World Journal of Zoology 9 (3): 155-161, 2014
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2014.9.3.84291

Toxic Effect of Alphamethrin on Catalase, Reduced Glutathione and Lipid Peroxidation in the Gill and Liver of Zebrafish, *Danio rerio*

Shabnam Ansari and Badre Alam Ansari

Zebrafish Laboratory Department of Zoology,
D.D.U. Gorakhpur University,
Gorakhpur - 273 009 (U.P.), India

Abstract: The indiscriminate use of the pesticides worldwide for agricultural and other activities is responsible for pollution which causes various deleterious effect on fish and ultimately on human. Alphamethrin, a synthetic pyrethroid intensively controls a wide range of pests in agriculture and animal breeding. The present study was aimed to investigate the changes due to alphamethrin exposure in the activity of catalase (CAT), reduced glutathione (GSH) and lipid peroxidation (LPO) in the tissues of zebrafish at different concentrations and exposure periods. A significant reduction was observed in the CAT activity and GSH level in the gill and liver, on the other hand there was an increase in LPO in both the tissues and the probable causes are discussed. The toxicity was time as well as concentration dependent. Thus, oxidative stress parameters may be highly recommended as an early-warning bio-indicator of environmental pollution using the zebrafish.

Key words: Alphamethrin · Zebrafish · Oxidative Enzymes · Gill · Liver

INTRODUCTION

Pesticides are unusual among environmental pollutants because they are used deliberately for the purpose of killing some form of life. In considering the use of pesticides, the benefits must be weighed against the risk to human health and environmental quality. A major risk is environmental contamination, especially translocation within the environment where pesticides might enter both food chains and natural water systems. The pesticides which are liberated into the aquatic environment causes various deleterious effect on fish and ultimately on human [1]. Alphamethrin, a synthetic pyrethroid used for the control of wide range of pests in agriculture and animal breeding. It consists of the active isomer of synthetic pyrethroid cypermethrin and is highly effective against wide range of chewing and sucking insects. It is also used to control mosquitoes, flies and other insect pests in public health programs and animal houses. Alphamethrin is practically non toxic to birds but is highly toxic to fish and aquatic invertebrates. This is mainly because it is metabolized and eliminated significantly more slowly by fish than mammals or birds [2].

The synthetic pyrethroids may damage the vital organs [3], reduced reproductive ability [4] and causes various biochemical alterations [5] of the non-target organisms. All aerobic organisms need molecular oxygen for their oxidative metabolic processes but as a consequence it also deal with the formation of dangerous reactive oxygen species (ROS) including superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) [6]. Due to their high reactivity, these species may damage lipids, proteins, carbohydrates and nucleic acids [7]. Under normal physiological conditions there is a balance between ROS generation and their elimination by different antioxidant scavengers. If pro-oxidant processes are enhanced or the power of the antioxidant defense system decreased, oxidative stress arises [8]. Many pollutants can also induce the formation of ROS [9, 10].

Corresponding Author: Badre Alam Ansari, Zebrafish Laboratory Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur - 273 009 (U.P.), India.
Tel: +91-9452448942.

Antioxidants provide chemical protection to the biological system against harmful effects of reaction that causes excessive oxidation, DNA damage and cell death [11]. Antioxidant enzymes of fishes that play a crucial role in maintaining cell homeostasis, have received much attention in ecotoxicology since oxidative damage was considered as mechanism of toxicity in aquatic organism exposed to environmental contaminants [12].

The biochemical changes induced by stress are described as secondary responses of the fish. The toxic pollutants affect the activity of enzymes and change the level of enzymes so they can be used as biomarkers [13]. It is known that antioxidant enzymes themselves are very sensitive to oxidative inactivation by ROS [14]. Catalase (CAT) is a common enzyme found in nearly all the organisms which are exposed to oxygen where it functions to catalyze the decomposition of H_2O_2 to O_2 and H_2O. Reduced glutathione (GSH) is an antioxidant which helps to protect the cells from the ROS such as free radicals and peroxides. The glutathione defense enzyme systems in living cell detoxifies and eliminates xenobiotics leading to the formation of products easily soluble in water and their rapid elimination from the organism. Lipid peroxidation (LPO) is a well-established mechanism of estimating cellular injury in both plants and animals. It is used as an indicator of oxidative stress in cells and tissues. The lipid peroxidation due to free radicals is considered as the main mechanism of cellular destruction [15]. Investigators showed that lipid peroxidation in cells increased with pyrethroid treatment, antioxidant enzyme activities and malonyldialdehyde (MDA) concentration were altered after exposure to pesticide in fishes [16, 17].

Fish, among the group of non-target aquatic organisms, represent the largest and most diverse group of vertebrates. Hence the present study was aimed to study the toxic effect of sub-lethal dose of alphamethrin on the activity of CAT, GSH and LPO in the gill and liver of zebrafish. This fish was selected for the present study because they are model organisms for toxicological research and also recommended by the Organization for Economic Co-operation and Development [18].

MATERIALS AND METHODS

Maintenance and Experimental Design: Zebrafish were collected, acclimatized for 15 days, stocked and bred under laboratory conditions. The aquaria were continuously aerated through stone diffusers connected to a mechanical air compressor. Water temperature was 25±2°C and pH was maintained between 6.6 and 8.5. The fish were fed twice daily alternately with raw chopped goat liver and brine shrimp pellets prepared in our laboratory. The diet was supplemented with *Drosophila* flies once daily.

For the present study matured laboratory bred adult zebrafish (3.62±0.04 cm length & 1.00±0.48 gm weight) were procured from the stock aquarium. The fishes were exposed to different concentrations viz. 0.03, 0.07, 0.10 and 0.14 µg/l (20%, 40%, 60% and 80% of 96-h LC_{50} value of alphamethrin) as calculated earlier [5] for 21 days continuously. Fifty fishes for each concentration of the pesticide were used. In these aquaria water was replaced daily with fresh treatment of pesticides. The experiment was accompanied by the control. After the expiry of the exposure periods (7, 14 and 21 days), required number of exposed fishes were taken out from experimental and control groups.

Biochemical Assays: Activity of catalase (EC 1.11.1.6) was estimated according to the procedure of Sinha [19]. This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate is measured colorimetrically at 620 nm. The catalase preparation is allowed to split H_2O_2 for different time interval by the addition of dichromic acetic acid mixture and the remaining H_2O_2 is determined colorimetrically. The results were expressed as micro-mol (µM) H_2O_2 utilized/min/mg protein.

GSH level was estimated according to the method of Paglia *et al.* [20] determined by its reaction with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) to yield a yellow chromophore which was measured spectrophotometrically at 412 nm and results were expressed as GSH mg/mg protein. LPO were estimated via the thiobarbituric acid reacting substances (TBARS) color reaction for malondialdehyde (MDA) according to the procedure of Placer *et al.* [21] and results were expressed as µM of MDA formed/30 min/mg protein.

Total Protein Determination: In tissues the protein contents were assayed using the method of Lowry *et al.* [22] with bovine serum albumin as standard.

Statistical Evaluation: The data were subjected to a two-way analysis of variance (ANOVA) and the significance of the differences between means ($p<0.05$) was determined by using StatPlus® version 2009 computer software. Values expressed are means (n=6) ± standard deviation (SD).

RESULTS

In the treated group of fishes abnormal behaviour such as restlessness, sudden quick and jerky movements were observed at low concentration of pesticide whereas, increased opercular movements accompanied with surface to bottom movements and loss of equilibrium was observed in the fishes exposed to high concentrations. There was a significant ($p<0.05$) alterations in the CAT activity, GSH and MDA level in gill and liver of zebrafish exposed to alphamethrin at different concentrations (0.03, 0.07, 0.10 and 0.14 µg/l) and exposure periods (7, 14 and 21 days).

Changes in Gills: The catalase activity was estimated after 7, 14 and 21 days of continuous exposure to four different concentrations of alphamethrin. After highest concentration i.e. 0.14 µg/l of alphamethrin the CAT activity was reduced gradually to 119.29±0.45, 94.14±0.73 and 86.79±0.73 µM H_2O_2 utilized/min/mg protein after 7, 14 and 21 days respectively. Analysis of variance showed that the inhibition was concentration and time dependent ($p<0.05$) i.e. increase in concentrations enhanced the inhibition of CAT (Table 1).

Alterations in GSH level after 21 days of continuous treatment at highest concentration a significant ($p<0.05$) decrease in GSH level was recorded i.e. 1.13±0.06 GSH mg/mg protein. However, at the lowest concentration after 7 days of exposure the changes were not so prominent but with the increase of the exposure period i.e. after 21 days there was a drastic change even at lower pesticide concentration (0.03 µg/l). Overall reduction in the GSH level after treatment was statistically significant ($p<0.05$) (Table 2). The effect of alphamethrin on LPO shows a significant ($p<0.05$) change at different concentrations and treatment period. The LPO in the gill of zebrafish exposed for 21 days showed remarkable increase. At 0.03 µg/l of pesticide the level was 7.92±0.79 µM of MDA formed/30 min/mg protein, at 0.07 µg/l the change was 8.51±0.45, at 0.10 µg/l was 9.91±0.38 and 10.66±0.15 at 0.14 µg/l (Table 3).

Changes in Liver: The effect of pesticide on the CAT activity showed significant ($p<0.05$) decrease after 7, 14 and 21 days of treatment period. Minimum change in CAT activity was observed after 7 days of treatment period at each concentration which was 160.55±0.33, 153.28±0.23, 142.55±0.33 and 119.06±0.24 µM H_2O_2 utilized/min/mg

Table 1: Effect of alphamethrin on catalase activity (µM H_2O2 utilized/min/mg protein) in the gill of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations(µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	170.46±0.28(100)	169.29±0.19(100)	168.37±0.19(100)	Variationsdue toconcentrations	2	10684.75	5342.38	21384.12	0.05
0.03	155.14±0.58(91)	142.93±0.41(84)	129.14±0.69(77)	Variationsdue tooperations	4	48291.91	12072.98	48324.94	0.05
0.07	150.88±0.30(89)	125.12±0.34(74)	113.96±0.39(68)	Interaction	8	2675.50	334.44	1338.67	0.05
0.10	138.13±0.63(81)	116.21±0.42(69)	104.32±0.64(62)	Residual	75	18.74	0.25		
0.14	119.29±0.45(70)	94.14±0.73(56)	86.79±0.73(51)	Total	89	61670.90			

-Values are mean±SD of six individual observations and significant at $p<0.05$ (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%.

*The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

Table 2: Effect of alphamethrin on GSH (GSH mg/mg protein) in the gill of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations (µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	2.32±0.15(100)	2.56±0.27(100)	2.18±0.07(100)	Variationsdue toconcentrations	2	6.06	3.03	15.85	0.05
0.03	2.13±0.52(94)	2.12±0.45(83)	1.54±0.18(71)	Variationsdue tooperations	4	7.10	1.77	9.29	0.05
0.07	2.08±0.41(90)	1.99±0.56(78)	1.37±0.17(63)	Interaction	8	0.80	0.10	0.53	NS
0.10	1.98±0.65(85)	1.81±0.55(71)	1.25±0.23(57)	Residual	75	14.32	0.19		
0.14	1.80±0.43(76)	1.64±0.44(64)	1.13±0.06(52)	Total	89	28.28			

-Values are mean±SD of six individual observations and significant at $p<0.05$ (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%. NS=not significant

*The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

Table 3: Effect of alphamethrin on LPO (µM of MDA formed/30 min/mg protein) in the gill of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations(µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	8.16±0.53(100)	7.76±0.42(100)	6.48±0.32(100)	Variationsdue toconcentrations	2	18.67	9.34	66.29	0.05
0.03	8.75±0.15(107)	8.76±0.17(113)	7.92±0.79(122)	Variationsdue tooperations	4	154.89	38.72	274.97	0.05
0.07	9.50±0.35(116)	9.68±0.18(125)	8.51±0.45(131)	Interaction	8	3.23	0.40	2.86	0.05
0.10	10.55±0.23(129)	10.61±0.37(137)	9.91±0.38(153)	Residual	75	10.56	0.14		
0.14	11.12±0.34(136)	11.72±0.17(151)	10.66±0.15(165)	Total	89	187.35			

-Values are mean±SD of six individual observations and significant at $p<0.05$ (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%.

*The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

Table 4: Effect of alphamethrin on catalase activity (µM H2O2 utilized/min/mg protein) in the liver of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations(µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	180.39±0.28(100)	180.49±0.20(100)	179.36±0.13(100)	Variationsdue toconcentrations	2	12787.38	6393.69	44897.89	0.05
0.03	160.55±0.33(89)	137.15±0.58(76)	129.15±0.09(72)	Variationsdue tooperations	4	63739.40	15934.85	111898.01	0.05
0.07	153.28±0.23(85)	126.32±0.64(70)	116.59±0.22(65)	Interaction	8	3169.42	396.18	2782.05	0.05
0.10	142.55±0.33(79)	111.92±0.67(62)	104.05±0.24(58)	Residual	75	10.68	0.14		
0.14	119.06±0.24(66)	95.64±0.55(53)	86.07±0.24(48)	Total	89	79706.88			

-Values are mean±SD of six individual observations and significant at p<0.05 (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%. *The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

Table 5: Effect of alphamethrin on GSH (GSH mg/mg protein) in the liver of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations(µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	3.42±0.19(100)	3.59±0.22(100)	3.83±0.11(100)	Variationsdue toconcentrations	2	4.25	2.13	16.31	0.05
0.03	3.15±0.09(92)	3.06±0.24(85)	2.45±0.18(64)	Variationsdue tooperations	4	29.00	7.25	55.57	0.05
0.07	2.94±0.64(86)	2.61±0.55(73)	2.12±0.65(55)	Interaction	8	3.49	0.44	3.34	NS
0.10	2.71±0.43(79)	2.39±0.28(67)	1.87±0.42(49)	Residual	75	9.78	0.13		
0.14	2.18±0.09(64)	2.06±0.24(57)	1.56±0.34(41)	Total	89	46.53			

-Values are mean±SD of six individual observations and significant at p<0.05 (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%. NS = not significant

*The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

Table 6: Effect of alphamethrin on LPO (µM of MDA formed/30 min/mg protein) in the liver of zebrafish

	Treatment period (days)			Summary of computation for ANOVA					
Concentrations(µg/l)*	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control	11.49±0.37 (100)	11.55±0.33 (100)	11.19±0.10 (100)	Variationsdue toconcentrations	2	43.92	21.96	150.23	0.05
0.03	12.8±0.42 (112)	13.64±0.55(118)	14.08±0.24 (126)	Variations due to operations	4	462.88	115.72	791.57	0.05
0.07	13.68±0.25(119)	14.89±0.38(129)	15.89±0.37(142)	Interaction	8	20.25	2.53	17.31	0.05
0.10	15.39±0.28(134)	16.64±0.56(144)	17.80±0.54(159)	Residual	75	10.96	0.15		
0.14	16.21±0.43(141)	18.23±0.42(158)	19.14±0.06(171)	Total	89	538.02			

-Values are mean±SD of six individual observations and significant at p<0.05 (two-way ANOVA). Numbers in parentheses indicate the percent change rounded to the nearest values in comparison with control value taken as 100%. *The exposure concentrations used were 20%, 40%, 60% and 80% (0.03, 0.07, 0.10 and 0.14 µg/l) of 96-h LC_{50} value.

protein as compared to control (180.39±0.28). But after 21 days of treatment period at all concentrations the maximum change in CAT activity were observed which shows a concentration and time-dependent action of alphamethrin (Table 4).

Alterations in the GSH level after 7 to 21 days of continuous treatment of pesticide are presented in Table 5. The reduction of GSH level was maximum at 21 days of treatment of 0.14 µg/l of alphamethrin and it was found to be only 1.56±0.34 GSH mg/mg protein. Analysis of variance confirmed that the inhibition was concentration and time dependent. The effect of alphamethrin on LPO also showed a significant (p<0.05) change at different concentrations and exposure periods. Contrary to CAT and GSH the MDA level exhibited enhancement after different treatment of pesticide (Table 6). At 0.14 µg/l of pesticide treatment for 21 days there was drastic increase of in the MDA level (19.14±0.06) as compared to control (11.19±0.10).

DISCUSSION

CAT is a common enzyme where it functions to catalyze the decomposition of H_2O_2 to molecular O_2 and H_2O. Our results showed a time and concentration-dependent decrease in CAT activity in gill and liver of zebrafish exposed to alphamethrin with respect to control. It might be due to binding of alphamethrin to CAT or by inhibiting CAT synthesis as reported by Tripathi and Verma [23]. The same decreased activity of CAT was observed in brain, liver and kidney of *Channa punctatus* exposed to triazophos [24]. Tripathi and Singh [25] observed the simultaneous reduction in CAT activity in the brain, gill, liver and skeletal muscles of alphamethrin treated *Channa punctatus* in comparison to control. According to Tripathi and Bandooni [26] the specific activity of CAT declined significantly (p<0.05) in liver of *Clarias batrachus* in response to treatment with alphamethrin (0.018 ppm) for 14 days. Also, Zhang *et al.*

[27] found that severe oxidative stress may suppress antioxidant defense enzyme activities. The same concentration-dependent decrease in the activity of CAT was observed by many other workers [9 & 28-30]. The degree of effects of alphamethrin on CAT and protein profile was different on different tissues because it may depend on the location and metabolic significance of the tissues [31]. In *Channa punctatus* the activity of CAT was reduced because pesticides initiate the enhancement of superoxide radicals that can inhibit the CAT activities [32].

The effect of the pesticide on cellular structures or its alternative effect on enzyme activities can explain the alterations in GSH activities. A significant decline in GSH level in gill and liver under the present experimental study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from alphamethrin. Previous investigators have also reported the significant concentration and time dependent decrease in the level of GSH [33- 35]. The decreased level of GSH was observed in the gill, liver, brain and muscle of *Oreochromis mossambicus* exposed to organophosphate (OP) insecticide [36]. Farombi *et al.* [37] observed the reduction in GSH level in kidney, gill and heart of African catfish, *Clarias gariepinus* treated with butachlor. Similarly, Jin *et al.* [38] studied the effect of cypermethrin on the induction of hepatic oxidative stress, DNA damage and the alteration of gene expression related to apoptosis in adult zebrafish. The decreased GSH level in gill and liver of treated fish indicates that protection against the ROS is more required.

LPO *in vivo* has been identified as one of the basic deteriorative reactions in cellular mechanism of the pesticide induced oxidative stress in fresh water fishes. Many other workers [17, 28, 30, 37, 39] observed a concentration-dependent increase in the level of LPO which is in support of our result. According to Sayeed *et al.* [10] increased LPO in *Channa punctatus* exposed to deltamethrin is the result of oxidative stress and causes several degenerations. Impairment of enzymatic antioxidant system may favour accumulation of free radicals that may be responsible for increased LPO on alphamethrin exposure. Sastry and Dasgupta [40] also reported the enhancement of LPO in the liver of *Channa punctatus* treated with monocrotophos. Elevation in the level of LPO was observed in the liver, gill, muscle and brain of OP insecticide-intoxicated *Oreochromis mossambicus* in comparison to control [36].

LPO was increased in the brain of a freshwater fish, *Oreochromis niloticus* treated with diazinon [41] which is in favour of our result. Similarly, Parthasarathy and Joseph [42] found that ë-cyhalothrin induced a significant increase in LPO in the liver of *Oreochromis mosambicus* as compared to control. Suneetha [43] observed the significant induction of LPO in the gill, kidney and liver of *Labeo rohita* treated with endosulfan and fenvalerate which may be due to ROS. Recently, Kakoolaki *et al.* [44] observed the significant increase ($p<0.001$) in LPO in cypermethrin exposed rainbow trout. Increased ROS production may, thus be associated with the metabolism of alphamethrin leading to the peroxidation of membrane lipids of both the tissues and finally cell damage or death. Thus, the reduction in CAT activity and GSH is probably related to the enhancement of LPO.

CONCLUSION

From the present study it is concluded that alphamethrin is harmful to the defense system of the fishes. The ecological contamination of such toxic chemicals should be avoided. Further research is necessary to study the effect of other pesticides on the antioxidant enzyme status of fish in the laboratory as well as field conditions before implementing such markers in the current scenario. Thus, these antioxidant enzymes may be highly recommended as early-warning bio-indicators of environmental pollution by alphamethrin in the areas where it is to be used for pest control.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Rajendra Singh, Head of the Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur for providing the laboratory facilities during this research work.

REFERENCES

1. Ansari, R.A., M. Kaur, F. Ahmad, S. Rahman, H. Rashid, F. Islam and S. Raisuddin, 2009. Genotoxic and oxidative stress-inducing effects of deltamethrin in the erythrocytes of a freshwater bio-marker fish species, *Channa punctatus* (Bloch). Environmental Toxicology, 24: 429-436.
2. Greulich, K. and S. Pflugmacher, 2003. Differences in susceptibility of various life stages of amphibians to pesticide exposure. Aquatic Toxicology, 65: 329-336.

3. Veronica, W. and P.A. Collins, 2003. Effect of cypermethrin on the fresh water crab, *Trichodactylus borellianus* (Crustacea: Decapoda: Braquiura). Bulletin of Environmental Contamination and Toxicology, 71: 106-113.
4. Sharma, D.K. and B.A. Ansari, 2010. Effect of the synthetic Pyrethroid Deltamethrin and the neem based pesticide Achook on the reproductive ability of zebrafish, *Danio rerio* (Cyprinidae). Archives of Polish Fisheries, 18: 157-161.
5. Ansari, S. and B.A. Ansari, 2012. Alphamethrin toxicity: Effect on the reproductive ability and the activities of phosphatases in the tissues of zebrafish, *Danio rerio*. International Journal of Life Science and Pharma Research, 2: 89-100.
6. Halliwell, B. and J.M.C. Gutteridge, 1989. Free Radicals in Biology and Medicine. 2nd Edn. Clarendon Press, London.
7. Hermes-Lima, M., 2004. Oxygen in biology and biochemistry: role of free radicals. In: Storey, K.B. (Ed.) Functional Metabolism: Regulation and Adaption. Wiley-liss, Hokoben, NJ, pp: 319-368.
8. Sies, H., 1991. Oxidative stress: Introduction. In: Sies, H. (Ed.) Oxidative Stress: Oxidants and Antioxidants, Academic Press, San Diego, pp: 21-48.
9. Ahmad, I., T. Hamid, M. Fatma, H.S. Chand, S.K. Jain, M. Atha and S. Raisuddin, 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochimica et Biophysica Acta, 1523: 37-48.
10. Sayeed, I., S. Parvez, S. Pandey, B. Bin-Hafeez, R. Haque and S. Raisuddin, 2003. Oxidative stress biomarkers of exposure to Deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicology and Environmental Safety, 56: 295-301.
11. Arnao, M.B., A. Cano and M. Acosta, 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry, 73: 239-244.
12. Santos, M.A., M. Pacheco and I. Ahmad, 2004. Antioxidants responses to *Anguilla anguilla* L. in situ bleached kraft pulp mill effluent outlet exposure. Environment International, 30: 301-308.
13. Mayer, F.L., D.J. Verteeg, M.J. Mckee, L.C. Folmar, R.L. Graney, D.C. McCume and B.A. Rather, 1992. Physiological and nonspecific biomarkers, in fishes. Comparative Biochemistry and Physiology, 94: 265-270.
14. Hermes-Lima, M. and K.B. Storey, 1993. In vitro oxidative inactivation of glutathione-S-transferase from a freeze tolerant reptile. Molecular and Cellular Biochemistry, 124: 149-158.
15. Yarsan, E., M. Tanyukesel, S. Celik and A. Aydin, 1999. Effects of aldicarb and Malathion on lipid peroxidation. Bulletin of Environmental Contamination and Toxicology, 63: 575-581.
16. Kale, M., N. Rathore, S. John and D. Bhatnagar, 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes a possible involvement of reactive oxygen species. Toxicology Letters, 105: 197-205.
17. Uner, N., E. Ozcan Oruc, M. Canli and Y. Sevgiler, 2001. Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). Bulletin of Environmental Contamination and Toxicology, 67: 657-664.
18. Organization for Economic Co-operation and Development, 1992. Guidelines for Testing of Chemicals, Guideline 210 "Fish, Early-life Stage Toxicity Test." Adopted July, 17.
19. Sinha, A.K., 1972. Colorimetric assay of Catalase. Analytical Biochemistry, 47: 389-394.
20. Paglia, D.E., W.N. Valentine and J.G. Dahlgren, 1975. Effects of low level lead exposure on pyrimidine 5'-nucleotidase and other erythrocyte enzymes. Possible role of pyrimidine 5'-nucleotidase in the pathogenesis of lead induced anemia. Journal of Clinical Investigation, 56: 1164-1169.
21. Placer, Z.A., L. Cushman and B.C. Johnson, 1966. Estimation of product of lipid peroxidation (Malonyldialdehyde) in biochemical systems. Analytical Biochemistry, 16: 359-364.
22. Lowry, O.H., N.J. Roserbrough, A.L. Farr and R.J. Randall, 1951. Protein measurements with Folin-phenol reagent. The Journal of Biological Chemistry, 193: 265-275.
23. Tripathi, G. and P. Verma, 2004. Fenvalerate-induced changes in catfish, *Clarias batrachus*: metabolic enzymes, RNA and protein. Comparative Biochemistry and Physiology Part C, 138: 75-79.
24. Naveed, A. and C. Janaiah, 2011. Effect of Triazophos on protein metabolism in the fish, *Channa punctatus*. Current Research Journal of Biological Sciences, 3: 124-128.

25. Tripathi, G. and H. Singh, 2013. Impact of Alphamethrin on biochemical parameters of *Channa punctatus*. Journal of Environmental Biology, 34: 227-230.
26. Tripathi, G. and N. Bandooni, 2011. Impact of Alphamethrin on antioxidant defense (Catalase) and protein profile of a catfish. The Environmentalist, 31: 54-58.
27. Zhang, J., H. Shen, X. Wang, J. Wu and Y. Xue, 2003. Effect of choric exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish, *Carassius auratus*. Chemosphere, 55: 167-174.
28. Gultekin, F., M. Ozsturk and M. Akdogan, 2000. The effect of organophosphate insecticide chlorpyrifosethyl on lipid-peroxidation and antioxidant enzymes (in vitro). Archives of Toxicology, 74: 533-538.
29. Pascual, P., J.R. Pedrajas, J. Lopez-Barea, F. Toribio and J. Peinado, 2003. Effect of food derivation on oxidative biomarkers in fish (*Sparus auratus*). Chemico-Biological Interactions, 145: 191-199.
30. Manna, S., D. Bhattacharya, D.K. Basak and T.K. Mandal, 2004. Single oral dose toxicity study of α-cypermethrin in rats. Indian Journal of Pharmacology, 36: 25-28.
31. Doss, P.J., P. Sreelatha, R.N. Sulthana and V.K. Lakshmi Bai, 2008. Effect of cypermethrin on selective biochemical parameters in a fish, *Heteropneustes fossilis*. Aquaculture, 9: 205-210.
32. Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiological Reviews, 74: 139-162.
33. Banerjee, B.D., V. Seth, A. Bhattacharya, S.T. Pasha and A.K Chakraborty, 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology Letters, 107: 33-47.
34. Menagaux, L., A. Baruchel, Y. Bertrandy, B. Lescoeur, G. Levergers, B. Nelken, D. Sommelet, D. Hemon and J. Clavel, 2006. Household exposure to pesticides and risk of childhood acute leukemia. Occupational and Environmental Medicine, 63: 131-134.
35. Sharma, D.K. and B.A. Ansari, 2013. Effects of Deltamethrin on CAT, LPO and GSH in Tissues of zebrafish, *Danio rerio*. Research Journal of Environmental Toxicology, 7: 38-46.
36. Rao, J.V., 2006. Toxic effect of novel organophosphorous insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pesticide Biochemistry and Physiology, 86: 78-84.
37. Farombi, E.O., Y.R. Ajimoko and O.A. Adelowo, 2008. Effect of Butachlor on antioxidant enzyme status and lipid peroxidation in freshwater African catfish, *Clarias gariepinus*. International Journal of Environmental Research and Public Health, 5: 423-427.
38. Jin, Y., S. Zheng, Y. Pu, L. Shu, L. Sun, W. Liu and Z. Fu, 2011. Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (*Danio rerio*). Chemosphere, 82: 398-404.
39. Maheshwari, S.L., R.V. Vankatakrishna Murali and R. Balaji, 2014. Aluminium induced cholinotoxicity in zebrafish - A sequel of oxidative stress. International Journal of Advanced Research, 2: 322-335.
40. Sastry, K.V. and A. Dasgupta, 1991. Effect of nuvacron on the nutritive value of freshwater teleost fish, *Channa punctatus*. Journal of Environmental Biology, 12: 243-248.
41. Uner, N., E. Ozcan Oruc, Y. Sevgiler, N. Sahin, H. Durmaz and D. Usta, 2006. Effects of diazinon on acetylcholine esterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. Environmental Toxicology and Pharmacology, 21: 241-245.
42. Parthasarathy, R. and P. Joseph, 2011. Study on the changes in the levels of membrane-bound ATPases activity and some mineral status in lamba-cyhalothrin induced hepatotoxicity in freshwater tilapia (*Oreochromis mossambicus*). African Journal of Environmental Science and Technology, 5: 98-103.
43. Suneetha, K., 2014. Toxic effects of endosulfan and fenvalerate on oxidative stress biomarkers and lipid peroxidation in freshwater *Labeo rohita* (Hamilton). International Journal of Biology and Pharmaceutical Research, 5: 156-160.
44. Kakoolaki, S., Z. Selamoglu, T. Oguz, O. Cakir, O. Cifti and I. Ozdemir, 2013. Role of Propolis on Oxidative Stress in Fish Brain. Basic and Clinical Neuroscience, 4: 47-52.