaches for mapping regenerating tropical forest classes from Landsat-TM data,” International Journal of Remote Sensing, vol.16, pp.747-759, 1995.
8. G.M. Foody,”land use classification by an ANN with ancillary information,” International Journal of Geographical Information Systems, vol.9, pp.527-542, 1995.
9. C.F. Hutchinson,”Techniques for combining landsat and ancillary data for digital classification improvement,” Photogrammetric Engineering and Remote Sensing, vol.8, pp.123-130, 1982.
10. B.G. Long and T.D. Skewes, "A technique for mapping mangroves with landsat tm satellite data and geographic information system,” Estuarine Coastal and Shelf Science, vol. 43, pp. 373-381, 1996.
11. J.F.Mas, “An ANNs approach to map land use/cover using Landsat imagery and ancillary data,” International Geoscience and Remote Sensing Symposium, vol.6, pp. 3498 – 3500, 2003.
12. S.Sayago, M.Bocco1, G. Ovando and E. Willington1, "ANN models for land use classification from satellite images,” Agriculture Tcnica, vol. 67. pp. 414-421, 2007.
13. URL: http://www.scielo.cl/pdf/agrtec/v67n4/at09.pdf. Accessed on: 15.02.2012
14. A. Ohkubo and K.Niijima, “New supervised learning of ANNs for satellite image classification,” International Conference on Image Processing, vol.1, pp.505-509, 1999.
15. R. Pu and P. Gong, "Predicting landcovar changes with gray systems theory and multitemporal aerial photographs,”. URL: http://www.cnr.berkeley.edu/~gong/PDFpapers/

PuGongGISChange.pdf. Accessed on: 07.01.2012
16. G.Josan. S.Jindal, "ANN and fuzzy logic approach for satellite image classification: A review," National Conference on Challenges and Opportunities in information Technology Proceeding of COIT 2007.
17. Tatem, A.J. Lewis, H.G. Atkinson, P.M and Nixon, M.S, "Super-resolution target identification from remotely sensed images using a Hopfield ANN," IEEE Transactions on Geosciences and Remote Sensing, vol.39, pp.781-796, 2001.
18. Y. Wang, Jamshidi. M, "Fuzzy logic applied in remote sensing image classification," IEEE International Conference on Systems, Man and Cybernetics, vol.7, pp. 6378 – 6382, 2004.
19. M. Jamshidi, Large-Scale Systems: Modeling, control, and fuzzy logic, Prentice Hall Inc., 1997.
20. Lobina P, Harold A, Melaine K, "Mapping Rural Savanna Woodlands in Malawi: a comparisons of Maximum Likelihood and Fuzzy Classifiers" Geoscience and Remote Sensing Symposium, pp.1260-1264, 2007.
21. Wataru M, Ryichi N, Senya K and Sueharu M, "Sub pixel Estimation of land cover in remotely sensed image using Spectral information of surrounding pixels" SICE Annual Conference, pp. 1781 – 1784,2007.
22. S. Kiyasu, K. Terashima, S. Hotta and S. Miyahara,"Adaptive Subpixel Estimation of Land Cover in a Remotely Sensed Multispectral Image," Proc.SICE-ICCAS2006, pp.1943–1946, 2006.
23. JongGyu.H, KwangHoon. C and YeonKwang. Y, "Land Cover Classification of IKONOS Multispectral Satellite Data: Neuro -fuzzy, Neural Network and Maximum Likelihood Methods" Springer Berlin / Heidelberg. vol. 3642, pp. 252-262, 2005.
24. J.Han, S.Lee, K.Chi and K.Ryu,"Comparison of Neuro-Fuzzy,Neural Network, and Maximum Likelihood Classifiers for land Cover Classification using IKONOS Multispectral Data" IEEE International Geosciences and Remote Sensing Symposium,vol.6, pp.3471 – 3473, 2002.
25. Ke Wu, Pingxiang Li, Liangpei Zhang."Sub-pixel Mapping of Remote Sensing Image Based on MAP model", Fourth International Conference on Image and Graphics, vol. pp. 742 – 746. 2007.
26. Bittner, T., Frank, A.U.: An Introduction to the Application of Formal Theories to GIS. In: Dollinger, F., Strobl, J. (eds.) Proc. Angewandte Geographische Information sverarbeitung IX (AGIT), Salzburg, Austria, pp. 11–22 (1997).
27. Khan, S.A and Zafar, N.A, Improving Moving Block Railway System using Fuzzy Multi-Agent Specification Language, Int. J. Innov. Computing, Inform. Control, 7(7(B)):4517-34, 2011.
28. Khan, S.A, Zafar, N.A and Ahmad, F, Petri Net Modeling of Railway Crossing System using Fuzzy Brakes, International J. Phy. Sci, 6(14): 3389-3397, 2011(a).
29. Zafar, N.A, Khan, S.A and Araki, K, Towards the Safety Properties of Moving Block Railway Interlocking System, Int. J. Innov. Computing, Inform. Control, 8(8): 2012 .
30. Khan, S.A, Zafar, N.A, Extending promotion for the management of moving block interlocking components., International J. Phy. Sci, 6(31), 7262-70. 2011(b).
31. Khan, S.A and Zafar, N.A, Promotion of Local to Global Operation in Train Control System, Journal of Digital Information Management (page 228-233).,2007.
32. Ahmad, F. Khan, S.A, Module-based Architecture for Periodic job-Shop Scheduling problem, Computers & Mathematics with Applications, 64(1), 1-10, 2012.
33. Ali, G., Khan, S.A., Ahmad, M.F. and Zafar, N.A, Visualized and Abstract Formal Modeling towards the Multi-Agent Systems, International Journal of basic and Applied Sciences 2(8)8272-8284, 2012
34. Ali, G., Khan, S.A., Ahmad, M.F. and Zafar, N.A, Formal Modeling towards a Dynamic Organization of Multi-Agent Systems Using Communicating X-Machine and Z-Notation, Indian Journal of Science and Technology, Vol. 5 No. 7, 2012(a).

10/7/2012

Kelussia odoratissima Mozaffarian inhibits ileum contractions through voltage dependent and beta adrenergic receptors

Sedighi M (MSc) [1], Rafieian-kopaei. M (PhD) [1]*, Noori-Ahmadabadi M (MD student) [1]

[1] Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
***Corresponding author**: Professor in Pharmacology, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
E-mail: rafieian@skums.ac.ir

Abstract: The anti-spasm properties of *Kelussia odoratissima* Mozaffarian have been mentioned in Iranian traditional medicine and it is used to gastrointestinal disorders treatment. The plant leaf alcoholic extract cumulative effect of this plant on Wistar rats ileum contractions and expression of its probable mechanism was investigated in this research. Hydro-alcoholic extract was prepared by maceration method using 70% ethanol. Forty eight male Wistar rats (150-200 g) were randomly designated to 6 random groups with 8 rats in each, in this interventional research as following. Control group, cumulative concentrations of *Kelussia odoratissima* Mozaffarian extract receiving group, propranolol receiving group, naloxone receiving group, L-NAME receiving group, and sodium chloride receiving group. Ileum samples were taken from rat and subjected to 1gr tension in tissue-bath containing tyrode solution. Isotonic contractions was recorded following addition of potassium chloride (60mM), saline or 10% and 20% cumulative concentrations of *Kelussia odoratissima* Mozaffarian extract. In order to understand the above mechanism, ileum was incubated with L-NAME, naloxone or propranolol and also affected by different doses of calcium chloride. Then, the observed effect was recorded and the variation percentage was calculated. Statistical analysis was performed by parametric test, repeated measures, ANOVA and t test. Findings: 10% and 20% cumulative concentrations from alcoholic extracts of *Kelussia odoratissima* Mozaffarian could reduce contractions caused by potassium chloride ($P<0.001$). There was a significant different between extracts of 10% and 20% groups ($P>0.05$). Beta adrenergic receptor blocker (1µM propranolol), significantly decreased the contractions caused by potassium chloride (P-value=0.013) but nitric oxide inhibitor (100 µM L-NAME) and opioid receptor blocker (1µM naloxone) had no effect on this contraction. Calcium was also caused tissue contraction depolarized by potassium chloride and this contraction effect reduced by cumulative concentration ($P<0.001$). In general it can be concluded that Kelussia alcoholic extracts can inhibit ileum contractions of rat through the effect on voltage dependent and beta adrenergic receptors and it might be used to relieve intestinal spasms.

[Sedighi M, Rafieian-kopaei M and Noori-Ahmadabadi M. ***Kelussia odoratissima* Mozaffarian inhibits ileum contractions through voltage dependent and beta adrenergic receptors.** *Life Sci J* 2012;9(4):1033-1038]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 157

Key words: Kelussia leaf, ileum, rat.

Introduction:

Plants can be a major source to supply drugs in traditional medicine (1). Among these plants, *Kelussia odoratissima* Mozaffarian from *Umbelliferae* family can be mentioned. Kelussia is a perennial, glabrous, erect and very aromatic and is harvested in April (2,3). Flavonoids have anti-inflammation (4), anti-allergy (5), anti-diabetes, anti-hyperlipidemic and anti-hypercholestrolemic (6,7) effects and improve spatial memory (8). Phthalides constitutes 70% of the plant essence with the effect of liver protection, prostaglandin F2α inhibition, cancer tumors prevention, epilepsy and liver disorders treatment and blood viscosity reduction (2, 10, 13, 14). The ligastilid extracted from Kelussia leads to relax rat's vessels (9). This plant is also used to cure some rheumatism disorders, common cold, cough, blood pressure, blood lipid and stomachache (15). But no research has been done concerning this plant effect on ileum motion activity. The smooth muscle motion activity can be changed by several factors. The contraction of smooth muscle can be affected by:

1. High increasing of extracellular potassium concentration and preventing the outward diffusion of potassium, depolarization, and subsequently contraction of smooth muscle.
2. Opening the slow sodium calcium channels and calcium entry into the cell and beginning the contraction.
3. Activating protein G binding receptors due to which, IP_3 and DG are produced. IP_3 affects sarcoplasmic proteins and results in releasing calcium.
4. Activating rhokinases (Rho) by arachidonic acid which dephosphorylate myosin phosphatase and pump calcium into sarcoplasmic reticulum.

In total it can be said, the factor that increases calcium concentration in cytoplasm and eventually deactivates myosin phosphatase, leads to contraction of the muscle and the factors that decreases cytosolic calcium and activates myosin phosphatase, leads to relaxation of the muscle (16, 17).

In addition to investigation of Kelussia hydroalcoholic extract anti contraction effect on motion activity in this study, the probable mechanism of the extract cumulative concentration anti contraction effect on motion activity of rats was examined including possible involvement of beta adrenergic receptors by propranolol (1µM) inhibitory effect, opioid receptors by naloxone (1µM) and nitric oxide synthesis inhibitory effect by L-NAME (100 µM). The effect of Kelussia hydroalcoholic extracts on ileum contractions of rats with the effect on voltage dependent receptors through calcium chloride (2 to 8mM) was studied cumulatively.

Materials and methods:

The applied materials:

The propranolol and L-NAME were prepared from Sigma Co.(USA), naloxone and all of the salts used in this research were prepared from Tolid Daru Co. (Iran) and Merk Co. (Germany) respectively.

The extraction method:

In order to prepare Kelussia extract the maceration method used in this study. After cleaning and separating extra parts, 1000 g of this plant was dried, powdered and 70% ethanol was added to it. After 72 hours, the obtained mixture filtered by Buchner funnel and the resulting solution was evaporated in 35 ˚C in a Rotary evaporator and dried completely in an incubator with maximum temperature of 40˚C (18). Finally, 35 g extract powder was obtained from 500 g of initial Kelussia. This powder was maintained in a refrigerator until the time of using.

Animals:

Forty eight male Wistar rats (150-200 g) were taken from the Animals Unit of Shahrekord University of Medical Sciences, and then randomly divided to 6 groups with 8 rats in each. The rats were kept in 12 h daylight situation in 24-20˚C and freely feed and watered. But the night before the experiment, they were deprived of food (19).

Ileum preparation and the procedure:

In the test day, the rats anesthetized with chloroform, a 2 cm piece was separated from their terminal part of ileum (except the last 2 cm) and the contractions of this piece was recorded under 1gr tension in tissue bath (500 ml) containing tyrode solution (pH 7.4, 37˚C), air bubble continuous flow and 60 minutes adoption period by potassium chloride (60 mM). The cumulative concentration of extract (10% and 20%) added to tissue bath when the contraction reached to pan mode (20). The obtained variation percentage was recorded by isotonic transducer (Harvard, UK) and a recording machine (Universal Harvard Osilloghraph). In order to study the involvement of beta adrenergic receptors, nitric oxide and opioid receptors, the effect of extract cumulative concentrations on potassium chloride induced contraction was recorded after incubating tissue with 1µM propranolol (21), 100µM L-NAME (22), or 1µM naloxone. To determine the role of extracellular calcium in extract performance, first tissue should be put in tyrode solution with no calcium and high concentration of potassium chloride (60mM) and calcium chloride added to the bath cumulatively (2 to 8 mM) (24). Then the extract was exposed to cumulative concentrations for 5 minutes and its effect was recorded on paper by a recording machine.

Composition of tyrode solution used in the bath (in mM) was:

NaCl (136), KCl (5), CaCl2 (2), NaHCO3 (11.9), MgCl2 (0.98), NaH2PO4 (0.36) and Glucose (5.55)

Data analyzing:

Contraction pan resulted from KCL then, the percentages of relaxation resulted from saline or extract were calculated as mean ± SD (standard deviation). Statistical analysis was performed by parametric test, repeated measures, ANOVA and t test.

Results:

Comparison the effect of Kelussia extract cumulative concentrations on contractions caused by potassium chloride in rat ileum.

Adding potassium chloride to tissue bath led to contraction due to potassium chloride on ileum in the entire period of experiment and after a short time contraction reached to pan mode in which contraction percentage of ileum was calculated. The variation mean of tissue contraction in response to saline was also calculated by adding saline after reaching tissue to the pan mode. Figure 1 shows that saline have low inhibitory effect on tissue contraction due to potassium chloride so that no significant difference observed between two groups. Additionally, although Kelussia hydroalcoholic extract cumulative concentrations (10% and 20%) has reduced ileum contraction caused by potassium chloride (60 mM) ($P<0.0001$), but no significant difference observed for contraction mean between 10% and 20% of Kelussia ($P>0.05$).

The effect of propanolol, L-NAME, and naloxone on extract inhibitory performance

The NO synthesis stimulation may cause reduction of extract contraction performance.

Incubating ileum in the presence of L-NAME (nitric oxide synthetase enzyme inhibitor) for 20 minutes was compared to incubating it in the absence of L-NAME with 15 minutes interval followed by washing tissue. But chart 2 indicates that the extract, has reduced the contraction effect of potassium chloride significantly (P-value=0.000) and there is no significant difference between the inhibitory effect of extract in the presence and absence of L-NAME (P-value=0.703).

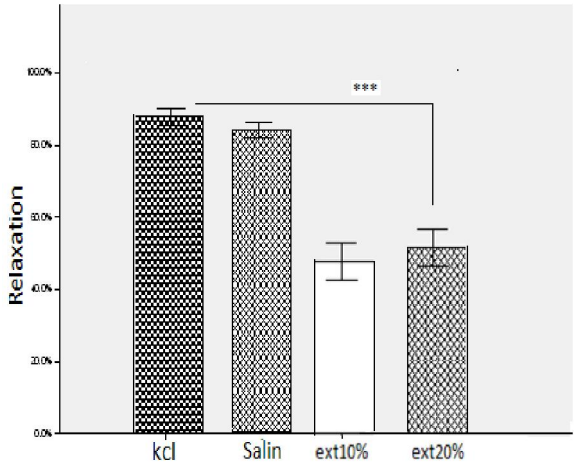


Figure 1: Comparison of ileum contractions between tested groups receiving saline and potassium chloride (60mM). No significant difference was observed between two groups. The relaxation effect of Kelussia extract cumulative concentrations (10% and 20%) on ileum contraction due to potassium chloride (60mM) in comparison with control group (*** P<0.0001, n=8, ANOVA).

Considering that the stimulation of opioid receptors makes intestinal movements reduced, so the inhibitory effect of extract on receptors in the absence of naloxone, was compared to the effect of extract on receptors when it was put in the presence of 1µM naloxone for 30 minutes with 15 minutes interval followed by washing tissue. Figure 2 shows that the contraction effect of potassium chloride significantly was reduced by the extract (P-value=0.000), but there is no significant difference between the inhibitory effect of extract in the presence and absence of naloxone (P-value=0.516).
Stimulation of beta adrenergic receptors also caused relaxation of small intestine. Effective materials may affect receptors and resulted in muscle relaxation. Incubating tissue without propranolol was compared to incubating it in the presence of 1µM propranolol for 30 minutes with 15 minutes interval followed by washing tissue. But figure 2 indicates that the contraction effect of potassium chloride significantly reduced by extract (P-value=0.000) and the inhibitory effect of extract in the presence and absence of propranolol was significantly different (P<0.001).

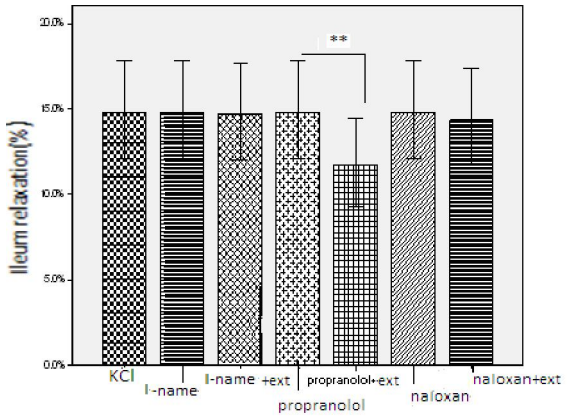


Figure 2: Comparison of the anti-contraction effect of Kelussia hydroalcoholic extract concentration (20%) on contraction caused by potassium chloride (60mM), in the presence and absence of L-NAME (1µM, 30 min, n=8), propranolol (1µM, 30 min, n=8) and naloxone (1µM, 30 min, n=8).
Propranolol+ext receiving groups and extract receiving group without propranolol was significantly different (**P<0/001, n=8).

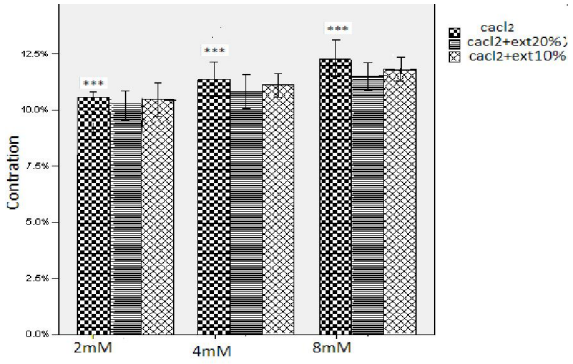


Figure 3: Comparison of calcium chloride cumulative concentrations (2, 4, 8 mM) on ileum in free calcium tyrode solution with high concentration of potassium chloride (60mM) in the absence of extract followed by incubating tissue in cumulative concentration of extract (10% and 20%) for 3 minutes.
Contraction responses of calcium chloride is dose dependent (** P<0.0001, n=8, ANOVA) and represents (P<0-001) significant different between groups with extract inhibitory response 10% and 20%) in the presence of calcium chloride.

The effect of Kelussia hydroalcoholic extract on contraction caused by depolarized calcium chloride through potassium chloride

Figure 3 shows that depolarized ileum of rat has contracted as of concentration dependant (2, 4, 8 mM) in the presence of cumulative concentration of calcium chloride, in tyrode solution with high concentration of potassium chloride (60mM) but without calcium (n=8,P<0.0001). After washing tissue in free calcium tyrode solution and tissue resting for 20 minutes, repeating these steps in the presence of cumulative concentration of extract (10% and 20%) for 3 minutes, decreased the contraction effect in ileum caused by calcium chloride (P<0.001).

Discussion:

This study was aimed to investigate the anti-contraction effect of Kelussia hydroalcoholic extract on ileum motion activity. The probable mechanism of the extract cumulative concentration anti-contraction effect on ileum activity of rats including possible involvement of beta adrenergic receptors, opioid receptors and nitric oxide synthesis inhibitory effect, as well as involvement of voltage dependent receptors in the effect of Kelussia hydroalcoholic extracts were also studied.

Cumulative concentrations of Kelussia hydroalcoholic extract, reduced contractions caused by potassium chloride in the ileum of rat and tissue washing and changing solution bath didn't remove the extract anti-contraction effect therefore; decreasing contraction in the presence of extract can't be due to exhausting muscle during contraction (25). Additionally, contraction occurred by potassium increasing out of smooth muscle cell along with depolarization despite adequate concentration of intracellular calcium ion. The major way for increasing calcium is opening voltage dependent calcium channels after depolarization. The existence of L type calcium channels has been proved in ileum smooth muscle of rat (26). Previous studies reported that contraction caused by potassium chloride is done by involvement of voltage dependent calcium channels (27). Considering the approval of L type calcium channels existence in smooth muscle of ileum, it seems that the observed extract effect is as a result of depolarization consequences inhibition and also increasing intracellular calcium prevention.

To understand the above mechanism, ileum was incubated with L-NAME, naloxone and propranolol and also affected by different doses of calcium chloride. Nitric oxide causes ileum relaxation through cAMP pathway (28). Incubating tissue with L-NAME was not enable to reduce inhibitory performance of extract which represents NO has no involvement in it. In addition, naloxone inability to reduce inhibitory performance of extract, demonstrates opioid receptors has no involvement in it.

Beta adrenergic receptors exist in rats ileum and their inhibitory effect has been proved (29). Propranolol has reduced extract inhibitory effect on contractions caused by potassium chloride in this research too, so probably the effective materials in Kelussia has activated receptors and relaxed muscle by affecting beta adrenergic receptors. Tissue depolarized by high concentration of extracellular potassium in free calcium tyrode, which is consistent with previous studies (30) and its contraction provided adding calcium to environment and the contraction level is depending on the environment calcium dose (31). The results also indicated that the extract inhibits calcium contraction performance in depolarized tissue so confirms calcium channels involvement in occurring extract inhibitory performance.

E-ligustilide Phthalide, 3- e -butyldiene phthalide and z-ligustilide are main chemicals in Koohrang Kelussia (9). Inhibitory and relaxant effects of butylidenephthalide and its z isomer were determined in tissues of many parts of body like different blood vessels, intestine, respiratory and genital system and it seems that inhibition of voltage sensitive calcium channels play a role in it (32). It has been determined in other studies that phtalids in Kelussia leaf have inhibitory effects on prostaglandin F2α (33). Contraction effects of prostaglandin F2α occurs through activation of EP1 receptors. Stimulation of these receptors activates phosphatidyl inositol pathway and increases intracellular calcium (34). So the phthalides in Kelussia extract stops activating phosphatidyl inositol pathway and reduction of cellular calcium as a result obviates contraction caused by potassium chloride in ileum by prostaglandin F2α. The anti-contraction effect of flavonoids on guinea pig was also reported (35). So it can be suggested that voltage dependant calcium channels have main involvement, beta adrenergic receptors have small involvement and nitric oxide synthesis and opiod receptors have no involvement in occurrence of this inhibitory effect and the extract anti contraction performance can be as a result of mentioned flavonoids and phthalides through voltage dependant calcium channels and beta adrenergic receptors.

General conclusion: Totally it can be concluded that alcoholic extracts of Kelussia leaf can inhibit ileum contractions of rat by affecting voltage dependant calcium channels and beta adrenergic receptors and can be used to relieve intestinal spasms.

References:

1-Khajehdehi, P. Turmeric: Reemerging of a neglected Asian traditional remedy. J Nephropathology. 2012; 1(1): 17-22.

2-Dadkhah Tehrani, Z. Phytochemical chemical study on Kelussia odoratissima Mozaffarian. Pharmacy thesis. School of Pharmacy, Isfahan University of Medical Sciences, 1999.

3-Gandomkar, M. Phytochemistry of the essential oil of wild celery plant. School of Pharmacy, Isfahan University of Medical Sciences. 1999.

4-Asgari, S. Naderi GhA. Garipour, M. Dashti Gh,R. Sadjadian , A. Effect of Amirkabiria Odoratissima on the development and progression of atherosclerosis in hypercholesterolemic rabbits. Iranian Journal of Diabetes and Lipid Disorders. 2003;3(1): 88-83.

5-Haj Hashemi, V. Ghannadi, AR. Soltani, L. Analgesic and anti-inflammatory effects of Amirkabiria Odoratissima. Journal of Research In Medical Sciences. 2003;7(4): 125-121.

6-Asgari, S. Naderi GhA. Jafariyan, A. Askari, N. Behagh, AR Fibrinolytic activity of Amirkabiria odoratissima Mozaffarian. Journal of Medicinal Plants .2005;4(13): 25-19.

7-Roghani, M. Baluchnejadmojarad, T. Ramazani ,M .The Effect of Chronic Oral Feeding of Apium graveolens on Learning and Memory in Diabetic Rats. Journal of Medicinal Plants.2008;7(27): 98-105.

8-Cao,YX. Zhang, W. Jian-Yu, He. Lang-Chong , He. Cang-Bao, XuLigustilide induces vasodilatation via inhibiting voltage dependent calcium channel and receptor- mediated Ca2+ influx and release. Vascul Pharmacol. 2006;45:171–176.

9-Sultana, S. Ahmed ,S. Jahangir, T. Sharma, S. Inhibitory effect of celery seeds extract on chemically induced hepatocarcinogenesis: modulation of cell proliferation, metabolism and altered hepatic foci development. Cancer Lett. 2005; 221(1):11-20.

10-Craker, LE. Herbs species and medicinal plants. Encanto. Oxyx Press. 1984; 2-15-16.

11-Harbrone , JB. The flavonoids advances in research science. London، Chapman S hall.1994، p: 1-20-5- 292- 480- 500.

12-Kaouadji , M. DE Pachtere, F. Pouget , C. Chulia ,AJ. Three additional phthalide derivatives، an epoxymonomer and two dimmers، from ligusticum wallichii rhizomes. J. Nat. Prod. 1986; 872-877.

13-Kerry, N. Rice Evans, C. Peroxynitrite oxydices catechols to quinnones. FEBS Lett.1998; 437(3): 167-171.

14-Beck, J. Investigation of the bioactive constituents of several herbal medicines. Doctoral dissertation. 2004; 27 (5): 255-7.

15-Iravani , M. Jaberol-Ansar , Z. Kelussia odoratissima, an overthrowing plant in Central Zagros region. Payam Sabz. 2005 Publ;39.[Persian].

16-Ratz, RH. Berg, KM. Urban , NH. Miner , AS. Regulation of smooth muscle calcium sensitivity KCL as a calcium sensitizing stimulus. J, Physiol. 2005; 288:2772-2783.

17-Ratz, RH. Berg, KM. , Urban, NH, Miner, AS. Role of Ca2+ and MLK in regulation of smooth muscle. Am J Physiol. 2005; 248:2769-2783.

18-Samsam Shariat, H. Quantitative evaluation of the active constituents and control method for medicinal. Plants. Mani. Publication, Isfahan.1992:13-17.

19-Gebhardt ,Y. Witte, S. Forkmann, G. Lukacin, R. Matern, U. Martens, S. Molecular evolution of flavonoid dioxygenases in the family Apiaceae. Phytochemistry. 2005; 66:1273-1284.

20-Gharib Naseri, MK. Pilehvaran , AA. Shamansouri, N. Investigating the spasmolytic activity of celery (Apium Graveolens) leaf hydroalcoholic extract on rat's ileum. Journal of Kashan School of Medical Sciences. 2007;11(3)

21-Storr, M. Franck, H. Saur, D. Schusdziarra, V. Allescher, HD. Mechanisma of alpha, beta-methylene ATP-induced inhibition in rat ileal smooth muscle: involvement of intracellular Ca2+ stores in purinergic inhibition. Clin Exp Pharmacol Physiol. 2000; 27:771-779 .

22-Gray, AC. White, PJ. Coupar, IM. Characterisation of opioid receptors involved in modulating circular and longitudinal muscle contraction in the rat ileum**.** Br J Pharmacol**.** 2005;144:687-694.

23-Andersson, A. Sundler, F. Ekblad, E. Expression and motor effects of secretin in small and large intestine of the rat. Peptides. 2000;21:1687-1694.

24-Gharib Naseri, MK. Anvari, A. Badavi , M. Spasmolytic effect of Cuscuta pentagona fruit aqueous extract on rat ileum Scientific. Journal of Kurdistan University of Medical Sciences. 2007;12(2): 9-20.

25-Madeira, SVF., Matos, FJA., Leal-Cardoso, JH. and Criddle, DN. Relaxant effects of the essential oil of Ocimum gratissiumum on isolated ileum of the guinea pig. Journal of Ethnopharmacology, 2002;81: 1-4.

26-El Bardai, S. Lyoussi, B. Wibo, M. Morel, N. Comparative study of the antihypertensive activity of Marrubium vulgare and of the dihydropyridine calcium antagonist amlodipine

in spontaneously hypertensive rat. Clin Exp Hypertens. 2004;26(6):465-74.
27-Bolton, T.B. Mechanisms of action of transmitters and other substances on smooth muscle. Physiological Reviews. 1979;59: 606-718.
28-Ekblad , E., Sundler, F. Motor responses in rat ileum evoked by nitric oxide donors vs. field stimulation: modulation by pituitary adenylate cyclase-activating peptide, forskolin and guanylate cyclase inhibitors. J Pharmacol Exp Ther. 1997; 283 :23-28.
29-Roberts, SJ. Papaionnou, M. Evans, BA. Summers, RJ. Characterization of β-adrenoceptor mediated smooth muscle relaxation and detection on mRNA for β1-, β2- and β3-adrenoceptors in rat ileum. Br J Pharmacol 1999; 127:949-961.
30-Fujimoto, S. Mori, M. Characterization of capsaicin-induced, capsazepine-insensitive relaxation of ileal smooth muscle of rats. Eur J Pharmacol. 2004 ;487(1-3): 175-82.
31-Zhang, WW., Li, Y., Wang, XQ., Tian, F., Cao, H., Wang, MW. et al. Effects of magnolol and honokiol derived from traditional Chinese herbal remedies on gastrointestinal movement. World J Gastroenterol. 2005; 11(28): 4414-8.
32-Rahmanro, A. Bioactive Natural Products. Stud Nat Prod Chem. 2005;32(Part L):1252-3.
33-Salimi, M. Ebrahimi, A. Shojaee Asadieh, Z. Saei Dehkordi, SS. Essential oil composition of Kelussia odoratissima Mozaff. Iran J Med Aromatic Plants. 2010; 26(2): 147-56. (Persian).
34-Phillippe, M. Saunders, T., Basa, A. Intracellular mechanisms underlying prostaglandin F2 alpha-stimulated phasic myometrial contractions. Am J Physiol. 1997; 273(4 Pt 1): E665-73.
35-Zhang, WJ., Chen, BT., Wang, CY., Zhu, QH, Mo, ZX. Mechanism of quercetin as an antidiarrheal agent. Di Yi Jum Ya Da Xue Xue Bao. 2003;23:1029-31.

8/13/2012

A New Coherent Technique for Real-Time Shadow Generation with Respect to the Sun's Position

Hoshang Kolivand [1], Mohd Shahrizal Sunar [2], Ayman Altameem[3], Amjad Rehman[4]

[1,2,4]UTM ViCubelab, Department of Computer Graphics and Multimedia, Faculty of Computer Science and Information System, Universiti Teknologi Malaysia 81310, Johor, Malaysia
[3]College of Applied Studies and Community Services King Saud University Riyadh KSA
shahinkey@yahoo.com

Abstract: Soft shadow with respect to the sun's position in virtual environments is a fascinating topic in outdoor rendering. A coherent mathematic formula to create shadow with respect to the sun's position in specific location, date and time can make outdoor rendering as easy as indoor rendering. A target of this study is to propose a new coherent formula to do this. Outdoor rendering using a coherent formula will eliminate worrying about the sun's position and shadow status during the daytime. Low frames per second (FPS) in real-time rendering is a crucial problem in computer graphics, especially in soft shadow generation. A novel technique to create soft shadow in virtual environments is proposed. Geometric progression to select the color and a combination formula using sequence progression are proposed to determine the sample size. The new soft shadow generation, in addition to increasing FPS, enhances the quality of soft shadows. Finally, we strongly contend that the proposed technique can be used in commercial gaming and virtual reality systems.
[Kolivand, H, Sunar, M.S., Altameem, A., Rehman, A. **A New Coherent Technique for Real-Time Shadow Generation with Respect to the Sun's Position.** *Life Sci J* 2012;9(4):1039-1045] (ISSN:1097-8135). http://www.lifesciencesite.com. 158

Keywords: real-time shadow, sun's position, projection shadow, geometric progression, real-time rendering

1. Introduction

Computer animation has become one of the most important components of online and off line games, advertisements and simulation in a virtual environment. Shadows are the main components of outdoor rendering used to make scenes realistic. After smooth motion, shadows are the second main effect used in computer animation to reveal information about the distance between objects and to show the complexity of objects in the scenes. Unfortunately, shadowing is still an expensive component of animation and games, especially in real-time rendering. In computer games, shadows give the gamers feelings that trigger the sense that they are playing in the real world, resulting in maximum enjoyment. Games which lack shadows are not seen as attractive, especially since gamers' have had a taste of virtual games and their imagination now requires more and more realistic situations when they are watching cartoons or playing games.

Soft shadow is another challenging factor in computer animation and computer games. Good quality and high frames per second are two components of soft shadow generation that require more improvement. Low rendering time required by projection shadowing leads us to extend this kind of shadow to create a high speed shadow generation method with respect to the sun's position during the daytime.

The sun's position and its effect on shadows during the daytime is another factor to consider when attempting creating a more realistic scene. Calculating the shadow status during the daytime encouraged us to prepare this study.

2. Previous work

The first researcher who worked on shadow was Leonardo Da Vinci in 1460 (Rautenbach 2002). He focused on painting and static images. Hard shadows are types of shadows with a point light source, which have a sharp brim, and they include fully shadowed regions without any soft edges. Hard shadows will be generated by a point light source. Soft shadows are another kind of shadow which is caused by a wide light source. Soft shadows are more realistic for virtual environments. In the real world, most light sources are wide and wide light sources lead to the creation of soft shadow; as a result, the focus on soft shadow will be more appreciated. Soft shadows include two parts, umbra and penumbra. Umbra includes those parts of the shadow region which do not see any portion of the light source and are fully shadowed; it is like hard shadow. Penumbra is the other part of a shadow region which can see some portion of the light source.

The first researcher who seriously worked on real-time shadow generation was Crow (1977). He proposed shadow volume, which is a geometry based technique used to create hard shadow. Currently, shadow volume is still a famous algorithm used to create precise shadow, but the main drawback is that silhouette detection is expensive. Shadow mapping is

a widely used image based technique proposed by Williams (1978). High speed rendering commonly uses this image based algorithm. Shadow maps suffer from aliasing and many improved algorithms have tried to remove aliasing but not completely been able to do so. (Scherzer, 2011)(Bittner, 2011)(Donnelly, 2006)(Lauritzen, 2008)(Liang, 2011) (Rehman et al., 2011) (Saba and Rehman, 2012).

Projection shadow proposed by Tessman et al. (1989) is the third type of shadow. Projection shadows are convenient for flat surfaces. High-speed rendering is the most important reason behind their use in recent games and animations. It is not so difficult to cast projection shadow on arbitrary objects.

Low speed rendering of soft shadow is a critical problem in computer graphics(Boulanger, 2008)(Wyman, 2004)(Zhang, 2007)(Liu, 2011)(Kolivand, 2013) Generating soft shadow using projection shadow with respect to the sun's position in a specific location, and specific date and time is the main purpose of this study.

The principle calculations of the sun's position have been very well known for a long time. The sun's position and the amount of sunshine has historically been a very attractive subject for most of researchers. For example Nawar et al. (1958) worked on the principle amount of sunshine in a day. Kambezidis et al. (1990) provided several functions to calculate the sun's position by focusing on factors such as light refraction.

A coherent formula for shadow and the sun's position in specific longitude, latitude, date and time can be used in virtual environments such as games and 3D animations.

This paper presents a coherence formula to create exact shadow with respect to the real sun's position in specific location, date and time that can be used in augmented reality to keep the virtual shadows without worrying about confusing between real shadow and virtual ones.

3. Material and Methods

3.1. The Sun's Position

Suppose that the earth is a sphere. Julian dating is a precise technique to calculate the sun's position. The sun's position relative to earth can be calculated for a specific longitude, latitude, date and time using Julian dating.

Assume that f is a function sphere:

$$f(\theta,\varphi)=\cos^2\theta\cos^2\varphi+\sin^2\theta+\sin^2\theta\cos^2\varphi-r^2$$

$$0\le\theta\le\frac{\pi}{2}$$

$$0\le\varphi\le 2\pi \qquad (1)$$

where θ and φ are zenith and azimuth respectively.

$$t=t_s+0.17\sin(\frac{4\pi(J-80)}{373})+0.129\sin(\frac{2\pi(J-8)}{355})+12\frac{SM-L)}{\pi} \qquad (2)$$

where
t: Solar time
t_s: Standard time
J: Julian date
SM: Standard meridian
LO: Longitude
The solar declination is calculated as follows:

$$\delta=0.4093\sin(\frac{2\pi(J-81)}{368}) \qquad (3)$$

The time is calculated in decimal hours and degrees in radians (Kolivand et al. 2011) (Zafar 2012). Finally, zenith and azimuth can be calculated as follows:

$$\theta_s=\frac{\pi}{2}-\sin^{-1}(\sin l\sin\delta-\cos l\cos\delta\cos\frac{\pi t}{12}) \qquad (4)$$

$$\varphi_s=\tan^{-1}(\frac{-\cos\delta\sin\frac{\pi t}{12}}{\cos l\sin\delta-\sin l\cos\delta\cos\frac{\pi t}{12}}) \qquad (5)$$

θ_s : Solar zenith

φ_s : Solar azimuth

l : Latitude

3.2. Shadow Calculation

To calculate the shadow on a plan *f*, very simple calculations are needed. *L* is light source, *P* is a point of occluder and *S* is the projection of *P* in the plan *f*. Figure 1 illustrates the generation of projection shadow.

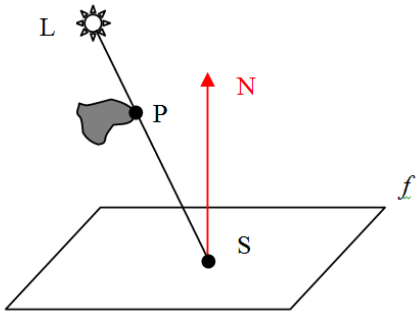


Figure 1. Shadow generation theory

where

$L=(l_x,l_y,l_z)$: light source point

$P=(p_x, p_y, p_z)$: point of occlude

$S=(s_x, s_y, s_z)$: projection of p

$N=(n_x, n_y, n_z)$: normal vector of f

$E=(e_x, e_y, e_z)$: a point of f

$L, P, S, E \in R^3$

The equation of a straight line from L to S is:

$$x = L + \alpha(P - L) \quad (6)$$

The formula off will be:

$$(x - E).N = 0 \quad (7)$$

By combining (6) and (7):

$$(L - E + \alpha(P - L)).N = 0 \quad (8)$$

Then

$$\alpha = \frac{E.N - L.N}{N.(P - L)} \quad (9)$$

Finally, for S we can have:

$$S = L + \frac{E.N - L.N}{N(P - L)}(P - L) \quad (10)$$

Assume that:

$$d = L.N \quad (11)$$

$$c = E.N - d \quad (12)$$

Then

$$S = L + \frac{c}{N.(P - L)}(P - L) \quad (13)$$

$$s_i = l_i + \frac{c(p_i - l_i)}{NP - d}$$

$\forall i =$ x, y and z (14)

$$s_x = \frac{l_x n_x p_x + l_x n_y p_y + l_x n_z p_z - d l_x + c(p_x - l_x)}{n_x p_x + n_y p_y + n_z p_z - d}$$

$$s_y = \frac{l_y n_x p_x + l_y n_y p_y + lyn_z p_z - d l_y + c(p_y - l_y)}{n_x p_x + n_y p_y + n_z p_z - d}$$

$$s_z = \frac{l_z n_x p_x + l_z n_y p_y + l_z n_z p_z - d l_z + c(p_z - l_z)}{n_x p_x + n_y p_y + n_z p_z - d} \quad (15)$$

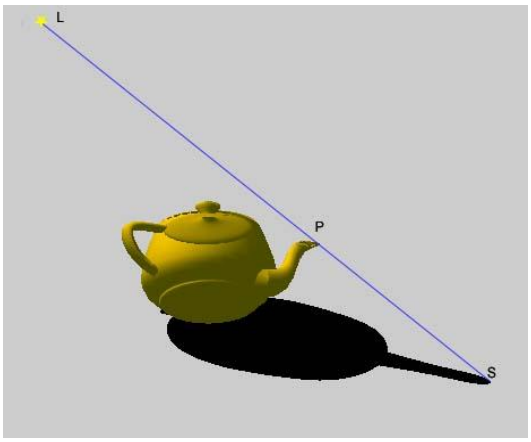


Figure 2. Projection shadow

3.3. Shadow Formula with Respect of the Sun's Position

To use the zenith and azimuth in specific location with radiance ρ of the sky dome the statements of (16) are used (Kolivand and Sunar, 2012).

$$x_s = \rho \sin\varphi_s \cos\theta_s$$
$$y_s = \rho \sin\varphi_s \sin\theta_s \quad (16)$$
$$z_s = \rho \cos\varphi_s$$

In this case x_s, y_s, z_s are the sun's position in specific location, date and time. Therefore, to calculate the projection of each pixel of occluder on the f the triple of (17) are obtained. In the other word, the projection of pixel $P= (p_x, p_y, p_z)$ With respect to specific longitude, latitude, date and time is pixel S.

$$S = (\frac{\rho \sin\varphi_s \cos\theta_s (n_x p_x + n_y p_y + n_z p_z - d - c) + c p_x}{n_x p_x + n_y p_y + n_z p_z - d},$$
$$\frac{\rho \sin\varphi_s \cos\theta_s (n_x p_x + n_y p_y + n_z p_z - d - c) + c p_y}{n_x p_x + n_y p_y + n_z p_z - d},$$
$$\frac{\rho \sin\varphi_s \cos\theta_s (n_x p_x + n_y p_y + n_z p_z - d - c) + c p_z}{n_x p_x + n_y p_y + n_z p_z - d}) \quad (17)$$

where

ρ: radiance of sky dome

θ_s : Solar zenith

φ_s : Solar azimuth

$n_i \forall i =$ x,y and z is i^{th} component of the normal vector of the plan

d and *c* are defined in (12).

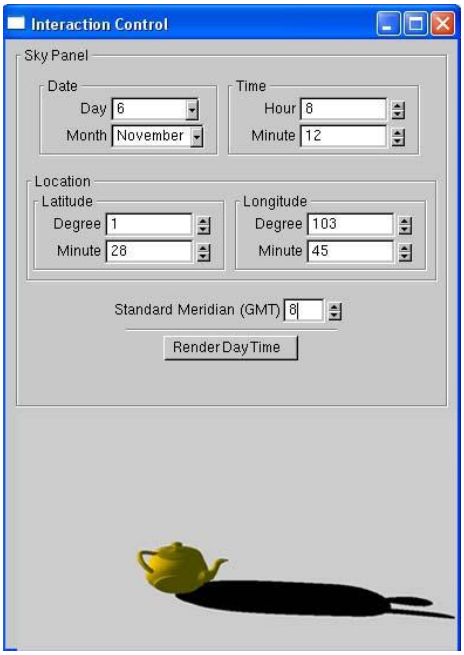


Figure 3. Projection shadow using new formula at 8:12 am of the day on 6th November in Universiti Teknologi Malaysia (latitude: 1.26 and longitude: 103.45)

4. Soft Shadow

Soft shadow will be produced if the light source is wide or there is more than one light source in the scene. Since most light sources in the real world are wide focus on soft shadow, using projection shadow due to low rendering time can be more beneficial (Bavoil, 2011).

4.1 Soft Shadow Sampling

Although soft shadow generation using hard shadow sampling is an old technique, improvements in projection shadow can produce a faster method to create soft shadow. This technique can be used for complex scenes and improves the quality of the results(Liu, 2011).

To create some hard shadow samples of different color, the smallest sample is created with less diffuse illumination (Forest, 2006). The largest sample is created with high diffuse illumination. In our method, to have different colors of different samples, a geometric progression is used.

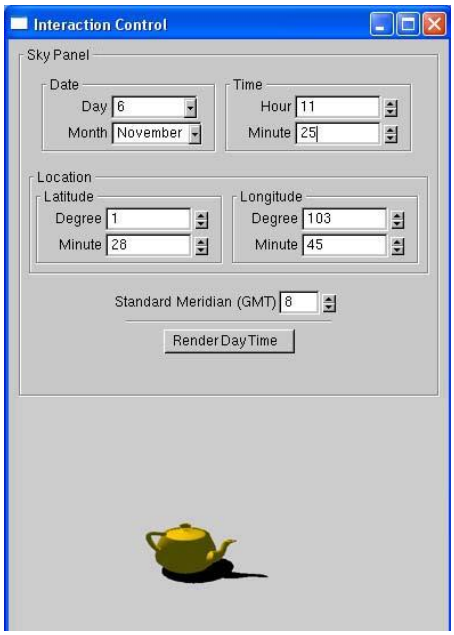


Figure 4. Projection shadow using new formula at 11: 25 am of the day on 6th November in Universiti Teknologi Malaysia

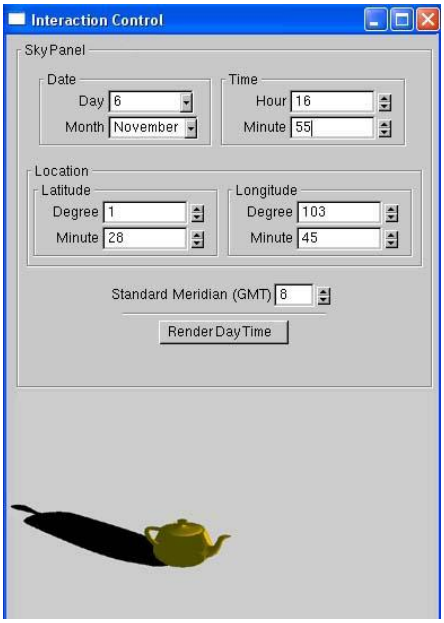


Figure 5. Projection shadow using new formula at 4:55 pm of the day on 6th November in Universiti Teknologi Malaysia

In projection shadow, the amount of color and samplesize are calculated using the (18) and (19) formulas.

$$S_n = S_0 + \sqrt{d}(\frac{\Omega}{\omega})(n-1) \quad (18)$$

$$C_n = C_0 q^{n-1} \quad (19)$$

where

S_0 =0.2 (can be changeable)

S_n is size of nth sample.

Ω is distance between occluder and shadow receiver.

ω is distance between light source and shadow receiver.

d is ratio between two consequent samples.

q is a decimal number to control the amount of penumbra.

C_0 is darkness of umbra.

C_n is color of nth sample.

In this case, the proposed technique is called hard shadow sampling using geometric progression.

Figure 6 illustrates that to control the size of umbra and penumbra, the ratio of Ω and ω is important. To contribute these main parameters Ω/ω in equation (18) is a convenient ratio. The sample size is related to the Ω. When Ω increases, sample sizes will be increased. On the other hand, when ω increases the region of umbra will be decreased. This converse relationship improves the main problem of soft shadow algorithms when the distance between occluder and light source is small.

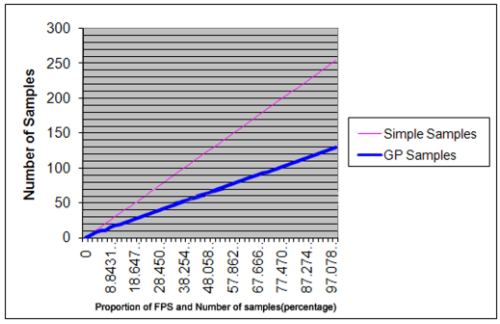


Figure 6. Soft shadow parameters.

4.2 Blending of Hard Shadow Samples

To create soft shadow, sample blending of hard shadow is common. The other contribution of this paper is to combine hard shadow samples using geometric progression to select the color and size of different samples in projection shadow.

In traditional sample blending, when the number of light or number of samples increases, the FPS decreases slightly.

The relationship between the number of hard samples and FPS is illustrates in Figure 7. The initial impression of Figure 7 is that almost half of the samples of traditional sample blending are required for geometric progression blending. This means that with each quality, the FPS of using geometric progression is higher than simple blending. FPS decreases slightly when the number of samples increases. A comparison between FPS without geometric progression and using geometric progression is shown in Figure 7. GP samples are the number of samples using geometric progression. Simple samples are the number of samples without the use of geometric progression. For example, to have good quality soft projection shadows like Figure 8, sample blending using geometric progression requires 130 samples, compared to 255 for soft projection shadow without using geometric progression.

The relation between the required number of samples with the same FPS in simple sample blending and sample blending using geometric progression is shown in Figure 7.

Figure 8 illustrates the relation between sample size and Ωwhere Ωis the distance between occluder and shadow receiver. It shows that when occluder is near the light source, the sample size enlarges faster but when occluder is near the shadow receiver, the sample size enlarges slowly.

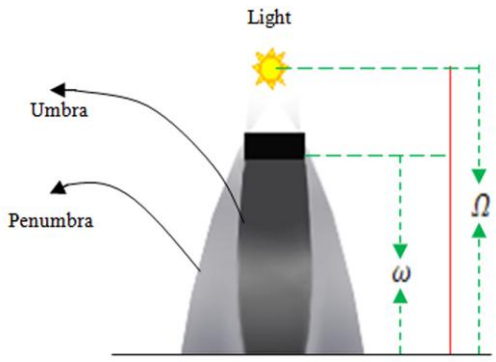


Figure 7. Compare FPS for simple sampling and sampling using proposed algorithm.

Using the proposed technique for samples size not only can improve the FPS but also affects the quality of shadow. As revealed in Figure 7, to create the same result as Figure 11 (Right) using traditional sampling, 780 samples are needed.

A big drawback of projection shadow is the inability to have shadow on other objects. Stencil buffer can solve this problem as well as shadow volume. Figure 9 is the result of soft projection shadow using stencil buffer with 360 samples. Figure 10 is the result of projection shadow using geometric progression and stencil buffer with 255 samples. The difference in quality can be seen in the comparison of 9 and 10.

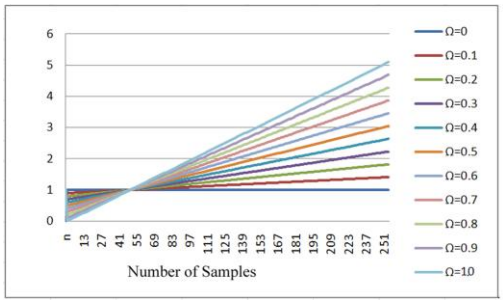


Figure 8. Relation between size of samples and Ω.

5. Conclusion and Future Work

Projection shadows are one of the well-known algorithms used to create shadow. Although it is convenient only for flat surfaces, rendering time is low enough. Projection shadows are not only suitable for flat surfaces but also, with some calculations and using stencil buffer, can be used for casting shadow on arbitrary surfaces. High rendering speed is the main reason we have tried to improve it for generating soft shadow.

Figure 9. Result of soft projection shadow with 360 samples using simple sampling and stencil buffer.

The sun's position is one of main factors in outdoor games. Outdoor rendering is widely used situation for game programmers. Combination of accurate position of the sun and shadows is necessary for mixed reality to cast shadows in precise position.

Soft shadows are the most important shadows and this paper has accordingly focused on them. Soft shadows need big improvements, which can be implemented in a real-time environment. Although sample blending of hard shadow is a common way to create soft shadow, the stability of FPS is very important. FPS is compared using zero to 256 samples without geometric progression and using geometric progression.

Figure 10. Result of soft projection shadow with 256 samples using geometric progression for choosing color samples and proposed formula to choose size sampling and stencil buffer.

To choose color for different samples, to control the sample size in projection shadow and to create soft shadow, geometric progression is used with acceptable results.

The new technique can be implemented in shadow volume to have shadow on arbitrary objects without using double blending.

Figure 11. Left, simple sampling using 360 samples; Right, GP Sampling using 360 samples

Acknowledgements:

This research was supported by UTMVicubeLab at the Department of Computer Graphics and Multimedia, Faculty of Computer Science and Information System, Universiti Teknologi Malaysia. Special thanks to Universiti Teknologi Malaysia (UTM) Vot. J13000.7282.4F085 FRGS Grant for providing financial support of this research.

Corresponding Author:
Hoshang Kolivand,
UTM ViCubelab, Department of Computer Graphics and Multimedia, Faculty of Computer Science and Information System, Universiti Teknologi Malaysia
81310, Johor, Malaysia

shahinkey@yahoo.com

References

1. Pierre (HR) Rautenbach. An empirically devived system for high speed shadow rendering. 2002.
2. Crow F. Shadow Algorithms for Computer Graphics. Computer Graphics 1977; 11(2): 242-247.
3. Williams L. Casting Curved Shadows on Curved Surfaces. SIGGRAPH '78 1978; 12(3).
4. Scherzer D, Wimmer M and Purgathofer W. A survey of real-time hard shadow mapping methods. Computer Graphics Forum, 2011; 30(1):169–186.
5. Kolivand Hm Sunar M S. A Survey on Shadow Volumes in Computer Graphics, IETE Technical Review, vol. 30, no 1, 2013,
6. Bittner J, Mattausch O, Silvennoinen A and Wimmer M. Shadow caster culling for efficient shadow mapping. In Symposium on Interactive 3D Graphics and Games, ACM, New York, NY, USA, I3D 11 2011; 81–88.
7. Donnelly W and Lauritzen A. Variance Shadow Maps. In Proceedings of the 2006 ACM SIGGRAPH Symposium on Interactive 3D graphics and games 2006:161–165.
8. Lauritzen A and McCool M. Layered variance shadow maps. In GI '08: Proceedings of Graphics Interface 2008 Toronto, Ontario, Canada), Canadian Information Processing Society 2008: 139–146.
9. Liang X-H, Ma S, Cen L-X and Yu Z. Light Space Cascaded Shadow Maps Algorithm for Real Time Rendering. Journal of Computer Science and Technology 2011; 26(1): 176–186.
10. Boulanger K. Real-Time Realistic Rendering of Nature Scenes with Dynamic Lighting. University of Rennes I, France, 2008.
11. Tessman and Thant. Casting Shadows on Flat Surfaces. Iris Universe, Winter 1989: 16-18.
12. Wyman C R. Fast Local Approximation to Gobal Illumination. ph.D Thesis, The University of Utah, 2004.
13. Zhang F, Sun H and Nyman O. Parallel-split shadow maps on programmable gpus. In GPU Gems 3. H. Nguyen (Ed.), 2007.
14. Liu L and Xiao S. Real-time soft shadows for large-scale virtual environments. In Multimedia Technology (ICMT), International Conference 2011; 5464-5467.
15. Nawar S, Morcos A and Mikhail J. Photoelectric Study of the Sky Brightness Along Sun's Meridian During the March 29. New Astronomy 2007; 1(12): 562-568.
16. Kambezidis K , Asimakopoulos D and Helmis C. Wake Measurements Behind A Horizontal-Axis 50 Kw Wind Turbine. Solar Wind Tech 1990 177–184.
17. Sugisaki E, Seah H S, Tian F and Morishima S. Interactive shadowing for 2D Anime. Computer Animation and Virtual Worlds 2009; 20: 395-404.
18. Kolivand H and Sunar M S. An Overview on Based Real-Time Shadow Techniques in Virtual Environment, TELKOMNIKA 2012; 10(1): 171-178.
19. Kolivand H and Sunar M S. Real-Time Outdoor Rendering using Hybrid Shadow Maps, International Journal of Innovative Computing, Information and Control (IJICIC) 2012; 8(10): 7169-7184.
20. Kolivand H, Amirshakarami A and Sunar M S. Real-time projectionshadow with respect to suns positionin virtual environments. International Journal of Computer Science Issues 2011; 8(6):80–84.
21. Forest V, Barthe L and Paulin M. Realistic soft shadows bypenumbra-wedges blending. In Proceedingsof Graphics Hardware 2006: 39-47.
22. Bavoil L. Multi-view soft shadows. NVIDIA, technical report, http://developer.nvidia.com, 2011.
23. Zafar H, Zuhairi M, Harle D and Andonovic I. A Review of Techniques for the Analysis of Simulation Output. IETE Technical Review 2012; 29(3): 223-228.
24. Rehman A, Kurniawan F and Saba T An Automatic Approach for Line detection and Removal without Characters Smash-up. Imaging Science Journal 2011;59:171-182.
25. Saba T and Rehman A. Effects of artificially intelligent tools on pattern recognition, International Journal of machine learning and cybernetics, 2012.

9/12/2012

The Comparison of Iranian School Children Performance in Self-concept, Self-efficacy, Self-esteem and Anxiety

Maryam Sahranavard[1], *Siti Aishah Hassan[2], Habibah Elias[1], Maria Chong Abdullah[1]

[1.] Department of Foundations of Education, Faculty of Educational Studies, Universiti Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia
[2.] Department of Counselor Education & Counseling Psychology, Faculty of Educational Studies, Universiti Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia
Sahra1102004@yahoo.com; * siti_aishahh@putra.upm.edu.my

Abstract: The main objective of the present study is to explore the differences between male and female school children performance in students' psychosocial factors; general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety, and science anxiety among lower secondary school children. The participants in the study consisted of 680 lower secondary school children, 14 year olds (317 male and 363 female) at Tehran and Shahriar city, the province of Tehran, Iran. Five valid and reliable instruments were used to assess Self-concept Attribute Attitude Scale, State-Trait Anxiety Inventory, Coopersmith Self-Esteem Inventory, General Self-Efficacy, and Science Self-Efficacy. Descriptive statistics, and to compare male and female students in different variables, MANOVA was used. The results showed that, except for self-concept, there are significant differences in science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety, and science anxiety between male and female students. This study supports the previous finding that boys perform better than girls in the courses related to science. This worthy performance has been reflected in their science self-concept and has resulted in larger mean score in boys in this psychological variable rather than girls.
[Sahranavard. M, Hassan. SA., Elias. H. Abdullah, MC. **Universiti Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia**. *Life Sci J* 2012;9(4):1046-1052] (ISSN:1097-8135).
http://www.lifesciencesite.com. 159

Keywords: self-concept, self-efficacy, self-esteem, anxiety, gender, school children

1. Introduction

Students who believe in their abilities tend to perform more successfully (Bandura, 1993). One of the most important issues of development, education and academic achievement is to consider the student's psychological dimensions in the curriculum. One of these dimensions is *self-efficacy* (first introduced in Bandura & Adams, 1977). Sue, Sue, and Sue (1986) stated that a person with high self-efficacy may engage in a more health-related activity when an illness occurs, whereas a person with low self-efficacy would harbor feelings of hopelessness, and following on from this, science self-efficacy is the belief in one's own capability to study science, in terms of organizing and executing the skills and knowledge needed to manage science content and processes (Miller, 2006).

Self-concept refers to the global understanding a sentiment has of him or herself (Fleming & Courtney, 1984). They also mentioned that it presupposes, but can be distinguished from self-consciousness, which is simply an awareness of one's self. It is also more general than self-esteem, which is the purely evaluative element of the self-concept. science self-concept is a term used to describe one's perception of the self in relation to achievement in science (Byrne & Shavelson, 1987) and one's confidence in science (Campbell, 1992). Self-esteem can generally be defined as the set of attitudes and beliefs that a person bears in relation to the outside world, which includes expectations of success/failure, the effort required for possible success and the reaction to possible failure (Coopersmith, 1967, 1981). Spielberger et al. (1983) state that anxiety is a psychobiological process involving stressors that evoke perceptions of threat, which culminate in an unpleasant emotional reaction. As its name would suggest, science anxiety in students is a debilitating fear of learning science but with the emotion processed on a cognitive level, and lastly, science anxiety manifests itself primarily during examinations, but is distinct from an apprehension towards examinations in general, since students who exhibit science anxiety often react normally in their non-science subjects (Mallow, 1994).

Therefore, Naderi, et al., (2009) indicated that there is no relationship between self-esteem and academic achievement ($r = .074$, $P > 0.05$). And also, Kennedy (1996) states that science self-efficacy does not significantly influence academic achievement. Meanwhile, Milford (2011) conducted a study that showed the relationship was negative between self-concept and academic achievement in science (i.e., countries with higher science self-concept tend to achieve lower on scientific literacy), while science

self-efficacy and science self-concept positively influenced science achievement.

Moreover, one of the most remarkable findings from TIMSS 2007 regarding Iranian Eighth Graders is that male students' science achievement scores have declined significantly (Sharanavard, et al., 2013), while female students' scores have shown a significant improvement compared to the scores of 1999 and 1995 (in Martin, Mullis, Foy, & Olson, 2008). Likewise, a study conducted by Comber and Keeves (1973) showed that girls consistently performed less well than boys in science (Sharanavard, et al., 2012). Similarly, Simpson and Oliver (1990) found that although, males achieved higher scores and possessed a more positive attitude than females, the females were significantly more motivated to achieve in science. Also, Tirri and Nokelainen (2011) show that females tend to attribute success to effort more than males. while totally the anxiety in girls is more than boys, but in science the anxiety of boys has a larger mean score than that of girls, while the research conducted by Spielberger (1972), report higher anxiety levels among females than males, vice versa.

Çakır, Şahin and Şahin (2000), in their study, found out that the variable of gender did not influence science self-concept. A study conducted by Davis (1980), asserted that there are not statistically significant relationship between the sex of the students at each of the four grade levels and their anxiety toward science (at the .05 level). And also, he stated that students of both sexes in each of the grades nine through twelve are equally anxious toward science. Whereas, several studies, for instance, Qi and Zhang (2010) showed that the interaction of students' gender and their self-esteem reached a significant level. And also they stated that the analysis of the interaction revealed that girls in the top and middle self-esteem groups score the teachers higher than the boys, while as for the low self-esteem group, the evaluation of girls and boys are quite similar. Besides, in the evaluation, the interaction of the students' self-esteem and their gender reaches a significant level. Preckel et al., (2008) in their study about gender differences in gifted and average-ability students assert that in both groups, boys earned significantly higher test scores in academic self-concept. A study done by Mahyuddin et al. (2006) showed that girls have higher self-efficacy ($t = -2.7$; $X = 35.5$; $SD = 4.5$; $p = 0.006$) in the English language compared to boys. The under representation of girls in science classes can cause the excluded group to suffer a loss of self-esteem and self-concept (Cohen & Cohen, 1980). Cipriani-Sklar (1996) revealed a significantly positive higher science self-concept in girls. The correlation of science self-concept and science self-efficacy was higher for girls ($r = .53$) than for boys ($r = .39$), as was the correlation of science self-concept and science achievement ($r = .53$ for girls, $r=.39$ for boys) (Britner, 2002). Gender has been found to be a significant factor in many studies of science anxiety (Mallow, 1994; Mallow, 1986).

There is a statistically significant relationship between gender and a person's self-esteem for his or her physical abilities (Longmire, 2008). Qi & Zhang (2010) indicated that the students with low self-esteem score of the teachers, and girls from the groups with high and middle self-esteem have a higher evaluation of the teachers than boys.

Based on to above mentioned and importance of the factors in academic achievement, this study generalized this information to specifically Iranian eighth grade lower secondary school students. Some studies obtained similar results and the other studies were showed different results. This study determined whether, the gender can influence on general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety, and science anxiety among Iranian eighth grade lower secondary school students.

2. Material and Methods

2.1. Sample

The sample for this study is selected from the total population of Eighth Grade students in lower secondary schools from large community schools in Tehran city as urban and Shahriar as suburban and the rural areas of Shahriar, during the academic year of 2010/2011. For the present study, stratified sampling was used, and therefore the sample of this study involves two centrally-located school districts among 21 districts of Tehran with 120 male and 160 female students, and also Shahriar lower secondary schools with 202 male and 198 female students.

2.2. Procedure

Data was collected by means of structured questionnaires and the questionnaires were applied in class. Based on verbal agreements of the training lecturers and participants, the questionnaire forms were distributed to the 680 participants and were asked to complete the questionnaires simultaneously at the start of a core lecture and return them to their lecturer Immediately after completing them. All completed questionnaires were passed on to the researchers.

2.3. Measures

All participants responded to Iranian translation of the instruments in this study which is listed below. They were translated into Persian and then the questionnaires were verified by the panel of lecturers and researchers to check the format, arrangement, appropriateness of the content and the language used in the instruments (Asghar-Nezhad,

Karimi Klwadapanahi, & HeydariI, 2004; Fathi-Ashtiani, Ejei, Khodapanahi, & Tarkhorani, 2007; Fathi, 2006b; Hayati & Ostadian, 2008; Khodarahimi, 2010).

2.3.1. Self-concept Attribute Attitude Scale (SaaS);

The SaaS instrument was developed by Campbell (1991). The response format is a five-point Likert scale. The first version of SaaS was developed by factor analyzing the data from 1300 high achieving high school students, with exploratory and confirmatory factor analyses determined for each sample. These factors were extracted by using the Principal Component Analyses with varimax iterations. The three factors that were produced from the factor analyses are math self-concept, science self-concept, and general self-concept. In the present study, only general self-concept and science self-concept were used which include 6 and 14 items relating to general self-concept, For example, I take a positive attitude toward myself and science self-concept, for example, I have a lot of self-concept in science. A major contribution to the validity of the self-concept scales comes from the extensive factor analyses used in the development of the SaaS. Most items had factor loadings in excess of .60 (Campbell, 1991). Alpha reliability values were calculated for general self-concept of 0.85 and a science self-concept of 0.89 were used, (Carmines & Zeller, 1979). In this study, the reliability coefficient for each subscale ranged between 0.87 for science self-concept and 0.61 for general self-concept.

2.3.2. State-Trait Anxiety Inventory (STAI);

The STAI developed by Spielberger (1970a) contains self-report scales for measuring both state and trait anxiety. The S-Anxiety Scale (STAI Form Y-1) used in this study consists of twenty statements designed to evaluate how a respondent feels at that particular time, for example, I feel calm in science. The T-anxiety (STAI Form Y-2) refers to the relatively stable-individual differences in anxiety proneness, i.e., the tendency of an individual to perceive stressful situations as a threat, and to then respond to these situations with a heightened S-anxiety reaction (O'Neil & Spielberger, 1979) and used in this study consists of twenty statements, for example, I feel pleasant. The S-Anxiety Scale required the respondent to determine how he or she feels at a particular moment in time. Evidence bearing on the construct validity of the state scales was derived from a sample of 977 undergraduate students at Florida State University with a median r of .73 for females and .60 for males (Spielberger, Gorsuch, & Lushene, 1983). Caldwell (1988) obtained an alpha coefficient of 0.94 for the S-Anxiety. T-Anxiety scores (Dreger, 1978; Katkin, 1978). In this study, the reliability coefficient for each subscale ranged between 0.88 for S-Anxiety and 0.85 for T-Anxiety.

2.3.3. Coopersmith Self-Esteem Inventory (CSEI);

The CSEI measures general self-esteem. Coopersmith's (1967) own inductive work examined CSEI scores as they related to other personality constructs. The present study has used the Adult Form of the CSEI, which is adapted from the School Short Form for children. The CSEI-A is a 58-item questionnaire completed by respondents by way of answering a five-point Likert scale. As Coopersmith (1967) claims, the questionnaire is designed to measure "the evaluation a person makes and customarily maintains with regard to him or herself". The CSEI has been the subject of many validity research studies (Taylor & Reitz, 1968). For example, I spend a lot of time daydreaming. A study by Kokenes (1978) confirmed the construct validity of the subscales used to measure of self-esteem that were proposed by Coopersmith. Test retest reliability for the CSEI was originally reported by Coopersmith to be 0.88 for a sample of 50 children in grade V and 0.70 for a sample of 56 children, 12 years old (Azar & Vasudeva, 2006). In this study, the Cronbach's coefficient alpha for CSEI was 0.86.

2.3.4. General Self-Efficacy (GSE);

General Self-Efficacy (GSE) developed by Sherer et al. (1982) is designed to gauge self-efficacy in clinical, educational, and organizational settings (Chen, Gully, & Eden, 2001). The measure contains items assessing GSE and social self-efficacy, but only GSE items be considered in the present study. As Sherer et al. (1982) claim, these items tap a "general set of expectations that the individual carries into new situations." The GSE Scale contains is 17-items, for example, When I make plans, I am certain I can make them, while the response format is a five-point Likert scale. The sum of item scores reflects general self-efficacy, meaning that the higher the total score, the more self-efficacious the respondent. Convergent validity has been established in studies comparing the general self-efficacy scale and similar clinical measures (Sherer, *et al.*, 1982). Reliability, measured with Chronbach's alpha, was found to be .86 for General Self- Efficacy (Sherer, *et al.*, 1982). In this study, the Cronbach's coefficient alpha for CSE was 0.79.

2.3.5. Science Self-Efficacy Questionnaire (SSEQ);

The SSEQ was developed by Smist (1993) to assess students' self-efficacy in science by measuring beliefs about competence in school science tasks (Smist, 1993). The SSEQ-A is a 27-item questionnaire completed by respondents by way of answering a five-point Likert scale. The SSEQ was developed to assess students' self-efficacy in science by measuring students' own beliefs about their competence to

perform or complete science-related tasks. This questionnaire includes physics, chemistry, biology, and laboratory. The researcher has used science totally. In the present study, only science self-efficacy was included which includes nine items related to science, for example, I can use a computer in science class. In this study, the Cronbach's coefficient alpha for SSEQ was 0.70.

3. Results

Data was analyzed by using statistical package for social sciences (SPSS 18.0). Besides descriptive statistics, MANOVA was also used in this study.

3.1. Descriptive statistics;

A perusal of table 1 reveals that the largest mean scores on self-efficacy for male students is 192.16 with the SD of 25.4 and the smallest mean scores on science anxiety for male students is 21.24 with the SD of 4.3. Also the largest mean scores on self-efficacy for female students is 185.82 with the SD of 24.91 and the smallest mean scores on science anxiety for female students is 20.30 with the SD of 4.34.

3.2. MANOVA

To compare male and female students in different variables, MANOVA (Multivariate Analysis of Variance) was used. First, the important assumptions for the method such as, outlier and homogeneity of variance-covariance matrices were investigated.

The results of normality show the distribution of science self-concept, self-concept and anxiety in boy and girl groups have non-normal distribution, but, the results of Shapiro Wilk show that all variables in two groups have normal distribution. The results of statistics of skweness and kurtosis show that all values of this statistics are common range ±1. Therefore, the assumption of normality can be accepted (Meyers, Gamset, & Guarino, 2003). Based on the results of Mahalanobis distances, there was no multivariate outlier data. The results of Box's Test shows that covariance matrix of dependent variables in different levels of independent variable of gender is not equal ($p<0.001$). Therefore, the assumption of equality of covariance of the matrix of dependent variables is not supported. The results of Levene's test shows that the assumption of equality of variance for all of the dependent variables in two groups (boy and girl) can be accepted.

Based on the results of Box's Test, Pillai's trace results is used instead of Multivariate Wilks' lambda results, in order to report significance of linear combination of the dependent variables in different levels of gender. Since, significant level is smaller than $\alpha = 0.01$, the null hypothesis stating that there is no significant difference between boys and girls in dependent variables can be rejected ($F_{(7,672)} = 5.182$, $P<0.0001$). Consequently, there is significant different between two groups of boys and girls in dependent variables.

Finally, based on the results of table 2, the investigation of performance of two groups in each variable shows that except for self-concept ($p>0.05$), there is significant difference in the other variables between two groups ($p<0.05$). Investigation of η coefficient for the significant variables shows that in science self-concept, science anxiety, anxiety, self-esteem, self-efficacy, and science self-efficacy respectively 2.5%, 1.2%, 4.3%, 2.7%, 1.6% and 1.5% of variance is due to independent variable of gender.

4. Discussion

The Investigation of the results of descriptive statistics in two groups shows that mean of science self-concept for boys is more than girls. These results also are in line with other studies (Cipriani-Sklar, 1996; Cohen & Cohen, 1980) indicated that science self-concept for boys is more than girls. While, some of other studies showed that this variable for girls is more than boys (Britner, 2002). Moreover, many studies showed that there is no difference between male and female students in science self-concept and science anxiety (Çakir, et al., 2000; Davis, 1980). This finding supports the old findings that boys perform better in the courses related to physics has been reflected in their science self-concept and has resulted in larger mean score in boys in this psychological variable rather than girls (Beaton, et al., 1996; Erickson & Farkas, 1991; Martin, et al., 2000).

Investigation of the results of the mean of self-esteem for girls is more than boys. This result is in line with other studies such as, (Qi & Zhang, 2010) indicated that this variable for girls more than boys. Although, in the other studies signified that self-esteem for boys more than girls (Cohen & Cohen, 1980) The greatest effect of gender was on anxiety and self-esteem. The gender was determined 4.3% and 2.7% variances respectively for those variables. Also, available evidences have supported gender differences in self-esteem (Sar-Abadani-Tafreshi, 2006). It could be said that the source of anxiety and self-esteem are different in girls and boys. If we accept that the ability in science is more important among boy groups, therefore, the amount of their science anxiety will be higher than that of girls but the source of anxiety or the feeling of self-efficacy in girls may be more related to the verbal domain.

Finally, the mean of self-efficacy and science self-efficacy for boys is more than girls. In line with this result, Mahyuddin et al. (2006) showed that similar results in their study. It could be said that the culture influences the relation between self-efficacy, self-esteem and achievement. According to Bandura

(1997), multicultural societies possess lower self-efficacy than individualistic ones. The investigation of cultural differences in social cognitive theory can serve as a sound basis for research. Understanding the relation between self-efficacy, self-esteem and achievement in Iran, as a more multicultural and traditional society than western societies, and comparing the results with previous research findings could pave the way for a better understanding of self-efficacy.

5. Conclusion

The results indicated that there is no statistically significant difference between boys and girls in the variable, self-concept however, in the science self-concept variable; the mean of the boys group is more than the girls. Therefore this finding supports the previous finding that, boys perform better in the courses related to physics and its worthy performance has been reflected in their science self-concept and has resulted in larger mean score in boys in this student's psychological variable rather than girls. While, there are several demographic that could be affect the preference for the relationship between self-concept, self-efficacy, self-esteem, anxiety and gender which should be studied in the future, in the present study focused on gender only. Implication of the study was derived from the fact that student's psychological factors are important indicators for quality learning outcomes, students who believe in their abilities tend to perform successfully. If students can be enabled to be more aware of their abilities and the ways in which they are likely to achieve better, they can be encouraged to develop more effective and more flexible psychological factors. Future research should examine whether the present findings generalize to other samples and settings. In addition, there may be cultural differences that influence the concept of self as it relates to achievement.

Table 1. Descriptive Statistics with respect to gender

Dependent Variables	Gender	Mean	Std	N
Science self-concept	Girl	46.49	10.05	364
	Boy	49.68	9.86	316
	Total	47.97	10.08	680
Self-concept	Girl	57.98	10.26	364
	Boy	59.33	9.89	316
	Total	58.61	10.11	680
Science anxiety	Girl	20.30	4.34	364
	Boy	21.24	4.3	316
	Total	20.73	4.35	680
Anxiety	Girl	46.20	10.86	364
	Boy	41.51	11.18	316
	Total	44.02	11.25	680
Self-esteem	Girl	47.06	10.9	364
	Boy	43.57	9.79	316
	Total	45.43	10.54	680
Self-efficacy	Girl	185.82	24.91	364
	Boy	192.16	25.4	316
	Total	188.77	25.32	680
Science self-efficacy	Girl	27.66	6.86	364
	Boy	29.29	6.33	316
	Total	28.42	6.67	680

Table 2. Tests of Between-Subjects Effects with respect to gender

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Gender	Science self-concept	1719.047	1	1719.04	17.304	.000	.025
	Self-concept	308.138	1	308.13	3.023	.083	.004
	Science anxiety	150.655	1	150.65	8.042	.005	.012
	Anxiety	3724.149	1	3724.14	30.711	.000	.043
	Self-esteem	2063.023	1	2063.02	19.054	.000	.027
	Self-efficacy	6804.681	1	6804.68	10.764	.001	.016
	Science self-efficacy	449.588	1	449.58	10.234	.001	.015
Error	Science self-concept	67355.869	678	99.34			
	Self-concept	69111.393	678	101.93			
	Science anxiety	12700.761	678	18.73			
	Anxiety	82216.635	678	121.26			
	Self-esteem	73408.747	678	108.27			
	Self-efficacy	428627.254	678	632.19			
	Science self-efficacy	29786.188	678	43.93			

Acknowledgments:

We would like to thank all the administration officers at all participating schools for giving the research team information about their students in the schools. We also appreciate the contribution of lower secondary schools students by participating in this research, thus allowing us to collect the necessary data for the study.

Corresponding Authors:
Siti Aishah Hassan
Maryam Sahranavard
Faculty of Educational Studies

Universiti Putra Malaysia (UPM)
43400 UPM Serdang, Selangor, Malaysia.
E-mail: siti_aishahh@putra.upm.edu.my
sahra1102004@yahoo.com

References

1. Asghar-Nezhad, T., Karimi Klwadapanahi, M., & HeydariI, M. (2004). The relationship between general self-efficacy, locus of control and academic achievement *Journal of Psychology*.
2. Azar, I. A. S., & Vasudeva, P. (2006). Relationship between Quality of Life, Hardiness, Self-efficacy and Self-esteem amongst Employed and Unemployed Married Women in Zabol. *Iranian Journal of Psychiatry, 1*(3).
3. Bandura, A. (1993). Perceived self-efficacy in cognitive development and functioning. *Educational Psychologist, 28*(2), 117-148.
4. Bandura, A. (1997). *Self-efficacy: The exercise of control*: Worth Publishers.
5. Bandura, A., & Adams, N. E. (1977). Analysis of self-efficacy theory of behavioral change. *Cognitive Therapy and Research, 1*(4), 287-310.
6. Beaton, A. E., Mullis, I. V. S., Martin, M. O., Gonzales, E. J., Kelly, D. L., & Smith, T. A. (1996). *Mathematics achievement in the middle school years: IEA's Third International Mathematics and Science Study (TIMSS)*: TIMSS International Study Center, Boston College, Chestnut Hill, MA.
7. Britner, S. L. (2002). *Science Self-Efficacy of African American Middle School Students: Relationship to Motivation Self-Beliefs, Achievement. Gender, and Gender Orientation. Published doctoral dissertation* Georgia State University.
8. Byrne, B. M., & Shavelson, R. J. (1987). Adolescent self-concept: Testing the assumption of equivalent structure across gender. *American Educational Research Journal, 24*(3), 365.
9. Çakir, Ö., Sahin, T., & Sahin, B. (2000). lkö retim 6. s n f fen bilgisi dersinde çe itli de i kenlerin ö rencilerin duyu sal alanlarini açiklama gücü. *Hacettepe Üniversitesi E itim Fakültesi Dergisi, 19*, 43-49.
10. Caldwell, L. M. (1988). *Preferences for information and self-care, stress, and coping with outpatient surgery: A descriptive correlational study.* Unpublished Dissertation Abstracts International., Boston University.
11. Campbell, A. M. (1992). Chromosomal insertion sites for phages and plasmids. *Journal of bacteriology, 174*(23), 7495.
12. Campbell, J. R. (1991). The roots of gender inequity in technical areas. *Journal of Research in Science Teaching, 28*, 251-262.
13. Carmines, E. G., & Zeller, R. A. (1979). *Reliability and validity assessment CA: Sage Publications*.
14. Chen, G., Gully, S. M., & Eden, D. (2001). Validation of a new General Self-Efficacy Scale. *Organizational Research Methods, 4*, 62-83.
15. Cipriani-Sklar, R. (1996). *A quantitative and qualitative examination of the influence of the normative and perceived school environments of a coeducational public school vs. A single-sex catholic school on ninth-grade girls' science self-concept and anxiety in the area of science education* St. John's University, Published doctoral dissertation, New York.
16. Cohen, R. F., & Cohen, M. R. (1980). Opening new doors: taking sex-role stereotyping out of science and mathematics. *School Science and Mathematics, 80*, 566-572.
17. Comber, L., & Keeves, J. P. (1973). Science Education in Nineteen Countries. International Studies in Evaluation I.
18. Coopersmith, S. (1967). *The antecedents of self-esteem*: WH Freeman San Francisco.
19. Coopersmith, S. (1981). *Self-esteem inventories*: Consulting Psychologists Press Palo Alto, CA.
20. Davis, G. R. (1980). *The relationship between science anxiety and formal reasoning in secondary students.* University of Northern Colorado, Dept. of Science Education,(Spring) quarter, 1981. Major advisor.
21. Dreger, R. M. (1978). *Review, state-trait anxiety inventory*. New Jersey: The Gryphon Press.
22. Erickson, G., & Farkas, S. (1991). Prior experience and gender differences in science achievement. *Alberta journal of educational research, 37*(3), 225-239.
23. Fathi-Ashtiani, A., Ejei, J., Khodapanahi, M. K., & Tarkhorani, H. (2007). Relationship Between Self-Concept, Self-esteem, Anxiety, Depression and Academic Achievement in Adolescents. *Journal of Applied Sciences, 7*, 995-1000.
24. Fathi, H. (2006). *A Study On The Relationship Between Self-Esteem And Academic Achievement Of Iranian Students [BF697. 5. S46 H615 2006H615 2006 ff rbrb]*. Universiti Sains Malaysia Penang, Published Master of thesis.
25. Fleming, J. S., & Courtney, B. E. (1984). The dimensionality of self-esteem: II. Hierarchical facet model for revised measurement scales. *Journal of personality and social psychology, 46*(2), 404-421.
26. Hayati, A. M., & Ostadian, M. (2008). The Relationship between Self-esteem and Listening Comprehension of EFL Students.
27. Katkin, E. S. (1978). *Review, state-trait anxiety inventory*. New Jersey: The Gryphon Press.
28. Kennedy, H. L. (1996). *Science learning: A self-efficacy study in higher education.* University of Southern California.
29. Khodarahimi, S. (2010). General self-efficacy and worry in an Iranian adolescents and youths samples.
.

30. Kokenes, B. (1978). A factor analytic study of the Coopersmith Self-Esteem Inventory. *Adolescence*.
31. Longmire, B. M. (2008). *Exploring the relations among racial identity, perceptions of family socioeconomic status, and self-esteem for emerging adults*. New York University.
32. Mahyuddin, R., Elias, H., Cheong, L. S., Muhamad, M. F., Noordin, N., & Abdullah, M. C. (2006). The Relationship Between Students' Self Efficacy And Their English Language Achievement. *Malaysian Journal of Educators and Education, 21*, 61-71.
33. Mallow, J. (1994). Gender-related science anxiety: A first binational study. *Journal of Education and Technology, 3*, 227-238.
34. Mallow, J. V. (1986). *Science Anxiety: Fear of Science and How to Overcome It* (Revised edition. Clearwater, FL ed.): H & H Publ.
35. Martin, M. O., Mullis, I. V. S., Foy, P., & Olson, J. F. (2008). *TIMSS 2007 international mathematics report: Findings from IEA's trends in international mathematics and science study at the fourth and eighth grades*.
36. Martin, M. O., Mullis, I. V. S., Gonzalez, E. J., Gregory, K. D., Smith, T. A., Chrostowski, S. J., et al. (2000). *TIMSS 1999 international science report: Findings from IEA's repeat of the Third International Mathematics and Science Study at the eighth grade*: International Study Center, Lynch School of Education, Boston College, Chestnut Hill, MA.
37. Meyers, L. S., Gamset, G., & Guarino, A. J. (2003). *Applied Multivariate research.* . Thousand Oaks. London. New Delhi.: SAGE Publications.
38. Milford, T. (2011). *An Investigation of International Science Achievement Using the OECD's PISA 2006 Data Set. Published doctoral dissertation.* University of Victoria.
39. Miller, M. D. (2006). *Science self-efficacy in tenth grade Hispanic female high school students.* University of Central Florida Orlando, Florida.
40. Naderi, H., Abdullah, R., Aizan, H. T., Sharir, J., & Kumar, V. (2009). Selfesteem, gender and academic achievement of undergraduate students. *American Journal of Scientific Research, 3*, 26-37.
41. O'Neil, H. F., & Spielberger, C. D. (1979). *Cognitive and affective learning strategies*: Academic Pr.
42. Preckel, F., Goetz, T., Pekrun, R., & Kleine, M. (2008). Gender Differences in Gifted and Average-Ability Students: Comparing Girls' and Boys' Achievement, Self-Concept, Interest, and Motivation in Mathematics. *Gifted Child Quarterly, 52*(2), 146.
43. Qi, G., & Zhang, W. (2010). A Study of Teaching Evaluation Based on Network: PE Majors' Evaluation of the Theory Course of College Teachers. *Asian Social Science, 6*(9), P100.
44. Sar-Abadani-Tafreshi, L. (2006). *The relationship between academic achievement, Self-Esteem and Gender with Anxiety of Computer among Postgraduate of Students in University of Tabeiyat Moallem Tehran. Unpublished Master of thesis.* University of Tabeiyat Moalem, Tehran, Iran.
45. Sahranavard, M., Hassan S. A., Elias, H., & Abdullah, M. C. (2013). Comparing the scores of 8th grade Iranian students at various income levels in psychological measurement tools. *Asia Life Science. 22(1): 61-73.*
46. Sahranavard, M., Hassan S. A., Elias, H., & Abdullah, M. C. (2012). Student's psychological factors and science performance: does gender matter for Iranian Students. *Life Science Journal. 9(3):2069-2075.*
47. Sherer, M., Maddux, J. E., Mercandante, B., Prentice-Dunn, S., Jacobs, B., & Rogers, R. (1982). The Self-Efficacy Scale: Construction and validation. *Psychological Reports, 51*, 663-671.
48. Simpson, R. D., & Oliver, J. S. (1990). A summary of major influences on attitude toward and achievement in science among adolescent students. *Science Education, 74*(1), 1-18.
49. Smist, J. M. (1993). General Chemistry and Self-Efficacy.
50. Speilberger, C. D. (1972). *Anxiety as an emotional state*. New York: Academic Press.
51. Spielberger, C. D., Gorsuch, R. L., & Lushene, R. (1983). Manual for the state-trait anxiety inventory.
52. Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *State-trait anxiety inventory-STAI manual: Calif: Consulting Psychologists Press*: Inc.
53. Sue, D., Sue, D. W., & Sue, S. (1986). *Understanding abnormal behavior*: Houghton Mifflin Boston.
54. Taylor, J., & Reitz, W. (1968). The three faces of self-esteem. *Research Bulletin #80. London, Ontario: University of Western Ontario*.
55. Tirri, K., & Nokelainen, P. (2011). The Influence of Self-Perception of Abilities and Attribution Styles on Academic Choices: Implications for Gifted Education. *Roeper Review, 33*(1), 26-32.

FLT3 internal tandem duplication and JAK2 V617F mutations in *de novo* acute myelogenous leukemia: relation with induction chemotherapy and overall survival

Magda M Assem[1], Magda M Noshy[2], Ghada M Elsayed[1], Hanan R Nassar[3] Gamal Thabet[1], Ghada M Sherif [4] and Aida K Ahmad[2]

[1]Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Egypt
[2]Zoology Department, Faculty of Science, Cairo University, Egypt
[3]Medical oncology, National Cancer Institute, Cairo University, Egypt
[4] Biostatistics and Epidemiology Department, National Cancer institute, Cairo University, Egypt
elsayed276@yahoo.com; magda_assem123@yahoo.com

Abstract: Molecular characterization of acute myeloid leukemia (AML) allows prognostic stratification and assessment of the chances of durable treatment response. The presence of FLT3 internal tandem duplication (ITD) mutation as well as the allelic ratio (ITD-AR) and JAK2 V617F mutation may be associated with clinical outcome in patients with AML. FLT3-ITD and JAK2 V617F mutation status was determined for 194 patients with de novo AML. ITD-AR was calculated for patients with FLT3- ITD. Clinical characteristics and outcomes for patients with different FLT3 genotypes were compared. In the total group of 194 patients, FLT3- ITD mutation was detected in 34 (17.5%) patients, 30 (18.8%) adults and 4 (11.8%) pediatric. JAK2-V617F mutation was detected in one patient (0.5%). Among the adult group, patients with FLT3/ITD had a significantly elevated diagnostic white blood cell count (WBC) compared to patients with FLT3 WT/WT genotype (p=0.02).Sixty three (61.2%) achieved complete remission (CR), 52 (82.5 %) were of the FLT3 WT/WT genotype and 11(17.5%) of the FLT3 WT/ITD genotype (p=0.75). Overall survival (OS) of patients with FLT3 WT/ITD group was shorter (28.5%) when compared with for the FLT3 WT/WT group (40.8%) although no significant difference was detected(p=0.2). The disease free survival (DFS) for patients with FLT3 WT/ITD genotype was (100%) compared to (86%) for patients with FLT3 WT/WT genotype, with no significant difference (p=0.3) between the two groups. In conclusion we found that FLT3-ITD mutation is a frequent finding in adult patients with *de novo* AML. There is a significant association between FLT3-ITD mutation with high WBC count and a tendency towards a worse prognosis. The ratio of mutant to wild allele level may have a strong relation to the patient outcome. JAK2-V617F mutation is infrequent finding in *de novo* AML.

[Magda M Assem, Magda M Noshy, Ghada M Elsayed, Hanan R Nassar, Gamal Thabet, Ghada M Sherif and Aida K Ahmad. **FLT3 internal tandem duplication and JAK2 V617F mutations in *de novo* acute myelogenous leukemia: relation with induction chemotherapy and overall survival.** *Life Sci J* 2012;9(4):1053-1060]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 160

Key words: AML, FLT3-ITD, JAK2 V617F

1. Introduction

Acute myeloid leukemia is being revealed as an increasingly heterogeneous entity as the molecular aberrations underlying it are defined. Such information is fundamental in assessment of the chances of durable treatment response. Morphological complete remission is now achieved in the majority of patients with current chemotherapeutic regimens, so the main determinants of prognosis are therefore those variables that influence treatment-related death or relapse risk [(1)].

The Fms-like tyrosine kinase 3 (FLT3) genes encodes a class III tyrosine kinas that plays important roles in cellular proliferation and differentiation. To date, 2 types of FLT3 mutations have been found to induce autophosphorylation through ligand-independent FLT3 dimerization, leading to uncontrolled hematologic progenitor cell proliferation and malignancy. One such mutation is an internal tandem duplication of the region between exon 14 and 15 encoding the juxtamembrane domain; the other, in exon 20, is a point mutation in aspartic acid residue 835 (D835) within the activation loop of the second tyrosine kinase domain [(2)]. FLT3-ITD mutation is a common finding in approximately 25% of younger adult AML patients. Many studies have found that FLT3-ITD mutation is associated with adverse prognosis [(3)].

An acquired mutation in the JAK2 gene has recently been described in human myeloproliferative disorders (MPD). JAK2 is a cytoplasmic tyrosine kinase that plays an essential role in the signaling pathways of cytokines and growth factors. The mutation 1849 G>T, which leads to amino acid substitution of phenylalanine for a highly conserved valine (V617F), renders JAK2 kinase constitutively active and leads to cell proliferation in the absence of the growth factors [(4)]. Now there is evidence in the

literature that patients with AML with an antecedent MPD often have JAK2 V617F mutations [5].

Only recently, a few patients with AML without previous hematologic disorders were found to have the JAK2-V617F mutation. It can be assumed that mutations of JAK2-V617F lead to a more aggressive subtype of leukemia because of the activation of the JAK2-STAT5 cascade which substantially alters apoptotic response, self-renewal and proliferative capacity of myeloid cells [6]. It is of note that a high prevalence of co-operating mutations of FLT3, KIT, or N-RAS in AML patients with the JAK2 mutation has been reported.

The aim of this study was to assess the frequency and the prognostic impact of FLT3-ITD and JAK2- V617F gene mutations on the outcome of patients with *de novo* AML.

2. Patients and methods

The study population

DNA was available from bone marrow (BM) or peripheral blood (PB) samples at diagnosis from 194 patients with *de novo* AML. All patients presented to outpatient clinic of the National Cancer Institute (NCI), Cairo University, in a six month period from May 2010 to December 2010. Thirty four patients were children (≤ 16 years) with a median age of 7.5 (0.5-16) years and 160 were adults (> 16 years) with a median age of 40 (18-80) years. This study was approved by the local ethics committee at the university. No potential conflicts exist.

All adult patients received the 3 and 7 protocols which consisted of adriamycin 30mg/m2 for 3 days and ARAC 100mg/m2 by continuous infusion for 7 days. Further treatment of AML patients was according to their risk group. Patients with favorable risk group were treated with high dose ARAC containing usually HAM protocol(ARAC 1 gm/m2 over 3 hours infusion every 12 hours day 1 to 3 and mitoxantrone 12mg/m2 short infusion days 3 to 5. If relapse occurred to these patients a second induction by HAM or AVVV protocol was given. Then if donor was available, an allogenic BM transplantation was carried out. For unfavorable risk group, allogenic BMT was carried out if a suitable donor was available, but if not HD-ARAC containing regimen was given for 3 cycles then autologous BMT was done. If relapse occurred for this group of patients they were treated with palliative care only.

Pediatrics protocol consisted of two induction courses of ADE protocol (doxorubicin 50mg/m2 D 1, 3, 5, ARAC 3.3 MG/KG D 1 to 10 and etoposide 100mg/m2 D 1 to D 5). Intensification course was done by 4 cycles of MIDAC (ARAC 1gm/m2 every 12 hours for 6 doses and mitoxantrone 8mg/m2 D 1to D3). For special subgroups of AML, special treatment was used. Patients with AML M5 have high risk of central nervous system disease, so they were given triple intrathecal prophylaxis (methotrexate 15mg, ARAC 40mg and dexamethasone 4mg) every 8 weeks for a total 6 injections with induction treatment. If CNS disease was diagnosed at presentation, triple intrathecal injections was given until CSF is free then craniospinal irradiation was given to be followed by intrathecal injection of ARAC and dexamethasone every 8 weeks for 7 doses. Patients with AML M3, induction treatment was consisted of ATRA 45mg/m2 oral daily in divided doses every 12 hours till complete remission or for maximum 90 days, and adriamycin 30mg/m2 for 3 days for every month for 3 courses. These patients received maintenance treatment after complete remission with ATRA (45mg/m2 oral daily for two weeks every 2 years) and 6 mercaptopurine and methotrexate for 2 years.

Morphologic analysis

Peripheral blood and BM smears were stained by standard techniques for diagnosis of AML and assignment of French- American- British (FAB) subtypes [7].

FLT3-ITD gene mutation analysis

High molecular weight DNA was extracted from EDTA anticoagulated BM samples using of QIAamp DNA blood Mini Kit (QIAGEN).Earlier studies have showed that the location of ITD of FLT3 gene is at exon 14 and exon 15 [8]. PCR amplification was performed on the 194 samples using the (11F): 5'-GCAATTTAGGTATGAAAGCCAGC -3' and (12R): 5'- CTTTCAGCATTTTGACGGCAACC -3'. In brief, 1ul DNA was amplified in a volume of 25 µl containing 50 mM KCl, 10 mM Tris-HCl, PH 8.3, 1.5 Mm MgCl2, 200mM dNTPs, 0.5uM of each primer and 1U Taq DNA polymerase (QIAGEN). The PCR consisted of an initial incubation step at 94 °C for 150 seconds followed by 35 cycles at 94 °C for 30 seconds, 57 °C for 60 seconds, and 72 °C for 120 seconds, and a final elongation step at 94 °C for 30 seconds and 60 °C for 10 minutes. The PCR product was analyzed on standard 3% agarose gel. A fragment of 328 base pair (bp) was produced from wild- type (WT) alleles. All patient with an additional higher molecular weight band was considered to be FLT3/ITD+.

Semiquantitative analysis of FLT3-ITD gene mutation

To calculate the allelic ratio (mutant/wild), densitometric estimations of ethidium bromide–stained agarose gels with (BioDocAnalyze (BDA) software (Biometra), Germany) which is included in BioDocAnalyze gel documentation system was performed. The intensity of the mutant and the wild fragments were measured and then the ratio of the mutant to the wild allele was calculated [9]. ITD-

allelic ratio threshold (>0.4) was used to separate FLT3-ITD population into risk groups [10].

V617F genotyping by amplification refractory mutation system (ARMS)

Genomic DNA was analyzed for V617F JAK-2 mutation as previously described by Jones et al [11]. PCR primers were: forward outer (FO), 5`-TCCTCAGAACGTTGATGGCAG-3`; reverse outer (RO), 5`ATTGCTTTCCTTTTTCACAAGAT-3`; forwardwild-type–specific(Fwt),5`-GCATTTGGTTTTAAATTATGGAGTATaTG-3`; reverse-mutant–specific (Rmt), 5`GTTTTACTTACTCTCGTCTCCACAaAA-3`. Amplifications were performed for 30 cycles with HotStar Taq polymerase (QIAGEN), an annealing temperature of 60°C, 25 ng genomic DNA, and standard amplification conditions, except that the final concentrations of the outer primers and the Mutant/wild-type–specific inner primers were 1μM and 0.5 μM, respectively. Products were resolved on 3% agarose gels and visualized after staining with ethidium bromide.

Statistical methods

SPSS package version 17.0 was used for data management. Non-parametric ANOVA and t- test compared means of independent groups. Chi-square tested proportion independence. Kaplan-Meier method was used to estimate survival and log rank compared curves. *P* value ≤ 0.05 is significance.

Endpoints

Complete remission required a normocellular BM containing fewer than 5% blasts with evidence of normal maturation of other marrow elements, a neutrophil count of 10^9/L and a platelet count of 100 > 10^9/L. Remission failures were classified by the clinicians as either partial remission (PR, defined as 5%-15% blasts or fewer than 5% blasts but a hypocellular BM), resistant disease (RD, more than 15% blasts in the BM), or induction death (ID; i.e., related to treatment or hypoplasia). Where the clinician's evaluation was not available, deaths within 30 days of entry were classified as ID and deaths later than 30 days after entry as RD. DFS was defined only for patients who achieved CR and was measured from the documented date of CR until date of relapse or death regardless of cause (death in first CR or relapse), censoring for patients alive in continuous CR. Overall survival was measured from the protocol on-study date until date of death, regardless of cause of death, censoring for patients alive [12].

3. Results

Frequencies, laboratory and clinical characteristics

The study included 194 *de novo* AML patients, 160 adults and 34 children. In the total AML group, FLT3- ITD mutation was detected in 34 (17.5%) patients. In the adult AML group, 30 (18.8%) were found to be FLT3-ITD positive, while in pediatric AML group; the mutation was detected in 4 (11.8%) patients. Details of clinical characteristics for the adult and pediatric groups are shown in table 1 and 2. In two of the 34 cases with FLT3-ITD more than one mutant allele was detected, and in one case no DNA PCR evidence of FLT3 (WT) allele was detected. This indicates that the mutant allele was predominant in three cases, figure1. Laboratory and clinical characteristics of adult patients in the FLT3 WT/WT and FLT3 WT/ITD genotype groups were compared in table 3. Patients with FLT3/ITD had a significantly elevated diagnostic white blood cell count (WBC) with a median of $36.5x10^9$/L compared with $19.4x10^9$/L for patients with FLT3 WT/WT genotype (*p*=0.02). No significant difference was found when we compared other clinical and laboratory parameters.

The mean value for the ITD-allelic ratio (ITD-AR) was 1.2± 0.75 and median was 1.14(0.13 -3.7). When we used the threshold (0.4) for risk stratification of FLT3-ITD positive patients, only three patients were found to have ITD- AR the (≤ 0.4). Statistical analysis of FLT3-ITD patients using ITD-AR threshold could not be done because of the small number of patients in the (≤ 0.4) group. Among the pediatric group FLT3- ITD was detected in 4 out of 34 patients (11.8%) which was a small number to compare among FLT3 genotypes.

JAK2-V617F mutation was detected in one out of 194 AML patients (0.5%). This patient was fifteen years old male, with AML M1 with no hepatomegaly or splenomegaly. WBC count was 30x 10^9/L, hemoglobin level was 7.9 g/dl, and platelet count was 13x 10^9/L, blast percentage was 80%. The patient was FLT-3 ITD negative.

Clinical outcome

Of the 103 adult patients, 63 (61.2%) achieved CR within 28 days of chemotherapy. Among these, 52 (82.5%) were of the FLT3 WT/WT genotype and 11(17.5%) of the FLT3 WT/ITD genotype. Although there was an apparent difference between the two groups, it was not statistically significant (*p*=0.75).Thirty two (24.8%) patients had treatment failure caused by an early death (death before evaluation of response) and 21 (16.2%) died within 7 days after completion of therapy. The main causes of deaths were uncontrolled infection, febrile neutropenia and cerebral hemorrhage. Median follow up period for all adult patients was relatively short, 2 months ranging from (0.03-15) months. Overall survival of adult patients with FLT3 WT/ITD group was shorter (28.5%) when compared with the FLT3 WT/WT group (40.8%) although no significant difference was detected(p=0.2), figure 2. The disease

free survival for patients with FLT3 WT/ITD genotype was (100%) compared to (86%) to patients with FLT3 WT/WT genotype, with no significant difference (p=0.3) between the two groups, figure 3.Overall survival for the pediatric group at 6 months period was 65.3±8.8 and DFS was 71.9±10.

Table 1 Clinical and laboratory characteristics for the adult group at presentation

	N=160
Age(years), median, range	40 (18-80)
Gender, (male) n. (%)	77 (48.1)
Hemoglobin (g/dl), median, range	7.3 (2.5-13.2)
Platelets (10^9/L), median, range	36(3-359)
TLC (10^9/L), median, range	23(0.8-312)
Percentage of bone marrow blasts, median, range	55(1-94)
FAB classification, n. (%)	
Mo	1 (0.7)
M1	19(12.8)
M2	64(43.2)
M3	23(15.5)
M4	34(23)
M5	6(4)
M7	1(0.7)
Hepatomegaly, n. (%)	39(30.7)
Splenomegaly, n. (%)	33(26)
DFS	
Mean (months) ±SE	10.6±5.7
Percentage at 6 months, (95% confidence interval)	88.2 (9.6-11.6)
Survival	
Mean (months)±SE	2 ±4.3
Percentage alive at 6 months, (95% confidence interval)	38.5 (1.6-2.4)
Clinical outcome	
Percentage of complete remission, n. (%)	63(61.2)

We had only one patient with JAK2-V617F mutation who had achieved complete remission after induction chemotherapy, DFS was three months and OS was seven months.

Table 2 Clinical and laboratory characteristic of the pediatric group at presentation

	N=34
Age(years), median, range	7.5 (0.5-16)
Gender, percentage (male)	21(61.8)
Hemoglobin (g/dl), median, range	7.1(2.6-10)
Platelets(10^9/L), median, range	35.5(7-164)
TLC (10^9/L), median, range	30(4.4-257)
Percentage of bone marrow blasts, median, range	70(1-96)
FAB classification, n. (%)	
Mo	1 (3.2)
M1	9(29)
M2	7(22.6)
M3	5(16.1)
M4	6(19.4)
M7	3(9.7)
Hepatomegaly, n. (%)	13(46.4)
Splenomegaly, n. (%)	12(42.9)
DFS	
Mean (months)±SE	7.7±0.9
Percentage at 6 months, (95% confidence interval)	71.9 (6.1-9.2)
Survival	
Mean (months)±SE	7.5±8.8
Percentage at 6 months, (95% confidence interval)	65.3 (5.8-9.2)
Clinical outcome	
Percentage of complete remission	23 (85.2)

Table 3 Clinical characteristics at presentation for adult patients in the FLT3 WT/WT and FLT3 WT/ITD genotype groups

	FLT3 WT/WT N=130	FLT3 W/ITD N= 30	*P*-value
Age(years)			
Median, range	40.5(18-80)	38.5(18-65)	0.29
Gender, n. (%)			
Male	61(46.9)	16(53.3)	0.53
Female	69(53.1)	14(46.7)	
FAB subtypes, n. (%)			
M1+M2	68(56.7)	15(57.7)	
M3	19(15.8)	4(15.4)	0.99
M4+M5	33(27.5)	7(26.9)	
Hemoglobin (g/dl), median, range	7.3(3.4-9.4)	6.9(2.5-11)	0.26
Platelets (10^9/L), median, range	37(3-359)	29(4-193)	0.35
TLC (10^9/L), median, range	19.4(0.8-257)	36.6(5.6-312)	0.02
Splenomegaly, n. (%)	29(28.4)	4(16)	0.20
Hepatomegaly, n. (%)	34(33.3)	5(20)	0.22
Percentage of BM blasts, n. (%)	50(1-94)	65(6-90)	0.42

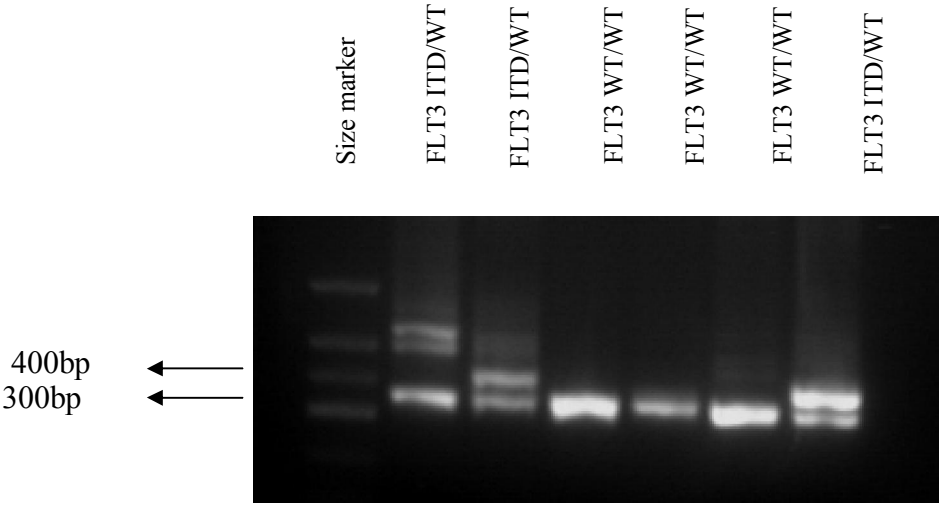


Figure 1: PCR detection of FLT3-ITD. The WT FLT3 genomic PCR product is indicated by the arrow at 330bp. Lane 1 is showing 100bp size marker, lane 2 is showing patient with FLT3 WT/ITD with two mutant alleles, lane 3 and 7 is showing FLT3 WT/ITD with one mutant allele, lanes 4,5 and 6 is showing FLT3 WT/WT.

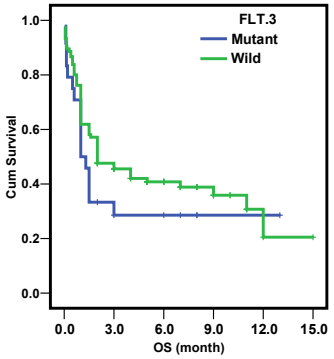


Figure 2: Comparison of OS between adult patients with FLT3 WT/WT and FLT3WT/ ITD mutation.

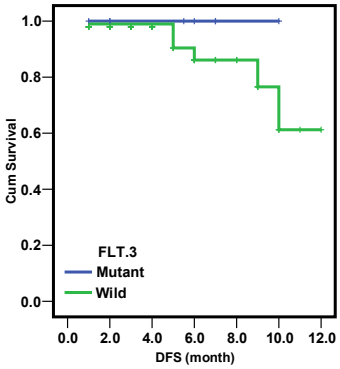


Figure 3: Comparison of DFS between adult patients with FLT3 WT/WT and FLT3WT/ ITD mutation.

4. Discussion

We examined the frequency and prognostic impact of FLT3-ITD and JAK-2 V617F mutations in 194 patients with *de novo* AML. The overall frequency of FLT3 mutation was (17.5%) and JAK-2 V617F mutation was (0.5%). The frequency of FLT3-ITD in the adult group of patients was (18.8%) which was lower than reported for other ethnic population, in Germans (31%, 53%) [13-15] and in Japanese (28.0%, 47.4%) [16-17], but approximated to those reported by Colovic *et al.,* [18] (17.7%). It is possible that studies using DNA banks slightly overestimate the true incidence of FLT3/ITD mutations because there may be bias toward availability of DNA from patients with higher peripheral WBC counts [19-20].

In this study, FLT3-ITD mutation was detected in 6/25 (24%) of elderly adult (≥60years) patients and 24/105 (22.8%) young adults (p>0.05). A higher frequency of FLT3-ITD mutation has been reported in elderly patients, although without these mutations having a prognostic impact [21]. Whereas, Schnittger *et al.,* [9] found that the median age of FLT3-ITD mutation was significantly lower than FLT3 negative patients.

In agreement with previous studies [22], the frequency of FLT3-ITD was higher (18.8%) in adult

compared to pediatric AML patients (11.8%). This observation may partially explain why adult AML has a poorer clinical outcome than pediatric AML

We found a significantly increased leucocyte count in adult patients with FLT3-ITD (p=0.02). This finding was consistent with some previous reports [17-18, 23-24]. Although the effect of FLT3-ITD on inducing leukomogenesis was not directly proved, the ligand – independent constitutive activation of FLT3 induced by ITD mutation could activate some downstream signal molecules including mitogen activated protein (MAP) kinase, signal transducer and activator of transcription 5 (STAT5), and serine-threonine kinases Akt, which contribute to cell proliferation and survival advantages [25-29]. These findings might partially explain the close relationship between FLT3/ITD and higher WBC count.

Furthermore, we found AML FLT3-ITD in all FAB subtypes but we could not reach a significant association with any FAB subtype. Previous studies demonstrated high percentage of FLT3-ITD in certain FAB subtypes, M3 especially the M3v group both with t (15; 17) [9, 30], M5 [15 -31] and M1 [32]. In this work, we had a small number of patients in each FAB subgroup of the FLT3-ITD AML patients, with which we could not reach a significant association.

In line with previous studies, there was no significant difference with respect to age, sex, or other clinical characteristics such as, splenomegaly or hepatomegaly [33].

Previous studies indicated that FLT3 mutation had no influence on complete remission rates and overall survival especially young adults with normal karyotype [9,15,32,33]. However, other studies have found that the presence of FLT3-ITD mutation and the relative level of FLT3-ITD allele have a major impact on long term outcome in predicting relapse from complete remission [12, 19, 34].

When we analyzed the clinical outcome of adult patients with and without FLT3-ITD mutation, patients with FLT3WT/ITD had tendency toward lower CR rate and shorter OS when was compared with the FLT3 WT/WT group although, no significant difference between the two groups could be reached. Moreover, we found no difference in DFS between FLT3 genotypes because of the small number of patients who achieved CR with FLT3WT/ITD genotype.

The prognostic impact of FLT3-ITD in this study was less pronounced than in other reports. The mutation status at diagnosis could not predict the clinical outcome and we could not evaluate the prognostic impact of the mutant allele. However, we cannot ignore that high level of mutant allele (> 0.4) in most of our ITD positive patients might explain the poor outcome of this group of patients.

One possible explanation for the discordant results is that , a large group of our patients (41%) died during induction therapy (ID) and with that short median follow up period (2, 0.03-15) months, CR rate and the long term outcome (OS and DFS) was hard to predict. Our analysis suffered from low statistical power due to limited number of patients and short follow up period.

Another explanation could be related to differences in the cohorts and treatment regimens of various intensities. Our adult group of patients was heterogeneous with respect to cytogenetic which was not available, and age (above and below 60 years) and these two factors are very important for risk stratification. Moreover, recent studies have found FLT3-ITD positive patients are heterogeneous with respect to mutant level, number and size of mutant and with the potential interaction of FLT3-ITD with other molecular abnormality such as nucleophosmin 1 gene (NPM1) [12].

In this study, JAK2-V617F mutation was found in one (0.5%) patient who had AML M1 subtype, high WBC and BM blast count, anemia and thrombocytopenia. The frequency in our patients was similar to that of Levine *et al.,* [35]. Previous studies from the literature have shown that V617F mutation to be distributed mainly among the more immature AML M1 and M2 subtype suggesting a correlation with less differentiated AML [36]. Although the mutation has been found in a small number of AML patients, a relatively high incidence of JAK2-V617F mutation was often seen in *de novo* and therapy-related t(8;21) AML patients and was associated with high WBC count in AML [37]. The small number of mutated cases in our study did not permit correlation with clinical or laboratory parameters.

In conclusion, we found that FLT3-ITD mutation is a frequent finding in adult patients with *de novo* AML. There is a significant association between FLT3-ITD mutation with high WBC count and a tendency towards a worse prognosis. The ratio of mutant to wild allele level may have a strong relation to the patient outcome. JAK2-V617F mutation is infrequent finding in *de novo* AML.

Corresponding author
Ghada M Elsayed
Clinical Pathology and Oncologic Laboratory
Medicine, National Cancer institute, Cairo
University, Egypt
elsayed276@yahoo.com

References

1- Smith ML, Hills RK, Grimwade D. (2011): Independent prognostic variables in acute myeloid leukaemia. Blood Rev., 25:39-51.

2- Park SH, Chi HS, Min SK, Cho YU, Jang S, Park CJ, Lee JH, Lee JH, Lee KH, Im HJ, Seo JJ.(2011): Prognostic significance of the FLT3 ITD mutation in patients with normal-karyotype acute myeloid leukemia in relapse. Korean J Hematol., 46:88-95.
3- Bienz M, Ludwig M, Leibundgut EO, Mueller BU, Ratschiller D, Solenthaler M, Fey MF, Pabst T. (2005): Risk assessment in patients with acute myeloid leukemia and a normal karyotype. Clin Cancer Res 11:1416-1424.
4- Chen Q, Lu P, Jones AV, Cross NC, Silver RT, Wang YL. (2007): Amplification refractory mutation system, a highly sensitive and simple polymerase chain reaction assay, for the detection of JAK2 V617F mutation in chronic myeloproliferative disorders. J Mol Diagn., 9:272-276.
5- Frohling S, Lipka DB, Kayser S, Scholl C, Schlenk RF, Dohner H, Gilliland DG, Levine RL, Döhner K. (2006): Rare occurrence of the JAK2 V617F mutation in AML subtypes M5, M6, and M7. Blood, 107:1242-3.
6- Illmer T, Schaich M, Ehninger G, Thiede C; DSIL2003 AML study group. (2007): Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias. Haematologica, 92:137-138.
7- Muñoz L, Aventín A, Villamor N, Juncà J, Acebedo G, Domingo A, Rozman M, Torres JP, Tormo M, Nomdedéu JF. (2003): Immunophenotypic findings in acute myeloid leukemia with FLT3 internal tandem duplication. Haematologica, 88:637-640
8- Kiyoi H, Towatari M, Yokota S, Hamaguchi M, Ohna R, Saito H, and Naoe T. (1998): Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product**.** Leukemia 12: 1333– 1337.
9- Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Löffler H, Sauerland CM, Serve H, Büchner T, Haferlach T, Hiddemann W. (2002): Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood 100:59-66.
10- Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, Kaspers GJ, Heerema NA, Gerbing R, Lange BJ, Radich JP. (2006): Clinical implications of FLT3 mutations in pediatric AML. Blood 108:3654-61.
11- Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, Score J, Seear R, Chase AJ, Grand FH,*et al.* (2005): Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 106:2162-8.
12- Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, Linch DC. (2008): Medical Research Council Adult Leukaemia Working Party. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood 111:2776-84
13- Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, Bullinger L, Fröhling S, Döhner H. (2005): Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 106:3740-3746.
14- Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, Haferlach T, Hiddemann W, Falini B. (2005): Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood 106:3733-9.
15- Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. (2006): Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 107:4011-20.
16- Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K. (2005): Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood 106:2854-2861
17- Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, Habdank M, Späth D, Morgan M, Benner A. (2008): Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N. Engl. J. Med 358:1909-1918.
18- Colovic N, Tosic N, Aveic S, Djuric M, Milic N, Bumbasirevic V, Colovic M, Pavlovic S .(2007): Importance of early detection and follow-up of FLT3 mutations in patients with acute myeloid leukemia. Ann. Hematol 86:741-747.
19- Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH, Linch DC. (2001): The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 98:1752-9.
20- Murphy KM, Levis M, Hafez MJ, Geiger T, Cooper LC, Smith BD, Small D, Berg KD. (2003): Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. J Mol Diagn 5:96-102.
21- Stirewalt DL, Radich JP. (2003): The role of FLT3 in haematopoietic malignancies. *Nat* Rev Cancer 3:650- 65.
22- Gregory TK, Wald D, Chen Y, Vermaat JM, Xiong Y, Tse W. (2009):Molecular prognostic markers for adult acute myeloid leukemia with normal cytogenetics. J Hematol Oncol 2:23.

23- Fröhling, S, Schlenk, RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, Döhner H, DöhnerK. (2002): Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood 100:4372-4380.

24- Huang Q, Chen W, Gaal KK, Slovak ML, Stein A, Weiss LM. (2008): A rapid, one step assay for simultaneous detection of FLT3/ITD and NPM1 mutations in AML with normal cytogenetics. Br. J. Haematol 142: 489-492

25- Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H, Naoe T. (2000):Tandem-duplicated *FLT3* constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3- dependent cell lines. Oncogene 19:624-631.

26- Kiyoi H, Naoe T. (2002): FLT3 in human hematologic malignancies. Leuk Lymphoma 43:1541-7.

27- Tse KF, Allebach J, Levis M, Smith BD, Bohmer FD, Small D. (2002): Inhibition of the transforming activity of FLT3 internal tandem duplication mutants from AML patients by a tyrosine kinase inhibitor. Leukemia 16: 2027-2036.

28- Grundler R, Miething C, Thiede C, Peschel C, Duyster J. (2005): FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. Blood 105:4792-4799.

29- Rocnik JL, Okabe R, Yu JC, Lee BH, Giese N, Schenkein DP, Gililand DG. (2006) : Roles of tyrosine 589 and 591 in STAT5 activation and transformation mediated by FLT3-ITD. Blood 108:1339-1345

30- Kuchenbauer F, Kern W, Schoch C, Kohlmann A, Hiddemann W, Haferlach T, Schnittger S .(2005): Detailed analysis of FLT3 expression levels in acute myeloid leukemia. Haematologica 90:1617-25

31- Koh Y, Park J, Ahn KS, Kim I, Bang SM, Lee JH, Yoon SS, Soon Lee D, Yiul Lee Y.(2009): Different clinical importance of FLT3 internal tandem duplications in AML according to FAB classification: possible existence of distinct leukemogenesis involving monocyte differentiation pathway. Ann Hematol., 88:1089- 97

32- Wang L, Xu WL, Meng HT, Qian WB, Mai WY, Tong HY, Mao LP, Tong Y, Qian JJ, Lou YJ, Chen ZM, Wang YG, Jin J. (2010): FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. J Zhejiang Univ Sci B11:762-70.

33- Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, Carroll AJ, Mrózek K, Vardiman JW, George SL, Kolitz JE, Larson RA, Bloomfield CD, Caligiuri MA. (2001): Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res., 61:7233-9.

34- Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhäuser M, Ritter M, Neubauer A, Ehninger G, Illmer T. (2002): Analysis of FLT3-activating mutations in 979 patients with acute myeloid leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 99: 4326-4335

35- Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E, Berger R, Clark JJ, Willis SG, Nguyen KT, Flores NJ, Estey E, Gattermann N, Armstrong S, Look AT, Griffin JD, Bernard OA, Heinrich MC, Gilliland DG, Druker B, Deininger MW. (2005): The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. Blood 106:3377-9.

36- Vicente C, Vázquez I, Marcotegui N, Conchillo A, Carranza C, Rivell G, Bandrés E, Cristobal I, Lahortiga I, Calasanz MJ, Odero MD. (2007): JAK2-V617F activating mutation in acute myeloid leukemia: prognostic impact and association with other molecular markers. Leukemia 21:2386-90.

37- Iwanaga E, Nanri T, Matsuno N, Kawakita T, Mitsuya H, Asou N. (2009): A JAK2-V617F activating mutation in addition to KIT and FLT3 mutations is associated with clinical outcome in patients with t(8;21)(q22;q22) acute myeloid leukemia. Haematologica 94:433-5.

9/22/2012

Current Research And Future Development In Leprosy And Tuberculosis Control

Esmaeilzadeh Mahdi[1], Kazemzadeh Fariba[2] and Borhani Mohammad [3]

1. Department of Basic Science , Nikshahr Branch, Islamic Azad University , Nikshar,Iran
   Email: mehdi_dna@yahoo.com (**Corresponding Author**); Phone: +98 (0) 935 979 3491
2. Department of Basic Science , Nikshahr Branch, Islamic Azad University , Nikshar,Iran
3. Department of Basic Science , Nikshahr Branch, Islamic Azad University , Nikshar,Iran

Abstract: During recent years we have witnessed a burst of activity in leprosy research. By definition, leprosy is an infectious disease, the causative organism being cobacterium leprae. The leprosy bacillus is virtually non-toxic and may occur in large amounts in tissues with only moderate clinical symptoms. In fact, leprosy may to a great extent be regarded as an immunological disease since most symptoms arc due to immune reactions against antigens liberated from the leprosy bacilli. During recent years leprosy research has been centred, to a great extent, around studies of immunological phenomena since a better understanding of basic immunological mechanisms would provide a rational basis for improved treatment of patients with established disease, and for advancements in our understanding of the epidemiology of leprosy and its control.
[Esmaeilzadeh M, Kazemzadeh F, Borhani M. **Current Research And Future Development In Leprosy And Tuberculosis Control**. *Life Sci J* 2012;9(4):1061-1064] (ISSN:1097-8135). http://www.lifesciencesite.com. 161

Keywords: Leprosy, Tuberculosis, Research and Development

1-Introduction

1-1-The Course After Infection With Mycobacterium Leprae

Figure 1 provides a schematic outline of the course after infection with M. leprae. This course is highly variable, and the figure points out several distinctive features of major importance for our understanding of the epidemiology of leprosy, its control, and variation in clinical picture in patients with established, chronic disease. Only a minority of the individuals who have been infected with M. leprae go from infection

to disease. Many of them have probably gone through a subclinical stage limited multiplication of M. leprae terminating without development of any clinical symptoms at all. Other individuals develop " indeterminate leprosy" with one, or a few, vague lesios that heal sponstaneously. To the right in the figure, we see the individuals who develop determinate. Persisting disease which is classified as a spectrum between the two polar forms.

Lepromnatous (LL) leprosy is characterised by lack of resistance, exten8ive bacterial multiplication with large amounts of bacilli in the lesions which are multiple and tend to become nodular in appearance. The other polar group, tuberculoid (TT) leprosy, is characterised by one or a few well-defined flat lesions, being hypopigmented in dark skinned people, containing few or no detectable acid fast bacilli as evidence of marked resistance.

The factors which determine whether an infected individual will develop no clinical symptoms of the infection or experience bacillary multiplication and eventual development of persisting, chronic disease are not well known. There is a great need for additional Information and for identification of risk factors favouring development of clinical disease after infection.

The present epidemiology of leprosy is, to a great extent, the epidemiology of disease. There is a great need for development of new methods by which infection with M. leprae could be reliably diagnosed. This would make us able to study both the epidemiology of infection and the epidemiology of clinical disease, and to define risk! factors favouring progression from infection to disease, In tuberculosis, development of the tuberculin reaction has been of fundamental importance, providing an indicator of infection permitting determination of annual incidence rates of infection in various populations.

A similar test is not yet available with regard to leprosy, but important information in this area was initially obtained by the lymphocyte transformation test (LTT) at the Armauer Hansen Research Institute in Addis Ababa (1,2) and through studies of more refiner! lymphocyte stimulation tests (LST) measuring lymphocyte proliferation by recording the in corporation of labelled thymidin into newly synthesised DNA after stimulation of lymphocytes in vitro with various antigen preparations derived from M. leprae (3).

The Iymphocyte transformation t('Sts provided evidence of immunological conversion in individuals coming from leprosy non endemic countries to work at leprosy hospitals in Ethiopia (1). The frequency of conversion in this group is much higher than the probability of subsequent development of clinical signs of the infection.

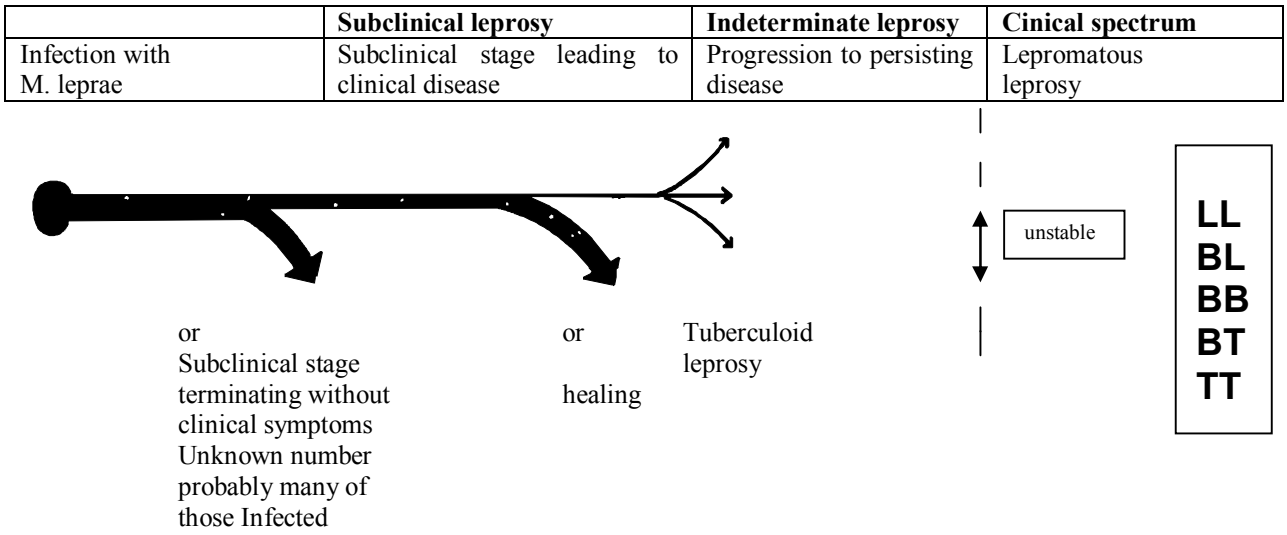


Figure I. The course after infection with *Mycobacterium leprae.* Reproduced from M. Harboe, The Immunology of Leprosy, Chapter 4 in *Textbook of Leprosy.* R.C. Hastings, Ed. Churchill, Livingstone, London, 1985.

So, further development of lymphocyte stimulation tests and corresponding delayed type hypersensitivity skin tests may result in methods for diagnosing infection with M. leprae, but at present it can not be foreseen how these tests might predict the probability of further development towards clinical disease. It is particularly important in this connection that patients with multibacillary leprosy usually have entirely negative tests both in vivo and in vitro for cell mediated immune responses towards M. leprae.

Extensive experiments have been carried out on various sensitive antibody assays, both with "global tests" demonstrating antibodies directed against a variety of different constituents of M. leprae (e.g. radioimmunoassay and ELISA tests for antibodies against total M. leprae sonicates), tests for antibodies against M. Leprae antigen 7 which cross reacts widely with other mycobacteria (4), and assays for antibodies against M. leprae specific antigenic determinants (5).

There are still problems with regard to development of sufficiently sensitive but nevertheless specific assays, and with false negative results since, even in patients with multibacillary disease, there is a wide variation in antibody content in individual patients and some of them have only low antibody activity (4,.5,6).

In experimental models, it has been demonstrated that there is a striking relationship between development of systemic infection after inoculation with M. leprae and, formation of an ti-M. lepra 7 antibodies (4). So it is e'xpected that antibody assays will turn out to be fairly reliable indicators of the total antigenic load in a given individual, and thus for thus extent of bacillary multiplication and probability of development of disease.

The combined use of a test for cell mediated immune reactions and antibody response against M. leprae antigens is expected to provide the best information on the occurrence of infection with M. leprae and the tendency to further development of clinical disease.

In patients with determinate, established clinical disease there is a wide variation in clinical appearance. The area between the polar groups denoted "B" for "Borderline" could also be seen as being denoted "B" for "Beware of reactions". In this area of the clinical spectrum, there is the greatest tendency for development of particular forms of reactions, denoted reversal reactions, due to delayed type hypersensitivity reactions against antigens 1iberated from M. leprae in the tissues.

The symptomatology depends greatly on the location of liberated antigen, the most severe results being extensive nerve damage due to local DTH reactions in nerves after release of antigen from M. leprae residing inside Schwann cells. Immunological studies of leprosy have, during recent years, been centred around the study of these reversal reactions providing improved understanding of their pathogenesis (3,7,8) and thus a more rational background for treatment.

Patients with lepromatous (LL) leprosy are characterised by a profound, specific cel-lular immuno-deficiency, detectable both by in vivo and in vitro tests (9). This lack of cell mediated immune reactions against M. leprae is probably directly responsible for the lack of resistance, extensive

multiplication of M. leprae, and the great risk of relapse after treatment since this specific immuno-deficiency persists after prolonged chemotherapy. Recent findings at the Armauer Hansen Research Institute in Addis Ababa have provided new insight into the mechanisms of this deficiency (10).

The development of cellular immune responses after antigenic stimulation depends upon activation and proliferation of reactive cell clones. This proliferation in turn depends upon production and release of cell growth factors being produced in some cells (particularly T helper cells) and affecting cells in the immediate neighbourhood. Interleukin-2 (IL-2) is of main importance in this regard.

In most instances, the addition of IL-2 to cultures of lymphoid cells from peripheral blood of patients with lepromatous leprosy exposed to M. leprae in vitro results in marked proliferation of lymphoid cells. This reconstitution of proliferation in response to M.leprae demonstrates clearly that patients with lepromatous leprosy do possess circulations M . leprae reactive T cells and that the lack of proliferation is due to deficient production of IL-2 (10). This demonstration of reversal of that specific immuno deficiency in vitro provided fundamental, new knowledge with regard to the mechanism of this immuno-deficiency.

Experiments with local injection of IL-2 into leprous lesions are expected to he performed soon and will he an important new feature in attempts at immunologic intervention to correct this basic immuno-deficiency which is of major importance for development of multi bacillary disease in the individual patient and for the total infectious load in the population.

2-Influence Of Treatment On Leprosy Control

The interaction between treatment and control is an essential issue in leprosy. At present, the major principle of leprosy control is to break the chain of infection by treating as many patients with multibacillary disease as effectively as possible and at an early stage. In the years to come, it will be essential to carefully follow up the effect of the new multiple drug therapy (MDT) regimens recommended by WHO and to assess their impact on the leprosy endemic in the population.

To assess the effect will be more difficult in leprosy than in tuberculosis since we have no practical means, at present, for direct determination of the annual incidence of infection in a population but would depend on observation of patients with established disease. The establishment of suitable epidemiological indicators is a matter of urgency.

Based on observation during the disappearance of the: leprosy endemic in Norway (11), it app4'ars that age specific incidence rates would provide important information, but it is a complicated project to obtain such data. Determination of the frequency and severity of deformity at the time of diagnosis is another obvious parameter which provides essential information for our evaluation of the extent of infection at the population level, and is concerned with matters of immediate relevance to the patients themselves.

The development of new technology is an obvious need has become a major problem during the era of dapsone monotherapy (12) and it is not clear how the new MDT regimens will cope with this problem under realistic field conditions. Dugs with bacteriocidal or acteriostatic effect on M. leprae are very few indeed. There is thus a great need for the development of new drugs and for the introduction of new techniques for primary intervention.

The development of a leprosy vaccine has been given top priority in the WHO Immunology of Leprosy (IMMLEP) Programme. Based on the use of M. leprae grown in vivo in armadillos, marked progress has been made in this area. However, it is not yet clear to what extent killed M. leprae will be able to induce protective immunity , that is, increased ability to limit bacterial multiplication. In humans, BCG must be given alive to induce protectivc immunity against tuberculosis.

At present, there are several candidate vaccines, killed armadillo-grown M. leprae given alone, killed M. leprae given together with live BCG, and the use of another carefully selected cross-reacting mycobacterium. Work on vaccine development occurs step-wise and will ultimately lead to field trials to determine the efficacy of the selected vaccination procedures. The essential field trials will be expensive and difficult to carry out in a disease like leprosy with a long incubation period and low incidence rates. The matter is further complicated considering prior experience in tests for efficacy of BCG vaccination against tuberculosis and leprosy .In both instances, the protective effect of BCG varies considerably in different populations, and we would therefore also expect the effect of new leprosy vaccines to differ markedly in different populations.

3-Trends In Tuberculosis Research

With regard to tuberculosis, much of the current interest is directed towards the development of new technology for prophylaxis. The effect of BCG vaccination against tuberculosis has been extensively discussed for many decades. In my opinion, one major point appears:

In some populations there is an indisputable, marked protective effect of BCG vaccination against subsequent development of infectious disease. fu other populations, the effect is less, or apparently nil.

The sad fact is that the smallest effect is obtained where an efficient vaccine is most nceded. In developing countries tuberculosis represents a major health problem, and there is an immediate need for improvements in strategies and techniques for tuberculosis control work (13).

Research is active and greatly needed for better characterisation of the antigenic structure of mycobacteria and to learn more about mechanisms for induction of effective protective immunity. In the WHO Immunology of Tuberculosis (IMMTUB) Programme, priority has been given to exploring the new biological technology, including DNA technology, to develop new principles for vaccine production.

These technologies have already proven to be very effective in the development of new vaccines against diseases in which humoral immunity is essential for resistance. It is hoped that this technology will also result in the development of new vaccines capable of inducting protective cellular immunity which is what is needed in the two most important mycrobacterial diseases, tuberculosis and leprosy.

REFERENCES

1. Godal, T., M. Lofgren and K. Negassi. 1972. Immune response to M. leprae of healthy leprosy contacts. Int. J. Leprosy. 40:243-250.
2. Godal, T. and K. Negassi. 1973. Subclinical infection in leprosy. Brit. Med. J. 3:557 -559.
3. Bjune, G., R.St C. Barnetson, D.S. Ridley and G. Kronvall. 1976. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. Clin. Exp. Immunol. 25 :85-94.
4. Harboe, M. 1981. Radioimmunoassay and other serologic tests and their application in epidemiological work. Symposium on the Epidemiology of Leprosy, Geilo, Norway, 1981. Lepr. Rev. 52, Suppl. 1:275-288.
5. Cho, S.N., T. Fujiwara, S.W. Hunter, T.H. Rea, R.H. Gelber and P.J. Brennan. 1984. Use of an artificial antigen containing the 3,6-di-O-methyl- -D-glucopyranosyl epitope for the sero-diagnosis of leprosy. J. Infect. Vis. 150:311-322.
6. Yoder, L., B. Naafs, M. Harboe and G. Bjune. 1979. Antibody activity against

Mycobacterium leprae antigen 7 in leprosy: Studies on variation in antibody content through-out the spectrum and on the effect of DDS treatment and relapse in BT leprosy. Lepr. Rev. 50:113-121.

7. Godal, T ., B. Myrvang, D.R. Samuel, W .F .Ross and M. Lofgren. 1973. Mechanism of «reactions» in borderline tuberculoid (BT) leprosy. Acta. Path. Microbiol. Scand., Sect. A, Suppl. 236 :45-53.
8. Barnetson, R.St C., G. Bjune, J.M.H. Pearson and G. Kronall. 1975. Antigenic heterogeneity in patients with reactions in borderline leprosy. Brit. Med. J. 4:435-437.
9. Harboe, M. 1980. Immunological aspects of leprosy: Ten-year activity at the Armauer Hansen Research Institute and prospects for further work. Int. J. Leprosy. 48:193-205.
10. Haregewoin, A., A.S. Mustafa, I. Helle, M.F .R. Waters, D.L. Leider and T. Godal. 1984.

Reversal by interleukin-2 of the T cell unresponsiveness of lepromatous leprosy to Mycobacterium leprae. Immunological Rev. 80:77 -86.

11. Irgens,L.M. 1980. Leprosy in Norway. Lepr. Rev. 51, Suppl. 1.
12. Pearson, J.M.H., J.A. Cap, G.S. Haile and R.J.W. Rees. 1977. Dapsone resistantleprosy and its implications for leprosy control programmes. Lepr. Rev. 48:83-94.
13. Styblo, K. 1983. Tuberculosis and its control: Lessons to be learned from past experience, and implications for leprosy control programmes. Eth. Med. J. 21:101-122.

9/29/2012

Health, Development And Primary Health Care

Esmaeilzadeh Mahdi[1], Kazemzadeh Fariba[2] and Borhani Mohammad [3]

1. Department of Basic Science, Nikshahr Branch, Islamic Azad University, Nikshar,Iran
   Email: mehdi_dna@yahoo.com (Corresponding Author); Phone: +98 (0) 935 979 3491
2. Department of Basic Science, Nikshahr Branch, Islamic Azad University, Nikshar,Iran
3. Department of Basic Science, Nikshahr Branch, Islamic Azad University, Nikshar,Iran

Abstract: The peoples and countries of the Third World are struggling to overcome the effects of centuries of colonial dependency and unequal world relationships. These effects can be seen clearly in the area of human health. For example, of the 1978 world total of 17 million early childhood deaths (i.e., those under five years of age) around 97% took place in the Third World (1). If all the countries of the world had the same early childhood mortality rates as those of Northern Europe there would have been only 2 million such deaths. The relationship between such appalling health conditions health conditions and wider social structures is highlighted in the Six World Health Situation Report (1973-1977), prepared by the World Health Organization (2).
[Esmaeilzadeh M, Kazemzadeh F, Borhani M. **Health, Development And Primary Health Care**. *Life Sci J* 2012;9(4):1065-1073] (ISSN:1097-8135). http://www.lifesciencesite.com. 162

Keywords: Health, Development, Primary Health Care

1- Introduction

The most important social trends during the period of this report are reflected in the still low, and in some areas worsening nutritional level of the bulk of the population. The employment situation, including access to land has not improved in many countries and is partly, but not primarily, affected by continuing high rates of population growth, although there are signs of slowing-down of such growth in many ports of the world. The decline of rural life in many countries has led to unacceptable rates of urbanization and social and health problems on a mass scale in the world's cities and larger towns. Although some progress has been made in reducing illiteracy, a significant proportion of children in developing countries still do not attend primary school. The needs of women are being discussed to a greater extent than every before but there has been little practical achievement in this domain.

The policy implications for the health sector which should arise out of the situation described above are considerable. Perhaps the most important of all is the explicit recognition now being given to the view that health development is a reflection of political, social and economic policy and planning and not merely an outcome of the application of health technologies. This recognition can be perceived as being at the heart of the intensive discussions now taking place, both among and within countries, about the political, social and economic links between health and development on the one hand, and the consequent health policies to be followed by government, on the other. It is worth nothing that it is the changing definition of development itself that is, something considerably more than the mere growth of national product - which is critical to the view that an increase in technological and economic capacities alone will not automatically produce health. A brief, health related historical exploration of the experiences of both industrialized and Third World countries is particularly important to an understanding of the present situation of health and disease in poor countries. The two basic points to be made are: one the countries of the Third World - the so-called developing nations - have not always been poor, or at least not in the sense that us implied by the terms "developing" or "underdeveloped" and, two, analysis of the historical experience of today's industralized countries in relation to improvements in the health status of their population leads to the conclusion that these were not especially dependent upon improvements in medical technology.

2-HISTORICAL EXPERIENCE

2-1-Third World

As background to the discussion it should be borne in mind that at the time of Europe an expansion in to the rest of the world there was relatively little to choose between the technological capacities and standards of life of the life of the mass of the population of Europe and the countries to which Europeans were going. This point is fairly well understood in relation to countries such as India or China, or much of the Middle East. It is less appreciated with regard to Black Africa. Latin America is in a rather different situation in that in almost all of those countries the precolonial societies have been almost totally destroyed.

Rather than citing a greeting deal of materials from many different countries with regard to the first issue raised above - that is, what has been called the development of under development (3) - it might be more useful to draw upon the example of one particularly poor, particularly dependent African country. [At the same time, for subsequent issues of the Journal, we would like to invite in-depth studies and analyses based on Ethiopia and other African countries. For the present purpose of general observations, we hope that the example from Lesotho will sufflce.] Lesotho, a small country with a population of somewhat over 1 million, is completely surrounded by South Africa. Formerly a British territory, having the name Basutoland, the country came to independence in 1966. Lesotho is largely rural and even its capital city has a population of only about 50,000. Life expectancy at birth is put at 52 years and infant mortality is probably underestimated at 106 per thousand. The pattern of disease is similar to most other third World countries in that it is shaped primarily by low incomes, in adequate diets, limited access to clean water, and a generally low standard of hygiene for the mass of the population. As in most countries the past emphasis on hospital medicine has been accompanied by neglect of preventive programmes and services (4). What is of interest here is not so much the distressing disease picture of Lesotho, but rather how Lesotho came to be this way.

The Basutoland Kingdom was founded under Moshoeshoe in the early part of the nineteenth century. Its prosperity was such that "by the end of the 1830s the Basotho had stored reserve supplies of millet sufficient for between four and eight years, while in the mid-1840s white farmers 'flocked' to them to buy wheat" (5). This prosperity was based upon Mosheshoe's control of the land and cattle resources if the Caledon Valley. In 1869 Lesotho was forced to cede a large part of its territory to the white-ruled Orange Free State. The loss of this territory coincided with the opening of the Kimberly diamond mines, and these events become a major stimulus for migration of the neighboring white-ruled southern African colonies of the Orange Free State and Cape Colony. At the same time Lestho continued to be a major supplier of grain to the diamond mines at Kimberly and the goldfields of the Rand. Lesotho was engaged in vigorous trade relations with her neighbours based upon the export of labour and grain and the import of manufactured goods. By the late 1880s the tide began to turn against the Basotho people. The Afrikaners of the Orange Free State increased their production of agricultural goods and placed restrictive tariffs on Lesotho grain imports passing through their territory en route to the mines, the railways began to bring in cheap Australian grain, and the coming of rinderpest cattle disease decimated the Basotho herds. By 1903, with the collapse of the trade boom and the onset of drought, Lesotho was obliged to import North American wheat in order to feed herself.

Increasing land pressure arising from the events described, plus growing population and soil erosion, coupled with the need for cash income for imports and colonially imposed hut taxes and school fees and by the early part of this century created a powerful stream of migratory labour from Basutoland to the mines and farms of South Africa. Furthermore, this movement was fostered by the policies of the colonial government, as indicated in the report of the (Basutoland) Resident Commissioner, Colonial Office Report No. 177, 1898/99 as follows:

Though for its size and population Basitoland produced a comparatively enormous amount of grain, it has an industry of great economic value to South Africa, viz., the output of native labour. It supplies the sinews of agriculture in the Orange Free State; to a large extent in keeps going railway works, coal mining, the diamond mines at Jagesfontein and Kimberly, the gold mines of the Tansvaal and furnishes, in addition, a large amount of domestic services in surrounding territories. The number of men who received passes for labour during the year under review amounted to 37,371 These factors are the best rejoinder to those who argue that Basuto land is a useless native reserve. To others, who urge higher education of the natives, it may be pointed out that to educate themabove labour would be a great mistake.

Primarily the native labour industry supplies a dominion want, and secondly it tends to fertilise native territories with cash which is at once diffused for English goods. From the 38,000 Lesotho nationals employed in South Africa at the turn of the century, it was estimated that around 150,000 were so employed by the late 19708 a total equivalent to about half the overall male labour force of the country.In addition, perhaps 100 of the female labor force was also outside the country.

The outmigration of the country's young men has contributed to the rapid decline in the output 0£ agricultural crops. Between 1950 and 1970 the production of all grains fell by 41%. During the.same time period the country's population grew by about 45%, putting additional pressure on available land. Increasing land pressure and soil deterioration encouraged further outmigration which, in turn, deprived Lesotho of manpower resources that could have been employed to reverse the pattern of diminishing agricultural outputs. Thus Lesotho, a "comparatively enormous" producer of grain in 1898,

had net imports amounting to one-fifth of its total consumption of cereals in 1970 (6). Food production is below requirements and this gap is growing while average food consumption is declining (7).

The continuing poverty and dependence of Lesotho can be seen clearly from the results of a 1970 study of the deployment of the labour force (8). It found that of a total population of over 1 million, only 216,000 were "adequately employed" (defined as earning U.S..9175 per annum). There were 640,000 dependents and almost 300,000 either inadequately employed or under employed; Of the 216,000 "adequately employed", 700/0 were engaged outside the country.The same study also makes apparent the gross overall poverty of the people of Lesotho. Of almost 5,200 families in Maseru (the capital city) in 1972/73, about one-fifth had a per annum income of less than U.S. $230; about three-fiths between U.S. 230 and U.S,,;$1,150; and only one-fifth more than U.S..Bl,150. of all rural households 90% had a per annum income of 1f-SS than U.S. $00 and only 2% more than U.S. $ 575 (the overall average was U.S. $250). The case of Lesotho isinstructive not because it is absolutely typical, but because it illustrates some of the most basic structural underpinnings of the development of underdevelopment, and con-sequent health problems of much of the Third World. Lesotho is today one of the poorest countries in the world, one of the most dependent, and one of the most completely integrated into the imperialist market system. It is one classic example of the development of under development.

2-2-Industrialized Countries

Until relatively recently the overall health status of the populations of the industrialized countries was not dissimilar to that of the poorest parts of today's Third World. For example, in the 1840s in Preston, Lancashire, northeastern England, Reverend Clay in his Report of the Commission for Inquiry into the State of Large Towns and Populous Districts found that the lowest social class grouping, "factory operatives", had an infant mortality rate of 312 per thousand, more than three times that of the highest social class grouping, the "gentry which had a rate of 92 per thousand (9). Clay also calculated that of every 1,000 children born to the families of operatives only 112 survived to age 60, in contrast to 451 children of the gentry.It is important to note that such disparities could not have been based upon differences in access to medical care, because at this time there was relatively little that medicine could positively accomplish, but rather upon the differing social and economic conditions of the classes concerned.

More recent historical experiences are reflected in changes in infant mortality rates in New York City over the first three decades of this century (10). From 1900 to 1930 these rates fell from 140 per thousandto around 55 per thousand, an overall fall of about 60%. Of that fall two-thirds occurred in the so-called "diarrhea-pneumonia" complex of childhood diseases. The most striking aspect of this rapid fall of infant mortality in New York City is that it occurred before there were any antimicrobial drugs, or vaccines with which to treat this particular disease complex. The sharp decrease in mortality resulting from control of the diarrhea-pneumonia disease complex in New York City took place in keeping with major social and political changes, particularly those leading to higher incomes for the poor, that were occurring in a society which had already reached a relatively advanced state of technological development. In addition, a number of more specifically public health related developments were taking place in areas such as nutrition, sanitation, education, visiting nurse services and well-baby clinics, etc. In the years since 1930 infant mortality has fallen in New York City from 55 per thousand to below 20. Studies of the effects of modern medical care (the last 50 years or so) on death rates indicate that despite the availability of powerful new drugs and more scientific medical procedures, improved socio-economic conditions still provide the basic preconditions for further significant and relatively rapid declines in infant (and more general) death rates (11). In any event, most scholars agree (12) that medicine per se had contributed little to the major improvements in health status which occurred prior to the 1930s. Rather, it was positive economic, social and political developments which led to improved diets, a cleaner environment, a better educated population etc., and thus more healthy people. In more recent years the experiences of countries such as China and Cuba have borne out the validity of this historical finding.

3- SOCIAL-POLITICAL BASES OF ILL HEALTH

In low income countries the major cause to the degree there is one major cause of early childhood death is malnutrition, which contributes to most other disease problems. A careful study of early childhood mortality in the Americas, where nutritional status is on average better than in Africa or Asia, showed that 57% of the children who died before reaching the fifth year of life had malnourishment as an underlying or associated cause of death (13). The dominant role of birth weight has been firmly established, with biological variables such as age of mother, parity and pregnancy interval playing

relatively minor roles (14). Not surprisingly, in poorer countries anyway, birth weight is primarily determined by the socio economic position of families. As many as 400/0 to 50% of the infants born to poor families in the Third World have low birth weights, as compared to only 5% to 100/0 of those born to richer families.

It is well known that many Third World countries, especially since their political independence, have experienced a fairly rapid fall in infant and child mortality rates, which in turn is linked to a significant growth of population. Although specific reasons for this fall are not precisely known, it is commonly thought to be primarily due to public health measures such as the international smallpox and malaria campaigns and possibly to increasing availability of supplies of clean water and improved nutritional status. Although death rates may have been affected by the smallpox, malaria, and other campaigns, the large fall that actually took place does not appear to be adequately explained by these alone. With regard to clean water, waste disposal and other aspects of sanitation, little has changed for the bulk of the population of the Third World which remains primarily rural; those who have migrated to the towns may have improved their position in this respect. With regard to the nutrition factor, it may be that its important contribution to falling especially) infant mortality rates has come about primarily through the more rapid availability of at least minimum quantities of foodstuffs at times of extreme food shortage and examine. Of course, famine still occurs in the Third World but no longer so regularly as during the colonial era -although recent African experience begins to call this observation into question. There is little evidence that average nutritional standards within much of the Third World are rising, within the context of relatively wide variations, but the very availability of national and international food stocks and the transport systems to move them quickly make it less possible under conditions of independent sovereignty to allow starvation to the point of immediate death. However, although fewer people may die outright from starvation many survive only at lowered nutritional and energy levels; thus undernutrition becomes a chronic process rather than an acute event.

The importance of malnutrition as the primary cause of early childhood mortality, as well as the essentially socio-political basis of malnutrition, can be clearly demonstrated in the context of the experience of oneparticularly poor country, Bangladesh. Per capita income in Bangladesh is around US $ 25 and is badly distributed. Approximately 80Jo of the labour force is engaged in agriculture. The potential capacity of the land and its people to produce food is very great, although only part of that potential is being realized. A major constraint to increased production is limited effective economic demand for foodstuffs. A very substantial proportion of Bangladeshi families either do not own enough land, or in the case of tenants are not allowed to retain for themselves enough of their harvest, or else do not earn enough wage income so as to be able to keep themselves alive. The very large volume of food imports and external contributions coming into the country helps to avoid many immediate deaths from starvation, although it cannot prevent a permanent condition of malnourishment for millions; It has been estimated that only half the population enjoys an adequate caloric intake and even less sufficent protien consumption. Some 1976 data from a fairly typical thana (district) in Bangladesh, Companiganj, illustrate then prevailing situation (5). Almost certainly the situation has worsened during more recent years (6).

Twenty-three percent of families have no land and no salaried income of any kind. Forty-eight percent have less than half an acre of land and less than US $3.60 per month salaried income. Thus, nearly half of the families of this thana do not have the resources within the family to support the family and are dependent upon income from casual labour, usually available only at the time of harvest. Fifty percent of families live off four percent of the land.

Not surprisingly the health-related effects of the situation described are very serious. Given the prevailing nutritional picture even in "no.nnal " years any economic shock will immediately cause grevious damage to the weakest parts of the population. Thus, the economic and political crises experienced by Bangladesh in 1975 increased the crude death rate to around 26 per thousand from an earlier 14 per thousand. As would be expected the increase in mortality was felt most keenly by the children. Infant mortality rose from 128 to 142 per thousand live births and mortality in the one to four years age group from 23 to 55 per thousand. Of all deaths in 1975 in Companiganj thana, 50% were of children under the age of four. Of these deaths 30% were ascribed to "primary malnutrition ", 27% to chronic or acute diarrhoea or dysentery, and close to 20% to other preventable illnesses. The study goes on to report that those in the "low economic group ", had mortality rates three times those in the "high economic group ". In absolute terms high income group experienced a death rate of 9.7 per thousand, the middle income group 16.6 per thousand, and the low incomegroup 31.3 per thousand.

Inequalities of power, influence, opportunity, and the ownership and distribution of assets and income

lie at the root of the much discussed "Bangladesh crisis" In this the country is not unlike many others in the Third World, only more so. Of course, sufficient inequality can kill children in any national environment, but in low income countries the margin of safety is much narrower. Beyond that, in the international area, the wasteful affluence of the industrialized countries is linked not only in a lufntitative sense to the poverty of Third World nations, but also in more subtle relationships which encourage both internal inequality and continuing external dependency on the part of these nations.

The socio-political bases of ill health in Bangladesh as in so many other countries are rooted in centuries of colonial exploitation, economic dependence and external control. The basic global inequaities created during those centuries continue despite political independence having been achieved by virtually all the people of the world. However, the particular patterns of development being followed by many of the newly independent countries, and in the case of the Americas by countries that have long been independent, have reproduced key areas of inequality which had been either initially created or encouraged during the colonial period. Land ownership is being concentrated in fewer hands with agricultural production oriented increasingly to market production, especially the global market for foodstuffs. Coupled with the loss of land by small holders is the breakdown of traditional family support systems which leave the weakest part of the family, the women and children, in the most vulnerable position. There are worsening life conditions for the mass of the population in many countries at the same time as a relative few appropriate the wealth of the nation to themselves. This process goes forward in the context of the general impoverishment of subsistence and other small peasants and farmers, rapidly growing and increasingly filthy urban environments, unemployment and underemployment of unprecedented proportions coupled with the importation of inappropriate capital intensive technologies, rates of illiteracy which often remain virtually constant together with growing university enrollments even in the face of graduate unemployment, and rapid population growth flowing out of continuing poverty and inequality.All of the above has led, inevitably, to increasingly militant and revolutionary reactions and, just as inevitably, in many countries to more and more unrepresentative and repressive regimes as the privileged struggle to protect their perceived interests.

4- FROM GROWTHMANSHIP TO BASIC NEEDS

The conditions described above have led to a re-examination on the part of many development theorists and some governments of the very definition of development. From the end of World War II until the late 1960s the prescription to the economic problems of the Third World advanced by the dominant circles in the advanced capitalist countries was what some have called "growthmanship" (17). The solution to underdevelopment was seen to lie in a relatively rapid growth of national product coupled with some sort of modernization which would lead to gradual amelioration of the situation of the mass of the population. The approach implied, at least in the short and medium term, curtailment of investment in sectors considered as "non productive " -health, education, etc. -until the economy was strong enough to support such "costs ". Even a more equitable spread of income was suspect on the same grounds.

By the late 19608 there was general dis illusionment in bourgeois economic circles with the appropriateness of pure growth models for developing as well as developed countries. Doubts about growthmanship as an appropriate model had been raised by the increasing volume of evidence pointing to the impossibility of repeating nineteenth century experiences in the conditions of twentieth century underdevelopment. Ithad also been perceived that, contrary to expectations and despite a certain amount of economic growth, the previous several decades had witnessed increasing impoverishment for a significant proportion of thepopulation of the Third World. In addition, and in sharp contrast, the experience of developing socialist countries, China in particular, in significantly reducing poverty and its effects was beginning to become more widely known in the non-socialist world.

The decade of the 1970s saw a move away from growth of national product as the unique development indicator. This in turn, opened the way for the needs of the poor, especially as seen in terms of income redistribution, employment creation and the provision of "basic needs ", to emerge as the immediate goal of an increasing number of development programmes. In addition, the need for genuine community participation for the achievement of these goals came increasingly to be part of the common development perspective. All of this, in conjunction with the New Inter national Economic order (NIEO), has come to dominate contemporary development discussion.

5-The New International Economic Order (Wealth) and Health.

As part of the current debate about political, social and economic relations between the developed capitalist countries and the underdeveloped countries little looms larger than the demand of the latter for the New International.economic Order (18). Its name makes clear what it is about. The leadership of the Third World is demanding a greater share of the benefits accruing from international economic relationships; in practice, an international redistribution of income among countries. The developed capitalist countries have responded with calls for a "new international development strategy, which while incorporating some (usually marginal) elements of the NIEO within it, concentrates primarily upon intra-national issues, especially the meeting of people's basic needs, including that of health care by the governments of the developing countries.

The nations which persistently demand the establishment of the New International Economic Order have also committed themselves, formally at least, to basic changes in their own countries in the approach to health development. At the First International Conference on Primary Health Care they joined with all the other countries of the world in stating that "existing gross inequality in the health status of the people, particularly between developed and developing countries as well as within countries is politically, socially and economically unacceptable and is, therefore, of common concern to all countries (19). And further that "economic and social development, based on a New International Economic Order, is of basic importance to the fullest attainment of health for all and to the reduction of the gap between the health status of the developing and developed countries. The promotion and protection of the health of the people is essential to sustained economic and social development and contributes to a better quality of life and to world peace " (19). Within their organization the Group of 77 has stated that the "central element of the international development strategy of the Third United Nations Development Decade (the 1980s) should be the implementation of the New International Economic Order and in this context action needs to be taken to enhance the share of the developing countries in the international decision making for management of the world economy (20). Also, that within. the new strategy adequate attention should be paid to the eradication of mass poverty and to raising the living standards of people in the developing countries" (20).

The major industrialized capitalist countries however, continue to oppose the basic concept of the NIEO in favour of a separate basic needs strategy which would be independent of any significant changes in the overall world order. In any event, it should be clear that achievement of the NIEO would not by itself guarantee either development in any real sense or improved health conditions for the world's poor. This flows from the fact that more material resources are not the unique basis of improved national health indices.

It is true that, historically, improved levels of income have led to improved health status, at least in the sense that morbidity and mortality experience has shifted from the more acute to the more chronic diseases. As discussed above, this change is not especially related to specific health care (preventive or curative) programmes as such, but rather to overall improvement in living standards. It is also true that dependent capitalist "development" in the neocolonial states has led to a deterioration of the health status of the majority. Thus to be really effective, the NIEO would have to correspond with changes in the internal order of most of the developing countries.

Primary Health Care

Primary health care is a concept based on a redefinition of health, expressing national political committments to a "new health order" and its goal of "health for all by the year 2000". PHC is not some special activity separate from the overall socioeconomic or health care system, nor it is some perfect solution to all health problems arid the sole creator of "health for all ". At the very least, though, it offers the health sector an instrument for the organization of more relevant and effective health care systems. Thus, a reorientation of the health care system, away from its typical hospital bias in favour of a widespread network of fully utilized basic health services, is a minimum goal for any health ministry. An adequate primary health care strategy would be broader than only an extension of the services offered by the conventional Ministry of Health, or even the development of new types of community health activities. The intrinsic philosophy of PHC would find application in terms of overall economic and social policy, specific ministerial and programme activity in all sectors influencing health, and all aspects of the work of the Ministry of Health itself. Planning for health requires a conscious review of the effects on the health of the entire population, and especially on its more vulnerable groups, of all governmental and governmentally influenced decisions and activities. Thisimplies a democratic, interrelated and generally decentralized approach to health related planning and decision making, programme management and implementation, and administration.

The PHC strategy in its more carefully elaborated form relates overall social change to such innovations as the use of village level workers or indigenous health practitioners in health

development. But many of these types of activities appear only as projects; that is, isolated activities carried out apart from conventional national health and other systems which continue to absorb virtually the whole of ministerial budgets. In the absence of changes in the whole of national health planning and delivery systems such projects cannot be expected to be successful. In fact, even apparently quite radical proposals directed toward the training of "bare-foot doctors " or other types of village workers, or concentrated activity directed towards scattered populations, avoid the more central question: the present unsatisfactory pattern of allocation of resources and resulting absence of care even for those who already have reasonably adequate physical access to, say, health or educational facilities. In some ways it appears that answers to long standing problems are being sought outside existing health, education and other systems thus reflecting either cynicism or disillusionment about the possibility of changing the way these structures now function. But such attitudes cannot be allowed to become the basis of two-tier systems, one for the minority with access to an expensive high technology system and one for the rest of the population. While village workers may well be potentially important instruments of change, they can only be so in the context of extensive political, social and economic adjustment at national and international level. At this moment there is at least as much possibility of the PHC strategy becoming in many countries nothing more than a dodge to avoid change, as of its becoming a positive instrument in the creation of change.

It is important to note that despite the almost universal rhetorical support being given to the PHC concept (although different definitions of PHC are being offered), many/most governments and agencies remain tied to more traditional views of the causes of disease and the best ways of organizing scarce resource for disease control activities. These' more traditional views are often expressed in the context of continuing support for categorical disease control programmes, and opposition to the integration of such programmes into more generalized PHC: activities. (Sometimes, however, certain of the apparent intellectual struggle between "verticalists" an integrationists " seem to be based more on empire protection and building than anything else. A particularly pernicious defense of traditional categorical disease control programmes goes under the name, or concept, of "selective primary health care ". Such programmes are vertical in character and are distinguished from, say, unipurpose malaria or family planning programmes only by the fact that they combine several activities within structures which remain essentially vertical in character. Such programmes make a mockery of the integrated PHC approach to health development (21).

Another important point in the PHC approach is community participation. Community control over local PHC activities offers, at least potentialy, the best bruarantee of their successful development. To besuccessflJI PHC will have to make use of technologies which result in services that are affordable to low income populations. It is precisely the fact of community development and control over limited resources,both those contributed directly by the local community itself and by higher levels of government, which offers the best assurance that appropriate technological choices will be made so as to assure the equitable and efficient use of those limited resources. There are many ways in which the community can participate in the different stages of PHC development and implementation, from the definition of problems and the setting of priorities through the planning and implementation of the entire range of locally based PHC activities. It is in the course of such participation that precise judgements could be made which would determine the nature, cost and utilization of PHC activities: judgements concerned with such activities as labour contributions to construction, the training of village health workers, collaboration with traditional healers and birth attendants, the creation of local social insurance funds, and so on. As already implied, it is the net results of such decisions which would determine the basic costs of PHC.

Technical supervision of community level health services in PHC comes from the more specialized levels of the health system, primarily through guidance, education and that supply of appropriate information. Managerial control of PHC activities comes primarily from the community itself; for example, with reward to staff discipline, the supply and safekeeping of drugs, and community links with traditional healers andbirth attendants. In addition to its contribution to the management of PHC, the community can contribute financial resources in a large number of different ways, or its labour for the construction of clinics, pit latrines, and the development of sources of clean water. Labour and time can also be contributed to the control of mosquitoes, snails and other disease carriers. Another important form of labour contribution by the community is the community health worker. If these health workers come from the community in which they live and are truly chosen by it they are likely to have its support.

6- CONCLUSION

Deeper understanding of the bases of good health coupled with changed ideas about economic and

social development are inherent to the primary health care approach to "Health for All by the Year 200". The slogan of Health For All necessarily provides sufficient ambiguity of definition to allow governments to pursue their preferred course of action in reaching their chosen health goals. Of course, many variations of interpretation reflecting different levels of development could properly be expected, but other differences are based primarily upon the degree of willingness to genuinely pursue the PHC approach. Perhaps the most obvious distinction is between, integrated national approaches to health development of the type to be found, typically, in countries maintaining socialist perspectives and the piecemeal, project type activities which characterize most such efforts in capitalist countries within the Third World. In this latter approach, PHC is usually equated with community health workers and isolated low cost community programmes which are distinct from the overall health system of the country.This approach has contributed to the view that PHC is a type of second class medicine reserved for the poor. It need hardly be said that this approach is completely at variance with the expressed purposes of the Declaration of Alma Ata. It is not surprising that in countries in which decisions are taken by and in the interest of only a small part of the population, little progress has been made in implementing the primary health care approach to health development although this has not stopped many such countries from waving the banner of Health for All. It should be clearly stated that primary health care is not, in the first place, about medical care; rather, it is about overcoming under development and thereby achieving health for all. Obviously health for all cannot be achieved in any country in the absence of an end to underdevelopment.

World Bank and other data indicate that in the absence of basic changes 600 to 1,000 million people will still be living in "absolute poverty "in the year 2000 and hundreds of millions of other will continue to live below the "poverty line". The contradiction between these figures and the goal of health for all offers a stark and incompatible contrast. Of course, the fact that such predictions are offered does not mean either that they must come to pass or that they will necessarily encompass the population of any particular country.The first task in each country is to fight against the forces of inequality and oppression which are opposed to a successful outcome of the struggle for Health for All. In fact, throughout the Third World millions already engaged in that struggle, supported by progressive forces and individuals all over that world. As always -the struggle continues.

REFERENCES AND NOTES

1. World Health Organization. 1979. Children and Health. In International Year of the Child Themes No0 5. Geneva: W.H.O. Figures calculated from data portrayed in Graphs I and 2.
2. World Health Organization. 1980. Sixth Report of the World Health Situation, 1973-1977. Part I: Global Analysis. pp. 1-2. Geneva: W.H.O.
1. For discussion of this concept and some of its antecedents see the work of such authors as. Samir Amin (Africa and the Arab World). P. Baran (Third World); B. Davidson (Africa); F 0 Fanon (Third World); A. Gunder Frank (Latin America); C. Furtado (Brazil); P.Jalee (Third World); W. Rodney (Africa); T. Szentes (Third World). Ethiopian Journal of Health Development. Vol.l No.1. 1984.
2. For further discussion see: Gish, 0. 1982. Economic dependence, health services and health: The case of Lesotho. Joumal of Health Politics, Policy and Law. 6(4):762-779.
3. Palmer, R. and N. Parsons. 1977. The Roots of Rural Poverty in Central and Southern Africa. p. 21.
4. Berkley and Los Angeles: University of California Press: This paragraph is based upon material con. tained in chapter 1. For more detailed discussion of this issue, see: Monyake, L.B. 1974. Lesotho-Land, Population and Food. In Report on the National Population Symposium. Maseru, Lesotho.
5. The September 14, 1981, issue of Africa News reports that "Half Lesotho's consumption of staple foods is now imported and the already poor level of health and nutrition is expected to worsen" p. 4. Africa News Durham, North Carolina.
6. Montsi, S. No date. Population Growth.. Labour Force and Employment. Maseru, Lesotho-Central Planning Office, Government of Lesotho. Mimeo.
7. Morris, j.N. 1975. The Uses of Epidemiology 5th edition. p. 144. Edinburgh, London and New York: Churchill Livingstone.
8. Based upon data from: New York City. Department of Health. Weekly Reports of the Department of Health.Vol. XXI. No. 50.December 17, 1932, p. 396.
9. For more on this question, see: Segall, M. 1983. The politics of primary health care. IDS BuUeti7L 14(4): 27-37. Institute of Development Studies. University of Sussex.
10. The best known work in this area is by Thomas McKeown. See: Medicine in Modem Society.

1965. London: Allan and Unwin. An Introduction to Social Medicine. 1974. Oxford: Blackwell; The Modem Rise of PopulatiO7L 1976. London: Edward Arnold. The Role of Medicine. 1979. Oxford: Blackwell.

11. Puffer, R.R. and C. V. Serrano. 1973. Patterns of Childhood Mortality. Washington, D.C.: Pan- American Health Organization/World Health Organization.
12. Sterky, G. and L. Mellander, eds. 1978. Birth-Weight Distribution -An Indicator of Social Development. Stockholm: SAREC Report No. R2:1978.
13. McCord, C. 1976. The Companiganj rural health project: A joint venture between government and voluntary agencies. Contact 34 (August 1976). See an excellent chapter by: Azizur Rahman Khan. 1977. Poverty and Inequality in Rural Bangladesh. In Poverty and Landlessness in Rural Asia. Geneva: International Labour Organization. 17. The literature on this subject is extensive. A useful starting point would be the document prepared for the 1976 World Employment Conference: Employment, Growth and Basic Needs: A One World Problem. 1976. Geneva: International Labour Organization. Discussion Article: Health, Development and PHC 29.
14. Useful publications on the NIEO include; Amin, Samir. 1977. Self-Reliance and the New International Economic Order. Monthly Review. 29(3):1-21;
15. Cox, R.W. 1979. Ideologies and the New International Economic Order: Reflections on recent literature. lhternational Organization. 33(2):257-301;
16. Shaw, T.M. 1978-79. Dependence to inter-dependence: Review of the debate on the (New) International Economic Order. Alternatives 4:557-578. With regard to health see: Gish, 0.1983. The relation of the New International Economic Order to health. Journal of Public Health Policy 4(2):207-221.
17. World Health organization. 1978. Declaration of Alma Ata. pp. 23. Geneva: W.H.O.
18. Ministerial Meeting of the Group of 77. 'Item 8". February, 1979. Arusha, United Republic of Tanzania. For more on this subject, see: Gish.O. 1982. Selective primary health care; Old wine in new bottles.Social Science and Medicine. 16:1049-1054.

9/29/2012