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## Investigating the effect of changing in aggregation of stone materials containing high specific gravity with constant fineness modulus on penetration of chloride ion, compressive strength and density of heavy concrete

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**Abstract:** This study analyzed some physical and mechanical properties of concrete produced with magnetite (Fe<sub>2</sub>O<sub>3</sub>) aggregate supplied from Khaf county of Iran. Since in the concrete mix design of sand gradation is controlled and applied just by fineness modulus and being in the range of standard gradation. In this paper with fixing these two factors, the effect of changing sand grading by decreasing or increasing the amount percent of remained in sift on changes of chloride ion penetration, compressive strength and density of heavy-concrete is investigated. For this purpose, four types of sand grading with constant fineness modulus in standard grading zone and two kinds of gravel with different maximum nominal size design of concrete mixes are probed. In these concrete samples, chloride ion penetration as one of the parameters related to the concrete durability and in the other hand 7, 28 and 42 days compressive strength, slump and density of concrete are studied.

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**Keywords:** Heavy concrete; Fineness modulus; Aggregation; Chloride penetration; Compressive strength

### 1. Introduction

Widespread and increasing use of radioactive materials in medicine, research, and industries particularly nuclear energy industry that is considered as basic needs of human existence today, along with increased risk of human exposure from ionizing radiation is due to these materials. To avoid the harmful environmental effects, protection against nuclear radiations is one of the most important problems in the use of radioactive materials for radiation therapy or nuclear technology. With the development of nuclear energy and particularly for protection from fatal rays such as neutron and  $\gamma$ , which have the ability to penetrate objects, heavyweight concrete began to be used as a shield [1]. The first step in any treatment room planning of radiotherapy services is to establish the design criteria. These criteria comprise (i) the limitations imposed by the allowed dose equivalent rates in different areas of the facilities and (ii) the existing space for construction [2]. Without losing the effect of shielding radiation, heavy concrete makes possible to reduce barrier thickness in limited spaces. It should be noted that in design of nuclear facilities with high production capacity reliable distance from residential areas and the population must be observed, in addition access to the vast resources of water particularly for use in various parts of these industries is very important. Therefore in most cases these facilities will be built adjacent seas and oceans. So the combination of strength and durability properties

of this particular type of concrete due to continuous exposure in the aqueous environment is very important. In fact the purpose of this type of structure is design and implementation of concrete that provide the proper compressive strength hand has lower permeability especially in chloride penetration, as long as the cement mortar is of sufficient quality, it can provide good lubrication and contraction [3]. The compressive strength of heavy concrete also increases with iron ore content, while the tensile strength declines [4]. Thus, investigation of the effect of grading aggregate particularly sand on pore structure of concrete and permeability is more important.

### 2. The test processing

#### 2-1- Consumable materials

##### 2-1-1- Cement:

Type I: Portland cement of Ghaen cement factory, according to ASTM C150 with 370kg/cm<sup>2</sup> compressive strength used in design of concrete mixes. To prevent heat of quick and high hydration and later potential cracks, guidelines of using concrete have recommended not using the type III cement and acceleration in the preparation of this particular kind of concrete [4-5].

##### 2-1-2- Aggregate:

Coarse and fine aggregates made of magnetite (Fe<sub>2</sub>O<sub>3</sub>) aggregate with 60% purity percent are gained from Khaf mine, one of the best mines of magnetite stone in the world, located at 10km of Torbat-e-Jam city. Completely broken down and washed sand and gravel are used. In order to create aggregates grading in

the standard range and constant fineness modulus, one ton of sand and gravel is separated into individual grains according to ASTM C33 via standard sifts [6]. In this laboratory study coarse aggregate with two maximum nominal sizes of 25 and 12.5 mm and fine aggregate with fineness modulus of 2.83 are selected in four different kind of grading and based on standard grading range. The most important factors for fixing fineness module are mentioned below:

- 1- Fixing volume amount of coarse aggregate and fine aggregate and stability of coarse aggregate to fine aggregate ratio in any mix design.
- 2- Stability of quality and softness aggregate in design of mentioned mixes in order to study changes of fine aggregate in identical conditions

for both aggregates with different maximum nominal sizes.

It is worth noting that these four different types of sand grading are created by changing remained amount on each sift and constant softness module of 2.83. Therefore, with considering the selection of two types of gravel gradation (the maximum sizes of grains are 25 and 12.5 mm) and 4 different types of sand gradation with constant fineness modulus of 2.8, totally eight different types of grading aggregates are used in concrete samples. Information relating to the tests carried out on gravel and sand with different nominal maximum sizes of 12.5 and 25 mm according to ASTM C637, are shown in Tables 1,2 and 3 their grading curves are presented in figures 1 and 2[7].

Table 1. The results of experiments conducted on aggregates with a maximum nominal size of 12.5 mm and 25mm

Nominal maximum size of coarse	12.5mm	25mm	Standard No.
Specific gravity of coarse aggregate	4092kg/m <sup>3</sup>	4288kg/m <sup>3</sup>	ASTM C127
Specific gravity of fine aggregate	4362kg/m <sup>3</sup>	4362kg/m <sup>3</sup>	ASTM C128
Water absorption of coarse aggregate%	1.55	1.59	ASTM C127
Water absorption of fine aggregate%	0.7	0.7	ASTM C128

Table 2. The results of the coarse aggregate gradation

Sieve size	coarse aggregate	4.75mm	9.5mm	12.5mm	19mm	25mm	37.5mm
Percent passing	D max= 25mm	0	39.697	48.092	73.302	96.238	100
Percent passing	D max=12.5mm	0	60.363	91.563	100	100	100

Table 3. The results of the fine aggregate gradation

Sieve size	Sand type	No.4	No.8	No.16	No.30	No.50	No.100
Percent passing	A	100	94	66.9	40.2	13.5	2.3
Percent passing	B	100	90.2	61.5	41	22.5	2.3
Percent passing	C	100	83.4	63.5	43.6	24.1	2.3
Percent passing	D	100	81.2	56.9	47.1	30	2.3

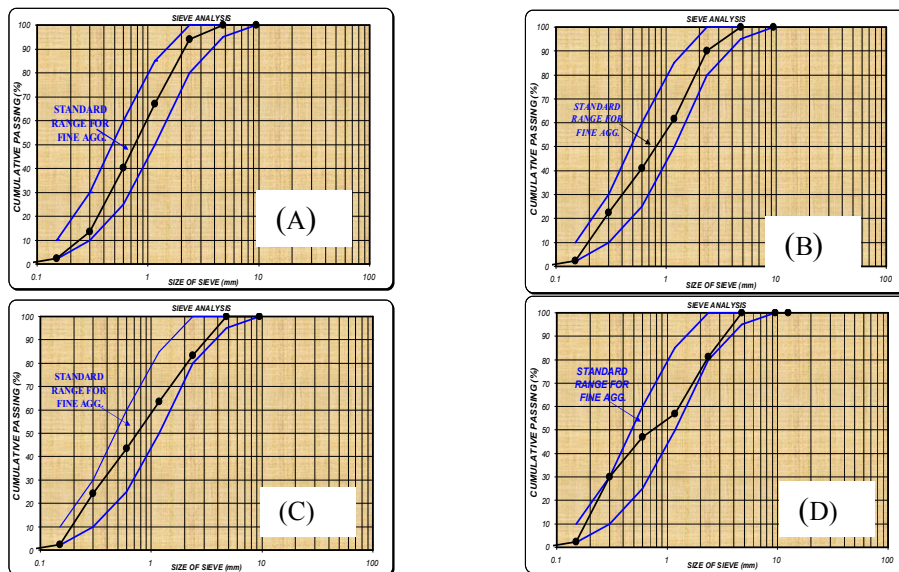


Fig 1. Fine aggregate gradation curves with different values of the rejection of standard sieve and constant Fineness modulus (2.83).

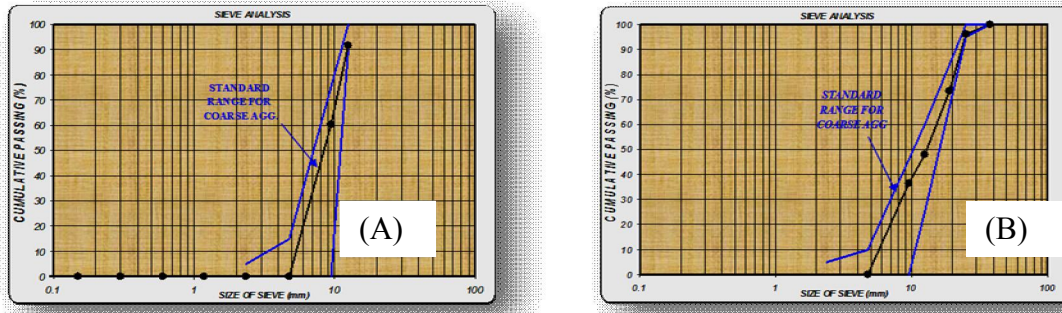


Fig2. Coarse aggregate gradation curves with a maximum nominal size of (A) 12.5 and (B) 25 mm

**2-2 The design of mix**

Heavy concrete mix design widely applied in radiation protection is much like the method used in the normal-weight concrete and The ACI 211.1 standard is described in Appendix 4, [8].According to the choice of two maximum sizes of 12.5 and 25 mm, we have used two mix designs. Since aggregates have special high weights and least water greatly helps reduce water of concrete and separation of aggregates,

water to cement ratio of 0.4is used in the design of mix. For each mix, four different types of sand gradation with constant fineness modulus of 2.83 presented in gradation tables and curves are used. The designs of Concrete mix are presented in Table 4. Finally, eight different types of concrete samples with different gradations were studied and their details are shown in Table 4.

Table4.Resultsofconcretemix design

Concrete mix design for maximum nominal size25mm					
Mix ID	Sand type	water(kg)	cement(kg)	Sand (kg)	Crushed stone(kg)
SP 25-A	A	202	505	1072.109	1616.141
SP 25-B	B	202	505	1072.109	1616.141
SP 25-C	C	202	505	1072.109	1616.141
SP 25-D	D	202	505	1072.109	1616.141
Concrete mix design for maximum nominal size12.5mm					
Mix ID	Sand type	water(kg)	cement(kg)	Sand (kg)	Crushed stone (kg)
SP 12.5-A	A	230	575	995.72	1367.5
SP 12.5-B	B	230	575	995.72	1367.5
SP 12.5-C	C	230	575	995.72	1367.5
SP 12.6-D	D	230	575	995.72	1367.5

Note: For example the code SP 12.5-A means coarse aggregate gradation curves with a maximum nominal size of 12.5 mm and A sand type.

**2-3- making mixes and preparation of samples:**

Aggregates such as crushed, washed and separated-grain gravel and sand (the remained amounts of each standard sieve are split apart to achieve a desired gradation) have been used in the design of concrete mixes. All concrete mixes are performed using a horizontal mixer and low speed.15cm × 15cm × 15cm cube mold sampling was conducted in three layers. Prepared samples are removed from molds after 24 h at laboratory temperature and maintained in a pool of processing water with a constant temperature of 23°C. In order to performance of Chloride ion penetration test, The specimens were then cured in water at 23°Cfor 28 days; five sides of one sample of each mix design are covered by Epoxy varnish to become completely impermeable. Samples prepared

according to ASTM C1152 were in a solution of water and salt 90 days (165 gr of salt per liter of water). So that the samples were completely under water and salt solution and the side without Epoxy varnish was free from the surrounding.

**2-4 Tests performed on samples**

**2-4-1 Experiment related to investigating the properties of fresh concrete**

**2-4-2 Experiment for determination of Slump**

To study the properties of fresh concrete, relative efficiency determining test is done according to ASTM C143 standard immediately after the preparation of concrete mix [9]. Results are presented in Table 5.

**2-4-1-2 Experiment for determination the Unit volume weight of concrete**

The average special weight of fresh concrete is obtained from molded samples immediately after molding and compressing concrete samples. The results are shown in Table 5.

**2-4-2 Experiment for investigation of hardened concrete properties**

**2-4-2-1 Experiment for determination of compressive strength of concrete**

In order to determine the average compressive strength of concrete over time, Cubic samples with dimensions of 15cm × 15cm × 15cm are

tested after 7, 28 and 42 days. Concrete breaker Jack is used to break down concrete samples. Force rate is kept about 680 to 700kgf/s. The results of this experiment are presented in Table 5.

**2-4-2-2 Experiment for determination percent of Chloride ion penetration in concrete**

After preparing powder from different samples in various depths 0-1, 1-2, 2-3 and 3-4cm (ASTM C1152) the amount of chloride of investigated samples is measured by using electro potentiometric (ASTM C114) and satisfactory results are achieved. Results of this experiment are presented in Table 5.

**Table 5.** Overall results of tests conducted on concrete samples

Mix ID	Specific gravity of concrete (kg/cm <sup>3</sup> )	slump (mm)	compressive strength (kg/cm <sup>2</sup> )			depth of Chloride ion penetration(cm)			
			7 day	28 day	42day	0-1	1-2	2-3	3-4
			percent of Chloride ion%						
SP 25-A	3334.07	30	349.33	463.18	480.23	0.75	0.45	0.35	0.25
SP 25-B	3349.62	40	360.35	486.15	489.56	0.8	0.65	0.45	0.35
SP 25-C	3351.55	45	369	489	505.6	0.65	0.45	0.3	0.2
SP 25-D	3356.77	30	384.10	503.21	515.33	0.55	0.35	0.25	0.15
SP 12.5-A	3205.29	40	367.42	467.50	484.32	0.55	0.40	0.30	0.20
SP 12.5-B	3233.33	44	372.07	477.35	489.26	0.75	0.55	0.35	0.30
SP 12.5-C	3257.77	70	361.73	483	498.53	0.55	0.35	0.25	0.15
SP 12.6-D	3291.85	60	392.2	493.3	510.87	0.45	0.30	0.20	0.10

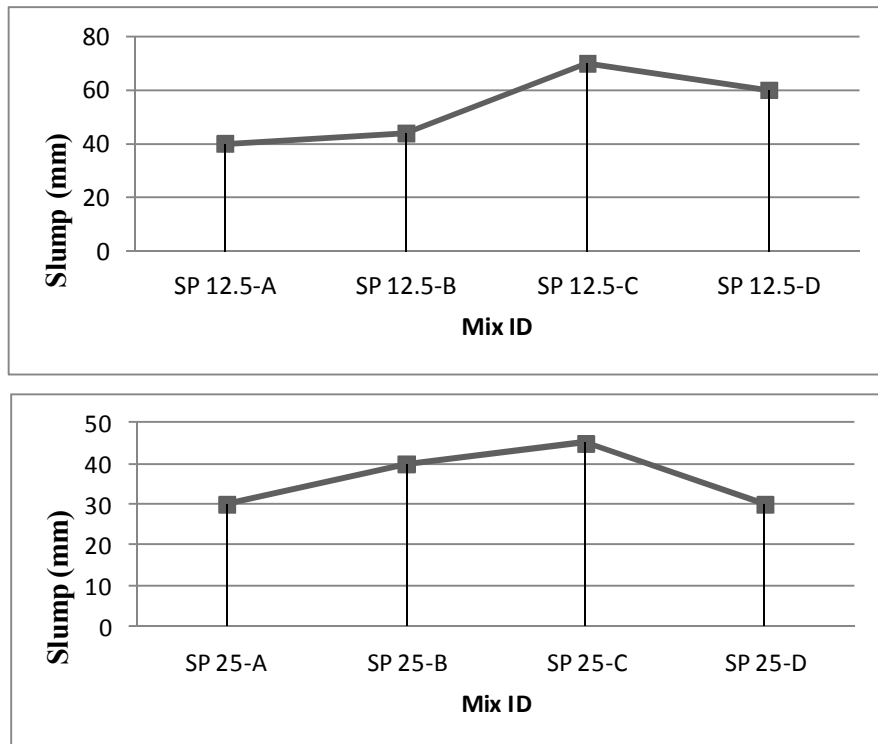


Figure 3. Graph showing change of slump in concrete, Based on the different aggregation.

**3. Result and discussion**

**3-1- Slump and flow of concrete**

One of the most important characteristic of aggregates affecting on properties of concrete is

shape of particles. Shape of Particles is the main factor in changes of mix performance. Due to the high special weight of materials, to water run Policy some measures such as increasing smallness of grading



tissue.(decreasing fineness modulus of aggregate mix), Increasing cement or adhesive materials, Using crushed aggregate, decreasing water-cement ratio and increasing powder materials can be useful to reduce bleeding. By considering the amount of allocated water of two mentioned designs of mix, slump higher than obtained values is expected (Expected slump = 100 mm).It should be noted that stone materials such as gravel and sand used are crushed and this factor is a clear reason for flowing concrete. The results presented in Table (7) indicate that the slump of concrete of SP 25-A samples increases from SP 25-A to SP 25-C by changing grading of sand respectively and the slump of SP 25-D sample is lower than SP 25-C sample. Changes of slump between SP 12.5 samples are the same as SP 25 samples. It is noteworthy that in general the slump of the concrete of SP 12.5 samples is more than SP 25 samples. Figure 3 shows changes of the slump of SP 25 concrete samples with the change of sand gradation and figure 4 shows changes of the slump of SP 12.5 concrete samples with the change of sand gradation.

**3-2- compressive strength of concrete**

According to the results presented in table (5), 7, 28 and 42 days compressive strengths are increased between SP25 samples by changing of grading from SP 25-A to SP 25-D. The increased amount of 7, 28 and 42 days compressive strengths of SP 25-D concrete samples are 9.95%, 8.64% and 7.3% respectively as compared with SP 25-A samples.in the other hand 7, 28 and 42 days compressive strengths are increased by changing of sand grading from SP 12.5-A to SP 12.5-D. The increased amount of 7, 28 and 42 days compressive strengths of SP 12.5-D concrete samples are 6.74%, 5.51% and 5.30% respectively as compared with SP 12.5-A samples. Considering various curves of sand grading and comparison with the compressive strength of concrete show that strength of concrete samples is increased by increasing the remained percent on No.100 sieve (from 13.5% to 30%) and fixing the fineness modulus of sand (FM=2.83). So the solid concrete is created. Moreover, as mentioned in the previous section, this increase of strength is also associated with the increase of slump. Figure 4 shows changes of 7, 28, 42 days compressive strengths of SP 25 and SP12.5 concrete samples with the change of sand gradation.

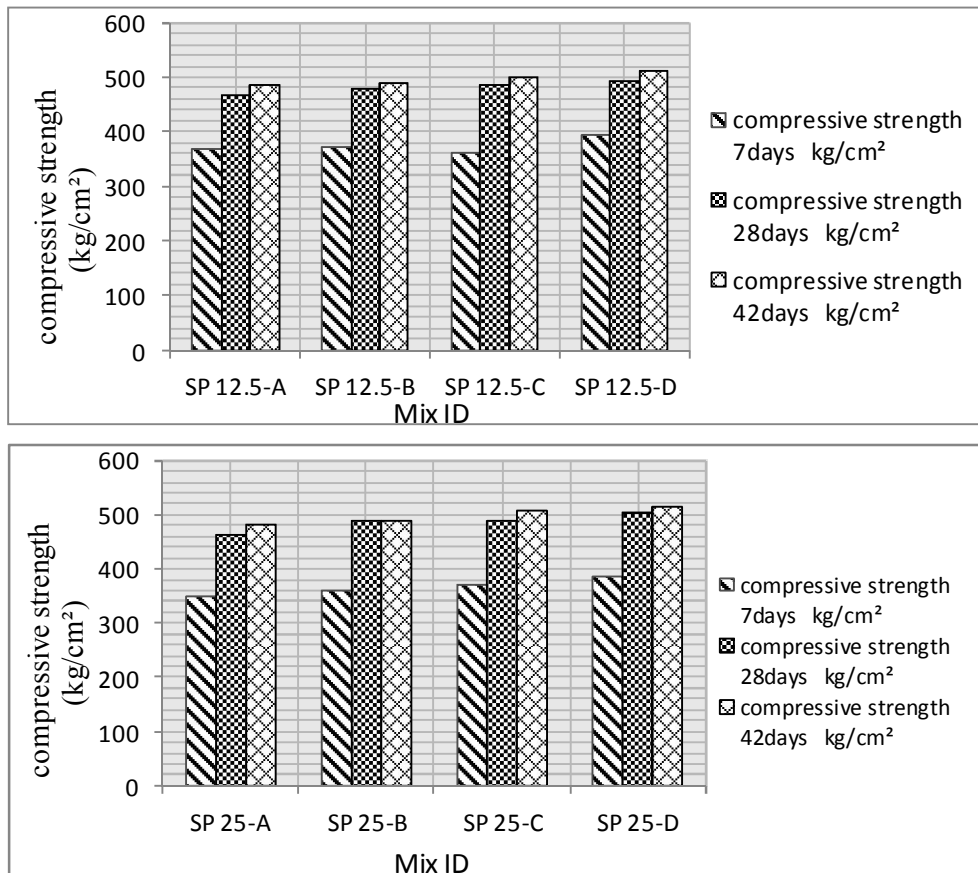


Figure 4. Graph showing change of compressive strength in concretes Based on the different aggregation.

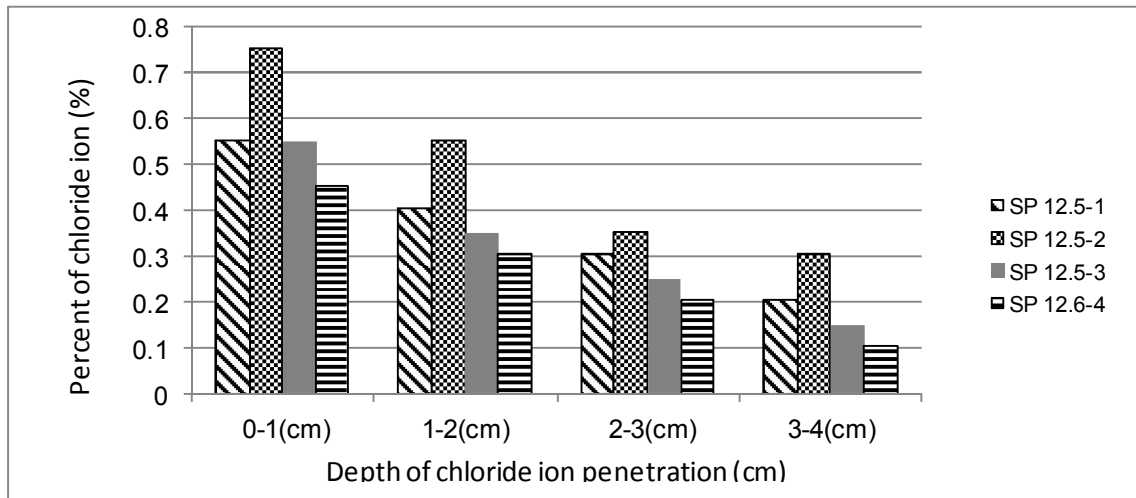


Figure 5. Graph showing change of chloride ion penetration in various depths of concrete samples, base on changes in aggregate.

#### 4. Conclusion

**Chloride ion penetration:** According to the results presented in table 5, the percent of chloride ion penetration of SP 25 samples is increased by increasing the remained percent on No.100 sieve from SP 25-A to SP 25-B and it is remarkably decreased in SP 25-C and SP 25-D samples as compared with SP 25-A and SP 25-B samples. Even as the permeability coefficient of SP 25-D is less than permeability coefficient of SP-25-A sample. Changes of concrete permeability coefficient among SP 12.5 are the same as SP 25 samples. This result is also true for different depths in discussed samples. In four types of sand grading mentioned in this paper, grading changes alters the grain distribution and leads to create concrete samples with different pore structures. This eventually causes a change of percent penetration of chloride ion in different samples of concrete. Figure 5 shows Changes of the percent penetration of chloride ion in SP-25 and SP 12.5 concrete samples with changes of sand grading.

According to the results presented in table (5), with the rise of remained percent on NO.100 sieve from 13.5% to 30%, Unit volume weight is increasing. Considering that the concretes with a unit volume weight greater than 2600 kg/m<sup>3</sup> are called heavyweight, all of the concrete samples in this study can be called heavyweight concrete. It is noteworthy that in general the unit volume weights of the concrete of SP 25 samples are more than SP 12.5 samples.

the rise of remained percent on NO.100 sieve (increase passed percent of NO.50 sieve) from 13.5% to 24.1%, sand acts as ball and the performance of concrete is increased. With increasing remained percent on the No.100 to 30% sieve, special surface of sand increases and leads to increase percent absorption of water by sand compared with ball performance and then the slump of concrete decreases.

With constant fineness modulus of sand in the range of standard grading curve, the change of sand grading with increasing and decreasing of remained amount on sieves significantly alters strength properties and permeability of the concrete.

Considering various curves of sand grading and comparison with the compressive strength of concrete show that strength of concrete samples is increased by increasing the remained percent on No.100 sieve (from 11.2% to 27.7%) and fixing the fineness modulus of sand (FM=2.83). So the solid concrete is created.

samples of concrete made of(D) type sand that have maximum remained percent on the NO.100 sieve(27.7%)includes the highest compressive strength and by comparing the changes of compressive strength in the ages of 7,28 and 42 days, we find that Difference of development of compressive strength decreases by increasing age of investigated samples from SP-A to SP-D. The percent development of strength in different ages are mentioned briefly: The percent development of compressive strength of SP-25-A compare with SP-



25-D: (9.95%, 7 days), (8.64%, 28 days) and (7.3%, 42 days) and the percent development of compressive strength of SP-12.5-A compare with SP-12.5-D: (6.74%, 7 days), (5.51%, 28 days) and (5.30, 42 days).

Samples of concrete made of (D) type sand that have maximum remained percent on the NO.100 sieve (27.7%) are selected as the most efficient kinds of Sand gradation. They have the best and lowest percentage of chloride ion penetration.

With comparing the changes of compressive strength and the percent of chloride ion penetration of SP-25 and SP-12.5 samples with each other, we find that the increase in compressive strength of SP 25-A to SP-25-D samples and SP 12.5-A to SP 12.5-D samples doesn't lead to same changes of the penetration of chloride ion. In fact porosity alone has no effect on the rate of chloride penetration of concrete but pore structure of concrete (size and connection of pores) affects on penetration parameters.

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## Responses of Antioxidative Protection to Varying Drought Stresses Induced by Micro-Ecological Fields on Desert C<sub>4</sub> and C<sub>3</sub> Plants in Northwest China

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**Abstract:** Desert plants are exposed to a combination of environmental stress conditions, including water deficit, high temperature and high irradiance. We focused on antioxidative protection systems of C<sub>4</sub> desert plant *Haloxylon ammodendron* and C<sub>3</sub> desert plant *Hedysarum scoparium* within arid dune ecosystem with artificial controlling water availability. The activities of antioxidative enzymes (SOD, POD and CAT) in *H. scoparium* were significantly higher than those in *H. ammodendron* under the same conditions; the activities of antioxidative enzymes in leaflets of *H. scoparium* were higher than those in rachis. With the increasing drought, the accumulation of the antioxidant (flavonoids) was increased in *H. ammodendron*. However, the amount of flavonoids was decreased in *H. scoparium*. Flavonoid contents in the leaflets and rachis of *H. scoparium* were higher than those in assimilating shoots of *H. ammodendron*. In conclusion, *H. ammodendron* and *H. scoparium* applied different strategies of protection against water deficit, high irradiance stress and high temperature stress during daily process in summer, and inducing high activities of antioxidative enzymes in C<sub>3</sub> desert plant was an important safeguard to live in desert condition, and the advantages of C<sub>4</sub> metabolism of *H. ammodendron* was that it was not easily influenced physiologically to the same level of stress as *H. scoparium*.

[Gong C, Ning P, Bai J. Responses of Antioxidative Protection to Varying Drought Stresses Induced by Micro-Ecological Fields on Desert C<sub>4</sub> and C<sub>3</sub> Plants in Northwest China. *Life Sci J* 2012; 9(4):2006-2016] (ISSN: 1097-8135). <http://www.lifesciencesite.com>.301

**Keywords:** antioxidative enzymes; antioxidant; desert C<sub>3</sub> and C<sub>4</sub> plants; *Haloxylon ammodendron*; *Hedysarum scoparium*; drought stress.

### 1. Introduction

Plants are subjected to several harsh environmental stresses that adversely affect growth, metabolism, and yield (Almaghrabi, 2012; Metwali, 2012). Especially, the desert plants are often exposed to all kinds of stressful conditions such as high irradiance, extreme temperature, water deficit and air-dryness. Water deficit is the principal impact on plants growth and development, causing physiological and biochemical responses. Generation of reactive oxygen species (ROS) is considered to be a primary event under a variety of stress conditions (Posmyk *et al.*, 2009). There is overwhelming evidence that many antioxidants play a key role in plant adaptation to both abiotic and biotic stresses (Burritt and MacK-enzie, 2003; Vranova' *et al.*, 2002). The sequence of events in the plant tissue subjected to drought stress are: (1) increased production of ROS and of oxidized target molecules; (2) increasing in the levels of antioxidative enzymes and antioxidants; and (3) increased scavenging capacity for ROS, resulting in tolerance against the drought stress (Mano, 2002). During the long term of evolution, higher plants have developed various defending systems to scavenge ROS, which helps them to survive under unfavourable conditions (Bowler *et al.*, 1992).

Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR)] and non-enzymatic (flavanones, anthocyanins, carotenoids and ascorbic acid) (Reddy *et al.*, 2004).

Antioxidant enzymes are known to increase in response to drought (Menconi *et al.*, 1995; Zhang and Kirkham, 1996; Sairam and Saxena, 2000; Sairam and Srivastava, 2001), high temperature (Upadhyaya *et al.*, 1990; Sairam *et al.*, 2000). High SOD activity has also been reported during the treatment of low temperature, high irradiance stress and drought (Dhindsa and Matowe, 1981; Burke *et al.*, 1985; Baisak *et al.*, 1994). CAT is irradiance-sensitive and easy to be inactivated by irradiating (Feierabend and Engel, 1986). Meanwhile plants typically produce a diverse group of antioxidants as a protective mechanism against oxidative compounds which are produced in response to various stresses and known to have a damaging effect on membranes, organelles and macromolecules (Mittler, 2002; Noctor and Foyer, 1998). A significant part of antioxidants produced by plants in response to stress is secondary metabolites, including a vast array of

simple and complex phenolic compounds derived primarily via the phenylpropanoid pathway (Dixon and Paiva, 1995). Flavonoids are a vast group of polyphenolic substances, with high antioxidant properties, found in most plant species (Moure *et al.*, 2001).

Studying the response of desert plants to the combined effect of these stresses within their natural habitat may therefore unravel complex relations between known mechanisms, and possibly reveal novel mechanisms and strategies that enable plants to resist stressful conditions. Mittler *et al.* (2001) found in response to prolonged exposure to extreme conditions, the upper part of a C<sub>3</sub> desert plant such as *R. raetam* enters a state of 'dormancy' that may protect the entire plant during stress. The two main strategies employed by desert plants to withstand the extreme environmental conditions of their natural habitat are avoidance, as with growth of winter annuals or resurrection plants only during the rainy season; and resistance, as in the survival of evergreens throughout the different seasons (Cushman *et al.*, 1989; Raven *et al.*, 1992). The level of response depends on the species, the development, and the metabolic state of the plant, as well as the duration and intensity of the stress. Many stress situations cause an increase in the total foliar antioxidant activity (Pastori *et al.*, 2000), the anatomical and physiological adaptations of evergreen desert plants have been the subject of numerous studies (Fahn and Cutler, 1992; Mittler *et al.*, 1991; Streb *et al.*, 1997), but little is known about the protected mechanisms of different carbon metabolism pathway of desert plants that enable these plants to withstand harsh desert environments.

In this research we aimed to investigate the anti-oxidative response of desert plants which were subjected to increasing water stress in natural combined conditions. In order to discuss environmental adaptation of the different carbon metabolism pathway of desert plants we have investigated respectively the change of protecting components such as SOD, POD, CAT and flavanones in desert C<sub>3</sub>, C<sub>4</sub> plants. *Haloxylon ammodendron* is a C<sub>4</sub> plant with assimilating shoots as photosynthetic organ, and *Hedysarum scoparium* is a desert legume C<sub>3</sub> plant with leaflets and rachis performing photosynthesis (Su *et al.*, 2003). *H. ammodendron* and *H. scoparium* are common in arid ecosystems in the desert regions of China. They are important components of the desert vegetation and even as a dominant population in arid areas in Northwest China. *H. ammodendron* and *H. scoparium* are distributed over areas where there is air-dryness, adequate sunshine and precipitation less than 130 mm. *H. ammodendron* is a priori species of desert vegetation

in Northwest China. In the present paper we will report putative different defense responses of *H. ammodendron* and *H. scoparium*. We detected activities changes of antioxidative enzymes in different organs at three depths of ground water (DGW) every three hours in *H. ammodendron* and *H. scoparium*, attempting to compare different mechanisms in the antioxidative system defending against high temperature, water deficit and high irradiance between C<sub>4</sub> and C<sub>3</sub> desert plants.

## 2. Material and Methods

**Habitat of trial site:** The study was carried out near the town of Minqin, northwestern China, which locates in the joint of Tengger Desert and Badain Jaran Desert (38°38'N, 103°05'E). The trial site lies in the Plant Transpiration Consume Water Observation Station which is in the Desert Plantation of Gansu Provincial Institute of Desert Control Research. The trial spot is in natural state where there are many sand dunes correlating to *Nitraria tangutorum* and *Reaumuria soongorica* communities in an enclosed and protected area. The average precipitation a year is 115 mm, with main precipitation converging in 7-9 month; dry degree is 5.3, and the average evaporation a year is 2643.9 mm which is 23 times to the average precipitation a year. The average temperature a year is 7.8 °C, with the highest temperature 38.1 °C, the lowest temperature – 28.8 °C. The non- frost season is 165 days, and the period of blowing sand is 139 days. the process of sandstorm is 37 days. The soil is sandy soil. The depth of ground water is 18 m (Zhao *et al.*, 2003). With the diurnal changes of photon flux density (PFD) and air temperature (T<sub>Air</sub>) of sunny day in early August, 2003 in Minqin, the highest value of photon flux density (PFD) increases at 13:00, which reaches 1638 μmol m<sup>-2</sup>s<sup>-1</sup>, and the highest temperature is 42.4 °C, appearing at 15:50 (Figure 1) (Gong *et al.*, 2006).

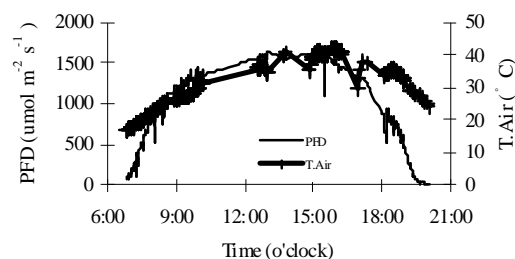


Figure 1. Diurnal changes of photon flux density (PFD) and air temperature (T<sub>Air</sub>) on sunny days in early ten days of August 2003 in Minqin, northwest China (Gong *et al.*, 2006)

**Plants growth conditions and sampling procedures:**

Description of the Plant Transpiration Consume Water Observation Station and transplant and treatment of *H. ammodendron* and *H. scoparium* was detailed by Gong *et al.* (2006). Water was supplied by groundwater eternal compensatory apparatus, which could balance water level of storage in a stable water level. The Plant Transpiration Consume Water Observation Station established 3 depths of ground water (DGW), 1.4 m, 2.4 m and 3.4 m. Soil water content increased gradually as soil depth increase at every DGW (Figure 2), resulting in light, moderate and severe water stress for plant growth (Gong *et al.*, 2006). There was no negative effect for plant's growth and development at 3 depths of groundwater in the Plant Transpiration Consume Water Observation Station (Gong *et al.*, 2006).

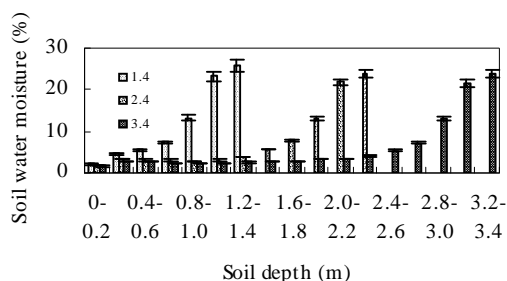


Figure 2. Soil water moisture at different depths of ground water (DGW) in root areas of *Haloxylon ammodendron* and *Hedysarum scoparium*. Each value represents mean ( $\pm$ s.e.) of three replications ( $P < 0.05$ ) (Gong *et al.*, 2006)

The sample of collected leaves and assimilating shoots was frozen in liquid nitrogen. We did this investigation in early August, 2003. The collected samples were all in the growing and developing stage.

**Determination of water content:** Relative water content (RWC) of the assimilating shoots in *H. ammodendron* and the leaflets and rachis in *H. scoparium* were estimated by recording the fresh mass (FM) and the saturated mass (SM) of 0.5 g fresh leaf samples by keeping in water for 24 h, followed by drying in hot air oven till constant dry mass (DM) is achieved according to the method of Barrs *et al.* (1962).

$$\text{RWC (\%)} = \left( \frac{\text{FM} - \text{DM}}{\text{SM} - \text{DM}} \right) \times 100\%$$

**Enzyme assays:** Leaf tissue, 0.5 g, was frozen in liquid nitrogen and ground to fine powder with a pre-cold mortar and pestle, the powder was homogenized in 1:5 (w/v) 50 mmolL<sup>-1</sup> phosphate buffer, pH 7.8, containing 1 % insoluble polyvinylpyrrolidone and 10 mmolL<sup>-1</sup> sulfhydryl alcohol. The homogenate was

centrifuged at 15000  $\times$  g for 20 min and the supernatant obtained was used immediately for assay as enzyme extract. All steps in the preparation of the enzyme extract were carried out at 4  $^{\circ}$ C. Determination of all enzyme activities was conducted through UV-751 ultraviolet spectrophotometer. An aliquot of the extract was used to determine its protein content by Bradford (1976) method using bovine serum albumin as a standard.

SOD (EC 1.15.1.1) activity was measured by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971) as modified by Dhindsa and Matowe (1981). The reaction mixture (3 ml) contained 50 mM phosphate buffer, pH 7.8, 0.1 mM EDTA, 13 mM methionine, 75  $\mu$ M NBT, 2  $\mu$ M riboflavin and 50  $\mu$ l of the supernatant diluted 5 times. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 15 W fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Reaction product was measured at 560 nm. The volume of the supernatant corresponding to 50% inhibition of the reaction was assigned a value of 1 enzyme unit. A non-irradiated reaction mixture did not develop colour and served as control. Enzyme activity was indicated unit mg<sup>-1</sup>protein.

POD (EC1.11.1.7) activity was assayed as increase in absorbance due to the formation of tetraguaiacol was recorded at 470 nm (Castillo *et al.*, 1984). The 3 ml reaction mixture contained 16 mM guaiacol, 2 mM H<sub>2</sub>O<sub>2</sub>, 70 mM phosphate buffer (pH 6.1) and 20  $\mu$ l enzymatic extract diluted 2 times.

CAT (EC 1.11.1.6) was assayed by measuring the initial rate of disappearance of hydrogen peroxide at 240 nm according to the method of Chance and Maehly (1955) as modified by Dhindsa and Matowe (1981) and Cakmak and Marschner (1992). The 3 ml contained 50 mM phosphate buffer, pH 7.0, 15 mM hydrogen peroxide, and 50  $\mu$ l enzyme extract diluted 10 times. The decrease in hydrogen peroxide was followed as a decline in 240nm. The activity was expressed in units where one unit of catalase converted one  $\mu$ mole of hydrogen peroxide per minute. Enzyme solution containing hydrogen peroxide-free phosphate buffer was used as control.

**Flavonoids contents assays:** The relative content of flavonoids was detected at 305 nm treating with acidified methanol according Mirecki *et al.* (1984). The fully expanded green assimilating shoots, leaflets and rachis (0.1 g) were cleaned and air-dried and

extracted with 20 ml acid methyl alcohol (methanol: water: hydrochloric acid = 79:1:20) to deal with 3000 × g centrifuge, the supernatants in the 305 nm light absorption value means the relative concentration of flavonoids.

**Statistical analyses:** Tukey's HSD (honest significant difference) tests were used to analyze the differences between activities of enzyme. Correlations between parameters were determined using least-squares linear regression. Data presented in the figures are means of three replications ( $\pm$  s.e.).

### 3. Results and discussions

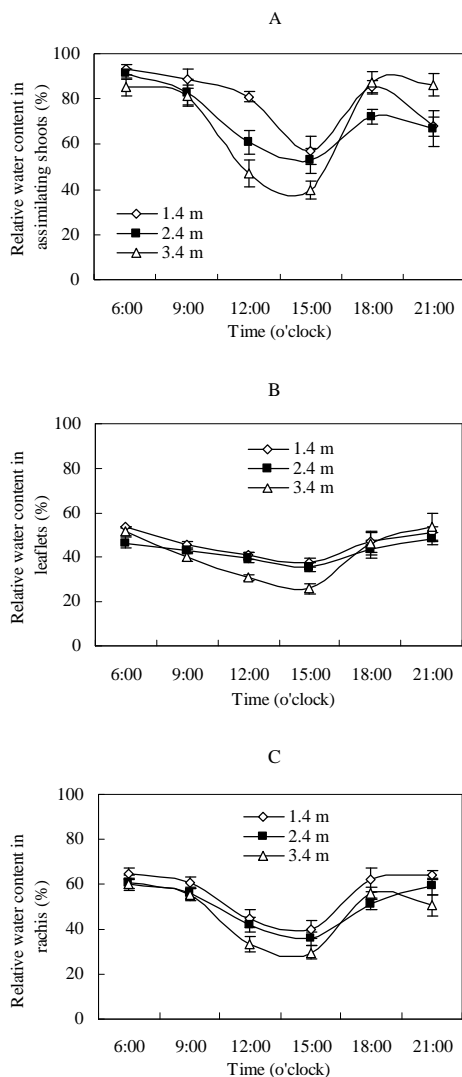


Figure 3. RWC in assimilating shoots of *Haloxylon ammodendron* (A), and in leaflets (B) and rachis (C) of *Hedysarum scoparium*. Vertical bars represent s.e. Each point represents mean  $\pm$  s.e. (n=3,  $P < 0.05$ )

**The higher RWC in *H. ammodendron* than in *H. scoparium*:** *H. ammodendron* true leaves are quite reduced and the cortex of young annual cylindrical shoots was the main photosynthetic tissue. *H. scoparium* contained two different assimilating organs (leaflets and rachis). As is shown in Figure.3, the RWC either in leaflets or in rachis of *H. scoparium* was very low compared with that of *H. ammodendron*. The high resistance of *H. ammodendron* to hot summer, drought conditions may be attributed in part to assimilating shoots which are succulency and fleshy (Pyankov *et al.*, 1999). Even higher RWC was recorded at 15:00 in the 1.4 m, RWC in 2.4 m higher than that in 3.4 m in assimilating shoots of *H. ammodendron* (Figure 3A). The lower RWC in *H. scoparium* may be one of survived mechanism of  $C_3$  plant in desert environment. Drought-related physiological changes, such as decrease in leaf water content and the accompanying stomatal closure, resulted in limited  $CO_2$  availability and the channeling of reducing equivalents from the photosynthetic apparatus to the production of ROS, rather than to  $CO_2$  fixation (Krause and Cornic, 1987). It will trigger the antioxidative protection. By controlling the availability of water, we were able to show that the lack of water, and high temperatures, excess light, or low relative humidity which occurred in the dry season, was the limiting environmental factor that triggered the plant to defend oxidative damage. The diurnal changes of RWC in leaflets were gentler and lower than those in rachis of *H. scoparium* in same condition (Figure 3B and C). It was more sensitive for RWC of cylindrical assimilating shoots in *H. ammodendron* and cylindrical rachis in *H. scoparium* to increase drought than that of rachis in *H. scoparium*.

#### Antioxidative enzymes system of *H. ammodendron*:

SOD activity tended to increase at a low level in a short period treatment of the combined stress (Ye *et al.*, 2000). We also detected that SOD activity unremarkably enhanced during early morning to afternoon and reached peak in 15:00 when in high irradiance and high temperature, the lowest RWC whatever any DGW (Figure 4A). *H. ammodendron* at 3 DGW from 1.4 m to 3.4 m showed increased SOD activity. Plants were resistant to single high irradiance, and many evidences showed that photochemical apparatus could keep their integrity and the recovery from photoinhibition is fast. Due to high temperature sustain from 10:00 which simplified conclusion that SOD activity in response to high irradiance was relatively high compared to water deficit, it was possible that the increase in SOD activity provided another pathway for the removal of



excess electrochemical energy (Ye *et al.*, 2000). High SOD activity has also been reported during the treatment of drought (Baisak *et al.*, 1994). Although activity of SOD in 3 DGW in response to high irradiance stress were probably sufficient to withstand the amount of oxidative stress, our results also clearly showed the ability of tolerant water deficit in *H. ammodendron*.

POD and CAT appeared to play an essential protective role in the scavenging processes when coordinated with SOD activity (Massacci *et al.*, 1995). Our data also indicated that, the activities of guaiacol peroxidase (POD) increased almost coordinately with SOD activity in all treatments (Figure 4B). POD activity increased at 12:00 when plants faced with high irradiance ( $> 1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) and high temperature ( $< 35^\circ\text{C}$ ) but decreased at 15:00 when plant resisted to high irradiance ( $> 1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) and high temperature ( $> 39^\circ\text{C}$ ) at 3.4 m. These indicated not only moderate irradiance could produce more  $\text{H}_2\text{O}_2$  under high temperature (Ye *et al.*, 2000), but also high irradiance could produce more  $\text{H}_2\text{O}_2$  under higher temperature. We thus found that POD activity was more inactive to temperature than to irradiance. In addition, a significant increase in the POD activity in *H. ammodendron* assimilating shoots accompanied the increase of water stress at noon, indicated that *H. ammodendron* is capable of effectively scavenging the ROS for the production of certain secondary metabolites to withstand during stresses of high irradiance and water deficit.

An increase in the activity of *H. ammodendron* CAT was observed from 1.4 m to 2.4 m treatment and a decline in 3.4 m from morning to afternoon (Figure 4C). There was no significant change in the activity of CAT at 3.4 m treatment from 12:00 to 15:00 in spite of high irradiance and temperature during this period. This further suggested that the responses of CAT activity to water deficit proved to be very complex, involving nearly every major function of plant growth. Under the double effect of irradiance and temperature, most decrease of CAT activity was caused by temperature, and irradiation exacerbated the inactivation of CAT. There was sustained increased in CAT activity at 3 DGW from 6:00-12:00 when temperature and irradiance were all moderate, however, CAT activity increased at 1.4 m, 2.4 m, but more decreased at 3.4 m. Moreover, during 12:00 (high irradiance) -15:00 (high temperature) only 7.8% of CAT activity were decreased at 2.4 m, far less than that at 1.4 m. This result supported that CAT activity was more sensitive to temperature than to irradiance (Ye *et al.*, 2000). After high temperature at 18:00, apparently, there was no significant difference in CAT activity change at 3 DGW under different irradiances. This further

suggested that concomitant resynthesis of catalase compensates for the loss of catalase and maintains a constant level under irradiation when plants were not exposed to stress (Feierabend and Engel, 1986).

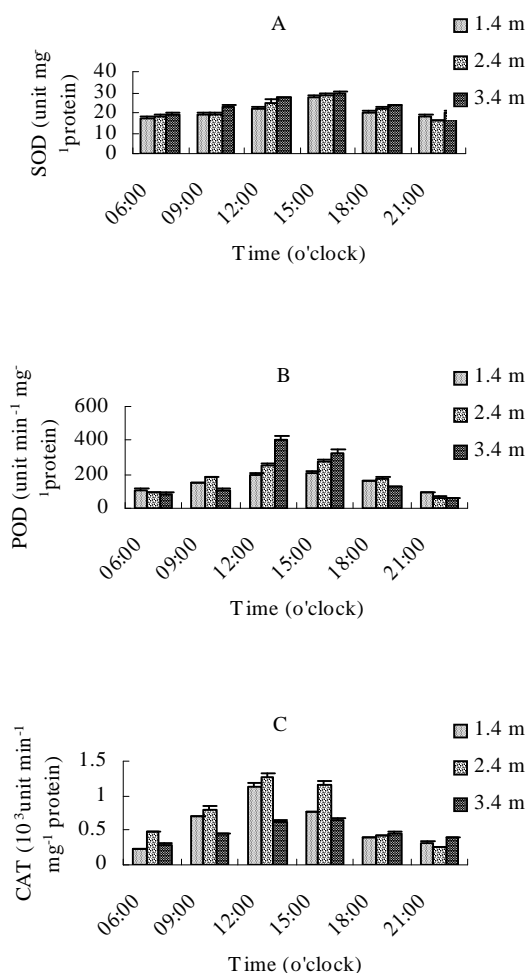


Figure 4. Diurnal changes of antioxidant enzymes activities in assimilating shoots of *Haloxylon ammodendron* at different DGW. (A) SOD activity, (B) POD activity and (C) CAT activity. Vertical bars represent s.e. Each point represents mean  $\pm$  s.e. (n=3,  $P < 0.05$ )

As is shown in Figure 4, the activities of the SOD, POD and CAT was upregulating during 6:00-15:00, suggesting that the intracellular level of ROS may increase in plants during these time points. It was also possible that changes in the activities level of these enzymes reflected a specific diurnal rhythm. Induction of oxidative stress in drought-stressed plants had also been well known (Ramachandra Reddy *et al.*, 2000; Chaitanya *et al.*, 2002; Mano, 2002). Antioxidant components were also known to



be distributed among all photosynthetic cells in higher plants. The distribution of antioxidative enzymes between mesophyll and bundle sheath cells of  $C_4$  plants has been described (Foyer, 2002). In maize leaves, most of the SOD and APX were localized in mesophyll and bundle sheath cells. CAT was approximately equally distributed between mesophyll and bundle sheath cells.  $H_2O_2$  was found to accumulate only in mesophyll cells (Doullis *et al.*, 1997; Foyer, 2001). These localization studies are very interesting, because enzymes of the PCR cycle, which are very sensitive to  $H_2O_2$ , are found only in bundle sheath chloroplasts. Such studies on  $C_4$  plants indicate that oxidative damage was not uniformly distributed between mesophyll and bundle sheath cells of  $C_4$  plants. Kingston-Smith and Foyer (2000) suggested that oxidative damage under stressful conditions in  $C_4$  plants is restricted to bundle sheath tissue because of inadequate antioxidant protection in this tissue. Regrettably, very little mechanistic information is available on drought-induced antioxidative metabolism between the two cell types in *H. ammodendron*.

**Antioxidative enzymes system of *H. scoparium*:** In general, activities of antioxidative enzymes were higher in leaflets than in rachis in *H. scoparium*, they are all higher than that in *H. ammodendron*. Changes of antioxidant enzymes were all undulated violently either in leaflets or in rachis in process of a day. Activities of antioxidative enzymes were higher at noon but lower in the early morning and evening. In order to compare activities of antioxidant enzymes in leaflets to in rachis we discuss specially the changes in activities of anti-oxidative enzymes at different DGW at 15:00 when plants exposure to high irradiance and high temperature.

SOD activity in leaflets and in rachis gradually increased with the deeper of DGW at 15:00, SOD activity was found to be much higher in leaflets than in rachis of *H. scoparium* at 3 DGW (Figure 5A), and the ratio of SOD activity of leaflets/rachis gradually decreased, which demonstrated that SOD activity in leaflets play a more significant role than in rachis when plants grow in the relative sufficient water condition and explained why appearance of assimilating shoots of *H. scoparium* is essential to defend oxidative stress in environment of water deficit.

A different trend in POD activity in leaves of *H. scoparium* was observed, POD activity in leaves increased slightly from 1.4 m to 2.4 m but declined at 3.4 m at 15:00. POD activity in assimilating shoots gradually increased with the deeper of DGW at 15:00, as well as the ratio of POD activity of leaves/assimilating shoots gradually decreased with the deeper of DGW (Figure 5B).

There was a familiar effect in POD activity of *H. scoparium* with in SOD activity of *H. scoparium*. High activity of antioxidative enzymes could be correlated to the process of differentiation that occurred during shoot induction (Thakar and Bhargava, 1999).

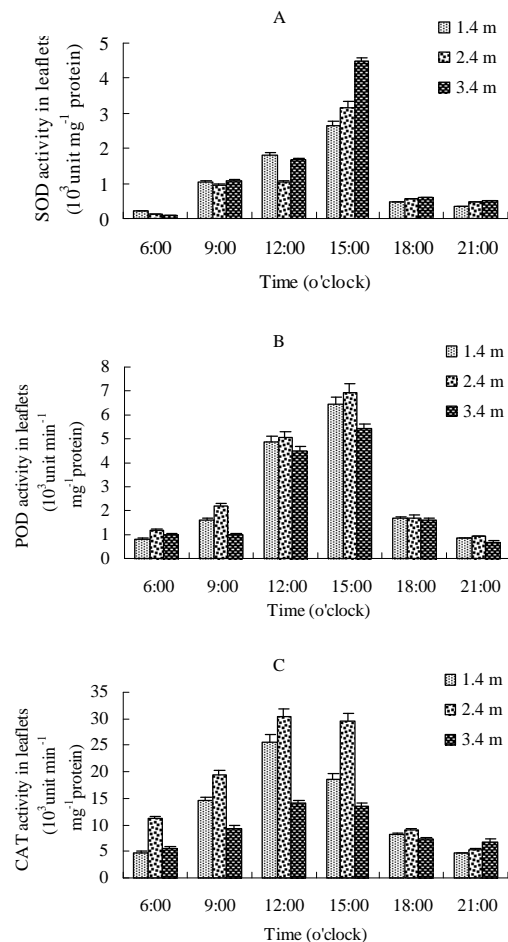


Figure 5. Diurnal changes of antioxidant enzymes activities in assimilating shoots of *Haloxylon ammodendron* and in leaflets and rachis of *Hedysarum scoparium* at different DGW. (A) SOD activity, (B) POD activity and (C) CAT activity. Vertical bars represent s.e. Each point represents mean  $\pm$  s.e. ( $n=3$ ,  $P < 0.05$ ).

There was only minor losses of CAT activity occurred in assimilating shoots of *H. scoparium*, but a marked apparent water deficit inactivation of catalase was observed in leaves of *H. scoparium*. This result consisted with CAT activity in stems of *R. raetam* and in leaves of *A. halimus* in natural daylight (Streb *et al.*, 1997). CAT activity was found to be higher in leaves than in assimilating shoots of *H. scoparium* under 3 DGW at 15:00,

however, increased CAT activity was observed either in leaves or in assimilating shoots from 1.4 m to 2.4 m but declined at 3.4 m at 15:00 (Figure 5C). The present findings further convinced that CAT activity not only subjected to high irradiance but also water deficit, moderate water stress could stimulate CAT activity but stronger water stress inactivated dramatically CAT activity. This finding supported that drought stress inactivated the key enzyme activity of light respiration-ethanol acid oxidative enzyme 44% (Morgan, 1994), leading to accumulating more NADPH and ATP, increasing the possibility of production of  $O_2^-$ ,  $H_2O_2$  in chloroplast. Production of  $H_2O_2$  during drought stress may also result from the catalytic activity of glycolate oxidase in peroxisomes during photorespiration (Osmond, 1981). CAT is also involved in  $H_2O_2$  scavenging during stress (Comba *et al.*, 1998). The activity of SOD increased during somatic embryogenesis while POD and CAT activities decreased (Gupta and Datta, 2003). The change of CAT activity in rachis is more smoothed than that in leaflets, suggesting that rachis was not subjected to a similar level of stress to these leaflets during the midday stressful hours.

#### Antioxidant in *H. ammodendron* and *H. scoparium*:

Plants containing flavonoids have been reported to possess strong antioxidant and hypolipidemic properties (Aviram, 2004; Sudheesh and Vijayalakshmi, 2005; Sweedy *et al.*, 2007). Previously, methanolic extract of this whole plant extract exhibited strong antioxidant activity (Surveswaran *et al.*, 2007). Flavonoids content was determined in *H. ammodendron* and *H. scoparium*, which may be how the plants to resist high temperatures, drought, ultraviolet radiation and removal of reactive oxygen species.

Flavonoid contents in the leaflets and rachis in *H. scoparium* was higher than that of assimilating shoots under the same DGW (Figure 6A, B and C). Flavonoids content in a different trend both in *H. ammodendron* and in *H. scoparium* under different DGW, in that other environmental conditions are the same, different DGW influence the accumulation of flavonoids. Flavonoid contents was the lowest and most stable at 1.4 m and was highest at 2.4 m in *H. ammodendron*. The drought increase, the accumulation of flavonoids was increase. Flavonoid content was higher at 1.4 m and was the lowest at 3.4 m in *H. scoparium*. The drought increase, the amount of flavonoids was decrease. But in the same environmental conditions  $C_3$  plants *H. scoparium* need to produce more amounts of flavonoids in order to protect them from higher temperature, stronger light and higher drought, to

absorb ultraviolet light, to prevent the evaporation of water, to regulate metabolism. This is also one of the  $C_3$  plants mechanisms adapting to adversity in the desert conditions. The flavonoid-rich fraction of *S. hispidum* seed possesses strong antioxidant properties as evidenced by a significant increase in the levels of enzymic and non-enzymic antioxidants (Kaviarasan *et al.*, 2008).

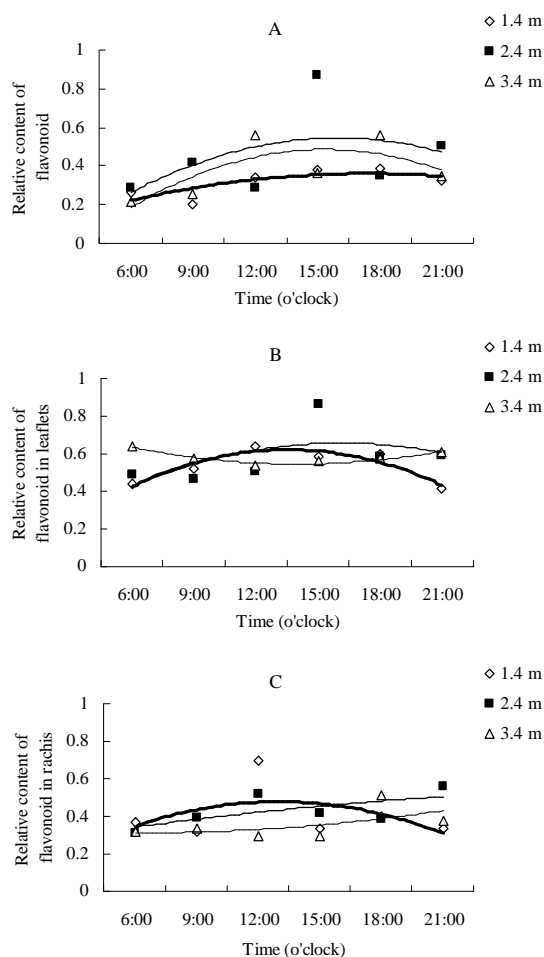


Figure 6. Diurnal changes of flavonoid in assimilating shoots of *Haloxylon ammodendron* (A) and in leaflets (B) and rachis (C) of *Hedysarum scoparium* at different DGW

Antioxidant levels and the activities of ROS scavenging enzymes have been correlated with tolerance to several different environmental stresses (Massacci *et al.*, 1995), because one of the biochemical changes subjected to environmental stresses in plants is the production of ROS (Dat *et al.*, 2000). As plants acclimatise or experience sub-lethal levels of stress like high temperature, potential to scavenge free radicals often increases (Bridger *et al.*, 1994). From the foregoing discussion it can be

concluded that antioxidant enzymes such as SOD, CAT play an important role in the regulation of senescence processes. So we prefer to conclude that antioxidant enzymes in naturally growing desert plants would respond to naturally occurring stresses and artificial controlling water content within their natural habitat, explaining the detail between the activities of ROS scavenging enzymes and several different environmental stresses. It seems quite plausible to assume that much of the increase in SOD activity observed in mature leaves could be increased in mitochondrial SOD (Sairam *et al.*, 2003). SOD activity has also been reported during high irradiance stress (Burke *et al.*, 1985). Certain POD isomers utilise the phenolic compounds and H<sub>2</sub>O<sub>2</sub> to initiate the biosynthesis of several secondary metabolites required for the plant growth, development and differentiation. H<sub>2</sub>O<sub>2</sub> of lower concentration will be scavenged by POD in the oxidant of somatic such as phenols (Mittler, 2002). Chowdhury and Choudhuri (1985) reported a larger decrease in the activities of SOD and CAT in a drought-sensitive line of jute than in a drought-tolerant line. CAT is irradiance-sensitive and easy to be inactivated by irradiating (Feierabend and Engel, 1986). Despite its restricted localization and photolability, CAT may play a significant role in scavenging H<sub>2</sub>O<sub>2</sub>, which can readily diffuse across the membrane (Bowler *et al.*, 1992). In addition, CAT was sensitive to longer daily exposures, evidenced by a decrease in its activity (Casati *et al.*, 2002).

Here we show that all these different antioxidative protection responses of the desert plants to combined environmental stress, which were tested in the laboratory, can be found in naturally growing desert plants that respond to naturally occurring stresses and artificial controlling water content within their natural habitat. The increased activities of ROS scavenging enzymes should have a greater significance as invaluable tools in the elucidation of plant metabolic regulation under stressful environment (Chaitanya *et al.*, 2001). As the results have shown, activities of antioxidant enzymes either in leaflets or in rachis of *H. scoparium* were higher than those of *H. ammodendron* (Figure 5 and Figure 6). In supporting of our observation, higher activity of SOD is one of the most important guarantees which defend high irradiance (Rabinowitch and Sklan, 1980). Leaves of plant in light were more sensitive than in dark either in high temperature or in low temperature (Feierabend *et al.*, 1992). Due to C<sub>4</sub> plant tending to occupy places which were in high irradiance, high temperature and water deficit but C<sub>3</sub> plant did not acclimatize well. However, we found C<sub>4</sub> pathway of photosynthetic carbon assimilation in leaflets and in rachis of *H. scoparium* (Gong *et al.*,

2006), which explained why *H. scoparium* as a kind of C<sub>3</sub> shrub could survive in desert environment. The observation that high temperatures stimulated the mulberry leaf chloroplasts to reduce oxygen to superoxide due to high temperature-induced electron transport system, prompted us to postulate that the over-expression of SOD might have improved the tolerance to high temperature (Chaitanya *et al.*, 2001), over-expression of SOD might have improved the adaptation of C<sub>3</sub> plants in desert conditions. It is conceivable that acquisition of the stress tolerance in any plant is a multi-factorial function and amelioration of ROS scavenging systems is an important index to assess the abilities of mulberry cultivars to tolerate the stressful conditions like high temperature. We presume that the metabolism of the reactive oxygen species under stressful environment is dependent on different functionally interrelated antioxidant enzymes. This finding may suggest that, physiologically, *H. ammodendron* is not subjected to the same level of stress as *H. scoparium*. It is possible that the additional anatomical and physiological adaptations of a C<sub>4</sub> plant better protect it from the stressful conditions in the desert ecosystem (Edwards and Walker, 1983).

#### 4. Conclusions

The above results suggested that the desert plants *H. ammodendron* and *H. scoparium* applied different strategies of protection against water deficit, high irradiance stress and high temperature stress damage. In the assimilating shoots of *H. ammodendron* antioxidative protection appears to play the major role, while *H. scoparium* appears to avoid oxidative damage in the field by leaflets more than rachis. The drought increase, the amount of flavonoids was increase in *H. ammodendron*. Whereas, the drought is increase, the accumulation of flavonoids is decrease in *H. scoparium*. Flavonoid content in the leaflets and rachis of *H. scoparium* were higher than those in of assimilating shoots of *H. ammodendron*. Our findings may provide the advantages of C<sub>4</sub> metabolism of *H. ammodendron* is not subjected physiologically to the same level of stress as *H. scoparium*. It is essential for us to provide with a theoretical foundation of rational exploitation and optimizable distribution for desert plants in Northwest China.

In conclusion, the present work compared the oxidative protection system of the C<sub>3</sub> desert plant with that of C<sub>4</sub> desert plant during the daily process in summer, and demonstrated high activities of antioxidative enzymes in C<sub>3</sub> desert plant was important safeguard of living in desert condition.

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## Cause related marketing campaigns and consumer purchase intentions: The mediating role of cultural values and perceptual brand equity

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**Abstract:** The purpose of this research is to investigate the kind of relationship between Cause Related Marketing (CRM) campaigns, brand awareness, brand image and perceived quality as possible antecedents of consumer purchase intentions in the country of Iran. An initial conceptualization was developed from mainstream literature to be validated through empirical research. The conceptualization was then tested with quantitative survey data collected from about 400 consumers studying in different purchase centers in Tehran. Structural Equation Modeling was used to test the hypothesis derived from literature. The findings indicate that consumer purchase intentions are influenced by the cause related marketing campaigns. Furthermore it was observed that the perceptual brand equity dimensions and cultural values partially mediate the impact of CRM campaigns on consumer purchase intentions. The data was gathered from purchase centers in Tehran only. Hence, future research could extend these findings to other cities in Iran to test their generalizing ability. This research has key implications for companies, charities and academic to effective use of cause related marketing campaigns in Iran. The study contributes to cause related marketing literature by indicating a mediating role of perceptual brand equity and cultural values on CRM campaigns and consumer purchase intentions. The mediating role of culture and perceived quality was ignored in previous studies. Moreover, it contributes to close the gap of empirical research in this field in Iran.

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**Key words:** cause related marketing, conceptual brand equity dimensions, cultural values, purchasing intentions, Iran.

### INTRODUCTION

Corporate social responsibility is one of the concepts of social marketing. As Maignan and Ralston (2002) propose social responsibility is, "A firm committed to CSR has principles and processes in place to minimize its negative impacts and maximize its positive impacts on selected stakeholder issues". Kotler and Lee (2005), identify six major initiatives under which social responsibility-related activities could fall: Cause promotions; Cause Related Marketing; Corporate Social Marketing; Corporate Philanthropy; Community Volunteering; Socially Responsible Business Practices. As above mentioned, Cause related marketing is one way to operationalise corporate social responsibility and Varadarajan and Menon (1988) state that a CRM campaign aims at two objectives: to support a social cause and to improve marketing performance. In fact cause related marketing is a pro-social strategy for influencing consumers (Osterhus1997) in which three different players intervene: the brand, the consumer and the social cause, usually a Non-Profit Organization (NPO).

Simply providing quality products or services does not suffice in today's competitive business environment to win the brand (Shabbir et al, 2009) and also, customers are instead more interested about finding of what the brand stands for and companies that take in consideration factors such as environmental and social have an impact in customers' decision(Fellman, 1999) has becoming the most

important strategic challenges of businesses and forced them to search unique strategies to overcome this challenge. Therefore because of growing public concern over environmental and social issues, corporations have begun to affiliate their products with a range of popular causes, including social and ecological issues(Yechiam et al, 2002). This not also help to companies create differentiate in competitive environment but also help them answer to social and environmental issues. Linking to good causes has become attractive to many businesses, especially those engaged in dealings with consumers (Till and Nowak, 2000). These associations can influence perceptions regarding the corporations and, consequently, have an effect on how consumers evaluate products or services offered by the corporation (Dacin and Brown, 1997). In this context, Cause Related Marketing (CRM) represents one of the modern marketing strategies (Taylor, 2007) which are being used by most companies in the western world (Shabbir et al, 2009). Cause related marketing campaigns can increase the sales of the company by increasing trial purchase, repeat purchase and / or promoting multiple unit purchase. Today, most of the companies are using Cause Related Marketing as a strategic tool (Till and Nowak, 2000) to build a positive corporate image in the minds of consumers (Chattananon et al., 2008) which results in enhancing the sales of a company's products (Varadarajan and Menon, 1988) and profits as well (Adkins, 2004). Also consumers have better

perceptual associations with those organizations that work with charities and good causes (Farache and Perks, 2008). Cause related marketing that is synonym with concepts like cause marketing (Varadarajan and Menon, 1988), Pro-social marketing (Berglind & Nakata, 2005), resembles marketing (Quinones & Rebollo, 2009), Passion Marketing (Berglind & Nakata, 2005), mission marketing (Duncan & Moriarty 1997), and affiliated marketing (Varadarajan and Menon, 1988), was successfully launched by the American Express company for the first time during 1983 (Akins, 2000). The 2008 Cone Cause evolution study constituted of 1071 adults indicated that about 85% of U.S. consumers are more likely to buy from corporations associated with a cause or issue. 79% would be likely to switch from one brand to another brand, about the same in price and quality, if the other brand is associated with a good cause. 38% have bought a product associated with the cause in the last 12 months.

Similarly, a lot of research work has been done to investigate the impact of cause related marketing on consumer attitudes in developed countries like in UK, USA, Canada, Australia, and New Zealand, illuminating the topic from different angles. This research has shown that CRM programs are very successful in developed countries and have produced tremendously beneficial results for the companies in the form of increasing awareness, sales, profits and building positive image in the minds of consumers (Shabbir et al, 2009). There is no literature available regarding the validation of the studies on a developing country of Iran and therefore this study was conceptualized to fill this void.

In Iran, very a little companies have used the simplest form of CRM strategy as a tactical marketing tool to enhance the sales and to differentiate themselves in the market place that by attention to the positive attitude of Iranian consumers towards cause related marketing was founded during the exploratory data gathering, it is anticipated that these companies might not be effective in achieving their objectives. Therefore suggests the need for more differentiated research in this context to better understand the impact of CRM campaigns on local consumer purchase intentions.

Summarizing, in an effort to gain new insight into the effect of CRM on consumer attitudes in Iran, this study examines the impact of CRM campaigns on conceptual brand equity dimensions and cultural values as moderating and effective variables on relationships between cause related marketing campaigns and consumer purchase intentions. Therefore it seems that this study by attention to cultural differences among countries, prepare valuable information to compare Iranian consumers with

countries in which has conducted similar studies. Although this study is similar to the previous studies on the impact of CRM on consumer attitudes, it will extend the existing literature on cause related marketing by investigating the mediating role of perceptual brand equity and cultural dimensions which was ignored in the previous studies. For this purpose, an integrated initial conceptualization of the proposed relationships was developed with the goal of answering two research questions:

- 1) Do Cause Related Marketing campaigns have a positive impact on consumer purchase intentions in less developed countries like Iran?
- 2) Is this relationship a direct one, or is it mediated by cultural values of consumers and perceptual brand equity dimensions of the company?

The remainder of the paper is organized as follows: the very next section defines cause related marketing. Consecutively, the next section provides a more detailed theoretical background being conditional for the hypotheses to be developed followed by the methodology and empirical analysis of the results. Conclusions and discussions come next and, finally, limitations and directions for future research are provided.

#### **Defining cause related marketing (CRM)**

The concept of Cause Related Marketing (CRM) was firstly introduced in 1998 by Varadarajan and Menon as “the process of formulating and implementing marketing activities characterized by an offer from a firm to contribute to a designated cause when customers make purchases” (Varadarajan and Menon, 1988). However after it has been offered a various definitions of CRM that intentioned to a aspect of CRM, but in my opinion the best and perfected definition has been offered by Adkins that states cause related marketing is about a win: win: win scenario where the charity or cause and business win and indeed where the benefits also extend to consumers and other stakeholders”, meaning that not only companies, nonprofit organizations and/or consumers, but all of them benefit from CRM programs (Adkins, 2000). Therefore the definition of CRM used for this research is according on Adkins’ definition as be profitable for all society.

#### **Brand awareness and purchase intentions**

In this paper, brand awareness is seen as an important concept for two reasons: first, brand awareness is one of the factors which affect the attitude of the consumers towards the purchase of products; second, the relationship between CRM and brand awareness had been established in previous research (Shabbir et al, 2009). Nedungad (1990) argued that consumers’ inclination to purchase a brand in the market place depends upon the prior knowledge about the brand. Keller (2003) defines brand

awareness as the ability of customers to recall a brand among the clutter of rival brands. In the same vein, Radder and Huang (2008) hold that, especially in highly competitive markets, awareness can strongly influence a customer while buying a product. Consequently, to better exploit the contributions of brand awareness companies are using different strategies to create brand awareness among the consumers (Schmitt and Geus, 2006). Using cause related marketing to create brand awareness (Varadarajan and Menon, 1988) leads to enhanced purchase intentions (Hoyer and Brown, 1990; Grewal, 1998). Accordingly, Kotler and Keller (2006) regard Cause Related Marketing as just one opportunity for the companies to enhance the brand awareness. Implying the importance of experience on memory building, Skory et al (2004) argued that most of the companies use CRM to increase brand awareness among the consumers through their participation. So companies use the cause-related marketing campaigns consistently over time to change the overall attitude of consumers towards the company and its brands (Till and Nowak, 2000).

#### **Corporate image and purchase intentions**

Corporate image can be defined as the perception/feelings of customers regarding the company's products and activities (Webb and Mohr, 1998). It requires a lot of time and enormous resources to build a positive corporate image (Markwick and Fill, 1997). In order to build a positive corporate image in the minds of customers, companies are using cause related marketing as a strategy (Varadarajan and Menon, 1988; Chattananon et al., 2008) to gain a competitive edge in market place (Anselmsson and Johansson, 2007). As a result of their studies, Webb and Mohr (1998) as well as Anselmsson and Johansson (2007) argued that customers' purchase intentions are influenced by the corporate image of a company involved in cause related marketing.

#### **Perceived Quality and purchase intentions**

Perceived quality can be defined as the customer's perception of the overall quality or superiority of a product or service with respect to intended purpose, relative to alternatives (Zeithaml, 1988). Aaker (1991) has suggested that perceived quality lends value to a brand in several ways: reason-to-buy, differentiation, price premium, channel member interest, and brand extensions. Marketers across all product and service categories have increasingly recognized the importance of perceived quality in brand decisions (Morton, 1994). However in the literature, nothing founded in relation between perceived quality and cause related marketing. Therefore can be claim that the above mentioned relation for the first time is stated and tested in current research.

#### **Perceptual brand equity dimensions**

In general, customer-based brand equity consists of four main components: brand awareness, perceived quality, brand image, and brand loyalty (Chen and Chang, 2008). Among them, the first three components belong to perceptual brand equity while the last component refers to behavioral brand equity (Cobb-Walgreen et al, 1995; Yoo and Donhu, 2001). As previous studies have determined, directional relationships exist among the brand equity dimensions. Perceived quality is based in part on brand awareness, as a visible brand might be considered more able to provide superior quality. Brand association relies on the establishment of brand awareness, because brand awareness indirectly affects behavior, having a positive influence on perceptions and attitudes toward the brand (Aaker 1996). It is usually wasteful to attempt to communicate brand attributes until a name is established with which to associate them. Moreover, the principal characteristic of a brand is its position on the perceived quality dimension. High quality enables consumers to recognize the brand's distinctiveness and superiority. Thus, there is no claim that the four major brand equity dimensions are independent. Results from Yoo et al. (2000) support the statements that there are significant inter correlations among the dimensions of brand equity.

#### **Cause related marketing and perceptual brand equity dimensions**

The brand knowledge-building process depends on three factors: choosing brand elements, designing and integrating marketing programs, and leveraging secondary knowledge to build brand equity. Among them, the primary input comes from the marketing activities related to the brand. A high level of brand awareness and strong, favorable, and unique brand associations can be created in a variety of different ways by marketing programs (Keller, 2002). According to Yoo et al.'s Brand Equity Creation Process Model, the effects of marketing activities are mediated by the dimensions of brand equity. To examine this relationship, we need to first investigate and determine the relationships between marketing activities and brand equity dimensions. This study has investigated cause related marketing as a basis to build brand equity

#### **Culture and consumer behavior**

There are large differences among the value systems of consumers in different countries, value systems that are strongly rooted in history and appear to be very resistant to change. Effectiveness in marketing means adapting to cultural values Mooij and Hofstede (2002). Culture has recently gained more attention from academics in the area of consumer behavior. Nevertheless, the relation between culture and consumer behavior seems debatable. In business

practice, ignoring culture's influence has led many multinationals to centralize operations and marketing, but instead of increasing efficiency this has resulted in declining profitability. Thus, many experts have expressed their confidence and consensus in the link between culture and consumer behavior (Yau, 1994).

### Consumer Reactions to CRM

Besides corporate interest, CRM has increasingly become the subject of scientific interest. A substantial body of research has emerged over the years, investigating the effects of CRM on a variety of consumer factors, including consumer choice, consumer purchase decisions and consumer attitudes towards CRM itself, as well as towards companies that engage in CRM (Brink et al., 2006; Webb and Mohr, 1998). CRM, as a type of CSR has received particular corporate interest, due to the fact that CRM might have positive effects on consumer behavior (Brink et al., 2006). Although there are people who are willing to accept a lower performance or even a higher price because they believe that a company's CRM efforts are well motivated, CRM is most effective when consumers do not feel that they are making any obvious trade-offs in exchange for participating in the CRM programs (Till and Nowak, 2000). Previous studies of CRM have demonstrated that it can impact consumer choice. Till and Nowak (2000) state that an increasing number of consumers express an intention to reward firms that are proactive towards environmental and/or social issues in the course of their marketing practices. According to Lorge in Till and Nowak, (2000), three-quarters of consumers are prepared to switch brands to one that is involved in the support of a cause, as long as price and quality are of a similar standard.

### Hypotheses and initial conceptualization

Based upon the literature review it is proposed that cause related marketing campaigns help the companies in order to enhance the brand awareness among the customers which leads to influence their purchase intentions. Most of the previous studies have only focused on the direct impact of cause marketing campaigns on consumer purchase intentions while ignoring the significant role of society cultural values and perceptual brand equity of the organization. To overcome the gap, the current study intended to confirm some previously established relationships in the setting of Iran. Following hypothesis can be drawn based on the literature review (figure 1):

- 1) Cause related marketing campaigns for a brand have a positive relation with the brand awareness.
- 2) Cause related marketing campaigns for a brand have a positive relation with the brand image.
- 3) Cause related marketing campaigns for a brand have a positive relation with the brand perceived quality.

- 4) Knowledge of a brand has a positive relation with the brand image.
- 5) Knowledge of a brand has a positive relation with the brand perceived quality.
- 6) Perceived quality of a brand has a positive relation with the brand image.
- 7) Perceived quality of a brand has a positive relation with the consumer buying decision.
- 8) Knowledge of a brand has positive relation with the consumer buying decision.
- 9) Brand image has a positive relation with the consumer buying decision.
- 10) Cause related marketing campaigns for a brand have a positive relation with consumer decision.
- 11) Cultural values of consumers have a positive relation with cause related marketing campaigns.

### METHODOLOGY

The main objective of the present study was to investigate the relationship between cause related marketing campaigns, perceived brand equity dimensions, and cultural values of the company (involved in cause related marketing) and ultimately, consumer purchase intentions. To prove these relationships data was collected from 400 consumers buying in different shopping center of Tehran city by using the convenience sampling technique. This city was selected due to high level of diversity of the respondents hailing from different areas of Iran. Before further data collection was started a pilot study was conducted to test the instrument. The sample for the pilot study consisted of 50 members. Further the questionnaire was designed in three parts. In the first part cultural values were investigated and in the second part different statements were solicited based upon the variables the study. In the third part the respondents were asked about demographic aspects like gender, age, income, and education. A five point likert scale was used to assess the respondents' level of agreement or disagreement with each statement by circling a number from 1 - 5. Before filling in the questionnaire the respondents were informed about the significance and purpose of the study. Cause marketing was found relatively a new concept for most of the respondents of the study. To overcome this impediment and to enhance the validity of the results of current study the respondents were also informed regarding the meaning of cause related marketing using examples to familiarize them with this concept.

### Measures

All measures for the constructs were taken from previous studies which were carried out from a marketing perspective. In order to measure the impact of cause related marketing campaigns on consumer purchase intentions scenarios were given. The scenarios were provided to get them in to a real time situation. Based upon the given scenarios and previous



studies, four items were developed to elicit the consumers' opinion about Cause Related Marketing campaigns (Shabbir et al, 2009). These items constitute: 1. "The company has selected an appropriate organization with a worthy cause"; 2. "The company is perceived as more democratic and humanistic"; 3. "The company can build long term customer relationships with CRM"; 4. "With CRM the company can achieve its commercial objectives". Furthermore, in order to measure the perceptual brand equity, eight items were adapted from the studies of Yoo et al, 2000; and pappu et al, 2005. Similarly, for measuring the cultural values of customers', 23 items were adapted from the study of tong and Hawley, 2009. Also for measuring the consumer purchase intentions five items were adopted from the study of Hou et al. (2008).

## DATA ANALYSIS

### Reliability analysis

A summary of the level of reliability achieved with the scales used to measure the dependent and independent variables in the pilot study is shown in Table 1. The reliabilities were acceptable as all scales achieve the Cronbach's alpha coefficient was greater than 0.7.

### Test of hypothesis

In this section hypothesis derived from literature review were investigated. As has shown in the figure 1, eleven hypotheses have been studied in this research. Based upon analysis, the relationships hypothesized in H1 through H11 were all confirmed to be positive and significant as predicted (table 2). The results provide an evidence that cause related marketing campaigns have a positive impact on brand awareness. The tested model shows that 43percent of brand awareness has been defined by CRM campaigns (Chi-square=9.08, df=4, p.value=0.05907, RMEA=0.056, H1 supported). The model also approved the second hypothesis and represent that 59percent of changes of brand image is caused by CRM campaigns (Chi-square=24.38, df=8, p.value=0.00198, RMEA=0.072). the processed model showed that CRM campaigns explained the 55percent of changes of perceived quality of brand and therefore the third hypothesis was approved (Chi-square=13.37, df=4, p.value=0.00961, RMEA=0.077). the forth hypothesis also shows that there is a positive relation between brand awareness and brand image and second power of correlation coefficient between two variables was .65 that means, 65percent of changes of brand image is stated by brand awareness (Chi-square=13.36, df=4, p.value=0.00965, RMEA=0.077). the fifth hypothesis has also been approved. The structural model related to fifth hypothesis is a suitable model and states relation between two variables is direct because 54percent of perceived quality changes

has occurred by brand awareness (Chi-square=3.77, df=1, p.value=0.05214, RMEA=0.083). the results also approved the sixth hypothesis with a correlation coefficient equal with .58 between two variables of perceived quality and brand image (Chi-square=18.10, df=4, p.value=0.00118, RMEA=0.094). base upon the seventh hypothesis, there is a positive relation between brand perceived quality and consumer's purchase decision and perceived quality explains 63percent of consumers decision's changes (Chi-square=55.32, df=13, p.value=0.00000, RMEA=0.090). The second power of correlation coefficient between brand awareness and consumer buying decision is .61 that means there is a positive relation between two variables and therefore the eighth hypothesis is approved (Chi-square=30.40, df=13, p.value=0.00412, RMEA=0.058). the model results also approve the ninth hypothesis and second power of correlation coefficient shows a very strong relation between brand awareness and consumer buying intention (Chi-square=11.18, df=8, p.value=0.19161, RMEA=0.032). the tenth hypothesis is also approved by model results. In this model, 47percent of changes of consumer buying intentions is caused by CRM campaigns (Chi-square=22.63, df=8, p.value=0.00388, RMEA=0.068). Eventually the eleventh hypothesis is approved by the model. It means that consumers cultural values have a positive relation with evaluating CRM campaigns (Chi-square=1406.59, df=284, p.value=0.00000, RMEA=0.100). in continue in order to evaluating the proposed model, we studied all hypotheses of the model simultaneously. all of the hypothesis were approved by the result except, the direct relation between CRM campaigns and consumer buying intentions. Therefore it can be stated that CRM campaigns have a positive impact on the consumer buying decision making through they're impact on perceptual brand equity dimensions and they have not a direct impact on consumer buying intentions.

## DISCUSSION AND IMPLICATIONS

The current study approved all hypotheses. Therefore it seems that by attention to conditions such as quality and competitive price that is almost equal for all competitors, and also consumers accessibility on companies information and their ability to select a brand among some brands, the proposed strategy will can a significant impact on establishing brand awareness, increasing brand image and perceived quality if effectively planed and performed. Being a developing country with a population of more than 70 million people, it was very important to investigate to what extent the customers' purchase intentions are influenced by the cause related marketing campaigns? The main objective of the present study was to investigate the relationship between cause related marketing campaigns, perceptual brand equity

dimensions and ultimately consumer purchase intentions under the effects of cultural values of consumers. Conclusively, through the testing of the proposed conceptualization, the study intended to confirm some previously established relationships in the setting of Iran, and answer the following questions:

(1) Do cause related marketing campaigns have a positive effect on consumer purchase intentions in Iran?

(2) What is the nature of the relationship of the aforementioned factors, i.e. is it direct, or mediated by perceptual brand equity dimensions or cultural values of consumers?

The provide strong evidence that cause related marketing campaigns positively influence the consumer purchase intentions in Iran which is the primary contribution of this research paper. These findings confirm the findings of Varadarajan and Menon (1988), Adkins (2004), Chattananon et al. (2008) and Shabbir et al, (2009). Contributing to the body of knowledge, the results also indicate that the effect of Cause Related Marketing campaigns on consumer purchase intentions is partially mediated by perceptual brand equity dimensions. The results of the structural model and mediation test provide new insight for the development of different strategies to make the Cause Related Marketing campaigns successful in less developed countries such as Iran. The findings suggest that the companies must concentrate on increasing the brand awareness among the customers so that they can recall the brands at the point of purchase. For this purpose, the effective use of media (electronic and print) for CRM campaigns can play a significant role but there must be a match between the objectives of the company and the promotional campaign design (Taylor, 2007). Additionally, brand-cause fit can also play a vital role to increase the brand awareness among the customers (Till and Nowak, 2000; Gupta and Pirsch, 2006). Selecting an appropriate cause for a brand can increase the interest of customers. This interest of customers can then lead to the purchase of the advertised brand. Also brand-cause-culture fit reduces the consumer skepticism and increase their intention to participate in Cause Related Marketing campaigns (Web and Mohr, 1998).

Similarly, the research suggests that firms need to undertake meaningful efforts to improve corporate image as it mediates the impact of CRM campaigns on consumer purchase intentions. Although CRM campaigns help the companies to improve their corporate image (Farache and Perks, 2008), there are also some other measures which must be taken into consideration, for example, the selection of the non-profit organization really matters a lot. The company must select an appropriate organization from the target

audience point of view (Boush et al., 1994. An appropriate selection of the cause can also support the company in terms of positive word of mouth from the consumer. Moreover, the use of an appropriate celebrity for overall communication strategy (Liu et al., 2007; Silvera and Austad, 2004) in CRM campaigns could produce better results for the company reflected in positive attitudes/feelings of the consumers towards the company. In addition, highlighting the contribution to society through electronic and print media (Farache and Perks, 2008) can help the companies not only in enhancing brand awareness but also in reducing consumer skepticism.

The study in the field of perceived quality shows that consumers have more positive evaluations toward the products of companies which use of CRM campaigns and they are ready to pay more money to them in compare with companies which are not involvement in CRM campaigns. Consumers when filling questionnaires show that have desire to buy products of such companies. They stated that their reason to do this is that those companies have an attention to society and therefore their products must have a good quality in compare with their competitors. This positive sense forces them to do an experimental buying but consumer's repurchasing depends on real quality of their products in compare with competitors. As stated, because of absence of such companies in Iran, we use of perceptual brand equity dimensions (it means brand equity dimensions minus brand loyalty) in current research. Therefore brand loyalty as one of the brand equity dimensions can be investigated in future studies.

Survey cultural values of the society and its impact on consumer are buying intentions was one of other difference of current study in compare with similar previous studies. However results show that cultural values have a positive impact on evaluations of Iranian's consumers from CRM campaigns. Therefore investigates the influences cultural differences among countries can be view as a basis for future researches.

#### **LIMITATION AND SUGGESTIONS FOR FUTURE RESEARCH**

This study has attempted to investigate the impact of cause related marketing campaigns on consumer purchase intentions and discovered the mediating role of brand awareness and brand image and perceived quality under the influences of the society cultural values. For this purpose, data was collected only from one main city of Iran (Tehran). Further research can be carried out in other cities of Iran to increase the generality of the results. Also, further research can be carried out to investigate other mediating variables such as age, education, income, and gender. The respondents of this study were



consumers who were unfamiliar with the CRM campaigns; further research could be conducted to investigate the response of those consumers who have actually participated in Cause Related Marketing campaigns or serving in public or private companies (Chattananon et al, 2008) to investigate the actual behavior rather than merely their purchase intentions. This research is also seen to be necessary because the original behavior of consumers can vary from their intentions towards some phenomena. Also, further

research can be carried out in other developing countries like Iran to compare the results and findings in other developing countries. Furthermore, the findings of this research may not relate to specific goods or services because this research did not focus on specific goods or services so future research is suggested to validate the findings of this research with specific goods or services.

Table 1: The Cronbach’s alpha coefficient related to pilot study

construct	Cultural values	Brand image	Perceived quality	Brand awareness	Purchase intentions	CRM campaigns
Cronbach’s alpha	.876	.747	.841	.722	.737	.735

Table 2: Structural model results

Hypothesized path	Path coefficients	P. value	R <sup>2</sup>
CRM → Brand awareness	0.87	0.05907	0.43
CRM → Brand image	1.19	0.00198	0.59
CRM → Perceived quality	1.11	0.00961	0.55
Brand awareness → Brand image	1.35	0.00965	0.65
Brand awareness → Perceived quality	1.09	0.05214	0.54
Perceived quality → Brand image	1.18	0.00118	0.58
Perceived quality → Purchase intentions	1.28	0.00000	0.63
Brand awareness → Purchase intentions	1.25	0.00412	0.61
Brand image → Purchase intentions	2.29	0.19161	0.84
CRM → Purchase intentions	0.94	0.00388	0.47
Cultural value → CRM	0.89	0.00000	0.45

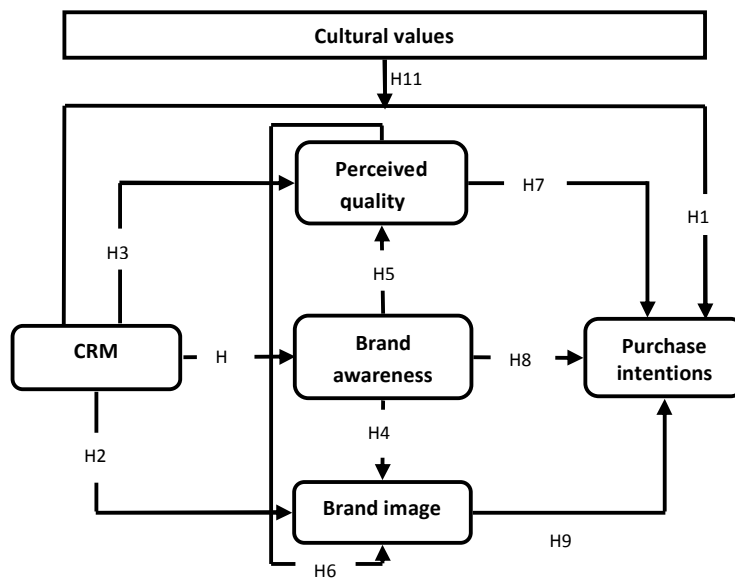


Figure 1: Conceptual model of the relationships between CRM, perceptual brand equity and consumer purchase intentions.

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**K<sub>ATP</sub> channel openers may protect MOG-G-UVW cells from hypoxia mimetic insult induced by CoCl<sub>2</sub>**

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**Abstract:** This study is going to find out whether K<sub>ATP</sub> channel is playing a protective role or not under neuronal insult model. A human astrocytoma cell line, MOG-G-UVW, was used in this study. RT-PCR was performed to find out whether K<sub>ATP</sub> channel exists or not at mRNA level and which subunits are the main compositions. Cell proliferation MTS assays were used to test the response of the cells to the insults and/or K<sub>ATP</sub> modulators. Hypoxia mimetic induced by CoCl<sub>2</sub> was applied as a cell insult model. Diazoxide, nicorandil and cromakalim were used as K<sub>ATP</sub> openers, and tolbutamide and glibenclamide were used as K<sub>ATP</sub> blockers. K<sub>ATP</sub> channel exists in MOG-G-UVW cell line at mRNA level and the main composition is K<sub>ir</sub>6.2 and SUR2A. 65 μM is the EC<sub>50</sub> of CoCl<sub>2</sub> insult to MOG-G-UVW cells and 125 μM can further induce cell death. K<sub>ATP</sub> openers cannot protect the cells from 65 μM CoCl<sub>2</sub> insult, but diazoxide and nicorandil can reduce the cell death under 125 μM CoCl<sub>2</sub>. K<sub>ATP</sub> blockers, tolbutamide and glibenclamide, cannot protect the cells at all, and tolbutamide may further reduce the cell numbers from the same dose of CoCl<sub>2</sub> insult. K<sub>ATP</sub> activation may protect the cells from hypoxia mimetic insult induced by CoCl<sub>2</sub> at a high dose, but K<sub>ATP</sub> blockers may further induce cell death.

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**Key words:** K<sub>ATP</sub> channel, neuroprotection, cell proliferation MTS assay

**1. Introduction**

Cellular neurodegeneration is a hallmark of many central nervous diseases including stroke, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). The primary insults leading to this degeneration are no doubt different but what may possibly prevent the neuronal death or protect the cells from neuronal insults are widely studied. Amongst these common features is surely the homeostasis of intracellular contents, such as calcium (Ca<sup>2+</sup>), potassium (K<sup>+</sup>) and adenosine-5'-triphosphate (ATP), and the functions of mitochondrial at a physiological level are essential to keep the neurones alive. For example, one hypothesis for AD asserts that β-amyloid (Aβ) peptides actually form ion channels in neuronal membranes transporting ions such as calcium (Ca<sup>2+</sup>) into the cell hence mediating cytotoxicity (Kagan et al., 2002, Arispe et al., 2007).

K<sup>+</sup> channels are purported to be involved in the life and death decisions that cells, and in particular neurones, make. The role of these ion channels in the cascade of events which leads to cell death is confusing. On the one hand it is argued that the activation of potassium channels can be neuroprotective (Coles et al., 2008, Henney et al., 2009) by virtue of the hyperpolarisation and resultant decrease in Ca<sup>2+</sup> entry into the cell. On the other hand there is a view that activation of K<sup>+</sup> channels can lead to K<sup>+</sup> efflux, water loss, cells shrinkage and apoptosis

(Burg et al., 2006). Regarding to the ATP-sensitive K<sup>+</sup> channel (K<sub>ATP</sub>), two opposite opinions were raised previously for its possible role in neuronal death.

One in vivo study on the K<sub>ir</sub>6.2 knocked out mouse model has found that the neurones were severely damaged after focal cerebral ischemia induced by middle cerebral artery occlusion, whereas few injured neurones were found in the wild type counterparts (Sun et al., 2006). Another study on a K<sub>ir</sub>6.2 mutant mouse model has also found that the activity of substantia nigra pars reticulata neurones, which plays an important role in the control of seizures, was enhanced during hypoxia, while the activity of the same neurones in normal mice was inactivated by the opening of the postsynaptic K<sub>ATP</sub> channel (Yamada et al., 2001). Similar neuronal activity has been found in the normal mice when tolbutamide, which is a K<sub>ATP</sub> channel blocker, was applied (Yamada et al., 2005). In addition, another study on rat hippocampal cells has found that diazoxide (a K<sub>ATP</sub> opener) treatment of neurones reduces neuronal death from insults of Aβ<sub>25-35</sub> or hypoxia induced by ferrous sulphate (FeSO<sub>4</sub>) (Goodman & Mattson, 1996). Also, data from mice administrated diazoxide has shown that the infarct volume in the brain under cerebral ischemia was reduced (Liu et al., 2002).

In the contrast, other studies have claimed the opposite. One study on mammalian central neurones has shown cell apoptosis induced by

cromakalim, a  $K_{ATP}$  channel opener, in electron micrographs (Yu et al., 1997), and another study in rat hippocampal neurones has found that 5-hydroxydecanoate, a mitochondrial  $K_{ATP}$  blocker, can increase cell survival from glutamate or staurosporine insult (Liu et al., 2003). In addition to neurones, another study on rat preadipocytes has found that diazoxide can reduce the relative cell number, while the  $K_{ATP}$  channel blocker glibenclamide can increase cell number, in MTT assays (Wang et al., 2007). For a possible mechanism, it was believed that  $K^+$  efflux from mitochondria can cause mitochondrial membrane depolarization, cytochrome c release, and induce subsequent extrusion of cytosolic  $K^+$  via a  $K^+$  channel on the cell membrane.

Since it is still unclear what the role of  $K_{ATP}$  channel is in neuronal death, it would be useful to further investigate its possible effects in cell death in this study. A functional  $K_{ATP}$  channel is constructed from four inward rectifier ( $K_{ir}$ ) subunits and four sulphonylurea receptor (SUR) subunits (Burg et al., 2006). Although the ion-conducting structure of  $K_{ATP}$  channel exists in the  $K_{ir}$  subunits (Ho et al., 1993), SUR subunits are also essential for  $K_{ATP}$  channel function, since SUR subunits represent the ATP binding area. There are several inward rectifier  $K^+$  channels found so far ( $K_{ir1}$  to  $K_{ir7}$ ), but only  $K_{ir6}$  is found to be ATP sensitive (Alexander et al., 2009), hence it is named the  $K_{ATP}$  channel. There are two subtypes of  $K_{ATP}$  channel,  $K_{ir6.1}$  and  $K_{ir6.2}$ , and there are three different SUR thus far, SUR1, SUR2A and SUR2B (Alexander et al., 2009).

A human brain astrocytoma cell line, MOG-G-UVW, was applied in this study since astrocytes were used to study anti-inflammation function and their relevant role in neuronal protection previously (Spillantini, 2011). Reverse transcription (RT)-polymerase chain reaction (PCR) was applied to find out whether  $K_{ATP}$  channel message exists in this cell line and which subunits are the main composition. The  $K_{ATP}$  modulators were applied in combination with a hypoxia mimetic insult model to find out the possible effect, if any, of this channel.

In this study, hypoxia mimetic insult induced by cobalt chloride ( $CoCl_2$ ) was applied to induce the cell death since it has been widely used in previous studies (Gasperi et al., 2010; Bautista et al., 2009). Particularly,  $CoCl_2$  can increase the generation of  $A\beta$  by up-regulating the expression of amyloid precursor protein (APP) as well as the expression of  $\beta$ -secretase and  $\gamma$ -secretase (Zhu et al., 2009). Then,  $K_{ATP}$  channel activators, diazoxide, nicorandil and cromakalim, as well as  $K_{ATP}$  channel blockers, glibenclamide and tolbutamide, were tested in combination with  $CoCl_2$  insult on MOG-G-UVW cells.

In previous studies, 10  $\mu$ M levcromakalim

evoked a hyperpolarisation under current clamp, and this effect of levcromakalim can be inhibited by glibenclamide with an  $EC_{50}$  of 55 nM. 10  $\mu$ M glibenclamide alone depolarises the neurones (Hogg et al., 2001). For diazoxide, 200 – 500  $\mu$ M can increase the channel opening probability by  $480 \pm 120\%$  (Allen & Brown, 2003). For the  $K_{ATP}$  blocker, it has been found in previous studies that the  $K_{ATP}$  channel current ( $I_{K_{ATP}}$ ) can be inhibited reversibly by tolbutamide with an  $EC_{50}$  at 34.1  $\mu$ M, and can be inhibited irreversibly by 0.3 – 3 nM glibenclamide (Allen & Brown, 2003). The inhibitory effect of tolbutamide was confirmed in a current clamp experiment suggesting that 100  $\mu$ M tolbutamide depolarises the neurones (Hogg et al., 2001). An irreversible effect of glibenclamide was argued by another study which suggested that the dose of glibenclamide to induce half-maximal inhibitory effect was increased from 9.1  $\mu$ M to only 10.6  $\mu$ M by 1 mM nicorandil (Obata & Yamanaka, 2000).

## 2. Methods and materials

### 2.1 Methods

MOG-G-UVW is a human astrocytoma cell line (from Sigma-Aldrich). It is cultured in a sterilised incubator at 37 centigrade ( $^{\circ}C$ ) with 5% of carbon dioxide ( $CO_2$ , p/p). The composition for the culture medium was Dulbecco's modified Eagle medium (DMEM) and nutrient mixture F10 ham (F10) (1:1, v/v) with 10% (v/v) of foetal bovine serum (FBS) and 100 units (u) of penicillin-streptomycin mixture (P/S). PBS and P/S were kept at  $-20^{\circ}C$  and all other mediums were kept at  $4^{\circ}C$ .

Ribonucleic acid (RNA) was extracted from a confluent flask (75 square centimetre,  $cm^2$ ) of cells with TRIzol reagent (from Life Technology, stored at  $4^{\circ}C$ ). DNase inactivation was performed to remove all the remaining deoxyribonucleic acid (DNA) with a DNase 1 kit (from Ambion, stored at  $-20^{\circ}C$ ). The RNA concentration (w/v) and quality (260/280) in the final product was determined with a spectrophotometer (DU730, from Beckman Coulter). The RNA product was kept at  $-80^{\circ}C$ . Thereafter, RT was performed to transcribe message RNA (mRNA) into DNA with a reverse transcriptase kit (from Promega, stored at  $-20^{\circ}C$ ). 1 microgram ( $\mu$ g) of RNA was added in each RT reaction and the RT programme was:  $25^{\circ}C \times 5$  minutes (min) +  $42^{\circ}C \times 1$  h +  $70^{\circ}C \times 15$  min. The DNA product was kept at  $-20^{\circ}C$ .

PCR was then applied to find out whether  $K_{ATP}$  channel exists at the mRNA level in MOG-G-UVW cells. The primers of  $K_{ATP}$  subunits were designed on Primer III website (<http://frodo.wi.mit.edu/primer3>) and the sequences of primers are listed in Table 1. PCR was performed



with a Go Taq DNA polymerase kit (from Promega, stored at -20 °C) and the PCR programme was: 95 °C × 2 min + [95 °C × 30 seconds (s) + annealing temperature × 45 s + 72 °C × 1 min] × 30 – 40 cycles + 72 °C × 5 min. Two different PCR conditions were applied: 55 °C as the annealing temperature for 30 cycles and 58 °C as the annealing temperature for 40 cycles. Primers were from Life Technology and kept at -20 °C. PCR thermocycler is from Biometra. PCR results were tested with electrophoresis (100 mV). The gel was 1% agarose and the buffer was 1 × TAE containing 40 milli mole (mM) Tris [(HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>2</sub>] base, 20 mM acetic acid (CH<sub>3</sub>COOH) and 1 mM ethylenediaminetetraacetic acid (EDTA). All these drugs were from Fisher Scientific.

Cell proliferation MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt, from Promega] assays were applied to test the possible effects, if any, of K<sub>ATP</sub> modulators in MOG-G-UVW cells. Unless specific information was given, cells were seeded as 3,000 per well in 100 micro litre (µL) per well of medium in 96 well plates, and were insulted with CoCl<sub>2</sub> and/or K<sub>ATP</sub> modulator for 24 hours (h) after being incubated overnight. To insult the cells, all drugs were dissolved in insult medium (culture medium without FBS). Then, 20 µL of MTS and phenazine methosulfate (PMS, from Sigma-Aldrich) mixture (20 : 1, v/v) was added into each well and the cells were incubated again for another 2 – 4 h. Finally the absorbance of each well

was read at 490 nanometre (nm) with a laser plate reader (from Tecan). In each experiment there were blank wells prepared for each sample. The blank wells were the wells with the same solution mix but with no cells present. The absorbance of the blank was subtracted from the absorbance of each relevant sample for data analysis. The net absorbance (where the absorbance of the relevant blank was subtracted) of each sample was thereafter compared with that of control, where the relative cell number was set at 1, and final ratio was the relative cell number of each sample. Both MTS and PMS were light sensitive and kept at -20 °C.

## 2.2 Drug solutions

CoCl<sub>2</sub>, cromakalim, diazoxide, nicorandil and tolbutamide are from Sigma-Aldrich, and glibenclamide is from Tocris. CoCl<sub>2</sub> was dissolved in water (H<sub>2</sub>O) at 10 mM for stock. Cromakalim, diazoxide and glibenclamide were dissolved in DMSO at 10 mM for stock. Nicorandil and tolbutamide were dissolved in C<sub>2</sub>H<sub>5</sub>OH at 10 mM for stock. Diazoxide and nicorandil stocks were stored at -20 °C. CoCl<sub>2</sub>, cromakalim, glibenclamide and tolbutamide stocks were stored at 4 °C. CoCl<sub>2</sub> is light sensitive.

## 3. Results

### 3.1 Primer design

The primers for K<sub>ATP</sub> channel are listed in Table 1:

Table 1 Primers for K<sub>ATP</sub> subunits

Primer		Sequence	Location
Kir6.1 NM_004982	Forward	5'-CATCTTTACCATGTCCTTCC-3'	560 – 893 bp in total 3,281 bp
	Reverse	5'-GTGAGCCTGAGCTGTTTTCA-3'	
Kir6.2 D50582	Forward	5'-ACTCCAAGTTTGGCAACACC-3'	1,197 – 1,550 bp in total 1,635 bp
	Reverse	5'-CTGCTGAGGCCAGAAATAGC-3'	
SUR1 L78207	Forward	5'-ATGAGGAAGAGGAGGAAGAG-3'	2,941 – 3433 bp in total 4,892 bp
	Reverse	5'-GCGATGGTGTACAGTCAGA-3'	
SUR2A NM_005691.2	Forward	5'-GGCCTTGCTTCACTGTCTC-3'	1,730 – 1,939 bp in total 4,670 bp
	Reverse	5'-TTGGCTGAACTCCAGTGTG-3'	
SUR2B NM_020297	Forward	5'-TGGGAACACATTTCTGCAA-3'	1,528 – 1,677 bp in total 4,650 bp
	Reverse	5'-CGCATGGGTCACAAATGTAC-3'	

### 3.2 RT-PCR

Two annealing temperatures, 55 °C and 58 °C, have been trialled on the amplification of K<sub>ATP</sub> subunits in MOG-G-UVW cell line. The figures are shown in Figure 1 and 2.

From the two figures, it can be seen that higher annealing temperature with more amplification cycles make the bands for Kir6.1, SUR1 and SUR2B visible and make the SUR2A band clearer, but this condition also causes the bands to be unspecific. From Figure 1, it may also be noticed that Kir6.2 with

SUR2A might be the main subunit composition of K<sub>ATP</sub> channel in MOG-G-UVW cells, but the existence of Kir6.1, SUR1 and SUR2B cannot be eliminated.

### 3.3 K<sub>ATP</sub> activators effect against CoCl<sub>2</sub> insult

Firstly, the possible effect, if any, of diazoxide alone on the cell proliferation was tested in MOG-G-UVW cells. The results are shown in Figure 3.



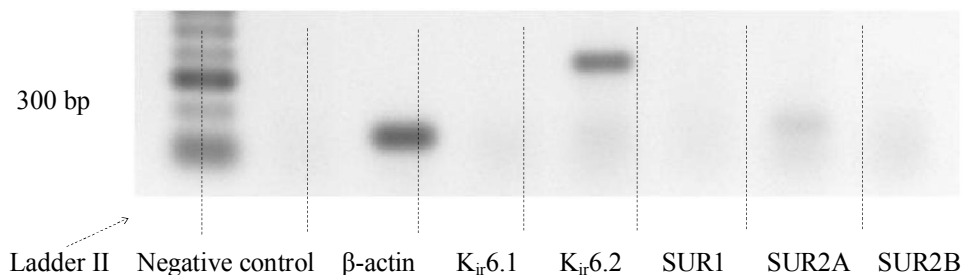


Figure 1.  $K_{ATP}$  subunits in MOG-G-UVW cells, 55 °C, 30 cycles.

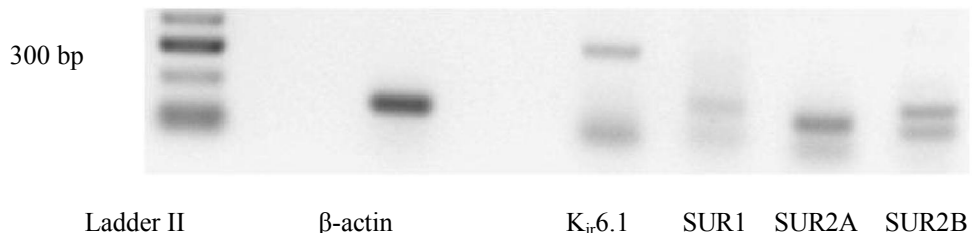


Figure 2.  $K_{ATP}$  subunits in MOG-G-UVW cells, 58 °C, 40 cycles. The bands were unspecific.

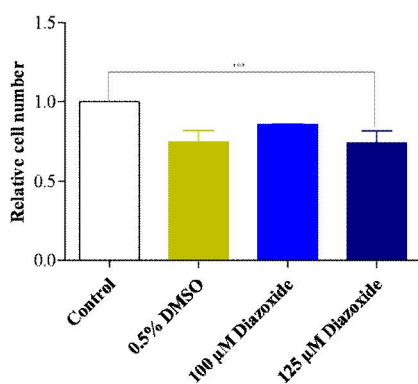


Figure 3. The effect of diazoxide alone in MOG-G-UVW cells. The results were the means of 9 for 125  $\mu$ M diazoxide and the control, and 3 for DMSO vehicle and 100  $\mu$ M diazoxide.

A one way ANOVA test for all the groups in Figure 3 suggested that the difference amongst those groups was significant ( $p < 0.05$ ), and the Dunnett's post test (comparing each group with the control) indicated that 125  $\mu$ M diazoxide caused a significant reduction in relative cell number from the control ( $p < 0.01$ ); the vehicle, DMSO and 100  $\mu$ M diazoxide did not have any significant effect on the relative cell number. It can be concluded that diazoxide cannot induce cell death in MOG-G-UVW cells at concentrations of 100  $\mu$ M or lower.

Diazoxide was then tested against two different doses of  $CoCl_2$  in MOG-G-UVW cells. The results are shown in Figure 4.

For the results shown in Figure 4A, a one way ANOVA test for all the groups showed that the

difference amongst those groups was significant ( $p < 0.001$ ), and the Tukey's post test (comparing every two groups) indicated that 65  $\mu$ M  $CoCl_2$  caused a significant reduction in the relative cell number from the control ( $p < 0.001$ ). A one tail t test suggested that 100  $\mu$ M diazoxide in combination with 65  $\mu$ M  $CoCl_2$  had a significant effect on the relative cell number from the same dose of  $CoCl_2$  alone ( $p < 0.05$ ).

Thus, it can be concluded that diazoxide cannot protect MOG-G-UVW cells from a low dose of  $CoCl_2$  (65  $\mu$ M), and may even further reduce the relative cell number from  $CoCl_2$  alone. However, in the presence of a high dose of  $CoCl_2$  (125  $\mu$ M), 10  $\mu$ M diazoxide can paradoxically protect the cells.

Besides diazoxide, another  $K_{ATP}$  activator, nicorandil was also tested on the  $CoCl_2$  insult in MOG-G-UVW cells. The results are shown in Figure 5.

For the results shown in Figure 5A, a one way ANOVA test for all the groups showed that the difference amongst all the groups was significant ( $p < 0.001$ ), and the Tukey's post test (comparing every two groups) indicated that 65  $\mu$ M  $CoCl_2$  caused a significant reduction in the relative cell number from the control. However the difference in the relative cell numbers between  $CoCl_2$  + nicorandil and  $CoCl_2$  alone was not significant.

For the results shown in Figure 5B, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ( $p < 0.001$ ) and the Tukey's post test (comparing every two groups) indicated that 125  $\mu$ M  $CoCl_2$  caused a significant reduction in the relative cell number from the control, and 10  $\mu$ M or 100  $\mu$ M nicorandil in

combination with 125  $\mu\text{M}$   $\text{CoCl}_2$  also had a significant effect on the relative cell number in comparison with the same dose of  $\text{CoCl}_2$  alone.

Thus, it can be concluded that nicorandil cannot protect MOG-G-UVW cells against a low dose of  $\text{CoCl}_2$  (65  $\mu\text{M}$ ), but protects the cells against a high dose of  $\text{CoCl}_2$  (125  $\mu\text{M}$ ).

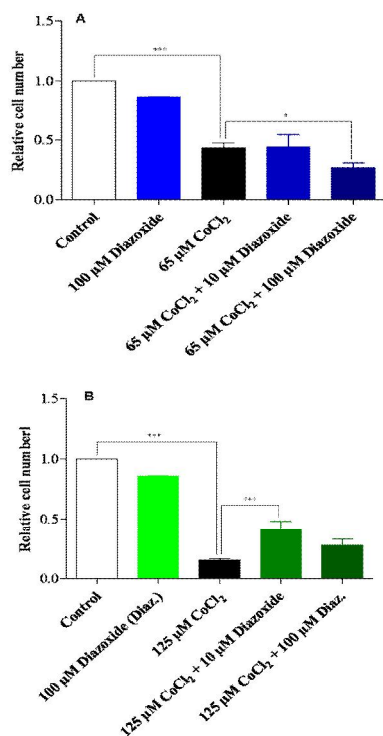


Figure 4. The effect of diazoxide (Diaz.) in combination with  $\text{CoCl}_2$  insult in MOG-G-UVW cells. A: 65  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 30 for  $\text{CoCl}_2$  alone and the control, 9 for  $\text{CoCl}_2$  + 10  $\mu\text{M}$  diazoxide, 6 for  $\text{CoCl}_2$  + 100  $\mu\text{M}$  diazoxide, and 3 for diazoxide alone. And B: 125  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 12 for  $\text{CoCl}_2$  alone and the control, 9 for  $\text{CoCl}_2$  + 10  $\mu\text{M}$  diazoxide, 6 for  $\text{CoCl}_2$  + 100  $\mu\text{M}$  diazoxide, and 3 for diazoxide alone seen with a high dose of  $\text{CoCl}_2$ . From the results shown in Figure 4B, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ( $p < 0.001$ ), and the Tukey's post test (comparing every two groups) indicated that 125  $\mu\text{M}$   $\text{CoCl}_2$  alone caused a significant reduction in the relative cell number from the control ( $p < 0.001$ ), and 10  $\mu\text{M}$  diazoxide in combination with 125  $\mu\text{M}$   $\text{CoCl}_2$  also had a significant effect on the relative cell number in comparison with the same dose of  $\text{CoCl}_2$  alone ( $p < 0.001$ ).

In addition to diazoxide and nicorandil, the third  $\text{K}_{\text{ATP}}$  activator, cromakalim, was also tested against the same doses of  $\text{CoCl}_2$  in MOG-G-UVW cells. The results are shown in Figure 6.

For the results shown in Figure 6A, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ( $p < 0.001$ ), and the Tukey's post test (comparing every two groups) indicated that 65  $\mu\text{M}$   $\text{CoCl}_2$  caused a significant reduction in the relative cell number from the control ( $p < 0.001$ ), but 10 – 100  $\mu\text{M}$  cromakalim in combination with 65  $\mu\text{M}$   $\text{CoCl}_2$  did not have any significant effect on the relative cell number from the same dose of  $\text{CoCl}_2$  alone.

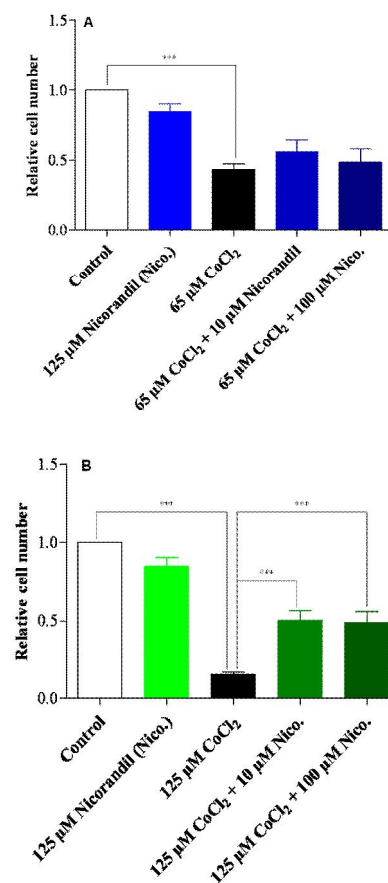


Figure 5 The effect of nicorandil (Nico.) in combination with  $\text{CoCl}_2$  insult in MOG-G-UVW cells. A: 65  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 30 for 65  $\mu\text{M}$   $\text{CoCl}_2$  alone and the control, and 9 for 125  $\mu\text{M}$  nicorandil alone, 65  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  nicorandil and 65  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  nicorandil. And B: 125  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 12 for  $\text{CoCl}_2$  alone and the control, and 9 for 125  $\mu\text{M}$  nicorandil alone, 125  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  nicorandil and 125  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  nicorandil. property is similar to the effect of diazoxide on  $\text{CoCl}_2$  in MOG-G-UVW cells.

From the results shown in Figure 6B, it can be seen that cromakalim did not protect the cells from a high dose of  $\text{CoCl}_2$  (125  $\mu\text{M}$ ) either. A one way ANOVA test for all the groups suggested that the

difference amongst those groups was significant ( $p < 0.001$ ), the Tukey's post test (comparing every two groups) indicated that 125  $\mu\text{M}$   $\text{CoCl}_2$  alone caused a significant reduction in the relative cell number from the control ( $p < 0.001$ ), and the Dunnett's post test (comparing each group with the  $\text{CoCl}_2$  alone) indicated that 100  $\mu\text{M}$  cromakalim in combination with 125  $\mu\text{M}$   $\text{CoCl}_2$  also had a significant effect on the relative cell number from the same dose of  $\text{CoCl}_2$  alone ( $p < 0.05$ ).

To summarise the cell proliferation MTS assays results on  $\text{K}_{\text{ATP}}$  activators, it can be reported that none of them can protect MOG-G-UVW cells against a low dose of  $\text{CoCl}_2$  (65  $\mu\text{M}$ ). However, against a high dose of  $\text{CoCl}_2$  (125  $\mu\text{M}$ ), diazoxide and nicorandil protect MOG-G-UVW cells, and cromakalim has the opposite effect by further reducing the relative cell number from 125  $\mu\text{M}$   $\text{CoCl}_2$  alone.

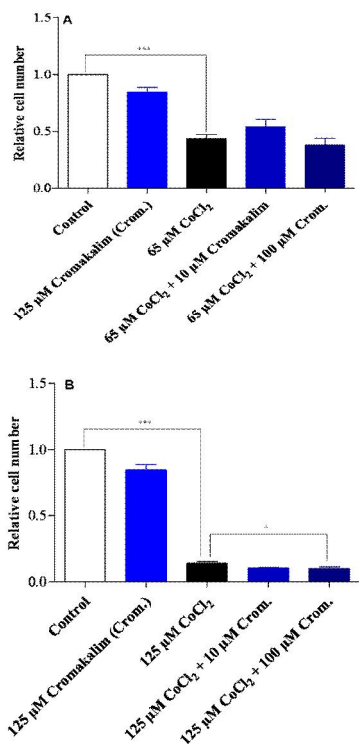


Figure 6 The effect of cromakalim (Crom.) in combination with  $\text{CoCl}_2$  in MOG-G-UVW cells. A: 65  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 30 for 65  $\mu\text{M}$   $\text{CoCl}_2$  alone and the control, and 6 for 125  $\mu\text{M}$  cromakalim alone, 65  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  cromakalim and 65  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  cromakalim. And B: 125  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 21 for 125  $\mu\text{M}$   $\text{CoCl}_2$  alone and the control, and 6 for 125  $\mu\text{M}$  cromakalim alone, 125  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  cromakalim and 125  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  cromakalim. combination with  $\text{CoCl}_2$  insult are shown in Figure 7.

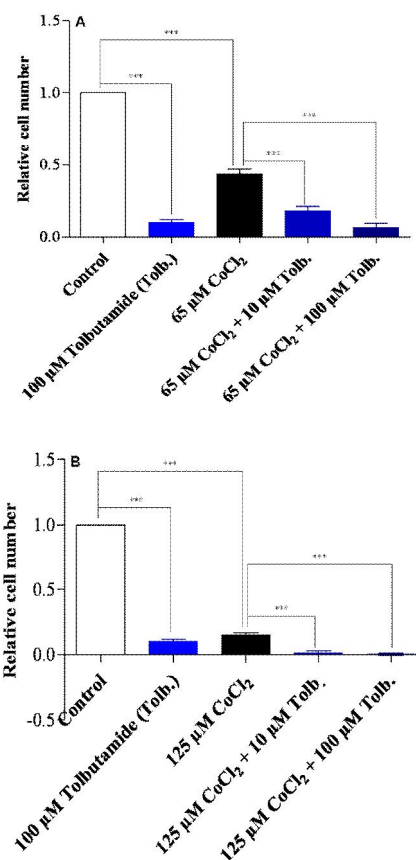


Figure 7 The effect of tolbutamide (Tolb.) in combination with  $\text{CoCl}_2$  in MOG-G-UVW cells. A: 65  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 30 for 65  $\mu\text{M}$   $\text{CoCl}_2$  alone and the control, and 6 for 100  $\mu\text{M}$  tolbutamide alone, 65  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  tolbutamide and 65  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  tolbutamide. And B: 125  $\mu\text{M}$   $\text{CoCl}_2$ , the results are the means of 12 for 125  $\mu\text{M}$   $\text{CoCl}_2$  alone and the control, and 6 for 100  $\mu\text{M}$  tolbutamide, 125  $\mu\text{M}$   $\text{CoCl}_2$  + 10  $\mu\text{M}$  tolbutamide and 125  $\mu\text{M}$   $\text{CoCl}_2$  + 100  $\mu\text{M}$  tolbutamide.

### 3.4 $\text{K}_{\text{ATP}}$ blocker effect on $\text{CoCl}_2$ insult

In addition to  $\text{K}_{\text{ATP}}$  activators, the blockers, tolbutamide and glibenclamide, were also tested in MOG-G-UVW cells. The results of tolbutamide in Figure 6.

For the results shown in Figure 7A, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ( $p < 0.001$ ), and the Tukey's post test (comparing every two groups) indicated that both 65  $\mu\text{M}$   $\text{CoCl}_2$  alone and 100  $\mu\text{M}$  tolbutamide alone caused significant reductions in the relative cell numbers from the control ( $p < 0.001$  for both), and 10  $\mu\text{M}$  or 100  $\mu\text{M}$  tolbutamide in combination with 65  $\mu\text{M}$   $\text{CoCl}_2$  also

had a significant effect on the relative cell number in comparison to the same dose of CoCl<sub>2</sub> alone ( $p < 0.001$  for both).

Similar effects can be seen with a higher dose of CoCl<sub>2</sub> (125  $\mu$ M). The Tukey's post test (comparing every two groups) for all the groups in Figure 7B indicated that 10  $\mu$ M or 100  $\mu$ M tolbutamide in combination with 125  $\mu$ M CoCl<sub>2</sub> caused a significant reduction in the relative cell number from the same dose of CoCl<sub>2</sub> alone ( $p < 0.001$ ). Therefore, it can be concluded that 100  $\mu$ M tolbutamide alone reduces the relative cell number, and 10  $\mu$ M – 100  $\mu$ M tolbutamide cannot protect MOG-G-UVW cells from either a low dose (65  $\mu$ M) or a high dose (125  $\mu$ M) of CoCl<sub>2</sub>, and may in fact further reduce the relative cell number from the same dose of CoCl<sub>2</sub> alone.

In addition to tolbutamide, another K<sub>ATP</sub> blocker, glibenclamide, has also been tested, but only negative results were achieved. The cell density was 5,000 per well in 24 well plates here. The EC<sub>50</sub> of CoCl<sub>2</sub> in these experiments was estimated at about 500  $\mu$ M. The glibenclamide results are shown in Table 2.

Table 2 The effect of glibenclamide in combination with an EC<sub>50</sub> of CoCl<sub>2</sub> insult in MOG-G-UVW cells (n = 2)

Glibenclamide concentration (in combination with 500 $\mu$ M CoCl <sub>2</sub> )	Relative cell number
10 $\mu$ M	0.621
50 $\mu$ M	0.586
100 $\mu$ M	0.428
500 $\mu$ M	0.348

The Bonferroni's post test (comparing every two groups) did not suggest any significant difference in the relative cell number. Hence, it can be concluded that glibenclamide cannot protect MOG-G-UVW cells from a CoCl<sub>2</sub> insult.

Therefore, to summarise the results of the K<sub>ATP</sub> channel blockers against the CoCl<sub>2</sub> insult, it can be concluded that neither tolbutamide nor glibenclamide was protective. In addition, tolbutamide in combination with CoCl<sub>2</sub> insult may even further reduce the relative cell number compared to the same dose of CoCl<sub>2</sub>.

#### 4. Discussion

From the PCR results shown above, it can be seen that the messages of K<sub>ATP</sub> subunits exist in the MOG-G-UVW cell line, and the subunit composition of K<sub>ATP</sub> channel in MOG-G-UVW cell line might (tentatively) be K<sub>v</sub>6.2 with SUR2A.

For the possible role, if any, of the K<sub>ATP</sub> channel in cell death, previous studies have reached different conclusions, as discussed in the introduction.

Some studies believe that the activation of K<sub>ATP</sub> channel can induce cell death when there was no insult applied to the cells, for example both diazoxide and cromakalim can cause a reduction in cell numbers (Burg et al., 2006, Wang et al., 2007), and the blockade of K<sub>ATP</sub> channel, with for example glibenclamide, may increase cell proliferation (Wang et al., 2007). However, in combination with insults, such as hypoxia and A $\beta$ , a K<sub>ATP</sub> channel activator like diazoxide might then be neuroprotective (Goodman & Mattson, 1996).

Interestingly, the results in this study did not exactly concur with those studies above. K<sub>ATP</sub> channel activators alone, such as diazoxide, nicorandil and cromakalim, tended to cause a reduction in the relative cell number from the control, but not significantly. Only 125  $\mu$ M diazoxide alone caused a significant reduction in the relative cell number compared to the control, and K<sub>ATP</sub> channel activators, like diazoxide and nicorandil, protected the cells from a high dose of CoCl<sub>2</sub> (125  $\mu$ M), although the results were negative in the case of a low dose of insult (65  $\mu$ M). In addition, K<sub>ATP</sub> channel blockers, for example tolbutamide and glibenclamide, cannot protect the cells from CoCl<sub>2</sub> insult, and tolbutamide in combination with CoCl<sub>2</sub> insult may even cause a further reduction in the relative cell number from the same dose of CoCl<sub>2</sub> alone.

From the results shown before, a K<sub>ATP</sub> channel blocker alone, such as tolbutamide, cannot increase cell proliferation in MOG-G-UVW, but causes a reduction in the relative cell number from the control. Additionally, not all K<sub>ATP</sub> channel activators may protect the cells from CoCl<sub>2</sub> insult. Cromakalim may somehow aggravate the insult of a high dose of CoCl<sub>2</sub> (125  $\mu$ M).

These results might be explained by considering the balance of [Ca<sup>2+</sup>]<sub>i</sub> or K<sup>+</sup> release from the cell. K<sub>ATP</sub> channel activator would reduce the open probability of Ca<sup>2+</sup> channels on both ER membrane and cell membrane (Brini, 2003). In normal conditions without any insult, such an effect might lower the [Ca<sup>2+</sup>]<sub>i</sub> to below a 'normal' physiological level. On the other hand, as introduced before, a K<sub>ATP</sub> channel blocker might reduce cellular K<sup>+</sup> release and might cause the mitochondrial membrane hyperpolarisation.

In the hypoxia environment, such as exists with a CoCl<sub>2</sub> insult, two opposite results might follow the activation of the K<sub>ATP</sub> channel. It can reduce the open probability of Ca<sup>2+</sup> channels, but can also cause a K<sup>+</sup> release or even efflux. Hence, whether the K<sub>ATP</sub> channel activator is neuroprotective or not may depend on the balance of those two opposing effects.

In addition, the difference in the effects of cromakalim and the other two K<sub>ATP</sub> channel activators



might be explained by the selectivity to different  $K_{ATP}$  subunits. From the RT-PCR results shown before, it can be seen that, in MOG-G-UVW cell line, the expression of  $K_{ir6.2}$  is higher than that of  $K_{ir6.1}$ , and the expression of SUR2A is higher than that of the other two SUR subunits. If there is any possible selectivity with respect to the different  $K_{ATP}$  channel subunits in the activators, it might explain the negative results shown by cromakalim.

### 5. Conclusions and future work

To summarise all the results and discussion, it may be suggested that:

The messages of  $K_{ATP}$  channel subunits exist in the mRNA of MOG-G-UVW cell line, and the main subunit composition of the  $K_{ATP}$  channel in this cell line might be  $K_{ir6.2}$  with SUR2A.

$K_{ATP}$  activators, such as diazoxide, nicorandil and cromakalim, cannot increase the cell proliferation of MOG-G-UVW cells and cannot protect the cells from a low dose of  $CoCl_2$  (65  $\mu M$ ). However, with a high dose of  $CoCl_2$  (125  $\mu M$ ), diazoxide and nicorandil protect the MOG-G-UVW cells, but cromakalim causes a further reduction in the relative cell number from  $CoCl_2$  alone.

$K_{ATP}$  channel blockers, such as tolbutamide and glibenclamide, cannot increase cell proliferation in MOG-G-UVW cells, and tolbutamide alone may even cause a reduction in the relative cell number. Neither of the two blockers can protect MOG-G-UVW cells from  $CoCl_2$  insult, and tolbutamide may even cause a further reduction in the relative cell number from the same dose of  $CoCl_2$  alone.

For the future work on  $K_{ATP}$  channel, the possible selectivity, if any, for different  $K_{ATP}$  subunits in the activators should be investigated to find out whether this is the reason for cromakalim to be different from the other two activators. If this is the case, the expression of different  $K_{ATP}$  subunits in neurones can be studied with Q-PCR to find out what the main subunit composition of  $K_{ATP}$  channel in neurones is. Furthermore, the  $K_{ATP}$  channel activators which can target those subunits, for example  $K_{ir6.2}$  and/or SUR2A might point the way to a  $K^+$  channel strategy for neuroprotection.

### Acknowledgements

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### Authors Contributions

Dr. ZHANG Jin wrote the paper and did the RT-PCR work. Dr. Zhang, Miss. Marwa

KHUDHEYER and Miss Jasmin NOWAK did the cell proliferation MTS assays. Dr. Zhang and Dr. Dwaine BURLEY analysed the data. Dr. Burley and Prof. Kenneth WANN designed the research. And, Prof. Wann supervised this study as the group leader.

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## Scenario analysis and rankings of Iran power plants using mathematical models of DEA with the factor of the type of chemical consumption fuel

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**Abstract:** In this article, considering the importance of the optimum utilization of feasibilities for gaining more efficiency, first, Iran power plants status is surveyed. Then the methodology of data envelopment analysis, taken as the suitable scientific and non-parametrical method of the performance assessment and determination of DMU (Decision Making Units) efficiency has been introduced for the performance improvising and rankings of electricity production; moreover, by using the improvised model the scenario analysis of the data envelopment for gaining the concerned targets will be assessed. Then the various kinds of the consumptive fuels undergo discussion.

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**Key words:** data envelopment analysis (DEA), efficiency, chemical consumption fuel, power plants, rankings models, scenario analysis

### Introduction

The basic efficiency management of any organization is to benefit wisely from the existent feasibilities in which the access to this goal requires overall thinking and scientific resolution. Considering the power industry being an expensive and important one and without suitable investment in developing this industry one cannot reach the economical development, so one of the related discussions is the assessment of the performance and measuring the efficiency of its units and the pursuant consequences and devices that affect on the operation of the electricity generating power plants.

The surveyed power plants in this article can be grouped in two main categories:

- Steam power plants
- Gas synthetic-cyclic power plants

All the steam power plants surveyed in this article ranging from the dominant gas consumption along with Mazut (black oil) fuel as the second one have used. The gas fuel in the warmer seasons and Mazut is mostly used in cold days of the year. Overall, the average efficiency of gas power plants is lower than the steam power plants. However, synthetic-cyclic power plants cause the increment of the efficiency median of these power plants. One of the points that can be effective in increasing the efficiency of the synthetic cyclic power plants is to re-heat through the duct burner in the retrieving boiler. This efficiency increment may be observed in Kerman and Neka synthetic-cyclic power plants. By using duct burner, the rate of gas consumption in torches is much lower in relation to the gas flow but its effect in efficiency increment is very considerable. Of the points both in the gas, unit's and steam units' efficiency is efficient percentage composition of the gas constituents, this

matter can be seen in the synthetic-cyclic power plant of Khayam and Ramin power plants. In Khayam power plant in spite of the fuel heating value, the efficiency increment is reduced compared to other power plants.

In this article, first, a general consideration concerning the country power plants including the steam, gas and synthetic-cyclic power plants will be presented ; and concerning the chemical fuel consumption and its heating value is discussed and then the mathematical models used in the article including CCR model, ranking model and the scenario envelopment analysis model will be reconsidered. Afterwards, the evaluation indexes of the power plants will be presented and finally the evaluation consequences using GAMS software will be discussed and conclusions will be drawn.

### Types of Power Plants

In generation of electricity the mechanical energy will transfer to electrical energy, in this field our country uses four types of steam, gas, diesel, and water power plants. Moreover, the synthetic-cyclic power plants are a combination of gas and steam power plants; and a nuke power plant has been considered in recent years too.

### Steam power plant

In this type, power plant for converting water to steam uses high pressure and heating so that the steam in passing through turbine can make circular mechanical energy and at the end the circular mechanical energy will change to electrical energy in the generator. The smallest unit of steam power plant is Tarasht power plant with production capacity of 12.5 Mega watts installed in 1338 and the biggest one is Neka power plant with the production capacity of 440 Mega watts installed in the year of 1358.

### Gas Power plant

In this type, the gasses produced from gasoil or natural gas under high pressure and temperature is coming out from the input route of the compartment in which the turbine is on the way and causes the turbine to rotate and in the consequence causes electrical energy in generator that its axis is connected to the turbine shaft. Due to the lower efficiency of these power plants, they are put in the electricity cycle net during the hours of high daily consumption.

### Synthetic-cyclic configuration Power plant

These types of power plants are combination of gas and steam units that are for efficiency increment, the total gas taken from the gas turbine enters an area of boiler steamed unit and of its heating energy is used for steam.

### Assessment of mathematical Models

Data envelopment analysis is a mathematical programmed method, which without the requirement of knowing the production function by using a border of non-parametric production can assess the efficiency in the relative form. This method was introduced called CCR Model through an article by Charnez and Roods (1987) [4]. In pursuant, Banker, Charnez and Copper (1984) [3] took the conception of return to scale in this method (BCC Model). And such the basis of a totality of the performance assessment methods took root which are disposed at the services of managers the suitable and efficient instruments for the assessment of industrial, cultural, economical and ... called in the literature of data envelopment analysis the decision taking units(DMU) .

### Ranking Model

Ranking of the decision taking units and determination of any units based on their activities in the direction of goals and taken policies, the result and objective of ranking incurring motives and sound competition in the decision making units in order to increase their efficiency for short and long terms objectives. The retrieval models to the decision-taking units allow that to compensate for their shortages or weakness of their inputs with the help of other inputs and or the extra consumption in some of their outputs is compensated with other inputs. In the retrieval models, which was presented, by Anderson and Peterson[2] the intervention of the unit under the survey was omitted in the assessment so that in this form in nature of input (output) the smaller (greater) grades or equitant one for the efficient units and the greater grades (smaller) than one for the inefficient units be obtained. In this respect the complete ranking model is:

$$\begin{aligned}
 & \text{Max} \quad \theta \\
 & \text{s.t.} \quad \sum_{\substack{j=1 \\ j \neq p}}^n \lambda_j y_{rj} \geq \theta y_{r0} \quad r = 1, \dots, s \\
 & \quad \quad \sum_{\substack{j=1 \\ j \neq p}}^n \lambda_j = 1 \\
 & \quad \quad \lambda_j \geq 0 \quad j = 1, \dots, n
 \end{aligned}$$

In the above model, giving weight or relative value to the recipients for determination of score to the units is not required. In consideration to this fact that the recipients of a unit is usually from the quality viewpoint may be of such difference which regarding any relative value would be illogical for them. In addition, due to the individual interest in relative giving value may intervene therefore this matter is open to discussion and cannot be optimum value giving.

The retrieval specification of this model to the decision-taking units makes it feasible to compensate the shortages. This specialty in many systems gives this possibility to the units to expand its abilities in special branches. In this article, this model has carried out for Iran power plants generation and so we analyze the resulted outputs.

### Data Envelopment Scenario Analysis

There are scenarios for objective determination, which enable the decision takers for utilization of the new policies in relation to the future performance of the organization. The design of these scenarios for determination of objects results in formulation of a data envelopment model.

- **The first scenario**

The inefficient decision-taking units should assume to obtain sub-optimal results with the performance improving methods. Finding the sub-optimal units should also be in accordance with the general strategy of the organization that that organization considers reaching the goal.

- **The second scenario**

The inefficient decision-taking units by simultaneous improvement of inputs/outputs can consider parts of the efficiency border by which create the increment of lower expenditures inputs and or reduction of lower value inputs to be in the privilege of the organization. In this respect, it can be said that the decision –taking units may reach greater input results to consume greater levels of inputs and or the decision-taking units can by choosing tactical decisions to contract inputs and output of a selected operational units.

I have presented a model, which is the improvised model of DESA[1] in which at onset we consider a set of  $n$  units of decision-taking units that any unit consume the input quantities of  $x \in R^m$  for

generation of output quantities of  $y \in R^n$ . We represent the output indices set by  $I = 1, 2, \dots, m$  and the set of input indices by  $O = 1, 2, \dots, s$ .

$$\begin{aligned} \gamma &= \text{Max} \sum_{r \in O} z_r - \sum_{i \in I} w_i \\ \text{s.t} \quad &\sum_{j=1}^n \lambda_j x_{ij} = w_i x_{ij_0} \quad i \in I \\ &\sum_{j=1}^n \lambda_j y_{rj} = z_r y_{rj_0} \quad r \in O \\ &w_i \leq 1 \\ &z_r \geq 1 \\ &\lambda_j \geq 0 \quad j = 1, \dots, n \end{aligned}$$

The duality between inputs and outputs in DESA will reduce considerably. In fact, it can be said that DESA considers the centers activities by pursuing a practical process obtained from input and output which some of them are for minimizing and some for maximizing. For example it can be said in the index of generating power pollution is an input, which should be minimized while the staff salary is an output that is better to be maximized. Therefore, in the DESA model, pollution is considered as output index and staff salary is an input index. The DESA model considers the decision takers actively in the process of object determination because the object determination is not irrelevant with the organization strategy and managers should be considered too for reaching the determined goals.

**Power Plants performance assessment indexes**

▪ **Inputs**

**1. Production conditions**

Production condition as first input equals to the reverse multiplication output of the numbers of emergency stops. The performance factor or the factor of depreciation (AS), the factor type of the consumptive fuel (FF), and the factor of work difficulty(Hardness) (HF) that these factors will be

calculated and put for power plants by weight combination of practical power of their units.

$$PRD = 1 / NFF \times FF \times HF \times (AS - 0.05)$$

This factor for the power, which has no emergency stops, the years of its performance more than 30 years all its units use Mazut and only has steam unit will be the number of 1 which represents the least input employed by this power plant. Moreover, if any one of these parameters change these numerical factor will be greater than one that is the representative of greater input consumption.

• **The factor of the emergency numbers of stops**

First, the emergency stops have bad effects on the main unit equipments. Namely by each stoppage the heating shocks will be imposed on the unit various points which in turn will decrease the life longevity of the unit. Second, any units by each emergency stoppage will cause disturbance in the network status and the control center should enterprise any readiness for this stopped compensations and return the network to a primary stable status. Therefore, it is logical to consider the numbers the stops in relation to performance time. This index depends on the factors such as the emergency stop time and the program stoppage time and the performance duration of the unit.

• **Performance efficiency or depreciation factor**

By the increase of a unit performance, the various systems will gradually depreciate and the probability of failures and unit stoppage will increase. On the other hand, through the passing of time the more advanced systems in various fields will be invented and are put at the disposal of this industry, which is more efficient, compared the old models. Therefore, one can discriminate from two dimensions viewpoints the old depreciated equipments and the accelerating progress in equipments. For this reason, by using the existing experiences depending on the unit age the efficiency factor of the years will be defined so that there would a balance between the old and new units.

Therefore, the following table in this respect is suggested:

Performance age(year)	More than 30	25-30	20-25	15-20	10-15	Less than10
Gas and Steam units	1.05	1.04	1.03	1.02	1.01	1
Water Units	1.025	1.02	1.015	1.01	1.005	1

**Factor of the type of fuel consumption**

All the steam power plants surveyed in this article have used ranging from the dominant gas consumption along with Mazut (black oil) fuel as the second one. The gas fuel in the warmer seasons and

Mazut is mostly used in cold days of the year. Since gas fuel has generally more heating value compared to Mazut; therefore, the usage is more often causes the efficient performance of the units of these power plants. However, in some cases in some power plants

the use of Mazut despite the lower heating value not only caused not the reduction of the performance compared to the times that gas has been used but also the efficiency has been similar to the units that had used gas and in some plants the efficiency has been even more. The chemical type of the consumption fuel of the heating units regarding to the existing impurities in these fuels is of importance. The steam units can simultaneously use Mazut or natural gas and or both. Regarding various fuels are used in a power

plant all can be measured equally by a common scale. Therefore, a factor can be considered for the user unit of different fuels. These factors can be considered in accordance with the following table so that by using the necessary scale factor among the power plants is balanced. For the units that use two types of fuels the above factor will be assessed by calculating the average related weight considering the used fuels during the course of assessment.

Consumption fuel type	Mazut	gasoil	gas	water
Consumption fuel factor	1	0.98	0.95	0.9

- **Work hardness factor**

Regarding the type of the employed equipments and operation complexity or simplicity, for water and gas units the number 0.8 and for the steam units, the number 1 are considered as the factors of work hardness.

2. **Personnel**

The number of the affiliated equipments to the power plants types are different compared to each other; and consequently, the required personnel number with the regard to production process are different. So that the steam power plants with consideration of the equipment number and different sections require more personnel for production and units maintenance and gas and water power plants with respect to the limitations in equipment number and the executive sections have less personnel relatively. Therefore regarding the country different power plants circumstances, a factor in conformity with the production capacity in any productive unit can be defined so that it would be possible to determine the same index as the personnel number factor while installing a new unit or removing the old units. Therefore, for steam units regarding the more required personnel the factor of 0.5 individual for each production capacity Mega watt and in gas and water units the factors of 0.3 and 0.25 can be taken respectively.

$$\text{Production factor} = \frac{\text{the produced gross energy during the evaluation}}{\text{Practical power performance hours during the evaluation}}$$

3. **Aptness Factor (AF)**

Aptness of the units from the viewpoint of control center for keeping the network stable status through coordination of the produced amount of consumption is of great importance. Actually, we can say the aptness of a unit is the actual percent of being apt to work. One aptness under one hundred percent shows that there is a default in getting target compliance. On

- **Outputs**

1. **Unit performance factor (UPF)**

In order to assess the power plants performance it is better to investigate the different power plants units one by one and to define the effective factors in their performance separately and at the end to intervene the factors that are related totally to the power plant. The unit performance factor involving the main performance factors are such as energy generation, energy power and production continuity in the peak time hours.

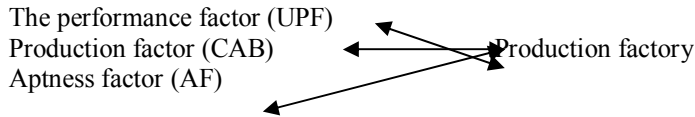
2. **Production Coefficient (CAB)**

Daily load consumption fluctuations cause any type of the power plants to follow a special pattern in the production times. The low load consumption hour during the day and night reaches to the half of the maximum load of consumption and this causes that some units to reduce their load and some to get totally out of the circuit. Therefore, the volume of the production total activities in any power plants should be defined during the assessment course as a production factor index. Since the produced energy volume in appropriation of useful performance time duration, considering the amount of produced energy during the course will be a correct criterion of the total produced activities volume, which in this relation the factor index is defined as follow:

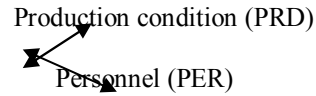
the other hand one aptness above one hundred percent for maximizing the benefit is not a bad index and this phenomenon occurs when between budget and real force demand there is a disfigure. Therefore, a lower expenditure factory can be put in the network for production and this is out of the devices that have been considered for them. Therefore, a factor can be considered as an index for aptness.



Considering the above-mentioned matters, the factors are defined for the evaluation of the power



plants performance as follows:



**Models executions, results and their analysis**

**Ranking Model execution**

By using this model, the power plants according to the defined indexes for the recipient units are ranking and the only used input in the ranking model equals to one. In the table number 3, the power plants are listed as in the first column. In the second column, the ranks related to every power plant, in the third col. The index (reference) power plants for ranking have been listed respectively. Regarding the obtained results, one can see that the power plant number 23 with the score of 1.44752 has an excellent grade and the power plant number 39 with the score of 0.92862 has the lowest grade. In addition, in the table 5 the power plants with greater score or equal one in one group and the power plants with the score less than one are classified which attribute the concept of first grade to the first group power plants and the second grade to the second group power plants. It can be observed that such power plants as the power plant number 23 which are excellent grade is relatively inefficient because this production unit has an upper expenditure that obviously can be seen from the table 1 quantities and explains clearly that this power plant has a big volume but cannot have maximum production from its existing resources. This analysis is of great importance for management points of view.

**The execution of DESA Model**

Scenario analysis of data envelopment aforesaid on the country power plants with two input indexes and three recipient indexes at first executed for the group one power plants and then for the group second power plants. In execution of model, we consider a state with equal priority to the all inputs and recipients. Out of this model analysis with regard to the obtained results one can suggest the recipients' increment and reduction of inputs regarding the  $w_i$  and  $z_i$  quantities which can deliver the rate of recipient expansion and input contraction. For the interpretation of the results obtained in the tables 5 and 6 for example the power plant number 16 with the reduction of the second its input that is the personnel factor can increase its first and third recipients to the rate of 1.25 and 1.13 respectively. We observe that the units number 1 and 7 as the

index (reference) units have acted as for the unit 16 and a weighted combination of units 1 and 7 with defined weights are introduced which both equals to 0.564, one figurative unit presents as the pattern are for performance improvement. In addition the  $z_1, z_2, z_3, w_1, w_1$  coefficients have presented the improvement rate on the unit 16 recipient and input vector. The interpretation of these coefficients is that if unit 16 reduces its second input up to 0.61 against previous input amount in the figurative level obtained from the linear combination will act as two efficient units 1 and 7. In other words with the change mentioned in the second input of unit 16, its recipient will equal to 1.25, 1 and 1.13 respectively. Therefore, we have the amounts of  $w_1 = 1$  and  $w_2 = 0.61$  for the unit 16 input:

$$\begin{pmatrix} x'_1 \\ x'_2 \end{pmatrix} = \begin{pmatrix} 1.19 \\ 0.16 \times 0.995 \end{pmatrix} = \begin{pmatrix} 1.19 \\ 0.60695 \end{pmatrix}$$

In addition, we have for its recipient vector:

$$\begin{pmatrix} y'_1 \\ y'_2 \\ y'_3 \end{pmatrix} = \begin{pmatrix} 1.25 \times 80.4 \\ 1 \times 81.21 \\ 1.13 \times 87.87 \end{pmatrix} = \begin{pmatrix} 100.5 \\ 81.21 \\ 99.2931 \end{pmatrix}$$

On the other hand, by calculating the amounts of the figurative unit input and recipient we have:

$$\begin{pmatrix} x''_1 \\ x''_2 \end{pmatrix} = 0.564 \begin{pmatrix} 1.06 \\ 0.333 \end{pmatrix} + 0.564 \begin{pmatrix} 1.05 \\ 0.75 \end{pmatrix} = \begin{pmatrix} 1.19004 \\ 0.610812 \end{pmatrix}$$

$$\begin{pmatrix} y''_1 \\ y''_2 \\ y''_3 \end{pmatrix} = 0.564 \begin{pmatrix} 88.4 \\ 64.35 \\ 86.27 \end{pmatrix} + 0.564 \begin{pmatrix} 90.22 \\ 79.65 \\ 89.97 \end{pmatrix} = \begin{pmatrix} 100.82 \\ 81.216 \\ 9.2931 \end{pmatrix}$$

We observe that the obtained quantities through the improvement of input-recipient and the figurative unit are approximately equal. Therefore, by changing the priorities on the input and recipient quantities, quantities that are more specific can be obtained for the improvement rate in order to be useful for managers' taking decisions. The results obtained in the tables 5 and 6 can generalize for all the power plants.

### Conclusion

What presented is the application of data envelopment analysis in the evaluation of Iran electricity generation power plants, the results obtained from this assessment through considering the scenarios that are dependent on objectives. It gives the feasibility to power plant management in order to take the different priorities to assess the performance improvement. The information obtained from the execution of the DEA models, first gives management a relatively specific recognition of system in order to control the parts under its surveillance with a specific and scientific conception. Secondly, we can recognize the power plants performance improvement ways, finding out capabilities and or barriers on the way of power plants development, presenting scientific pattern for control centers decision takers, dedication of resources to the power plants etc.

### Data and Resources

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## Agricultural development through information and communication technologies (ICT)

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**Abstract:** The concept of development of the rural, today is not just project initiatives and governance; it is much more beyond that. This paper uncovers a whole plethora of ICT emergence as a technology of the new millennium. Against the backdrop of the ongoing ICT boom, this paper makes an attempt towards studying its applications and usage planning process and policy making for the rural communities focusing on how it helps in aligning the key factors and reduce the problems of alienation, fragmentation and dislocation of knowledge. Review of literature shows that intervention of information and communication technologies (ICT) in rural development initiatives are capable of development, but are not successful. Lack of community participation, absence of an integrated approach and non-inclusion of traditional knowledge systems (TKS) in the project designs are the major impediments. We therefore suggest a systems-based approach in the design of e-Governance projects, and brief some future directions. Sustained development using rural informatics is possible, only if ICT interventions are able to respond to the local needs and re-adjust as per the prevailing knowledge (Traditional Knowledge Systems- TKS) of the rural areas.

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**Keywords:** information and communication technologies (ICT), rural development

### Introduction:

Direct or indirect application of ICT, in rural development sector has also been referred to as "Rural Informatics". Rural economies can be benefited from ICT by focusing on social production, social consumption and social services in the rural areas (Malhotra, 2001). The inculcation of a Citizen-to-Government (C2G) and Citizen-to-Citizen (C2C) interface would provide this link that would also lead to community participation in design and implementation of ICT interventions. This in return could promise better economic opportunities as well as social inclusion of rural people in the processes of governance. Such attributes in the social set up are essential prerequisites for good governance and rural development.

Globalization and technological changes, the processes in the past fifteen years have been quickly lead to a new global economy have been driven with the reinforced technology and fuel (energy) that by providing information and knowledge.

The global economy requires the kind of necessity and purpose of educational institutions. Since the current trend towards reducing incomplete information and access to accurate information is growing, other schools can not control time to transfer a set of prescribed information from teacher to student during a fixed time point are, but schools must to promote Culture of "Teaching for Learning For example, acquisition of knowledge and continuous learning skills which make possible during the individual's life. According to Alvin Toffler, illiterate in 21st century, who was not read and write but those who do not know which fail to learn or remember are illiterate. (Jauhari, 2004).

Concerns about educational quality and educational opportunities with the necessity of developing those most vulnerable are the accumulation of globalization is symbiotic. Generally, "the changes of globalization in developing countries, on low-income groups, especially women and girls and" low skill workers, as well as all groups applying for and obtaining new skills to press. (Bellamy and Taylor, 1998).

In the rural context, development involves use of physical, financial and human resources for economic growth and social development of the rural economies (Burkey, 2000). The term rural development also represents improvement in quality of life of rural people in villages. As per Chambers (1983) "Rural Development is a strategy to enable a specific group of people, poor rural women and men, to gain for themselves and their children more of what they want and need." Singh (1999) defines Rural Development as "A process leading to sustainable improvement in the quality of life of rural people, especially the poor". The fact of the matter is that three quarters of the world's poor, about 900 million people are in rural areas, and the Millennium poverty target set by Millennium Development Goals (MDG), cannot be met unless the world addresses rural poverty. "Sustainable Rural Development can make a powerful contribution to four critical goals of: Poverty Reduction, Wider shared growth, Household, national, and global food security and Sustainable natural resource management" (World Bank, 1997). Hence worldwide there is a growing emphasis on development of rural economy of the countries. Any improvement, in the social or economic status of rural areas would not just directly benefit rural

poor but would also bring down the migration-pressures on cities and contribute by positive ripple effect in global stride towards development.

The process of development in a country is to be aided by its governance. The goal of governance "should be to develop capacities that are needed to realize development that gives priority to the poor, and creates needed opportunities for employment and other livelihoods" (The World Bank, 1992, UNDP, 1994). Increased number of poor, hungry or marginalized people in a country represents decrease in its quality of governance. To promote development, various studies have proposed governance in the contextual realities of each country, including veritable participation of citizens in the governmental decision-making process (Grindle, 2004; Evans and David, 2006). Several

Institutions and experts accept Governance as a reflexive process, wherein policies, institutions, outcomes and analysis interact, to maximize the process of participatory development (UNDP, 1997; Ludden, 2005; Mehta, 2006).

Information and communication technologies (ICT), including radio and television and the newer digital technologies like computers and the Internet as potentially are introduced powerful tools and activators of educational reform and changes. different ICT, when properly applied can be developed to help access to education and the relationship between training and workshops to strengthen the increasingly digital, the quality of education also helped to create teaching and learning in an active process connected to real life high take. However, the experience of being raised by ICT in the classroom and other educational sites around the world during the last few decades proves that is not automatic fully realize the potential benefits of ICT training. (Guptaand et al, 2004)

Effective integration of ICT in the educational system is a complex process that involves not only technology but also involves educational and technical training, institutional readiness, teacher competencies and long-term investment. In fact the subject of such vital importance is that the technology to get the easiest part of it. Introduced ICT information and communication technologies are for this purpose, as a different set of tools and technology resources, used to information communicate, create, release, storage and management have been defined. These technologies are including computers, internet, broadcasting technologies (radio and television) and telephone. In recent years started a wave of intense public interest about how computers and the Internet can become a better control to the efficiency and effect of education at all levels and in both formal and informal development. (Rogers and Shukla, 2001).

But nowadays, ICT is more than a technology. Although the old technologies such as telephone, radio

and television, will be less attention in the past but were used as educational tools. For example, "radio and television are used for over forty years to open and distance education. In this regard, although print remains the most expensive method and therefore available, but in developed and developing countries is provided the most prominent mechanism.

ICT for developing countries, are associated a potential for increased availability and quality of training and development. ICT basis and attract a lot of knowledge and its acquisition, providing unprecedented opportunities for developing countries, adding and expanding educational systems, improve policy formulation and implementation of opportunities to expand scope of work and gives poor facilitation. One of the biggest hardships that the poor are bearing the other people, who live in the poorest countries, is the sense of isolation. Communication technologies such sensory loss, are guaranteed and also has been unthinkable facilitate access to knowledge through the ways that already. However, the reality of the digital divide (the gap between those who control access technology and those who do not have access) means that the introduction and integration of ICT are challenging at different levels and in various types of training, most commitments. Failure in this struggle to become more significant gap of knowledge and the deepening economic and social inequalities (UNDP, 1997; Ludden, 2005; Mehta, 2006).

How ICT can help developing access to education?

ICT is a potentially powerful tool for developing educational opportunities, whether formal or informal is for areas already "stated (rural and dispersed populations) ethnic minorities, women, girls, disabled and old people traditionally excluded from education groups because of cultural or social reasons are also all those financial reasons or time constraints can not register in educational centers. Any time, anywhere (defining feature ICT) capability in ICT is a passing of time and place. ICT, education or training with asynchronous features provide a time delay between education and its acceptance by students makes it possible.

#### **ICT applications in education:**

Organizations and educational policy planners should first of all about the desired educational outcomes (mentioned above) is straightforward. The broad objectives must choose different technologies used to go and how to apply the guidance to go. Potential of each technology varies according to how to use. Haddad and Draxler have been identified IT application in education at least five levels of:

1. present,
2. experimental proof,

3. practice and practice,
4. interaction,
5. collaboration

Each of the different ICT tools (print, audio cassette and video, broadcast radio and television, computer or Internet) may provide the most basic means and surfaces used to go to prove. Except for visual technology, practice and practice the maximum use of both technologies may be offered. Each of the different ICT (print, audio cassette and video, broadcast radio and television, computer or Internet) used to may provide the most basic means and surfaces. Except for visual technology, may be offered practice and practice the maximum use of both technologies. The other network computers and Internet, ICT interactive learning that are provided and they if only used for providing proof or go, was not realized can better their full potential (Jauhari, 2004)

### Results:

This paper is a multidisciplinary study of ICT initiatives for rural development. It emphasizes adoption of a more systematic approach for integrating Traditional Knowledge Systems (TKS) and ICT inputs to ensure sustainability of rural e-governance projects. The study of literature related to rural development and e-governance has indicated various issues impeding success of such initiatives. The main issues are lack of localization of content for rural communities and inadequate participation of rural communities in design of rural ICT initiatives. The study therefore suggests the use the systems-approach to integrate the relevant TKS along with ICT initiatives in the design of e-governance systems for rural development. This participatory approach can lead to creation of more acceptable and sustainable e-governance projects.

Regardless of the wide differences in ICT access between rich and poor countries and between different groups in the country, there are concerns that challenge the application of ICT in education with the existing differences among the lines of economic, social, cultural, geographic and gender will be broader. Everyone equal opportunities in terms of suitability for participation are necessary, but access to various factors, either as users or as producers through their sources is difficult and heavy. Therefore, the primary differences enhance and even grow. Consequently, programmers' international education is faced with a difficult challenge and how to help solve the problem and its development.

Promoting ICT in education, when done without careful study, can lead to the marginalization of those with more favorable conditions are unknown. For example, "women compared with men, because of illiteracy, lack of higher education, lack of time and

mobility and poverty, controlling access to ICT and fewer opportunities for training are relevant. Also, more boys than girls' access to computers at home and school are not strange to say that if more boys than girls are willing to work with computers. The report of the University Association of American Women is that "Although some girls have an important gender gap have been limited, but today's technology, technology club, and boys in public schools while its own problems and programs are settled girls use computers for word processing the brand". In an assessment in four African countries, the activities organized by World links remote international cooperation on projects between teachers and students in developing countries will promote, despite creating programs without regard to sex contacts, sexual inequalities remain Uganda and Ghana. In addition, while more girls than boys in relation to academic performance and advanced communication skills program will enjoy more than boys, but they were unable to perform their technological skills were. A set of economic factors, organizational and cultural differences involved in the social.

"The high ratio of students to computers and politics, whoever came first, the first is used in accordance with the girls wanted it." Girls travel restrictions in the early hours of daily work and home responsibilities are that this will limit their access. Also because local patriarchal beliefs dominate the boys are in the computer lab environment. Including proposed measures to address this discrimination, strategies to encourage schools to create "fair use" in the computer labs and the holding of meetings and sexual sensibilities conductivity decreased defense duties after school girls. ICT provides access to only a small part of the action is created equal. Equal attention should also be applied to ensure the technology really "is used by learners and ways of how well their needs will cure.

An educational program that reinforced this approach shows the overall program is bilingual. The program seeks to establish technology learning centers for bilingual teachers, students, teachers, parents and community members. Technical teams from each center three students, two teachers and the director of the Center with at least one female student and a teacher are female.

Another example of a general approach to the application of ICT in education, radio education project Gobi Women of Mongolia, which seeks to provide professional and educational structure of women's favorite courses around the nomads and their opportunities for income generation.

It contains topics such as livestock rearing, family support (family planning, health, nutrition and health) to create income in the application of local raw materials and basic skills for the job is a new market.



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## Micropropagation of Ornamental Plant *Musa Beccarii* through Tissue Culture Technique Using Suckers and Male Buds as Explants

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**Abstract:** *Musa beccarii* is grouped under wild bananas and endemic in Borneo. This ornamental plant is among the most unique ornamental bananas that produce colorful and attractive flowers with small plant compared to the cultivated bananas for fruits. Micropropagation is a laboratory based tissue culture technique commonly used to propagate any plants. Micropropagation studies of these species have become important in order to increase the productivity for mass propagation in floriculture industry. This study was conducted to observe and determine the optimum sterilization procedure and media requirement for the regeneration of explants using *in vitro* method. Sterilization procedure for both suckers and male buds inflorescence for this ornamental banana were almost the same, whereas for suckers, 100% Clorox with tween 20 and 70% ethanol were used and for male buds, 70% ethanol was only used. It is found out that prolific multiple shoot formation and elongation were obtained from the suckers and male flower buds cultured on MS basal medium with 0.014mg/L BAP. Charcoal, coconut water, and gelrite (agar) were also added into the medium and PH was adjusted to 5.8. Unfortunately, the final phase was not able to perform as the plantlets were died after 5 weeks transplanting from media culture to the soil. It might be because of the sunlight that was too hot or the plant could not acclimatize to the new condition. So, the plantlets were not capable of supporting themselves in the soil.

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**Keywords:** Micropropagation; Tissue culture; Explant

### 1. Introduction

Banana is the fourth most important fruit crop in the world. They are grown in 132 countries worldwide, more than any other fruit crop ever grown. Banana is believed to be originated from the Southeast Asia and is well known among tropical countries around the world through the trading activities. In general, banana grows well in temperature which ranges from 13°C – 38°C with relative humidity regime of 75-85%. The normal growth of the banana begins at 18°C, reaches optimum at 27°C, then declines and halts at 38°C (Simmonds, 1966). The requirements for growing ornamental bananas are the same as growing other types of bananas. The original bananas contain rather large seeds and cannot be eaten while cultivated bananas are parthenocarpic, which makes them sterile and unable to produce viable seeds. There are many types of ornamental bananas such as *Musa ornata*, *Musa beccarii*, and *Musa velutina* (Anon, 1997). Ornamental banana such as *Musa beccarii* produces seeds but cannot be eaten. In the recent years, tissue culture propagation of banana has been utilized to increase banana production. Tissue culture is the propagation of a plant by using a plant part or single cell or a group of cells in a test tube under very

controlled and hygienic conditions. Tissue culture of banana has been widely used these days due to its many advantages. Through tissue culture, elite banana varieties that are disease free can be produced because tissue cultured bananas are made under an aseptic condition where any form of contaminations from microorganisms are eliminated. Rapid multiplications of tissue cultured bananas enable early harvesting and bring a lot of benefits to the growers. The banana plants that are the products of tissue culture, have uniform size and age and the fruit bunches are of higher quality. Since tissue culture ensures continuous multiplication of plant parts in the laboratory and is not limited by the time of year or the weather, so that tissue cultured bananas are available throughout the year. This method eliminates the transmission of diseases from parent plants to offspring as the external contaminants are removed when explants are cleaned. Despite all the advantages of tissue culture, there can be a few disadvantages when it is not properly handled which can cause severe loss to a grower. For example, if a mutation occurs during mitosis, it is carried in all future divisions. A defect due to mutation could be multiplied in culture and results in thousands of cultured plants with the same defects, which can be

visible as they are planted and grown in the field. *Musa beccarii* is a type of ornamental banana under the section of *Callimusa*. The plants are small, the pseudostems are about 1-1.5m tall, sheaths are bright green, devoid of wax and somewhat polished. They are also tinged with brownish-purple at the edges. Petioles are up to 35 cm long, tightly clasping below, with erect or slightly incurved, narrowly purple and scarious margins above. Leaves are up to 100 x 30 cm, oblong-lanceolate, obtuse, the lamina halves slightly unequal, rounded or sharply cuneate at the base, bright green in color and devoid of wax. Inflorescence is small, erect, borne on a minutely hairy peduncle, and 2 cm in diameter. Basal hands are 2 - 5 in number, each bearing 1 - 3 uniseriate female flowers. Female flowers have the ovary 5 - 6 cm long, trilocular with 170 - 250 biseriate ovules. Male bud is spindle-shaped, broadest about the middle, rounded-acute at the apex strongly imbricate in the distal one third of its length. Male bract is deciduous, lanceolate, obtuse, 10 x 3 - 4 cm, almost flat, thick and leathery in the center, thinner near the edges, polished scarlet and greenish at the tip without, scarlet and duller within, persistent for a day after the flowers fall, deflexed at flowering and slightly recurved at the margins but not rolled back. Male flower is uniseriate, each 4 - 5 cm long. The stamens are 35 - 40 mm long, white, with copious chalky-white pollen. Fruit-bunch is small, erect and loosely packed, consisting of 2 - 5 hands of 1 - 3 fruits each. Fruit is shortly pedicellate, erect, cylindrical, bottlenecked at the tip, 5 - 15 x 2 cm (Simmonds, 1962). Seeds are subglobose, 4 - 5 mm in diameter, light brown in color. Somatic chromosome number is  $2n = 18$  (Simmonds, 1960). It takes about 6-8 months for the male buds of *Musa beccarii* to be produced. *Musa beccarii* was treated as *incertae sedis* (a term used to define a taxonomic group where its broader relationships are unknown or undefined) until Simmonds and Weatherup (1990) detected numerical taxonomic analysis of wild bananas and placed it in *Callimusa* section. However, the seed shape is not typical of *Callimusa* section and the chromosome number ( $2n = 18$ ) was new to the genus *Musa*. Simmonds and Weatherup (1990) did not consider chromosome number as an important determinant of section but on this basis, Jong and Argent (2001) maintained *Musa beccarii* as *incertae sedis*. Objectives of the study were to develop *Musa beccarii* (Ornamental banana) through tissue culture population from suckers and male buds. Moreover, to make a comparison between the sterilization technique and the micropropagation process among male buds and suckers. Finally, study the micropropagation process in tissue culture of *Musa acuminata* var. *Berangan* and *Musa beccarii* (Ornamental banana).

## 2. Materials and Methods

### 2.1. Source of explants

The samples for *Musa beccarii* were prepared from Kalis Nursery and Landscaping in Janda Baik, Pahang, Malaysia. Explants that were used in this study were the suckers and male buds. For *Musa beccarii*, because of limited of sources, there were only 6 clones of suckers and 4 clones of male buds that have been cultured. Each of the samples were coded and marked.

### 2.2. Preparation of media

The media used in this study was the Murashige and Skoog (1962) medium, or commonly referred to as MS medium. Stock solutions of macronutrient, micronutrient, vitamin source, and Iron (Fe) sources were prepared in relatively large batches and stored in the refrigerator. Other ingredients such as Sucrose, BAP (6-Benzyl aminopurine), coconut water, and Gelrite (Agar) were also added to make the final medium.

### 2.3. Preparation of aseptic condition

Explants were cultured under the laminar flow cabinet. Before using the laminar flow, they were exposed to UV light for about 30 minutes to eliminate or kill all the microorganisms (pathogens). Each time the laminar flow was used, the surface of the hood wiped down with 70% alcohol. Instruments like forceps and knife (scalpel) that were going to be used were sterilized by dipping them in alcohol by flaming (burning off the alcohol) with the use of spirit lamp. Alcohol used for disinfecting instruments was 95% alcohol because it was able to burn off more easily than a more dilute form. The instruments were placed in a hot-bead sterilizer after use and then they were dipped in alcohol and flamed again before each use.

### 2.4. Production of *in vitro* complete plantlet from suckers

The suckers were washed to clean the remaining soil from the roots and corms. The outer layers of the suckers were removed and trimmed until the size became about 9 cm in length. Explants were put under running water for 30 minutes and rinsed with distilled water. After that, they were brought to laminar flow and were soaked in 100% Clorox and Tween-20 for 15 minutes. Then, they were rinsed with sterilized distilled water for three times, followed by soaking in 70% ethanol for 5 minutes. After this step, they were rinsed again with sterilized distilled water three times, trimmed, cut and cultured in MS media.

### 2.5. Production of *in vitro* complete plantlet from male buds



The outer layers of the male buds were removed up to 3-4 cm in length and put into a beaker. Explants were put under running water for 30 minutes and rinsed with distilled water for once. They were soaked in ethanol 70% for three times and rinsed with sterilized distilled water for three times. The outer layers were removed until reaching the size of 1cm × 0.5cm. Finally, explants were cut into two portions and cultured in MS media.

### 3. Results

In this study, only 6 suckers and 4 male buds had been cultured from the total amounts of samples from suckers of *Musa beccarii*. Only two sub-culturing in suckers and one sub-culturing in male buds of *Musa beccarii* were managed. The first batch of culture for explants (one suckers and two male buds) of *Musa beccarii* were all contaminated. In the sterilization procedure, it could be observed that the explant from suckers got contaminated easily in comparison with the male buds.

In plantlets produced from suckers, after one week of culturing, the explant turned green in color. After another week, the explant started to swell and a shoot was seen growing from the tip of the explant. The explant was then sub-cultured by excising the shoot and divided into two equal halves to induce multiple shoots formation.

In plantlets produced from male buds, the first batch of culture for male buds (two clones) of *Musa beccarii* were all contaminated because of infection by bacteria. The second batch of culture, which consisted of another two clones, however, was able to survive and this was occurred by the condition of the explants after two weeks of culture. After about two weeks, the male buds swelled a little and had turned reddish in color, just like the original color of the male buds.

Figures 1 to 7 below show the micropropagation in suckers and male buds of *Musa beccarii*. The micropropagation of the explants could be observed by counting the number of shoots formed for suckers and the number of propagules for male buds.

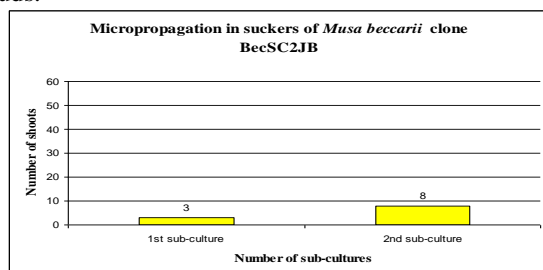


Figure 1. The number of shoots produced after two sub-cultures in suckers of *Musa beccarii* clone BecSC2JB

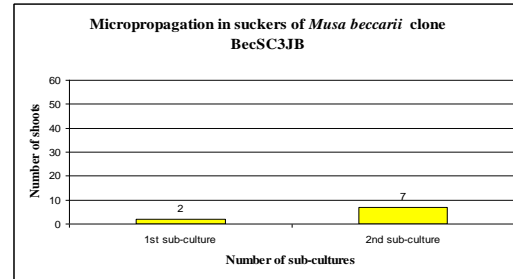


Figure 2. The number of shoots produced after two sub-cultures in suckers of *Musa beccarii* clone BecSC3JB

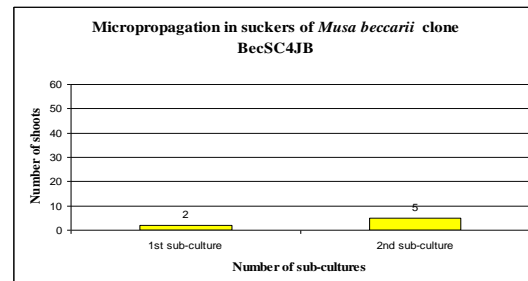


Figure 3. The number of shoots produced after two sub-cultures in suckers of *Musa beccarii* clone BecSC4JB

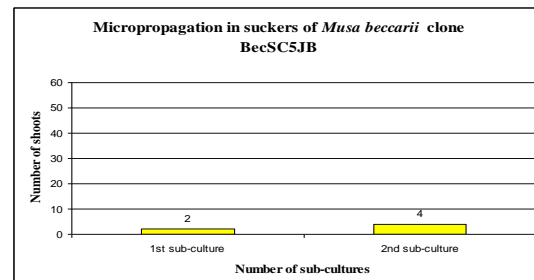


Figure 4. The number of shoots produced after two sub-cultures in suckers of *Musa beccarii* clone BecSC5JB

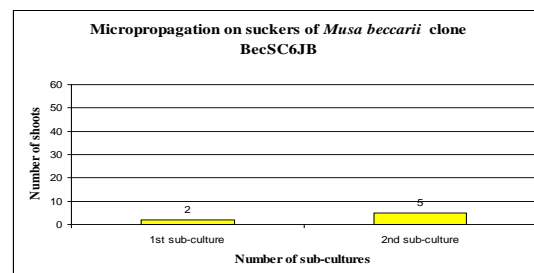


Figure 5. The number of shoots produced after two sub-cultures in suckers of *Musa beccarii* clone BecSC6JB



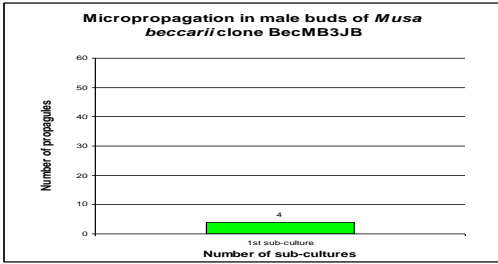


Figure 6. The number of propagules produced after one sub-culture in male buds of *Musa beccarii* clone BecMB3JB

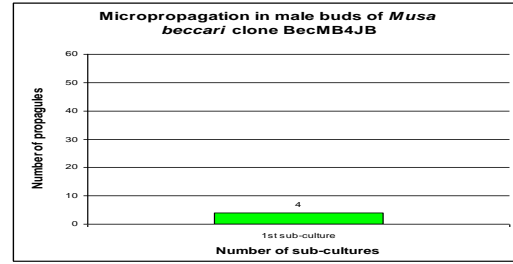


Figure 7. The number of propagules produced after one sub-culture in male buds of *Musa beccarii* clone BecMB4JB



Plate 1. *Musa beccarii* at the nursery



Plate 2. Male bud of *Musa beccarii*



Plate 3. Male bud of *Musa beccarii* before sterilization



Plate 4. Explant from male bud of *Musa beccarii* (Stage 1)



Plate 5. Explant from male buds of *Musa beccarii* after two weeks of culture (Stage 1)



Plate 6. Suckers of *Musa beccarii* before sterilization

Plate 7. Explant from suckers of *Musa beccarii*Plate 8. Explant from suckers of *Musa beccarii* after two weeks of culturePlate 9. Seeds of *Musa beccarii* floated when soaked in waterPlate 10. The absence of embryo in seeds of *Musa beccarii*

#### 4. Discussions

Suckers spring up around the main plant forming a clump or "stool". The eldest sucker replaces the main plant when it fruits and dies, and this process of succession continues indefinitely. Male buds or inflorescence on the other hand, is formed from the transformed growing point in the heart of the pseudostem and undergo much of its development before emergence. Cultures initiated from explants of juvenile seedlings were easier to stabilize than those from explants of older materials. It is also important to ensure that the chosen suckers and male buds are not over matured or too old.

Size of plant materials in this study was paid much attention as it is important in determining a suitable sterilization procedure in the tissue culture of banana. The average size for suckers of *Musa beccarii* was about 80cm in length and the male bud was about 12cm in length and 4cm in wide.

It was also intended to culture embryo using the seeds of *Musa beccarii*. However, it could not be done because the seeds were not viable. They all had floated when soaked in water (Plate 9). When the seeds were opened up, no embryo was found (Plate 10). Due to time constraints, further study on embryo rescue of the seeds of *Musa beccarii* could not be done.

In this study, sterilization process was done properly as it is critical in determining the successful of a tissue culture procedure. Without proper

sterilization process, explants will not be able to survive long in culture media due to presence of microorganisms that might invade them. Plant surfaces are habitats for microorganisms (Campbell, 1985). A proper sterilization process will ensure that almost all microorganisms that might present on the explants will be destroyed or killed. The sterilization steps for suckers are more extended and comprehensive compared to male buds. In sterilization of suckers, they had to undergo a series of surface sterilization using Clorox and ethanol before being rinsed in sterilized distilled water while for male buds; sterilants such as Clorox were not used. This is because suckers had originated from soils that are rich with soil-borne microbes. So, their surfaces must be thoroughly sterilized using Clorox to kill all attached microbes.

To improve wetting of the tissue surface, treatment with hypochlorite is often preceded by a detergent or alcohol wash. Ethanol partially removes hydrophobic waxes and resins which protect microorganisms from contact with aqueous sterilants. Furthermore, ethanol is a potent phytotoxic agent on its own. However, it does not kill all microorganisms; some bacteria survive when they are exposed to 96% ethanol for at least 40 min (Kunneman and Faaij-Groenen, 1988). Surface sterilization of male buds required simpler steps compared to that of suckers. This is maybe because the suckers had originated from the soil which is full of microorganisms and thus, more complex sterilization method is required.

#### 4.1. Micropropagation

Micropropagation generally involves four distinct stages namely; initiation of cultures, shoot multiplication, rooting of *in vitro* grown shoots, and acclimatization. The first stage, culture initiation (Plates 5 and 8) depends on explants type or the physiological stage of the donor plant at the time of excision. In this stage, the innermost tissue of surface sterilized plant material was aseptically dissected and put directly into a growth medium.

In the second stage of micropropagation, the shoot multiplication is crucial and is achieved by using plant growth regulators namely; auxin and cytokinin. When the tissues started to grow in the first stage and started to form shoot, they were transferred to another medium. Difference in the manner of growth in suckers of *Musa beccarii* was observed after the initiation of culture in Stage 1. After about a week of culturing, the tissues of explants in both types of banana started to turn green in color and swollen a little. This indicated that nutrient uptake had occurred and the explant had adapted to the new environment. However, after about two weeks of culturing, the explants of *Musa beccarii*, primordial shoot started to form at the tip of the explant. The explants were then excised or cut out to initiate more shoot formation. The number of shoots increased with subsequent subcultures on the fresh culture. This was caused by the elimination of some contaminated explants. Using suckers as explants is an example of a micropropagation process via meristem culture or shoot tip culture. *In vitro* propagation through meristem culture is the best possible means of virus elimination and produces a large numbers of plants in a short span of time. After the first sub-culture, shoots started to form at the region of the apical meristem. This condition was observed in the shoot-tip culture of suckers. As the clusters of shoots are divided and re-cultured, the numbers of microshoots multiply at an exponential rate. The growing shoot multiplies and forms a clump of 3-4 shoots per clump. The pattern of multiplication and sub-culturing continues until a population of microshoots is obtained.

In the third stage, the elongated shoots derived from the multiplication stage, are subsequently rooted. After multiplication, the single shoots are separated and placed into a rooting medium. The media used in this stage is the same as MS media used in the previous stages except for the addition of activated charcoal in it. Activated charcoal was added to the medium primarily to absorb unwanted exudates, but in addition, it removed some essential chemicals from the medium such as phenolics, auxins and cytokinins. The shoots were elongated and new root came up. After about two or three months of rooting, a

well-developed root system and leaves had been achieved.

The fourth stage of micropropagation involves acclimatization of *in vitro* grown plants to the natural environment. The plantlet was taken out of the culture jar and the media adhering to the root system was washed. After one week of transplanting, the plantlet had adapted to the new environment seeing to their healthy condition.

In this study, only 6 clones of suckers and 4 clones of male buds of *Musa beccarii* had been cultured. This is because, it was quite hard to find samples of ornamental banana since this type of banana is a new attractant in Malaysia besides they also needed to be bought with quite a high price. Thus, only two subcultures were done on the suckers and once in male buds of *Musa beccarii*. Plates 1, 2, 3, 4, 6, and 7 also show various details about materials in this study.

#### 4.2. Contamination and formation of phenolic compounds

Losses can be readily ascribed to overrunning of cultures in Stage 1, which may prevent the culture of specific genotypes, or laboratory contamination due to systems failure, or infestation with mites and thrips (Blake, 1988) or poor sterile technique. Microorganisms may be rapidly expressed when they transfer to new media, for example, in the transfer from Stage 2 to 3, particularly where the concentration of salt and sucrose in the medium is reduced (Cassells, 1988; Cassells, 1991; Cassells et al., 1988).

Contamination in tissue culture can originate from two sources, through existence of microorganisms on the surface and in the tissues of explants, or through faulty procedures in the laboratory. A lot of contaminations had occurred throughout this study. Bacteria and fungi have destroyed many cultured clones of suckers and male buds.

Contamination rates are often high when explants from field-grown trees are used. From observation, I discovered that suckers were easier to get contaminated compared to male buds. The contamination rate in suckers was higher compared to that of male buds because the suckers originated from soil. Improper sterilization procedure cannot kill some of the soil microorganisms that might attach to the suckers and this may results in exogenous infection of bacteria.

According to Cassells (1991), it is more difficult to interpret the results with respect to bacterial contamination, since most often it is impossible to distinguish between endogenous and exogenous bacteria. In this study, in the case of suckers' infection by bacteria, it was impossible to determine whether the



cause of the infection was due to endogenous bacteria that had already present in the suckers even before being cultured or whether it was due to exogenous bacteria that might invade the suckers' *in vitro*.

When plant tissues are exposed to stress situations such as mechanical injury, which might happen after the isolation of an explant from the stock plant, metabolism of phenolic compounds is stimulated. This intervention leads to hypersensitivity reactions such as the release of the content of broken cells, reactions in the neighboring cells but without showing symptoms of injury themselves, and/or to the premature death of specific cells in the environment of the wound or the place of infection. It was observed that the formation of phenolic compound was less in the explants of suckers compared to that of male buds. Insufficient heating of excision tools can also result in contaminated cultures. Excision, like other actions that cause wounding, initiates the production of polyphenol oxidases which cause browning of the tissue (Vaughn and Duke, 1984; Marks and Simpson, 1990).

The production of phenolic compounds was clearly seen in the culture of male buds. The media turned brown in color and the tissues of the explants were blackened. The growth of the explant became somewhat inhibited or slowed down. This was proven by the observation made on the development of male buds in comparison with that of suckers. After a period of about nine months of culturing, a complete plantlet with an efficient root system and a distinct leaf structure had been obtained from the culture of suckers. For the male buds on the other hand, within the same period of culturing, only a flowery structure or somatic embryos were observed forming on the surface of the explants. About four to five flowery structure formed on the clump of tissues. The formation of phenolic compounds in the culture might contribute to this slow growth or development of explant from male buds.

George and Sherrington (1984) mentioned that the possibility of preventing or reducing the activity of enzymes concerned with both the biosynthesis and oxidation of phenolic, by keeping the cultures in the dark. Lowering the temperature can also reduce their activity. Most of this hypersensitivity reaction of explant was observed in the second stage of micropropagation, during the initiation of culture.

## 5. Conclusions

There are many advantages of micropropagation namely; the production of more uniform plants, produced plants often grow faster and show improved vigor, and plants tend to mature earlier than when propagated by seed. Banana cultivation and production are threatened by many pests and diseases. By using tissue culture, this problem can be reduced

since production of disease-free plantlets can be achieved.

Ornamental banana is a new attractant in Malaysia. Planting material of ornamental plants is in great demand for commercial production as well as for domestic gardens and landscaping. So, if the planting material for ornamental banana can be produced in high amount through micropropagation, a lot of profits can be obtained.

Ornamental plants are produced mainly for their aesthetic value, thus the propagation and improvement of quality attributes such as leaf types, flower color, longevity and form, plant shape and architecture, and the creation of novel variation are important in terms of economic goals for floriculturists. Successful *in vitro* propagation of ornamental plants is now being used for commercialization.

Tissue culture population of *Musa beccarii* can be developed using suckers and male buds as explants. Multiple shoots of *Musa beccarii* were obtained. The development or growth of explant from suckers was faster compared to male buds. Within the same period of culturing, a complete plantlet can be obtained from the culture of suckers but only somatic embryos were obtained in the culture of male buds. Different sterilization technique needs to be used in culturing male buds and suckers since both explants have different degree of contamination rates because of the difference in size and background. Different types of micropropagation process can be observed in male buds and suckers. The male buds undergo direct somatic embryogenesis while the suckers were derived from the organogenesis pathway. Only two sub-cultures were done on the suckers and once on male buds of *Musa beccarii*. From observation, it can be concluded that suckers were more prone to contamination than male buds. The formation of phenolic compound on the other hand, was more visible in male buds.

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## Extent of Injury of Gastrointestinal tract due to accidental ingestion of chemicals among children at Bandar Abbass Children Hospital 2009-2011

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**Abstract:** Accidental Ingestion of burning material could lead to serious injury in different part of the gastrointestinal tract, mostly esophagus as it has the most contact with the swallowed material. The best diagnostic method is Endoscopy and Esophagoscopy. This study intended to assess the severity and extent of burning, its chronic complications and recommend appropriate preventive method of such catastrophe, occurring mostly in children 1-16 years of age. This cross-sectional study was carried out between March 2009 and February 2011, in which 78 cases of children who had accidentally ingested burning material and undergone therapy were under research. The type of ingestion material, the extent of esophagus injury, number of chest and abdomen radiographies, extent of exposure to X-ray, the applied therapeutic method and the long term complications were recorded and then assessed. 37.2% of the 78 children were girls, 62.8% boys 71.8% urban, 28.2% rural. 47 cases of children were poisoned with stronger materials and 29% with higher ones. Clinical signs included vomiting, oral irritation, bloody discharge, abdominal pain, coughing, loss of consciousness and respiratory distress respectively. 45% of the cases had normal degree of burning, 20% G1 burning, 12.5% G2a burning, 1/2% G2b burning, 6.2% G3a and 7.5% G3a burning. 11 children undergone surgery, 7 were diagnosed with esophagostenosis in a year follow-up and the extent of exposure to X-ray for each chest and abdomen graphy was 1/. This study concluded that accidental ingestion of chemical detergent by children lead to esophagus injury with different degree and extents. The complications are not limited to a period of time and might be life-long. Preventing would be a comprehensive solution in this regard which can be emphasized by ministry of health, media, manufacturers and sellers of chemical detergents be supplied in secure and tightly-capped containers with advise tags for families. Comical material must be sold in specific shop under supervision and limitation for stronger detergents.

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**Keywords:** Endoscopy, Esophagoscopy, Burning material, Accidental ingestion, Esophagostenosis, X-ray

### 1. Introduction

The chemical ingestion by children include acidic and alkaline material such as detergent, all type of pipe openers, paint solvent surface cleansers, bleach and dishwasher liquid. Esophagus is the most vulnerable part of digestive tract which is injured following accidental ingestion of chemicals due to early and lasting contact with the swallowed materials. Its subtle mucosa and thin wall along with the lack of strong Immune mechanisms make it even more

susceptible to get injured (1-4). Esophagus injuries due to the alkaline ingestion are more common in southern urban areas of Iran where air conditioner are cleaned by such material. Once these chemicals are ingested accidentally by children, esophagostenosis is developed which is not treated easily. Dilatation using stent is the fundamental therapy for stenosis (5-9).

Incidence of esophagus injury due to the ingestion of chemicals is 15.8 in 10000(10). During the acute phase of poisoning by chemicals there exists

the risk of bleeding esophagus perforation and respiratory distress (11-12). Long-term complications of ingesting chemicals include stenosis in different part of the alimentary canal, theracho esophageal fistula, shortening of the esophagus, lower sphincter incompetence, dismotility, false intramural diverticulum and SCC (10, 13, and 14). Swallowing stronger material in greater amounts, esp. alkaline which is tasteless and odorless, could cause severe burnings and be fatal (15). Some countries have restricted the density of chemicals in household stuff and their selling as well. Some others have decreased the density to less than 10%, stored in secure standard containers, while in developing countries (such Iran) no restriction laws have been passed in this regard (16,17).

In Iran such chemicals are stored in shiny colorful containers which attract children to attention and are uncapped quite easily (18). Different types of chemicals could lead to deep injuries in esophagus. The solid types are instantly spit out since they cause pain and irritation in mouth, but injure esophagus by sticking to oropharynx and proximal esophagus (17). Normal sodium hydroxide 10% causes the necrosis of mucosal, sub-mucosal and some fibers of the muscle layer in 10 second. 3n or 10.7% solution causes tranmural esophagus (10, 18). Acid causes ophagus perforation less often. Pyloric stenosis might lead to gastric outlet abstraction 3-10 weeks following acid ingestion (10, 19). Clinical manifestations include vomiting, bloody discharge, abdominal pain, diarrhea, oral and reparatory passage irritation and frequent coughing. Some patients might have no symptoms and complaints. Secondary complication of ingesting chemicals includes dysphagia and progressive vomiting which imply stenosis that is sometimes undetectable until a year after ingestion (11, 14, 19, 20, and 21). There are various methods to estimate the complications of chemical ingestion. Taking necessary precautions and the suspected cases of perforation, contrast radiography, contrast and non-contrast CT scan, radio nuclide scan and endoscopy are the diagnostic methods to estimate primary injuries during the acute phase after ingestion (1-3, 23, 24).

The extent of exposure to X-Ray is 0.02 for each chest radiography and 1.00 for each abdominal radiography. The period during which radiation effects remain in body is up to 3 days for chest and 6 days for abdominal (25). This study intended to determine the demographic characteristics of poisoning with chemicals and the effect of such material along with the extent of exposure to X-Ray for every digestive tract radiography in children. The findings could enhance the awareness of medical staff and consumers of such material. Also, it can help governments take

necessary steps regarding restrictions on household detergents.

## 2. Material and Methods

This was a cross- sectional study during which all 16-year-old and younger children who had accidentally ingested chemicals and referred to Bandar Abbess children Hospital between 2009-2011 were under research. The data was collected using a questionnaire which was designed by researcher. The items about the chemical included the type, the form (solid, liquid, crustal), characteristics (acid, alkaline) and burning power (PH from neutral range). The ones about patient included age, sex, place of living. Extent of exposure to X-Ray through radiography, number of visits to hospital and follow ups. The primary symptoms included drooling, dysphagia, respiratory distress, vomiting, digestive bleeding, coughing, hoarseness, abdominal pain and orophageal irritation.

By referring to the patient's records the items were checked. The collected data was analyzed with SPSS software version 16, descriptive statistics (frequency, percentage, mean and standard deviation) and chi-square test. The mucosal lesions of patients who had undergone Endoscopy were categorized according to ZARGE: grade zero normal mucosa, mucosal edema, and hyperemia were considered as G1a, also surface laceration and with membrane bleeding as G2a. Findings from G2a along with deep or G2b sigmoidal ulceration and multiple ulceration were assigned as G3a necrosis and G3b necrosis. Materials divided into the two groups of acid and alkaline based on their effects on digestive tract. Among these pipe opener as a strong kind of alkaline cause the most damage to the alimentary tube while dishwasher liquid, thinner and bleach caused the least damage as weaker chemicals.

Patients with no or slight G1 esophageal lesions were discharged from hospital after having received primary care and being under supervision for several hours. Patients with G2a and more severe cases were hospitalized to be treated. All patients with G2a and G3b got dilation every time they referred to hospital. Statistical findings were determined as relative frequency of multiple variables such as age, sex, type of burning material, form of ingested chemical, the degree of esophagus tissue burning, place of living. Number of referring to hospital, chest and abdominal radiograph. The mean of quantitative variables were determined.

## 3. Results

62.8% of 78 patients were boys and the rest were girls. The mean age was  $3.01 \pm 2.6$ . The youngest one was 2 months and the oldest was 16. 71.8% were citizens and the rest were rural. Pipe opener chemicals

are used to open sinkholes and clean air conditioners. 48 patients (61.5%) were poisoned with opener chemicals (strong alkaline), 18 patients (23.1%) with thinner, 11 patients (14.1%) with bleach and only one (1.3%) with dishwasher liquid (weak acid). 60.3% of the burning materials or chemicals were solid, 37.2% liquids, 2.6% crystal. Vomiting, drooling, oral irritation, abdominal pain, respiratory distress, frequent cough and loss of consciousness were most typical clinical symptoms. 25.6% of patients had not undergone chest and abdominal radiography. 74.4%

had a radiograph for every time they had referred to hospital. Estimating the degree of burning, 46.2% were diagnosed as normal, 25.6% as G1 and 28.2% as G2a and more. 67.4% were hospitalized once, 27.2% between 2-8 times and 3.4% between 13-47 times.

Based on the finding on the table 1, Results of chi-square test indicates a significant relationship between type of burning material and degree of burning ( $P=0.03$ ) in the way that the stronger burning material, the greater the degree of burning.

**Table 1.** Frequency and percent of burning based on the ingested burning material

	Normal	G1	G2a	G2b	G3a	G3b
Pipe opener	12(25%)	14(29.2%)	10(20.8%)	5(10.4%)	1(2.1%)	6(12.5%)
Bleach	9(81.8%)	2(18.2%)	0(0%)	0(0%)	0(0%)	0(0%)
Thinner	14(77.8%)	4(22.2%)	0(0%)	0(0%)	0(0%)	0(0%)
Dish wash liquid	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Chi-square result		$P=0.031$		$df=15$		$X^2=26.78$

#### 4. Discussions

Chemicals which are used as household components are accidentally at children's access due to the parents' inattention. Such detergents toxin to some extent (16, 26). The damage to digestive tract because of accidental chemical ingestion is emergency worldwide (27). Compact detergents cause acidosis, incompetence of nervous system, lung diseases and damage to liver and kidney (26). Chemicals are divided into two major groups: Acid and alkaline. Alkaline penetrates deep tissues and can irritate full thickness of esophagus due to the liquefying necrosis and mixing with cell wall lipid (1,2,27-30). The extent of damage to esophagus depends on the quantity, thickness, exposure to the chemical and secondary infections. The most common chemical ingested by children was alkaline (cleanser) in this study and other studies (32-34). Esophagoendoscopy that is done during the first 48 hours is very important in identifying the degree of damage to mucosa and treatment protocol (35-37). Similar studies were carried out in Turkey and Egypt which detect that the poisoning was more frequent in boys than girls and they were mostly 4 years of age (38-39). Statistical numbers of poisoning which chemical ingestion in developed countries are lower than developing countries (40). In an attempt to identify the degree of burning, 46.2% of patients had normal esophagus while 53.8% had abnormal results from endoscopy (G3b, G3a G2b). In similar study in U.S.A 82% of patients had normal or G1 burning, 18% G2 and no cases of G3 were reported. The study results are significantly lower than that of this study. Having no restriction or laws for manufacturing and selling strong household chemicals and detergents in developing

countries could account for such a difference in findings of these two studies. Most of the patients, who developed esophagostenosis in this study, had digested strong alkaline (pipe opener). Identifying the extent of damage due to direct contact with chemicals along with exposure to X-Ray for every visit at hospital was under research in this study. Patients who referred to hospital frequently due esophagostenosis were more probable to develop chest and abdominal consortium because of too much exposure to X-Ray. Clinical manifestations of damages due to accidental ingestion of chemicals have a significant role in predicting the range and depth of esophageal injury (32, 41-43). While other studies recommend endoscopy only for patients with clinical symptom

It seem easy prevent ingestion burning and keep children more safe. Health ministry should give explicit direction to keep burning material out of rich of children, for family, factories, health care personnel and vender. There also some law should be added to constitution, by parliament to force producer moderate and modify PH, concentration and so provide sufficient information on production label. House and its furniture and air conditioner also should be cleaned by specific companies, as limitation of powerful detergent purchasing and usage. Broadcast corporations (TV, radio and magazine) could play essential role to inform people how to contract against toxicity. Performing above-mentioned would help to decrease whether governmental or personal recompense due to the burning materials.

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## ***In Vitro* Shoot Regeneration from Stem Internodes of *Polygonum tinctorium***

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**Abstract:** An efficient and simple protocol for shoot organogenesis and plant regeneration from the stem internodes of *Polygonum tinctorium* was developed. The stem internodes were cultured on media containing different concentrations of cytokinins. Thereafter, the optimum concentration of cytokinin was selected and supplemented with various concentrations of auxins, to determine the potential regeneration capacity and shoot growth from excised stem internodes. Of the cytokinins, 6-benzylaminopurine exhibited the highest efficiency for shoot initiation, followed by zeatin, kinetin, and 1-phenyl-3-(1, 2, 3-thiadiazol-5-yl)-urea. In this study, 2.0 mg/L of 6-benzylaminopurine was highly effective for shoot initiation, in terms of regeneration efficiency, shoot number, and shoot length. Among the cytokinin/auxin combinations, 2.0 mg/L of 6-benzylaminopurine with 0.5 mg/L indole-3-butyric acid provided the optimal conditions for yielding the highest number of shoots (5.6) per explant, greatest shoot length (19.8 mm), and the highest regeneration rate (98%). The protocol developed here is potentially useful for studying the *in vitro* organogenesis of *P. tinctorium*.

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**Keywords:** auxin, cytokinin, *in vitro*, stem internode, *Polygonum tinctorium*

### **1. Introduction**

*Polygonum tinctorium*, which is commonly known as subtropical indigo, is an herbaceous annual plant belonging to the family Polygonaceae (Angelini et al., 2004). The indigo plant has been regularly used as an important source of dye, due to the beautiful blue color that it produces (Jang et al., 2012; Gilbert et al., 2004). The indigo plant has various pharmacological and medicinal properties, exhibiting antianaphylactic, antibacterial, antiviral, anti-inflammatory, cytotoxic, and apoptotic actions (Kim et al., 1998; Ishihara et al., 2000; Kataoka et al., 2001; Zhong et al., 2005). Moreover, anti-tumor and anti-oxidative activities have been identified in ethyl acetate extracts of this plant. According to observations, the anti-tumor and anti-leukemia activities are attributable to tryptanthrin, whereas the anti-oxidative activity has been attributed to gallic acid and caffeic acid, which are also present in ethyl extracts of the indigo plant (Kimoto et al., 2001; Koya-Miyata et al., 2001; Honda et al., 1980).

It is possible to propagate indigo plants from seeds in the natural environment. However, this method requires time, with scarification (i.e., breaching the seed coat by mechanical, thermal, or microbial techniques) sometimes being necessary because of the plant's hard seed coat. Therefore, *in vitro* propagation and regeneration has been developed for the effective use of *in vitro* grown plants that are used for many purposes in molecular biology. Plant tissue culture is the *in vitro* culture of plant cells,

tissues, and organs, and this process may regenerate the whole plant in the culture medium. This technique serves an important tool for both basic and applied studies, as well as in the commercial production of economically important crops (Chandra et al., 2010; Debnath et al., 2006; Thorpe, 2007). The technique also provides an effective means for studying the rapid propagation of plants, because of advantages such as a large propagation coefficient, short reproductive cycle, and year-round production and supply (Nge et al., 2006). An efficient technique for the regeneration of plant parts via tissue culture is therefore critical for multiple micropagation (i.e., rapidly multiplying stock) and genetic transformation. In this study, we developed an efficient method for the regeneration of *P. tinctorium* plants by using different quantities and combinations of plant hormones.

### **2. Experimental Procedures**

#### **Seed Sterilization and Germination**

Seeds of *P. tinctorium* were procured from the experimental farm of Chungnam National University (Daejeon, Korea). The seeds were disinfected with 70% (v/v) ethanol for 30 s and 4.5% (v/v) sodium hypochlorite aqueous solution, together with a few drops of Tween 20 for 15 min. Subsequently, the seeds were thoroughly rinsed with sterilized distilled water under aseptic conditions, and then cultured on a solid basal medium for germination. The basal medium consisted of Murashige and Skoog (MS, Murashige and Skoog, 1962) salt and vitamins

supplemented with 3% sucrose, and solidified with 0.7% (w/v) plant agar. The medium was adjusted to pH 5.8 before adding the plant agar, and was then sterilized by autoclaving at 121°C for 20 min. Ten seeds were placed on each Petri dish, and these were placed in a growth chamber at 25 ± 1°C under standard cool white fluorescent tubes with a flux rate of 35  $\mu\text{mol s}^{-1} \text{m}^{-2}$  and 16-h photoperiod. The seeds germinated within 1 week of culture, and the seedlings were then transferred to a Magenta box containing the same MS solid medium, and cultured for 3 weeks for the establishment of plant material.

#### **In Vitro Plant Regeneration**

Stem segments of *P. tinctorium*, approximately 2.0 cm in length, and each including one internode, were aseptically cut from plants grown *in vitro*. The explants were cultured on 50 mL of the respective medium in a Magenta box. For shoot regeneration from stem internodes, the MS medium was supplemented with 0, 0.1, 0.5, 1, 2, or 4 mg/L BAP (6-benzylaminopurine), kinetin ( $\text{N}^6$ -furfuryladenine), TDZ (1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea; thidiazuron), and zeatin. To improve shoot regeneration, the medium was optimized by testing the effect of different concentrations of 0, 0.1, 0.5, and 1.0 mg/L IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), and NAA ( $\alpha$ -naphthalene acetic acid) in combination with 2 mg/L of BAP on shoot formation and growth. Plant hormones were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). Cultures were maintained at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35  $\mu\text{mol s}^{-1} \text{m}^{-2}$ ) for 5 weeks.

#### **Rooting of Regenerated Shoots**

Regenerated shoots (~2.0 cm long) were placed in MS medium for root induction. The medium was solidified with 8 g/L of plant agar and dispensed at 50 mL per culture vessel, with 4 shoots being cultured in each culture vessel. Regenerated shoots were incubated at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35  $\mu\text{mol s}^{-1} \text{m}^{-2}$ ) for 4 weeks. After 4 weeks of culture, the rooted plants were washed with sterile water to remove plant agar, transferred to pots containing autoclaved vermiculite, and covered with polyethylene bags for 1 week to maintain high humidity. The plants were then transferred to soil, and maintained in a growth chamber with a 16-h photoperiod, and a night/day temperature of 18–20°C for 2 weeks. These hardened plants were then transferred to the greenhouse for further use.

#### **Statistical Analysis**

The experimental data derived from the 50 tested stem internodes were expressed as the mean ± standard deviation.

### **3. Results and Discussion**

#### **Effect of Cytokinins on Shoot Growth**

The rapid development of *in vitro* shoot regeneration from the stem internodes of *P. tinctorium* was investigated. The efficiency of shoot regeneration, shoot number, and shoot length were determined by using MS basal medium supplemented with different concentrations of cytokinins. Shoot development was successful under all cytokinin regimes. Increased shoot regeneration was observed with increasing concentrations of BAP, kinetin, and zeatin from 0.1 to 2.0 mg/L for all measured parameters. However, shoot regeneration decreased significantly when these cytokinin concentrations were raised to 4 mg/L (Table 1). The trend for TDZ was slightly different compared to the other cytokinin groups. This hormone produced a gradual increase in shoot organogenesis up to 1.0 mg/L of TDZ concentration, and decreased beyond this. At a concentration of 2.0 mg/L, BAP produced the highest frequency of shoot formation (96%), the maximum number of adventitious shoots (5.2) per explant, and the longest shoot lengths (18.8 mm), compared to all other concentrations of BAP and compared to the other cytokinins used in this study.

The plant hormone, BAP, has also been found to be the most effective hormone for shoot organogenesis in other plants, such as *Nicotiana glauca* (Marcotrigiano, 1986) and *Cucumis melo* (Marta and Andrés, 2009). Eckardt (2003) reported that cytokinin deficiency causes a major decline in shoot development, consequently leading to dwarfism, late flowering plants, enhanced root growth, and changes in reproductive development. Furthermore, cytokinins are required in plant tissues to maintain both cell division and leaf primordium formation. Therefore, cytokinins are considered important hormones for plant growth.

#### **Shoot Promotion by Auxins in Combination with Cytokinin**

Previously cultured *P. tinctorium* stem internodes were grown for 5 weeks on MS basal medium containing 2.0 mg/L BAP supplemented with various concentrations (0, 0.1, 0.5, and 1.0 mg/L) of different auxins (IAA, IBA, and NAA) to evaluate shoot regeneration and multiplication of stem internodes. Among the treatments, 0.5 mg/L IAA and 0.5 mg/L IBA in combination with cytokinin (BAP 2.0 mg/L) produced more shoots from the explant compared to the control (cytokinin alone). At all concentrations of auxins, NAA exhibited a negative response with the cytokinin combination (Table 2). As NAA concentration increased from 0.1 to 1.0 mg/L, it caused a decreasing trend in shoot growth. Moreover, the lowest regeneration efficiency, shoot number (3.6 shoots per explant), and shortest shoot length (13.2 mm) were observed at 1.0 mg/L NAA compared to all other auxin treatments.

Table 1. Effect of different concentrations of cytokinins on the shoot regeneration of stem internode explant cultures of *Polygonum tinctorium* after being grown for 5 weeks on MS medium

Cytokinins (mg/L)	Regeneration (%)	Shoot no. per explant	Shoot length (mm)
BAP	0.1	72	2.4 ± 0.1
	0.5	84	4.2 ± 0.3
	1	94	4.8 ± 0.3
	2	96	5.2 ± 0.2
	4	81	2.4 ± 0.1
Kinetin	0.1	74	2.0 ± 0.2
	0.5	79	3.6 ± 0.1
	1	84	4.0 ± 0.2
	2	89	4.2 ± 0.3
	4	85	2.0 ± 0.2
TDZ	0.1	68	1.8 ± 0.1
	0.5	75	2.6 ± 0.1
	1	89	2.8 ± 0.3
	2	75	2.4 ± 0.1
	4	53	1.6 ± 0.1
Zeatin	0.1	76	1.4 ± 0.1
	0.5	85	2.4 ± 0.1
	1	88	3.4 ± 0.3
	2	92	4.6 ± 0.1
	4	79	3.6 ± 0.1

Table 2. Effect of different concentrations of auxins on the shoot regeneration of stem internode explant cultures of *Polygonum tinctorium* after being grown for 5 weeks on shoot regeneration medium (MS medium with 2 mg/L BAP)

Auxins (mg/L)	Regeneration (%)	Shoot no. per explant	Shoot length (mm)
Control	96	5.2 ± 0.2	18.8 ± 0.08
IAA	0.1	95	5.0 ± 0.4
	0.5	96	5.4 ± 0.3
	1	97	5.0 ± 0.1
IBA	0.1	97	4.8 ± 0.2
	0.5	98	5.6 ± 0.1
	1	96	5.2 ± 0.1
NAA	0.1	95	5.2 ± 0.2
	0.5	93	5.0 ± 0.1
	1	86	3.6 ± 0.1

Therefore, NAA did not enhance shoot growth in this study. Overall, the highest number of shoots (5.6 shoots per explant), longest shoot length (19.8 mm), and the best regeneration efficiency (98%) were obtained from 0.5 mg/L IBA in combination with 2.0 mg/L BAP.

In this study, BAP and IBA played an important role in the shoot regeneration of *P. tinctorium*. It is well known that cytokinins stimulate plant cell division and contribute to the release of lateral bud dormancy, induction of adventitious bud formation, growth of lateral buds, and cell cycle control. In comparison, auxins exert a strong influence on the initiation of cell division, meristem organization giving rise to un-organized tissue (callus) or defined organs (shoots), cell expansion, cell wall acidification, apical dominance, promotion of vascular differentiation, and root formation (Gaspar et al.,

1996). Therefore, manipulation of the exogenous cytokinin:auxin balance might influence the pattern of plant development, thus facilitating the development of an organogenic program (Gaspar et al., 2003).

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## A Study on the Relation between Yield and Some Maize Genotypes Traits in the Presence of Humic Liquid Fertilizer Based on Peat

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**Abstract:** In order to study the response of maize genotypes against the application of peat based liquid humic fertilizer. The Experiment was conducted as Split Plot in the form of randomized complete block design with three replications. The main factor included two conditions (with the application of humic fertilizer; without humic fertilizer) and the sub factor included 6 maize genotypes. Considering the compound ANOVA results in studied traits, it was observed that there is a significant difference between all traits at probability level of 1% in experimental conditions. Results indicate that among studied genotypes Golden West with a mean of 19.80 ton per hectare had the highest biological yield and OS 499 genotype with a mean of 14.56 ton per hectare had the lowest biological yield. According to data, it can be suggested that applying humic fertilizer based on peat had good effects on studied traits.

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**Keyword:** Maize, Humic, Correlation, Yield

### Introduction:

Maize (*Zea mays* L.) is C<sub>4</sub> plant in terms of photosynthesis and has a better growth in tropical and subtropical (Emam, 2008) areas and native regions of South and Central America (Khodabandeh, 1998). The maize role in providing seeds, forage and livestock feed and its industrial use has increased the importance of the product in Iran. Hence, by implementing programs to increase maize seed production during recent years, this crop has quickly grown in cultivation, production and performance (Cakir, 2004).

Humic substances (HS) are the components of organic decomposition and they are the natural organic compounds which comprise 50 to 90 % of the organic matter of peat, lignites and sapropels, as well as of the non-living organic matter of soil and water ecosystems. These substances are the source of the humates used in agriculture. According to the classical definition, HS are "a general category of naturally occurring heterogeneous organic substances that can generally be characterized as being yellow to black color with high molecular weight and they are refractory" (Kulikova et al. 2005). Yang et al (2004) has stated that the humic materials can affect physiological processes of plant

growth directly and indirectly. Their direct effects include increase in cell membrane permeability, respiration, nucleic acid biosynthesis, ion uptake, enzyme activity and sub-enzyme activity. The biological activity of HS encompasses all activities of HS in regulating plant biochemical and physiological processes, irrespective of their stimulatory or inhibitory roles. Soil organic matter is one of the important indices of soil fertility, since it interacts with many other components of the soil. Soil organic matter is a key component of land ecosystems and it is associated with the basic ecosystem processes for yield and structure (Pizzeghello et al, 2001). In spite of numerous studies on the biological effects of HS, the mechanism of their action remains unclear<sup>4</sup>. However, farmers use humates to accelerate seed germination and improve rhizome growth. These materials are able to stimulate oxygen transport, accelerate respiration and promote efficient utilization of nutrient by plants (Islam et al. 2005). Nevertheless, humic acid in proper concentrations can enhance plant and root growth (Bacilio et al. 2003). Presence of HS is important during all stages of plants' development but particularly vital in the early stages. That is why the pre-planting



treatment of seeds is very important. Even before germination begins, vital forces are awakened, and the immune system is stimulated (Levinsky, 2009). Numerous researches have demonstrated conclusively that HS have significant impacts on the soil structure and plant growth (Fong et al. 2007).

A proper method for identifying effective traits on yield is determination of the simple correlation between those traits and yield. Traits which are not in a significant relation with yield do not have practical application in modification programs (Wallace et al. 1972).

Correlation analysis provides the information of interrelationship of important plant characters and also leads to a directional model for direct and/or indirect improvement in grain yield. Although direct selection for various parameters could be misleading, indirect selection via related parameters with high heritability might be more effective than direct selection (Shahryari and Mollasadeghi, 2011).

Shahryari (2010) expressed that a biometric procedure such as Path analysis leads us to understanding of the genetic association of traits and their contribution to yield. Comparison of path coefficients in two different conditions of that study revealed there were more complex relations between characters at presence of potassium humate. And cumulative effects (significantly direct and indirect effects) of traits caused increase in yield.

The following research tries to compare the humic liquid fertilizer based on peat effects on biological yield and some yield components in six maize genotypes in Ardabil region.

#### **Materials and Method:**

In order to study the response of maize genotypes against the application of peat base liquid humic fertilizer (Table 1), an experiment was conducted at experimental field of Islamic Azad University, Ardabil

Branch (5 km west of Ardabil City). The Region has a semi-arid and cold climate, where the temperature during winter season usually drops below zero. This region is located 1350 m above the sea level with longitude and latitude being 48.2° eastern and 38.15° northern, respectively. Average annual minimum and maximum temperatures are -1.98 and 15.18°C respectively; whereas maximum absolute temperature is 21.8 °C; and mean annual precipitation has been reported to be 310.9 mm. The soil of the field was alluvial clay with a pH ranging from 7.8-8.2.

Vegetative material included 6 maize genotypes prepared from Agriculture and Natural Resources Research Center of Ardabil Province. The Experiment was conducted as Split Plot in the form of randomized complete block design with three replications. The main factor included two conditions (by application of humic fertilizer; without humic fertilizer) and the sub factor included 6 maize genotypes (ZP677, Golden west, OS499, ZP434, Ns540 and Single Cross 704). Each experimental plot included 3, 320 cm long rows recurring 80 cm from each other containing plants recurring at 20cm distance. Pretreatment of seeds were done on the basis of 220 mL per 10 L of water to be applied for 1 ton of seeds.

Weed-fighting was done both mechanically and manually during all growth stages. Liquid humic fertilizer was prepared and applied based on 400 mL per 50 L of water for 1 hectare of maize plantation. The prepared solution was sprayed upon the aerial part of the plants during 4-5<sup>th</sup> leaf stage, appearance of reproductive organs, flowering and grain filling stages. All the samples were taken randomly from competitive plants at middle rows.

Studied traits included Length of ear at the harvesting time, number of grains per ear, grain weight per ear, grain weight, ear dry weight and biological yield.

Analysis of variance of data and mean comparison of them was done using MSTATC, SPSS and Minitab programs. Mean comparison was done using Duncan's Multiple Range Test, at 5% probability level.

**Table 1.** Compounds of liquid humic fertilizer based on peat

Parameter name	Dry residue (%)	pH	Total nitrogen (mg/l)	Phosphorus (mg/l)	Potassium (mg/l)	Nitrate nitrogen (mg/l)
amounts	56.304	10.08	1318	97	10845	78.81
Parameter name	Total carbon (g/l)	Humic acids carbon (g/l)	Humic acids (g/l)	Fulvic acids carbon (g/l)	Fulvic acids (g/l)	Sum of humic and fulvic acids (g/l)
amounts	21.69	17.68	33.23	4.01	9.02	42.25

### Results and Discussion

Considering the compound ANOVA results (Table 1) in studied traits, it was observed that there is a significant difference between all traits at probability level of 1% in experimental conditions. Also, there was a significant difference between studied genotypes based on all evaluated traits at probability level of 1%. This indicates that the genetic diversity between genotypes to choose the desired traits. Also genotype effect  $\times$  experimental conditions in biologic yield terms were significant at probability level of 5% and other traits were significant at probability level of 1%. According to Shahryari et al(2009) this means that under study genotypes had the same responses to potassium humate.

According to data mean, which compare (Table 2) the studied genotypes, NS 540 genotype with a mean of 21.12 cm had the highest ear at the harvesting time while OS 499 genotype with a mean of 18.57 cm had the lowest length of ear. ZP 677 and Single Cross 704 genotypes formed a group and showed no differences in the studied traits. Based on number of grains in ear, ZP 677 genotype with a mean of 420.50 was the highest and OS 499 with a mean of 303 was the lowest. Golden West and NS 540 genotypes formed one group and showed no differences in the studied traits. ZP 677 genotype with a mean of 48.97gr had the highest grain weight per ear and NS 540 genotype with a mean of 34.57 gr had the lowest weight per ear. OS 499, ZP 434, Golden West, Single Cross 704 and NS 540 genotypes formed a group and showed no differences in the studied grain weight per ear. Based on grain weight among the studied genotypes, ZP 434 with a mean of 159.41 gr was the best genotype and Single Cross 704 genotype with a mean of 101.65 gr was the lowest studied genotype. ZP 677 ad Golden West and also OS 499 and NS 540 genotypes formed a groups and showed no differences in the studied traits. Based on ear dry weigh, ZP 677 with a mean of 132.48 gr was the best genotype and OS 499 genotype with a mean of

92.97gr was the lightest. ZP 434, Single Cross 704 and NS 540 genotypes formed a group and showed no differences in the studied traits. Golden West with a mean of 19.80 ton per hectare had the highest biological yield and OS 499 genotype with a mean of 14.56 ton per hectare had the lowest biological yield. ZP 434, Single Cross 704 and NS 540genotypes formed a group and showed no differences in the studied traits.

Shahryari and Shamsi (2009) reported that potassium humate increased the rate of biological yield of wheat from 3.26 to 3.72 gr/plant; but it had not effect on harvest index. Also they found that uses of potassium humate increased grain yield. Results mean comparison (Table 1) in experimental conditions suggested that applying humic fertilizer based on peat had good effects on studied traits.

Ayas and Gulser (2005) reported that humic acid caused to increase growth and height and subsequently increase biological yield through increasing nitrogen content of the plant. It has been reported that application of humic acid in nutritional solution led to increased content of nitrogen within aerial parts and growth of shoots and root of maize(Tan, 2003). In another investigation, the application of humic acid led to increased phosphorus and nitrogen content of bent grass plant and increased the accumulation of dry materials(Mackowiak et al. 2001). Humic acid leads to increased plant yield through positive physiological effects such as impact on metabolism of plant cells and, increasing the concentration of leaf chlorophyll(Naderi et al. 2002). Also, spraying humic acid on wheat crop increased its yield by 24%(Delfine et al. 2002). Simple correlation coefficients between various traits without humic fertilizer application are presented in Table 3. In these conditions, the relation between dry ear weight and Length of ear at the harvesting time was positively significant at probability level of 1% ( $r=0.923$ ) and the relation between dry ear weight and number of grains in ear was positive significant at

probability level of 5% ( $r=0.816$ ). Other traits correlation coefficients are presented in Table 3.

Shahryari and Mollasadeghi(2011) reported that all possible correlations were worked out to determine the relationship between harvest index with economic yield and biological yield separately in four studied experimental conditions.

Correlation between economic and biological yield was positively significant for all of four irrigation and humate levels. Thus, there is a linear relationship between these traits. Also, there was positively significant linear relationship between economic yield and harvest index for well-watered and drought stress+humic fertilizer levels while no correlation was observed among them in the other two conditions.

Correlation of biological yield and harvest index was negatively significant for well watered+humic fertilizer. But relationships between these characters did not have a linear correlation for other conditions.

Simple correlation coefficients between various traits with humic fertilizer application are presented in Table 4. In these conditions, the relation between dry ear weight and Length of ear at the harvesting time and number of grains in ear was positive significant at probability level of 5%. Also, the relation between grains weight in ear and number of grains in ear was positive significant at probability level of 5% ( $r=0.844$ ).

Shahryari (2010) reported that seed weight per spike had the most effect on yield increase with direct effect ( $r = 0.576$ ) at presence of potassium humate. After that spike length ( $r = 0.337$ ), biomass ( $r = 0.254$ ) and seed number per spike ( $r = 0.175$ ) had total correlation effects on increasing of grain yield.

### Conclusion

Results of this experiment indicated that the application of liquid humic fertilizer can positively affect the maize biological yield and some agronomic traits related to it. These desirable effects can be a consequence of its effect on the physiology of the maize. In general, application of humic acid can lessen the need for using chemical fertilizers and subsequently reduce environmental pollution, and it is also cost effective since the desired target is achieved by using less amount of it. Finally, it can be suggested that application of humic fertilizer not only increases the yield of maize, but also can play a significant role in achieving the goals of sustainable agriculture.

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**Table 1.** Combined Analysis of variance of evaluated traits under various experimental conditions for 6 maize genotypes.

Source of Variations	df	Mean Square						
		Length of ear at the harvesting time	Number of grains per ear	Grain weight per ear	Grain weight	Ear dry weight	Biological yield	
Replication	2	2.70	90.58	2.18	143.09	32.88	20.98	
Experimental conditions (E.C.)	1	95.58**	156025.00**	2086.21**	24666.79**	14733.11**	318.03**	
Error 1	2	0.96	22.75	4.38	3.21	5.91	3.75	
Genotype (G)	5	5.17**	10089.60**	172.74**	2407.44**	1114.95**	23.27**	
G × E. C.	5	6.73**	10192.33**	112.58**	1336.44**	725.04**	14.02*	
Error 2	20	0.76	956.13	21.18	30.97	63.37	5.09	
CV (%)		4.38	8.59	11.97	4.53	7.04	12.74	

\* and \*\*: Significant at  $p < 0.05$  and  $< 0.01$ , respectively

**Table 2.** Mean comparison of Studied traits of maize genotypes

Genotypes	Characters					
	Length of ear at the harvesting time(cm)	Number of grains per ear	Grain weight per ear(gr)	Grain weight(gr)	Ear dry weight(gr)	Biological yield (ton/h)
<b>OS 499</b>	18.57 d	303.00 d	35.26 b	112.60 c	92.97 d	14.56 c
<b>ZP 677</b>	20.34 ab	420.50 a	48.97 a	123.95 b	132.48 a	16.34 bc
<b>Golden West</b>	19.00 cd	359.50 bc	38.77 b	126.82 b	102.69 c	19.80 a
<b>ZP 434</b>	19.93 bc	334.50 cd	36.15 b	159.41 a	115.75 b	18.98 ab
<b>Single Cross 704</b>	20.17 ab	388.50 ab	36.83 b	101.65 d	117.54 b	18.97 ab
<b>NS 540</b>	21.12 a	354.00 bc	34.57 b	112.95 c	116.76 b	17.63 ab

Differences between averages of each column which have common characters are not significant at probability level of 5%.

**Table 3.** Simple correlation coefficients between traits for maize genotypes at absence of humic fertilizer

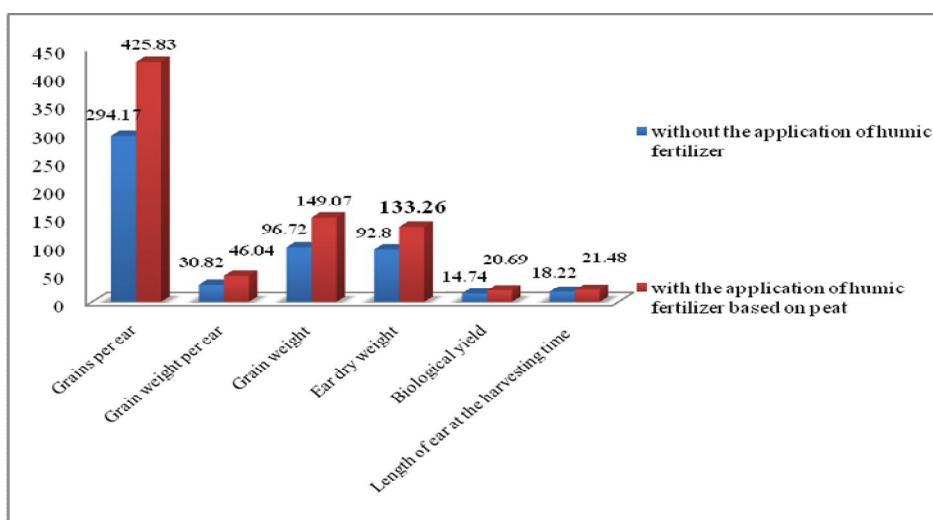
	Length of ear at the harvesting time	Number of grains per ear	Grain weight per ear	Grain weight	Ear dry weight	Biological yield
Length of ear at the harvesting time	1					
Number of grains per ear	0.666	1				
Grain weight per ear	-0.038	0.692	1			
Grain weight	-0.757	-0.350	0.336	1		
Eardry weight	0.481	0.923**	0.816*	-0.027	1	
Biological yield	0.578	0.578	0.102	-0.665	0.239	1

\* and \*\*: Significant at  $p < 0.05$  and  $< 0.01$ , respectively

**Table 4.** Simple correlation coefficients between traits for maize genotypes at presence of humic fertilizer

	Length of ear at the harvesting time	Number of grains per ear	Grain weight per ear	Grain weight	Ear dry weight	Biological yield
Length of ear at the harvesting time	1					
Number of grains per ear	0.726	1				
Grain weight per ear	0.521	0.844*	1			
Grain weight	-0.003	-0.403	-0.369	1		
Ear dry weight	0.915*	0.867*	0.704	-0.084	1	
Biological yield	0.034	0.031	-0.355	0.499	0.101	1

\* and \*\*: Significant at  $p < 0.05$  and  $< 0.01$ , respectively



**Figure 1.** Effect of the usage of humic fertilizer derived from peat on measured traits of maize genotypes

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## Total Parenteral Nutrition, Metabolic Consequences, Liver Complication and Role of Some Natural Extracts

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**Abstract:** Liver is negatively affected by using of total parenteral nutrition (T) for long time. It has most recently been found that T is associated with recognizable loss of body weight when diuretic drug (TD) is used to minimize water retention. This has been maximally resolved when green tea was added to parsley extract (TPG). A negative role for T administrations on liver health statues included a remarkable histopathological subclinical disorder such as local monocular inflammatory and vacuolations for application of T alone in addition to dissociation hemolysed RBCs, deposition of golden brown hemosidrine pigment, necrosis of hepatocytes with psychosis of the nuclei and necrosis of sporadic were recorded when TD used. These hepatic cells were partially or even totally recovered upon the intervention with natural extract. This additional extract was also relatively stronger in controlling blood sugar, blood cholesterol, Fe level in serum, liver enzymes, WBCs as well as RBC and kidney function whose reversely affected by T administration alone. In fact, this internal metabolic homeostatic role powered by the natural mixture of extract is an added bioingredient value could be attributed to the complementary action of a unique combination of polyphenols, flavones and vitamins provided by this mixture. In essence, the highly mutual biological intervention appeared for parsley is of important medical role, but the presence of tea solution has magnified its curing effect. Yet, it may concluded that T application cannot be improved using lasix except for water retention, but health complications, especially those of liver, can be avoided by an especial mixture of the natural preparation of parsley and green tea since, tea + parsley extract was an excellent dietary therapy recorded here. Further biological evaluation for T complication recorded for other organs using its effect on their histopathology will appear soon.

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**Keywords:** Total parenteral nutrition, parsley, green tea, polyphenols.

### 1. Introduction

The concept of feeding patients entirely parenterally by injecting nutrient substances or fluids intravenously was advocated and attempted long before the successful practical development of total parenteral nutrition (T) four decades ago. Realization of this 400 year old seemingly fanciful dream initially required centuries of fundamental investigation coupled with basic technological advances and judicious clinical applications (Dudrick, 2009). Most clinicians in the 1950's were aware of the negative impact of starvation on morbidity, mortality, and outcomes, but only few understood the necessity for providing adequate nutritional support to malnourished patients if optimal clinical results were to be achieved. The prevailing dogma in the 1960's was that, "Feeding entirely by vein is impossible; even if it were possible, it would be impractical; and even if it were practical, it would be unaffordable." Major challenges to the development of T included: (1) formulate complete parenteral nutrient solutions (did not exist), (2) concentrate substrate components to 5–6 times isotonicity without precipitation (not easily

done), (3) demonstrate utility and safety of long-term central venous catheterization (not looked upon with favor by the medical hierarchy), (4) demonstrate efficacy and safety of long-term infusion of hypertonic nutrient solutions (contrary to clinical practices at the time), (5) maintain asepsis and antisepsis throughout solution preparation and delivery (required a major culture change), and (6) anticipate, avoid, and correct metabolic imbalances or derangements (a monumental challenge and undertaking). This presentation recounts approaches to, and solution of, some of the daunting problems as really occurred in a comprehensive, concise and candid history of parental nutrition. (Dudrick, 2009). Practically, and in connection of some organ health, the influence of TPN was studied in 67 patients with severe acute pancreatitis having three or more criteria. Although TPN has been reported to not be of benefit in the progress and severity of the disease, it has found that the time T is started is important in influencing the course of the disease and in the development of local complications, as well as in the mortality rate. Patients whose TPN was started within the first 72 hours of the disease had a 23.6%

complication rate and 13% mortality, in comparison with patients whose TPN was started later in the course of the disease, who had a 95.6% complication rate and a mortality rate of 38% (Kalfarentzos et al., 1991). The nutritional status of the patients during T administration of 28.4 days was maintained either steady or was improved, as assessed by nitrogen balance, serum levels of transferrin, and albumin. The administration of fat solution derangement in protein metabolism with the standard T solution in current use suggests that either a modification of amino acid composition or an increase in total energy to protein energy ratio in T solution may be necessary to obviate such a consequence either to prevent essential fatty acid deficiency or to provide part of the caloric requirements, was found to cause neither clinical nor laboratory worsening of the disease. All pancreatic fistulae that developed during the course of the disease spontaneously closed in patients

receiving TPN without operation in a mean period of 33.3 days, and all pseudocysts subsided in an average of 18.3 days. Those who died (overall mortality rate 24%) had had uncontrollable sepsis, which resulted in hypercatabolism and multiple system organ failure (Kalfarentzos et al., 1991). Therefore, to assess the effect of (TPN) on macronutrient metabolism in obstructive jaundice, forty adult mongrel dogs were equally divided into four groups: group I (PO-control) received sham ligation of common bile duct (CBDL) and was fed dog chow and water ad libitum; group II (PO-CBDL) underwent CBDL and was fed dog chow; group III (T-control) received sham CBDL and TPN ; and group IV (T-CBDL) underwent CBDL and received TPN. Blood chemistries, plasma amino acids and liver histologies were studied before (Day 1) and at the end (Day 14) of the experiment.

**Table (1): The chemistry and usage of T (Cited from Hayes et al. 2000).**

Nutrient	Amount
Water (kg body wt/day)	30–40 mL
Energy* (kg body wt/day): <b>Medical patient</b>	30 kcal
<b>Postoperative patient</b>	30–45 kcal
<b>Hypercatabolic patient</b>	45–60 kcal
Amino acids (kg body wt/day): <b>Medical patient</b>	1.0 g
<b>Postoperative patient</b>	2.0 g
<b>Hypercatabolic patient</b>	3.0 g
Minerals: <b>Acetate/gluconate</b>	90 mEq
<b>Calcium</b>	15 mEq
<b>Chloride</b>	130 mEq
<b>Chromium</b>	15 µg
<b>Copper</b>	1.5 mg
<b>Iodine</b>	120 µg
<b>Magnesium</b>	20 mEq
<b>Manganese</b>	2 mg
<b>Phosphorus</b>	300 mg
<b>Potassium</b>	100 mEq
<b>Selenium</b> <span style="border: 1px solid black; padding: 2px;">SOME TRADE NAMES</span>	100 µg
<b>Sodium</b>	100 mEq
<b>Zinc</b>	5 mg
<b>Vitamins : Ascorbic acid</b>	100 mg
<b>Biotin</b>	60 µg
<b>Cobalamin</b>	5 µg
<b>Folate (folic acid)</b>	400 µg
<b>Niacin</b> <span style="border: 1px solid black; padding: 2px;">some trade names niacorniaspanslo-niacin</span>	40 mg
<b>Pantothenic acid</b>	15 mg
<b>Pyridoxine</b>	4 mg
<b>Riboflavin</b>	3.6 mg
<b>Thiamin</b>	3 mg
<b>Vitamin A</b>	4000 IU
<b>Vitamin D</b>	400 IU
<b>Vitamin E</b>	15 mg
<b>Vitamin K</b>	200 µg

\*Requirements for energy increase by 12% per 1° C of fever.

A significant elevation of bilirubin and alkaline phosphatase was observed in dogs with

CBDL. Blood glucose was not changed significantly in any group. Significant increases in triglyceride and

cholesterol were present in CBDL dogs. Significant differences in the concentrations of a few plasma amino acids, including an elevation of phenylalanine, were found in TPN dogs. A significant increase in aromatic amino acids (AAA) and a noticeable depression of the molar ratio of branched-chain amino acids (BCAA) to AAA was present in T-CBDL dogs, as was a significant increase in blood ammonia. In the presence of obstructive jaundice, TPN does not significantly affect carbohydrate or lipid metabolism (Chuang et al., 1995). However, a derangement in protein metabolism with the standard T solution in current use suggests that either a modification of amino acid composition or an increase in total energy to protein energy ratio in T solution may be necessary to obviate such a consequence (Chuang et al., 1995). Amino acids, as

mentioned before, are essential for enterocytes, but the luminal supply is compromised with changes in dietary intake. The importance of maintaining amino acid supply for intestinal mucosal cells is illustrated (Howard et al., 2004). Previously lipid emulsions were given separately but it is becoming more common for a "three-in-one" solution of glucose, proteins, and lipids to be administered (Rollins et al., 1990 and Didier et al., 1998). In most hospitals, clinical pharmacists evaluate the patient's individual data and decide what T formula to be used. The use of standardized T is cost effective and may provide better control of serum electrolytes, e.g. in Tables 1 and 2 (Hayes et al., 2000). Standardized solutions may also differ between developers (Howard et al., 2004).

**Table2. Examples of T solutions (after Hayes et al., 2000)**

Substance	Normal patient	High stress	Fluid-restricted
Amino acids	85 g	128 g	75 g
Dextrose	250 g	350 g	250 g
Lipids	100 g	100 g	50 g
Na <sup>+</sup>	150 mEq	155 mEq	80 mEq
K <sup>+</sup>	80 mEq	80 mEq	40 mEq
Ca <sup>2+</sup>	360 mg	360 mg	180 mg
Mg <sup>2+</sup>	240 mg	240 mg	120 mg
Acetate	72 mEq	226 mEq	134 mEq
Cl <sup>-</sup>	143 mEq	145 mEq	70 mEq
P	310 mg	465 mg	233 mg
MVI-12	10 mL	10 mL	10 mL
Trace elements	5 mL	5 mL	5 mL

Still TPN is an important way of nutrition when no food is given by other injection routes (Kozier et al., 2004) and long-term TPN is occasionally used to treat people suffering the extended consequences of an accident, surgery, or digestive disorder. TPN has extended the life of children born with nonexistent or severely deformed organs. Surgically, the effect of preoperative TPN on morbidity and mortality was studied in medical records of discharged surgical patients. Nutrition parameters measured included serum albumin, total lymphocyte count, hemoglobin, weight, and percent weight loss. Major septic complications (MSC) considered were intra-abdominal sepsis, wound dehiscence, septicemia, and pneumonia (Grimes et al., 1987). Other complications included respiratory failure, congestive heart failure, fistulas, urinary tract infection, shock, and death. The experimental group showed improvements after surgery in the nutritional parameters listed and had a lower incidence of morbidity and mortality. Although TPN is sometimes a must, Ahmed et al., (2009) stated that the TPN by its slandered solution complication on rat's main blood constituents is vital. In connection to liver

health, a recent small-scale study at Children's Hospital Boston on the cause of liver failure suggests it may be due to a large difference in omega-6 to omega-3 ratio and some patients were able to recover from their condition (Gura et al., 2006). Two related complications of TPN are venous thrombosis and rarely priapism TPN, in addition, increases the risk of acute cholecystitis due to complete abusage of gastrointestinal tract, which may result in bile stasis in the gallbladder. Other potential hepatobiliary dysfunctions include steatosis, steatohepatitis, cholestasis, and chole-lithiasis (Quigley et al., 1993). Such complications are suggested to be the main reason for mortality in people requiring long-term T, such as in short bowel syndrome (Vanderhoof and Langnas, 1997). Although TPN was considered to be a dangerous form of therapy, critical review of the data suggests that, in humans, TPN does not cause mucosal atrophy or increase bacterial translocation. Increased sepsis with TPN can be ascribed to overfeeding and the dangers of T-induced complications have been exaggerated. TPN is an equally effective alternative to enteral nutrition (EN) when a risk of malnutrition is present and EN is not

tolerated or when gut failure is present (Jeejeebhoy, 2001). As a background, food in the intestine drives the enterohepatic circulation of bile components. To investigate whether parenteral or enteral delivery of nutrients alters serum and biliary lipids in critically ill patients in an intensive care unit (ICU), patients who had received  $\geq 5$  d of T were compared with those who had fasted for  $\geq 5$  d. Both groups were studied before and after 5 d of EN. Each patient served as his or her own control. Duodenal bile was analyzed for biliary lipid content and serum lipids were determined simultaneously. Duodenal bile samples from 18 healthy persons were served as controls. As a result, bile salt concentrations in all ICU patients were 17% of control values before EN and 34% of control values after 5 d of EN. Phospholipids concentrations were 12% of control before EN, but increased almost 4-fold after EN. Biliary cholesterol concentrations were 20% of control values before EN and did not improve afterward. No difference in bile composition was observed between fasted ICU patients and those who received T. The inverse correlation between the severity of illness and biliary lipid concentrations observed before EN disappeared with enteric stimulation. The low serum concentrations of HDL cholesterol and apolipoprotein A-I increased significantly with EN in all ICU patients. In conclusion, lack of EN during critical illness was associated with profound decrements in biliary lipid concentrations that normalized partially after 5 d of EN. Therefore, it hypothesize that loss of enteric stimulation in ICU patients impairs hepatic lipid metabolism (De Vree *et al.*, 1999). Moreover, Raina *et al.* (2000), in an animals treated with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), noticed developed severe metabolic abnormalities despite receiving sufficient protein and energy by T. It has been sought that it is the nutritional and metabolic effects of bacterial lipopolysaccharide (LPS) in rats. In brief, biochemical abnormalities and plasma corticosterone concentrations were greater in the T+LPS group than in the other groups. These data suggest that provision of sufficient protein and energy by T does not prevent general carcass wasting induced by LPS but may protect individual muscles. However, compared with an oral ad libitum diet, T providing sufficient protein and energy worsens the biochemical abnormalities induced by LPS. More rapid clearance of TNF- $\alpha$  and low corticosterone concentrations in weight-losing animals may help reduce the severity of the metabolic effects of LPS. Earlier, hyperglycemia was noticed as common at the start of therapy, but can be treated with insulin added to the T solution. Most serious of which is liver failure, often related to fatty liver that may sometimes occur. Liver dysfunction can be limited to a

reversible cholestatic jaundice and to fatty infiltration demonstrated by elevated transaminases. Severe hepatic dysfunction is a rare complication (Horattas, *et al.*, 2001). Typical T induced fatty liver tissue is shown in the figure below.

From the nutritional point of view, although T is a nutritional tool to keep someone who is under critical condition alive, there is great need to biologically control its clinical and nutritional complications. As a result, hence T responses on rat's main blood chemistry, organs functions and blood cells were explored to be somewhat biologically remarkable Ahmed *et al.*, (2009), the reverse action on some organs histology is necessary to measure these organs longevity upon T long term application. Interaction of some plant extract rich of polyphenols was assessed. As an example, diets rich in fruits and vegetables (FV) have been associated with a reduced risk of chronic disease, including cardiovascular disease. Unfortunately, public health campaigns to increase FV intake have had limited success. None of the studies reported any serious adverse effects. However, health advantages on markers of inflammation, immunity, and endothelial function are promising. Limitations of the available studies were related to the diversity of studies conducted with respect to design and study population and the variability in the measured outcomes and assays utilized. While mixed FV supplements may serve as an efficacious complement for individuals who have difficulty achieving their daily FV intake requirement, further research on additional retail preparations is warranted (Esfahani *et al.*, 2011). Based on the antioxidant activity of tea, which was recognized earlier, effect of green tea and black tea have been shown to reduce blood glucose and blood triglycerides. The green tea was higher than that of black tea in the aged rats but that the antioxidative ability of black tea was better than that of green tea in the aged rats (Zeyuan *et al.*, 1998). Furthermore, antioxidant Activity of various tea extracts in relation to their antimutagenicity was conjugated to the electrocatalytic oxidation of (-)-epigallocatechin gallate (EGCG), the main monomer flavanol found in green tea (Yen and Chen 1995). It seems that natural antioxidants such as phenols and flavones may have strong health capacity in controlling T complications. Although parsley is well known as diet of highly therapeutic potential, its companion with tea may considered a real therapy in case of T administration.

## 2. Materials and Methods

Experimental animal design: To test the biological effect of TPN, 30 male Sprague-Dawley aged rats of initial body weight  $301 \pm 3.0$ g in a 5 X 6 animal's group system were conducted (Table 3).



The animals were fed on basal diet for 10 days for adaptation, and then divided into those groups taking casein as negative control (*Bowman et al., 1990*). Rats have been kept individually in plastic cages oriented to the experimental procedures described as the established guidelines for the care and handling of laboratory animals. The preferred method of normal patient's TPN delivering (*Hayes et al., 2000*) was infusion procedure with a medical infusion pump used. Sterile bag of the nutrient solution, a 50 mL each animal groups from 2 to 5, were used. The pump infused a small amount (0.15 to 0.25 ml/hr) continuously in order to keep a rate of around 4 ml/d and the vein open. TPN was performed through a central intravenous catheter, through the subclavian vein, with the tip of the catheter at the superior vena

cava without entering the right atrium. Animal movements were totally minimized up to the end of experiment including the control group. The entire time of this trail was 5 days, then animal was killed after blood collection, and then organs were separated, weighed and kept to be treated afterwards. The blood analysis of total cholesterol, Fe and glucose were performed according to *Allian et al., (1974)*; *AOAC, (1984)*; and *Dacie and Lowis (1984)*, respectively. The blood cell counts were counted according to the method of Van Der Zijpp and Leenstra (1980). The liver enzyme function: GPT and GOT in rat serum were determined according to the method described by *Reithman and Frankel (1957)*. A mean of six samples was used as a final figure.

**Table (3): Systemic administrations of T (G2 to G5).**

#	Group	Description
G1	NC	Basel diet ( <b>Bowman et al., 1990</b> ).
G2	T	50 ml of T solution ( <b>Hayes et al., 2000</b> ) each animal.
G3	TP	G2 + 1% Parsley curly 40% extract in cold water.
G4	TPG	G3 + 1% green tea 20% extract in hot water.
G5	TD	G2 + 1% an artificial common diuretic drug.

Where: NC negative control basel diet; T, total parental nutrition; P, Parsley curly; G, green tea; and D, Lasix.

Histopathological examination of liver: The organs were collected and post-mortal examination was done as soon as possible. Fixation was carried out in 10% of natural formalin, dehydrated, cleared, and ended paraffin then sectioned at (4-6 mm), and stained with harris hematoxylin, and casein for histopathological examination (*Frankel and Reitman, 1963*).

### 3. Results and Discussion

Total parental nutrition (T) response on rat's liver is recorded in Table (3). Exceptional T alone, all other treatment causes 20% reduction in liver masse; surprisingly this was around to be 40% under the effect of TD.

**Table (4): Effect of T administration on body and liver weights as percent control group.**

Group	BW changes		Liver changes	
	g	%	g	%
NC	304.0 ± 3.52	100	8.36 ± 0.23	100
T	295.2 ± 2.69	97.1	8.45 ± 0.19	101.1
TP	282.1 ± 3.06	92.8	6.80 ± 0.32	81.3
TPG	285.2 ± 2.87	93.8	6.85 ± 0.28	81.9
TD	278.8 ± 2.59	91.7	5.15 ± 0.24	61.6

Where: NC, Basel diet; P, Parsley curly; PG, Parsley curly + green tea; D, Lasix; T, total parental nutrition and BW, body weight.

This, however, was correlated with body weight loss that ranged between 3 to 9% for T to TD, respectively. In accordingly, most probably, most of this total weight reduction is water.

More biological evaluation for T complication related to liver tissues health is appeared in Table (5). As seen, the nearest liver health profile to the control was TPG and the most reversely affected one was

TD. However, Fig 1 is showing some liver histological complication caused by T application. Here, these histological findings summary are clearly pictured in Figure 2 where liver of control, untreated rat (Fig. 2.1) showing the normal histology of liver parenchyma; small local mononuclear inflammatory cells infiltration that has been caused by T (Fig. 2-2). Additional vaculation of hepatocytes was occurred

under the effect of T alone, small and large arrows, respectively (Fig. 2-3). Comparatively, TP treatment only made a dissociation of hepatocytes (Fig. 2-4), meanwhile the TPG has appeared to be normal hepatocytes parenchyma (Fig. 2-5). Concurrently, TD section included serious histopathological disorders such as hemolysed RBCs and deposition of golden brown hemosidrine pigments, as seen in Fig. 2-6, in addition to necrosis of hepatocytes with pyknosis of their nuclei (Fig. 2-7).

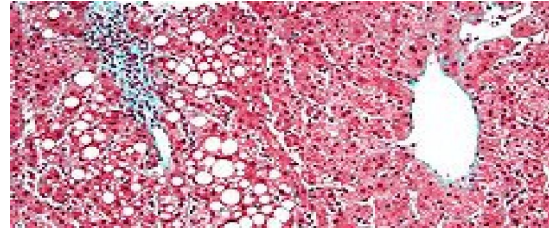


Figure (1) Micrograph of periportal fatty liver as may arise due to T (Gura et al., 2006).

Table (5): Effect of T administrations on liver histopathology as compared with control group

Liver complication	NC	T	TP	TPG	TD
1- Local monocular inflammatory	-	++	-	-	+
2- Vacuulations	-	++	-	-	++
3- Dissociation hemolysed RBCs	-	-	++	-	++
4- Deposition of golden brown hemosidrine pigment	-	-	-	-	++
5- Necrosis of hepatocytes with pyknosis of the nuclei	-	-	-	-	++
6- Necrosis of sporadic	-	-	-	-	+
Ranking No.	1	3	2	1	4

Where: NC, Basel diet; P, Parsley curly; PT, Parsley curly + green tea; D, Lasix and T, total parental nutrition.

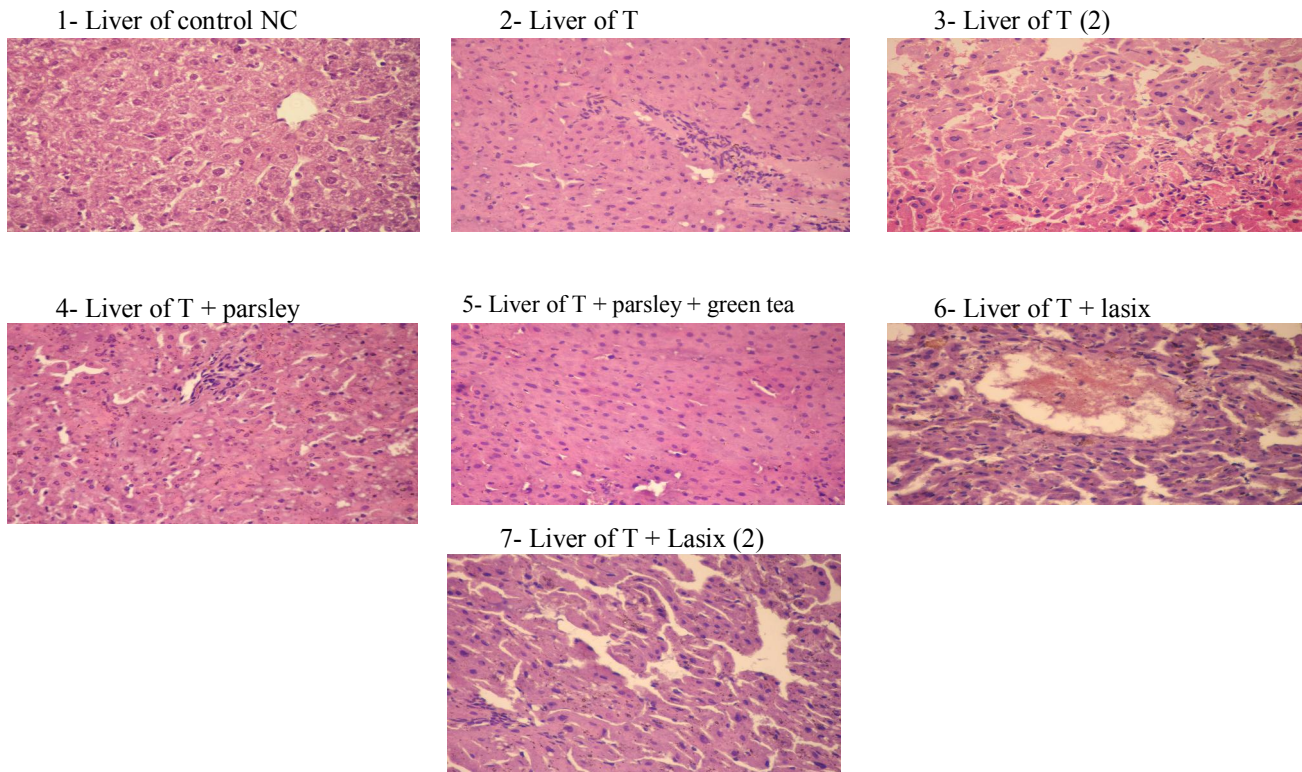


Fig. 2, 1: Liver of control, untreated rat showing the normal histology of liver parenchyma, 2: Liver of rat treated with T showing local mononuclear inflammatory cells infiltration, 3: Liver of rat treated with TP showing small local mononuclear inflammatory cells infiltration (small arrow) and vacuulations of hepatocytes (large arrow), 4: Liver of rat treated with TP showing dissociation of hepatocytes, 5: Liver of rat treated with TPG showing apparent normal hepatocytes, 6: Liver of rat treated with TD showing hemolysed RBCs and deposition of golden brown hemosidrine pigments, 7: Liver of rat treated with TD showing necrosis of hepatocytes with pyknosis of their nuclei. (H and E ×400)

Again, Table (5) and Figure 2 showed the exact negative role of T administrations on liver histopathology where several clinical remarks included local monocular inflammatory and vacuulations for application of T alone in addition to dissociation hemolysed RBCs, deposition of golden brown hemosidrine pigment, necrosis of hepatocytes with pyknosis of the nuclei and necrosis of sporadic in case of adding artificial drug to solution, e.g. TD treatment were occurred. These hepatic cells are partially or even totally recovered when the drug intervention was replaced with P or actually PG treatments, in the same respect. As an example to assess the liver response to T application, endotoxemia effect on hepatic lipid, male experimental rats received T or T plus a continuous infusion of *E. coli* lipopolysaccharide (LPS), liver weight increased significantly by 60% from  $7.5 \pm 0.6$  g. Therefore, endotoxin, when given concomitantly with T, increases hepatic lipid accumulation and thus augments the development of T-associated fatty liver in rats (*Dickerson and Karwoski 2002*). Moreover, pathohistologic and electron microscopy examination discovered liver damage, similar to that caused by TD in the present study, with typical congestive changes mainly manifested local erythremia and a reduced fluid content of the blood in the liver with blockage in the sinusoidal pole of hepatocytes. There were also focal micronecrosis, considerable reduction of glycogen and slight centrolobular steatosis. The possibility is discussed for usage of hepatotoxicity, induced by furosemide, in examining the effects of some drugs with potential hepatoprotective activity (*Klouček and Popv 1989*). Likewise drugs, localization of some important nutrients upon T administration were estimated in thin sections at higher magnification to show that most of the lipid droplets containing vitamin A were localized near the sinusoidal side. There were very few lipid droplets in the stellate cells and none in the Kupffer cells. The results indicated that vitamin A infused in T solutions is stored in the same cellular location as orally ingested vitamin A (*McKenna et al., 1983*). Liver health is sensitive to several T content but postnatal

hepatic fatty acid oxidative capacity of preterm pigs receiving T does not differ from that of term pigs and is not affected by supplemental arachidonic and docosahexaenoic acids (*Campbell et al., 2010*). In harmony with what recorded in the present study, physicians claimed that parsley is very effective in remedying liver disease. It enriches the liver and nourishes the blood. Parsley helps reduce liver congestion, clearing toxins and aiding rejuvenation. In women, parsley improves estrogen and nourishes and restores the blood of the uterus. Conditions like delayed menstruation and the menopause, dry skin, irritability, depression and hair loss can often improve (*McKeith, 2000*). Furthermore, the present results and some earlier one suggested that the drinking of green tea with high catechin content may help to prevent and/or attenuate the development of a certain type of hepatitis (*Abe et al., 2005*). In a rats fed 2.5% green tea leaves, for 27 and 63 weeks trail; the changes of GOT, GPT,  $\gamma$ -GT, and creatinine were not significant in the treated group as compared with the control. These results suggested that long-term feeding of green tea leaves was not toxic to the liver or kidney. Serum total cholesterol, triglyceride, and LDL-C were decreased in the tested group. Interestingly, the dietary intakes of the two groups were approximately the same, but the body weights of the tea-fed group were decreased 10-18% compared with those of the control (*Lin et al. 2003*). This data is going in line with that tabulated in Table6. Similar to what given in Table 6, treatment with green tea extract significantly prevented the increases in the GOT, GPT and ALP activities in a dose-related manner. It also significantly prevented the decreases in serum albumin and total cholesterol, although not in a dose-related manner. A tendency to prevent the increase in Lecithin: cholesterol acyltransferase (LCAT) activity and the decrease in liver microsome P-450 were also noted. Little effect was found on the other abnormal changes in the serum lipids and proteins and the organ weights. These results suggest that green tea may have an ameliorating effect on hepatic dysfunction (*Hayashi et al., 1992*).

**Table (6): Effect of TPN administration on rat's BW, blood constituents and liver functions as % control.**

T Solution	BW	T C mg/dl	Glucose mg/dl	WBCs	RBCs	Iron mg/dl	GOT	GPT
Control	+	93.50	77.500	1155	17.55	184.9	326.0	114.0
T	—	132.5	104.5	29.9	97	80.6	168.9	70.6
TP	—	128.3	100.1	98.3	94.7	95.3	177.3	102.2
TPG	—	102.1	87.7	103.9	100	101.1	102.3	102.2
TD	—	146.4	114.2	33.3	93.9	71.6	187.7	127.2

Where: NC, Basel diet; P, Parsley curly; PG, Parsley curly + green tea; D, Lasix and T, total parental nutrition, body weight (BW changes; TC, total cholesterol, and W and R blood cells.



Parsley, on the other hand, prevents salt from being reabsorbed into the body tissues; thus parsley literally forces debris out of the kidneys, liver and bladder. It helps improve oedema and general water retention, fatigue and scanty or painful urination and used in conjunction with complete nutritional programmes to aid the dissolving of gall stones and in cases of gout (McKeith *et al.*, 2000). In most cases, as seen also in Table 6 and Fig. 2, dietary therapy stood as a promising health agent. Moreover, the above mentioned data went in line with some former studies on biological complication for T including rat's body weight (BW), Total Cholesterol (TC), glucose, blood cell counts. A recognizable loss of body weight; similar to what happened to organ size, reached 8.3% in 5 days using the artificial diuretic drug (TD) that used to minimize water retention. Water retention was maximally resolved also in the presence of G. In this regard, it has been suggested that patients with small-bowel syndrome who are currently on T may be at greater risk for atherosclerosis. Since T has restored a reasonably normal life expectancy for these patients, long-term follow-up will likely provide answers (Badimon *et al.*, 1987).

As general discussion and conclusion, addition to these histological impairments of liver caused by TD, the blood conservative power showed a great iron sweeping off. This was noticed under the same treatment; meanwhile, a noticeable reverse action was accomplished when T was applied. Although phenolic-rich extracts used as antioxidants in foods reduce the utilization of dietary iron, this data goes in reverse way and tea seems to synergist parsley action on Fe retention. Furthermore, blood enzymes and cell count, liver enzymes, as seen also in Table (6), were in better condition under TPG application. Moreover, artificial drug highly caused a severe reduction of WBCs and RBCs. Better results for these blood constants were found also for TPG. Although a better result was recorded for TP, TPG was the best over all including the control. Seemingly, a combined phenolic compound of the two plants is considering the main factor in this positive health role. In fact, this internal metabolic homeostatic power of PG mixture special extract is an added medicinal value to be further studied in the area of nutritional biochemistry and dietary therapy. However tea is reach of specific polyphenols. The native occurrence of tea polyphenols, namely, (-)-epicatechin, (+)-catechin, (-)-epigallocatechin 3-gallate, (-)-epicatechin, and (-)-epicatechin 3-gallate, and caffeine in tea flowers was assessed by an isocratic HPLC procedure. The levels of total catechins ranged from 10 to 38 mg/g, whereas the level of caffeine ranged from 3 to 8 mg/g. Levels of catechins and

caffeine in tea leaves and various teas were also determined and ranged from 2 to 126 mg/g and from 23 to 49 mg/g, respectively. Both tea flower and tea leaf extracts exert their strong hydroxyl radical scavenging effects in the Fenton reaction system and nitric oxide suppressing effects in LPS-induced RAW 264.7 cells. Most tea flowers contain less caffeine, but comparable amounts of total catechins, compared to tea leaves and teas (Lin *et al.*, 2003). Likewise, greater polyphenols content are determined in parsley (Halvorsen *et al.*, 2002). Tea also is an important dietary source of flavanols and flavonols. In vitro and animal studies provide strong evidence that tea polyphenols may possess the bioactivity to affect the pathogenesis of several chronic diseases, especially cardiovascular disease and cancer. However, the results from epidemiological and clinical studies of the relationship between tea and health are mixed. International correlations do not support this relationship although several, better controlled case-referent and cohort studies suggest an association with a moderate reduction in the risk of chronic disease. Conflicting results between human studies may arise, in part, from confounding by socioeconomic and lifestyle factors as well as by inadequate methodology to define tea preparation and intake. Clinical trials employing putative intermediary indicators of disease, particularly biomarkers of oxidative stress status, suggest tea polyphenols could play a role in the pathogenesis of cancer and heart disease (McKay and Blumberg 2002). Hormonal imbalance followed T application induces biliary dilatation, sludge and formation of gallstones. Cholecystokinin (CCK) induces gallbladder (GB) contraction. During thyrotropin-releasing hormone (TRH) testing for thyroid function patients felt a strong micturition reflex attributable to smooth muscle contraction of the bladder. The possibility of GB contraction after TRH administration was studied compared to cholecystokinin-octapeptide (CCK-OP) and/or fatty meal administration. The effect of intravenous (IV) CCK-OP, TRH and a combination of the two on GB volume was studied in normal volunteers without GB or liver disease. Gallbladder contraction was estimated by ultrasound prior to and after administration of the fatty meal; in the other 36 subjects, GB contraction was calculated prior to and after administration of CCK-OP, TRH, or both. Results are expressed as a percentage of the GB basal volume using each subject as his or her own control (Kalfarentzos *et al.*, 1992). The overall biological concept existed here and in several other experiments can be facilitated by the theory of hormonal oxidative balance for food and drug (Ahmed *et al.*, 2003). The naturals are always support health comparing to

synthetic drugs that may impair the oxidative hormonal balance.

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## Using bicarbonates for controlling late blight disease of potato plants under field conditions

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**Abstract:** Late blight disease caused by *P. infestans* of potato plants was controlled under field conditions using potassium or sodium bicarbonates alone or in combination with Citral. In laboratory experiments, results indicated that complete inhibition in linear growth of *P. infestans* was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.5 %. Moreover, in field experiments, results indicated that the most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons. The highest reduction was obtained with potassium or sodium bicarbonate at 1.0 or 2.0 respectively plus citral at 0.5 % and Redomil which reduced the early blight incidence more than 61.8 % as compared with untreated plants. As for potato yield the highest increased was obtained with potassium bicarbonate at 2.0 % plus Citral at 0.5 % which increased tuber yield by 76.0 and 67.9 % during two growing seasons. All treatments significantly increased the chitinase and  $\beta$ -1,3 – glucanase activities. The great increased was obtained with potassium bicarbonate at 2.0 or 1.0 % plus Citral which increased the chitinase and  $\beta$ -1,3 – glucanase activities more than 120.0 and 220.1 % respectively. It could be suggested that combined treatments between potassium bicarbonate plus Citral might be used for controlling late blight disease of potato plants under field conditions.

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**Keywords:** Late blight – Citral – Potassium bicarbonate – Potato Sodium bicarbonate

### 1. Introduction

Potatoes (*Solanum tuberosum* L.) are considered one of the most important vegetable crops in Egypt. Late blight caused by *Phytophthora infestans* is the most important disease attacking potato plants (Bad-El-Kareem, *et al.*, 2001; Pilet *et al.*, 2006; El-Gamal *et al.*, 2007 and Skelsey *et al.*, 2009).

There has been considerable interest in the use of baking soda (sodium bicarbonate,  $\text{NaHCO}_3$ ) and potassium bicarbonate ( $\text{KHCO}_3$ ) for controlling various plant fungal diseases (Karabulut *et al.*, 2003 and Smilanick *et al.*, 2006). Bicarbonates are widely used in the food industry (Lind say, 1985) were found to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). Baking soda sprays provided good control of several plant diseases (Horst *et al.*, 1992; Arimoto *et al.*, 1997; Palmer *et al.*, 1997; Janisiewicz, and Peterson. 2005). Also potassium bicarbonate provided the best protection against plant diseases (Smilanick and Margosan, 1999; Fallilk *et al.*, 1996 and Smilanick, *et al.*, 2006). Sodium or potassium bicarbonates combined with oil were effective in the control plant diseases (Horst *et al.*, 1992 and Ziv and Zitter, 1992). Abd-El-Kareem (2007) reported that potassium bicarbonates combined with Nerol significantly reduce the early blight disease and increased the tuber yield of potato plant under field conditions. Citral as one fractions of

citrus essential oil caused complete inhibition of the linear growth of *Giotrichum candidum*, *Penicillium digitatum* and *P. italicum* as causal agents of fruit citrus diseases (El-Mohamedy *et al.*, 2002).

The main objectives of the present research are studying the effect of sodium or potassium bicarbonates alone or in combination with Citral against late blight disease of potato plants under field conditions.

### 2. Materials and Methods

#### Source of pathogenic fungi and potato tubers

Pathogenic isolate of *Phytophthora infestans* the causal agent of late blight diseases was kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt. Meanwhile, Potato tubers cv. Sponta were obtained from Dept., of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

#### Laboratory experiments

Effect of different concentrations of potassium, sodium bicarbonates or citral on linear growth of *Phytophthora infestans*

Different concentrations of potassium or sodium bicarbonates and Citral as one fractions of citrus essential oil (obtained from Delta Aromatic Company, Egypt) were tested to study their inhibitory effect on linear growth of *P. infestans*. Five concentrations of potassium or sodium bicarbonate

i.e. 0.0,0.25,0.50, 1.0 and 2.0 % (w / v) and Citral at 0.10, 0.25, and 0.50 % (v/ v) were added individually to conical flasks containing sterilized rye agar medium (Cohen *et al.*, 1991) to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri dishes (9 cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10-days old culture of *P. infestans*. Five plates were used as replicates for each particular treatments. Inoculated plates were incubated at  $25 \pm 2$  °C. The average linear growth of fungus was calculated after 10 days.

#### Field experiments:

Effect of potassium or sodium bicarbonates alone or in combination with Citral on late blight severity of potato plants under field conditions. Experiments were carried out, in Experimental Farm of National Research Centre at El-Noubareia, Behera governorate.

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.5 % in addition to Fungicides (Redomil® – plus at 2 g / l) were applied under field conditions to study the possibility of their effect against late blight disease during tow cultivation seasons. Potato yield was also determined at two cultivation seasons.

Field experiments were conducted under natural infection in plots (4x8 m) each comprised of 8 rows and 32 holes / row, was conducted in a completely randomized block design with three replicates (plots) for each particular treatment.

#### Treatments:

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.5 % in addition to Fungicides Redomil® – plus at 2 g / l (2,6 dimethyl phenyl methoxyacetyl amino) From Syngenta Crop Protection, Company, Australia were applied as follow: -

Treatments	
Single	Combined
1- KHCO <sub>3</sub> 1 %	5- KHCO <sub>3</sub> 1 % + Citral
2- KHCO <sub>3</sub> 2 %	6- KHCO <sub>3</sub> 2 % + Citral
3- NaHCO <sub>3</sub> 1 %	7- NaHCO <sub>3</sub> 1 % + Citral
4- NaHCO <sub>3</sub> 2 %	8- NaHCO <sub>3</sub> 2 % + Citral
Control	
Redomil® plus 2 g / l	
Un-treated plants	

#### Application:

All treatments were applied as foliar application on potato plants which had 4-5 compound leaves and every 15 days up to 90 days of planting.

#### Disease assessment

Late blight scale from 0 to 4 according to Cohen *et al.*, (1991) was used, as follows:

0 = No leaf lesions.

1 = 25 % or less.

2 = 26 – 50

3 = 51 –75

4 = 76-100 % infected area of plant leaf.

Late blight disease was recorded up to 90 days of planting.

#### Determination of tuber yield

Tuber yield of potato (kg /m<sup>2</sup>) for each treatment was determined.

Effect of potassium or sodium bicarbonates alone or in combination with Citral on enzymes activity of potato plants.

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.5 % were tested to study their effect on chitinase and  $\beta$ -1,3 -glucanase activities of potato plants.

Extraction of enzymes .Chitinase and  $\beta$ -1,3 -glucanase activities was determined after 40 days of planting. The enzymes extracted of potato leaves and the supernatant was prepared according to method of (Tuzun *et al.*, 1989)

Chitinase assay: chitinase activity was determined by colourimetric method of Boller and Mauch (1988). Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

#### $\beta$ -1,3 -glucanase assay:-

The method of Abeles and Forrence (1970) was used to determine glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

The method was carried out as 0.5 ml of enzyme extract was added to 0.5 ml of 0.05 M of potassium acetate buffer (pH 5) containing 2% laminarin. The mixture was incubated at 50°C for 60 minutes. The reaction was stopped by adding 1 ml of dinitrosalicylic acid reagent and heating the tubes for 5 minutes at 100°C. The tubes were cooled and 3 ml of distilled water were added before assay. The optical density was read at 500 nm. 1  $\beta$ -1,3-glucanase activity was expressed as mM glucose equivalent released gram fresh weight tissues / 60 minutes.

#### Statistical analysis :

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

### 3. Results

#### Laboratory experiments

#### Effect of potassium or sodium bicarbonate and citral on linear growth of *P. infestans*

Potassium or sodium bicarbonates at five concentrations *i.e* 0.0,0.25, 0.50, 1.0 and 2.0 % (w / v) and Citral at 0.1, 0.25, and 0.50 % (v/v) were

tested to study their inhibitory effect on linear growth of *P. infestans* *in vitro*. Results in Table (1) indicate that all treatments significantly reduced the linear growth of *P. infestans*. Complete inhibition was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.50 %. The highest reduction was

achieved with potassium bicarbonate at 1.0 % which reduced the linear growth by 93.3 % as compared with untreated plates. Meanwhile, potassium or sodium bicarbonates at 0.50 and 1.0 % respectively and Citral at 0.25 % had moderate effect. Other treatments showed less effective.

**Table(1) Linear growth (mm) of *P. infestans* as affected with different concentrations of potassium or sodium bicarbonates and citral**

Treatments	Concent. %	Linear growth (mm)	Reduction %
KHCO <sub>3</sub>	0.25	42.0 <sup>c</sup>	53.3
	0.50	31.0 <sup>d</sup>	65.6
	1.0	6.0 <sup>f</sup>	93.3
	2.0	0.0 <sup>f</sup>	100.0
NaHCO <sub>3</sub>	0.25	53.0 <sup>b</sup>	41.1
	0.50	37.0 <sup>c</sup>	58.9
	1.0	12.0 <sup>e</sup>	86.7
	2.0	0.0 <sup>f</sup>	00.0
Citral	0.1	32.0 <sup>d</sup>	64.4
	0.25	10.0 <sup>e</sup>	88.9
	0.50	0.0 <sup>f</sup>	0.0
Control		90.0 <sup>a</sup>	----

Figures with the same letter are not significantly different ( $P=0.05$ )

### Field experiments

#### Effect of potassium or sodium bicarbonate alone or in combination with citral on late blight incidence of potato plants under natural infection

The promising treatments in laboratory experiments were applied under field conditions during two successive growing seasons.

Potassium or sodium bicarbonate at 1.0 or 2.0 % alone or in combination with Citral at 0.50 %. In addition to Fungicides (Redomyl<sup>®</sup> – plus at 2 g / l) were applied to study their effect on late blight severity of potato plants. Results in table (2) indicate that all treatments significantly reduced the disease

incidence. The most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons. The highest reduction was obtained with potassium or sodium bicarbonate at 1.0 or 2.0 respectively plus Citral at 0.5 % and Redomyl<sup>®</sup> which reduced the early blight incidence more than 61.8 % as compared with untreated plants. Single treatments with potassium bicarbonate at 2.0 % reduced the disease incidence by 47.1 and 50.0 % during two growing seasons. Other treatments showed moderate effect.

**Table (2) Late blight incidence<sup>(2)</sup> on potato plants as affected with potassium or sodium bicarbonates alone or in combination with citral under field conditions**

Application (%)	First season		Second season	
	Disease incidence	Reduction %	Disease incidence	Reduction %
Single treatment				
KHCO <sub>3</sub> 1.0	2.0 <sup>b</sup>	37.5	2.3 <sup>b</sup>	32.4
KHCO <sub>3</sub> 2.0	1.6 <sup>c</sup>	50.0	1.8 <sup>c</sup>	47.1
NaHCO <sub>3</sub> 1.0	2.3 <sup>b</sup>	28.1	2.3 <sup>b</sup>	32.4
NaHCO <sub>3</sub> 2.0	1.9 <sup>b</sup>	40.6	1.9 <sup>c</sup>	44.1
Citral 0.5	2.1 <sup>b</sup>	34.4	2.0 <sup>c</sup>	41.2
Combined treatment				
KHCO <sub>3</sub> 1.0 + Citral	1.1 <sup>e</sup>	65.6	1.3 <sup>ed</sup>	61.8
KHCO <sub>3</sub> 2.0 + Citral	0.5 <sup>f</sup>	84.4	0.6 <sup>f</sup>	82.4
NaHCO <sub>3</sub> 1.0 + Citral	1.5 <sup>d</sup>	53.1	1.6 <sup>d</sup>	53.0
NaHCO <sub>3</sub> 2.0 + Citral	1.0 <sup>e</sup>	68.8	1.2 <sup>e</sup>	64.7
Redomyl – plus 2 g / l	1.0 <sup>e</sup>	68.8	1.1 <sup>e</sup>	67.6
Control	3.2 <sup>a</sup>	—	3.4 <sup>a</sup>	—

(1) Figures with the same letter are not significantly different ( $P=0.05$ )

(2) Late blight scale from 0 to 4 according to Cohen *et al.* (1991).

### Effect of potassium or sodium bicarbonates alone or in combination with Citral on tuber yield of potato plants under field conditions

Potassium or sodium bicarbonate at 1.0 or 2.0 % alone or in combination with Citral at 0.50 %. In addition to Fungicides (Redomyl® – plus at 2 g / l) were applied to study their effect on tuber yield of potato plants. Results in table (3) indicate that all treatments significantly increased the tuber yield. The

high increased was obtained with potassium bicarbonate at 2.0 % plus citral at 0.5 % which increased tuber yield by 76.0 and 67.9 % during two growing seasons. Moderate increased was obtained with potassium bicarbonate at 2.0 as single treatment, potassium bicarbonate at 1.0 % plus citral at 0.5 %, sodium bicarbonate at 2.0 plus Citral at 0.5 % and Redomyl® which increased the potato yield more than 40.0 %. Other treatments showed moderate effect.

**Table (3) Tuber yield of potato plants as affected with different concentrations of potassium or sodium bicarbonates alone or in combination with citral under field conditions.**

Application (%)	First season		Second season	
	Yield (kg /m2) Mean	Increase %	Yield (kg /m2) Mean	Increase%
Single treatment				
KHCO <sub>3</sub> 1.0	3.0 df	20.0	3.6 cd	28.6
KHCO <sub>3</sub> 2.0	3.7 bc	48.0	4.2 b	50.0
NaHCO <sub>3</sub> 1.0	3.3 cd	32.0	3.5 cd	25.0
NaHCO <sub>3</sub> 2.0	3.3 cd	32.0	3.8 c	35.7
Citral 0.5	3.0 df	20.0	3.2 f	14.2
Combined treatment				
KHCO <sub>3</sub> 1.0 + Citral	4.0 b	40.0	4.2 b	50.0
KHCO <sub>3</sub> 2.0 + Citral	4.4 a	76.0	4.7 a	67.9
NaHCO <sub>3</sub> 1.0 + Citral	3.5 cd	40.0	3.8 cd	35.7
NaHCO <sub>3</sub> 2.0 + Citral	3.7 bc	48.0	4.2 b	50.0
Redomyl – plus 2 g / l	3.7 bc	48.0	4.2 b	50.0
Control	2.5 g		2.8 g	

Figures with the same letter are not significantly different ( $P= 0.05$ )

### Effect of potassium or sodium bicarbonates alone or in combination with Citral on chitinase activity of potato plants under field conditions

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.50 %. were applied to study their effect on chitinase activity of potato plant. Results in table (4) indicate that all treatments significantly increased chitinase activity. The most effective treatment was potassium bicarbonate at 2.0 or 1.0 % plus Citral at 0.5 % which increased the activity by 126.7 and 120.0 % respectively as compared with untreated plants. Potassium bicarbonate at 2.0 as single treatments and sodium bicarbonate at 2.0 % in combination with Citral increased the enzyme activity by 100.0 %. Other treatments show moderate effect.

### Effect of potassium or sodium bicarbonates alone or in combination with Citral on $\beta$ -1,3 – glucanase activity of potato plants under field conditions

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with citral at 0.50 % were applied to study their effect on  $\beta$ -1,3 – glucanase activity of potato plant. Results in table (5) indicate that all treatments significantly increased  $\beta$ -1,3 – glucanase activity. The most effective treatment was potassium bicarbonate at 2.0 or 1.0 % plus Citral at 0.5 % which increased the activity by 333.3 and 220.1 % respectively as compared with untreated

plants. Potassium bicarbonate at 2.0 as single treatments and sodium bicarbonate at 2.0 % in combination with Citral increased the enzyme activity by 195.8 and 200.0 %. Other treatments show moderate effect.

**Table (4) Chitinase activity of potato plants as affected by potassium or sodium bicarbonate alone or in combination with citral**

Application (%)	Chitinase activity <sup>(2)</sup> Mean	Increase %
Single treatment		
KHCO <sub>3</sub> 1.0	2.7 <sup>c</sup>	80.0
KHCO <sub>3</sub> 2.0	3.0 <sup>b</sup>	100.0
NaHCO <sub>3</sub> 1.0	2.6 <sup>c</sup>	73.0
NaHCO <sub>3</sub> 2.0	2.6 <sup>c</sup>	73.0
Citral 0.5	2.5 <sup>c</sup>	66.7
Combined treatment		
KHCO <sub>3</sub> 1.0 + Citral	3.3 <sup>a</sup>	120
KHCO <sub>3</sub> 2.0 + Citral	3.4 <sup>a</sup>	126.7
NaHCO <sub>3</sub> 1.0 + Citral	2.6 <sup>c</sup>	73.0
NaHCO <sub>3</sub> 2.0 + Citral	3.0 <sup>b</sup>	100.0
Control	1.5 <sup>d</sup>	—

(1) Figures with the same letter are not significantly different ( $P= 0.05$ )

(2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released / gram fresh weight/ 60 min.



**Table (5)  $\beta$ -1,3 – glucanase activity of potato plants as affected by potassium or sodium bicarbonate alone or in combination with Citral**

Application (%)	$\beta$ ,1-3 – glucanase (2) Mean	Increase %
Single treatment		
KHCO <sub>3</sub> 1.0	6.5 <sup>c (1)</sup>	170.8
KHCO <sub>3</sub> 2.0	7.1 <sup>b</sup>	195.8
NaHCO <sub>3</sub> 1.0	6.4 <sup>c</sup>	166.7
NaHCO <sub>3</sub> 2.0	6.6 <sup>c</sup>	175.0
Citral 0.5	6.0 <sup>d</sup>	150.0
Combined treatment		
KHCO <sub>3</sub> 1.0 + Citral	7.7 <sup>a</sup>	220.1
KHCO <sub>3</sub> 2.0 + Citral	8.0 <sup>a</sup>	233.3
NaHCO <sub>3</sub> 1.0 + Citral	6.4 <sup>c</sup>	166.7
NaHCO <sub>3</sub> 2.0 + Citral	7.2 <sup>b</sup>	200.0
Control	2.4 <sup>e</sup>	—

(1) Figures with the same letter are not significantly different ( $P=0.05$ )

(2)  $\beta$ -1,3-glucanase activity was expressed as mM glucose equivalent released gram fresh weight tissues / 60 minutes.

#### 4. Discussion

Potatoes (*Solanum tuberosum* L.) are considered one of the most important vegetable crops in Egypt. Late blight caused by *Phytophthora infestans* is the most important disease attacking potato plants (Pilet *et al.*, 2006; El-Gamal *et al.*, 2007 and Skelsey *et al.*, 2009)

Controlling this disease depends mainly on fungicidal treatments (Pasche *et al.*, 2005). Bicarbonates are widely used in the food industry (Lindsay 1985) have antifungal activity (Ziv and Zitter, 1992). In present study, results indicate that potassium or sodium bicarbonates and Citral showed great inhibitory effect in linear growth of *P. infestans*. Complete inhibition was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.50 % *in vitro*. In this respect Ziv and Zitter (1992) reported that Potassium and sodium bicarbonates have inhibitory effects against several pathogenic fungi. In addition to bicarbonates salts has been shown to have a profound inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan, 1982 and Ziv and Zitter, 1992).

Potassium and sodium bicarbonates were found to control several plant diseases (Smilanick and Margosan, 1999; Janisiewicz and Peterson, 2005; and Smilanick *et al.*, 2006). In present study, in field experiments, results indicate that all treatments significantly reduced the disease incidence. The most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons. As for potato yield the highest increase was obtained with potassium bicarbonate at 2.0 % plus Citral at 0.5 % which increased tuber yield by 76.0

and 67.9 % during two growing seasons. In the present study bicarbonate and Citral oil are effective for controlling early blight disease. Their effectiveness is quite good when applied as single treatments but improved when used in combination. This result may be due to: the antifungal activity of bicarbonate and citral, synergistic effect between bicarbonate and citral and increased the activity of chitinase enzyme of potato against fungal pathogen. In this respect Punja and Grogan (1982) reported that the improved effectiveness of control with combinations of bicarbonates plus oil was attributed to fungicidal characteristics of bicarbonates ions. Moreover, the fungicidal and spreader sticker characteristics of oil that keep the bicarbonate ions on foliar surfaces (Homma *et al.*, 1981 and Ziv and Zitter, 1992).

Hypothesis have been proposed for the inhibitory mechanisms of bicarbonates and oil. Hydrogen ion concentration of bicarbonates salts has been shown to have a profound inhibitory effect on sclerotia and conidia germination of *S. rolfesii* and *S. fuliginea* respectively (Punja and Grogan, 1982 and Homa *et al.*, 1981). Furthermore, film-forming polymers may form a physical barrier on leaf surfaces against germ tube penetrations (Elad *et al.*, 1989 and Ziv, and Zitter 1992). The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia, (Punja and Grogan, 1982 and Ziv and Zitter, 1992). On the other hand the role of potassium (Potassium bicarbonate) in increasing crop resistance to diseases caused by bacteria and fungi was widely reviewed by Perrenoud (1990). In general, potassium application improves plant health and vigour, making infection less likely or enabling a quick recover (Perrenoud, 1993). Potassium probably exerts its greatest effects on disease through specific metabolic functions that alter compatibility relationships of the host-parasite environment and increases the production of disease inhibitory compounds, such as phenols, phytoalexins and auxins around infection sites of resistant plants. (Kiraly, 1976). In the present study results indicate that all treatments increased the chitinase and B -1,3-glucanase activities. In this respect, B-1,3-glucanases and chitinases are able to hydrolyze B -1,3-glucan and chitin, respectively, the major components of fungal cell walls (Kauffmann *et al.*, 1987, Legrand *et al.*, 1987 and Abd-El-Kareem *et al.*, 2009). Sodium or potassium bicarbonates combined with oil were effective in the control of several plant fungal diseases (Horst *et al.*, 1992 and Ziv and Zitter, 1992).

It could be suggested that combined treatments between potassium bicarbonate plus citral might be used for controlling late blight disease of potato plants under field conditions.

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## Rural Households' Awareness and Willingness to Pay for National Health Insurance Scheme (NHIS) in Ilesha West Local Government Area, Osun State Nigeria: A Recursive Bivariate Probit Approach

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**Abstract:** The Nigerian health policy seeks to ensure adequate access of the population to affordable and quality healthcare services. The NHIS seeks to complement efforts already put in place for achieving this goal. This paper therefore analyzed the factors influencing awareness and willingness to pay for NHIS in rural Nigeria. The data were collected with structured questionnaires using multi-stage sampling method. Data were analyzed using Recursive Bivariate Probit model. Results show that 47.27 percent of the respondents were using general (public) hospital while 15.45 percent were not using medical treatments. Also, 54.55 percent of the households were aware of NHIS whereas 71.82 percent were willing to pay. Awareness and willingness to pay were negatively correlated although both were positively and significantly influenced by years of education and sick time ( $p < 0.10$ ). It was concluded that efforts to properly educate rural people can facilitate subscription into the program.

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**Keywords:** health insurance, willingness to pay, awareness, Recursive Bivariate Probit

### 1. Introduction

The objective of the Nigerian national health policy is attainment of a level of health that will enable all citizens to achieve socially and economically productive lives. In order to achieve this, the National Health Insurance Scheme (NHIS) was established under the Federal Government of Nigeria Act 35 of 1999. Health insurance is social security system that guarantees access to healthcare services from funds created by pooling monthly contributions of all participants. Thus, health insurance scheme aims to mobilize resources in a sustainable manner for the provision of accessible and quality health care for every Nigerian irrespective of status (Benneth and Gilson, 2001; Bossert *et al.*, 2003).

Conceptually, health is a fundamental dimension of human well-being. The World Health Organization (WHO) defined it as a state of complete physical, mental and social well-being and not merely absence of diseases or infirmities (WHO, 1946). Good health is instrumental for improving other dimensions of human life (Dollar, 2001). Although traditional economists measured well-being in monetary term, health indicators are direct measures of well-being. Other factors that impact households' health include household assets, human capital, leisure time use, structure and stability of family, healthy practices, income level and stability, relative prices of essential goods, public health expenditure and uptake of health insurance.

In an economic perspective, an individual's propensity to utilize healthcare is determined by the costs of utilization and the perceived benefits of health care. Since costs are mainly determined by the allocation of healthcare resources, utilization will in practice be determined by the interaction between demand and provision of health care (Mc-Guire *et al.*, 1988). The provision of health care determines the availability of health care resources. The price of health care is occasionally a result of market "outcome" but more commonly the government regulates it. Similarly, public policies often determine the location of facilities, budgets, waiting times, among others.

Demand for health care, on the other hand depends on several factors of which some are interrelated. The individual's attitudes, perceptions and decisions are important determinants of health care utilization, with respect to the initial contact with a healthcare provider. Influential factors on demand for health care are health status, income and education (Grossman, 1992). An individual's health status will of course greatly affect her or his perceived benefit of medical treatment. However, perceived benefit is also influenced by education (Habtom and Ruys, 2007).

In developing countries, households that are headed by an individual with secondary level of education or higher would more likely be willing to seek health care (Diop *et al.*, 1998). Income is important since it determines ability to pay and it

influences earnings foregone when seeking care. Benefits and costs of treatment may be expected to vary with age and gender because of differences in health status and work productivity. Finally, individual differ in preferences and personal taste, and to the extent that education influence preferences this may lead to systematic socio-economic differences (Gottret and Schreber, 2006).

Many production and consumption decisions are made at the household decisions. This implies that the utilization of individual household members depend on household factors, including relation to other household members and their characteristics. The family can be viewed as the producer of health rather than the individual (Jacobson, 1999) and utilization of health care depends on household income etc. Furthermore, decisions are influenced by several intra-household factors. It has been shown that the education of the member of a household have an influence on the other household members health-related behaviour, such as health care utilization (Gilson and McIntyre, 2005).

Most researches on utilization of healthcare are concentrated on the individual as the customer of health, however much speak for viewing the household as the main producer of health and consumer of health care. This may be even more important to take into account when analyzing utilization in African countries, where the family union is stronger and the dependency ratio higher. Utilization is measured in terms of expenditure on health care. However, the determination of health care expenditure is done by individuals and the households in which they reside, given their resources and the prices that they face. Studies of utilization of health care generally use the individual as the unit of analysis. Much of this work has taken its point of departure in Grossman's seminar work (1972), where he argued that the individual produces the commodity "good health". This commodity is part of the individual's human capital and affects the total amount of time the individual can spend on productive activities. Even if Grossman's work provided the field of health economics with great input, it lacked the fact that individuals are household members and take much influence from (willing or not willing) from other household members.

Income and education are among the most important factors influencing health care utilization. However, other factors such as age also influence utilization since age reflects on perceived benefit and income. There have been a number of studies showing relationship between household income and utilization, of health care. However, all these have shown that the income elasticity is rather high, in developing countries. Utilization of healthcare is in

practice very much influenced by decisions by the provider, physicians' advice among others (Grossman, 1972).

The objective of this paper is to determine the interrelationship between awareness about NHIS and willingness to pay among rural households. This can be motivated from the fact that as a new policy initiative in the Nigerian health sector, NHIS adoption can boost access of people to healthcare services with immense welfare impact. The paper therefore determined the key factors that influence awareness and willingness to pay for NHIS with adequate correction of endogeneity. In the remaining parts of the paper, the methodology, results and finding and conclusion have been presented.

### **Materials and methods**

#### **Area of study and sampling procedures**

The study was conducted in Ilesha West Local Government which was created from the old Ilesha local government in November 1996. Ilesha enjoys an average rainfall of 1300 mm per annum. The local government is geographically located on latitude  $7^{\circ} 37' 12''$  North and longitude  $4^{\circ} 44' 24''$  East. The local government is divided into 10 wards namely, Egbedi (ward 1), Cocacola (ward 2), Okeola (ward 3), Omofe (ward 4), Isokun (ward 5), Ikoti (ward 6), Ilaje (ward 7), Odo Esira (ward 8), Ereja (ward 9) and Oke-Ese (ward 10). The inhabitants are predominantly Yoruba speaking people with Ijesha accent.

The target populations for this study were the households in Ilesha West Local Government. Multi stage stratified random sampling technique was used. The first stage was selection of one local government area (Ilesha West) from the two local government areas that make up Ilesha town. The second stage involves the selection of ten political wards that make up Ilesha West Local Government, while the third stage involved selection of eleven (11) households from each of the ward by random sampling procedure. A total number of one hundred and ten households were selected randomly, with sample size distributed proportionate to size of the wards. Willingness of the respondents (household heads) to pay for health insurance scheme was elicited by randomly giving them bids that ranged between ₦120- 150.

#### **Estimated model**

The Seemingly Unrelated Bivariate Probit (SUBP) was used to determine the factors that influence the probability of rural households' awareness and willingness to pay for NHIS. It was noted that willingness to pay and awareness can operate in a recursive manner, thereby implying that one of them is endogenous dependent variable. We first tested the endogeneity of awareness in the



willingness to pay model, which statistical insignificance of rho rejected. It was then proposed that awareness could as be influenced by willingness to pay. Therefore, if this holds, our estimated parameters from Probit regression will not meet the conventional conditions for being Best Linear Unbiased Estimate (BLUE). We estimated a recursive bivariate model based on propositions by Maddala (1983). The structural form of the model can be stated as:

$$Q_{i1} = \alpha + \beta_i \sum_{i=1}^n X_i + v_i \tag{1}$$

$$Q_{i2} = \gamma + \delta_i Q_{i1} + \delta_i \sum_{i=1}^n X_i + z_i \tag{2}$$

$Q_{i1}$  and  $Q_{i2}$  are latent variables of willingness to pay and awareness about NHIS respectively. These variables are dummy variables with values of 1 if willing to pay for NHIS and 0 otherwise for equation 1 and values of 1 if aware of NHIS and 0 otherwise for equation 2. Also,  $\alpha, \beta, \gamma, \delta$  are the estimated parameters and  $X_i$  are the socio-economic variables of rural households. Included explanatory variables are farming (yes = 1, 0 otherwise), sex (male =1, 0 otherwise), age (years), years of education, household size, total monthly earnings (N), sick time in a month, malaria in the past one week (yes = 1, 0 otherwise), high blood pressure in the last one month (yes = 1, 0 otherwise), treatment cost of disease (N), affordability of the scheme (yes = 1, 0 otherwise), workability of the scheme (yes = 1, 0 otherwise), visit hospitals (yes = 1, 0 otherwise), scheme has coverage for all family members (yes = 1, 0 otherwise), cost is okay (yes = 1, 0 otherwise) and prefers pre-paid health system (yes = 1, 0 otherwise). The error terms of the model are dependent and distributed as a bivariate normal such that:  $E(v_i) = E(z_i) = 0$ ,  $var(v_i) = var(z_i) = 1$  and  $\rho = cov(v_i, z_i)$ . The Wald test, which is reflected by statistical significance of  $\rho$  was used to determine whether the models would be best estimated jointly in a recursive manner or not.

**Results and Discussions**

**Descriptive Analysis of Household Head Socio-Economic Characteristics**

Table 1 shows that 80.9 percent of the respondents are males. This is expected in typical Nigerian family setting where the man heads the house except he is dead or on transfer. Also, 18.2 percent of the respondents had no formal education, 26.4 percent had primary education, while 32.7 percent had tertiary education. Average year of

schooling is 8.97 for all the respondents with standard deviation of 5.96. The age distribution of the respondents is also provided in table 1. It shows that 34.55 percent of the household heads falls into age group 40<50 years while 30.00 percent belongs to 30<40 years. Also, the aged in the range of  $\geq 60$  years constitute 14.55 percent whereas the youth that were less than 30 years accounts for 7.27 percent of the respondents. Average age of all the respondents is 44.37 years with standard of 11.86. Distribution of household size shows that majority of the households (52.73 percent) had 1-4 members, while 37.27 percent had 5-8 members. Average household size is 5.00 with standard deviation of 3.23.

Table 1: Socio-economic characteristics of the respondents

Socio-economic characteristics	Frequency	Percentage
<i>Sex of household heads</i>		
Male	89	80.9
Female	21	19.1
<i>Education</i>		
No formal education	20	18.2
Primary school	29	26.4
Secondary school	25	22.7
Tertiary	36	32.7
<i>Age</i>		
20<30	8	7.27
30<40	33	30.00
40<50	38	34.55
50<60	15	13.64
$\geq 60$	16	14.55
<i>Household size</i>		
1-4	58	52.73
5-8	41	37.27
9-12	7	6.36
$\geq 13$	4	3.64

Source: Field survey, 2008

**Healthcare choices and assessment of their present state**

Table 2: Healthcare service choices

Healthcare Provider	Frequency	Percentage
General hospital	52	47.27
Mission hospital	11	10.00
Private hospital	24	21.82
Primary health care centers	6	5.45
None	17	15.45

Source: Field survey, 2008



Results in table 2 show that 15.45 percent of the respondents was not using any health centers and therefore unable to provide assessment of health facilities. Also, 47.27 percent of the households was using publicly owned general hospitals, while 21.82 percent was using private hospitals. Similarly, 10.00 percent and 5.45 percent of the respondents had preferences for mission hospitals and public primary

healthcare centers respectively. This shows that public hospitals were in highest demand possibly due to lowest cost charges and efficiency of services. It should be noted that implementation guidelines of NHIS requires availability of public hospitals. Therefore, there is not going to be implementation difficulty in the local government.

Table 3: Adequacy of healthcare centers based on buildings

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	0.91	3.64	8.18	28.18	6.36	47.27
Mission hospital	0.00	0.00	1.82	3.64	0.00	5.45
Private hospital	0.00	0.91	5.45	7.27	5.45	19.09
Private maternity/clinics	0.00	0.91	3.64	5.45	0.00	10.00
Primary health care centers	0.91	0.00	1.82	2.73	0.00	5.45
Total	1.82	5.45	20.91	47.27	11.82	87.27

Source: Field survey, 2008

Table 3 shows that 28.18 percent of the respondents indicated that the building structures of general hospitals are good while 6.36 percent ranked them as excellent. Also, 3.64 percent of the respondents ranked general public hospitals as fair while 0.91 percent indicated that it was poor. The private hospitals were ranked as good by 7.27 percent

of the respondents, while 5.45 percent indicated that it was excellent. Private maternity/clinics were ranked as good by 5.45 percent of the respondents. The table also shows that 47.27 percent of the respondents ranked the health centers buildings as good, while 11.82 percent ranked them as excellent.

Table 4: Adequacy of healthcare center ranking based on number of staff

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	0.00	1.82	11.82	30.00	3.64	47.27
Mission hospital	0.00	0.91	0.91	3.64	0.00	5.45
Private hospital	0.00	0.91	2.73	10.91	4.55	19.09
Private maternity/clinics	0.91	0.00	1.82	7.27	0.00	10.00
Primary health care centers	0.91	0.00	0.91	3.64	0.00	5.45
Total	1.82	3.64	18.18	55.45	8.18	87.27

Source: Field survey, 2008

Table 4 shows that 30 percent and 3.64 percent of the respondents ranked general hospital as good and excellent respectively based on adequacy of number of staff. Also, 10.91 percent and 4.55 percent of the respondents indicated that private hospitals

were in good and excellent conditions respectively. In all, 55.45 percent indicated that health centers were in good condition while 8.18 percent noted that they were in excellent condition.

Table 5: Adequacy of healthcare center ranking based on staff competency

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	1.82	7.27	4.55	30.00	3.64	47.27
Mission hospital	0.00	0.00	0.91	4.55	0.00	5.45
Private hospital	0.00	3.64	1.82	9.09	4.55	19.09
Private maternity/clinics	0.00	1.82	1.82	5.45	0.91	10.00
Primary health care centers	0.00	0.00	0.00	4.55	0.91	5.45
Total	1.82	12.73	9.09	53.64	10.00	87.27

Source: Field survey, 2008

Table 5 shows that 30 percent and 3.64 percent of the respondents ranked general hospital as good and excellent respectively based on staff competency. Also, 9.09 percent and 4.55 percent of the respondents indicated that based on staff

competency private hospitals were in good and excellent conditions respectively. In all, 53.64 percent of the respondents ranked health centers' staff to be good while 8.18 percent noted that they were excellent.

Table 6: Adequacy of healthcare center ranking based on standard of services

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	2.73	5.45	9.09	22.73	7.27	47.27
Mission hospital	0.00	0.91	0.00	4.55	0.00	5.45
Private hospital	0.91	0.91	4.55	10.00	2.73	19.09
Private maternity/clinics	0.00	0.91	1.82	7.27	0.00	10.00
Primary health care centers	0.00	0.00	3.64	1.82	0.00	5.45
Total	3.64	8.18	19.09	46.36	10.00	87.27

Source: Field survey, 2008

Table 6 shows that 22.73 percent and 7.27 percent of the respondents ranked general hospital as good and excellent respectively based on adequacy of standard of services. Also, 10.00 percent and 2.73 percent of the respondents indicated that standard of

services in private hospitals were in good and excellent conditions respectively. In all, 46.36 percent indicated that standard of services in the health centers were in good condition, while 10.00 percent noted that they were in excellent condition.

Table 7: Adequacy of healthcare center ranking based on cost of services

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	2.73	6.36	10.00	23.64	4.55	47.27
Mission hospital	0.00	0.91	1.82	2.73	0.00	5.45
Private hospital	1.82	0.00	7.27	5.45	4.55	19.09
Private maternity/clinics	1.82	0.91	5.45	1.82	0.00	10.00
Primary health care centers	0.00	1.82	0.91	2.73	0.00	5.45
Total	6.36	10.00	25.45	36.36	9.09	87.27

Source: Field survey, 2008

Table 7 shows that 23.64 percent and 4.55 percent of the respondents ranked general hospital as good and excellent respectively based on cost of services. Also, 5.45 percent and 4.55 percent of the respondents indicated that cost of services in private

hospitals were good and excellent respectively. In all, 36.36 percent indicated that cost of services in the health centers were good, while 9.09 percent noted that they were excellent.

Table 8: Adequacy of healthcare center ranking based on drug availability

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	3.64	6.36	10.00	19.09	8.18	47.27
Mission hospital	0.00	0.91	0.00	4.55	0.00	5.45
Private hospital	0.00	0.91	1.82	10.91	5.45	19.09
Private maternity/clinics	0.91	2.73	1.82	4.55	0.00	10.00
Primary health care centers	0.91	1.82	0.91	1.82	0.00	5.45
Total	5.45	12.73	14.55	40.91	13.64	87.27

Source: Field survey, 2008

Table 8 shows that 19.09 percent and 8.18 percent of the respondents ranked general hospital as good and excellent respectively based on drug availability. Also, 10.91 percent and 5.45 percent of the respondents indicated that drug availability in

private hospitals were good and excellent respectively. In all, 40.91 percent indicated that drug availability in the health centers were good, while 13.64 percent noted that it was excellent.

Table 9: Adequacy of healthcare center ranking based on timeliness in patient attendance

Healthcare Providers	Poor	Fair	Average	Good	Excellence	All
General hospital	7.27	5.45	10.00	12.73	11.82	47.27
Mission hospital	0.91	0.00	0.00	1.82	2.73	5.45
Private hospital	2.73	4.55	1.82	4.55	5.45	19.09
Private maternity/clinics	0.91	0.00	5.45	2.73	0.91	10.00
Primary health care centers	0.00	1.82	0.91	1.82	0.91	5.45
Total	11.82	11.82	18.18	22.73	21.82	87.27

Source: Field survey, 2008

Table 9 shows that 12.73 percent and 11.82 percent of the respondents ranked general hospital as good and excellent respectively based timeliness in attending to patients. Also, 4.55 percent and 5.45 percent of the respondents indicated timeliness in attending to patients in private hospitals were good and excellent respectively. In all, 22.73 percent indicated that timeliness in attending to patients in the health centers were good, while 21.82 percent noted that it was excellent.

#### Awareness, willingness to pay for NHIS and their correlates

Table 10: Awareness about National Health Insurance Scheme (NHIS)

Awareness	Frequency	Percentage
Yes	60	54.55
No	50	45.45
Total	110	100

Source: Field survey, 2008

Table 10 shows that 54.55 percent of the respondents had heard about National Health Insurance Schemes (NHIS), while 45.45 percent were not aware at all. This means that more effort should be put in place to sensitize the aware of NHIS through the media, and social agents to most especially the farmers who may not have access to television or radio.

Table 11: Level of awareness of the respondents about NHIS

Level of Awareness	Frequency	Percentage
Very aware	20	18.18
Aware	22	20.00
Average aware	18	16.36
Not aware	50	45.45
Total	110	100

Source: Field survey, 2008

The level of awareness of National Health Insurance Schemes (NHIS) among the respondents as revealed in table 11 shows that only 18.18 percent of the sampled respondents were very aware of the schemes, while 20.00 percent were aware. Also,

16.36 percent indicated that they were just averagely aware. This could mean that the impact of NHIS is not felt by the respondents in Ilesha West.

Table 12: Willingness to pay for NHIS

Willing	Frequency	Percentage
Yes	79	71.82
No	31	28.18
Total	110	100

Source: Field survey, 2008

In table 12, 71.82 percent of the respondents were willing to pay for the scheme while 28.2 percent were not willing to pay. The respondents that did want to pay considered the scheme as a waste of money and time. Therefore, the sampled respondents that indicated willingness to pay considered the scheme to add value to their households and were optimistic about the workability of the scheme. Those that were not willing to pay viewed the scheme in the opposite.

The results of econometric analysis of the determinants of awareness and willingness to pay are presented in table 13. The model produced a good fit of the data as evidence by statistical significance of the Wald Chi square parameters ( $p < 0.01$ ). Inclusion of willingness to pay variable in the awareness model as an explanatory variable is also justified by the statistical significance of rho ( $p < 0.01$ ). This implies that estimation of the models as ordinary Probit regression would have yielded inefficient parameters. The results of the awareness model show that awareness significantly reduces willingness to pay. This implies that those households that were aware of the scheme prior to the research were not willing to pay. This may have resulted from detailed information already had. However, years of education parameters in the two models are with positive sign and statistically significant ( $p < 0.05$ ). This implies that education increases awareness and willingness to pay. Also, the parameters of sick time have positive sign and statistically significant ( $p < 0.10$ ) in the two models. This implies that as sick time increases, awareness and willingness to pay for NHIS increases. However, those households that

perceived that the scheme is workable in Nigeria have significantly higher probability of willingness to pay ( $p < 0.05$ ). This is expected because workability is going to be a major issue of concern for subscribing. Also, those that were of the opinion that the cost is okay have significantly higher probability ( $p < 0.05$ ) of being aware about NHIS. This is also expected because access to some information about cost

denotes that the person is aware and would have compared the cost across time. Also, households that indicated that they have preference for health insurance have significantly higher probability of willing to pay. This is expected because individual's preference will connote usage and willingness to subscribe.

Table 13: Recursive bivariate results of factors influencing awareness and willingness to pay for NHIS

Variables	NHIS Awareness			Willingness to pay		
	Coef.	Standard Error	z	Coef.	Standard Error	z
wtp	-1.156183	.2583424	-4.48	-	-	-
farming	-.0256672	.8390677	-0.03	-.0062623	.8025374	-0.01
sex	.0975138	.4676084	0.21	.1287649	.573509	0.22
age	.0196959	.0140394	1.40	.0309801	.0217185	1.43
yredu	.1169209	.0293944	3.98***	.0968292	.0416303**	2.33
size	-.0052113	.2746187	-0.02	-.0072302	.3472274	-0.02
totalear	.0000185	.0000109	1.69	.0000175	.0000154	1.14
sicktime	.2684846	.1331731	2.02**	.2956125	.1573923*	1.88
malaria	-.0432113	1.119365	-0.04	9.989241	5276725	0.00
hbp	-.6864567	.4447723	-1.54	.3208926	.5234628	0.61
treatcos	-9.42e-06	.0000155	-0.61	4.80e-06	.0000161	0.30
afford	-.3539805	.5822471	-0.61	-.301715	.6468158	-0.47
workable	.6378509	.4207933	1.52	1.298384	.5524058**	2.35
visithop	.347439	.3345236	1.04	.3315481	.4696631	0.71
coverage	-.0527206	.4278181	-0.12	.1033798	.5146399	0.20
costokay	.7957769	.4030503	1.97**	.6514492	.5288412	1.23
prefer	.4781107	.4410356	1.08	1.274108	.5428131**	2.35
cons	-2.119348	.7988408	-2.65***	-3.391445	1.357496***	-2.50
athrho	15.85368	566.1558	0.03			
rho	1	3.85e-11				
Likelihood-ratio test of rho=0, $\chi^2(1) = 12.7228$ Prob > $\chi^2 = 0.0004$						
Wald $\chi^2(33) = 71.47***$						
Log likelihood = -83.772487						

## Conclusion

Workability of NHIS will offer a lot of impacts on health service utilization and delivery in Nigeria. There is no reason to doubt the fact that implementation of such scheme was long overdue. However, sustainability is a major issue that can ensure that lasting impacts are felt by the scheme. This will also translate into better well-being by the people. The major findings of this paper have pointed to the need for better awareness creation and proper education of the people about potential efficiency gains in health service delivery and utilization. Awareness was still low in the study area although this also reduced willingness to pay. There is the need for adequate education through different media for addressing low willingness to pay among those that were ever aware. Also, ensuring workability of the scheme will lead to utilization of health services through the scheme. This is vital because if the

people are not sure about the extent of its workability, it will be difficult to persuade many to subscribe.

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## Stigma, Discrimination and Willingness to Buy Vegetables from People Living With HIV&AIDS (PLWHA) in Rural Nigeria

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**Abstract:** Stigma and discrimination have been identified as key propelling forces that fuel HIV transmission. This study analyzed factors explaining willingness of rural households to buy vegetables from PLWHA. The data were the Nigeria's 2008 Demographic and Health Survey (DHS) and were analyzed with the Multinomial logit regression. The results show that awareness about HIV&AIDS is high (83.60 percent). Misconceptions about HIV&AIDS included belief that HIV can be transmitted by mosquito bites (24.90 percent), sharing of food (18.00 percent) and witchcraft (18.1 percent). Only 29.1 percent of the respondents would buy vegetable from PLWHA and stigmatization is reduced with age, being tested, knowing someone that had died of HIV&AIDS ( $p < 0.01$ ). It was concluded that HIV stigma and discrimination can be reduced by properly educating people on the potential harms of discriminating attitudes, especially by targeting the youth.

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**Keywords:** stigma and discrimination, PLWHA, HIV&AIDS, rural households, Nigeria

### Introduction

With estimated 3.1 million people living with HIV&AIDS in 2010, Nigeria has the third highest number of people living with HIV&AIDS in the world after South Africa and India. Nigeria is experiencing multiple HIV epidemics which show different features across demographic groups, economic settings and geopolitical zones. The reasons for these differences are difficult to understand although socio-cultural factors may play a key role. The major mode of transmission among adult population in Nigeria is heterosexual intercourse although other forms of transmission like injecting drug users (IDU) and homosexuality cannot be completely eradicated (UNGASS, 2010). Since HIV is mostly transmitted through unprotected sexual intercourse, the most vulnerable are people in their sexually active age. Incidentally, these groups are those in the agriculturally productive age. Food and Agriculture Organization (FAO, 2000) estimated that out of the 27 most affected countries in Africa, 7 million agricultural workers had died of AIDS and more are likely to die in the next few decades.

HIV&AIDS affect economic growth by decreasing availability of human capital (Bell *et al*, 2003). Without good nutrition, health care and medicine that are accessible in developed countries, huge numbers of people suffer and die from AIDS-related problems. They will not only be incapable to work, but will also need substantial medical care. The prediction is that this will most likely cause a collapse of economies and societies in countries with

substantial AIDS population. The outbreak had left behind many orphans that are now looked after by aged and poor grandparents (Greener, 2002). The increased mortality has resulted in a smaller skilled population and labour force. This smaller labour force consists of increasingly younger people, with reduced knowledge and work experience causing decreased productivity. An upsurge in workers' spare time to take care of sick family members or for sick permission decreases productivity. Escalated mortality decreases the means that generate human capital and investment in people, via loss of income and the demise of parents.

According to Mann (1987) the phases of HIV&AIDS epidemic transmission are three vis-à-vis: the epidemic of HIV, the epidemic of AIDS, and the epidemic of stigma, discrimination and denial. However, it was noted that the third phase is "as central to the global AIDS challenge as the disease itself". Stigma refers to processes of devaluing, labelling, and stereotyping that result in loss of status, unfair and unjust treatment and social isolation (Campbell and Deacon, 2006). Discrimination against people with HIV&AIDS (PLWHA) results in oppression or unfair treatments. Within sociology, 'discrimination' is the prejudicial treatment of an individual based on their membership in a certain group or category. Discrimination is the actual behaviour towards members of another group. It involves excluding or restricting members of one group from opportunities that are available to other groups (Giddens *et al*, 2009).

Moral philosophers have defined discrimination as disadvantageous treatment or consideration. This is a comparative definition. An individual need not be actually harmed in order to be discriminated against. He or she just requires to be treated worse than others for some obvious or arbitrary reasons. Discrimination is often equated with stigma although not always so in reality. Some researchers have argued that discrimination is similar to enacted stigma which denotes the 'real experience of discrimination' (Brown *et al*, 2003; Jacoby, 1993). Major and O'Brien (2005) have argued that discrimination is an instrument of stigmatization, while Collymore (2002) stated that stigma and discrimination are two separate entities but closely linked.

Despite international efforts to tackle HIV&AIDS, stigma and discrimination remain among the most poorly understood aspects of the epidemic. Stigma had been identified as a challenge that prevents concerted action at community, national, and global levels (Piot, 2000). AIDS stigma occurs all over the world in a variety of ways, including ostracism, rejection, discrimination and avoidance of HIV testing without prior consent or protection of confidentiality; violence against HIV infected individuals or people who are alleged to be infected with HIV; and the isolation of HIV infected individuals (UNAIDS, 2006). Stigma-related violence prevents many people from seeking HIV testing, returning for their results, or securing treatment, probably changing what could be a controllable chronic disease into a death sentence and perpetuating the spread of HIV (Ogden and Nyblade, 2005).

Stigma and discrimination are principal factors contributing to HIV epidemic. For example, HIV-related stigma increases vulnerability to HIV infection by reducing access to HIV prevention and testing and presenting barriers to treatment, care and support for PLWHA (Mahajan *et al*, 2008). Morrison (2006) found that stigma and discrimination are recognized as two key factors that need to be tackled to create an effective and sustained response for HIV prevention, care, treatment and impact mitigation.

This paper seeks to assess the extent of stigmatization towards PLWHA in rural Nigeria. Specifically, we identified some misconceptions and knowledge gaps among rural households about HIV&AIDS. The paper also explored the socio-economic factors that influence discrimination against PLWHA by not willing to offer patronage if the person is selling vegetables. In the remaining parts of the paper, the materials and methods, results and discussion and conclusion were presented.

## Materials and Methods

## Area of the Study

Nigeria is in the West African sub-region, on the Gulf of Guinea, lying between latitudes 4°16' and 13°53' north and longitudes 2°40' and 14°41' east. It is bordered by Niger in the north, Chad in the northeast, Cameroon in the east, and Benin in the west. To the south, Nigeria is bordered by approximately 850 kilometres of the Atlantic Ocean, stretching from Badagry in the west to the Rio del Rey in the east. With a total land area of 923,768 square kilometres, Nigeria is the fourteenth largest country in Africa. The 2006 population and housing census put the population at 140,431,790.

## Methods of Data Collection

The study used the 2008 Demographic and Health Survey (DHS). These are survey-based secondary data that were collected with samples selected using stratified two-stage cluster design. There were 888 clusters comprising of 286 in the urban areas and 602 in rural areas. When the number of households was assigned to individual state, the numbers of clusters, which was calculated based on an average sample take of 41 completed interviews, was calculated by dividing the total sample in the state. The data that were used consist of 12766 individuals in the rural areas.

## Methods of Data Analysis

### Estimated model

Multinomial logistic regression analysis was used to determine the factors explaining willingness not to buy vegetables from PLWHA. Multinomial logistic regression method was used because it provides an interpretable linear model for a categorical dependent variable and allows the testing of the significance of a given predictor whilst controlling for all other predictors in the model. The model can be expressed as:

$$Z_i = \ln \frac{P(Y_i=m)}{P(Y_i=n)} = \beta_o + \sum_{k=1}^K \beta_k x_{ki} + \varepsilon \quad \dots 1$$

Where  $Z_i$  – Discriminatory attitudes of people to PLWHA which are would not buy vegetables from vendor with AIDS,  $P$  is probability,  $Y_i$  is dependent variable for any observation  $i$  with  $m$  being response of 1 if "yes", 2 if "no" and 3 if "don't know".  $\beta_o$  is the baseline constant and  $\beta_k$  are the corresponding vector of estimated regression coefficients.  $x_{ki}$  is an array of  $k$  independent variables which are  $x_1$  for age (years);  $x_2$  is sex of the respondent (female = 1, 0 otherwise);  $x_3$  is educational level (years);  $x_4$  is North East regional dummy (yes = 1, 0 otherwise);  $x_5$  is North West regional dummy (yes = 1, 0 otherwise);  $x_6$  is South East regional dummy (yes = 1, 0 otherwise),  $x_7$  is South West regional dummy (yes = 1, 0 otherwise),  $x_8$  is South South regional dummy (yes = 1, 0

otherwise);  $x_9$  – Ever heard of AIDS (yes = 1, 0 otherwise);  $x_{10}$  is ever been tested for AIDS (yes = 1, 0 otherwise);  $x_{11}$  is know someone who has or died of AIDS (yes = 1, 0 otherwise);  $x_{12}$  is reduce the chances of AIDS by having only one sex partner (yes = 1, 0 otherwise);  $x_{13}$  is get AIDS from mosquito bites (yes = 1, 0 otherwise);  $x_{14}$  is reduce chances of AIDS by always using condoms during sex (yes = 1, 0 otherwise);  $x_{15}$  is get AIDS by sharing food with person who has AIDS (yes = 1, 0 otherwise);  $x_{16}$  is reduce risk of getting AIDS by not having sex at all (yes = 1, 0 otherwise);  $x_{17}$  is can get AIDS by witchcraft or supernatural means (yes = 1, 0 otherwise);  $x_{18}$  is can a healthy person have AIDS (yes = 1, 0 otherwise);  $x_{19}$  is can AIDS be transmitted during pregnancy (yes = 1, 0 otherwise);  $x_{20}$  is can AIDS be transmitted during delivery (yes = 1, 0 otherwise);  $x_{21}$  is can AIDS be transmitted during breastfeeding (yes = 1, 0 otherwise); and  $\varepsilon$  is the error term.

The  $\beta_s$  are typically estimated by the maximum likelihood (ML) method, which is preferred over the weighted least squares approach (Schlesselman, 1982). The ML method is designed to maximize the likelihood of reproducing the data given the parameter estimates. Data are entered into the analysis as 0 or 1 coding for the dichotomous outcome, continuous values for continuous predictors, and dummy coding (e.g., 0 or 1) for categorical predictors. The value of the coefficient  $\beta$  reveals the direction of the relationship between  $x$  and logits of  $Z$ . When  $\beta$  is greater than 0, larger (or smaller)  $x$  values are associated with larger (or smaller) logits of  $Z$ , and the curve will resemble an increasing sigmoid (or S-shape). Conversely, if  $\beta$  is less than 0, larger (or smaller)  $x$  values are associated with smaller (or larger) logits of  $Z$ . Such a relationship is shown in data in the form of a reverse sigmoid curve. In other words, an increase in  $x$  is associated with a decrease in logits of  $Z$  and vice versa.

The dependent variable categories are ‘yes’, ‘no’ and ‘don’t know’ with ‘no’ being the reference point. One regression was run for each category (yes and don’t know) to predict the probability of  $Y_i$  (dependent variable for any observation  $i$ ) being in that category. Then the probability of  $Y_i$  being in category 0 is given by the adding up constraint that the sum of the probability of  $Y_i$  being in the various categories equals 1.

## Results and Discussion

### Respondents’

### Characteristics

The age group of the respondents were presented in table 1. This shows that the highest

### Socio-Economic

percentage of respondents interviewed were in the age group of 25 – 29 years with 17.7% followed by age group of 30 – 34 with 16.9%, 16.1% were aged 35 – 39 years, 12.9% were aged 40 – 44 years, 11.1% were aged 20 – 24 years, 10.3% were aged 45 – 49 years, 5.5% were aged 15 – 19 & 50 – 54 years, and 3.9% were aged 55 – 59 years. However, respondents in the age group of 50 – 54 and 55 – 59 years were only male. This shows that that only female respondents between 15 and 49 years were interviewed. The median age of the respondents is 34 years. It also showed that respondents of 30 years of age were the ones that were mostly interviewed.

Table 1: Frequency distribution of respondents by their age group

Age group	Frequency	Percent
15 – 19	707	5.5
20 – 24	1417	11.1
25 – 29	2262	17.7
30 – 34	2160	16.9
35 – 39	2058	16.1
40 – 44	1650	12.9
45 – 49	1319	10.3
50 – 54	701	5.5
55 – 59	492	3.9
Total	12766	100.0

Source: NDHS, 2008

Respondents with no education constitute majority of the sample population (table 2). The distribution indicated that 53.3% were not educated, 23.8% had primary education, and 17.9% had secondary education while the least were those with higher with 5.0%. Lack of education ultimately brings about misconception about the disease and the unwillingness to take care of PLWHA which made them to be treated as a plague.

Table 2: Frequency distribution of respondents by their educational level

Education Level	Frequency	Percent
No education	6806	53.3
Primary	3041	23.8
Secondary	2281	17.9
Higher	638	5.0
Total	12766	100.0

Source: NDHS, 2008

With respect to the region (table .3), respondents in the North West part of the country were the most interviewed with 33.6%, followed by North East with 24.9%, North Central with 18.9%, South West with 9.4%, South South and South East with 8.0% and 5.2% respectively. This indicated that greater part of the sample population interviewed came from the northern part of the country.

Table 3: Frequency distribution of respondents by their region

Region	Frequency	Percent
North Central	2410	18.9
North East	3178	24.9
North West	4290	33.6
South East	664	5.2
South West	1200	9.4
South South	1024	8.0
Total	12766	100.0

Source: NDHS, 2008

Muslims constituted over half of the sample population. This could most likely be due to the fact that a high population of the respondents were in the northern part of the country. The distribution (table 4) indicated that 63.1% were Muslims and 2.3% were adherent to traditional faiths. Christianity was further divided into two groups: Catholics and other Christians (which can be referred to as the Protestants). The Protestants constituted the majority (27.4%) of the Christian respondents, while the rests were Catholics (7.1%). However, the last group was others with 0.1% of the respondents. This could be those who did not have a major belief.

Table 4: Frequency distribution of respondents by their religion

Religion	Frequency	Percent
Catholic	899	7.1
Islam	8012	63.1
Other Christians	3473	27.4
Traditionalist	296	2.3
Others	18	0.1
Total	12698	100.0

Source: NDHS, 2008

As regards wealth index, table 5 indicated 35.8% of the respondents were in the poorest category, which accounted for the majority of the respondents interviewed, followed by those in the poorer category with 29.2%, the middle category with 19.2%, the richer category with 11.1%. This revealed that majority of the rural population interviewed were living in poverty.

Table 5: Frequency distribution of respondents by their wealth index

Wealth Index	Frequency	Percent
Poorest	4570	35.8
Poorer	3722	29.2
Middle	2454	19.2
Richer	1412	11.1
Richest	608	4.8
Total	12766	100.0

Source: NDHS, 2008

### Levels of Knowledge and Misconceptions

The levels of knowledge and misconceptions were indicated in table 6. It showed that 16.4% of the respondents had not heard of the disease while 83.6% had heard about it. This indicated that a large

proportion of the population were conscious of the presence of the disease. However, 90.2% of these respondents had not been tested for the disease while 9.8% had been tested. This could arise due to the fear of discrimination if eventually they had the disease.

Likewise, 64.5% of the respondents did not know anyone with the disease or anyone that died of the disease, 33.3% knew someone with the disease while 2.2% did not know whether they knew anyone or not. 83.9% believed that someone can reduce the chances of getting AIDS by having only one sex partner who has no other partner, 7.2% did not believe it can be reduced, while 8.9% did not know whether it can be reduced or not by having just one sex partner. Furthermore, 24.9% indicated that a person can get AIDS from mosquito bites, 56.9% showed that one cannot get the disease from mosquito bites while 18.3% did not know whether it can be transferred through this method or not.

Also, 63.7% of the respondents knew that chances of getting HIV/AIDS can be reduced by using condoms during sex, 13.9% said no while 22.4% said they did not know. 67.8% indicated that they would not get AIDS by sharing food with PLWHA, 18.0% said they would get AIDS by sharing meals with PLWHA while 14.2% said they did not know. With respect to the knowledge about reducing the risk of getting AIDS by not having sex at all, 82.1% said it can be reduced, 10.9% of the respondents said it cannot be reduced, while 7.0% said they did not know. Fascinatingly, 60.6% of the respondents believed that a person cannot get AIDS by witchcraft or supernatural means, 18.1% said a person can get AIDS by witchcraft or other supernatural means while 21.3% said they did not know.

Furthermore, 74.5% of the respondents believed that a healthy looking person can have AIDS, 15.1% believed otherwise, while 10.4% did not know. 51.7% reported that AIDS can be transmitted from a mother to her unborn child while in the course of pregnancy, 19.8% and 28.5% said that no and don't know respectively. About 46.4% of the respondents indicated that AIDS can be transmitted during childbirth, 23.4% showed that AIDS cannot be transferred from mother to child at birth while 30.2% said they did not know. Likewise, 55.7% of the respondents believed that AIDS can be transmitted during breastfeeding, 16.2% believed otherwise, while 28% said they did not know anything about it.

The findings revealed that majority of the respondents in this study had heard of the disease and an encouraging percentage also had good knowledge about its mode of transmission.



Table 6: Frequency distribution of respondents by their knowledge and misconceptions about HIV transmission

Characteristics	Frequency	Percent
Have you ever heard of AIDS?		
No	2087	16.4
Yes	10676	83.6
Total	12763	100.0
Have you ever been tested for AIDS?		
No	10743	90.2
Yes	1171	9.8
Total	11914	100.0
Did you know someone who has or died of AIDS?		
No	6853	64.5
Yes	3538	33.3
Don't know	234	2.2
Total	10625	100.0
Can a person reduce HIV/AIDS infection by having one partner?		
No	771	7.2
Yes	8920	83.9
Don't know	944	8.9
Total	10635	100.0
Can a person get AIDS from mosquito bite?		
No	6060	56.9
Yes	2649	24.9
Don't know	1947	18.3
Total	10656	100.0
Can condom reduce contracting HIV/AIDS infection?		
No	1478	13.9
Yes	6777	63.7
Don't know	2383	22.4
Total	10638	100.0
Can a person get HIV infection by sharing meal with PLWHA?		
No	1921	18.0
Yes	1508	14.2
Don't know	10652	100.0
Total	1159	10.9
Total	8732	82.1
Can a person reduce of getting AIDS by not having sex at all?		
No	745	7.0
Yes	10636	100.0
Don't know		
Total	6450	60.6
Total	1925	18.1
Total	2260	21.3
Can a person get AIDS through witchcraft or other supernatural means?		
No	10635	100.0
Yes		
Don't know		
Total	1606	15.1
Total	7913	74.5
Total	1102	10.4
Total	10621	100.0
Can a healthy looking person be infected with AIDS?		
No	2112	19.8
Yes	5518	51.7
Don't know	3034	28.5
Total	10664	100.0
Can HIV/AIDS be transmitted during pregnancy?		
No	2498	23.4
Yes	4945	46.4
Don't know	3218	30.2
Total	10661	100.0
Can HIV/AIDS be transmitted during child birth?		
No	1732	16.2
Yes	5941	55.7
Don't know	2991	28.0
Total	10664	100.0
Can HIV/AIDS be transmitted during breastfeeding?		
No		
Yes		
Don't know		
Total		

Source: NDHS, 2008.

### Stigma and Discriminatory Attitudes

Table 7 shows that a high proportion of the respondents (68%) would not buy vegetables from vendors with AIDS indicating farmers with AIDS that sell their produce are at a loss as people would not from them after knowing their AIDS status.

Table 7: Percentage distribution of people that would buy vegetables from PLWHA

Characteristics	Frequency	Percent
No	7243	68.0
Yes	3105	29.1
Don't know	311	2.9

Source: NDHS, 2008.

### Multivariate Analysis of Discriminatory Attitudes towards PLWHA

The multinomial regression analysis carried out in this study had dependent variables with three categories which were ordered (i.e. yes, no, don't know). These were the responses with the answer "no" as the reference category. Table 8 shows the relationship between the respondents' willingness to buy vegetables from vendor living with HIV/AIDS and selected characteristics. The model specification was statistically significant ( $\chi^2 = 11154.53$ ;  $p < 0.00001$ ).

The two equations in the table were labelled by the group they contrast to the reference group. The first equation was labelled "Yes", and the second equation was labelled "Don't know". The variables that had a statistically significant relationship to distinguish respondents that answered yes from those that answered no; at 1% were age, education level, North West region, South South region, ever heard of AIDS, ever been tested for AIDS, knows someone who has or died of AIDS, a healthy looking person can be infected with AIDS and HIV/AIDS can be transmitted during child birth; at 5% was sex and at 10% was a person can get AIDS through witchcraft/other supernatural means. The variables that had a statistically significant relationship to distinguish respondents that answered don't know from those that answered no; at 1% was ever heard of AIDS: at 5% were sex, North East region, South East region, ever been tested for AIDS and a person can reduce risk of getting AIDS by not having sex at all; and at 10% were a person can reduce HIV/AIDS infection by having one partner, a person can get HIV infection by sharing meal with PLWHA, a person can get AIDS through witchcraft/other supernatural means and HIV/AIDS can be transmitted during child birth.

Respondents that answered yes revealed that older people show more willingness to buy vegetable from vendors with HIV/AIDS than younger people. Male respondents also showed more willingness than their female counterparts, indicating that female respondents were not willing to buy vegetables from



vendors with AIDS. Educated populations with presumably greater knowledge of the disease were expected to be less hostile to PLWHA i.e. discriminatory attitudes towards vendors with AIDS reduces with increasing levels of education.

Furthermore, the findings indicated that population in the North Western part of the country would be willing to buy vegetables from vendors living with AIDS than their counterparts from the North Central region while the South South region showed discriminatory attitudes. Respondents who had heard of AIDS are less likely to buy vegetables from vendors with AIDS showing a misconception about the disease. Also, respondents who had been tested for AIDS were willing to buy vegetables from vendors with AIDS indicating that they had enough knowledge of the disease. Respondents that knew people who had AIDS or died of AIDS would be willing to buy vegetables from vendors with AIDS.

Interestingly, respondents who believed that a person can get AIDS from witchcraft or other supernatural means would show discriminatory attitudes towards vegetable vendors with AIDS. This means that they had misconception about the disease which is due to their cultural beliefs. A statistically significant proportion of the population who believed that a healthy looking person could be infected with AIDS were more likely, than those who believed

otherwise, to buy vegetables from vendors with AIDS. Also, respondents who had the knowledge that AIDS can be transmitted through breastfeeding would be willing to buy vegetables from vendors with AIDS. Although this did not apply to those that believed that AIDS can be transferred during pregnancy or at childbirth as these were insignificant.

For respondents that answered ‘don’t know’, it was revealed that male respondents do not know whether to buy vegetables from vendors with AIDS or not, while female respondents would show discriminatory attitudes. Respondents in the South East were more likely not to know whether to buy vegetables from vendors with AIDS than those in the North Central region while respondents from the North East would show discriminatory attitude than those of North Central.

Furthermore, respondents that had ever heard of AIDS, been tested for AIDS, that believed that AIDS can be reduced by having sex with only one partner, that believed that AIDS can be transmitted by sharing meals with PLWHA, that confirmed that AIDS can be reduced by having no sex at all, that believed that a person can get AIDS from witchcraft or other supernatural means and those that believed that AIDS can be transferred at childbirth would not buy vegetables from vendors with AIDS. This showed that exhibit discriminatory attitude.

Table 8: Factors explaining willingness to buy vegetables from vendor living with HIV/AIDS

Explanatory Variables	Yes		Don't know	
	Coefficients	Standard errors	Coefficients	Standard errors
Age	0.008***	0.003	-0.003	0.007
Sex	-0.113**	0.052	-0.299**	0.144
Education level	0.075***	0.005	-0.025	0.017
Region				
North East	0.103	0.074	-0.549**	0.241
North West	0.484***	0.071	0.253	0.201
South East	0.025	0.105	0.560**	0.304
South West	-0.137	0.090	0.424	0.262
South South	-0.382***	0.091	0.083	0.283
Ever heard of AIDS	-1.199***	0.145	-25.782***	0.365
Ever been tested for AIDS	0.433***	0.071	-0.869**	0.374
Known someone who has or died of AIDS	0.414***	0.060	-0.002	0.212
A person can reduce HIV/AIDS infection by having one partner				
A person can get AIDS from mosquito bite	-0.067	0.0073	-0.291*	0.159
Condom can reduce contracting HIV/AIDS infection	-0.300	0.056	-0.137	0.161
A person can get HIV infection by sharing meal with PLWHA	0.379	0.055	-0.093	0.144
A person can reduce risk of getting AIDS by not having sex at all				
A person can get AIDS through witchcraft/other supernatural means	-0.529	0.067	-0.343*	0.182
A healthy looking person can be infected with AIDS				
HIV/AIDS can be transmitted during pregnancy	-0.012	0.068	-0.388**	0.154
HIV/AIDS can be transmitted during child birth				
HIV/AIDS can be transmitted during breastfeeding	-0.105*	0.063	-0.358*	0.198
Constant				
	0.830***	0.062	0.051	0.139
	-0.061	0.066	-0.214	0.207
	0.217***		-0.416*	
	-0.005	0.067	0.119	0.216
	-1.087	0.060	23.804	0.187

Significance level: \*\*\* p<0.01, \*\* p<0.05, \*p<0.1

Chi-square (42) = 11154.53; p<0.00001; -2 log likelihood = -7220.2724; Pseudo R<sup>2</sup> = 0.4358.

## Conclusion

AIDS poses serious threat to agricultural production in Nigeria. Stigma and discriminatory attitudes towards people living with HIV/AIDS in rural Nigeria reduces their productivity as well as having serious economic implications on the part of the producers, the sellers and the nation at large. A vegetable seller with AIDS (in most cases could be farmer) who could not sell his/her products and whose family are not willing to take care of him/her because of the discriminatory attitudes they have against him would be financially and economically unbalanced.

There is need for programmes to educate the general public that while AIDS remains incurable, it is possible for people living with the virus to live functional and economically viable lives with adequate nutritional and psychosocial support, rest, treatment of opportunistic infections and antiretroviral therapy. Traditionally, women are the primary care givers in the African community (Nigeria inclusive); hence the greater burden of providing care falls on them. Their increased domestic work load and insufficient psychological and social support may lead to resentment that involuntarily reinforces stigmatizing behaviour. Efforts need to be made to ensure that gender inequalities are not heightened while designing care and support programmes. Further qualitative research is needed to give better understanding of the reasons why females appear to have more stigmatizing attitudes.

Public education must play a key role in the success of prevention programmes and increase in the uptake of HIV testing. Behavioural change programmes must encourage individuals to reduce their risk of HIV acquisition. The stigmatization and discrimination that have typified societal responses must be dealt with promptly, as they compromise the effectiveness of prevention programmes. The early involvement and support of the government in the HIV/AIDS campaign can set the necessary groundwork for a continuing strong leadership that will be critical for initiating and sustaining an effective nationwide prevention programme. Stigmatization and discrimination against PLWHA are common in Nigeria. Often both Christian and Muslim religious leaders view immoral behaviour as the cause of the HIV/AIDS epidemic. PLWHA often lose their jobs or are denied health care services because of the ignorance and fear surrounding the disease. There is need for increased national campaigns and more visible and vocal societies and support groups for people infected with or affected by HIV as well as education of the public about HIV/AIDS in a bid to dispelling myths and giving the disease a human face.

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## Factors Explaining Acute Malnutrition Among Under-Five Children in Sub-Sahara Africa (SSA)

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**Abstract:** Child malnutrition poses serious challenge to economic development in many SSA countries. This study analyzed the factors predisposing children to wasting in selected SSA countries. Anthropometrics data for children in Gambia, Niger, Comoros, Central African Republic, Lesotho and Swaziland were analyzed using Z-score and Probit regression. Results show that wasting is highest in Niger (12.69 percent). Probit analysis shows that attainment of secondary education by the mothers, urbanization, presence of pipe water, vaccination, and mother's access to radio and television significantly reduce the probability of wasting, while infection with diarrhea, fever and age at first polio vaccine significantly increase it ( $p < 0.10$ ). Concerted efforts to reduce malnutrition must focus on provision of health facilities in the rural areas, promotion of women education, promotion of enlightenment programs on the need for child immunization and ensuring cleanliness in caring, among others.

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**Keywords:** wasting, secondary education, immunization, SSA

### 1. Introduction

The state of child malnutrition in many developing countries has significantly worsened over the past few decades (ACC/SCN, 2000). Because mothers were either ill or malnourished, about 24 million babies are annually born with small weight unable to sustain healthy living (de Onis *et al*, 1998, UNICEF, 1998). Out of under-five children in developing world, 206 million are stunted (low height for age), 50 million are wasted (low weight for height), and 167 million are underweight (low weight for age) due to lack of sufficient food and the presence of diseases (de Onis *et al*, 1997).

In Africa, several studies have shown that the goal of overcoming malnutrition among children, which has recurrently featured in several policy statements is yet to be achieved (WHO, 2000; UNICEF, 2000; World Bank, 2004; Silva, 2005). Despite several national and international awakening and assistance, child's health is still in a state of crisis in many SSA countries. Although some improvements had been recorded over the last fifty years, these have been slower than what was had in other regions of the world.

The incidence of wasting, which reflects the acuteness of malnutrition had been widely used to characterize the nutritional status of children (DCS, 2003; Wise, 2004; Silva, 2005). Wasting reflects a deficit in weight relative to height due to a deficit in tissue and fat mass (Fernandez *et al*, 2002). Epidemiological evidence suggests that the first response to a nutritional deficiency and/or disease infectious is weight loss (wasting), and this will be followed by retardation in linear growth (stunting). If

the infection persists, children will cease to grow in height and will lose weight, thus augmenting the process and prevalence of wasting (Fernandez *et al*, 2002; Wise, 2004).

This study is important because in the United Nations MDGs, which was unanimously adopted in September 2000, and latter accepted as a policy guide and framework for economic planning in many developing countries, child matters on issues of nutrition, health and mortality are very prominent. The objective of the study is to analyze the factors predisposing under-five children in some SSA countries to wasting? This will provide the needed directions to African policy makers on the way of reducing the problem of wasting in order to reduce child mortality and work towards meeting the MDGs.

### 2. Theoretical Framework and Literature Review

UNICEF (1990; 1998) analyzed the determinants of wasting within a framework that incorporate some underlying causes like inadequate health services, unhealthy environment, inadequate household food security, and inadequate mother- and child-caring practices. It was noted that most of the underlying causes are directly linked to some basic (or structural) factors that are political, legal, and cultural in nature. In like manner, Chevassus-Agnès (1999) presented a framework for understanding the causes of malnutrition (fig. 1). This identified key factors as access to food, care practices, and health and sanitation as factors ultimately determining nutritional status.

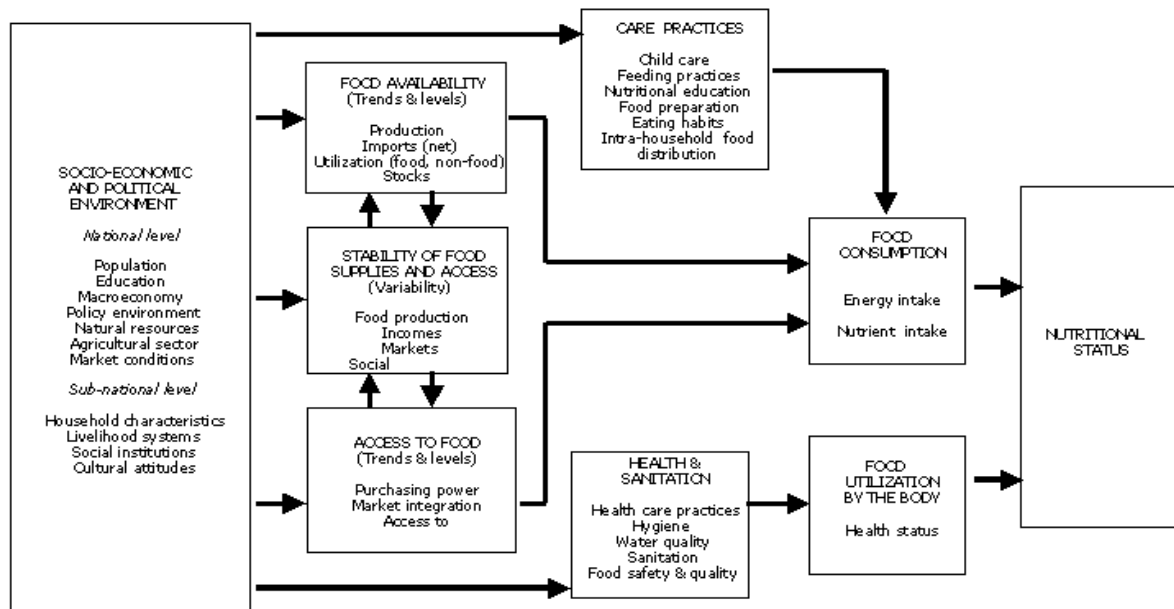


Figure 1: A conceptual framework for the determinants of child nutrition  
Source: Chevassus-Agnès, (1999).

Based on the framework presented above, many studies have analyzed the determinants of malnutrition. Specifically, Gunasekara (1999) used anthropometrics indices of weight-for-height to assess the nutritional status of children in Sri Lanka. It was found that employment status of mother, number of living children, the level of education of the mother and non-involvement of the mothers in the rural labour market significantly affected wasting problem in children.

ORC Macro (2001) found that in Africa, Malawi (2000 data), Tanzania (1999 data), Uganda (2000/2001 data) and Zimbabwe (1999 data) have prevalence of wasting being 5.5 percent, 5.4 percent, 4.0 percent, and 6.4 percent respectively. It is only in Malawi that wasting prevalence was higher among female children.

MINECOFIN (2002) also found that in Rwanda (using 2001 dataset), prevalence of wasting was 3.4 percent, but male children have a slightly higher prevalence. Wise (2004) further reported that using the same dataset, approximately 39 percent of Rwanda children between 12-17 months and 28 percent of children between 18-23 months were reported to have suffered from diarrhea two weeks prior to the survey. These two age groups also reported the highest prevalence of wasting (6.8 percent and 6.6 percent respectively). After the second year of life, the percentage of children reported to have suffered from diarrhea declines with

age. However, urban children experience a higher prevalence of wasting (4.1 percent) than children in rural areas (3.2 percent).

Fernandez *et al* (2002) found that low birth weight (LBW), measles incidence, and access to a safe water supply explained 64% of wasting variability in Asia. In Latin America, LBW and survey year explained 38% in Africa, LBW, survey year, and adult literacy explained 7%. Also, vitamin A had been identified as the growth-promoting factor "A." (Rivera *et al*, 2003).

Department of Census and Statistics (DCS) (2003) found that in Sri Lanka, the key factor explaining wasting in children were age of the child, sector of residence (urban or rural), work status of mother, access to media by the mother, mother's educational level and type of toilet. Also, wasting is influenced by number of living children in the family and whether the mother washes her hand after the child defecated, access to safe drinking water and sex of the child.

Ekanayake *et al* (2004) determined the factors influencing child malnutrition in Sri Lanka. Their findings revealed that while mother's nutritional awareness and total income are negatively related to child's malnutrition, mother's age and interest in media have positive significant relationship. Silva (2005) also analyzed the determinants of child malnutrition in Ethiopia. The study revealed that child's age,



mother's height, household wealth, education of mothers and access to good water explain to a large extent the nutritional status of children.

### 3. Methodology

#### Source of data

The study used the data set available for some developing countries on child's anthropometrics information. The data were collected in 2000 End-Decade Multiple Indicator Cluster Survey with the financial support from the United Nations International Children Emergency Funds (UNICEF). The requested information is robust in every respect comprising of child anthropometrics data, disease infection, mother's socio-economic characteristics, housing condition and participation in different forms of vaccination programs, among others. The samples in all the countries were selected in two stages. At the first stage, clusters were selected with probability proportional to size. After a household listing was obtained for the selected clusters, systematic samples were drawn. African countries for which data were analyzed in this study are Gambia, Niger, Central Africa Republic, Comoros, Lesotho, and Swaziland (see table 1 for the sample distribution). These countries were selected based on region and completeness of the needed variables.

Table 1: Distribution of Samples and Household Members in the Selected SSA Countries

Country	Gambia	Niger	Central Africa Republic	Comoros	Lesotho	Swaziland
Completed Samples	4190	4321	11452	3677	7471	4190
Household members	24213	26256	92466	27060	32744	24213
Total Women	5271	5664	14300	5242	6741	5271
Women included for analysis	4256	4331	12636	3687	4709	3730
Total Children	3509	5080	16964	4870	3737	3509
Children included for analysis	2474	4545	12094	3044	3083	3163

#### Analytical Models

##### Z-Score indices of prevalence of malnutrition

The z-score was used to carry out the analysis of child's malnutrition. This is represented as:

$$Z_i = \frac{X_i - \mu}{\sigma}$$

Where i refers to individuals (children) and j = 1...3 with  $Z_i$  = weight-for-height nutrition index, and  $Z_i$  = z-score weight-for-age nutrition index,  $X_i$  = observed value for the ith child,  $\mu$  = mean value of the reference population, and  $\sigma$  = standard deviation of the reference population. A z-score of -2 standard deviation is the most commonly adopted cut off for all nutrition indicators. Consequently, children with z-scores below (-2) are considered to be moderately or severely malnourished.

#### Probit Analytical Approach

The Probit regression was used to analyze the socio-economic determinants of the health outcomes using Limdep 7.0 software. The dependent variables ( $Y_{ij}$ ) were binary variables with value of 1 if malnourished in respect of wasting (z-score less than -2) and 0 otherwise. Following Zere and McIntyre (2003), outliers were excluded based on the recommendation of WHO (1995). Therefore, weight-for-height z score less than -4 and greater than +5 were excluded. The estimated model is stated as:

$$Y_{ij} = \phi_j + \sum_{i=1}^6 \beta_i M_i + \sum_{i=1}^4 \phi_i H_i + \sum_{i=1}^4 \gamma_i D_i + \sum_{i=1}^5 \theta_i V_i + \sum_{i=1}^4 \mu_i P_i + \sum_{i=1}^8 \rho_i A_i + v_j$$

Where:

- $M_1$  = sex (male 1, 0 otherwise)
- $M_2$  = urbanization (urban centers = 1, 0 otherwise)
- $M_3$  = age of child (months)
- $M_4$  = hour of early learning (minutes)
- $M_5$  = mother's secondary education ( $\geq$  secondary = 1, 0 otherwise)
- $M_6$  = mother's primary education ( $\geq$  primary = 1, 0 otherwise)
- $H_1$  = ever received vitamin A (yes =1, 0 otherwise)
- $H_2$  = month since last dose of Vitamin A
- $H_3$  = ever breastfed (yes =1, 0 otherwise)
- $H_4$  = currently breast-feeding (yes =1, 0 otherwise)
- $D_1$  = diarrhea in last 2 weeks (yes =1, 0 otherwise)
- $D_2$  = cough in last 2 weeks (yes =1, 0 otherwise)
- $D_3$  = fever in last 2 weeks (yes =1, 0 otherwise)
- $D_4$  = sleep under bed net (yes =1, 0 otherwise)
- $V_1$  = ever given BCG vaccines (yes =1, 0 otherwise)
- $V_2$  = age at first polio vaccine
- $V_3$  = number of polio vaccine
- $V_4$  = number of DPT vaccine
- $V_5$  = ever given measles vaccine (yes =1, 0 otherwise)
- $P_1$  = mother alive (yes =1, 0 otherwise)
- $P_2$  = mother at home (yes =1, 0 otherwise)
- $P_3$  = father alive (yes =1, 0 otherwise)
- $P_4$  = father at home (yes =1, 0 otherwise)
- $A_1$  = pipe water (yes =1, 0 otherwise)
- $A_2$  = sanitary toilet (yes =1, 0 otherwise)
- $A_3$  = non-iodized salt (yes =1, 0 otherwise)
- $A_4$  = tile as floor material (yes =1, 0 otherwise)
- $A_5$  = electricity (yes =1, 0 otherwise)
- $A_6$  = radio (yes =1, 0 otherwise)
- $A_7$  = television (yes =1, 0 otherwise)
- $A_8$  = refrigerator (yes =1, 0 otherwise)
- $v_j$  = error term

The first hypothesis is that education of mothers does not significantly decrease wasting



among children, while the second is that number of polio vaccine does not significantly reduce wasting.

#### 4. Results and Discussions

##### *Wasting Prevalence*

Table 2 shows the indices of wasting computed for some countries in SSA. The mean of the weight-for height z-score (WHZ) is highest in Swaziland with 0.4953 and the percentage of wasting is lowest there with 1.13 percent. The wasting

prevalence were also computed for rural children and urban children. Among all the countries, wasting prevalence is highest among all the children, rural children and urban children in Niger with 12.69 percent, 14.19 percent and 10.21 percent respectively. Whether for rural or urban children, Swaziland has the lowest wasting prevalence.

Table 2: Prevalence of Wasting Based on Child's Sex and Sector of the Economy

Malnutrition Variable/ Country	Gambia (n = 2474)	Niger (n = 4545)	Central African Republic (n = 12094)	Comoros (n = 3044)	Lesotho (n = 3083)	Swaziland (n = 3163)
Mean Z score (WHZ)	-0.4978	-0.8616	-0.1314	0.1811	0.3179	0.4953
Std deviation	1.1647	1.0605	1.3969	1.7692	1.4435	1.13
All children	8.60	12.69	8.19	10.22	4.96	1.20
Rural Sector	8.58	14.19	8.36	10.34	4.95	1.31
Urban Sector	8.75	10.21	7.91	9.97	5.08	0.69

##### *Determinant of Wasting*

The analysis of the socio-economic factors explaining wasting in under-five children is presented in tables 3a and 3b. The results show that male children in Niger and Central Africa Republic have significantly higher probability of wasting, while the opposite applies for Swaziland. This goes in line with the finding of ORC Macro (2001). In Comoros and Swaziland, children from urban centers have significantly lower probability of wasting than those

in the rural areas. This difference had been traced to the presence of health facilities and income generating opportunities in the urban areas {United State Agency for International Development (USAID) (2000)}. This goes in line with the findings of Yimer (2000), but contradicts that of Wise (2004) for Rwanda.

Also, as the children grow older, their probability of wasting significantly reduces in Niger and Comoros. Wise (2004) had similar finding and explained Table

3a: Probit Regression of the Determinants of Wasting (WHZ) Among Children in Sub-Saharan Africa

Country/Variables	Gambia		Niger		Central African Republic	
	Parameter	t-value	Parameter	t-value	Parameter	t-value
Constant	0.110953**	2.196	0.043876	0.742	0.053873**	2.471
Sex	0.001632	0.186	0.030855*	3.455	0.009379*	3.065
Urbanization	0.011081	0.823	-0.005992	-0.396	0.003397	0.906
Age of child	-0.000052	-0.118	-0.001065**	-2.344	0.000072	0.710
Hour of early learning	-0.00167***	-1.860	0.000993	0.723	-0.000212**	-2.538
Mother's primary education	-0.03366***	-1.778	0.016108	0.955	-0.010077*	-2.829
Mother's secondary education	-0.022526*	-2.815	-0.021965*	-2.706	-0.002836	-0.528
Vitamin A	0.003470	0.219	-0.000912	-0.220	-0.000708**	-2.162
Month since last dose of Vitamin A	-0.001585	-1.136	0.001855*	2.634	-0.000811	-1.495
Ever breast fed	-0.032786	-0.738	-0.000901	-0.030	-0.024876**	-2.548
Currently breast feeding	-0.007795	-0.536	0.062385*	3.941	-0.004880	-1.235
Diarrhea in last 2 weeks	0.01022**	1.981	0.038717*	3.895	0.010056*	2.770
Cough in last 2 weeks	0.01537***	1.828	0.012495**	2.129	0.001556**	2.458
Fever in last 2 weeks	0.001223**	2.094	0.037970*	3.786	0.001939	0.556
Sleep under bed net	-0.005832*	-2.657	-0.022007**	-1.961	0.001372	0.392
Ever given BCG vaccines	-0.003830**	-2.103	-0.005607**	-2.255	-0.000600**	-2.098
Age at first polio vaccine	0.018551*	2.677	0.042681*	2.753	0.007931*	2.577
Number of polio vaccine	-0.005158**	-2.561	0.003444	0.500	-0.00335***	-1.809
Number of DPT vaccine	0.008593	0.665	0.012900	1.602	0.001993	0.696
Ever given measles vaccine	-0.005476**	-2.290	-0.001716**	-2.249	-0.005989**	-2.055
Mother alive	-0.012249**	-2.326	0.024832	0.521	0.006872	0.432
Mother at home	0.003065	0.121	0.007674	0.307	-0.006903	-0.919
Father alive	0.028419	0.882	-0.025026	-0.621	0.01713***	1.947
Father at home	-0.021821**	-2.383	-0.014846	-0.842	-0.005721**	-2.358
Pipe water	-0.022683**	-2.318	0.000271	0.024	-0.003651**	-1.993
Sanitary toilet	-0.000071	-0.696	-0.048304***	-1.819	-0.014117*	-2.909

Non-iodized salt	0.027217*	2.906	0.018943**	2.109	-0.002487	-0.452
Tile as floor material	-0.004470**	-2.500	0.019914	1.349	-0.006444**	-2.579
Electricity	0.000556	0.041	0.032687***	1.893	0.013063*	1.689
Radio	0.004710	0.462	-0.006674	-0.691	0.00592***	1.795
Television	0.01494***	1.727	-0.021179*	-2.987	-0.01301***	-1.816
Refrigerator	-0.04402***	-1.727	0.026119	1.104	0.006201	0.446
F- value	9.03*		8.93*		8.62*	

\* - statistically significant at 1 percent level, \*\* - statistically significant at 5 percent level

\*\*\* - statistically significant at 10 percent level

Table 3b: Probit Regression of the Determinants of Wasting (WHZ) Among Children in Sub-Saharan Africa

Country/Variables	Comoros		Lesotho		Swaziland	
	Parameter	t-value	Parameter	t-value	Parameter	t-value
Constant	0.009608	0.641	0.00456	0.2345	0.017245	1.444
Sex	-0.001553	-0.412	0.00522	1.077	-0.00274***	-1.671
Urbanization	-0.007247*	-2.577	-0.004611	-0.696	-0.006393**	-2.481
Age of child	-0.000090*	-2.587	0.000075	0.345	-0.001191	-1.020
Hour of early learning	0.000257	0.629	-0.00283	-0.811	-0.000135	-0.364
Mother's primary education	0.001640	0.145	-0.067364*	-2.693	-0.008806**	-2.472
Mother's secondary education	-0.003070*	-2.714	-0.011207**	-2.104	0.001057**	-2.320
Vitamin A	0.015701*	2.597	0.003655	0.463	-0.003920*	-2.586
Month since last dose of Vitamin A	-0.001279	-1.008	-0.000140	-0.148	0.000093	0.795
Ever breast fed	0.00656	0.122	-0.007955*	-2.642	0.005649	0.910
Currently breast feeding	-0.004585	-0.837	0.020951*	2.843	0.004895	1.481
Diarrhea in last 2 weeks	0.012840**	2.479	0.0068311	0.993	0.004049	0.990
Cough in last 2 weeks	-0.005946	-1.263	-0.010335*	2.623	-0.001137	-0.547
Fever in last 2 weeks	0.002226**	2.500	-0.000421	0.325	0.036777*	5.447
Sleep under bed net	-0.006149**	-2.467	-0.006984**	-2.365	-0.012109	-0.341
Ever given BCG vaccines	-0.006997*	-2.671	-0.005701**	-2.222	0.016558*	-2.644
Age at first polio vaccine	-0.002945	-0.413	0.014990**	2.290	-0.004365*	-2.972
Number of polio vaccine	-0.000822**	-2.249	0.000138	0.019	0.002824*	-2.579
Number of DPT vaccine	0.002628	0.606	-0.003016	-0.308	-0.00353***	-1.703
Ever given measles vaccine	0.011233	1.156	0.015261	0.875	-0.008720	-1.459
Mother alive	-0.01228***	-1.866	-0.056487*	-2.647	0.004250	0.440
Mother at home	0.006264	0.660	-0.007697*	-2.844	-0.00864***	-1.702
Father alive	0.015384	1.308	0.000813	0.087	0.000126	0.018
Father at home	-0.016840*	-2.897	-0.001610**	-2.268	-0.001572**	-2.461
Pipe water	0.00800***	1.954	0.002224	0.415	-0.010334*	-2.582
Sanitary toilet	0.002075	0.214	0.060926*	2.711	0.002306	0.332
Non-iodized salt	-0.008442	-1.477	0.007474**	2.256	0.01137***	1.923
Tile as floor material	0.004906	1.193	0.0026052	0.181	0.004336	1.063
Electricity	0.007431	1.398	0.003340	0.221	-0.006778	-1.039
Radio	0.0026300	0.609	-0.004090	-0.744	-0.002744	-0.605
Television	-0.007934**	-2.255	-0.01551***	-1.739	-0.003072*	-2.638
Refrigerator	-0.002040	-0.312	0.000394	0.037	0.00623	0.118
F value	7.98*		8.06*		8.14*	

\* - statistically significant at 1 percent level, \*\* - statistically significant at 5 percent level

\*\*\* - statistically significant at 10 percent level

that it was due to high vulnerability of children to illness at the early stage of growth. Example is teething, which disturbs the growth processes if the child has diarrhea. The more the hours of early learning, the less the probability of wasting in Gambia and Central Africa Republic. This can also be linked to the fact that it is older children that are kept in most of these kindergarten classes.

Possession of at least a primary education significantly reduces the probability of wasting incidence in Gambia, Central Africa Republic, Lesotho and Swaziland. Also, possession of at least a secondary education significantly reduces the probability of wasting in all the countries except Central Africa Republic. Education is expected to

broaden the knowledge of the mothers on the best way to take care of children. Similar findings had been reported (Yimer, 2000; C). Hypothesis 1 is to be rejected for those countries with statistical significance.

Fortification of food with vitamin A significantly reduces the probability of wasting in Central Africa Republic, Comoros and Swaziland. However, the longer the time that children last took vitamin A, the higher the probability of wasting in Niger. This finding supports the finding of Rivera *et al* (2003) because vitamin A had been identified as a growth-promoting factor in children.

Children who ever breastfed have significantly lower probability of wasting in Central

Africa Republic and Lesotho. However, those children who were currently breast-feeding have significantly higher probability of wasting in Niger and Lesotho. Many factors like inability to fully breast-feed children, diseases and poor nutrition of the mothers may be responsible (Fernandez, 2002).

Infection with diarrhea significantly increases the probability of wasting in all the countries except Lesotho and Swaziland. Frogillo *et al* (1997) had a similar result. This is because of low food consumption and body dehydration associated with diarrhea. Also, infection with cough significantly increases wasting in all the countries with the exception of Comoros and Swaziland. Fever infection significantly increases wasting in all the countries except Central Africa Republic and Lesotho. Children that were sleeping under bed nets have significantly lower probability of wasting in all the countries except Central Africa Republic and Swaziland. This possibly results from prevention of malaria that may result from mosquito bites.

Children who ever took BCG vaccination have significantly lower probability of wasting in all the countries. The older the children are at the first time of taking polio vaccination, the higher the probability of wasting in all the countries except Comoros. The higher the number of polio vaccines that children took, the lower the probability of wasting in all the countries with exceptions in Niger and Lesotho. Therefore, hypothesis 2 is to be rejected for those countries with statistical significance. Also, it is only in Swaziland that increasing the number of DPT vaccines taken by the children results in significant reduction in the probability of wasting. This is because these vaccines reduce incidence of diphtheria, polio and tuberculosis in children. Children who ever took vaccination against measles have significantly lower probability of wasting in Gambia, Niger and Central Africa Republic. Frogillo *et al* (1997) observed a negative correlation between immunization rate and prevalence of wasting in Asia.

Children whose mothers are alive have significantly lower probability of wasting only in Gambia, Comoros and Lesotho. However, probability of children becoming wasting significantly decreases when mothers are at home in Lesotho and Swaziland. The parameter of the variable, father alive does not have the expected sign for many of the countries and it is statistically significant only in Central Africa Republic. However, children whose fathers were at home have significantly lower probability of wasting in all the countries except Niger. Presence of father is expected to guarantee food provision and this may make children non-vulnerable to acute growth disturbances.

UNICEF (1990) noted that economic status of households could be assessed by access to basic health facilities, safe water and sanitation facilities. Children from homes where there is safe (pipe) water have significantly lower probability of wasting in all the countries except Niger and Lesotho. The presence of sanitary toilet also significantly reduces the probability of wasting in Niger and Central Africa Republic. Contrary to expectation, the parameter for Lesotho has positive sign and it is statistically significant ( $p < 0.05$ ). Consumption of non-iodized salt leads to significant increase in the probability of child wasting in all the countries except Central Africa Republic and Comoros. Children from households where floors are covered with tiles have significantly lower probability of wasting only in Gambia and Central Africa Republic. The parameters of presence of electricity for Niger and Central Africa Republic have positive sign and statistically significant ( $p < 0.10$ ) showing that children from houses where there is electricity have significantly higher probability of wasting. Similar contrary observation was recorded for radio parameter, which has positive and statistically significant variable ( $p < 0.10$ ) for Central Africa Republic. However, households with television have significantly lower probability of wasting in all the countries except Gambia. Children whose households have refrigerator have lower probability of wasting in Gambia. This might result from increase in the ability to preserve food.

##### 5. Recommendations and Conclusion

Wasting is one of the major indicators of malnutrition in SSA. The MDGs cannot be fully achieved if this issue is not tactically addresses given its direct linkage with child mortality. To therefore work towards ensuring increased welfare for SSA children, the following policy statement can be derived from this study: Wasting worst affects rural areas and infection with diseases like diarrhea and fever increases the incidence of wasting. Immunization with BCG, polio, DPT and measles vaccines significantly reduce probability of wasting. These underscore the need for provision of basic health facilities in the rural areas and strengthening the existing ones in urban areas with necessary facilities. Health services should also include educating women on the need for child immunization, causes of major child illnesses and the way of preventing them. These will help reduce child wasting.

Wasting is more pronounced among younger children and those currently breast-feeding. The need to ensure that mothers have the know-how of discharging cares to children at early age is

underscored. This involves understanding the recommendations for exclusive breast feeding and nutritional requirements of mothers during breast-feeding.

Results for some countries revealed that children whose parents were alive and at home are nutritionally better than those who might have lost their parents or staying with guardians. This finding presupposes that setting up appropriate institutions to address issues like HIV/AIDS, malaria, tuberculosis, accidents etc. that often lead to untimely death of parents is a way of addressing malnutrition among children in SSA. Access to potable water reduces wasting in under-five children. There is therefore need to resuscitate water management schemes and their institutions to be able to cope with the water needs of the rural and urban people. These will lead to drastic reduction in the incidence of such diseases as cholera and diarrhea among children.

This study captures sanitary environment with inclusion of sanitary toilet and floor type variables, which significantly reduce probability of wasting in some SSA countries. Rural and urban sanitary officers should be strengthened to ensure that every household has a sanitary toilet for appropriate disposal of children and adult stools.

Finally, improved living condition with access to electricity, radio and television was found to significantly reduce probability of wasting in some SSA countries. This vividly underscores the important role media plays in improving the nutrition conditions of children in SSA. Therefore, radio and television programs on childcare and nutrition requirements of children should be sponsored and promoted.

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## Behavior of Skew Reinforced and Prestressed Concrete Composite Decks

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**Abstract:** Over the past fifty years, many countries have recognized benefits of using composite concrete slabs in building construction and highway bridges. In this paper, experimental study was carried out to study the effect of skew angle, percent of concrete shear keys and prestressing on behavior of skew composite decks with reinforced or prestressed concrete precast corrugated panels under cyclic loading. A skew folded corrugated panel was proposed and prestressed to allow its use as integral part of bridge composite deck. The proposed precast panels are of high strength concrete with small thickness while, the cast in situ top slab is of normal concrete strength. The experimental program consists of six specimens of 3100 mm long (3 with traditional reinforcement and 3 with post-tensioned reinforcement) with overall height of 250 mm. The precast panel is of thickness 50 mm for both inclined and top parts of specimens, and thickness of 50 mm for the top layer. The corrugation angle of the web and skew angle of the longitudinal direction are 60° and 20° respectively. The post-tensioned panels are prestressed by two 15.24 mm diameter strands. From experimental results, it was observed that no visible rotational effect occurred through the test for angles less or equal to 20°. No longitudinal cracks appeared in all tested specimens and no failure occurs at the bearing area of the specimen, the failure mode was flexure mode. The use of concrete shear keys as a shear connector affects its behavior (concrete and steel strains, deflection, and failure load).

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**Keywords:** Slab, Concrete, Prestressing, Skew, Post-tensioned, Experimental, Nonlinear.

### 1. Introduction

The composite concrete-concrete deck slab is that type of structural system which consists of a precast panel of either reinforced or prestressed concrete with a cast-in-situ layer. The composite deck slab has been widely used in buildings and bridge constructions since the last fifty years. The advantages of using composite slab were saving in cost and construction time.

Flat precast prestressed panel is the convention type of panels currently used in bridge deck construction due to its easy production sequence. The use of composite deck with reinforced concrete corrugated precast panel has been proposed in 1995 [1], and the dynamic behavior of prestressed composite girder bridges strengthened with external tendons were investigated in 2000 [2], but the use of composite deck with prestressed concrete corrugated precast panel has been proposed in 2002 [3]. The researchers approved the actual pronouncement of using concrete in corrugated shape rather than to be used in flat shape but they ignored the effect of skew.

The effect of torsion on externally prestressed segmental concrete bridge with shear key were studied experimentally in 2009 [4]. The effect of skew angle on determination the need for continuity diaphragms in skewed precast prestressed concrete girder bridges was investigated in 2007 [5] and its

effect on live load reactions at piers of continuous prestressed concrete bridges was studied in 2007 [6].

In this paper, the last studied corrugated panel was modified, prestressed and made with a skew angle of 20° as a simulation of construction requirements. Figures 1, 2 and 3 show the dimensions and reinforcement for all the test specimens.

Experimental investigation was carried out on skew composite deck with precast prestressed folded corrugated panel to examine its behavior under cyclic loading using different percentage of shear keys relative to the contact surface as shown in figure 2. The experimental results were compared to similar deck but with reinforced concrete precast panel.

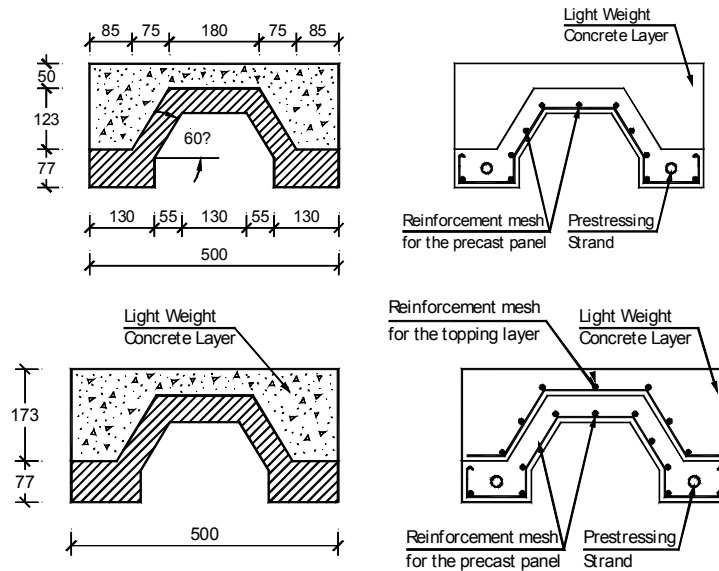
### 2. Experimental Work

To study the behavior of skew composite deck with reinforced and prestressed corrugated precast panels with different interface conditions (0, 20 and 40 % as shear area) under cyclic loading, six numbers of 3100 mm long specimens, (3 with traditional reinforcement and 3 with post-tensioned reinforcement) with dimensions shown in figure 1. The post-tensioned specimens were prestressed by two 15.24 mm diameter strands were prepared and tested. The precast panels were covered by a light weight reinforced concrete topping layer with thickness of 50 mm and reinforced with minimum reinforcement as shown in figure 1.

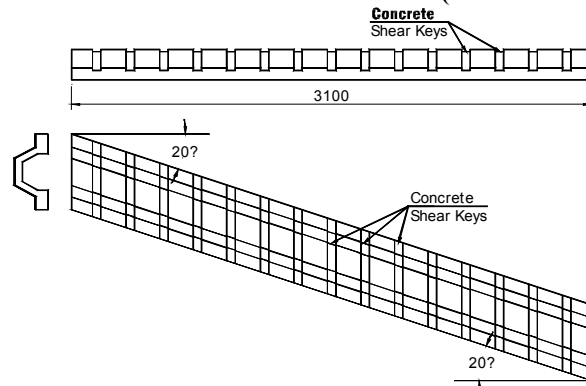


The reinforcement mesh for all precast panels were of 11 bar with 6 mm in diameter in the longitudinal direction supported on transverse bars of 6 mm in diameter spaced at 15 cm along the panel length. The reinforcement mesh for all cast in-situ layer were of 7 bar with 6 mm in diameter in the longitudinal direction supported on transverse bars of 15 bars with 6 mm in diameter along the panel

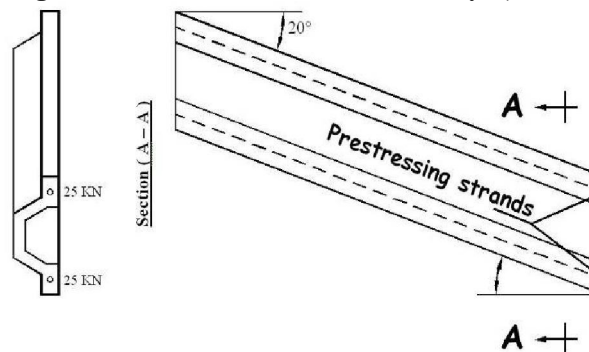
length. The labels of the specimens with reinforced concrete precast panels were (OR00, OR20 and OR40) and (PR00, PR20 and PR40) for specimens with prestressed concrete precast panels. The numbers (0, 20 and 40) refer to the percentages of the concrete shear keys relative to the contact surface area between the two layers.



**Fig. 1: Dimensions and reinforcement for all slabs (all dimensions are in mm)**



**Fig. 2: The skew angle of the tested slabs and the shear keys (all dimensions are in mm)**



**Fig. 3: Prestressing forces for all specimens**

### Materials

The materials used in the preparation of the tested specimens were locally produced. Tests were carried out to determine the mechanical properties of the materials according to the Egyptian Standard Specifications. The used fine aggregate was sand and coarse aggregate was crushed stone with percentages of 34 % and 66 % respectively. The cement used of type “Egyptian Ordinary Portland Cement”. The amount of cement used for concrete mixes were 500 kg/m<sup>3</sup> and 350 kg/m<sup>3</sup> for the precast and cast in-situ concrete layers respectively while, the water cement ratios (w/c) were 0.38 and 0.55 by weight of cement respectively.

### Steel Reinforcement

The used steel reinforcements for both precast and cast in-situ layers are mild steel bars of 6 mm in diameter in the longitudinal and transverse directions with yield strength of 355 Mpa and ultimate strength of 495 MPa.

### Prestressing steel

The three panels were prestressed by two 15.24 mm diameter 7-wire strands with tensile strength of 1860 MPa.

### Prestressing technique

### Construction of specimens

For the cast in-situ layer, the precast slab was prepared by removing any loose particles from its top surface using steel brush. The precast slab was

The prestressing system consists of a hollow core single acting piston of 200 KN in capacity as shown in figure 4, a hand pump of 400 KN maximum in capacity and a pressure sensor attached at the pressure line connected to a digital indicator which shows the pressure value in form of force value. The steel strands were prestressed to reach 25 KN for each one. The strands were over-tensioned by about 10 % of the required prestressing force for about 4 minutes and then relaxed to the original value to avoid any losses due to relaxation.



**Fig. 4: Prestressing Process**

simply supported on its shorter sides on very rigid steel beams to simulate the actual site behavior of such type of slabs as shown in figure 5.



**Fig. 5: Preparation and curing of composite decks**

### Preparation and testing of control specimen

Quality control during the mix of the concrete for the slab layers was made by the determination of the mechanical properties of the concrete by testing cubes with dimensions 15 x 15 x 15 cm and standard cylinders of 15 cm in height and height of 30 cm. A plain concrete beam with dimensions equal to 10 x 10 x 70 cm was prepared. The control specimens were cast at the same time of casting each layer of the composite deck. Tables 3 summarize the mechanical

properties of hardened concrete for both precast and cast in-situ layers.

Six cubes have been prepared with each of the composite slab component. Three cylinders of 15 cm in diameter and 30 cm in height were casted with each layer of the composite deck and cured in its condition. The cylinders were tested according to the standard specifications for determining the concrete tensile strength as shown in figure 6.

**Table 3: Properties of materials for both precast panels and topping layer**

Group	% Top Surface	28-day compressive strength MPa	Flexural Strength MPa	Splitting Tensile Strength MPa	Young's modulus MPa
Prestressed Panels	00	37.6	8.8	3.3	36700
	20	34.1	7.6	3.0	35100
	40	36.4	7.9	3.2	36300
R.C. Panels	00	37.8	8.3	3.3	36900
	20	36.0	7.9	3.2	36200
	40	35.2	8.4	3.1	35700
Topping Layer Over Prestressed Panels	00	22.9	6.8	2.6	28700
	20	21.8	6.6	2.5	28100
	40	21.0	6.5	2.4	27600
Topping Layer Over R.C. Panels	00	21.2	6.5	2.4	27700
	20	20.8	5.9	2.3	27400
	40	22.1	6.8	2.5	28200

**Fig. 6: Plain Concrete Beam and Concrete Cylinders under Test****Testing equipment and loading arrangement**

Hydraulic jack with a maximum capacity of 300 KN was used for applying concentrated load to the loading arrangement. Figure 7 shows the used loading system set up to perform uniformly distributed load. All the six specimens were supported along their short sides on very rigid steel beams while the long sides of the composite deck specimen were left free.

The tests were conducted in two stages, in the first stage; a slab was loaded with small load increments. The small loading increments were chosen to provide a good record of the slab behavior before and after cracking and to determine the first crack's load. The load was removed and the slab was loaded and unloaded for three times to study the behavior of these slabs under cyclic loading. In the second stage, the load increments were based on deflection measurement rather than load. The loading was gradually increased until the slabs failed completely.

**Instrumentation**

Three electrical strain gauges of 10 mm length and average resistance  $120.2 \pm 0.2$  ohms, were installed to measure the strains of the reinforcement mesh of the precast and cast in-situ layers.

The strain gauges were continuously attached to the data acquisition system while testing and checked by voltmeter before attaching. Mechanical dial gauges with a sensitivity of 0.01 mm were used in measuring the vertical displacements. Concrete compressive and tensile strains were measured using demec-point station by using a dial gauge with  $0.8 \times 10^{-5}$  mm/mm accuracy to measure the deformation along a 200 mm gauge length.

(LVDT) of accuracy 0.001 mm were placed at two locations at distance of 150 mm from the support line.

**Fig. 7: Test setup before loading****3. Results and Discussion**

Table 4 summarizes the results of testing of six specimens of composite decks with reinforced or

prestressed concrete precast panels. The comparison between results were made by means of cracking load, failure load, maximum deflection, steel tensile strain and end-slip at the end of the specimens between the two layers of concrete by using two (LVDT) of accuracy 0.001 mm.

**Deformation characteristics**

Figure 8 shows the load vertical movements due to prestressing effect at mid span of each precast unit by using dial gauges with a sensitivity of 0.01 mm at the bottom surface of different precast unit surface shape. The shape of the top surface of the precast unit affects the estimated camber due to prestressing process.

**Crack pattern and mode of failure**

The first crack was observed at mid-span section at loads equal to (14.3, 15.0, 16.0, 18.0, 20.6 and 21.8 KN) for (OR00, OR20, OR40, PR00, PR20 and PR40), respectively which approved that the presence of shear keys as well as its percentage and the presence of the prestressing force increased the first crack's load. The cracks were extended vertically

through the precast layer only. The load was increased until the failure occurs to the specimen. No bearing failure occurs at the bearing area of all specimens.

The mode of failure for all specimens was flexural behavior as shown in figure 9. The cracks pattern at the bottom surface of the specimens extended with skew equal to the skew angle of the specimens as shown in figure 10.

**Load deflection relationship**

Figures 11 and 12 show the load mid-span deflection relationship for all slabs and it was observed that the presence of shear key affects the slope of the load deflection curve which means that the stiffness is increased with the increase of the shear keys. It was observed that the stiffness of the member was decreased due to the effect of cyclic loading on specimens.

From the results of measured deflection at mid span of the composite slabs with prestressed concrete precast units it can be observed that, the shear key percentage affects the maximum deflection of the specimens by (29.86 % and 43.89 %) for (20 % and 40 %) percentage of shear keys from surface area.

**Table 4: Test Results at Final Loading**

Type of Composite Slab	Specimen	% Shear Keys	Cracking Load (KN)	Failure Load (KN)	Maximum Deflection (MM)	Tensile Steel Strain at Maximum Load	End Slip (MM)
e with Reinforced Precast	OR00	00	14.30	51.94	10.260	0.0021	0.0131
	OR20	20	15.00	53.18	16.125	0.0021	0.0040
	OR40	40	16.00	55.22	19.85	0.0058	0.0029
e with Prestressed Precast	PR00	00	18.00	82.00	44.288	0.0145	0.0170
	PR20	20	20.60	85.74	31.063	0.0130	0.0089
	PR40	40	21.80	88.67	24.850	0.0125	0.0057

**Load steel tensile strains relationship**

Figures 13 and 14 show the load longitudinal steel tensile strain relationship for all slabs and it can be seen that the stiffness of the slab decrease due to cyclic loading and the presence of shear key affects the tensile strains of the longitudinal reinforcement.

**Load concrete compressive strains relationship**

Figures 15 and 16 show the load concrete compressive strain relationship for all slabs and it was observed that increase in shear keys decreased the concrete compressive strains at the same load.

**Load end-slip relationship**

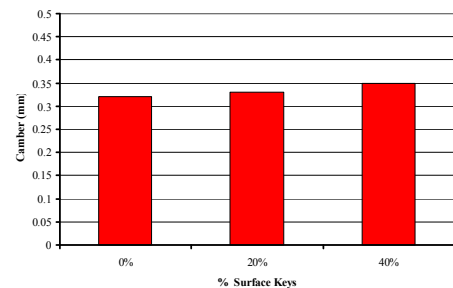
Figures 17 and 18 show the load end slip relationship for all slabs. The presence of prestressing affects the value of end slip on specimens and the increase in shear keys decreased the measured end-slip between the two layers at the same load

**Maximum load**

The capacity of the slab increases with the increase of shear key area. The presence of 20 % shear key increase the capacity of the slab with 2.4 %

and with 4.6 % for composite slabs with reinforced and prestressed concrete precast panels respectively. The presence of 40 % shear key increase the capacity of the slab with 6.3 % and with 8.1 % for composite slabs with reinforced and prestressed concrete precast panels respectively.

The prestressing force affects the capacity of the specimens by 57.9 %, 61.2 % and 60.6 % for specimens with 0 %, 20 % and 40 % shear keys respectively.

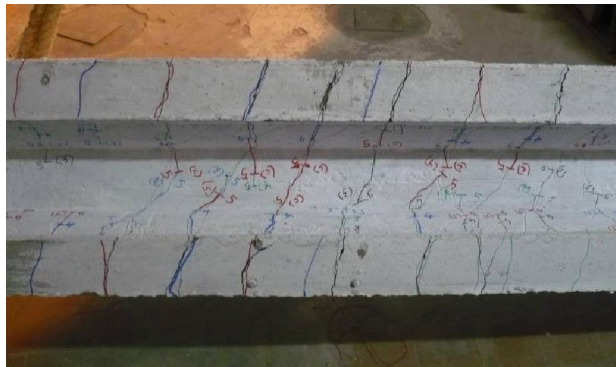


**Fig. 8: Measured Camber Experimentally**

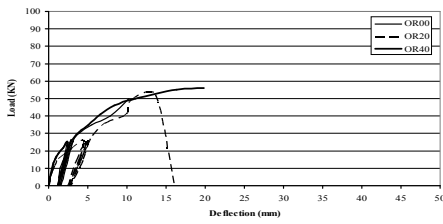




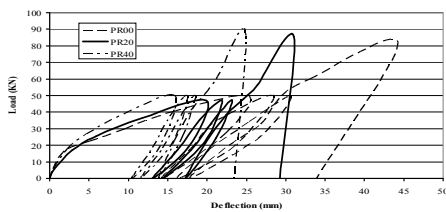
**Fig.9: Flexural Behavior of Specimen**



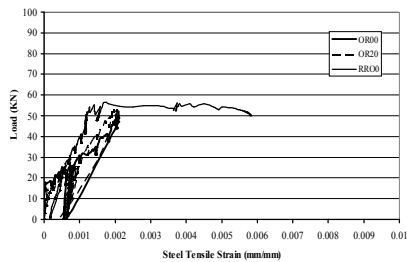
**Fig.10: Crack Distributions at the Bottom Surface**



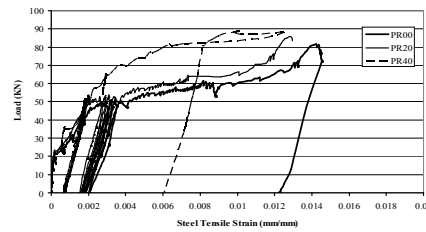
**Fig.11: Load Mid-span Deflection Relationship for Slabs with R.C. Precast Panels**



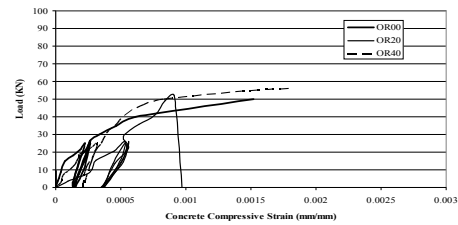
**Fig.12: Load Mid-span Deflection Relationship for Slabs with Prestressed Concrete Precast Panels**



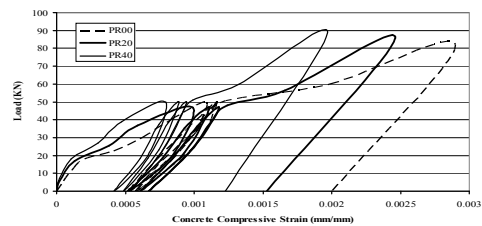
**Fig.13: Load Steel Tensile Strain Relationship for Slabs with R.C. Precast Panels**



**Fig.14: Load Steel Tensile Strain Relationship for Slabs with Prestressed Concrete Precast Pane**

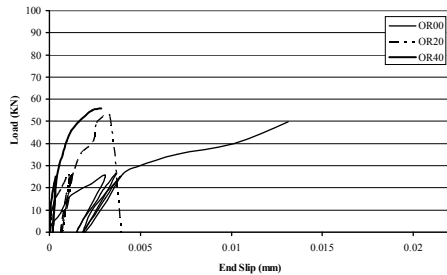


**Fig.15: Load Concrete Compressive Strain Relationship for Slabs with R.C. Precast Panels**

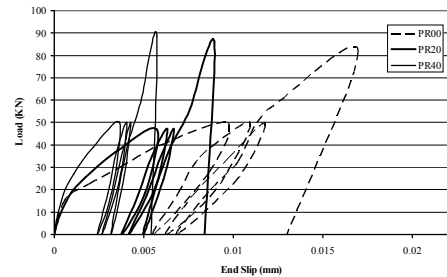


**Fig.16: Load Concrete Compressive Strain Relationship for Slabs with Prestressed Concrete Precast Panels**





**Fig.17: Load End-Slip Relationship for Slabs with R.C. Precast Panels**



**Fig.18: Load End-Slip Relationship for Slabs with Prestressed Concrete Precast Panels**

### Conclusions

In the limitation of this study, experimental investigation has been carried out to study the behavior of composite slab with reinforced and prestressed concrete precast skew panels.

A number of tests were conducted to study the behavior of all specimens due to the effect of skew angle, shear keys and prestressing under cyclic loading. The following conclusions were obtained:

1. The mode of failure for all specimens was flexure.
2. The increase in percentage of shear keys increases capacity of specimens and decreases tensile and compressive strains.
3. The cyclic loading decreases stiffness of specimen with increase of number of cycles at the same load.
4. The skew and geometry of the composite deck affects crack pattern on the bottom surface of specimens.
5. The presence of prestressing affects the behavior of specimens that it increases capacity of composite slab, and decreases end slip between the two surfaces of concrete.

6. No bearing failure occurred at supports due to loading or due to cyclic loading effect.

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## Assessment of Quality of Nursing Care Provided Immediately After Birth At University Hospital

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**Abstract:** The poor quality of care offered to neonates in hospital has been widely reported. The challenge is now therefore to define interventions that might improve this care. Aim. This study was designed to assess quality of nursing care provided immediately after birth for newborn and mothers, Methodology, Descriptive non participatory observational research design was utilized at OB- GYN department (Maternity unit) at University hospital in Shebin El-Kom Menoufiya University, through observational checklist and structured interview questionnaire with a convenient nurses in charge in delivery room were chosen by non probability purposive sampling technique. Data was entered and analyzed using SPSS-16 Descriptive statistics cross tabulation, correlation and graphs were used to illustrate the results. **Results:** The findings in this study indicate that nurses have an around average of knowledge and quality of practice regarding total score of knowledge of birth preparation, initiating attachment and breast feeding, While much still needs to be done to improve the quality of maternal and newborn care regarding responding to mothers questions, examining baby and mothers before discharge. A combination of factors in this study, inhibit the provision of quality care such as educational levels and lack of training courses that nurses received: Study, displaying the need for protocols in labor room, and post natal ward. There is no data available regarding the infection arising from the lack of care immediately after the delivery, since mothers get discharged within few hours after delivery. However efforts will be taken in future researches to assess the consequences of lack of care on neonate's outcomes.

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**Keywords:** quality, immediate care

### 1. Introduction

Immediate proper care of newborn is vitally important for survival, growth and development of a newborn. Despite several studies conducted about maternal and child health care practices, little is known about factors that determine behaviours related to immediate care of newborn. Most people are unaware of importance of immediate care of newborn and many unsafe behaviour do exist such as common use of untrained attendants, unsafe cord care, immediate bathing of baby (**Gurung, 2008**).

Maintaining and improving patient care requires active involvement of everyone in health care system, in order to meet the needs for evaluating health care in its totality as well as to identify whether effective and appropriate care has been provided. The quality is "the major component of neonatal health care, and it demands participation from nurses rendering care. (**Dalia, 2011**).

According to the **World Health Organization, (2010)** the majority of all neonatal deaths (75%) occur during the first week of life. Of those deaths, between 25% and 45% occur within the first 24 hours. Further, the neonatal period—which comprises of the first 28 days of life—accounts for 37% of all

deaths among children under five. **Abdel-Kareem (2008)**.

Each year 62,000 newborn babies die and an additional 43,000 are stillborn. Most of these babies die from preventable or treatable causes, and it is estimated that up to two-thirds could be saved if essential care reached all mothers and newborns. Maternal and newborn survival is interconnected and the most dangerous time in a child's life is during birth, as the majority of newborns die due to stressful events surrounding delivery. Newborn babies account for more than 40% of deaths amongst children under age five. The Lancet Newborn Series (**Darmstadt et al., 2005**) emphasizes essential newborn care including hygienic cord care, maintenance of warmth, and immediate and exclusive breastfeeding as a means to reducing newborn deaths. All of the immediate newborn care interventions observed are simple to perform and use minimal resources.

Directly after birth there should be attention to the condition of the newborn. **The World Health Organisation (2010)** states that such attention is an integral part of care in normal birth. Immediate care involves: drying the baby with warm towels or cloths, while being placed on the mother's abdomen or in her arms. This mother-child skin-to-skin contact is

important to maintain the newborn's temperature, encourage bonding and expose the newborn to the mother's skin bacteria. Ensuring that the airway is clear, removing mucus and other material from the mouth, nose and throat with a suction pump. Taking measures to maintain body temperature, to ensure no metabolic problems associated with exposure to the cold arise. Clamping and cutting the umbilical cord with sterile instruments, thoroughly decontaminated by sterilisation. This is of utmost importance for the prevention of infections. A few drops of silver nitrate solution or an antibiotic is usually placed into the eyes to prevent infection from any harmful organisms that the newborn may have had contact with during delivery (e.g. maternal STDs). Vitamin K is also administered to prevent haemorrhagic disease of the newborn. The newborn's overall condition is recorded at 1 minute and at 5 minutes after birth using the Apgar Scale. Putting the newborn to the breast as early as possible. Early suckling/breast-feeding should be encouraged, within the first hour after birth. About 6 hours or so after birth, the newborn is bathed, but the vernix caseosa (whitish greasy material that covers most of the newborn's skin) is tried to be preserved, as it helps protect against infection (Hon, 2002).

The health care professional in general and nurses in particular play a vital role to ensure that the new born has best possible beginning of life and the nurse must be aware of the potential problems and be alert to the infant's changing condition and to intervene appropriately when necessary. The nurse is the first health care provider who has direct contact with the neonate during birth. Hence nurses require the knowledge and skill to take care of the babies keeping in mind the basic principles so that many complications can be prevented. All staff involved with the clinical care of the newborn immediately following delivery are must be competent in newborn life support and neonatal resuscitation. Consultant obstetrician-led maternity services ensure that a professional who is competent in advanced newborn airway skills is immediately available (within five minutes). All staff has undertaken training appropriate to their role, supporting mothers to feed their infant appropriately and promote the use of breast milk and breastfeeding, preparation of babies and families for discharge, supporting families in acquiring the knowledge and skills they will need to care for their baby at home, assessing developmental needs, providing emotional and psychological support to families, safeguarding children and supporting families during bereavement (Royal College of Speech and Language Therapists, 2006; Health Professions Council, 2007 and *High Quality Care*

*For All – NHS Next Stage Review Final Report (DH, 2008)*

According to (Lhynelli, 2011), APGAR Scoring System was developed by Dr. Virginia Apgar as a method of assessing the newborn's adjustment to extra uterine life. It is taken at one and five minutes after birth. With depressed infants, repeat the scoring every five minutes as needed. The one minute score indicates the necessity for resuscitation. The five minute score is more reliable in predicting mortality and neurological deficits. The most important is the heart rate, and then the respiratory rate, the muscle tone, reflex irritability and colour follows in decreasing order. A heart rate below 100 signifies an asphyxiated newborn and a heart rate above 160 signifies distress.

Nurses have an important, enabling role to help the woman during childbirth. There must be a high percentage of interpersonal skills in the care of the woman in addition to being technically competent. Also, Evaluation is one of the most critical phases of the nursing process because it supports the basis of the usefulness and effectiveness of nursing practice.

In addition, it is known that nursing services are the backbone of the healthcare system in almost all countries in the world. They represent between 60-70% of the health personnel So, It is thus important that we assess quality of nursing care we offer in order to improve on it. Also, was found that there is a relationship between quality of care and performance of nurses in the delivery room and several hours later on at neonatal unit (Abdel-Kareem,2008).

Therefore, it is important to assess the nurse's performance in the delivery room based on a group of standards of care which are documented and developed by standard care.

So it important to have a well-trained nurse to provide proper immediate care for the newborn and prevent any complication to be arises.

#### **Significant of the study:**

Nurses will never know the quality of care they offer until it is being assessed. This study measures quality of care provides, will provide a means of evaluating the care and will provide suggesting recommendations for its improvement.

#### **Aim of the study:**

This study was designed to assess quality of nursing care provided immediately after birth for newborn and mothers.

#### **Research questions:**

1. Are the nurses having a poor knowledge or practice regarding immediate care of the newborn?
2. Are the nurses having a poor quality of care regarding immediate care of the newborn?

3. What is the current level of standards of quality care provided immediately after birth and up to discharge for mothers and neonatal?

## 2. Methodology:

### Research design:

Descriptive non participatory observational research design was utilized to achieve the aim of the study

### Setting:

The study was conducted in OB-GYN department (Maternity unit) at University hospital in Shebin El Kom - Minoufiya

### Sample and sampling technique

Non probability purposive sampling technique was utilized. Twenty three (23) nurses was observed during they provide an immediate newborn care and after 2 hours from delivery and interviewed later.

In this study, the quality achieved is evaluated by comparing what was actually achieved with the targets set criteria for quality of care must be provided. The nurses' application manner, a comprehensive overview can be provided for the newborn.

### Inclusion criteria:

Each nurse working in OB-GYN department at Maternity unit (which includes settings for normal delivery and C- section) and provide immediate care of the newborn

Sample included, neonates born within 37 to 42 weeks of gestation, through vaginal delivery (including low forceps and suction cups), weighing 2.5 to 3.8 kg, with Apgar score of 9 and 10 at 1 and 5 minutes.

### Exclusion criteria:

Any nurse working in OB-GYN department at Maternity unit but not participating neither in delivery unit work nor providing immediate care of the newborn. Also, neonates with prematurely, birth asphyxia and congenital malformation and babies born to high risk mothers such as who had pregnancy induced hypertension, heart disease, ante-partum hemorrhage, diabetes mellitus, jaundice, and anemia.

### Data collection tools:

Two tools were used in this study, namely structured interview questionnaire and an observational checklist. These tools were used to collect data from each study subject in the study settings.

### Tool I

#### Part I

It includes, biosocial data of nurses like age, years of experience, levels of education and marital status

## Part II

### Structured interview questionnaire:

It consists of 40 items contains questions related to nurses knowledge about the immediate newborn care with multiple form of qualitative and quantitative data, some questions responded by yes or no, with score one or zero, other questions were a multiple choices or open end questions. Every question was rated as poor, fair or good knowledge according to its content items, then the total score was calculated from 40 degree and classified into good knowledge (30-40) fair knowledge (29-20) and poor knowledge  $\geq 20$ .

The tool was developed by the researchers, and tested for content validity and reliability by a jury of 3 experts in the field of specialization.

### Tool II

#### Observational check list:

It formulated on the basis of standard intervention to be performed by the health personal Standards of Performance of the Immediate Neonatal Nurses Care. It was prepared using *Neonatal Nursing: Scope and Standards of Practice*, published by the American Nurses Association (ANA) consists of different tasks that are to be performed immediately after delivery checked by done fair, done poor or not done. The total quality score was calculated by converting score into percentage % of performed care as follows.

Done good < 75%, done fair 74-51% and done poor < 50% .

Pilot study:

A pilot study was conducted to test the applicability of the tools, and to estimate the time needed. It was carried out on 5 nurses. The results of the pilot study helped in refining the interview questionnaire and the observational checklist to set the final form of it.

#### Development of the tool:-

##### Validity:-

Content validity of the questionnaire sheet was determined through an extensive review of literature about the immediate care of the newborn. Modification to the tools were made according to the panels' judgment on clarity of sentences, appropriateness of content, sequence of items, and accuracy of scoring and recording of items (content validity)

##### Reliability:

Reliability analysis was used to determine the extent to which the items in the questionnaire are related to each other. Results of the pilot study were also used to confirm reliability (test-retest reliability). Cronbach's co-efficiency alpha for the questionnaire was 97. Pearson correlation co-efficiency indicated high internal consistency, which was 78,38 for all items of the questionnaire. The findings from validity



and reliability suggested that the current questionnaire and observational check list could be used as a viable tool for data collection in this study.

#### **Human Rights and ethical consideration:**

Before starting any steps in the study, official letter was issued from the faculty of nursing to the director of the hospital. The letter explained the aim of the study, clarified its procedures, and sought permissions to conduct it in this setting. Before inclusion in the sample, each nurse was observed for conducting the study 2 times without her consent and checking her application for the second time. Then her consent was assured oral and written to be participated in the study. The investigators approached each of them by giving an overview of the study, clarifying that participation was voluntary, total confidentiality of the obtained information, as well as respect of the subject privacy was ensured. Finally Health education and professional help was provided in case of need.

#### **Procedure:**

Field work was done three days per week to collect the data. Three to five cases were recruited per day.

#### **Statistical Analysis:**

After completing of the field work, data were processed, extensively reviewed. Each answer sheet was coded and scored, So that data could be prepared for computer use. Data were statistically analyzed using SPSS Version 16.0 statistical software packages. Data were presented using descriptive statistics in the form of frequencies and percentages for qualitative variables, and cross tabulation variables. Test of significance was used and level of significance is  $P < 0.05$ , is used if  $P$  value is less than 0.01, it was highly significance if  $P$  value is  $< 0.001$ .

#### **Limitations of the study:**

Small number of nurses participated the study was studied.

### **3. Result:**

To identify practices regarding immediate care of newborn, information were collected related to wiping, cleaning and placing of newborns at birth; material used for cord cutting, stump care, bathing, constraints regarding immediate care of newborn and suggestion to promote modern practices.

Table (1) Distribution of biosocial characteristics of studied nurses" The mean age were  $26.18 \pm 6.1$ , more than half of them (57.1%) were have diploma education, (66.6%) of them were married. and about half of them (48.2%) have 1 – 5 years of experience However, there were a significance difference ( $P < 0.05$ ) between their marital status versus to their age, levels of education, and years of experience ( $P > 0.05$ )

Table (2) Showed nurses knowledge related to birth preparation. More than half of nurses 60.9% didn't get any courses about delivery. However from their experience majority of them has a good knowledge to prepare labor (91.3%) or cesarean (78.4%) equipment as well as more than half of them (60.9%) have a good knowledge for preparing mothers for birth. So, there were a significance difference ( $P < 0.001$ ) for most of items of nurses knowledge to prepare mothers for birth.

Table (3) Nurses knowledge about care provided for mother and newborn care immediately after birth. Good and poor nurses knowledge about immediate care were nearly distributed (43.5% & 47.8%). Also, more than half of them (56.5%) respond poorly to mothers questions and majority of them were have poor knowledge regarding care provided for mothers and neonates before discharge. However the total knowledge score was good for less than half of them (48.2%). So, there were insignificance difference ( $P > 0.05$ ) for their knowledge about immediate care provided for mothers and newborn care.

Table (4) Relationship between nurses' knowledge and their practice of immediate neonatal care. More than half of nurses (52.6%) have a good knowledge and practice for hand washing, prepare labor equipment, receive the baby and clean the airway, apply Apgar score, clamp, sterile the cord, as well as put eye drop to the neonate. Unfortunately majority and/or more than half of them (69.5%, 52.2%, 56.2%, & 60.9%) were didn't administer Vitamin K to the baby, didn't examine or observe the body of the baby or their genitalia. However, there were no significance difference ( $P > 0.05$ ) between nurses knowledge and their practice for immediate neonatal care

Table (5): represent relationship between nurses' knowledge and their practice for newborn and their mothers care. Approximately three quarter of nurses have good practice for measuring baby weight, while more than half of them (52.2%, 66.2%, 56.5 and 52.2) didn't measures newborn head circumference, length, identify the baby or respond to mothers questions appropriately. Also, 69.5% of them didn't prepare the newborn or their mother for discharge. On the other hand more or less than (40%) of nurses have a good knowledge about newborn and mother care after birth. However there is no significance difference  $P > 0.05$  between nurses' knowledge and practices regarding newborn and mother care after birth.

Table(6) Correlation between nurses' education and years of experience to their knowledge of immediate care, total knowledge score, and responses to mother questions. There were a significance relationship between nurses education and their knowledge of immediate newborn care, total



knowledge score as well as responses to mothers questions, while a negative correlation were found regarding to their years of experience.

Figure (1) represent quality control chart for nurses knowledge of immediate care. It represented that however nurses have a good knowledge but they didn't catch the maximum quality for their knowledge, the greater number of them were around the average.

Figure (2) represent quality control chart for nurses' skills of immediate care. It represented that nurses didn't catch the maximum quality for their skills, the greater number of them were around the average

Figure (3) illustrated quality of control for nurses' total knowledge with their years of experience. It is obvious that the total quality of nurses' knowledge increased with their years of experience.

Table (1) Biosocial characteristics of studied nurses at University hospital

Biosocial characteristics of studied sample	Number of studied sample		X <sup>2</sup>
	No=21	%	
Nurses age			.6674 P=.955
>20	4	19.0	
21-25	4	19.0	
26-30	7	23.8	
31-35	5	23.8	
36-40	3	14.3	
<b>X and ±SD</b>	26.18±	6,1	
Levels of education			4.261 P=.119
Diploma	14	57.1	
Specialty	4	19.0	
Baccalaureate	5	23.8	
Marital status			14.174 P<0.001
Single	5	23.8	
Married	16	66.7	
Divorced	2	9.5	
Years of experience			10.348 P=.858
1-5	11	48.2	
6-10	5	21.7	
11-16	5	21.7	
17-21	1	4.3	
22-30	1	4.3	
Pleasure from carrier			X <sup>2</sup> =5.261 P=.022
Yes	17	73.2	
No	6	26.8	
Total	23	100.0	

Table (2) Nurses knowledge related to birth preparation

Birth preparation	No N=23	%	X <sup>2</sup>
<b>Getting birth training course</b>			X <sup>2</sup> = 4.261 P = .119
Yes	9	39.1	
No	14	60.9	
<b>Number of training courses</b>			X <sup>2</sup> =15.783 P=001
Non	13	56.5	
one	7	30.4	
Two	2	8.6	
Three	1	4.3	
<b>Prepare labor equipment</b>			X <sup>2</sup> =37.783 P=000
Poor	1	4.3	
Fair	1	4.3	
Good	21	91.3	
<b>Prepare caesarian section equipment</b>			X <sup>2</sup> =48.957 P=000
Poor	3	13.0	
Fair	2	8.6	
Good	18	78.4	
<b>Birth Preparation knowledge</b>			X <sup>2</sup> =27.826 P=004
Poor	6	26.8	
Fair	3	13.0	
Good	14	60.9	
Total	23	100.0	

Table (3) Nurses knowledge about care provided for mother and newborn care immediately after birth

Nurses knowledge of immediate care of newborn	No N=23	%	X <sup>2</sup>
<b>Knowledge of immediate care</b>			X <sup>2</sup> =19.304 P=.004
Poor	11	47.8	
Fair	2	8.6	
Good	10	43.5	
<b>Breast feeding initiation</b>			X <sup>2</sup> =5.304 P=.070
After half an hour	12	52.2	
After one hour	8	34.8	
After two hours	3	13.0	
<b>Neonates attachment</b>			X <sup>2</sup> = 8.997 P=011
Put baby with his mother	12	52.2	
Put baby in the incubator	10	43.5	
Put the baby in the nursery	1	4.3	
<b>Responses to mothers questions</b>			X <sup>2</sup> =4.087 P=.665
Poor responses	13	56.5	
Good responses	10	43.5	
<b>Knowledge of newborn examination before discharge</b>			X <sup>2</sup> =4.652 P=.199
Poor knowledge	18	78.3	
Good knowledge	5	21.7	
<b>Knowledge of mothers examination before discharge</b>			X <sup>2</sup> =5.696 P=.127
Poor knowledge	19	82.6	
Good knowledge	4	17.4	
<b>Total knowledge Score</b>			X <sup>2</sup> =11.565 P=.641
Poor	3	13.4	
Fair	9	39.2	
Good	11	48.2	
<b>X and SD total knowledge</b>	30.2 ± 8.3		
Total	23	100.0	

Table (4) Relationship between nurses knowledge and their practice of immediate neonatal care										
Nurses practice of immediate care	Total knowledge Score						Total			
	Poor		Fair		Good		N0	%		
	No	%	No	%	No	%				
<b>Hand washing</b>										
Done poor	2	8.6	1	4.3	4	17.4	7	30.4	41.394 <sup>ns</sup>	
Done fair	4	17.3	2	8.6	5	21.8	11	48.2		
Done good	0	0.0	2	8.6	3	13.4	5	21.8		
Total	6	26.8	5	21.7	12	52.6	23	100.0		
<b>Prepare Labor equipment</b>									28.146	
Done poor	0	0.0	4	17.3	2	8.6	6	26.8	0547	
Done fair	1	4.3	2	8.6	3	13.04	6	26.8		
Done good	1	4.3	2	8.6	8	34.8	11	48.2		
Total	2	8.6	8	34.8	13	56.5	23	100.0	16.073	
<b>Receive the baby and clean the airway</b>									.309	
Done fair										
Done good	0	0.0	5	21.8	2	8.6	7	30.4		
Total	3	13.4	2	8.6	11	48.2	16	69.5		
Total	3	13.4	7	30.4	13	56.5	23	100.0	50.507	
<b>Take Apgar Score</b>									.173	
Done poor	2	8.6	3	13.0	6	26.8	11	47.8		
Done fair	0	0.0	1	4.3	0	0.0	1	4.3		
Done good	1	4.3	3	13.4	7	30.0	11	47.8		
Total	3	13.4	7	30.4	13	56.5	23	100.0	24.495	
<b>Clump, sterilize and dress the umbilical cord</b>									.655	
Done poor										
Done fair	0	0.0	3	13.0	2	8.6	5	21.8		
Done good	0	0.0	1	4.3	2	8.6	3	13.04		
Total	2	8.6	5	21.8	9	39.2	16	69.5		
Total	2	8.6	9	39.2	13	56.5	23	100.0		
<b>Put eye drop to the baby</b>									38.759	
Not done	9	39.2	0	0.0	0	0.0	9	39.2	.614	
Done fair	0	0.0	1	4.3	2	8.6	3	13.4		
Done good	0	0.0	1	4.3	10	43.4	11	48.2		
Total	9	39.2	2	8.6	12	52.5	23	100.0	21.487	
<b>Administer Vit. K</b>									.805	
Not done	7	30.4	5	21.8	4	17.3	16	69.5		
Done good	3	13.4	2	8.6	2	8.6	7	30.4		
Total	10	43.4	7	30.0	6	26.8	23	100.0		
<b>Examine head and chest</b>									45.936	
Not done	6	26.8	3	13.4	4	17.3	12	52.2	.312	
Done good	5	21.8	4	17.3	1	4.3	13	56.5		
Total	11	48.2	7	30.0	5	21.7	23	100.0		
<b>Examine hip and back</b>									39.461	
Not done	7	30.4	2	8.6	4	17.3	13	56.5	.583	
Done good	8	34.8	1	8.6	1	8.6	12	52.2		
Total	15	65.2	3	13.4	5	21.8	23	100.0		
<b>Examine genitalia</b>										
Not done	3	13.4	3	13.4	8	34.8	14	60.9		
Done good	2	8.6	2	8.6	5	21.8	9	39.2		
Total	5	65.2	5	65.2	13	56.5	23	100.0		
<b>Nurses practice of care after birth</b>	Total knowledge Score						Total		X <sup>2</sup>	
	Poor		Fair		Good		N0	%		
<b>Measuring weight</b>										
Done fair	2	0.0	1	4.3	1	4.3	4	17.3	43.723	
Done good	9	39.1	4	17.3	4	17.3	17	73.9	.398	

Not done	0	0.0	1	4.3	1	4.3	2	8.6	
Total	11	47.8	6	26.8	6	26.8	23	100.0	
<b>Measuring head circumference</b>									41.802
Done fair	0	0.0	2	6.8	2	8.6	4	17.3	.480
Done good	1	4.3	5	21.7	1	4.3	7	30.4	
Not done	2	8.6	2	8.6	8	34.8	12	52.2	
Total	3	13.4	9	39.2	11	47.8	23	100.0	50.835
<b>Measuring length</b>									
Done fair	0	0.0	3	4.3	1	4.3	2	8.6	.165
Done good	1	4.3	5	21.7	2	8.6	8	34.8	
Not done	2	8.6	5	21.7	5	34.8	15	66.2	
Total	3	13.4	13	56.5	8	34.8	23	100.0	
<b>Baby identification</b>									37.257
Done fair	0	0.0	2	8.6	4	17.4	6	26.2	.679
Done good	0	0.0	2	8.6	2	8.6	4	17.3	
Not done	3	13.04	4	17.3	6	26.8	13	56.5	
Total	3	13.4	8	34.8	12	52.5	23	100.0	1.023
<b>Responses to mother questions</b>									.051
Done fair									
Done good	4	17.3	6	26.8	2	8.6	13	56.5	
	1	4.3	4	17.3	6	26.8	11	48.2	
Total	5	21.7	10	43.4	8	34.8	23	100.0	39.586
<b>Prepare newborn for discharge</b>									.577
Done fair									
Done good	4	17.3	7	30.4	5	21.7	16	69.5	
	1	4.3	1	4.3	5	21.7	7	30.4	
Total	5	21.7	8	34.8	10	43.4	23	100.0	37.107
<b>Prepare mother for discharge</b>									.005
Done fair									
Done good	3	13.4	9	39.2	4	17.3	16	69.5	
	0	0.0	0	0.0	7	30.4	7	30.4	
Total	3	13.4	9	39.2	11	47.8	23	100.0	

**Table (5): Relationship between nurses knowledge and their practice for newborn and mothers care after birth**

Nurses practice of care after birth	Total knowledge Score						Total		X <sup>2</sup>
	Poor		Fair		Good				
<b>Measuring weight</b>									23.8
Done fair	2	0.0	1	4.3	1	4.3	4	17.3	
Done good	9	39.1	4	17.3	4	17.3	17	73.9	
Not done	0	0.0	1	4.3	1	4.3	2	8.6	
Total	11	47.8	6	26.8	6	26.8	23	100.0	
<b>Measuring head circumference</b>									41.802
Done fair	0	0.0	2	6.8	2	8.6	4	17.3	
Done good	1	4.3	5	21.7	1	4.3	7	30.4	
Not done	2	8.6	2	8.6	8	34.8	12	52.2	
Total	3	13.4	9	39.2	11	47.8	23	100.0	
<b>Measuring length</b>									37.257
Done fair	0	0.0	3	4.3	1	4.3	2	8.6	
Done good	1	4.3	5	21.7	2	8.6	8	34.8	
Not done	2	8.6	5	21.7	5	34.8	15	66.2	
Total	3	13.4	13	56.5	8	34.8	23	100.0	
<b>Baby identification</b>									1.023
Done fair	0	0.0	2	8.6	4	17.4	6	26.2	
Done good	0	0.0	2	8.6	2	8.6	4	17.3	
Not done	3	13.04	4	17.3	6	26.8	13	56.5	
Total	3	13.4	8	34.8	12	52.5	23	100.0	
<b>Responses to mother questions</b>									.051
Done fair									
Done good	4	17.3	6	26.8	2	8.6	13	56.5	
	1	4.3	4	17.3	6	26.8	11	48.2	
Total	5	21.7	10	43.4	8	34.8	23	100.0	
<b>Prepare newborn for discharge</b>									39.586
Done fair	4	17.3	7	30.4	5	21.7	16	69.5	
Done good	1	4.3	1	4.3	5	21.7	7	30.4	
Total	5	21.7	8	34.8	10	43.4	23	100.0	
<b>Prepare mother for discharge</b>									37.107
Done fair	3	13.4	9	39.2	4	17.3	16	69.5	
Done good	0	0.0	0	0.0	7	30.4	7	30.4	
Total	3	13.4	9	39.2	11	47.8	23	100.0	

<sup>ns</sup>  $P \geq 0.05$  \*  $P < 0.05$  \*\*  $P < 0.001$

**Table (6)** Correlation between nurses education and years of experience to their knowledge of immediate care, total knowledge score, and responses to mother questions

**Correlations**

Co relational items		knowledge of immediate care	Response to mothers questions	Total knowledge score	Years of experience	Nurses education
knowledge of immediate care	Pearson Correlation	1	.467*	.307	.336	.478*
	Sig. (2-tailed)		.025	.155	.117	.021
Response to mothers questions	Pearson Correlation	.467*	1	.415*	-.004-	.459*
	Sig. (2-tailed)	.025		.049	.985	.028
Total knowledge score	Pearson Correlation	.307	.415*	1	-.191-	.284
	Sig. (2-tailed)	.155	.049		.383	.189
Years of experience	Pearson Correlation	.336	-.004-	-.191-	1	.311
	Sig. (2-tailed)	.117	.985	.383		.149
Nurses education	Pearson Correlation	.478*	.459*	.284	.311	1
	Sig. (2-tailed)	.021	.028	.189	.149	

\*. Correlation is significant at the 0.05 level (2-tailed).

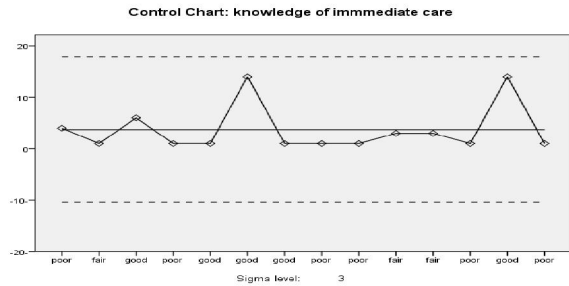


Figure (1) represent quality control chart for nurses knowledge of immediate care

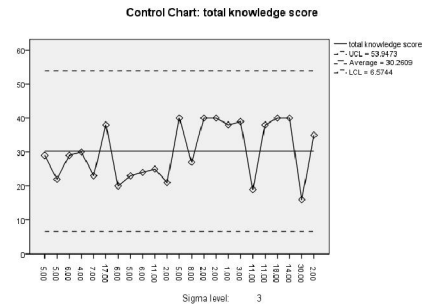
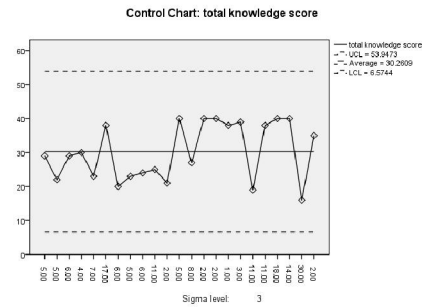


Figure (3) quality of care for nurses' total knowledge with their years of experience

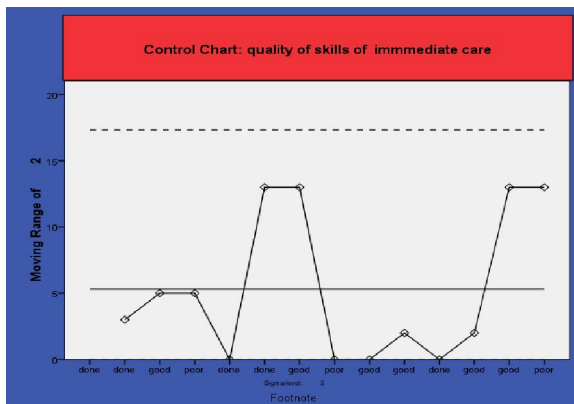


Figure (2) represent quality control chart for nurses' skills of immediate care

**4. Discussion:**

Birth is a major challenge for the newborn to negotiate successfully from intrauterine to extra uterine life. The first few hours since birth is the most crucial period in the life of an infant for further growth and development ,which is largely determined by the quality of care that the newborn receives

(Mangala and Bpkihs, 2009). The poor quality of care offered immediately after birth in many hospitals has been widely reported. The challenge is now therefore to define interventions that might improve nurses' knowledge and practice to improve their performance (Mike, etal, 2009).

The purpose of this study was to determine the quality of care provided by the nursing personnel in the selected aspects of care to the newborn babies from birth to two hours of age in the labour room and up to discharge. Adaptation of newborn baby to extra uterine life to a large extent is determined by the quality of care that he or she receives immediately after birth. The overall quality of knowledge and practices presented that nurses have a good quality of care in relation to birth preparation, (60,9%), preparing equipment for normal and cesarean, receiving the baby delivery (91,3% & 78,4%) and taking Apgar Score and clumping the umbilical cord (56.5%). During the birth of a newborn and immediately after delivery, if proper measures are not taken to establish and maintain an open airway, the newborn may aspirate. Prolonged exposure, keeping wet for long time with amniotic fluid and improper drying and wrapping may result in hypothermia and metabolic problems by cold stress. In addition only more than half of nurses (52,5%) highly skilled and knowledgeable about the importance of hand washing. This results was consistent with Shrestha et al, (2009) who found that tasks regarding washing and drying hands before receiving the baby was found poor for 76% of the babies. Even though almost all staff (99.9%) were wearing the gloves only 24% staff were wearing gown before receiving the newborn babies. So, quality of care provided in relation to the prevention of infection was average in more than half of nurses. Lack of proper hand washing before and after handling the baby and lack of proper aseptic techniques lead to infection and so the hospital stay will be prolonged which increases economical as well as psychological effects on the parents and hospital expenditure

(Gurung, 2008), mentioned that several studies have been conducted in Nepal collecting information on maternal and child health care practices. However, very few studies have been done specifically in the area of newborn care practices. Among a few study conducted, a hospital based study showed that birth asphyxia, low birth weight, hypothermia and infection were most common causes of neonatal death and most could be reduced by better care during delivery and after birth. Newborn care start before birth and among different stages of care, immediate care of newborn is equally important for newborn survival. With proper immediate care, newborn life

can be saved form untimely death due to the different causes

(Gurung, 2008) also mentioned that practices regarding newborn care are largely governed by various factors such as knowledge of caretaker, traditional beliefs and practices, socio-economic status of family, accessibility of health services and handling by trained birth attendants. From this concept, the total knowledge score was good in about half of the nurses (47,9%) as well as the biosocial characteristics of studied nurses reflected that more than half of them were have diploma in nursing education and have 1 – 5 years of experience. The study revealed also a significance relationship between nurses' education and their knowledge of immediate newborn care which affecting in the total quality of care provided for neonates and their mothers.

According to the current study, which obtained the relationship between nurses' knowledge and their practice of immediate care of the newborn. More than half of nurses have a good knowledge and practice for hand washing, prepare labour equipment, receive the neonate and clean the airway, apply Apgar score, clump, sterile the cord, as well as put eye drop to the neonate. This is in consistent with the Military Obstetrics and Gynaecology, 2009; which indicated that newborns, during the first few hours of life, have some difficulty maintaining their body heat and may develop hypothermia if not attended to carefully. During receiving the newborns replaces the wet towels; because the newborns can lose a tremendous amount of heat very quickly, particularly if they are wet. By removing the wet towels and replacing them with dry towels, it will reduce this heat loss.

As regard to clean the airway, the study is in concurrent with (Children's Hospital and Health System (2012) which reported that some newborns have excess amounts of fluid in their lungs. Stimulating the newborn to cry by massage and stroking the skin can help bring the fluid up where it can be suctioned from the nose and mouth. (Military Obstetrics and Gynaecology, 2009) added the importance of head pposition of the newborn, newborn should be kept on their backs or tilted to the side, but not on their stomachs. The orientation of the head relative to the body is important for breathing. In adults, this orientation is not usually crucial; adults tolerate a relatively wide range of head positioning without compromising their airway. Not so with newborns that have a relatively narrow range of head positioning that will permit air to move unimpeded through the trachea. The optimal position for the baby is with the head neither markedly flexed against the chest, nor extended with the chin up in the air. Instead, the head should be in a "military" attitude,



looking straight up. Position the newborn on its' back with the head looking straight up. This will usually provide for good airflow. If there is any airway obstruction, make small adjustments to the head position to try to straighten the trachea and eliminate the obstruction (Lhynneli, 2011).

As mentioned, more than half of nurses who participated in the current study, have a good knowledge and practice for applying Apgar score, clump, sterile the cord, as well as put eye drop to the neonate. This is similarly in agreement with (Lhynneli,2011) who highlighted that, it is part of the routine care of the newborn to give prophylactic eye treatment against gonorrhoea conjunctivitis or ophthalmia neonatorum. *Neisseria gonorrhoea*, the causative agent, may be passed on the fetus from the vaginal canal during delivery. This practice was introduced by Crede, a German gynaecologist in 1984. Silver nitrate, erythromycin and tetracycline ophthalmic ointments are the drugs used for this purpose. These ointments are the ones commonly used now a days for eye prophylaxis because they do not cause eye irritation and are more effective against Chlamydial conjunctivitis. Apply over lower lids of both eyes, then, manipulate eyelids to spread medication over the eyes. (Lhynneli, 2011) also emphasized the importance of administration of Vitamin K, because the newborn has a sterile intestine at birth; hence, the newborn does not possess the intestinal bacteria that manufacture vitamin K which is necessary for the formation of clotting factors. This makes the newborn prone to bleeding. As a preventive measure, 0.5 (preterm) and 1 mg (full term) Vitamin K or aquamephyton is injected IM in the newborn's vastus lateralis muscle. Unfortunately majority of the study sample were didn't administer Vitamin K to the newborns, didn't examine or observe their bodies or their genitalia. This also in contrast with (Children's Hospital and Health System, 2012) which describe the physical examination of the newborn in the delivery room; as a brief, it performed to check for obvious signs that the newborn is healthy. Other necessary procedures will be done over the next few minutes and hours. These may be done in the delivery room or in the nursery, depending on hospital policy and the condition of the newborn. Some of these procedures include the following: measurement of the temperature, heart rate, and respiratory rate, measurement of weight, length, and head circumference. These measurements help determine if a newborn's weight and measurements are normal for the number of weeks of pregnancy. Small or underweight newborns, as well as very large ones, may need special attention and care. Regarding the study results, approximately three quarter of nurses have good practice for measuring

newborn's weight, while more than half of them didn't measures newborn head circumference, length and identify the newborn.

Another positive finding of this study is that, quality of care was good for almost more than half of nurses in promoting attachment and initiating breast feeding from the first half an hour from delivery of the babies which reflected the importance of promoting intimate relationship between mothers and baby among nurses. This results was consistent with Shrestha ( 2009) who mentioned that over all care in relation to the initiation of breast feeding soon after delivery was average (76.9%). Informing the mother to initiate breast-feeding was found to be good (85%). These findings are not consistent with the study findings of Malar who reported that the implementations of the Baby friendly initiative policies were poor in the maternity wards of Mangala, and Bpkihs (2009). Invariably numerous literatures as Awasthi et al.,(1991), and Reeder *et al.* (1992), strongly advocate breast feeding because of its health benefits of the child as well as the mother herself, and also for the advantages of the bonding which it facilitates. These findings emphasize the need for developing protocol and checklist, which will include all-important essential newborn care.

According to (Ladewig *et al.*, 2006 & Mellisa, 2006)The postpartum period is a time of readjustment and adaptation for the entire childbearing family, but especially for the mother. The woman experiences a variety of responses as the mother adjusts to a new family member. Maternal role attainment is the process by which a woman learns mothering behaviors and becomes comfortable with her identity as a mother. Mothers are the primary caregivers of newborns. Thus, any factors that impact mothering affect the newborn and have public health significance. Mercer (1995) in (Ladewig *et al.*, 2006) mentioned that maternal role attainment often occurs in four stages: The anticipatory stage; which occurs during pregnancy. The woman looks to role models, especially her own mother, for examples of how to be a mother. The formal stage; begins when the newborn is born. The woman is still influenced by the guidance of others and tries to act as she believes others expect her to act. The informal stage; begins when the mother begins to make her own choices about mothering. She begins to develop her own style of mothering and finds ways of functioning that work well for her. The personal stage; is the final stage of maternal role attainment. When the mother reaches this stage, she is comfortable with the nobtion of herself as a mother (Ladewig *et al.*, 2006). Unfortunately The existing study reflected that more than half of the nurses respond poorly to mother's questions and majority of them were have poor

knowledge regarding care provided for mothers and newborns before discharge. Also, the majority of them didn't prepare the newborn or their mother for discharge. This is in contrast with (Lhynnelli, 2011) that provides Instructions to the mother especially on cord care; no tub bathing until cord falls off. Do not sponge bath to clean the newborn. That cord does not get wet by water or urine. Do not apply anything on the cord such as powder or antibiotic, except the prescribed antiseptic solution which is 70% alcohol. Avoid wetting the cord. Fold diaper below, it does not cover the cord and does not get wet when the diaper soaks with urine. Leave cord exposed to air. Do not apply dressing or abdominal binder over it. The cord dries and separates more rapidly if it is exposed to air. If notice bleeding from the cord, apply firm pressure and check cord clamp if loose and fasten. Report any unusual signs and symptoms of infection. Foul odor in the cord, presence of discharge, redness around the cord, the cord remains wet and does not fall off within 7 to 10 days and or newborn fever.

In addition to, Quality of care in examining newborn or their mothers were poor among (78,3% & 82,7%) of nurses in terms of physical assessment of head, back, extremities, genitalia, measuring length and head circumference as well as examining mothers for her, general condition, bleeding etc.. which reflect needs of those nurses for in-service program to fulfill those deficiency to reach the maximum quality of care provided to all neonates and their mothers, this is in contrast to the finding of Shrestha (2009) Study highlight fact that quality of care was good in areas such as initiation of breathing and physical assessment of newborn care was found to be good by (90%) of nurses. These findings emphasize the need for developing protocol and checklist, which will include all-important essential newborn care to be followed by the nurses.

### Conclusion

The findings in this study indicate that nurses have an around average of knowledge and quality of practice regarding total score of knowledge of birth preparation, initiating attachment and breast feeding. While much still needs to be done to improve the quality of maternal and newborn care regarding responding to mothers questions, examining baby and mothers before discharge. A combination of factors in this study, inhibit the provision of quality care such as educational levels and lack of training courses that nurses received: Study, displaying the protocols in labor room, and post natal ward. There is no data available regarding the infection arising from the lack of care immediately after the delivery, since mothers get discharged within few hours after delivery. However efforts will be taken in future researches to

assess the consequences of lack of care on neonates' outcomes.

### Recommendation

Based on the results of this study, it recommended the following:

Raising the awareness of nurses in delivery unit and nursery about the efficient examination of the newborns and mothers after delivery up to discharge.

### Management

- Hospital management should develop standards of quality care and procedures in the form of a manual to be available in delivery and neonatal units to all staff.
- The standards of quality care and performance should be reviewed regularly and developed according to international standards for quality of care and performance.
- Evaluating the quality of nursing care through outcomes-analysis activities

### Training

- providing continuous education that is based on systemic needs evaluation for nurses working in delivery and neonatal units.
- Providing quality improvement training programs for all nurses working in delivery and neonatal units.

Allow for competency-based in-service/refresher training that can be offsite, as well as more flexible on-the-job training. Training should include clinical simulations and other activities to encourage teamwork and improve efficiency in dealing with clinical efficiency needs

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10/10/2012

## Routine Evaluation Conducted on Registered Drugs (RECORD) – Experience of Glimpiride Oral Tablet in Treatment of Patients with Type 2 Diabetes Mellitus

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**Abstract: Objective** Patients were observed for improvement of diabetic symptoms and/or laboratory parameters in the form of improved RBS, FBS, 2 hr postprandial (PP) reduction rate in blood sugar, HbA1c reduction rate, or improvement in lipid profile. Patients were also assessed for the level of diabetes education they had received. **Methods** Three visits were scheduled for each patient, Baseline visit (visit 1), visit 2 (at 3 months from baseline), and visit 3 (at 6 months from baseline), during which observational data were collected; including patients demographics, diabetes/anti-diabetics history, symptoms & signs of diabetes, diabetes education, concomitant diseases, vital signs, HbA1c, FBG, 2 hr- postprandial & RBG values, lipid profile & creatinine (baseline visit). Changes to anti-diabetic therapy, HbA1c, FBG, 2 hr-postprandial & RBG values were evaluated at 3 & 6 months (visit 2 & 3). Lipid profile and creatinine were evaluated at 6 months. **Results:** The RECORD study demonstrated a statistically significant reduction in the baseline mean RBS level (from 275±86 to 162±43 mg/dl), FBS level (201±60 to 128±32 mg/dl), 2-hr postprandial blood glucose level (282±80 to 172±46 mg/dl) and HbA1c percentage (9.4±2 to 7.4±1.5 %) at the study endpoint (6 months duration), p value < 0.001 after a mean glimepiride daily dose of 2.36 ± 1.04 mg. Diabetic symptoms, especially; polyuria, polyphagia, polydipsia, numbness/tingling, burning sensation, and visual disorders showed significant improvements throughout the study duration. Glimpiride was well tolerated by the study population, and adverse events (AEs) were reported in 22 (1.35%) patients. All AEs were mild to moderate in intensity, none were serious and all AEs recovered without any sequelae. AEs included headache (8 patients), nausea (5 patients), dizziness (4 patients), diarrhea (3 patients) and hypoglycemia (2 patients). There was no causal relationship of the AEs to the study medication except in 2 patients who experienced hypoglycemia (one graded as mild and the other as moderate). **Conclusion** Glimpiride therapy in type 2 diabetic patients, showed safe and significant reduction in blood glucose parameters including HbA1c values, with significant improvements in diabetes symptoms over 6 month duration; demonstrating high effectiveness and tolerability among study population.

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**Keywords:** Routine Evaluation; Registered Drugs; Glimpiride Oral Tablet; Patient; Type 2 Diabetes Mellitus

### Abbreviation

ADA	American Diabetes Association	BMI	Body Mass Index	CAD	Coronary Artery Disease
FBS	Fasting Blood Sugar	GIT	Gastrointestinal Tract	HbA1c	Glycated Hemoglobin
HDL	High Density Lipoprotein	LDL	Low Density Lipoprotein	LPO	Last Patient Out
OAD	Oral Anti-Diabetic(s)	RBS	Random Blood Sugar		
SmPC	Summary of Product Characteristics	SU	Sulfonylurea(s)	T2D	Type 2 Diabetes

### 1. Introduction

Type 2 diabetes (T2D) is a progressive disorder with a consistent and steady increase in glycosylated hemoglobin (HbA1c) over time, associated with enhanced risk of micro- and macrovascular complications and a substantial reduction in life expectancy.<sup>1</sup>

It is a chronic disease associated with insulin resistance and a progressive failure of the pancreatic beta cells.<sup>2-4</sup> T2D is believed to account for about 90% of all cases of diabetes.<sup>5</sup> The American Diabetes Association (ADA) reported that, in the USA in 2007, 17.5 million people were diagnosed with diabetes.<sup>6</sup>

The 2010 International Diabetes Federation IDF atlas, indicates that the total number of diagnosed cases of diabetes (20-79 years age group) in 2010 is 17.96 (6.6%) millions in south America and Canada, 37.36 (10.2%) millions in north America and Caribbean region, 76.71 (4.7%) millions in Western Pacific region, 58.67 (7.6%) millions in South-East Asian region, 12.09 (3.8%) in Africa, and 26.65 (9.3%) in Middle East and North Africa; totaling to a global prevalence of 284.81 (6.4%) millions. According to the IDF 2010 atlas, there are 4.79 (11.4%) million case diagnosed with diabetes in Egypt, and the number is increasing at a rate of 8 new cases in every



100,000 person per year, with a mean yearly health expenditure of 0.55 billion USD (116 USD/person with diabetes) — all IDF percentages are for comparative prevalence adjusted to the world population.<sup>7</sup>

The number of people with diagnosed diabetes is growing at a rate of 1 million per year<sup>8</sup>, and is projected to reach over 48 million by 2050.

<sup>9</sup> The impact of diabetes on the US economy is alarming, with a total estimated cost of US\$174 billion in 2007. A majority of these costs are for treatment of complications of the disease.<sup>10-13</sup>

Large population-based studies have established that diabetes is associated with increased rates of cardiovascular morbidity and death. Clinical trials have shown the benefits of intensive glucose lowering therapies to reduce the risk of microvascular disease, cardiovascular events and death, or the combined risk of micro- and macrovascular events, in diabetic patients. Diabetes-related complications greatly diminish patients' health-related quality of life.<sup>14</sup>

The combination of dietary measures, body weight control and physical exercise is known to promote glycaemic control in patients with T2D. In 40% to 60% of these patients, however, this combination appears to be insufficient to achieve adequate blood glucose control and, in such cases, administration of one or several oral anti-diabetic drugs (OADs) is then added on to the initial measure.<sup>15</sup>

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) published an expert consensus statement on the approach to management of hyperglycemia in individuals with type 2 diabetes. Highlights of this approach are: intervention at the time of diagnosis with metformin in combination with lifestyle changes (MNT and exercise) and continuing timely augmentation of therapy with additional agents (including early initiation of insulin therapy) as a means of achieving and maintaining recommended levels of glycemic control (i.e., A1C <7% for most patients). As A1C targets are not achieved, treatment intensification is based on the addition of another agent from a different class. The overall objective is to achieve and maintain glycemic control and to change interventions when therapeutic goals are not being met.<sup>16</sup>

Most clinical evidence shows that the glucose-lowering effect of sulfonylureas and metformin is not durable and that the loss of glycemic control is associated with progressive  $\beta$ -cell failure. Metformin is traditionally known for its

metabolic effects on the liver; and other metformin target tissues include skeletal muscle and adipose tissue. Metformin is a useful adjuvant to lifestyle modification in overweight and obese patients with type 2 diabetes mellitus, metabolic syndrome, or impaired glucose tolerance (IGT).<sup>17</sup>

Sulfonylureas, which have been used for many years to treat T2D, decrease blood glucose by stimulating insulin secretion from pancreatic  $\beta$  cells. The extra-pancreatic actions of these drugs also contribute to their hypoglycemic action.<sup>18</sup>

Major complications associated with SU agents are hypoglycemia, weight gain and the exhaustion of beta ( $\beta$ ) cells with its hyperinsulinemic effect. Insulin resistance ultimately involves the development of obesity, metabolic disorder, hypertension and atherosclerotic diseases.<sup>19</sup>

Glimepiride is a long-acting SU of recent origin. The glucose-lowering effects are well documented and comparable with those of other long-acting SUs.<sup>1</sup>

In comparison with conventional sulfonylurea drugs, glimepiride has several benefits: rapid and complete absorption after oral administration, a lower dose, long duration of action, and possible insulin-sensitizing effect. In addition, previous clinical studies demonstrated that glimepiride once-daily dose, which is a common usage of this agent in Europe and the US, provided a good glycemic control of T2D as well as twice-daily doses.<sup>20</sup>

As monotherapy, glimepiride is well tolerated and effective in achieving metabolic control in treatment of patients with T2D.<sup>1</sup>

Although glimepiride has lesser insulin secretion action from the pancreatic  $\beta$ -cells compared with the conventional SU agent, its hypoglycemic action is equivalent to conventional SU agents.<sup>19</sup> It has recently been reported that plasma adiponectin levels increase after the administration of glimepiride<sup>21</sup>. Adiponectin shows anti-atherosclerotic effects on the vascular wall, and insulin sensitivity is increased in skeletal muscle and liver. The unique effects of this drug might be useful in patients with metabolic syndrome.<sup>19</sup>

There are clinical trial outcomes suggesting that glimepiride contributes to the improvement of hyperinsulinemia, visceral fat accumulation and atherosclerotic suppression, and that glimepiride was found to improve insulin resistance, consistent with results reported in previous studies.<sup>19</sup>

Without markedly increasing plasma insulin concentrations, glimepiride has a more prolonged hypoglycemic effect than glibenclamide, suggesting that glimepiride might have more



potent extra-pancreatic action.<sup>18</sup>

Glimepiride differs from other sulfonylureas in that it reportedly binds to a different receptor at the  $\beta$ -cell membrane than other agents in this class. Animal studies demonstrate greater reductions in plasma glucose per increment in plasma insulin with glimepiride than with glibenclamide or glipizide, suggesting that glimepiride may have direct extra-pancreatic effects that stimulate an improvement in insulin sensitivity.<sup>22</sup>

Glimepiride is characterized by a long duration of action, conferring effective glycaemic control over a 24-hour period with a single daily dose. The efficacy and good safety profile of glimepiride in patients with type 2 diabetes have been clearly demonstrated in previous studies employing doses ranging from 1 mg to 6 mg/day.<sup>15</sup>

Some clinical researchers have also reported that neither weight nor BMI is increased after the administration of glimepiride.<sup>23-24</sup> In a recent study, in agreement with these reports, results have shown no significant change in BMI.<sup>19</sup>

An intervention targeting reduction in glycaemia levels to current guidelines, as well as improving concomitant risk factors, such as blood pressure, lipid levels and bodyweight might prevent and reduce the risk of micro- and macro-vascular complications. This intervention has recently been endorsed by a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association.<sup>25</sup>

Improvements in the cardiovascular event rates are important as these events are the main contributor to death and increased cost of treating T2D.<sup>14</sup>

Recent drug strategies are also targeted to improve insulin resistance rather than the promotion of insulin secretion.<sup>19</sup>

Glimepiride leads to an improvement in lipid metabolism<sup>26</sup>, improves endothelial function due to the biosynthesis of nitric monoxide, and has anti-oxidative effects in addition to the anti-atherosclerotic effects mediated by cytokines.<sup>27,28</sup> Furthermore, glimepiride also has an inhibitory effect on the initiation and development of atherosclerosis.<sup>19</sup>

This study observed the effectiveness and safety of Glimepiride treatment in type 2 diabetes mellitus, and assessed how challenging it can be to achieve targeted treatment outcomes, safely and effectively within a practical clinical setting.

## 2. Patients and Methods

### Patients

Two thousand patients were planned for enrollment. Physicians were guided by the SmPC (Summary Of Product Characteristics) in deciding on the treatment regimen and patient selection. All patients presenting with type 2 diabetes mellitus (T2D) for whom the physician decided to prescribe Glimepiride were enrolled in the study.

### Study Design

The RECORD study was designed as a national, multicentre, prospective, observational study, and was conducted in Egypt over 4 years duration. The study duration for any one patient was 6 months, starting from the recruitment day (individual baseline), till visit 3 (6 months from individual baseline).

### Observations

Three visits were scheduled for each patient, baseline visit (visit 1), visit 2 (at 3 months from baseline), and visit 3 (at 6 months from baseline), during which observational data were collected; including patients demographics, diabetes/anti-diabetics history, symptoms & signs of diabetes, diabetes education, concomitant diseases, vital signs, HbA1c (glycosylated hemoglobin), FBG (Fasting Blood Sugar), 2 hr-postprandial & RBG (Random Blood Sugar) values, lipid profile & creatinine (baseline visit). Changes to anti-diabetic therapy, HbA1c, FBG, 2 hr-postprandial & RBG values were evaluated at 3 & 6 months (visit 2 & 3). Lipid profile and creatinine were evaluated at 6 months. Adverse events were monitored throughout the course of the study. The usual initial dose was 1 mg once daily, and was allowed to be gradually up titrated (1mg - 2 mg - 3 mg - 4 mg - 6 mg), as required based on regular monitoring of blood glucose levels.

### Efficacy Outcome

Patients were observed for improvement of diabetic symptoms and/or laboratory parameters in the form of improved RBS, FBS, 2 hr postprandial reduction rate in blood sugar, HbA1c reduction rate, or improvement in lipid profile. Patients were also assessed for the level of diabetes education they had received.

### Safety Outcome

Patients were observed for the occurrence of any adverse events, including causal relationship to study medication, measures taken, and outcome.

### Statistical Analysis

Descriptive methods were used for the analysis of the study outcomes, including calculation of appropriate measures of the empirical distribution

(mean, standard deviation, median, minimum, maximum, for continuous variables, and frequencies & percentages for categorical variables) as well as calculation of descriptive p-values for group comparisons. Quantitative data were analyzed for normal distribution using paired t-test and repeated measures analysis. Qualitative data were analyzed using Chi square test.

### 3. Results

#### Patients' Baseline Characteristics and Demographics

A total number of 1629 patients with T2D mellitus were enrolled in the study. The study population had a balanced contribution from both male and female patients (49.8%, n=811 males and 50.2%, n=818 females) with a mean age of  $55.23 \pm 10.49$  (years), a mean weight of  $85.74 \pm 15.16$  (kg), a mean height of  $1.67 \pm 0.08$  (m), a mean BMI of  $30.86 \pm 5.9$  ( $\text{kg}/\text{m}^2$ ), and a mean duration of diabetes of  $10.36 \pm 5.83$  (years).

The mean baseline random, fasting and 2 hours postprandial blood glucose levels (mg/dl) levels were  $275.3 \pm 86.4$ ,  $200.7 \pm 60.2$  and  $282.3 \pm 80.4$  respectively. The mean HbA1c level was  $9.4 \pm 2.0$ .

The most frequently reported diabetic symptoms among the study population at visit 1 were polyuria, polyphagia, polydipsia and numbness constituting 89.2% (n=1453), 75.3% (n=1226), 77.5% (n=1262) and 57.9% (n=943) respectively, and less frequently burning sensation 50.7% (n=826) and visual disorders 31.1% (n=507).

The highest rate of concomitant diseases among the study population was for hypertension (45.2%, n=737), hepatic disorders (12.5%, n=204) GIT (Gastro-Intestinal Tract) disorders (11.4%, n=185). Cardiovascular and renal diseases were less frequently recorded at 9.9% (n=162) and 7.7% (n=125) respectively.

Analysis of data concerning diabetes education received by patients revealed that 56.6% (n=922) of patients had been previously educated on disease background, 68.1% (n=1110) had been educated on diet, 55.9% (n=910) on exercise, 44.9% (n=731) on foot care, 35.6% (n=580) on eye care and 30.4% (n=496) on diabetic complications.

The mean gliclazide daily dose prescribed at baseline was  $2.36 \pm 1.043$  mg where the most frequent dosage forms were **3 mg** (25.4%) then **2 mg** (21.5%) while the most frequent concomitant OADs were metformin (18.4%), gliclazide (12.9%) and amophage by 8.9%.

#### Outcomes

The RECORD study demonstrated a

significant reduction in the baseline mean RBS (from  $275.3 \pm 86.4$  to  $187.4 \pm 57.3$  at visit 2 (3 months from baseline) to  $162.3 \pm 42.9$  mg/dl with mean percent reduction 41% at visit 3 (end of study),  $p < 0.001$ ).

Baseline mean fasting blood glucose levels also showed a significant reduction (from  $200.7 \pm 60.2$  to  $145.5 \pm 42.7$  at visit 2 to  $128.4 \pm 31.9$  mg/dl with mean percent reduction of 36% at visit 3,  $p < 0.001$ ), with similar statistically significant reduction in the mean baseline 2-hr postprandial blood glucose levels (from  $282.3 \pm 80.4$  to  $202.0 \pm 59.7$  at visit 2 to  $171.7 \pm 46.2$  mg/dl with mean percent reduction of 39.2 % at visit 3,  $p < 0.001$ ).

The mean value of HbA1c percentage showed statistically significant reduction from  $9.4 \pm 2.0$  at baseline  $8.0 \pm 1.6$  at visit 2 to  $7.4 \pm 1.5$  and mean percent reduction of 21.3% at visit 3 ( $p < 0.001$ ).

Diabetic symptoms, especially; polyuria, polyphagia, polydipsia, numbness/tingling, burning sensation, and visual disorders showed statistically significant improvements with time through visit 2 (3 months) and visit 3 (6 months) as outlined in Table 2 below.

Both mean systolic (SBP) and diastolic blood pressure (DBP) values at baseline ( SBP =  $139.7 \pm 20.3$  mmHg, DBP =  $86.9 \pm 10.4$  mmHg), showed significant gradual reduction, to reach  $130.98 \pm 13.92$  mmHg, and  $82.1 \pm 7.0$  mmHg, for SBP and DBP respectively by end of study; at visit 3. ( $p < 0.001$ ), representing a reduction of 8.72, and 4.77 mmHg, in SBP and DBP, respectively, compared to baseline values.

In the other hand, the BMI (Body Mass Index) was significantly decreased from  $30.86 \pm 5.89$   $\text{kg}/\text{m}^2$  at visit 1 to  $30.65 \pm 5.59$   $\text{kg}/\text{m}^2$  at visit 3 ( $p < 0.05$ ).

Concerning the lipid profile values, there were statistically insignificant reduction in the mean values of LDL (Low Density Lipoprotein) from  $148.8 \pm 42.9$  at visit 1 to  $146.1 \pm 42.8$  at visit 3 ( $p = 0.183$ ), for triglycerides there were statistically significant reduction from  $209.8 \pm 79.9$  at visit 1 to  $190.1 \pm 63.5$  at visit 3 ( $p < 0.001$ ), and for cholesterol from  $228.9 \pm 44.3$  at visit 1 to  $221.9 \pm 36.6$  at visit 3 ( $p < 0.001$ ). While the mean value of HDL (High Density Lipoprotein) showed an insignificant slight increase from  $47.5 \pm 26.6$  at visit 1 to  $47.7 \pm 23.8$  at visit 3 ( $p = 0.802$ ). Regarding the level of serum creatinine (mg/dl), the results revealed a very trivial decline in the mean value from  $1.1 \pm 0.3$  at visit 1 to  $1.0 \pm 0.3$  at visit 3 ( $p < 0.001$ ).

**Safety Profile**

Glimepiride was well tolerated by the study population, with 22 patients (1.35%) showing adverse events of mild to moderate intensity, mainly in the form of headache, nausea, dizziness, diarrhea and hypoglycemia (2 patients), all recovered with no sequelae. Both hypoglycemic events were of mild to moderate intensity and recovered with no sequelae, and one patient with hypoglycemic event

required dose reduction. There was no causal relationship of AEs (Adverse Events) to the study medication except in 2 patients who experienced hypoglycemia. There have been no serious adverse events recorded.(all adverse events were defined according to International Conference for Harmonization-Good Clinical Practice guidelines—ICH-GCP).

**Table (1) The mean values of Lab. results (Random Blood Sugar (mg/dl), Fasting Blood Sugar (mg/dl), 2 hrs-postprandial Blood Sugar (mg/dl) and HbA1c %) at different study visits.**

Laboratory Test	Visit	Mean Value (SD)	% Mean Change	P Value*	P Value**
Random Blood Sugar level mg/dl	Visit 1 Baseline	275.3 (86.4)	NA	NA	<0.001
	Visit 2 3 Months	187.4 (57.3)	31.9	<0.001	
	Visit 3 6 Months	162.3 (42.9)	41	<0.001	
Fasting Blood Sugar level mg/dl	Visit 1 Baseline	200.7 (60.2)	NA	NA	<0.001
	Visit 2 3 Months	145.5 (42.7)	27.5	<0.001	
	Visit 3 6 Months	128.4 (31.9)	36	<0.001	
2 hrs-postprandial Blood Sugar level mg/dl	Visit 1 Baseline	282.3 (80.4)	NA	NA	<0.001
	Visit 2 3 Months	202.0 (59.7)	28.4	<0.001	
	Visit 3 6 Months	171.7 (46.2)	39.2	<0.001	
HbA1c %	Visit 1 Baseline	9.4 (2.0)	NA	NA	<0.001
	Visit 2 3 Months	8.0 (1.6)	14.9	<0.001	
	Visit 3 6 Months	7.4 (1.5)	21.3	<0.001	

\* Using paired t-test

\*\* Using repeated measure analysis

**Table 2: Diabetes History of symptoms at visit 1 and sequelae of symptoms at visits 2 and 3**

	Visit 1		Visit 2			Visit 3		
	Freq.	%	Improved %	No change %	Worsened %	Improved %	No change %	Worsened %
<b>Polyuria</b>	1453	89.2	86.6%	10.2%	3.2%	90.1%	6.9%	3.0%
<b>Polyphagia</b>	1226	75.3	85.5%	10.3%	4.2%	90.0%	6.8%	3.2%
<b>Polydipsia</b>	1262	77.5	86.0%	12.0%	2.0%	89.7%	7.3%	3.0%
<b>Numbness/Tingling</b>	943	57.9	83.4%	11.9%	4.7	85.7	12.4	1.9
<b>Burning sensation</b>	826	50.7	68.2%	26.0	5.8%	83.6	13.6	2.8
<b>Visual disorders</b>	507	31.1	71.2%	23.7%	5.1%	82.0	15.3	2.7

**Table 3: Adverse events characteristics**

Adverse events characteristics	Frequency		
	Adverse events	22 (1.35%)	
Seriousness	Yes	0	
	No	22	
		Mild	Moderate
	Headache	3	5
	Hypoglycemia	1	1
	Nausea	3	2
	Dizziness	1	3
	Diarrhea	2	1
Intensity	Moderate	12	
	Mild	10	
Causal relationship	Yes	2*	
	No	20	
Remedial Measures	Dosage decreased	5	
	No change	17	
Outcome	Recovery without sequel	17	
	No change	22	

\* Two patients suffered hypoglycemia related to Amaryl® administration

### 3. Discussion

The study population demonstrated a clinical setting very similar to what is encountered in practice. The majority of the patients had been diabetics for long (mean duration 10.36 years), having a combination of concomitant diseases, notably hypertension (45.2%), hepatic disease (12.5%), or cardiovascular disease (9.9%). The majority of the patients received education about their disease, however, in most cases information was lacking; especially regarding eye care and diabetic complications, where two thirds of the patients were never educated in these two particularly important topics. Glimperide, demonstrated significant improvements in all diabetic symptoms after visit 2 (3 months), and improvements continued further as recorded in visit 3 (6 months).

All diabetic parameters (RBS, FBS, 2hrs pp levels, HBA1c) showed marked improvement, with good control of the disease, and excellent tolerability. No serious adverse events were recorded, and side effects were either mild or moderate, and recovered with no sequelae.

In a retrospective cohort study conducted using an academic health center enterprise-wide electronic health record (HER) system to identify 11,141 patients with T2DM with or without a history of previous CAD (Coronary Artery Disease), treated with either glipizide, glibenclamide, or Glimperide.

Patients were followed for mortality rates. Subanalysis of patients with documented CAD, showed a strong trend toward a reduced risk with Glimperide, and suggested that glimepiride may be the preferred sulfonylurea in those with underlying CAD.<sup>29</sup> In this study, both systolic and diastolic blood pressure values showed significant improvement by end of study, together with similar improvements in lipid profile with a cardiovascular protective attitude (increased HDL, and decreased LDL, cholesterol, and triglycerides). Creatinine was also reduced from 1.1mg/dl at baseline to 1.0 mg/dl on visit 3 (end of study), indicating an improvement in renal functions. Tsunekawa et al.<sup>30</sup> clearly demonstrated that glimepiride actually increases insulin sensitivity in type 2 diabetic patients. They also proposed that the increase in insulin sensitivity might be associated with increased adiponectinemia. The improvement in glycemic control, insulinemia, and adiponectinemia by glimepiride is of potential benefit to decrease risk factors of atherosclerosis in type 2 diabetic patients. The mechanisms of the increased adiponectinemia by glimepiride may be complex and multifactorial.<sup>31</sup>

In this study Glimperide has shown to be a safe and effective oral anti-diabetic agent, with good control of symptoms, and provided additional improvements to other organ systems, especially cardiovascular and renal functions.

In addition to safety and efficacy of glimepiride in T2D, it is worth mentioning that glimepiride's ease of use provides an affordable solution to "medicated compliance", which is a common problem among diabetic patients. Once-daily dose compared with more frequent dosing regimen promises to improve compliance among patients with NIDDM (Non-Insulin Dependent Diabetes Mellitus)<sup>19</sup>. In addition, glimepiride is available in different preparation concentrations (1,2,3,4, and 6 mg), which further adds to its ease of use. A recent randomized crossover study has shown that pharmacodynamic and safety profiles in once-daily dose of glimepiride in type 2 diabetic patients are not different from those in twice-daily dosing, and suggested that once-daily dosing is more suitable for the type 2 diabetic patients treated with glimepiride<sup>19</sup>.

Further patient education is recommended in order to maximize the individual benefits, and minimize the potential risk imposed on disease ignorant or misinformed patients.

### Conclusion

This study showed that glimepiride was



effective as an antidiabetic therapy for type 2 diabetic patients, who were able to achieve target metabolic control in terms of reduction of blood glucose parameters, including HbA1c values. Moreover, glimepiride therapy improved diabetic symptoms, and all reported AEs (1.35%) were of mild to moderate intensity, indicating high tolerance to glimepiride therapy.

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**The role of Ectonucleotidases Pathway (CD39/CD73) in Childhood Renal Failure****Nahla M Abd-Elaziz<sup>1</sup> and Ateyat A Ateya<sup>2</sup>**Department of Clinical Pathology<sup>1</sup> and Pediatrics<sup>2</sup>, Faculty of Medicine for girls, Alazhar University  
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**Abstract:** Background and Aim: Chronic renal failure (CRF) is defined as an irreversible reduction in glomerular filtration rate (GFR). In children, CRF may be the result of congenital, acquired, inherited or metabolic renal disease. In addition to progressive injury with ongoing structural/ metabolic genetic diseases, renal injury may progress despite removal of the original insult. The clinical presentation of CRF is quite varied and dependent on the underlying renal disease. Hypoxia is considered as the master factor in the pathogenesis of renal failure. In the last few years, studies have demonstrated the protective anti-inflammatory role of CD39/CD73 molecules pathway during hypoxia. CD39 and CD73 molecules have been described on circulating T lymphocytes, endothelial cells and minimally, expressed on granulocytes. CD39, (ectonucleotidase triphosphate diphosphohydrolase 1, ENTPD1) is responsible for the conversion of proinflammatory ATP to ADP and AMP whereas CD73 (ecto-5'-nucleotidase) converts AMP into adenosine. Adenosine plays a central role in tissue protection via anti-inflammatory and immune modulatory effect. We aimed to assess the role of CD39/CD73 axis in immuno-inflammatory pathogenesis of renal failure. Also, we aimed to define the expression pattern of both phenotypes on circulating T lymphocytes. Patients and method: This cross sectional study was conducted at Al Zahraa University Hospital from December 2011 to May 2012. An informed consent was obtained from parents of all children. The study included twenty children with chronic renal failure, (on regular hemodialysis) and twenty apparently healthy children as control group. The routine laboratory investigations were performed for patients (blood urea, serum creatinin, serum alkaline phosphatase and complete blood count). Using flowcytometry, the patients and control groups were investigated for the frequency of CD39 and CD73 T lymphocytes. In addition, C-reactive protein (CRP) was performed for patients as inflammatory marker. Results: We found that both CD39% and CD73% of patient group were significantly lower than those of control Group. In patient group, we observed that both CD39% and CD73% were negatively correlated to blood urea. Also, CD73% was negatively correlated to serum creatinine. In addition, patient group showed negative correlation between CRP as an inflammatory marker and the percentage of both CD39/CD73. Furthermore, there was negative correlation between CD73% and serum alkaline phosphates. Conclusion: The significant decline in both CD39 and CD73 molecules on patients T lymphocytes confirmed the relation between down modulation of ectonucleotidases and deregulation of renal immunological/inflammatory cascade. Also, the negative correlation between both molecules and blood urea proved that CD39/CD73 deficiency can impact renal function. Moreover, the negative relation between CRP and the mean percentages of both phenotypes revealed the protective anti-inflammatory role of the pathway.

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**Key words:** CD39 –CD73 –ectonucleotidases- chronic renal failure

**1. Introduction**

Extra cellular nucleotides and nucleosides act as signaling molecules involved in a wide spectrum of biological effects. Their levels are controlled by a complex cell surface-located group of enzymes called ectonucleotidases. There are four major families of ectonucleotidases, nucleoside triphosphate diphosphohydrolase (ENTPD/CD39), ectonucleo-hydrophosphatase, alkaline phosphatase and ecto-5'-nucleotidase (5'NT/CD73) (Rosa et al., 2008).). It is generally accepted that ectonucleotidase, CD39 (ENTPD1) hydrolyse pro-inflammatory adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) whereas CD73 (5'NT)

catalyses the dephosphorylation of AMP into adenosine (Hilaire et al., 2011).

Ectonucleotidases pathway participates in numerous important immunological functions, It has been appreciated that adenosine attenuates potentially harmful aspects of inflammation via controlling interaction between lymphocytes and vascular endothelium (Shirley et al., 2009).

The vascular endothelium is the primary interface between tissue inflammatory signals and circulating leukocytes (Colgan et al., 2006). As such, the endothelium is central to orchestration of lymphocytes activation in response to chemotactic stimuli (Hasko et al., 2011).

The ectonucleotidases (CD39/CD73) pathway has been widely implicated as an adaptive response to hypoxia (Robson, 2011).

It has been suggested that hypoxia up-regulates the expression of CD39 /CD73 molecules on T lymphocytes (Hernandez et al., 2011). CD39 and CD73 deficient T lymphocytes seem to occupy the central role in the pathogenesis of hypoxia-induced inflammatory disorders. They act as mediator of ischemic injury and modulator of immune response. The mechanism by which T lymphocytes exert their modulatory effect remain poorly understood (Thompson et al., 2004, Friedman et al., 2009).

Many experimental studies demonstrated the protective and modulatory role of ectonucleotidases (CD39 and CD73) in different inflammatory models. In human, controversial results have been published about their function and their pattern of expression on circulating leukocytes (Nitschk et al., 2011).

Previous studies reported that CD39 and CD73 deficient T lymphocytes is considered as proinflammatory phenotype of circulating lymphocytes, enhanced expression of adhesion molecules and predisposed to autoimmune inflammation (Zernecke et al., 2005)

In murine model, it was demonstrated that chronic lack of CD73 leads to interstitial nephritis with subsequent renal impairment. The underlying mechanisms depend upon activation of lymphocytes to secrete excessive inflammatory cytokines (Blume et al., 2012).

Research on adenosinergic mechanisms of inflammation, revealed that CD39 deficiency impacts nephropathy in murine models and CD39 gene mutation; also appear to influence renal outcomes (Shirley et al., 2009).

Chronic renal failure is considered as inflammatory disorder. It may results from various forms of glomerulonephritis. The prevalence of chronic renal failure in the pediatric population is approximately 18 per 1 million (Vogt and Avner, 2004). Therefore, we aimed to assess the contribution of CD39/CD73 pathway to the immuno-inflammatory pathogenesis of renal failure.

## 2. Patients and Methods

The study group consisted of 20 patients with chronic renal failure (on regular hemodialysis), they were 11 males and 9 females with ages ranged from 7 to 16 years. They were recruited from the pediatric nephrology and dialysis unit at Al zahraa Hospital. Inclusion criteria were patients on regular dialysis, with CRF caused by various forms of

glomerulonephritis. Exclusion criteria were CRF caused by congenital abnormalities such as renal hypoplasia, dysplasia and/or obstructive uropathy, CRF related to metabolic disorders (cystinosis, hyperoxaluria) and CRF resulted from inherited disorders (Alport syndrome, polycystic kidney disease). Twenty age- and sex- matched apparently healthy children were included as control group. All children included in the study were subjected to complete history taking, thorough clinical examination and routine laboratory investigations as blood urea, serum creatinine, serum alkaline phosphatase, CBC and CRP.

The patients and control groups were investigated for the frequency of CD39 and CD73 T lymphocytes using flowcytometry. C-Reactive protein (CRP) was performed for patients group by using Teco diagnostics (latex slide test)

### Methodology:

Venous samples were taken in the plain vacutainers for C-reactive protein and sterile EDTA vacutainers for immunophenotyping.

### Immunophenotyping:

We used one test tube for each sample (20 patients tubes and 20 control tubes). Each tube contain 20 microliteres of conjugated fluorescein isothiocyanate (FITC) labeled CD39 monoclonal antibodies and 20 microliteres conjugated phycoerythrin (PE) labeled CD73 monoclonal antibodies 100 microliteres of the test samples was add to each tube, vortex the tubes gently.

The tubes were incubated 20 minutes at room temperature in the dark, then add 1 mL of fix and lyse mixture, vortex the tubes immediately for one second and incubated again for 10 minutes in the dark at room temperature centrifugation of tubes at low speed for 5 minutes followed by aspiration of supernatant and resuspension of pellet in residual fluid. 2 mL of phosphate buffer saline was add to each tube, the suspension was centrifuged at low speed. The supernatant was discarded, then the residual suspension were passed through the flow-cytometer.

For analysis, gats were set around lymphocytes on the bases of the forward and side scatter profile.

## 3. Results

Twenty children with chronic renal failure, on regular hemo-dialysis and twenty apparently healthy children were included in our study. To assess the expression pattern of CD39/CD73 on T lymphocytes during renal failure, we compared the expression of both phenotypes in patients group and control group. Table and figure (1) show highly statistically significant decrease in patients compared to control group regarding CD39 and

CD73 T lymphocytes (P<0.001, P <0.002, respectively).

In order to detect the influence of CD39 and CD73 T lymphocytes on renal function, correlation study between the two parameters was performed.

As shown in Table (2) and figures (2, 3), both phenotypes (CD39 and CD73) were negatively correlated to blood urea (P <0.01, P P <0.001, respectively).

Also, figure (4) show statistically significant negative correlation between CD73 T lymphocytes and serum creatinine (P <0.012).

To evaluate the role CD39/CD73 in inflammatory pathogenesis of renal failure, correlation study between both phenotypes and C-reactive protein (CRP) as inflammatory marker was performed.

Table (3) and figure (5) show statistically significant negative correlation between the percentages of both CD39, CD73 T lymphocytes and CRP values (P <0.001, P <0.05, respectively).

The relation between CD39, CD73 T lymphocytes and serum alkaline phosphatase was studied. Table (3) shows statistically significant negative correlation between CD73 T lymphocytes and serum alkaline phosphatase, whereas no correlation between CD39 T lymphocytes and serum alkaline phosphate (P <0.05, P = 0.630, respectively).

In summary, the comparison between the two studied groups showed a highly statistically significant decrease regarding to CD39 and CD73 in patients compared to controls.

There was a statistically significant negative correlation between CD39 and urea. Also there was a statistically significant negative correlation between CD73 and S. creatinine and a highly statistically significant negative correlation between CD73 and urea.

There was a highly statistically significant negative correlation between both CD39 and CD73 T lymphocytes and CRP.

**Table (1) and figure (1): Comparison between the studied groups regarding CD39% and CD73%.**

Parameters	Groups				Independent t-test	
	Patients		Control		T	p-value
	Mean	±SD	Mean	± SD		
CD39%	5.44	±1.90	16.20	±1.62	-15.283	<0.001
CD73%	9.74	±2.95	18.66	±1.13	-9.160	<0.002

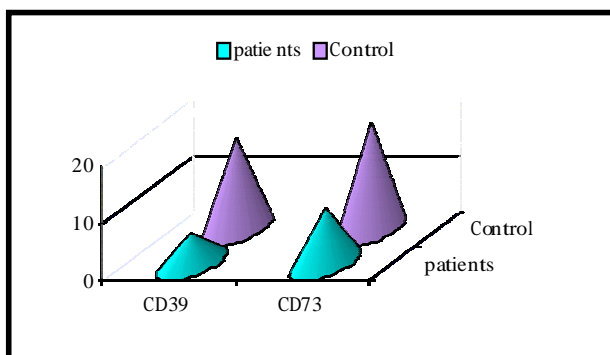


Table 2: Correlation between CD39% and CD73% with blood urea and Serum creatinine

Parameters	CD39%		CD73%	
	R	P	R	P
Urea( mg/dL)	-0.543*	0.01	-0.708**	<0.001
Serum creatinine(mg/dL)	0.579	20	-0.552*	<0.012

\* Correlation is significant at the 0.05 level (2- tailed).

\*\* Correlation is significant at the 0.001 level (2- tailed).

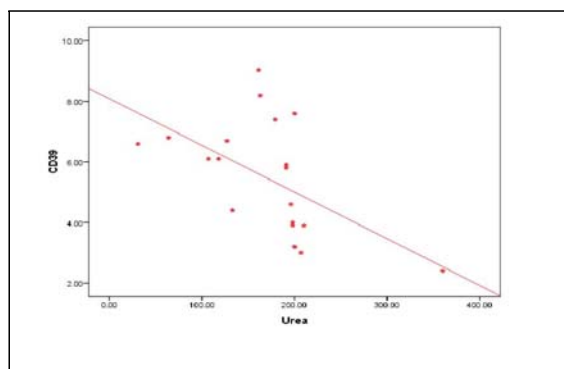


Figure 2: Correlation between CD39 and urea

Table 3: Correlation between CD39% and CD73% with alkaline Phosphatase and C-reactive protein (CRP)

	CD39%		CD73%	
	R	P	R	P
Alkaline phosphatase (u/l)	-0.115	0.630	-0.441*	< 0.05
CRP (mg/dl)	-0.801**	<0.001	-0.429*	< 0.05

\* Correlation is significant at the 0.05 level (2-tailed).

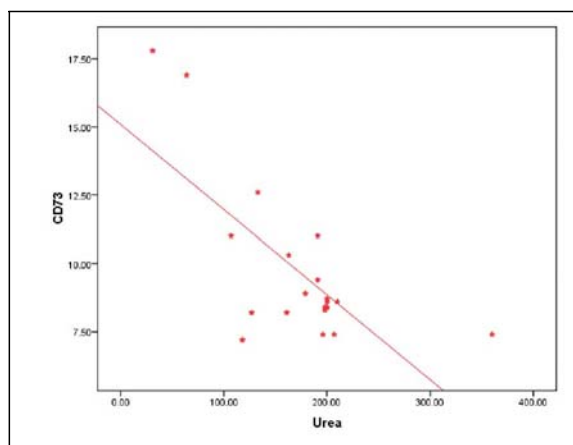


Figure 3: Correlation between CD73 and urea

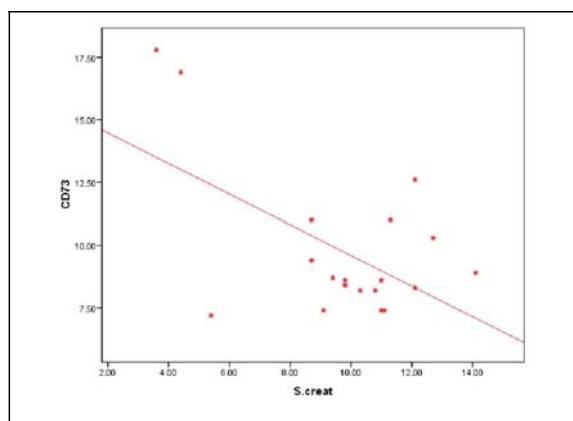


Figure 4: Correlation between CD73 and S. creat

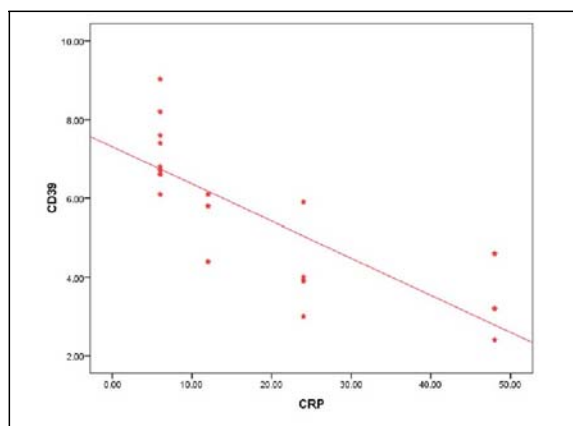


Figure 5: Correlation between CD39 and CRP

#### 4. Discussion

Cells with anti-inflammatory and immunosuppressive functions raise particular interest in renal impairment because of their potential role in determination of disease course and their prospective use in therapy. CD39 and CD73 T

lymphocytes are believed to be one of the immunosuppressive cells, contradictory reports are, however, available describing their frequency and function during ischemic diseases (Allam et al., 2009).

CD39 and CD73 molecules are members of ectonucleotidases family, it has been reported that both phenotypes are expressed on human and murine T lymphocytes. In particular, these molecules suppress T lymphocytes functions and control inappropriate or exaggerated immune response (Nicolova et al., 2011).

Also, CD39 molecule in concert with CD73 molecule generate adenosine which is important to balance between activation and regulation of immuno-inflammatory cascade (Kasper et al., 2007 and Cekic., 2012).

Many experimental studies have demonstrated the anti-inflammatory role of CD39/CD73 pathway in murine model (Blume et al., 2012).

In vitro tissue culture studies revealed that the lack of CD39/CD73 pathway is likely to predispose to increased inflammatory activity via disruption of cytokines expression and dysregulation of cell adhesion molecules cascade (Bell et al., 2010, Libra et al., 2011 and Zhang et al., 2011).

The in vivo studies on human CD39/CD73 axis are scarce and contradictory (Bonner et al., 2010). Therefore, our study aimed to define the expressive pattern of both phenotypes on circulating lymphocytes. Also, to assess the role of CD39/CD73 pathway in the immuno-inflammatory pathogenesis of renal impairment.

In the present study, we noted the down-modulation of CD39 in tandem with CD73. These findings may be due to deficiency of CD39 and subsequent accumulation of ATP which exhibits feed forward inhibition of CD73 (Robson, 2012).

On comparing the frequency of CD39 and CD73 T lymphocytes in patient group and control group, we observed that T lymphocytes of patients group displayed lower expression of both CD39 and CD73 molecules as matched to control group. These observations were in agreement with Blume et al. (2012), who demonstrated that CD39/CD73 deficiency exhibit glomerular injury and renal impairment.

Interestingly, Romio et al. (2011) have suggested the trafficking of murine circulating T lymphocytes into lymphoid tissues during inflammation.

Moreover, it was demonstrated that CD73 deficiency is associated with pro-inflammatory phenotype of the vasculature; increased attachment of lymphocytes to the endothelium and enhanced



expression of cell adhesion molecules (Zernecke et al., 2005 and Lecka et al., 2010).

Our results showed significant negative correlation between the percentages of both phenotypes and blood urea; also, CD73 was negatively correlated to serum creatinine. These findings confirmed that CD39/CD73 deficiency can impact renal function.

In consistent with our findings, Grenz et al. (2008) showed deterioration of renal hemodynamics including serum creatinine and blood urea in CD73 deficient mice. Also, Shirley et al. (2009) suggested that deficiency of ectonucleotidase, CD39 impacts nephropathy and renal impairment in murine model.

As regard, the role of CD39/CD73 pathway in inflammatory pathogenesis of renal failure, our finding proved significant negative relation between both phenotypes and CRP as inflammatory marker. Melbourne et al. (2012) suggested the anti-inflammatory role of AMP derived ectonucleotidases in ischemic induced renal impairment.

Also, the nephropathy of CD73 null mutant mice revealed marked tubulonephritis and glomerulonephritis (Grenzi et al., 2008).

In addition, in vitro studies found that the lack of adenosine derived ectonucleotidases pathway enhance release of inflammatory cytokines and chemokines by T lymphocytes (Romio et al., 2011 and Bonner et al., 2012).

In another inflammatory disorder model, Ham and Rees (2008), supported the hypothesis that CD39/CD73 which metabolize pro-inflammatory and pro-coagulant nucleotides into anti-inflammatory adenosine might have fundamental role in the pathophysiology of inflammatory disorders.

The association between deficiency of CD73 and activation of inflammatory process was reported by Bell et al. (2010).

Similar to the previous experimental models, we demonstrated significant negative correlation between the proportion of patients CD73 T lymphocytes and the mean value of serum alkaline phosphatase. Hilarie et al. (2011) proposed the link between down modulation of CD73 and increased alkaline phosphatase level in mutant mice.

Our data were similar to the previous findings by Bell et al. (2010) and Robson et al. (2011), who clarified the association between CD39 and CD73 deficiency and developing renal impairment. Also, they confirmed the protective immunomodulatory role of ectonucleotidases during ischemic renal injury.

We concluded that the down modulation of CD39 and CD73 molecules by patients T lymphocytes may predispose to ischemic nephritis and subsequent renal impairment via dysregulation of trafficking inflammatory mechanism. Also, the significant relation between the deficiency of both phenotypes and CRP may support the notion that the pathway is able to modulate inflammatory process via degradation of proinflammatory ATP as well as production of AMP and adenosine. Our results confirmed the association between the deficiency of the two molecules and deterioration of renal function.

In fact, the ectonucleotidases therapy might be promising in the future for nephroprotection during kidney inflammation.

Of note, our study was limited to circulating lymphocytes while, the CD39 and CD73 molecules were also expressed by secondary lymphoid organs. Thus, further future studies on tissue ectonucleotidases are recommended.

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## Antidiabetic and Hypocholesterolemic effect of Different Types of Vinegar in Rats

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**Abstract:** Vinegar is a traditional remedy for ailments including diabetes. This study was conducted to investigate the effects of different types of vinegar (sugarcane, apple, grape, coconut, artificial and palm vinegar) on serum Biochemical and Histopathological of pancreas and stomach of diabetic rats for 6 weeks at 15% concentration. The results indicated that, all of vinegar caused significant decrease  $P < 0.05$  in glucose, TC, LDL-c and significant increase in HDL cholesterol. Apple vinegar was the most effective to decrease glucose, TC and LDL-c followed by grape, sugarcane, coconut, artificial and palm vinegar. Apple vinegar contained the higher concentration of organic acid and phenolic compound compared to other vinegar. Apple vinegar and grape vinegar were the most effective to decrease liver and kidney function. Administering 15% vinegar with diet for 6 weeks decrease the food intake and feed efficiency ratio compared to control group. Moreover, administration different types of vinegar showed that no histopathological change in stomach and has protected effect of pancreas from undesirable change in B cells. In conclusion, using the different types of vinegar with diet for 6 weeks have beneficial effects on diabetic rats and have hypocholesterolemic effect. The vinegar did not effect on stomach histopathological structure and have protective effect of pancreas from damage.

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**Keywords:** Diabetes mellitus, Apple vinegar, Grape vinegar, Coconut vinegar, Organic acid in vinegar, Histopathology of pancreas and stomach.

### 1. Introduction

Diabetes mellitus has been defined as a chronic disease with persistently elevated blood glucose concentration (*Greenbaun and Harrison, 2008*). It is a major and growing public health problem throughout the world. Diabetes is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have diabetes mellitus and 300 million will subsequently have the disease by 2025 (*Wild et al., 2004 and Hamden et al., 2011*). Diabetes is the sixth most important cause of disability burden in Egypt (NICHP, 2004). Over the last century changes in human behavior and lifestyle have resulted in a dramatic increase in the incidence of diabetes in the world (*Kaushik et al., 2010*). The burden of the disease is increasing both for the progressive aging of population and for the worsening of lifestyle (*Zimmet, 2000*). Dietary and lifestyle factors play an important role not only in the etiology but also in the management of diabetic patients. In addition to the drug treatment, simple and inexpensive diet strategies should aid in achieving and maintaining optimal control of diabetes and diabetic complication (*Xuemei et al., 2012*).

Vinegar is a liquid product from fermentation of carbohydrate. It has been made and used dating from around 300 BC and is an important element in Asian, European, Western and other traditional cuisines of the

world. Vinegar has been used for various foods for preservation and often used for flavoring food and pickling. Moreover, diluted unpolished rice vinegar has been drunk as a health food in Japan and its antioxidant activity has been reported (*Nishidai et al., 2000; Shimoji et al., 2002*).

Many medicinal components that are good for health have been reported in natural vinegar, such as carbohydrates (*Johnston et al., 2004 and Leeman et al., 2005*), organic acid (acetic, formic, lactic, malic, citric, succinic and tartaric), alcohols and amino acids and peptides (*Cocchia et al., 2006; Fushimi et al., 2006*), vitamins, mineral salts, amino acids, polyphenolic compounds (e.g., Gallic acid, catechin, caffeic, ferulic acid) (*Morales et al., 2002; Natera et al., 2003*). Traditional vinegar is produced from regional foods according to well-established customs. The balsamic vinegar of Modena, Italy is made from the local white Trebbiano grapes. Traditional rice wine vinegar is produced in Asia, coconut and cane vinegar is common in India and Philippines and date vinegars are popular in the Middle East. Some scientific investigation clearly benefits of vinegar such as: antimicrobial properties (*Vijayakumar and Wolf-Hall., 2002; Sengun and Karapinar., 2005*), prevent inflammation and hypertension (*Murooka and Yamshita, 2008*), lower serum cholesterol (*Fushimi et al., 2006*), treatment of ear infection (otitis external,

otitis media) (*Aminifarshidmehr, 1996; Jung et al., 2002*), treating mal fungus and warts (*Takano- Lee et al., 2004*), reduction in systolic blood pressure (*Kondo et al., 2000*), enhanced calcium absorption and retention (*Kishi et al., 1999*), decrease the glycemic index of carbohydrate food for people with and without diabetes (*Sugiyama et al., 2003; Johnston et al., 2004*). Antiglycemic effects of vinegar have been known for more than a century and have been demonstrated in animal as well as human studies (*Salbe et al., 2009*). So that the objective of this work was to investigate the antidiabetic effects and hypocholesterolemic effect of different types of vinegar (sugarcane, apple, grape, coconut, artificial and palm vinegar) in rats.

## 2. Materials and Methods

### Materials

Fructose sugar was purchased from Sigma-Aldrich, St., and Louis, Mo, USA. Natural sugar vinegar (Sugarcane vinegar, 6% acetic acid), Natural apple vinegar (6% acetic acid), Natural grape vinegar (6%), Coconut vinegar (6% acetic acid), artificial vinegar (6% acetic acid) and Palm vinegar (6% acetic acid) were purchased from local market Cairo, Egypt. Kits for blood analysis were purchased from Biodiagnostic 29 Tahreer St., Dokki, Giza, Egypt.

### Methods

#### HPLC analysis of organic acids in different types of vinegar

Organic acids of different types of vinegar were determined by a HPLC according to the method by *Zbigniew et al., 1991*. 1ml of each sample was diluted by 10 ml water and take 35 µl for injection into HPLC Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 210 nm and quaternary HP pump (series 1100). Packed column Hypesil BDS- C18, 4.0 x 250 mm was used to separate organic acid. The column temperature was maintained at 55°C, at flow rate 1ml/min. Organic acid standard from Sigma Co. were dissolved in a mobile phase (phosphoric acid) and injected into HPLC. Retention time and peak area were used to calculation of organic acids concentration by data analysis of Hewllet Packard software.

#### HPLC analysis of phenolic compound in different types of vinegar

Phenolic compound in different types of vinegar were determined by HPLC according to the method of *Coupy et al., 1999*. 1ml of sample was diluted by 10 ml water and take 100µl for injection into HPLC Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet detector set at 280 nm and quaternary HP pump series

1100). Packed column Hypesil BDS- C18, 4.0 x 250 mm was used to separation phenolic compound. The column temperature was maintained at 35 °C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co. were dissolved in mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by data analysis of Hewllet Packard software, Germany.

### Animals and treatment

Normal forty eight male albino rats weighing 80-100 grams were used for the study. They obtained from animal house of El-Salam Farm, Giza, Egypt. The animal housed individually in stainless steel cages under controlled condition at constant temperature (22 °C) and lighting (12 h. light- dark cycle) and given free access to food and water at all times. The rats were divided randomly into eight groups, six rats each and were fed on the following diets for six weeks:

Group1: Rats were fed on standard diet as served as normal control (negative control group). Standard diet was prepared according to *Reeves et al., 1993*. It contained 14% casein, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.25% choline, 0.3% DL-methionine, 5% oil and 65% starch.

Group2: Rats were fed fructose rich diet (66% fructose) as diabetic group (positive control group). Fructose diet was prepared according to *Yador et al., 2004; Veerapur et al., 2010*.

Group 3: Rats were fed on fructose rich diet +15% natural sugarcane vinegar

Group 4: Rats were fed on fructose rich diet + 15% natural apple vinegar

Group 5: Rats were fed on fructose rich diet + 15% natural grape vinegar

Group 6: Rats were fed on fructose rich diet + 15% natural coconut vinegar

Group 7: Rats were fed on fructose rich diet + 15% artificial vinegar

Group 8: Rats were fed on fructose rich diet + 15% Palm vinegar

Each rat has been weighted at the beginning and the end of experimental and food intake was daily recorded. At the end of experimental period (six weeks), rats were sacrificed after overnight fasting. The blood of each rat was collected in two tubes. The first tub was containing sodium fluoride to preserve glucose (to determination of glucose). The blood in the second tube was centrifuged at 3000 rpm for 20 minutes to obtain the serum, which is kept at -20 °C until analysis.

### Chemical analysis

A- Serum glucose was determined according to *Trinder, 1969*

**B- Lipid Profiles**

Serum total cholesterol (TC), LDL-c, HDL-c and Triacylglycerol (TG) were measured by enzymatic method using commercial kits according to *Richmond, 1973, Burstein et al., 1970, Wieland and Seidel, 1983, Jacobs and Vandermark, 1960.*

**C -Kidney function**

Serum creatinine and serum Urea were determined according to *Larson, 1972; Patton and Crouch, 1977*

**D- Liver enzyme**

ALT and AST were determined by the method of *Reitman and Frankal, 1957.*

**E- Antioxidant enzyme in liver**

Glutathione in liver was determined according to the method by *Beulter et al., 1963.*

F- Hemoglobin concentration was performed using a UDI- HMI automatic hematology analyzer (France).

**Histopathological assessment**

At necropsy, stomach and pancreas were fixed in 10% buffered formalin until analysis. Tissue of stomach and pancreas were routinely processed for paraffin embedding and sections were prepared and stained with hematoxylin and eosin (using light microscopy). Histopathological assessment was performed on all tissues of control group and treatment

**Statistical analysis**

Analysis of the data was of preventative variable in the form mean  $\pm$ SD by SPSS version 17.0 according to *Snedecor and Cochran, 1967.*

**3. Results and Discussion**

Data in Table (1) revealed that all of samples vinegar was contained acetic acid and oxalic acid. Acetic acid and succinic acid was major organic acid in sugarcane, apple and grape vinegar. Oxalic, citric, formic, ascorbic, acetic, succinic and malic acid could be found in apple vinegar. These results are agreement with *Shahidi et al., 2008* indicated that acetic, citric, malic, lactic, succinic, tartaric and fumaric acid could be found in fruit vinegar including apple and grape. *Giumanin et al., 2001* found that apple vinegar contained succinic, malic, glutaric, lactic, citric and tartaric acid. Nevertheless, lactic, glutaric and tartaric acids could not be detected in our apple vinegar. Organic acid in fruit vinegar might source from original material and be generated during fermentation process (*Shahidi et al., 2008*). Artificial vinegar and palm vinegar contained acetic acid and oxalic acid only. Other organic acid could not be detected. Meanwhile, oxalic, formic, ascorbic and acetic acids could be found in coconut vinegar. Ascorbic acid

could be found only in sugarcane, apple, grape and coconut.

Data in table (2) illustrated that the higher concentration of catechin was detectable in apple vinegar (13.24mg/100ml) followed by grape vinegar (9.21mg/100ml), coconut vinegar and sugarcane vinegar (0.43 and 0.21mg/100ml). Meanwhile, it is not detectable in artificial and palm vinegar. Pyrogallol was only identified and major compound in apple vinegar (37.05mg/100ml). Higher concentration of Salycillic was observed in artificial vinegar (13.25mg/100ml) followed by palm vinegar (8.50mg/100ml), grape vinegar (3.13mg/100ml), apple vinegar (1.52mg/100ml), and coconut vinegar (0.21mg/100ml). Different in phenolic compounds may be due to different source of fruit used to produce vinegar. Phenolic compounds have been shown to good markers of the quality and origin of vinegar (*Galvez et al., 1995*).

The serum blood glucose concentration elevated from 134.0 $\pm$ 4.94 mg/dl of control group to 187.53 $\pm$ 4.75 mg/dl of diabetic group rats (Fig 1). Vinegar reduced glucose concentration, rate of decrease was 28.59%, 30.48%, 29.15%, 28.45%, 25.38% and 26.46% of sugarcane, apple, grape, coconut, artificial and palm vinegars respectively. All of types vinegar showed that significant decrease of glucose compared to the diabetic group. Apple, grape and sugarcane vinegar were the most effective decrease of glucose. This could be due to possibility the active ingredient in vinegar (acetic acid and organic acid) to enhanced secretion of insulin from beta cell. The higher effective of apple, grape and sugarcane vinegar may be they contained more organic acid than other types of vinegar. It is not known how vinegar alters blood glucose concentration, but several mechanisms have been proposed. Acetic acid in vinegar may interfere with digestion of starch molecules there by reducing the amount of glucose absorbed into the blood stream after meal (*Ogawa et al., 2000*). Other suggest that vinegar slows the rate of gastric emptying and thus delays carbohydrate absorption and improves satiety (*O'Keefe et al., 2008*), and or acetic acid enhances uptake of glucose from the blood stream into tissues thereby keeping blood glucose concentration (*Fushimi et al., 2001*). Other investigation for human found that 10 grams with a meal was the most effective dose to lower blood glucose levels (*Johnston et al., 2010*). Also the consumption of apple cider vinegar slowed the rise of blood sugar after the high carbohydrate vinegar breakfast (*Johnston et al., 2004*).

Data in Table (3) revealed that there are no significant differences in initial body weight  $P < 0.05$  of eight groups. While different types of vinegar administrated for 6 weeks demonstrated decreased in



body weight gain compared to the control group. Since the decrease in body weight gain was non significant between diabetic group and treatment vinegar groups. Acetic acid was considered to be the active ingredient in vinegar that effected reduction body fat and body weight gain (Kondo *et al.*, 2009). These results are in agreement with (Moon *et al.*, 2010) who reported that there were no significant difference in weight gain among mice groups intake different diet with persimmon – vinegar. Another investigator examined the effect daily vinegar ingestion on body weight of human, he found that the health adults ingested 2 tables spoons of apple cider vinegar (1 g acetic acid) twice daily for 4 week (Johnston, 2006) lead to body loss an average of 1.6 pound where the control subject gained 0.6 pound. Data in the same Table illustrated that there are significant decrease  $P < 0.05$  in food intake and feed efficiency ratio of all groups were administrated of vinegar compared to the control group. Meanwhile, no significant difference was observed between diabetic group and other groups were intake different types of vinegar. Decrease of food intake may be due to decrease appetite of food because a strong acidic taste and pungent smell of vinegar. These results are in line with the results by Moon *et al.*, 2010 reported that there were no significant different in feed consumption among all the vinegar administrated groups.

Table (4) illustrated that the effect of different types of vinegar on weight of organs. There are no significant change in weight of organs for all rats was administrated different types of vinegar.

Serum lipid profiles are shown in Table (5). Serum TC and LDL-c concentration significantly decreased  $P < 0.05$  in all types of vinegar administrated groups. Apple, grape, sugarcane and coconut vinegar revealed reduction of TC and LDL-c more than artificial and palm vinegar. These results may be due to the apple, grape, sugarcane and coconut were contained ascorbic acid (20.05%, 10.23, 2.33 and 0.34 mg/100ml) respectively behind acetic acid. McRac, 2008 reported that the supplementation with ascorbic acid lower serum low density lipoprotein and total cholesterol. These data may be due to the acetic acid (active component in vinegar) reduced serum cholesterol via the inhibition of hepatic lipogenesis and the promotion of fecal bile acid excretion. Acetic acid is converted to acetate in vitro, and acetate metabolism by tissues activates AMPK which play a key role in lipid homeostasis which may explain the lipid lowering effects of ingested acetic acid in animals (Yamashita *et al.*, 2007). While HDL-c concentration showed significantly increase compared to the diabetic group, but there was no significant difference seen among the vinegar administrated groups. Improved in lipid profiles by vinegar were also observed in another

study with rats. Fushimi *et al.*, 2006 reported that serum TC decreased when 0.3% (w/w) acetic acid was administrated for 19 days routine diet containing 1% cholesterol. Moon *et al.*, 2010 have reported similar finding that a persimmon- vinegar decrease serum TC concentration in mice. Shishehbor *et al.*, 2008 reported that apple cider vinegar improved the serum lipid profile in normal and diabetic rats by decreasing serum LDL, TG and increasing serum HDL. TG in the same Table revealed that increase in diabetic group  $60.10 \pm 5.91$  compared to the control group  $40.37 \pm 7.45$ , but there was no significant difference seen among the vinegar administrated groups. These results not on line with Fushimi *et al.*, 2006 who reported that the vinegar decrease TG.

Effect of administration of vinegar on liver function and kidney function are shown in Table (6). ALT and AST increased in diabetic group ( $48.30 \pm 2.67$  and  $49.07 \pm 1.43$ ) compared to the control group ( $30.60 \pm 2.37$  and  $30.57 \pm 6.6$ ) respectively. Administration of vinegar was decrease liver function when compared to the diabetic group. The best results of ALT and AST was observed in apple vinegar and grape vinegar (rate of decrease was (52.23%, 26.77% and 50.94% and 49.86%). Also, creatinine and urea increased in diabetic group ( $3.97 \pm 1.0$  and  $23.73 \pm 2.16$ ) when compared to the control group ( $1.67 \pm 0.44$  and  $19.87 \pm 1.42$ ) respectively. Vinegar administrations lead to significant decrease in creatinine and urea  $P < 0.05$  of all treatment when compared to the diabetic group. The apple vinegar was the most efficiency in kidney function. This results may be due to apple vinegar have high levels from phenolic compounds specifically catechin and pyrogallol, which prevent kidney from destroyed induced by diabetic disease. Pitchai and Manikkam, 2011 reported that the administration catechin lowered urea and creatinine in diabetic rat. Our results disagreement with Kondo *et al.*, 2009 who reported that there are no significant change in measurements of liver function (AST and ALT) or kidney function of two doses of apple vinegar. Data in the same Table showed that there are no significant differences between control, diabetic, sugarcane vinegar and apple vinegar groups in hemoglobin concentration. Meanwhile, grape vinegar group, coconut vinegar group, artificial vinegar group and palm vinegar group showed that significant decrease  $P < 0.05$  in hemoglobin concentration compared to the diabetic group. Data in (Fig 2) showed that significant decrease of glutathione in diabetic group, coconut vinegar group, palm vinegar group ( $3.19 \pm 0.52$  mM/L)<sup>bc</sup>, ( $3.0 \pm 0.72$ )<sup>c</sup> and ( $3.20 \pm 0.10$ )<sup>bc</sup> compared to the control group ( $4.95 \pm 0.65$ )<sup>a</sup>. Decrease of glutathione in diabetic group may be due to increase in lipid oxidation in fructose induced diabetic rats (Suwamaphat *et al.*, 2010). Decrease in glutathione of

coconut vinegar group and palm vinegar group may be due to increase in catabolism of fructose caused the reduction of total glutathione levels (*Oda et al., 1994 and Reddy et al., 2009*). Meanwhile, there are no significant difference between other vinegar groups and control group.

### Histopathological Assessment

#### Pancreas

Microscopically, pancreas of rat from group 1 (control group) revealed no histopathological changes (Fig 3). Meanwhile, pancreas of rats from group 2 (diabetic group) showed atrophy of islets of langerhan's and hyperplasia of  $\beta$  cells of islets of langerhan's (Fig 4). This result agreement with *Riccillo et al., 2012* and *Verma et al., 2012* reported that the type-2 diabetic induce markedly abnormal change in rat islets. Also, *Balamurugan and Ignacimuthu, 2011* found that small atrophies islets cells in diabetic control, whereas rats from groups 3 to

7 showed no histopathological changes (Figs 5 - 9). Moreover some section from group 8 rats was fed on palm vinegar revealed slight hyperplasia of  $\beta$  cells of islets of langerhan's (Fig 10). These results are on line with *Xuemei, et al., 2012* reported that vinegar improved pancreatic  $\beta$  cell deficit in STZ- induced diabetic in rats.

#### Stomach

Microscopically, stomach of rats from group 1 (control group) and diabetic group (group2) revealed no histopathological changes (Figs 11 and 12). Meanwhile, stomach of the rats from group 3 fed on 15% sugarcane vinegar showed few sub mucosal inflammatory cells infiltration (Fig 13). However, stomach of rats from group 4, 5, 6, 7 and 8 revealed no histopathological changes (Figs 14 - 18). These data suggest the vinegar intake at 15% concentration did not effect on stomach tissues.

**Table (1):** HPLC analysis of organic acids in different types of vinegar

Organic acids	Types of vinegar					
	Sugarcane mg/100ml	Apple mg/100ml	Grape mg/100ml	Coconut mg/100ml	Artificial g/100ml	Palm mg/100ml
Oxalic	47.65	12.47	23.82	2.40	1.13	5.41
Citric	54.97	95.70	19.70	-	-	-
Formic	-	96.85	-	10.80	-	-
Acetic	6380.32	6499.33	7336.27	8816.95	7210.37	7807.99
Ascorbic	20.05	10.23	2.33	0.34	-	-
Succinic	133.94	202.77	133.53	-	-	-
Malic	-	5.58	2.35	-	-	-

**Table (2):** HPLC analysis of phenolic compounds in different types of vinegar

Phenolic compounds	Types of vinegar					
	Sugarcane mg/100ml	Apple mg/100ml	Grape mg/100ml	Coconut mg/100ml	Artificial mg/100ml	Palm mg/100ml
Gallic	0.03	-	-	0.03	-	0.02
Catechin	0.21	13.24	9.21	0.43	-	-
Ferulic	0.01	-	-	0.01	0.03	0.02
Benzoic	0.36	-	-	0.36	-	-
Pyrogallol	-	37.05	-	-	-	-
Protocatechuic	-	1.48	-	-	-	-
Catechol	-	1.08	-	-	-	-
Vanillic	-	0.52	0.73	-	-	-
P-Coumaric	-	0.24	-	-	-	-
Salycilic	-	1.52	3.13	0.21	13.25	8.50
Chlorogenic	-	-	2.16	-	-	-
Caffeic	-	-	0.70	0.01	-	0.01
Caffien	-	-	0.50	-	-	-
Coumarin	-	-	-	-	0.46	0.29

**Table (3):** Effect of different types of vinegar on body weight, food intake and feed efficiency

Groups	IBW* (gm) Mean ±SD	FBW° (gm) Mean ±SD	BWG‡ (gm) Mean ±SD	Food intake daily Means ±SD	Feed efficiency ratio (FER) Means ±SD
1- Control	84.15±3.11 <sup>a</sup>	141.65±16.3 <sup>a</sup>	57.5±17.4 <sup>a</sup>	8.47±0.67 <sup>a</sup>	6.79±1.09 <sup>a</sup>
2-Dabetic	85.17±1.91 <sup>a</sup>	99.13±4.7 <sup>b</sup>	13.97±5.12 <sup>b</sup>	5.56±1.3 <sup>bc</sup>	2.64±2.09 <sup>b</sup>
3-Sugarcane vinegar	83.53 ±1.61 <sup>a</sup>	102.67±7.05 <sup>b</sup>	19.13±6.50 <sup>b</sup>	4.73±0.66 <sup>c</sup>	4.13±1.55 <sup>b</sup>
4- Apple vinegar	83.0±3.12 <sup>a</sup>	104.03±11.30 <sup>b</sup>	21.03±11.37 <sup>b</sup>	5.98±0.61 <sup>b</sup>	3.62±2.06 <sup>b</sup>
5- Grape vinegar	86.07±3.6 <sup>a</sup>	104.22±5.97 <sup>b</sup>	18.20±6.67 <sup>b</sup>	5.27±0.32 <sup>bc</sup>	3.89±0.97 <sup>b</sup>
6- coconut vinegar	85.86±4.03 <sup>a</sup>	104.57±9.03 <sup>b</sup>	18.70±9.90 <sup>b</sup>	5.82±0.21 <sup>bc</sup>	3.24±1.81 <sup>b</sup>
7- Artificial vinegar	84.63±1.73 <sup>a</sup>	101.10±16.47 <sup>b</sup>	18.77±13.57 <sup>b</sup>	5.95±1.2 <sup>b</sup>	3.23±2.20 <sup>b</sup>
8- Palm vinegar	84.0±2.12 <sup>a</sup>	97.97±3.19 <sup>b</sup>	13.97±5.12 <sup>b</sup>	6.23±1.2 <sup>b</sup>	2.25±0.81 <sup>b</sup>

Initial body weight\* Final body weight°, Body weight gain‡

**Table (4):** Effect of different types of vinegar on weight of organs

Group	Weight of Heart g/kg	Weight of Liver g/kg	Weight of Kidney g/kg	Weight of Spleen g/kg
1- Control	0.366 ± 5.16 <sup>a</sup>	4.80± 0.32 <sup>a</sup>	0.767±5.16 <sup>a</sup>	0.267±0.02 <sup>a</sup>
2-Diabetic	0.366 ± 5.16 <sup>a</sup>	4.53 ± 0.65 <sup>a</sup>	0.733±5.16 <sup>a</sup>	0.233±0.02 <sup>a</sup>
3- Sugarcane vinegar	0.400 ± 0.0 <sup>a</sup>	4.30±0.98 <sup>a</sup>	0.733±1.37 <sup>a</sup>	0.267±0.02 <sup>a</sup>
4- Apple vinegar	0.367± 0.15 <sup>a</sup>	5.10± 0.62 <sup>a</sup>	0.767±5.16 <sup>a</sup>	0.233±0.02 <sup>a</sup>
5- Grape vinegar	0.367±0.10 <sup>a</sup>	4.30±1.03 <sup>a</sup>	0.700±0.0 <sup>a</sup>	0.267±0.02 <sup>a</sup>
6- Coconut vinegar	0.400±8.9 <sup>a</sup>	4.53±0.41 <sup>a</sup>	0.800±8.9 <sup>a</sup>	0.267±0.02 <sup>a</sup>
7- Artificial vinegar	0.400 ± 8.9 <sup>a</sup>	4.833±0.45 <sup>a</sup>	0.762±4.60 <sup>a</sup>	0.233±0.02 <sup>a</sup>
8- Palm vinegar	0.400 ± 0.0 <sup>a</sup>	4.47±0.22 <sup>a</sup>	0.767±5.16 <sup>a</sup>	0.300±0.02 <sup>a</sup>

**Table (5):** Effect of different types of vinegar on lipid profile

Group	Total Cholesterol mg/dl	Total Triacylglycerol mg/dl	HDL Cholesterol mg/dl	LDL Cholesterol mg/dl
1- Control	200.27 ± 0.95 <sup>e</sup>	40.37± 7.45 <sup>b</sup>	44.83±6.91 <sup>a</sup>	147.36±8.23 <sup>d</sup>
2-Diabetic	260.93 ± 1.37 <sup>a</sup>	60.10±5.91 <sup>a</sup>	35.0±3.34 <sup>b</sup>	213.91±2.04 <sup>a</sup>
3- Sugarcane vinegar	233.57 ± 12.5 <sup>cd</sup>	55.07±11.09 <sup>a</sup>	44.83±6.20 <sup>a</sup>	177.72±14.8 <sup>bc</sup>
4- Apple vinegar	220.4 ± 23.5 <sup>d</sup>	52.73± 3.13 <sup>a</sup>	46.67±10.6 <sup>a</sup>	163.19±12.7 <sup>c</sup>
5- Grape vinegar	225.23±9.48 <sup>d</sup>	53.83±9.7 <sup>a</sup>	46.37±4.60 <sup>a</sup>	167.86±20.9 <sup>c</sup>
6- Coconut vinegar	242.87±10.8 <sup>bc</sup>	58.97±5.38 <sup>a</sup>	44.03±4.19 <sup>a</sup>	189.82±12.6 <sup>b</sup>
7- Artificial vinegar	244.47±9.86 <sup>bc</sup>	55.03±9.4 <sup>a</sup>	40.53±4.2 <sup>ab</sup>	191.57±12.9 <sup>b</sup>
8- Palm vinegar	253.83±9.48 <sup>ab</sup>	53.07±6.59 <sup>a</sup>	34.80±5.41 <sup>a</sup>	207.22±6.11 <sup>a</sup>

**Table (6):** Effect of different types of vinegar on liver and kidney functions and Hemoglobin

Group	ALT (µl/dl) Mean± SD	AST (µl/dl) Mean± SD	Creatinine mg/dl) Mean± SD	Urea mg/dl) Mean± SD	Hemoglobin mg/dl Mean± SD
1- Control	30.60±2.37 <sup>c</sup>	30.57±6.6 <sup>c</sup>	1.67±0.44 <sup>e</sup>	19.87±1.42 <sup>bcd</sup>	13.03±0.37 <sup>abc</sup>
2-Diabetic	48.30±2.67 <sup>a</sup>	49.07±1.43 <sup>a</sup>	3.97±0.10 <sup>a</sup>	23.73±2.16 <sup>a</sup>	13.57±0.68 <sup>a</sup>
3- Sugarcane vinegar	37.20±4.43 <sup>b</sup>	33.53±4.72 <sup>bc</sup>	2.65±0.41 <sup>cd</sup>	19.57±0.77 <sup>cd</sup>	13.70±1.08 <sup>a</sup>
4- Apple vinegar	23.07±2.04 <sup>c</sup>	24.07±4.83 <sup>d</sup>	2.90±0.02 <sup>bc</sup>	17.93±1.65 <sup>e</sup>	13.40±0.70 <sup>ab</sup>
5- Grape vinegar	35.37±5.48 <sup>bc</sup>	24.60±6.60 <sup>d</sup>	2.97±0.10 <sup>bc</sup>	18.97±1.25 <sup>de</sup>	11.37±0.67 <sup>c</sup>
6- Coconut vinegar	37.40±3.03 <sup>b</sup>	33.43±3.58 <sup>bc</sup>	3.83±0.49 <sup>a</sup>	20.73±0.52 <sup>bc</sup>	12.13±1.15 <sup>bcd</sup>
7- Artificial vinegar	39.50±6.35 <sup>b</sup>	38.87±5.23 <sup>b</sup>	3.17±0.42 <sup>b</sup>	20.30±0.72 <sup>bcd</sup>	11.90±1.09 <sup>cd</sup>
8- Palm vinegar	38.13±3.10 <sup>b</sup>	23.17±5.09 <sup>d</sup>	2.30±0.47 <sup>d</sup>	21.47± 1.28 <sup>b</sup>	12.47±1.76 <sup>abcd</sup>

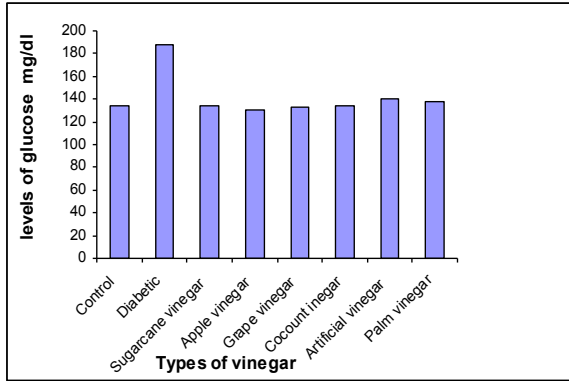


Fig (1): Effect of different types of vinegar on glucose level

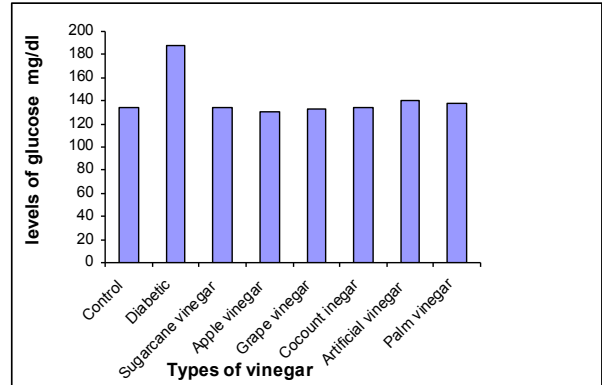


Fig (2): Effect different types of vinegar on glutathione levels

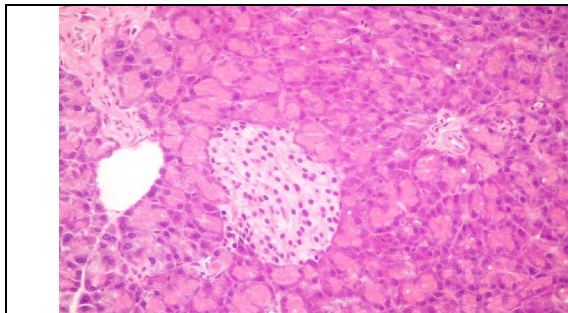


Fig (3): pancreas of control group (1) showing no histopathological Change (X-400)

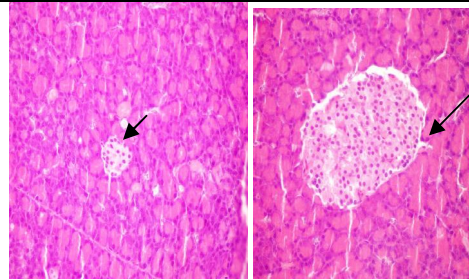


Fig (4): Pancreas of diabetic rat (group2) showing atrophy of Islets and hyperplasia of B cells of langerhans (X-400)

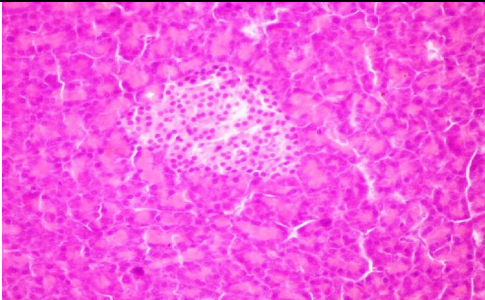


Fig (5): Pancreas of rats from group 3 fed sugarcane vinegar showing no Histopathological changes (X-400)

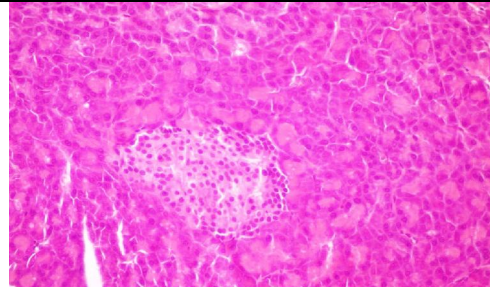


Fig (6): Pancreas of rats from group 4 fed on apple vinegar showing no Histopathological change (X-400)

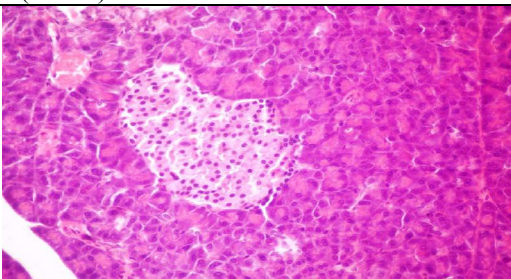


Fig (7): Pancreas of rats from group5 fed on grape vinegar showing no Histopathological changes (X-400)

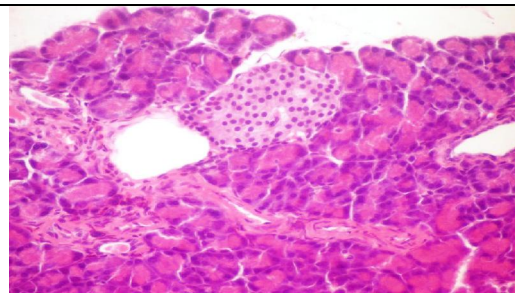


Fig (8): Pancreas of rats from group 6 fed on coconut vinegar showing no Histopathological change (X-400)



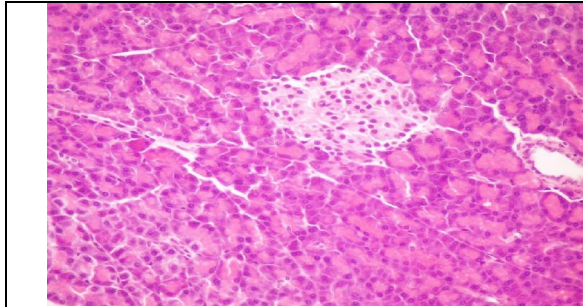


Fig (9): Pancreas of rats from group 7 fed On artificial vinegar showing no Histopatological changes (X-400)

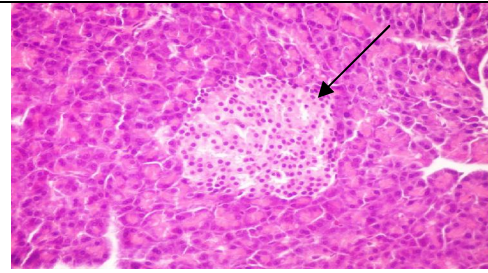


Fig (10): Pancreas pf rats from group 8 fed on palm vinegar showing slight Hyperplasia of B cells of islets of langerhan's (X-400)

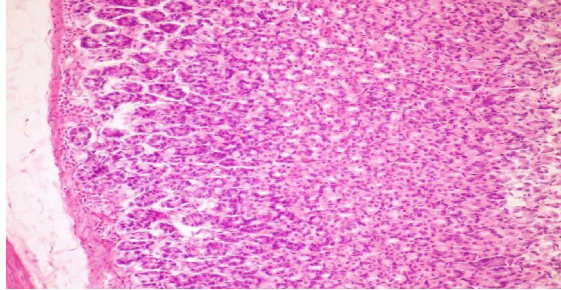


Fig (11): Stomach of control group (1) showing no histopathological Change (X-400)

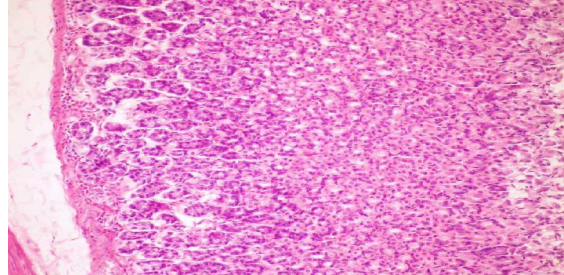


Fig (12) Stomach of diabetic rat (group2) showing no histopathological Change (X-400)

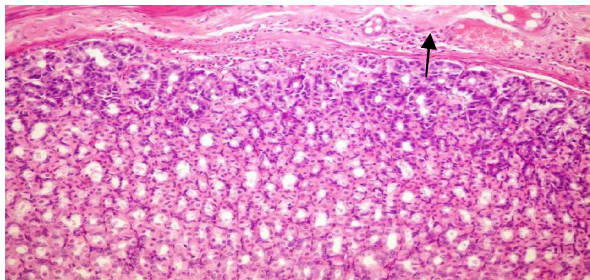


Fig (13): Stomach of rats from group 3 fed on sugarcane vinegar showing few sub mucosal cells infiltration (X-400)

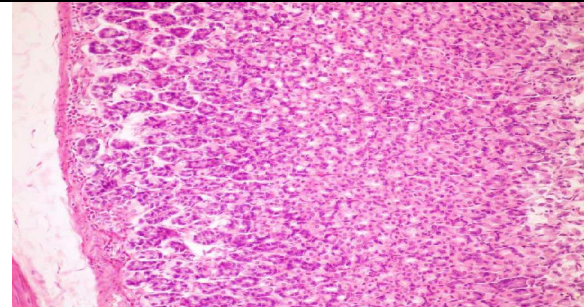


Fig (14): Stomach of rats from group 4 fed on apple vinegar showing no Histopathological changes (X-400)

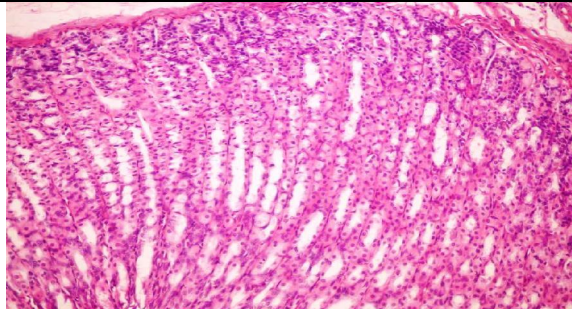


Fig (15): Stomach of rats from group5 fed on grape vinegar showing no histopathological changes (X-400)

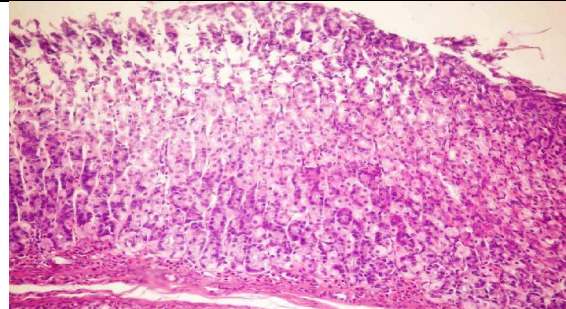


Fig (16): Stomach of rats from group 6 fed on coconut vinegar showing no histopathological change (X-400)



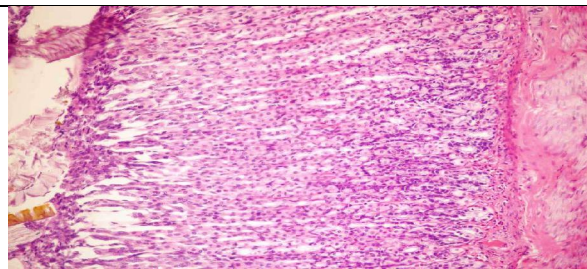


Fig (17): Stomach of rats from group 7 Fed on artificial vinegar showing no hiatopathological change (X-400)

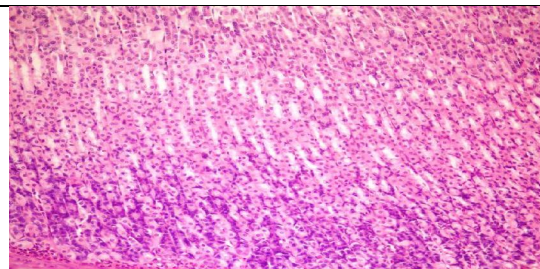


Fig (18): Stomach of rats from group 8 fed on palm vinegar showing no histopathological changes (X-400)

### Conclusion

Vinegar has potential benefits of diabetic rats thought decrease glucose concentration and cholesterol. Apple vinegar and grape vinegar were the more effective to decrease total cholesterol and LDL-c than the other types of vinegar. Moreover, they were caused increase of HDL-c more than the other types of vinegar. Apple vinegar and grape vinegar decreased AST, ALT, urea and creatinine more than the sugarcane, coconut, artificial and palm vinegar. They contained more organic acid and phenolic compound than the other vinegar. Apple vinegar contained the highest concentration of catechin. Pyrogallol, protocatechuic, catechol, p-coumaric was only detectable in apple vinegar. Apple and sugarcane vinegar has no effect on hemoglobin concentration. Glutathione was decrease in diabetic group, coconut vinegar group and palm vinegar group. Apple vinegar followed grape, sugarcane, coconut, artificial and apple vinegar consider antidiabetic and hypocholesterolemic effect in diabetic rat. Different type of vinegar has protective effect of pancreas and did not effect on stomach with 15% concentration for 6 weeks. So that using vinegar has a beneficial effect of diabetic disease in rats.

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