Ann. For. Res. 65(1): 663-677, 2022
DOI: 10.5281/zenodo.7231877

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

NEPHROPROTECTIVE EFFECT OF *TRIBULUS TERRESTRIS* ON ATRAZINE EXPOSED FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS* (W. K. H. Peters, 1852)

V.Nimavathi[1], J.Jayanthi[2] and M.G.Ragunathan[3]

1. Ph.D. Research Scholar, Department of Advanced Zoology and Biotechnology, Guru Nanak College, Affiliated to University of Madras, Chennai
2. HOD and Associate Professor, Department of Advanced Zoology and Biotechnology, Guru Nanak College, Affiliated to University of Madras, Chennai
3. Principal and Associate Professor, Department of Advanced Zoology and Biotechnology, Guru Nanak College, Affiliated to University of Madras, Chennai

1. INTRODUCTION

Atrazine is used as a selective pre and post-emergence herbicide to control the weeds in asparagus, maize, sorghum, sugarcane, pineapple and paddy fields. It is also used in forestry for non-selective weed control on non-crop areas. Its intensive usage in agriculture has led to the accumulation of atrazine in the soil and water if it exceeds the permissible limits (Vasanth *et al.,* 2013). The impact of atrazine is high toxic which has been resulted in the application of herbicides for varies agricultural crops lead to toxicity in aquatic living organisms. Atrazine rapidly metabolizes in the liver and the kidney and at the same time it rarely leaves any symptoms in the fish tissues. Some experimental studies also documented the alterations of the gill tissues because of their direct contact with water, which allows entering the substance through them into the fish body (Jana Blahova *et al.,* 2014; Solomon *et al.,* 2008; Graymore *et al.,* 2001). Therefore it is most often associated with the degenerative changes in the kidney, gills and also with the alteration in the liver tissues of fishes exposed to atrazine (Jana Blahova *et al.,* 2014; Yang *et al.,* 2010; Fischer-Scherl *et al.,* 1991)

Different studies have been reported the atrazine effects on fish, as atrazine affecting the hematological activity, locomotors activity, immune systems, metabolism, oxidative stress, osmoregulatory disturbance and reproduction (Nascimentoc *et al.,* 2012; Tillittd *et al.,* 2012). Bioaccumulation of atrazine in different organs of fish have been noted and thus concluded that the environmental pollutants would affect the fish by entering into and pollutants can enter fish through the gills and skin (Ahsan Khan *et al.,* 2016; Ortiz *et al.,* 2002). Exposing fish to different concentrations of atrazine can in accumulation bring out changes in fish behavior, like unequal movement, better opercular movement, floating on the side, vertical movement, fast swimming, often coming towards the water surface, etc., and such abnormal behavior indicates that herbicides affect the CNS of the fish (Ahsan Khan *et al.,* 2016; Antychowicz *et al.,* 1979). Atrazine is toxic to aquatic animals and most studies have noted that exposure of fish to atrazine results in the biochemical alteration, behavioral abnormalities and structural deformalities and plus stress on

reproduction. The alternation the immune system is assessed by quantifying white blood cells (Ahsan Khan *et al.,* 2016; Fu *et al.,* 2013; Solomon *et al.,* 2008).

Analysis of biomarkers in aquatic organisms particularly in fish is a validated approach for early warning of chemical exposure (Van der Oost *et al.,* 2003; Osman *et al.,* 2010). During the stress situation fish change due to the inhibition or induction of the enzymes and adapt their metabolic functions (Malarvizhi *et al.,* 2012; Abhijith *et al.,* 2016). Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals or xenobiotics. Oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants ratio, leading to the generation of ROS. ROS such as hydrogen peroxide (H_2O_2), superoxide anion O_2-and hydroxyl radical ($OH\cdot$) at supernormal levels can react with biological macromolecules potentially leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and even cell death but at low concentrations their effects are less pronounced.

2. MATERALS AND METHODS

2.1. Chemicals

Atrazine and other necessary reagents of analytical grade were bought form Hi-Media laboratories Ltd, Mumbai, India.

2.2. Fish Maintenance and Acclimatization

The fresh water fish *Oreochromis mossambicus* were collected from Cheyyar surroundings Thiruvannamalai District. The collected fish were acclamatized to laboratory condition for 15 days with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24°C for 15 days. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% KMnO4 solution for 15 min, they were placed in nine plastic pools (500L) containing non-chlorinated water. Animals were fed with ad labium Fishes measuring 11-14cm in length and 40-60g weight were selected irrespective of their sex for the experiments. Solutions were renewed once in every 24 hours. After exposure period, animals were sacrificed and isolated to the kidney tissues, which went homogenized and stored at −80 °C for further biochemical analyses.

2.3. Supplement feed

Healthy disease free plant of *T. terrestris* was collected from in and around Cheyyar and identified the plant with botany department. The plants, washed in running tap water for 10 minutes, were dried, 1kg of *T. terrestris* were macerated thrice at room temperature and prepared powdered form and equal amount of rice bran was mixed well and small amount water added and prepared small pellet form as feed.

2.4. Experimental Design

Fishes were divided into four equal groups each comprising of 25 fishes.

Group I	Fish exposed to freshwater for 120 hours (Control)
Group II	Fish exposed to sublethal concentration of atrazine (16.5 mg/L) for 120 hours
Group III	Fish exposed to atrazine (16.5 mg/L) and *Tribulus terrestris*(1.2g/L) for 120 hours

Group IV *Tribulus terrestris* alone(1.2 g/L) for 120 hours

Protein content in the kidney tissue was estimated by the method of Lowry *et al.,* (1951). Total free amino acids and content of the kidney tissue were estimated by the method of Stein and Moore, (1954). Lipid content was estimated by the semi-micro determination method of Pande *et al.,* (1963). The estimation of tissue glucose and glycogen was done by following the methods of Kemp and Kits, (1954). The concentration of TBARS in the kidney tissue was estimated by adopting the method of Nichans and Samuelsen (1968). The level of the reduced glutathione in kidney tissue was estimated by the method of Ellaman (1959). Superoxide dismutase in the kidney tissue was assayed by adopting the method of Kakkar (1984). The activity of catalase in the kidney tissue was determined by the method of Sinha (1972).The activity of GP_X in the kidney tissue was measured by the method of Rotruck (1973).The activity of alkaline phosphatase was assayed by the method of King and Armstrong (1988). The activity of AST and ALT was determined by adopting the method of King (1965).The activity of lactate dehydrogenase was assayed by the method of King (1965).

Statistical analysis

Values are given as mean ± S.D. for 25 fish in each group. The data for various biochemical parameters were analyzed using analysis of 't'-test and group means was compared by Duncan's multiple range test (DMRT) 1957. Values were considered statistically significant when $p<0.05$ and the values sharing a common superscript did not differ significantly.

Results

The results showed the total protein, amino acid, lipid, glucose and glycogen content in kidney tissue of control and experimental fish. It is clear from the results that there is an appreciable decreased the level of protein, lipid and glycogen content and increased level of amino acid and glucose level in the atrazine exposed animals when compared to control. If during the recovery period, Figures 1to5, the atrazine exposed fish were treated with *Tribulus terrestris and* they reached the normal level. An insignificant alteration was observed between the fish in *Tribulus terrestris* group and the control one. In this experiment the results show the enhanced level of LPO content and simultaneously decreased level of GSH content and enzymatic antioxidant (SOD, CAT, GPx) activity was noticed in atrazine exposed fish kidney tissue when compared to control. During the recovery period, the supplement feed of *Tribulus terrestris* on atrazine exposure indicates the restoration of both oxidant and antioxidant (non-enzymatic) activity in near normal level. A insignificant alterations were observed between the fish in *Tribulus terrestris* group and the control one Figures 6 to 10.

The level of ALT, AST, ALP, LDH, Urea, Uric acid and creatinine discloses the significant increase in the atrazine exposed fish kidney tissue and at the same time, during the recovery period, the administration of *Tribulus terrestris* after atrazine exposure proved the restoration and reached near the normal level. A insignificant alterations were observed between the fish in *Tribulus terrestris* group and the control one. Figures 11 to 17.

Discussion

Fish is one of aquatic inhabitant and is sensitive to environmental pollution that may lead to responses to biological pollution in water. Fishes exposed to the environment elicit directly in the form of responses to environmental by physical and chemical process. The effect of atrazine toxicity with particular reference to kidney was assessed in this present study, in terms of tissue antioxidant markers, peroxidation markers, and functional markers in kidney tissues.

The biochemical parameters either increased or decreased in the metabolic rate depending on the site of action. Toxic exposure of organisms interferes with organ integrity at the biochemical level and unlimitedly gives rise to affect at the individual levels (Smolders *et al.,* 2002). In the present investigation freshwater fish, *Oreochromis mossambicus* exposed to sub lethal concentrations of atrazine for the periods of 24, 48, 72, 96 and 120 h showed the decreased level of protein and the increased level of amino acid in kidney tissues. The depletion of protein contents induces diversification of energy to meet the impending energy of the animals when it is in stressful condition. Since proteins are being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism and catabolism. Catabolism of proteins and amino acids make a major contribution to the total energy production in fish when they are facing the energy demand or maintaining the normal life. The depletion of protein content observed in this investigation can be correlated to this fact. The present work agree with Tulasi and Jayantha Rao (2013) reported that total protein content is decreased and it may be due to breakdown of proteins in the fabrication of some amount of energy for organism. The level of amino acid was drastically increased and simultaneously, the level of protein content was decreased. Prabakaran *et al*., (2014) have reported that the increase in concentration of amino acid attributed to stepped up of proteolysis or increased synthesis of free amino acid by transaminase reaction. This result suggested that an enhanced level of proteolysis activity may be occurred in the respective tissues and it is also possible to utilize its degraded products for their metabolic purpose. The amino acids are the building blocks of protein (Tripathi and Rajesh Kumar Yadav, 2015).

Different amino acids are found in protein, every protein has a unique genetically defined amino acids sequence which determines its specific shape and function. The degree of increase in free amino acids has been corroborated with the decreased protein level. Bhaskaran (1980) and Manoharan and Subbiah (1982) noticed that depletion in protein level was due to diversification of energy to meet out the impending energy demand when the animals were under toxic stress. Karuppasamy (1990) reported the decrease in protein content of liver, muscle and kidney in *Channa punctatus* when exposed to sub lethal concentration of sugar mill waste. It may be inferreded that *Oreochromis mossambicus* exposed to sub lethal concentrations of atrazine causes energy crisis and alter the protein metabolism.

Most of the pesticides act as metabolic depressor in the environment and generally causes pressure on biologically active molecules such as glycogen, glucose and lipids (Agrahari and Gopal, 2009; Tripathi and Rajesh Kumar Yadav, 2015). Glycogen is a sub divisional polysaccharide and major storage of glucose that serve as a source of energy in animals. It plays

an important role in the glucose cycle that can be quickly mobilized to meet a sudden need for glucose (Sadava *et al.,* 2011; Tripathi and Rajesh Kumar Yadav, 2015). The glycogen content in kidney tissues of *Oreochromis mossambicus* decreased and glucose level was increased in the in the present experiment. A remarkable depletion in kidney glycogen shows an extensive utilization of energy stores under toxic stress. Depletion of glycogen content in all the tissues might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia. Chandrasekhar and Jayabalan, (1993) have also reported the same result was similar report was observed in common carp *Cyprinus carpio* exposed to sub lethal concentrations of endosulfon and showing a decreased in levels of blood glucose and little variation in the serum protein. Tripathi and Rajesh Kumar Yadav, (2015) also reported that the same result of the depletion of glycogen in the organophosphorus exposed fish *labeo rohita*. In the kidney tissue glycogen content has been reduced progressively in the present study due to the entry of toxic substances into the body and failure of the normal functional mechanism of routine metabolism processes. In the present study the significant increase in glucose level during sub lethal treatment may be due to gluconeogenesis to provide energy for the increased metabolic demands imposed by the pesticide atrazine. Al-Akel *et al.,* (2000) showed that the increasing of glucose level is due to high secretion of hormones like catecholamines, glucocorticoids and that lead to increasing of glycogenolysis and this lead to high glucose level in blood. Abou EL-Naga *et al.*, (2005) observed that glucose recorded high values than control group level; also muscle glycogen content was increased at the same time intervals. This high level was explained through gluconeogenesis, which mean formation of glucose and glycogen from non-carbohydrate source. Koundinya (1979) has reported the increase in glucose level in *Saratherodon mossambicus* exposed to pesticide. Saha and Kaviraj (2009) have reported that the increased glucose is usually observed in fish under undesirable conditions and it helps the animal by providing energy substrates to vital organs to cope with the better energy demand. Lipid may be very transient body materials but they are an important source of potential chemical energy and their presence or absence reflects the physiological capacity of fish (Schreck and Moyle, 1990). In the present work the level of lipid was decreased at 120 hrs of sub lethal concentration of atrazine exposed fish. Virk and Sharma (1999) have studied the biochemical changes induced by nickel and chromium in the liver of *Cyprinus carpio* and observed significant decline in the lipid level of liver and stated that this may be due to toxicity stress which suppresses the activity of a number of enzymes responsible for the lipid transformation and ultimately causing disturbance in the lipid metabolism. Tulasi *et al.,* (1992) have noticed a significant decrease of lipid content in the gill, liver and kidney of Anabas testudineus (Bloch, 1792) exposed to lead. Similar views have been recommended recently by Kumar *et al.,* (2010) after sub lethal exposure of *Clarias batrachus* to fluoride. In order to wriggle relies upon lipid metabolism as an energy source for its muscular activity which is evident by a considerable decrease in lipid content. Jha and jha (1995) has also observed the decreased levels of liver and muscle lipid content in *Channa punctatus* intoxicated with lead nitrate. This has reported the metabolism of lipid to meet the energy requirements in the toxic medium either through Beta-oxidation or a process of gradual instauration of lipid molecules.

Decrease in the lipid content in the present study might be due to utilization of lipid for the energy demand associated with the situation of stress (Rao, 2006).

Toxicity with herbicides causes severe stress involved in ROS production, leading to oxidative stress with lipid peroxidation (Chen *et al.,* 2015; Paulino *et al.,* 2012b). Atrazine can induce oxidative damage in cells and tissues of organisms via generating a huge amount of reactive oxygen species (ROS), such as superoxide anion, peroxyl radicals, hydroperoxyl radical, and hydrogen peroxide (Sandhya Bharti and Fazle Rasool, 2021). In chronic toxicity cases, the high ROS levels develop antioxidative enzyme production (SOD, CAT, and GPx) to cope with the impacts of oxidative stress (Chen *et al.,* 2015). The accumulation of free radicals and lipid peroxides led to inflammation with the loss of cell functions. (Blahová *et al.,* 2013; Xing *et al.,* 2012; Doherty *et al.,* 2019). It has been reported that atrazine may induce oxidative damage in a variety of tissues by enhancing peroxidation of membrane lipids due to inhibition of the antioxidant enzymes (Shukla *et al.,* 2000; Ozlem Tezcan *et al.,* 2012). LPO is considered the primary mechanism for atrazine toxicity, despite its inability to directly generate free radicals under physiological conditions (Eneman *et al.,* 2000). This causes oxidative stress through the Fenton reaction, producing hydroxyl radical species that are believed to initiate LPO. Doherty *et al.,* (2019) also reported to similar result of decreased level of lipid peroxidation in atrazine exposed *Clarias gariepinus* fish. Significantly higher lipid peroxidation and lower activities of CAT, GPx, and SOD in human erythrocytes were observed with the increasing concentrations of atrazine treatment. Antioxidant enzymes, such as CAT, GSH-Px and SOD, are known as the first fline of defense against oxidative stress caused by herbicides (Coelho *et al.,* 2011; Xulu Chang *et al.,* (2021). Antioxidant enzyme system plays an important role in maintaining the balance of oxygen free radical metabolism. CAT is important components of antioxidant enzymes in organisms. Under normal physiological conditions, antioxidant system can effectively remove reactive oxygen species (ROS) and protect cells from injury of lipid peroxidation (Zhu *et al*., 2008; Ni *et al.*, 2019; Guo *et al.,* 2020). The SOD is a group of metalloenzymes that play a crucial role as antioxidants and constitute the primary defense system against the toxic effects of superoxide radicals (O2−) in organisms.SOD detoxifies superoxide radicals and thus provides cytoprotection against free radical induced damage. Reports about SOD activity in atrazine exposed fishes are contradictory; some studies reported an increase (Ogjanovic *et al.,* 2003; Zikic *et al.,* 1998) and some others reported a decrease (Stajn *et al.,* 1997; Yalin *et al.,* 2006) in activity. The results showed markedly the impaired oxidative and antioxidative capacity in *Oreochromis mossambicus* exposed to atrazine; however, dietary *Tribulus terrestris* caused up regulated antioxidative capacity. In the same sense fish fed dietary *Tribulus terrestris* showed improved antioxidative capacity (Song *et al.,* 2018). During the present investigation, significant decrease in GSH level was observed in kidney of *Oreochromis mossambicus* at atrazine exposures which could be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from pesticide exposure. Reduced GSH and its metabolizing enzyme provide the chief defense against ROS induced cellular damage. Apparently GSH is important in protecting against

deleterious effects of the cell exposed to ROS by reacting with them to form through glutathione disulphide (GSSG). This antioxidant provides defensive effect defense effect occurs spontaneously. It acts as cofactor for glutathione transferase, which facilitates the removal of certain chemicals and other reactive molecules from the cells (Gate *et al.,* 1999; Mandeep Kaur, Rajinder Jindal, 2017). Thus a change in GSH levels may be an important indicator of detoxification ability of an organism (Cheung *et al.,* 2001; Mandeep Kaur, Rajinder Jindal, 2017). The level of GSH in kidney showed declining trend throughout the exposure periods. Similarly, there were significant decreases in the SOD, CAT activities, and GSH level due to nephrotic injury caused by atrazine. However, treatment with *Tribulus terrestris* significantly reached near normal level of SOD and CAT activities in fish. Also, a significant increase in GSH content was observed after post-treatment with *Tribulus terrestris*.

In our study, significant changes were observed in the levels of LDH, ALP, AST and ALT. A significant decrease is experimental in all electrolytes during the four experimental groups. A significant increase was observed in ALT and AST. ALT is a transaminase enzyme also known as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALAT). It is similar to aspartate transaminase (AST) because they are found in the liver and in various bodily tissues. The enhancement of oxidative stress causes cell damage or tissue injuries. Furthermore, these tissue injuries caused functional impairment as evidenced with hepatic function tests, like elevation of AST, ALT and ALP activities demonstrated the severity of atrazine induced tissue damage. However, ALT has a higher concentration in the liver compared to other tissue in normal health conditions. Even though these biomarkers are not specific for liver damage, the increase in their activities reflects active hepatic dysfunction. The hepatic injury caused by atrazine may be attributed to an enhanced oxidative stress. The induced hepatic damage mainly related to the lipid peroxidation which causes rapid breakdown of the structure and function of the membranes such as those of the endoplasmic reticulum, mitochondria, and lysosomes, leading to plasma membrane damage (Kumar *et al.,* 1997; Hosam Toughan *et al.,* 2018).LDH is a tetrameric glycolytic enzyme and recognized as a potential marker of tissue damage (Diamantino *et al.,* 2001). Moreover, pesticide induced decrease in glycolytic process may also result in its decrease due to low metabolic rate. Prevalence of anoxia during stress conditions may lead to an increase in LDH activity in tissues (Das *et al.,* 2004).Stoyanova *et al.,* (2015), Stela Stoyanova *et al.,* (2020)). LDH activity is usually related to cellular metabolic activity, and serves as the major enzyme between glycolysis and the citric acid cycle (Popoola Omoniyi Michael, 2018). LDH is an important enzyme, due to its function in the anaerobic pathway of energy production.In general, LDH activity is commonly used as diagnostic tool and demonstrates an increase in the anaerobic metabolism due to the depletion of energy and stress caused by environmental changes. LDH is an important glycolytic enzyme in anaerobic pathway of carbohydrate metabolism. In this study, the trend in LDH activity is in contrast to level noticed in the activity of aminotransferase enzymes. During prolonged exposure, the impact of atrazine became overwhelming as evidenced in decreased activities of LDH in tissues that also corroborated the reduction of protein in these tissues.

Conclusion

The experimental investigation clearly demonstrates the occurrence of oxidative stress induced nephtotoxic effects of atrazine in *Oreochromis mossambicus* fish. The acute exposure of atrazine proved to be toxic to fresh water fishes and induced cumulative deleterious effect at various functional sites like metabolic rate, biochemical activity, oxidative and antioxidant activity. And it is an evident of protective role of *Tribulus terrestris* in neutralizing atrazine exposed toxicity by improving the ability of the body to protect against oxidative damage through the modulation of antioxidant status. It is concluded that the parameter studied in present investigations can be used effectively as potential biomarkers of pesticides toxicity to the fish as well as other aquatic organisms in the field of environmental biomonitoring.

Acknowledgment

The authors are thankful to Professor and Head, P.G and Research Department of Advanced Zoology and Biotechnology, Guru Nanak College for providing necessary laboratory facilities to carry out this experimental work successfully.

References

Abhijith. B.D., Ramesh, M., Poopal, R.K., 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of Catla catla during methyl parathion exposure. The Journal of Basic & Applied Zoology.77, 31–40.

Abou EL-Naga EH, EL-Moselhy, KM, Hamed, MA., 2005. Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish Mugil seheli. Egyptian J. Aquat. Res.31(2),60-71.

Agrahari S., Gopal,K., 2009. Fluctuations of Certain Biochemical Constituents and Markers Enzymes as Consequence of Monocrotophos Toxicity in the Edible Freshwater Fish, Channa Punctatus, Pesticide Biochem. Physiol. 94,5-9.

Ahsan Khan., Nazish Shah., Muhammad., Mian Sayed Khan., Munawar Saleem Ahmad., Muhammad Farooq., Muhammad Adnan., Sahibzada Muhammad Jawad., Hayat Ullah., Ali Muhammad Yousafzai., 2016. Quantitative Determination of Lethal Concentration Lc50 of Atrazine on Biochemical Parameters; Total Protein and Serum Albumin of Freshwater Fish Grass Carp (Ctenopharyngodon idella). Pol. J. Environ. Stud. 25(4), 1555-1561.

Al-Akel, A., Shamsi, M.J.K., 2000. A comparative study of the toxicity of lead and its impact on the carbohydrate metabolism and some haematological parameters of cichlid fish Chromisniloticus and catfish clarius garlepinus.Saudi Arabia Toxicol. Environ. Chem.l74,19-28.

Ana Luiza F., Destro Stella, B., Silva, Kemilli P., Gregorio, Jerusa M., de Oliveira, Amanda A., Lozi, Jener Alexandre, S., Zuanon, Ana Lúcia Salaro, Sergio Luis P., da Matta, Reggiani V., Gonçalves, Mariella B., Freitas, 2021.Effects of subchronic exposure to environmentally relevant concentrations of the herbicide atrazine in the Neotropical fish Astyanax altiparanae. Ecotoxicology and Environmental Safety. 208(2021),111601.

Antychowicz J., Szymbor E., Roszkowski J., 1979. Investigations upon the effects of some pesticides on carp (*Cyprinus carpio*). Bulletin of veterinary Institute in Pulawy 23, 124.

Baker, F.J., Silverton, R.E., Pallister, CJ., 2001. Introduction to medical Laboratory Technology. 7th edn. Bounty Press Limited, Ibadan, Nigeria, 48.

Banaee, M., Sureda, A., Zohiery, F., Hagi, B.N., Garanzini, D.S., 2014. Alterations in biochemical parameters of the freshwater fish, Alburnus mossulensis, exposed to sub-lethal concentrations of Fenpropathrin. Int J Aquat Biol. 2(2), 58-68.

Benthesda, Maryland, USA.

Bhaskaran, R., 1980. Biological studies on a chosen thermo conformer Channa striatus. Ph.D. Thesis, Madurai Kamaraj University.

Blahova J ,Chromcova L, , Plhalova L, Zivna D, Stepanova S, Praskova E, Zelníckova L, Skoric M, Svobodova Z., 2013. The effects of atrazine exposure on early life stages of common carp (Cyprinus carpio). Neuroendocrinol Lett . 34(Suppl 2):95-101. PMID: 24362100.

Calbreath, D.F., 1992. Clinical Chemistry: a Fundamental Textbook, second ed., W.B.Saunders Co, Philadelphia, US, p. 490. ISBN-13: 978e0721626215, ISBN-10:0721626211.

Chandrasekhar, S., Jayabalan, N., 1993. Hematological Responses of the Common Carp, Cyprinus Carpio L. Exposed to the Pesticide Endosulfan, Asian Fisheries Science. 6(3), 331-340.

Chen, F.F., Huo, F.Q., Xiong, H., Wan, Q., Zheng, Y.N., Du, W.J., Mei, Z.N., 2015. Analgesic effect of total flavonoids from Sanguis draxonis on spared nerve injury rat model of neuropathic pain. Phytomedicine. 22(12),1125–1132.

Cheung, CCC., Zheng, GJ., Li AMY., 2001. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, Perna viridis. Aquat Toxicol. 52(3-4), 189–203.

Coelho, S., Oliveira, R., Pereira, S., Musso, C., Domingues, I., Bhujel, R.C., Soares, A.M.V. M., Nogueira, A.J.A., 2011. Assessing lethal and sub-lethal effects of trichlorfon on different trophic levels. Aquat. Toxicol. 103, 191–198.

Das, P.C., Ayyappan, S., Jena, J.K., Das, B.K., 2004. Acute toxicity of ammonia and its sublethal effects on selected haematological and enzymatic parameters of mrigala, Cirrhinus mrigala (Hamilton). Aquacult. Res. 35, 134–143.

Diamantino,T.C., Almeida, E., Soares, A.M.V.M., Guilhermino, L.,2001. Lactate dehydrogenase activity-an effective parameter in aquatic toxicity tests. Chemosphere. 45, 553–560.

Doherty, V.F., Aneyo Idowu., Abdullahi Adeola., Oluwatobi Owolabi., 2019. Comparative Toxicological Effects of the Herbicide, Atrazine, on Fingerlings and Juveniles of African Catfish, Clarias gariepinus (Burchell, 1822). Asian Fisheries Science. 32(2019), 48–55.

Ellaman, G.L., 1959. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 82,70-2.

Eneman, J.D., Potts, R.J., Osier, M., Shukla, G.S., Lee, C.H., Chiu, J.F., Hart, B.A., 2000. Suppressed oxidant induced apoptosis in cadmium adapted alveolar epithelial cells and its potential involvement in cadmium carcinogenesis. Toxicology. 7, 215.

Fazle Rasool., Sandhya Bharti., 2021. Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on Channa punctatus (Bloch) fish. Toxicology Reports. 8, 443–455.

Fischer-Scherl,T.A.,Veeser,R.W., Hoffmann, Kuhnhauser, C., Negele, R., Ewringmann,T., 1991. Morphological effects of acute and chronic atrazine exposure in rainbow trout (Oncorhynchus mykiss). Archives of Environmental Contamination and Toxicology, 20(4), 454–461.

fresh water catfish (Clarias batrachus, Linn.)Research report Fluoride 40(1),37–41.

Fu.Y., Lim., Liuc., 2013.Effect of atrazine and chlorphyrofos exposure on cytochrome p450 contents and enzyme activities in common carp gills. Ecotoxicology and Environmental Safety. 94, 28.

Gagnon, M.M., Holdway, D.A.,1999. Metabolic enzyme activities in fish gills as biomarkers of exposure to petroleum hydrocarbons. Ecotoxicol. Environ. Saf.44, 92-99.

Gate L., Paul J., Ba, GN., 1999. Oxidative stress induced in pathologies: the role of antioxidants. Biomed Pharmacother. 53(4),169-180.

Graymore, M., Stagnitti, F., Allinson, G., 2001. Impacts of atrazine in aquatic ecosystems, Environment International, 26(7-8), 483-495.

Guo, Y. Wang Y. and B.Huang, 2020.The acute toxicity effects of hexavalent chromium in antioxidant system and gonad development to male clam Geloina coaxans. The European Zoological Journal, 87(1), 325–335.

Hosam Toughan, Samah R. Khalil, Ashraf Ahmed El-Ghoneimy, Ashraf Awad, A.SH. Seddek., 2018. Effect of dietary supplementation withSpirulina platensison Atrazine induced oxidative stress- mediated hepatic damage and inflammation in the common carp (Cyprinus carpio L.). Ecotoxicology and Environmental Safety.149,135-142.

Houjuan Xing., Shu Li., Zhilei Wangb., Xuejiao Gao., Shiwen Xu., and Xiaolong Wang., 2012. Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Pesticide Biochemistry and Physiology.103(2012),74–80.

Jana Blahova, Lucie Plhalova, Martin Hostovsky., Lenka Divisova., Radka Dobsikova., Ivana Mikulikova., Stanislava Stepanova., Zdenka Svobodova., 2013.Oxidative stress responses in zebra fish Danio rerio after subchronic exposure to atrazine. Food and Chemical Toxicology. 61, 82–85.

Jana Blahova., Helena Modra., Marie Sevcikova., Petr Marsalek., Lenka Zelnickova., Misa Skoric., and Zdenka Svobodova., 2014.Evaluation of Biochemical, Haematological, and Histopathological Responses and Recovery Ability of Common Carp (Cyprinus carpioL.) after Acute Exposure to Atrazine Herbicide. BioMed Research International, 1-8, http://dx.doi.org/10.1155/2014/980948.

Jha, BS., Jha, MM., 1995. Biochemical effects of nickel chloride on the liver and gonads of the fresh water climbing perch, Anabas testudineus (Bloch). Proceedings of the National Academy of Sciences, India. Section B (Biological sciences). 65(1),39-46.

Kakkar, P,, Das, B., Viswanathan, P.N., 1984. A Modification Spectrophotometric assay of SOD. Ind J Biochem Biophys. 211,731-132.

Karuppasamy, R., 1990. Toxicity impact of sugar mill effluent on freshwater fish Channa punctatus (Bloch). M.Phil Thesis, Annamalai University.

Kemp, A., Kits, J.M.V.H., 1954. A colorimetric micromethod for the determination of glycogen in tissues. Bichem. J.56, 646-648.

King, J., 1965. Practical Clinical Enzymology.Van D. (Ed). Nostrand Co. London, pp. 83-93.

King, J., 1965.The dehydrogenase of oxido-reductase-lactate dehydrogenase, In: Practical Clinical Enzymology. Van D, (Eds). Nostrand Co. Ltd, London: pp. 93-193.

King, KJ., Armstrong, AL., 1988. Calcium, phosphorus and phosphatase. In: Varley, H., Ed. Practical Clinical Biochemistry. 4th ed. New Delhi: CBS Publishers.pp.457-461.

Koundinya, R.P., Ramamurthi, R., 1979. Effect of Organophosphate pesticide sumithion(Fenitrothion) on some aspect of carbohydrate metabolism in Fresh water fish, *Sarotherodon* (Tilapia) *mossambicus* (Peters). Experientia, 35, 1632-1633.

Kumar, A., Nalini Tripathi, Madhu Tripathi, 2007. Fluoride-induced biochemical changes in

Kumar, S., 2010. Mode of action of pyrethroid on energy dependent molecules and inorganic ions in Clarias batrachus. International. Journal of Biological, Ecological and Enviromental Sciences, 1(1), 21–26.

Kumar, V., Cotran, R.S., Robbins, S.L., 1997. The Liver and the Billary Tract in Basic Pathology. Saunders, Philadelphia, pp. 518–535.

Kumar. R., Banerjee, T. K., 2012. Study of sodium arsenite induced biochemical changes on certain biomolecules of the freshwater catfish Clarias batrachus. Neotropical Ichthyology, 10(2),451-459.

Lowry, OH, Rosenbrough, NJ, Farr, AL., Randall, RJ., 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem. 193,265.

Malarvizhi, A., Kavitha, C., Saravanan, M., Ramesh, M., 2012.Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, Cyprinus carpio. J. King Saud Univ. 24,179-186.

Mandeep Kaur, Rajinder Jindal, 2017. Oxidative stress response in liver, kidney and gills of ctenopharyngodon idellus (cuvier & valenciennes) exposed to chlorpyrifos.MOJ Biology and Medicine, 1(4), 103-112.

Manoharan, T., Subbiah, 1982. Toxic and sublethal effect of endosulfan on Barbus stigma. Proc. Ind. Acad., Anim., Sci., 91,523–532.

McPhalen, C.A., Vincent, M.G., Picot, D., Jansonius, J.N., Lesk, A.M., Chothia, C.,1992. Domain closure in mitochondrial aspartate aminotransferase. Journal of Molecular Biology. 227(1), 97-213.

Nascimentoc, R.B., Souzam, M., Martinezc. B.R., 2012. Copper and the herbicide atrazine impair the stress response of the freshwater fish Prochilodus lineatus. Pharmacol. Toxicol. Endocrinol., 155, 456.

Neeraj Kumar, K.K., Krishnani, K.K., Meena, Sanjay Kumar Gupta, N.P., Singh, 2017. Oxidative and cellular metabolic stress of Oreochromis mossambicus as biomarkers indicators of trace element contaminants. Chemosphere. 171(2017), 265-274.

Ni H., Peng L, Gao X., Ji H., Ma J., Li Y., Jiang S., 2019. Effects of maduramicin on adult zebra fish (Danio rerio):Acute toxicity, tissue damage and oxidative stress. Ecotoxicology and Environmental Safety.168, 249-259.

Nichens, WG., Samuelson, B., 1968.Formulation of malondialdehyde from phospholipid arachidouate during microsomal lipid peroxidation. Eur J Biochem. 6, 126-130.

Ogjanovic B. I., Pavlovic S. Z., Maletic S. D., Zikic R. V., Stajn A.S., Radojicic R. M.., Saicic Z. S., Petrovic V. M., 2003. Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. Physiol. Rev. 52, 563.

Olufemi David Owolabi and James Sunday Omotosho, 2017. Atrazine-mediated oxidative stress responses and lipid peroxidation in the tissues of Clarias gariepinus. Iranian Journal of Toxicology, 11(2),29-38.

Ortiz J.B., Gonzalez M.L., Sarasquete C., 2002. Histological alterations in different tissues of fish under the impact of persistent chemical pollution. Ecotoxicology Environmental Restontion. 54, 45.

Osman, A.G.M., Reheem, A.E.B.M.A.E., Abuel Fadl, K.Y., GadElRab, A.G., 2010.Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes. Nat. Sci. 2(11), 1302–1311.

Ozlem Tezcan, Dilek Pandır, Hatice Bas, 2012. The effects of cadmium on enzymatic antioxidant system and lipid peroxidation of human erythrocytes in vitro and the protective role of plasma level of vitamins c and E.Pol. J. Environ. Stud. 21(6), 1849-1854.

Pande, S.V., Parvin Khan, R., Venkitasubramanian, T.A., 1963.-Microdetermination of lipids and serum total fatty acids. Analyt. Biochem. 6, 415.

Paulino. MG., Souza, NES., Fernandes, MN., 2012. Subchronic exposure to atrazine induces biochemical and histopathological changes in the gills of a Neotropical freshwater fish, Prochilodus lineatus. Ecoto. Environ Saf. 80:6-13. doi: 10.1016/j.ecoenv.2012.02.001

Popoola Omoniyi Michael., 2018. Toxicity effect of atrazine on histology, haematology and biochemical indices of Clarias gariepinus. International Journal of Fisheries and Aquatic Studies.6(3),87-92.

Prabakaran. S., Pugazhendy, K., Revathi, A., 2014. Therapeutic efficacy of Pisonia alba and Cardiospermum halicacabum on the biochemical parameters of atrazine intoxicated liver tissue in fresh water fish Labeo rohita. Asian Journal of Biochemical and Pharmaceutical Research Issue. 3(4), 27-36.

Rao, JV., 2005. Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, Oreochromis mossambicus. Comp Biochem Physiol Part C: Toxicol Pharmacol.143(4), 492-498.

Reddy, J.S., Reddy, K.T., Reddy, C.D., 2011. Influence of heavy metals on biochemical and metabolic biomarkers of Indian major carp, Labeo rohita. The Bioscan: Int.Quart. J. Life Sci. 6(1), 167-173.

Reddy,S.J.,Kalarani,V.,Tharakanatha,B.,Reddy,D.C.,Ramamurthi,R.,1998.Changes in energy metabolism of the fish, Labeo rohita, in relation to prolonged lead exposure and recovery. J. Ecotoxicol. Environ. Monit. 8(1), 43-53.

Rotruck., 1973. Selenium biochemical role as a component at glutathione peroxidase. *Science*. 179,588-590.

Sadava, E.D., Hillis, . M. D and H. Craig Heller, Life (9th, International Ed.), W. H. Freeman. 2011.

Saha. S., Kaviraj, A., 2009. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. Chemosphere.74(19),1254–125.

Schreck, C.B., Moyle, P.B., 1990. Methods for Fish Biology. American Fisheires Society,

Senthil Elango, P., Muthulingam, M., 2014. Impact of heavy metal chromium on protein and aminoacid contents in brain and muscle of freshwater fish Oreochromis mossambicus (PETERS). International Journal of Current Research, 6(01),4841-4845.

Shukla, G. S., Chiu, J., Hart, B. A., 2000. Cadmium induced elevations in the gene expression of the regulatory subunit of g-glutamylcysteine synthetase in rat lung and alveolar epithelial cells. Toxicology. 151,45.

Sinha, KA., 1972. Colorimetric assay of catalase. *Anal Biochem*. 47,389-394.

Smolders, R., Bervoets, L., De B.G., Blust, R., 2002. Integrated condition indices as a measure of whole effluent toxicity in Zebra fish (Danio rerio).Environ Toxicol.Chem. 21(1),87-93.

Solomon. K.R., Carr, J.A., Du Preezetal L.H., 2008.Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review, Critical Reviews in Toxicology. 38(9),721–772.

Song, S., Fajol, A., Chen, Y., Ren, B., Shi, S., 2018. Anticonvulsive effects of protodioscin against pilocarpineinduced epilepsy. Eur. J. Pharmacol. 833, 237–246.

Stajn A., Zikic R. V., Ognjanovic B., Saicic Z. S., Pavlovic S. Z., Kostic M. M., Petrovic V. M., 1997. Effect of cadmium and selenium on the antioxidant defense system in rat kidneys. Comp. Biochem. Physiol. C 117(2), 167.

Stein, W. H., Moore, S., 1954.The free amino acids of human blood plasma. J. biol. Chem. 211, 915.

Stela Stoyanova., Elenka Georgieva., Iliana Velcheva., Ilia Iliev., Tonka Vasileva., Veselin Bivolarski., Stoil Tomov., Krisztian Nyeste., Laszlo Antal., and Vesela Yancheva., 2020. Multi-Biomarker Assessment in Common Carp (Cyprinus carpio, Linnaeus 1758) Liver after Acute Chlorpyrifos Exposure.Water, 12, 1837. doi:10.3390/w12061837.

Stoyanova, S., Yancheva, V., Iliev, I.,Vasileva, T., Bivolarski, V., Velcheva, I., Georgieva, E., 2015.Glyphosate induces morphological and enzymatic changes in common carp (Cyprinus carpio L.) liver. Bulg. J. Agric. Sci. 21,409-412.

Tillittd, E., Papouliasd, M., Whytej, J., Richter, C.A., 2012. Atrazine reduces reproduction in fat head minnow (Pimephales promelas). Aquatic Toxicology. 99,149.

Tripathi V.K., Rajesh Kumar Yadav., 2015. Effect of Pesticide (organophosphorus) on aquatic fish labeo rohita. Int. J. Chem. Sci. 13(2), 625-640.

Tulasi, G., Jayantha Rao, K., 2013. Effect of chromium on protein metabolism in different tissues of fish, Cyprinus carpio. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 4(1), 143-148.

Tulasi, SJ., Reddy PUM., Rao, JR., 1992.Accumulation of lead and effects on total lipids and lipid derivatives in the freshwater fish, Anabas testudineus (Bloch). Ecotoxicology and Environmental Safety. 23, 33-38.

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.

Vasanth. S., Arul Ganesh, T., Siva Vijayakumar, S., Karthikeyeni, M., Manimegalai, P., 2013. Subramanian. Impacts and Impairments of Atrazine on Male Poecilia Sphenops. Indian Journal of Advances in Chemical Science. 2(1), 62-70.

Virk, S., Sharma, RC., 1999. Biochemical changes induced by Nickel and Chromium in the liver of *Cyprinus carpio* L. *Poll. Res.,* 18(3), 217-222.

Wilkinson, J., 1976. The principles and practice of diagnostic enzymology. Fundam.Clin. Chem. 30, 379-385.

Xing H, Li S, Wang Z, Gao X, Xu S, Wang X. 2012.Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Pestic Biochem Phys.103,74-80. doi: 10.1016/j.pestbp.2012.03.007.

Xing, H., Li, S., Wang, Z., Gao, X., Xu, S., Wang, X., 2012, Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. Chemosphere. 88, 377-383. doi: 10.1016/j.chemosphere.2012.02.049.

Xulu Chang., Yongyan Chen., Junchang Feng., Mengyuan Huang., Jianxin Zhang., 2021.Amelioration of Cd-induced bioaccumulation, oxidative stress and immune damage by probiotic Bacillus coagulans in common carp (Cyprinus carpio L.). Aquaculture Reports 20 (2021),100678.

Yalin, S., Comelekoglu, U., Bagis, S., Sahin, N. O., Ogenler, O., Hatungil, R., 2006. Acute effect of single dose cadmium treatment on lipid peroxidation and antioxidant enzymes in ovariectomized rats. Ecotoxicol. Environ. Saf. 65(1),140.

Yang. L., Zha, W., Li., Z. Li., Wang, Z., 2010. Atrazine affects kidney and adrenal hormones (AHs) related genes expressions of rare minnow (Gobiocypris rarus). Aquatic Toxicology, 97(3), 204–211.

Zhu, X., Zhu, L., Lang,Y., Chen,Y., 2008. Oxidative stress and growth inhibition in the freshwater Fish Carassius auratus induced by chronic exposure to sub lethal fullerene aggregates. Environmental Toxicology and Chemistry.27, 1979–1985.

Zikic, R. V., Stajn, A. S., Ognjanovic, B. I., Saicic, Z. S., Kostic, M. M., Pavlovic, S.Z., Petrovic V. M., 1998. The effect of cadmium and selenium on the antioxidant enzyme activity. J. Environ. Pathol. Toxicol. Oncol.17,259.