

ORIGINAL RESEARCH ARTICLE

Chelating Properties of *Delonix elata* Against Cypermethrin Induced Oxidative Stress and Antioxidant Enzyme Activity in *Cyprinus carpio* (Linn)

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ABSTRACT

In the present investigation, the toxic effect of cypermethrin on antioxidant enzyme activity in fresh water fish *Cyprinus carpio*. Cypermethrin was applied 96 hrs LC₅₀ (250µg/l), sub lethal concentration (120 hours 50µg/l). The fish were treated with cypermethrin for five days, after that at the end of the fifth day treated fish were separated into two group one group is feed with *Delonix elata* supplementary fed for five days, organs like gill, liver and kidney were examined 24, 48, 72, 96 and 120 hours The antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (Gpx), Lipid peroxidation (TBARS) level changes occurs due to toxic effect of cypermethrin. Antioxidant enzymes are biomarkers used to indicating the cypermethrin toxic effect. SOD and CAT are decreased during exposure period. TBARS level increased negative correlation was observed in the treated group.GP_x level increased in the treated group. In the recovery group Novelities antioxidant enzymes were increased and TBAR activity was decreased. Present study to know the toxic effect of cypermethrin on *Cyprinus carpio* fish and chelating property of *Delonix elata* supplementary feed.

Key words: Cypermethrin, *Cyprinus carpio*, Antioxidant enzymes and *Delonix elata*.

1. INTRODUCTION

Synthetic pyrethroid insecticides are introduced over the past two decades for agriculture and domestic use ^[1]. Pyrethroid insecticides are extensively used to replace the organochlorin, organophosphorus insecticides and carbamates, to control various types of pests and increased agricultural production ^[2]. Cypermethrin most commonly used in agricultural and animal husbandry for lice infest. Cypermethrin also used fish culture to eradicate the lice in the form ^[3]. Cypermethrin is a neurotoxic chemical; the major target site is sodium channel of the nerve membrane. Cypermethrin enter into the cell along with Na⁺ ion so cell excitation prolonged several seconds ^[4].

According to environmental quality standard, the maximum allowable concentration of cypermethrin is 1ng/l ^[5] cypermethrin is one of the most common contaminants in fresh water aquatic system ^[6]. Owing the excessive use of synthetic pyrethroids in the environmental and water resources are being polluted thus endangering aquatic life directly and human life indirectly ^[7]. Cypermethrin is release directly into the environment it enters the water body mainly as

runoff ^[8]. USDA national agricultural pesticide impact assessment program's document reports that cypermethrin cause acute toxicity in fish in laboratory test at an average range of 1.8-8.2µg/l ^[5]. Exposure to toxic chemical may produce an unbalance between endogenous and exogenous ROS and subsequently induce decrease in antioxidant defense or cause out right oxidative damage to organisms ^[9] WHO guideline recommend no specific antidotes but symptomatic and supportive measures for this type of poisons ^[10] which have been shown that antioxidant of fish could be used as biomarkers of exposure to aquatic pollutants ^[11]. The deltamethrin induces oxidative stress in *Channa punctatus bloch* fish ^[12] deltamethrin induced a distinct oxidative stress response in the liver and intestine of *Carassium aciratus gibelio*. The antioxidant defense system is an integral part of the biochemical adaptive mechanisms of tolerance to deltamethrin ^[13]. Cypermethrin induced oxidative stress in fresh water mussels at various concentrations has been reported ^[14]. The changes in activity of antioxidant enzymes such as SOD, CAT, GP_x is considered as an effective method of denoting oxidative stress ^[15]. Study of cypermethrin

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induced oxidative stress and its influence on various antioxidants of fish and other aquatic organisms could provide useful information on the ecotoxicological consequences of cypermethrin use. Several studies have shown that the levels of antioxidants in fish and mussels could be used as biomarker of exposure to aquatic pollution [16,17]. In the present study, we have analysed the toxic effect of cypermethrin on *Cyprinus carpio* fish, lipid peroxidation and activity of enzymes such as super oxide dismutase, catalase and glutathione peroxidase in gill, liver, and kidney from fish treated with 5 days sub lethal concentration of this insecticide, and also providing data on toxic counteract of the *Delonix elata* leaf pellet supplementary feed recorded the chelating property.

2. MATERIALS AND METHODS

The fish *Cyprinus carpio* 75g±5 of weight 15±5cm length were obtained from the Navarathna fish farm nearby Pinnalloor fish were safely transferred to the laboratory. They were kept in the cement tank filled with dechlorinated water and continuous aeration. Acclimatization to experimental condition for 15 days at room temperature fish were feed artificial libitam during acclimatization and tank water was renewed every day after feeding, food was withheld from before 24 hours to the experiment. Cypermethrin technical trade 98.8% pure were obtained from Harda chemicals Mumbai, 1g of cypermethrin dissolved into 100 ml technical grade acetone this stock is used for daily requirement. The toxicant used for determine the sub lethal concentration of cypermethrin.

Cypermethrin toxicity

Toxicity of cypermethrin was evaluated by static bioassay method of [18] and the LC₅₀ value of 96 hour to be 250µ/l. One fifth of LC₅₀ was selected as nominal sublethal concentration (50µg/l) as used in the present investigation to analyze the sub acute effect at exposure period of 24, 48, 72, 96 and 120 hours respectively.

Group I -Fish reared in cypermethrin free water.

Group II -Fish exposed is 50 µg /l cypermethrin for five days.

Group III -Exposed its 50 µg/l cypermethrin for five days, fed with supplementary feed *Delonix elata*.

Group IV -Exposed to cypermethrin free water and feed with *Delonix elata* supplementary feed alone.

Delonix elata leaves were collected around university campus. Leaves are washed with water

and dried in room temperature; dried leaves were powdered than mixed with rice bran powder 1:1 ratio adds some ml of distilled water make small pellets. It's also dried inside the room with better aeration. Dried pellet feed with Group III and IV were given at 10g daily during the experimental period. Aquaria, water were renewed cypermethrin was added every day for Group II and III exposed to 120 hours. Six fish of each group were sacrificing and removed the organs immediately within an hour for the assay of antioxidant enzyme activity and lipid peroxidation using gill, liver and kidney tissues of the fish.

Assay method

TBARS in the liver gill, kidney were estimated by the method of [19] In this method, malondialdehyde and other thiobarbituric acid reactive substance (TBARS) react with thiobarbituric acid is an acidic condition to generate a pink colour chromophore which was read at 535 nm. Assay of superoxide dismutase (EC 1.15.1.1) activity was determined by the method of [20]. This assay is based on the inhibition of the formation of NADH- phenazinemetosulphate, nitro blue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90 seconds adding glacial acetic acid stops the reaction. The color developed at the end of the reaction was extracted into n- butanol layer and measured in a spectrophotometer at 520 nm. The activity of catalase CAT (EC 1.11.1.6) in the liver, gill, and kidney was determined by the method of [21]. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H₂O₂. The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H₂O₂ for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate- acetic acid mixture and remaining H₂O₂ as chromatic acetate was determined calorimetrically. Estimation of glutathione peroxidase GPX (EC.1.11.1.19) activity of GP_X in the liver, gill and kidney were measured by the method of [22]. A known amount of enzyme preparation was allowed to react with H₂O₂ in the presence of GSH for a specified time period. Then the remaining GSH content was measured.

Statistical analysis

All the data statistical analyses were performed with the SPSS version 11.5. All quantitative data were expressed as the mean ± S.E of the mean (SEM) statistical analysis of the T test were

calculated. A probability of $p < 0.05$ was considered as statically significant. Percent changes were calculated.

3. RESULTS

Response of an antioxidant enzymes activity in various organs like gill, liver and kidney of the fish at different exposure period of the cypermethrin was observed. Based on the organs and period exposure the enzyme activity changes were represented in the (Fig1-12).

Lipid peroxidation

The level of TBARS were increased in the treated group when compared to control, group III TBARS level gradually changed in 24 hours to 120 hours statically significant at $p \leq 0.05$ level. 120 hours Treated group TBARS level increased in gill (53%), Liver (33.34%) and kidney (20.51%). Increasing of TBARS elevating the ROS level in the tissues leads to cellular damage. Group III gradually decrease the TBARS level over the treated group gill (30%), liver (20.12%), and kidney (14.55%). Supplementary group slightly increased TBARS statistically insignificant. (Fig 1-3).

Superoxide dismutase

SOD activity in treated fish decreased gradually during exposure period when compared to control group. SOD decrease at 120 hours gill (29.99%), liver (37%) and kidney (32%) statically significant at $p < 0.05$ level. The group III rapidly increasing SOD level over the treated group at 120 hours gill (22%) liver (44%) and kidney (12%), supplementary feed group nearer to control group there is no significant changes in the SOD activity. SOD decreased in treated liver, kidney, and gill respectively. SOD enzyme converts the superoxide radicals into H_2O_2 (Fig 4 - 6).

Catalase

Catalase level in treated fish decreased gradually during exposure period. CAT decreased in the 120 hours gill (19%), liver (32%) and kidney (23%). The group III catalase increased gradually in liver, kidney and gill. CAT level increased over the treated group gill (19%) liver (32%) and kidney (22%) statistically significant. Supplementary feed group also slightly increased the CAT activity statistically insignificant. Antioxidant enzyme CAT removed the SOD generating H_2O_2 by converting H_2O_2 into O_2 and water molecule (Fig 7-9).

GP_X

GP_X level also increased in the treated group II and gradually decreased in the group III. In the treated group II 120 hours GP_X increased in gill

(40%), liver (57%) and kidney (35%). In group III decrease GP_X level over the treated group II like gill (25%) liver (26%) and kidney (23%). GP_X react to the free radicals and H_2O_2 in the cells. Supplementary feed group GP_X level nearer to control group (Fig 10-12).

Fig 1: shows TBARS activity in gill of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*.

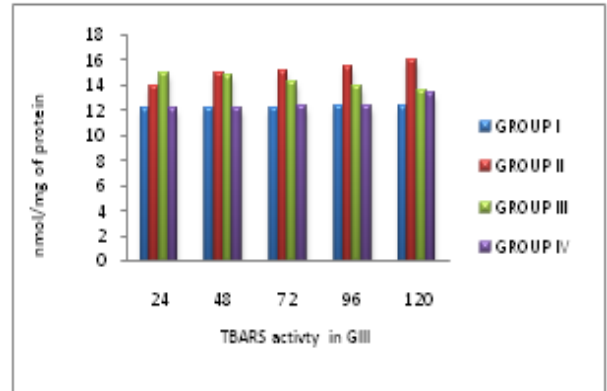


Fig 2: shows TBARS activity in liver of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

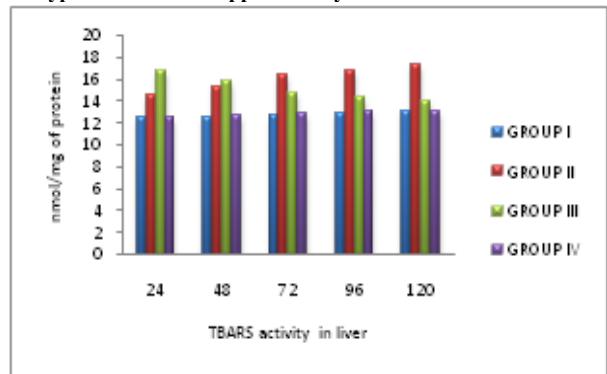


Fig 3: shows TBARS activity in kidney of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

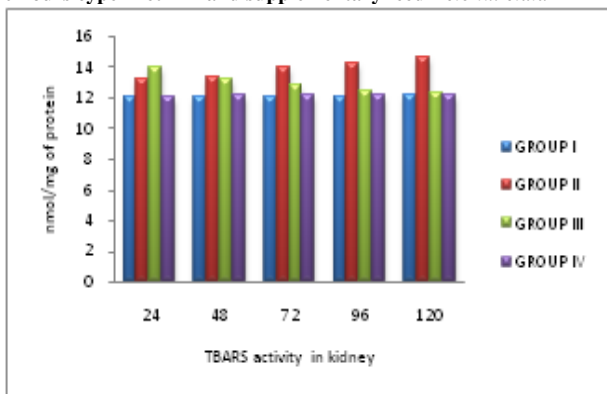


Fig 4: shows SOD activity in gill of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

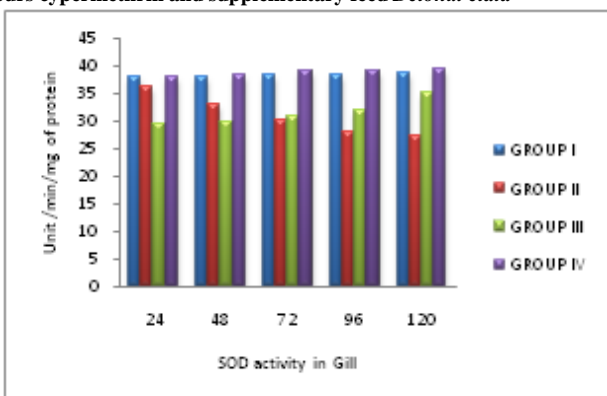


Fig 5: shows SOD activity in liver of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

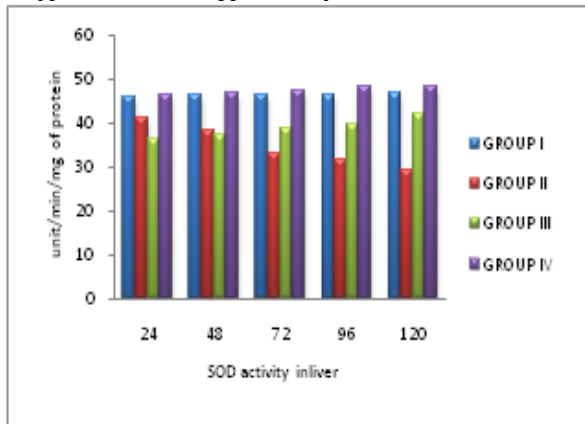


Fig 6: shows SOD activity in kidney of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

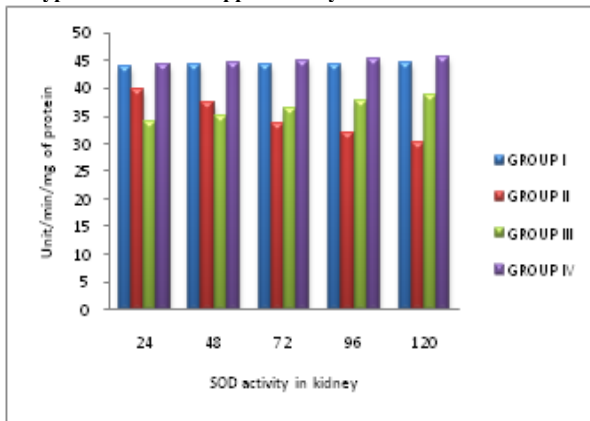


Fig 7: shows CAT activity in gill of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

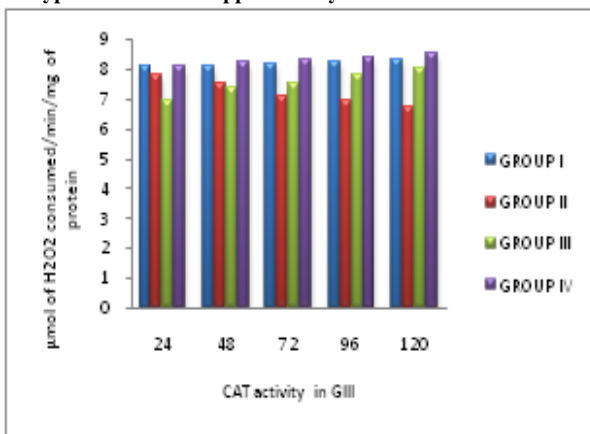


Fig 8: shows CAT activity in liver of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

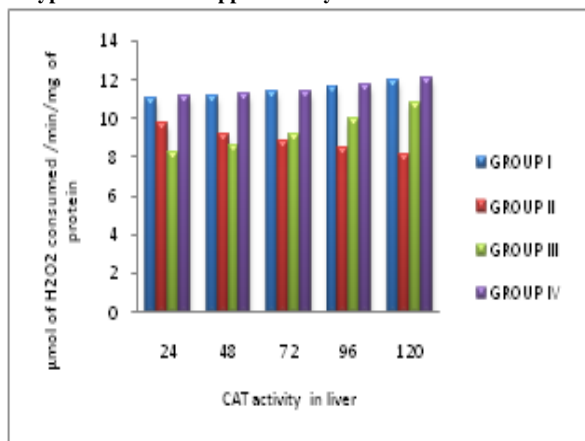


Fig 9: shows CAT activity in kidney of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

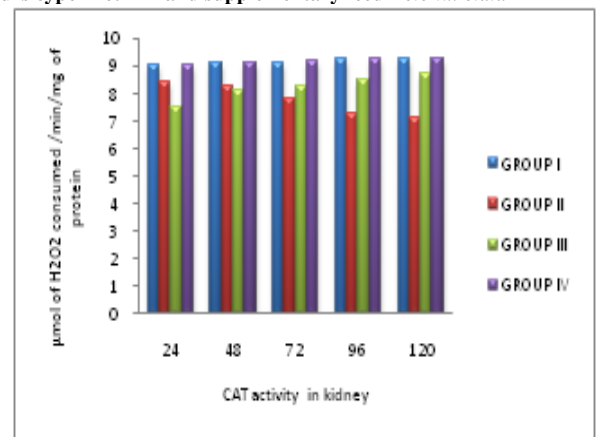


Fig 10: shows GP_x activity in gill of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

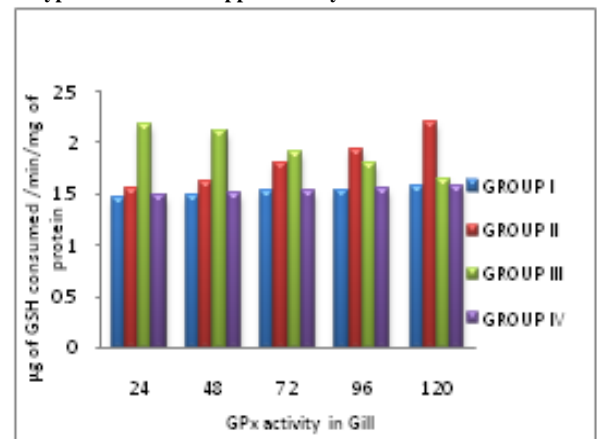


Fig 11: shows GP_x activity in liver of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*

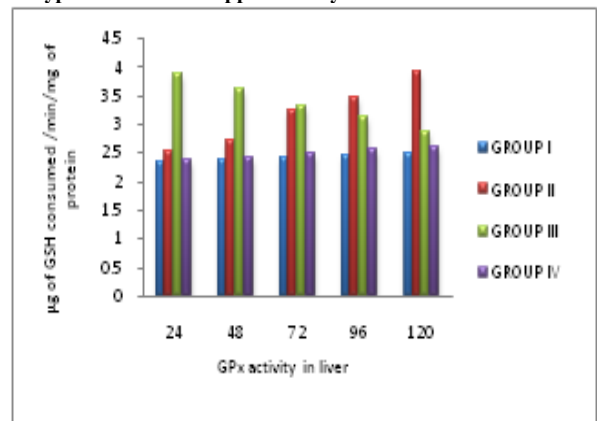
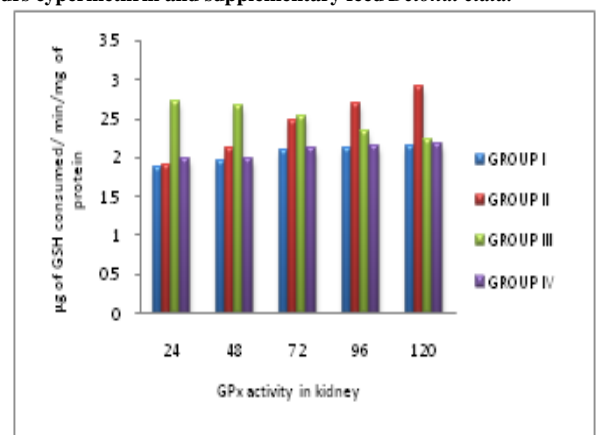


Fig 12: shows GP_x activity in kidney of *Cyprinus carpio* exposed to 120 hours cypermethrin and supplementary feed *Delonix elata*



4. DISSCUSSION

Lipid peroxide formation may be enhanced by the unbalance between pro- and antioxidant molecules (e.g. antioxidant enzymes) [23]. The extent of lipid per oxidation is determined by the balance between the production of removal and scavenging of those oxidants by antioxidants [24]. In the present study, LPO level of the fish organs increased mainly in the gill, liver followed by kidney. Gill was directly exposed to the toxin, so that gill TBARS increased than liver and kidney. Cypermethrin induced oxidative stress in all the tissues. Lipid per oxidation has been extensively used as a marker of oxidative stress [25]. Basically, the main mechanism of the toxic effect of pesticides involves the generation of a high level of free radicals, and thereby the damage of tissues and organs throughout this process [26,27]. These radicals attack the cell membrane and lead to destabilization and disintegration of cell membranes a result of lipid per oxidation [28]. TBARS is a major oxidation product of per oxidized polyunsaturated fatty acids, and increased TBARS content is an important indicator of lipid per oxidation [29].

Superoxide dismutase activity was observed at 24, 48, 72, 96 and 120 hours. SOD activity decreased gradually in the treated group compared to control group. Recovery group SOD activity increased in liver, kidney and gill. Supplementary feeder group observed slightly increased statistically insignificant. The enzyme SOD is known to provide cytoprotection against free radical induced damage by converting superoxide radicals (O_2^-) generated in peroxisomes and mitochondria to hydrogen peroxides. Treated group decrease the SOD level due to toxic effect of cypermethrin. Defense mechanism gradually failed for protection. The recovery group increasing the SOD activity due to plant pellet supplementary feed. Animal develop the defense mechanism. Catalase protects the system to converts H_2O_2 into O_2 and water molecules.

Activity of CAT was decreased significantly in all the organs during the cypermethrin treated period. In the similar observation was made [30,31]. Another similar observation of a decrease in CAT activity followed by is an inhibition of the activity of enzyme SOD has been reported by Fatima and Ahmad [32]. Recovery group gradually increased CAT activity at the end of the treatment due to plant pellet feed .supplementary group CAT increased slightly but statistically insignificant. The activity of GPx can be induced by

xenobiotics, and detoxification of peroxides can be achieved by this induction [33]. The biological function of GSH-Px is to reduce H_2O_2 and lipid hydro peroxides [34]. GPx enzymes play a critical role in the defense against oxidative stress. GPx level gradually increased in the treated group during exposure period .Recover group GPx level gradually decreased statistically significant. Supplementary feed group slightly increased statistically insignificant. We observed the decreased activities of antioxidant enzyme SOD, CAT and increasing of GPx in the all tissues of cypermethrin treated fish, which indicated the failure of antioxidant defense system to overcome the influx of ROS induced by cypermethrin. Uner [35] reported that CYP caused an increase in GSH-Px activity. While it caused a decrease in CAT activity in the liver and kidney of some fresh water fish species. Because GSH-Px is found mainly in cytosol and mitochondria, it is widely affected by xenobiotics. *Delonix elata* plant leaves contains rich content of edible protein, soluble fibers, minerals like calcium, magnesium, phosphorus, iron, potassium, and trace elements. Vitamins like thiamine, β carotene and ascorbic acid content was higher in *Delonix elata* have been reported. *Delonix elata* contains crude protein and phenolic compounds had been reported [36]. In animal studies ascorbic acid and phenolic compounds play an antioxidant activity. Several studies had reported ascorbic acid prevent cytotoxicity of the cell improving antioxidant defense it is very well possible to assume that vitamin 'C' can provide antioxidant-cellular protection against the oxidative toxicity of cypermethrin [37]. Administration of l-ascorbic acid (vitamin C) had the ability to reduce the mutagenic effect of Cypermethrin, as indicated by the significant reduction in micronucleated polychromatic erythrocytes and structural chromosomal aberration [38]. The presence of all compounds in the plant could be reason for recover from the toxic impact of cypermethrin.

5. CONCLUSION

In the present investigation, cypermethrin treated fish shows gradually lost its antioxidant defense during treated time. At the same time recover group increasing antioxidant defense and decreasing lipid peroxidation. This may be due to *Delonix elata* leaves supplementary feed detoxifying the cypermethrin toxicity and improved the antioxidant defense. Future studies isolating the bioactive compound of the *Delonix elata* and its antioxidant property.

REFERENCE

1. Sanchez -Fortun S., Barahona, M.V. 2005. Comparative study on the environmental risk Induced by several pyrethroids in estuarine and fresh water Invertebrate organisms. *Chemosphere*, 59: 553-559.
2. Malla Reddy, P and Bashamohiden. M. 1989. Fenvalarate and cypermethrin induced changes in the hematological parameters of *Cyprinus carpio*. *Acta. Hydrochim. Hydrobiol.*, 5 (1): 101-107.
3. Sommerville, C. 1995. Latest weapon in the war on lice fish former, 18: 53-55.
4. Hef, F. 1994. Chapter 6. Synthetic pyrethroids toxicology, 91: 43-49.
5. United States Environmental protection agency. 1989. Pesticide fact sheet number 199: Cypermethrin Office of pesticides and toxic substances, Washington, DC. PP: 2-9.
6. Carriquiriborde, P., Diaz, J., Mugni, H., Bonetto, C., Ronco, A.E. 2007. Impact of cypermethrin on stream fish populations under field – use in biotech -soybean production *Chemosphere*, 68: 613-621.
7. Hill, J.R. 1989. Aquatic organisms and pyrethroids. *Pestic sci.*, 27: 429-465.
8. Li, X., Ping, X., Shong, X., Wu, Z and Xie. 2005. Toxicity of cypermethrin on growth, pigments and superoxide dismutase of *Scenedesmus obliquus*. *Ecotoxicol. Environ saf.*, 60:188-192.
9. Valavanidis, A., Vlahogianni, T., Dassenatis, M and Scoullou, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol. Environ. Saf.*, 64: 178-189.
10. WHO, 2002. World health report 2002 reducing risk promoting health life Geneva.
11. Ahamad, I., Hamid, T., Fatima, M., Chand, H. S., Jain, S. K., Athar, M., Raisuddin, S., 2000. Induction of hepatic antioxidants in fresh water catfish *Channa punctatus bloch* is a biomarker of paper mill effluent exposure. *Biochem. Biophys. Acta.*, 1523: 37-48.
12. Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol Environ. Saf.*, 56: 295–301.
13. Dinana Dinu., Diana Marinescu., Maria Cristina Munteanu., Andreea Cristina Staicu., Marieta Cortache and Anca Dinischiotu. 2010. Modulatory effect of deltamethrin on antioxidant defense mechanisms and lipid per oxidation in *carassius auratus gibelio* liver and intestine. *Arch Environ contam. Toxicol.*, 58: 757-764.
14. Kenan Koprucu., Serpil Mise Yonar and Engin Seker. 2010. Effects of cypermethrin on antioxidant status, oxidative stress biomarkers, behavior, and mortality in the fresh water mussel *Unio elongatulus eucirrus*. *Fish Sci.*, 76: 1007-1013.
15. Jin, Y.X., Zheng, S.S., Pu, Y., Shu, L.J., Sun, L.W., Liu, W.P and Fu, Z.W. 2011. Cypermethrin has the potential is induce hepatic oxidative stress, DNA damage and apoptosis in adult zebra fish (*Danio rerio*). *Chemosphere*, 82: 398-404.
16. Cossu, C., Doyotte, A., Jacquin, M.C., Babut, M., Exinger, A and Vasseur, P. 2000. Antioxidants biomarkers in fresh water bivalves *uniotumidus*, in response to different contamination profiles of aquatic sediments. *Ecotoxicol. Environ. saf.*, 45: 106-121.
17. Jeferson, L.F., Trivella, D.B.B., Trevisan, R., Dinslaken, D.F., Marques, M.R.F., Bainya, CD and Dafre, A.L. 2006. Antioxidant status and stress protein in the gill of the brown mussel *Perna perna* exposed to zinc. *Chem Biol Interact.*, 160: 232-240.
18. Finney, D.J. 1971. Profit Analysis Cambridge university press, Cambridge, London. 1964 p.20.
19. Niehaus, W.G and Samuelson, B. 1968. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem.*, 6:126-130.
20. Kakkar, P., Das, B and Visvanathan, A. 1984. Amodified spectrophotometric assay of superoxide dismutase. *Ind, J. biochem. biophys.*, 21: 130-132.
21. Sinha, K.A. 1972. Colorimetric assay of catalase. *Anal. Biochem.*, 47: 3889-3894.
22. Rotruck, J.T., Pope, A.L., Gauthier, H.E., Swanson, A.B., 1984. Hafeman, D.G.,

- Hoekstna, W.G., 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 179: 588-590.
23. Almeida, E. A., Bairy, A.C.D., Loureiro, A.P.M., Martine, G. R, Miyamoto, S., Onuki, J, et al., 2007. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation and DNA damage. *Comp Biochem Physiol A Mol Integr Physiol.*, 46: 588 –600.
24. Filho, D.W. 1996. Fish antioxidant defences a comparative approach. *Braz J Med Biol Res.*, 29: 1735–1742.
25. Huggett, R.J., Kimerle, R.A., Mehrle, P.M and Bergman, H.L. 1992. Biochemical, physiological and histological markers of anthropogenic stress. Lewis publishers Boca Raton, F.L.
26. Yousef, M.I., El-Demerdash, F.M., Kamel, K.I and Al-Salhen, K.S. 2003. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. *Toxicology*, 189: 223–23.
27. Manna, S., Bhattacharyya, D., Mandal, T.K and Das, S. 2004. Repeated dose toxicity of alpha-cypermethrin in rats. *J. Vet. Sci.*, 5: 241–245.
28. Stajn, A., Zikic, R.V., Ognjanovic, B., Pavlovic, S.Z., Kostic, M.M and Petrovic, V.M. 1997. Effect of cadmium and selenium on the antioxidant defense system in rat kidneys. *Comp. Biochem. Physiol.*, 2, 167–172.
29. Celik, I and Suzek H. 2009. Effects of sub acute exposure of dichloros at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Ecotoxicol. Environ. Saf.*, 72, 905–908.
30. Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol Environ. Saf.*, 56: 295–301.
31. Tripathi, G and Verma, P. 2004. Fenvalerate-induced changes in a cat fish, *Clarias batrachus*: metabolic enzymes, RNA and protein. *Comp Biochem Physiol C Toxicol Pharmacol.*, 138: 75–79.
32. Fatima, R. A and Ahmad, M. 2005. Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in waste water. *Science of the Total Environment*, 346: 256–273.
33. Hamed, R.R., Elawa, Sh. E., Farid, N.M., Ataya and F. Sh 1999. Evaluation of detoxification enzyme levels in Egyptian catfish, *Clarias lazera*, exposed to dimethoate. *Bull. Environ. Contam. Toxicol.*, 63: 789.
34. Verma, R.S., Mehta, A and Srivastava, N. 2007. *In vivo* chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. *Pestic Biochem Phys.*, 88: 191–196.
35. Uner, N., Zcan Oru, E., Canl, M and Sevgiler, Y. 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.). *Bull. Environ. Contam. Toxicol.*, 67: 657-664.
36. Shayoa, C. M and Ude An, P. 1999. Nutritional uniformity of crude fraction in some tropical browse plant estimated by two *in vitro* methods. *Animal Feed science and Technology*, 78: 141-151.
37. Grajeda-Cota, P., Ramirez-Mares, M.V and de Mejia, E.G. 2005. Vitamin C protects against *in vitro* cytotoxicity of cypermethrin in rat hepatocytes. *Toxicol. In vitro*, 18: 13 –19.
38. Geetanjali, P. Rita and P.P. Reddy. 1993. Effect of ascorbic acid in the detoxification of the insecticide dimethoate in the bone marrow erythrocytes of mice. *Food Chem. Toxicol.*, 31(6): 435–437.