Toxicity of *Tecurium polium* extract using micronucleus and biochemical analysis in *Oncorhynchus mykiss* fish.

Ali Mirzaei[1], Nooshin Mirzaei[1], Majid amouei[2], Seyed Abdolmajid Khosravani[3], Zeynab Salehpour[1]*

[1]Medicinal plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
[2]The ministry of health and Medical Education, Tehran, Iran
[3]Research center of microbiology Yasuj University of Medical Sciences, Yasuj, Iran
*Corresponding Author: zeynab.salehpour@gmail.com

Abstract: *Teucrium polium* L. (Lamiaceae) is a plant that has been used for over 2000 years in traditional medicine due to analgesic, anti-spasmodic, hypolipidemic, Anti-bacterial, anti-inflammatory potential. The aim of the present study was to obtain a basic knowledge of *Tecurium polium* toxicity using micronucleus analysis in *Oncorhynchus mykiss* fish.**Material and Methods:** Fish were collected from locations that display no environmental stresses. Fish were divided to five groups (10 each one) including positive, negative control and three groups of treatments which received (100, 150 and 200 µg/ml) of plant extract. *Oncorhynchus mykiss* fish were exposed for 8 weeks with different methanol extract of *T. Polium*. In the end of study blood samples were collected, fixed for 24 h and then were stained with Giemsa.The biochemical parameters and micronucleus frequencies were measured. **Results:** the median lethal concentration (LC_{50}) of *T. polium* extract to *Oncorhynchus mykiss* fish for 24 h was found more than 2g/L. In the biochemical parameters increase of plasma glucose, blood urea nitrogen and protein levels were noticed. The glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were increased significantly in treated fish compared to their control groups.The normal concentration of plasma electrolytes also reported. *polium* extract associate with significant increase of micronucleus formation and nuclear disturbance.**Conclusion:** *T. polium* plant extract affects the biochemical parameters and micronucleus formation of fish. Change of these markers on *Oncorhynchus mykiss* fish was indicator of moderate toxicity *of T. polium.*
[Ali Mirzaei, Nooshin Mirzaei, Majid amouei, Seyed Abdolmajid Khosravani, Zainab Salehpour**. Toxicity of *Tecurium polium* extract using micronucleus and biochemical analysis in *Oncorhynchus mykiss* fish.** *Life Sci J* 2013;10(7s):1102-1108] (ISSN:1097-8135). http://www.lifesciencesite.com. 176

Keywords: *Oncorhynchus mykiss*, micronucleus, *Tecurium polium,* Biochemical parameter.

1. Introduction

Aquatic organisms such as fish are excellent genetic models for the screening of water genotoxicity in the field and in the laboratory. They have also become important for distribution and toxic effects of chemical pollution in aquatic ecosystems. Fish can fast response to toxins on low levels in comparable to higher vertebrates and also metabolize, accumulate and store waterborne pollutants (Mitchell and Kennedy, 1992; Park *et al.,* 1993; Hayashi *et al.*, (1998). A variety of teleost fish, during the early stages of life, have been used for the study of the mutagenic, clastogenic and teratogenic effects of environmental contaminants. In one experiment the fish *Oncorthychus mykiss* and *Oryzias latipes* were used as test organisms for the study of carcinogenesis and model systems (Metcalfe, (1988). Micronuclei (MNs) are small chromatin derived - fragments are formed in cytoplasm during cell division. Micronucleus is a useful and most popular tool for evaluation of cytogenetic damage, ecogenotoxicity studies and water quality monitoring (Fenech *et al.*, 2003). The micronuclei analysis in interphase time is rapid, much faster and technically easier than scoring of chromosomal aberrations. Erythrocytes of *Teleost* fish have nucleus and excellent device for the evaluation of initial step in clastogenic potential in water and also widely used to evaluate of pollutants (Hayashi *et al.*, 1998). Biochemical parameters are useful for evaluating of animals that expose to the toxin due to blood parameters are highly sensitive to environmental or physiological changes and health status of higher vertebrates (Talas and Gulhan 2009; Suvetha *et al.,* 2010). *Teucrium polium* L. (Lamiaceae) is a plant that has been used for over 2000 years in traditional medicine due to analgesic, anti-spasmodic, hypolipidemic, Anti-bacterial, anti-inflammatory potential. In spite of biological activity of *T. polium* in litrature, there are some reports in toxicity potential such as hepatotoxicity in living organisms (zal *et al*., 2001; Rasekh, 2005; Shahraki *et al*., 2007). According to few controversial reports about the *T. polium* genotoxicity, the main purpose of this study was to evaluation of *T. polium* hytroalcoholic extract toxicity with special reference to micronucleus, biochemical tests *by using Oncorhynchus mykiss* fish.

2.Materials and Methods

2.1.Plant collection

After collection of aerial plant parts of *T. polium*, botanical identification was conducted. The voucher sample was kept at the herbarium of the medicinal herb research center at Yasuj University of Medical Sciences.

2.2.Plant extraction

The aerial plant was dried in the shade and then ground using a mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany). The ground materials were extracted with methanol and water (70+30) by maceration method for 24 hours and filtered through Whatman No.1 filter paper. The extracts were collected and concentrated using a rotary evaporator (Heidolph Laborota, model 4000; Germany) and remained frozen prior to the study.

Adult specimens of *Oncorhynchus mykiss* obtained from the Mottaheri research center of cold aquaculture fisheries in Yasuj, Iran for study. They were safely brought to the laboratory by careful netting and handling and stocked in aquaria with 200 litter's capacity. They were allowed to acclimate for a week prior to taking the treatment. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. In this study physico-chemical characteristics of water (Tap water free from chlorine) in aquaria were temperature 22 o^c, pH 7.3, with 12:12 h photoperiod and an average density of 6.4 g/l.

Fish were fed *ad libitum* once a day with commercial pelleted (Khorak Dam food sticks) before the start of experiment.

2.3.Determination of 24 h LC_{50} value of Tecurium polium hydroalcoholic aerial parts extracts by fish

A static acute toxicity (24 h) test was conducted to determine the LC_{50} value of *T. polium* extract under laboratory condition. Different concentrations of the extract at 0.50, 1.0, 1.50 and 2 g/liter were prepared from the stock solution. For each concentration 10 Acclimated fish with average weighing 82-120 grams and average size 25.5±1.21 cm in length randomly selected from the stock were introduced and kept in separate glass tanks (120 cm X 80 cm X 40 cm). Three replicates were maintained for each concentration. A concurrent control was also maintained throughout the experimental period under identical conditions. The mortality/survival of fish was recorded after 24 h. The dead fish were removed from the tanks instantly. Feeding was stopped through the experiment. The level at which 50% mortality of fish happened after 24 h was taken as the medium lethal concentration (LC_{50}) for 24 h. The LC_{50} was calculated by the probit analysis method (Finney, 1978). Homogeneity of the population used in the present investigation was verified by chi-square test.

2.4.Chronic toxicity studies

Five aquariums were used in the experiment, being two for negative and positive controls and the others for each concentration of plant extract as treatment groups.Ten Acclimated fish *Oncorhynchus mykiss* were randomly collected for each aquarium in order to study of the genotoxicity effects of *T. polium*.Fish in treatment groups were received daily, 100, 150 and 200, µg/ml of hydroalcoholic extract of *T. polium* in aquariums water respectively. Positive groups receive Cyclophosphamide 2 mg/kg body weight by injection in the trunk muscle 3 times in week. The negative control group received the sterilized water by injection in the trunk muscle. Fish were fed by commercial pelleted food (Fagr Kh *et al*., 2008).At the end of 8 weeks, the blood was collected by puncture of the caudal vein with a heparin-coated 25 gauge×0.5 in. needle, attached to a 1 ml syringe and immediately smeared. After the sacrifice of fish cephalic kidney was dissected, and prepared a smear on clean slide and allowed to dry for 2–4 h. (Barsien *et al*., 2004). Blood and cephalic kidney smears was prepared from each fish and fixed in absolute methanol for 10 min at room temperature and stained with dilute Giemsa. The slides were subsequently examined for each fish by light microscopy. Only cells with intact cellular and nuclear membranes were estimated. The incidence of micronuclei (MN) was scored by a manual method. Three criteria for identification and scoring of MN were selected. The diameter of the MN should be less than one-third of the main nucleus, MN should be well separated or marginally overlap from main nucleus and the color of a MN should be same as the main nucleus (Fenech *et al*., (2003).Plasma was prepared by centrifuging the blood sample at 5,000 rpm for 10 minutes. Plasma biochemical parameters including glucose, total protein, Blood urea nitrogen (BUN), The glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were measured with national pars azmoon kits. Sodium and potassium was estimated by (Medica easylyte, USA, SNC 95 4A ca) electrolyte analyzer.

2.5.Statistical analysis

The data were statistically analyzed at $p<0.05$. Analysis of variance (ANOVA) was used to test their significance.

3.Result

LC_{50} (24h) value of *T. polium* extract on *Oncorhynchus mykiss* was more than 2 g /L.In during acute treatment mild behavioral responses such as body imbalance, surface floating, restlessness and loss of equilibrium were observed in last concentration of plant extract.

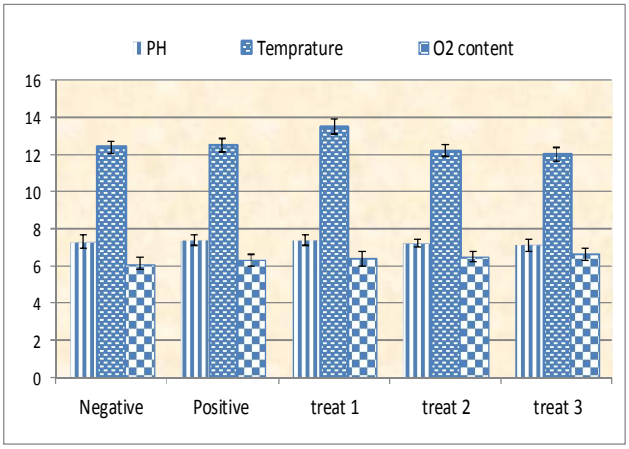

Fig. 1: Physico-chemical features of water (tap water free from chlorine) used for controls and the experiments groups of freshwater fish *Oreochromis niloticus* in 8 weeks. In treatment groups different concentration (100, 150 and 200 µg/ml) of *Tecurium polium* extracts (LC_{50}>2 g/ l; 24 h) was used. In positive and negative controls received cyclophosphamide (2 mg/kg) and sterilized water by injection in the trunk muscle 3 times in week respectively. Values are means ± S.D. of five individual observations; Significant ($p < 0.05$).

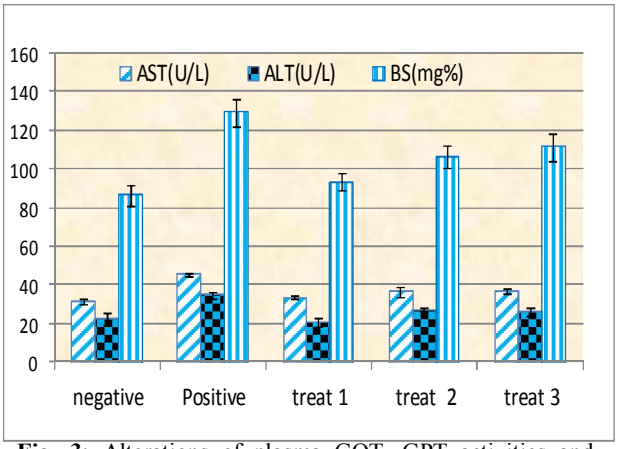

Fig. 3: Alterations of plasma GOT, GPT activities and blood glucose of freshwater fish *Oreochromis niloticus* in 8 weeks. In treatment groups different concentration (100, 150 and 200 µg/ml) of *Tecurium polium* extracts (LC_{50}>2 g/ l; 24 h) was used. In positive and negative controls received cyclophosphamide (2 mg/kg) and sterilized water by injection in the trunk muscle 3 times in week respectively. Values are means ± S.D. of five individual observations; Significant ($p < 0.05$).

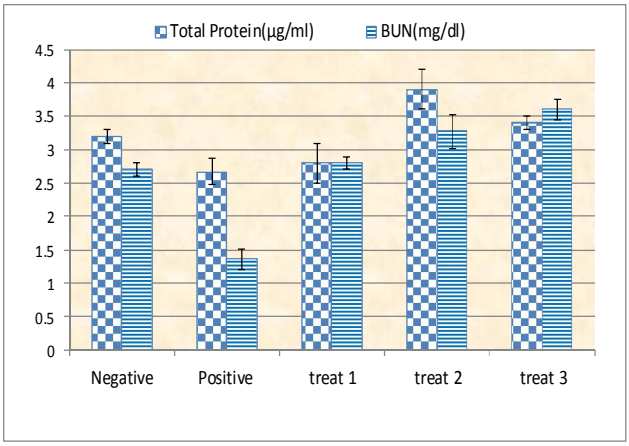

Fig. 2: Changes in plasma blood urea nitrogen (BUN) and protein content in control and treatment groups of a freshwater fish *Oreochromis niloticus* in 8 weeks. In treatment groups different concentration (100, 150 and 200 µg/ml) of *Tecurium polium* extracts (LC_{50}>2 g/ l; 24 h) was used. In positive and negative controls received cyclophosphamide (2 mg/kg) and sterilized water by injection in the trunk muscle 3 times in week respectively. Values are means ± S.D. of five individual observations; Significant ($p < 0.05$).

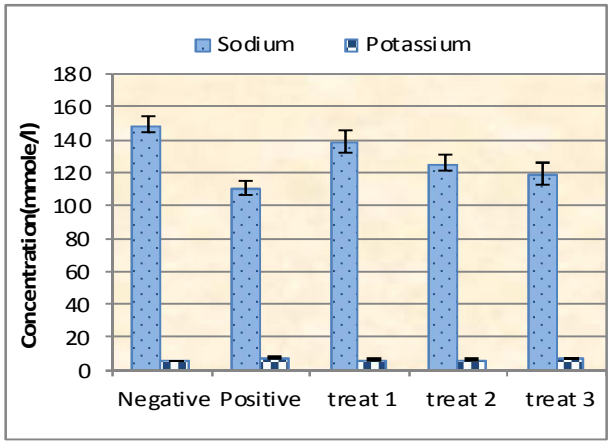

Fig. 4: Alterations in plasma sodium and potassium levels of controls of freshwater fish *Oreochromis niloticus* in 8 weeks. In treatment groups different concentration (100, 150 and 200 µg/ml) of *Tecurium polium* extracts (LC_{50}>2 g/ l; 24 h) was used. In positive and negative controls received cyclophosphamide (2 mg/kg) and sterilized water by injection in the trunk muscle 3 times in week respectively. Values are means ± S.D. of five individual observations; Significant ($p < 0.05$).

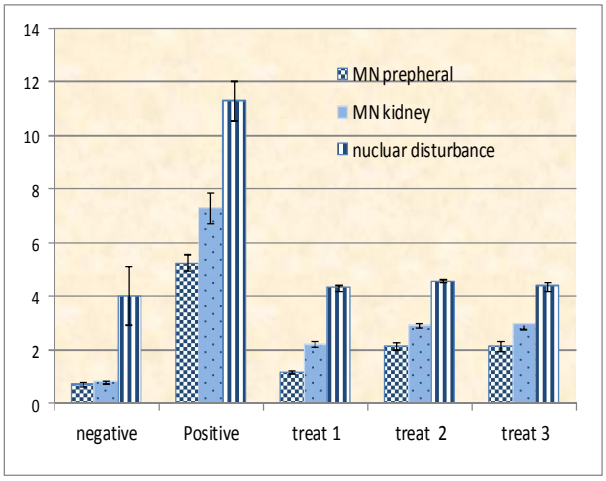

Fig. 5: peripheral and kidney micronuclei (MN) and nuclear disturbance of a freshwater fish *Oreochromis niloticus* in 4 weeks. In treatment groups different concentration (100, 150 and 200 µg/ml) of *Tecurium polium* extracts (LC_{50}>2 g/ l; 24 h) was used. In positive and negative controls received cyclophosphamide (2 mg/kg) and sterilized water by injection in the trunk muscle 3 times in week respectively. Values are means ± S.D. of five individual observations; Significant ($p < 0.05$).

Physico-chemical property of experiment water (tap water free from chlorine) including. PH=7.2, temperature =12.52 and O_2 content = 6.38 was shown (Fig. 1). In biochemical parameters, plasma BUN was increased in treated fish groups (3.7-33%) when compared to control group (Table 4).There was a significant difference in plasma BUN between treatment groups 2 and 3 compared to control group p<0.001 (Fig. 2). Plasma protein level decreased up to 17% in treatment group 1 and increased in treatment groups 2 (25%) and 3 (12.9%) compared to control group.There was a significant difference in plasma protein between treatment groups 2 compared to control group p<0.01 (Fig.2). There was a significant difference in plasma GOT activity between treatment groups 2 and 3 compared to control group p<0.05.AST activity was increased in treatment groups1 (10%), 2 (26.6%) and 3 (20%) compared to control group (Fig. 3). GPT activity was decreased up to 7.7% in treatment group (1) and increased in treatment group 2 (22.7%), and group 3 (13.6%) compared to control group. There was no significant difference in GPT activity between treatment groups (Fig. 3). Plasma glucose was increased in treated groups (treat 2, 3) when compared to negative (Fig. 3). There was no significant difference in plasma ionic level (Na^+ and $K^{+)}$ between treatment groups and negative control (Fig. 4).There was a significant difference in peripheral MN between treatment groups 2 and 3 compared to control group p<0.01. Peripheral level of MN increased in treatment groups 1 (57%) and 2 (200%) and 3 (204%) compared to control group (Table 4).There was a significant difference in kidney MN between treatment groups compared to control group p<0.001 (Fig. 5). MN in kidney increased in treatment groups 1 (175%) and 2 (262%) and 3 (266%) compared to control group. In this study, MN were significantly (p<0.001) higher in kidney, than that of the peripheral blood. There was no significant difference in nucleus disturbance between treatment groups compared to control group. Nucleus disturbance were increased in treated fish groups up to (7.5-15%) when compared to control group (Fig. 5).

4. Discussion

The different kinds of stressors in marine environments and aquaculture systems produce change in the physiological parameters (Martinez and Souza, 2002). Fish is the most prone to water pollution than any other aquatic organism. Newly, biomarkers are broadly used as early diagnostic tools for estimation of ecological quality in polluted water (Cajaraville *et al.,* 2000). In this study the median lethal concentration (LC_{50} for 24h) of *T. polium* hydro - alcoholic extract on *Oncorhynchus mykiss* fish was more than 2g/l.Behavioral toxicology is a means for risk evaluation of water pollution. In the present study, in duration of treatment, some mild to moderate behavioral responses such as body imbalance, surface floating and loss of equilibrium were observed. Biochemical tests can be useful to determining of toxicity effects of the target organs and the common health status of living organisms. They also provide early warning of potentially harmful changes in stressed organisms (Ferreira *et al.,* 2007). In this study, the increase in blood glucose level might be resulted from an increase in plasma catecholamine and corticosteroid hormones (Pickering, 1981). The hyperglycemic condition was observed which may be due to coping up the stress caused by hydro - alcoholic *T. polium* extract. Among the biochemical tests, plasma glucose and protein concentration are most important indicators for stress evaluation in fish (Ramesh, 2001). Fish under stress may mobilize protein to gather energy requirements and to maintain increased physiological activity (Martinez *et al*, 2004). The decrease in protein level may be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis (Singh and Singh, 2002). In the present study a similar mechanism may be operated.

Blood urea nitrogen (BUN) or Urea is the major excretory product of protein metabolism.it is formed in liver from amino groups of amino acids and free ammonia. Determination of urea is used to assess renal function and aid in the diagnosis of renal disease (Oh MS, 2006). The significant increase in blood urea concentration was seen in groups 2 and 3 compare to control group. Increase of plasma urea level may accompanied by stress induced by *T.polium* in high concentration. Enzyme activities have also been used as sensitive indicators of stress in fish. In recent study fish were exposed to different concentration of hydro - alcoholic *T. polium* extact for to predict the possible levels of threat to life. Measurement of plasma GOT and GPT activity in toxicant fish serve as a valuable indicator for physiological changes or stress state. (Knox and Greengard, 1965; Remyla *et al.,* 2008). Manavalaramanujam and Ramesh (1996) reported that the elevation of GOT and GPT activity in pesticide treated fish indicates the increased energy demands under pesticide stress. Also in hepatic tissue damage GOT and GPT activities will be increase (Agrahari *et al.,* 2007).In the present study, the activity of both GPT and GOT were increased in peripheral blood by treatment groups, indicating low damage of the organs due to accumulation of extract or increased metabolism as the organism tries to mitigate the induced stress. Moreover, detoxification may not be adequately effective to prevent the effect of extract on the system, result in an increase in GOT and GPT activities in peripheral blood of fish was induced. The major functions of kidney in fish are filtration and erythropoiesis. The immature red blood cells (RBC) with high proliferation are found in the kidney however, mature red blood cells are circulated through all organs as blood circulation. Immature RBC in kidney was more affected by toxin than matures RBC in blood circulation. So, genotoxicity in immature RBC displays a sensitivity and a higher frequency than blood RBC (Palhares and Grisolia, 2002). Determination of ions concentration is useful parameter for evaluation of stress and ionic regulation on ion regulatory organs (Barcarolli and Martinez 2004; Mathan et al. 2010).

In fish, when concentration of electrolyte in plasma decreases, sodium and chloride in tissue will be increase (McCarty and Houstan (1976).Decreased of sodium and potassium may be due to osmoregulatory failure or inhibition of the Na, K ATPase activity (Suvetha *et al*., 2010). In the present study, the normal concentration of plasma electrolytes might have resulted from the safety of *T. polium* extract. Some pollutants in aquatic organisms can make genotoxic effect on erythrocyte system and cause micronucleus formation (Campana *et al*., 1999). Micronucleus formations as well as induction of nuclear alterations were considered to be the consequences of genotoxic events in fish (Pacheco and Santos, 2002).

Micronucleus estimation in gill cells is more sensitive monitor than hematopoietic system to stressors. (Fagr *et al*., 2008). Micronuclei frequencies were reported in the fish red blood cells which increased with longer exposures with toxic substances. According to several research finding some chemicals can induce nuclear abnormalities in the gill and renal erythrocytes of fish (Palhares and Grisolia, 2002; Cavas *et al*., 2005). In recent study *T. polium* extract associate with micronucleus formation and nuclear disturbance.

There was correlation with incidence of micronuclei in fish peripheral erythrocytes with time exposure by different pollutants in literatures. This is in good agreement with present results (Minissi *et al*., 1996).

In the present experiment in cyclophosphamide as a positive control a significantly ($P < 0.001$) difference in all parameters was reported compared to control and treatment groups. This finding shows similarities with the results of other researchers (Ahmad, 2002).

5. Conclusion

T. polium plant extract affects the biochemical and micronuclei parameters of *Oncorhynchus mykiss* fish.Change of these parameters was indicator of mild toxicity *of T. polium.* Peripheral bloods as well as kidney cells were shown to be sensitive for detecting T. *polium*_toxicity that induced by the aquatic environment.

Acknowledgment

The authors thank to Mottaheri research center of cold aquaculture fisheries in Yasuj and Mohsen Haidari for their guideline, providing and the identification of fish samples.

References

1. Agrahari, S.,C., Kashev, C., Pandey and K.Gopal, 2007.Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). *Pestic. Biochem. Physiol.,* 268-272.
2. Ahmad, W., MN., Ali, M.A. Farah and B. Ateeq, 2002.Computerized automated morphometric assay including frequency estimation of pentachlorophenol induced nuclear anomalies (micronucleus) in catfish Heteropneustes fossilis. Chromosoma., 110(8): 570-4.

3. Al-Sabti, K., and C.D., Metcalfe, 1995.Fish micronuclei for assessing genotoxicity in water. Mutat. Res., 343: 121-135.
4. Barcarolli, I.F., C.B.R., Martinez, 2004. Effects of aluminium in acidic water on hematological and physiological parameters of the Neotropical fish Leporinus macrocephalus (Anostomidae). Bull. Environ. Contam. Toxicol. 72: 639-646.
5. Barsien, E, J. Lazutka, J. Syvokien, J. Dedonyt, V. Rybakovas, A.Bjorn-stad and O.K. Andersen, 2004.Analysis of micronuclei in blue mussels and fish from the Baltic and the North Seas. Environ. Toxicol., 19: 365–371
6. Cajaraville, M.P., M.J. Bebianno, J.Blasco, C. Porte, C.Sarasquete and A. Viarengo, 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci. Total. Environ., 247:295-311.
7. Campana MA, A.M. Panzeri, V.J. Moreno and F.N. Dulout 1999. Genotoxic evaluation of the pyrethroid lambda-cyhalothrin using the micronucleus test in erythrocytes of the fish *Cheirodon interruptus*.. Mutat. Res., 438(2): 155-61.
8. Cavas, T. and S. Ergene-Gozukara, 2003. Micronuclei, nuclear lesions and interphase silver-stained nucleolar organizer regions (AgNORs) as cyto-genotoxicity indicators in Oreochromis niloticusexposed to textile mill effluent. Mutat. Res., 538:81–91.
9. Cavas, T., N.N. Garanko and V.V. Arkhipchuk, 2005. Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate Food Chem. Toxicol., 43: 569–574.
10. Fagr Kh. Ali, A.M. El-Shehawi and M.A. Seehy. 2008. Micronucleus test in fish genome: A sensitive monitor for aquatic pollution. African Journal of Biotechnology 7 (5): 606-612.
11. Fenech, M., W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi and E. Zeiger, 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mut. Res., 534: 65–75.
12. Ferreira, J.G., A.J.S., Hawkins and S.B. Bricker, 2007. Management of productivity, environmental effects and profitability of shellfish aquaculture-the Farm Aquaculture Resource Management (FARM model. Aquaculure, 264:160-174.
13. Finney, D.J., 1971. Probit analysis.University Press, Cambridge, UK., pages: 333.
14. Hayashi M, T.Ueda, K. Uyeno, K. Wada,N. Kinae and K. Saotome, A. Tanaka N Takai, YF. Sasaki, N. Asano, T.Sofuni, Y. Ojima, 1998. Development of genotoxicity assay systems that use aquatic organisms Mutat. Res., 399(2): 125-33.
15. Knox, W.E. and O. Greengard, 1965.In: An introduction to enzyme physiology, Weber, G. (Edn.), Advances in enzyme regulation. Pergamon Press, New York, pages: 247-248.
16. Manvalaramanujam, R.and M.Ramesh, 1996. Sublethal toxicity of phosalone on transaminases of a freshwater fish *Labeo rohita*. *J. Ecol. Environ. Conserv.,* 2: 179-181.
17. Martinez, C. B. R., M.Y. Nagae, C.T.B.V. Zaia and D. A. M. Zaia, 2004. Morphologicaland physiological acute effects of lead in the Neotropical fish Prochilodus lineatus.Brazilian J. Biol., 64:797-807.
18. Martinez, C.B.R. and M.M. Souza, 2002.Acute effects of nitrite onion regulation in two Neotropical fish species. Comp. Biochem. Physiol., 33:151-160.
19. Mathan, R., A., Senthil Kumar, K., Mahalashmi Priya, 2010. Alterations in plasma electrolyte levels of a Freshwater fish. Cyprinus carpio exposed to acidic pH. Toxicol. Environ. Chem., 92: 149-157.
20. McCarty, L.S., A.H.,Houston, 1976.Effects of exposure to sublethal levels of cadmium upon water-electrolyte status in the gold fish, Carassius auratus. J. Fish Biol. 9: 11-19.
21. Metcalfe, C.D., 1988. Induction of micronuclei and nuclear abnormalities in the erythrocytes of mudminnows and brow bullheads. Bull Environ Contam Toxicol 40:489-495.
22. Minissi, S., E., Ciccotti andM., Rizzoni,1996. Micronucleus test in erythrocytes of Barbus plebejus (Teleostei, Pisces) from two natural environments; a bioassay for thein situ detection of mutagens in freshwater. Mutation Res 367:251-254
23. .Tissue concentrations of organochlorine compounds in common seals Mitchell,S. and S. Kennedy, 1992.Total from the coast of Northern Ireland. Sci., 115: 235-240.
24. Oh,M.S., 2006. Evaluation of renal function, water, electrolytes and acid-base balance.In Pincus MR, Lifshitz MS, eds. Henry's clinical diagnosis and management by laboratory methods. 21th ed., Philadelphia, Pa., WB Saunders, pages: 147.
25. Pacheco, M. and M.A.Santos, 2002. Biotransformation, genotoxicand histopathological effects of environmental contaminants in European eel(*Anguilla anguillaL.*). Ecotoxicol. Environ. Saf.,53:331–347.

26. Palhares, D, and C.K. Grisolia, 2002. Comparison between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mytomycin C treatment. Genet. Mol. Biol., 25:281–284.
27. Pandey R, Kalra A (2003). Root knot disease of ashwagandha Withania somnifera and its ecofriendly cost effective management. J. Mycol. Pl. Pathol. 33(2): 240-245.
28. Park, E., J. Lee, and H. Etoh,1993. Fish cell line (ULF-23HU) derived from the fin of the central mudminnow (Umbra limi): suitable characteristics for clastogenicity assay.In Vitro Cellular & Developmental Biology, 1993; 25: 987-94.
29. Pickering, A.D., 1981. Stress and compensation in teleostean fishes. Response to social and physical factors, in: Pickering, A.D. (Edn.), Stress and Fish, Academic press, New York, USA., pages:295-322.
30. Ramesh, M.,2001.Toxicity of copper sulphate on some haematological parameters of a freshwater teleost *Cyprinus carpio* var. *communis*. J.Indian Fish. Assoc. 28: 131-136.
31. Rasekh HR, H. Yazdanpanah, L. Hosseinzadeh, N. Bazmohammadi, and M. Kamalinejad, 2005.Acute and Subchronic Toxicity of *Teucrium polium* Total Extract in Rats.Iranian J. of Pharmaceutical Research, 4: 245-249.
32. Remyla, S.R., M. Ramesh, K.S. Sajwan and K. Senthil, 2008. Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish *Catla catla. Fish. Physiol. Biochem.,* 34: 169-174.
33. Shahraki MR, M.R. Arab and E. Mirimokaddam, 2007.The Effect of *Teucrium polium* (Calpoureh) on Liver function, Serum Lipids and Glucose in Diabetic Male Rats. Iranian Biomedical J., 11 (1): 65-68.
34. Singh, D., and A.Singh, 2002.Biochemical alteration in freshwater fish *Channa punctatus* due to latices of *Euphorbia royleana* and *Jatropha gossypifolia. Environ. Toxicol. Pharmacol.,* 12: 129-1.
35. Suvetha, L., M. Ramesh, and M. Saravanan, 2010.Influence of cypermethrin toxicity on ionic regulation and gill Na+/K+-ATPase activity of a freshwater teleost fish *Cyprinus carpio. Environ. Toxicol. Pharmacol.,* 29: 44-49.
36. Talas, Z.S and M.F.Gulhan, 2009. Effects of various propolis concentrationson biochemical and hematological parameters of rainbow trout (*Oncorhynchusmykiss*). *Ecotoxicol. Environ. Safe,* 72: 1994-1998.
37. Zal, F., M. Vasei, M. Rasti and M.Vessal,2001. Hepatotoxicity associated with hypoglycemic effects of *teucrium polium* in diabetic rats. Arch. Irn. Med., 4: 188-192.

5/15/2013