International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2020; 8 (2):291-298
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Studies on the toxicity of 4- Nonylphenol on the biochemical parameters of African catfish *Clarias gariepinus (*Burchell, 1822)

Zade SB[1*], Shinkhede[2] MM, Nagwanshi[1] AM and Shahare[1] SS

[1]PGTD Zoology, Mahatma Jyotiba Fule Educational Campus, RTM Nagpur University Nagpur-440033, Maharashtra, India.
[2]Dada Ramchand Bakhru Sindhu Mahavidyalaya, Nagpur, Maharashtra, India
*Corresponding author Email: profsbzade@rediffmail.com

Manuscript details:

Received: 18.02.2020
Revised : 29.02.2020
Accepted: 08.03.2020
Published: 29.06.2020

Cite this article as:
Zade SB, Shinkhede MM, Nagwanshi AM and Shahare SS (2020) Studies on the toxicity of 4- Nonylphenol on the biochemical parameters of African catfish *Clarias gariepinus (*Burchell, 1822). *Int. J. of. Life Sciences*, Volume 8(2): 291-298.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Aquatic system is more vulnerable to the pollution as most of the toxicants are released into it. These toxicants cause harmful effects on the life of organism and create a toxic stress. Because of these stress, the physiology of the organism changes and as results of this all the metabolic activity alters. The biochemical markers are a good indicator of changes in the metabolic activity caused by the aquatic toxicants. These markers provide one of the most reliable means for assessing the stress caused by the toxicants. Keeping in view, the present study was undertaken to investigate the effects of 4-nonylphenol (4-NP) on the biochemical parameters of African cat fish, *Clarias gariepinus*. The results of the present study showed that there was decrease in the level of protein and cholesterol in the fishes exposed to different concentrations 4-NP for 10 days as compared to the control. Based on the results it can be concluded that the 4-NP alters the metabolic activity of African catfish, *Clarias gariepinus* (Burchell, 1822) in response to stress caused by the 4-NP.

Keywords: Biochemical markers, aquatic toxicants, 4-nonylphenol (4-NP), *Clarias gariepinus* (Burchell, 1822), protein and cholesterol.

INTRODUCTION

The aquatic organisms are sensitive to environmental changes. They exhibit different degree of changes in the behavioral pattern when their habitat is polluted. The development of agricultural and industrial activities contributes to production of xenobiotics that can cause undesirable effects in aquatic systems depending on their toxicity and concentration (Havelková *et al.,* 2008). The widespread use of exogenous manmade chemicals not only brought adverse influence on agro ecosystems but also caused alteration in physiological processes of non-target organisms. These xenobiotics through surface runoff reaches to the unrestricted areas like ponds and rivers which alters the physicochemical properties of water and is toxic to aquatic organism and cause deleterious effect or even death to the aquatic animals. In many countries, large scale mortality of fishes has been recorded due to pesticides in water bodies as pollutants (Nikam *et al.,* 2011).

The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic fauna in the near future. Among the several aspects of toxicity studies, the bioassay constitutes one of the most commonly used methods in aquatic environmental studies with suitable organisms. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management.

Fishes are valuable sources of high grade proteins, mineral and salts including calcium, phosphorus and iodine essential amino acids, omega 3 fatty acids and vitamins A, B, D and E. Fish proteins occupy an important place and it constitutes about 17-20%. Moreover, carbohydrate content of the fish flesh is very low and hence, fish can make valuable contribution to any diet (Holt, 1967). Besides providing food to man, fishes are sources of numerous byproducts such as fish liver oil, fish flour, fish silage, fish glue etc. which have medical and economic importance. That's why it must be included in human diet at least 1.3 kg per week (FAO, 1989).

However, the fish habitats are being contaminated alarmingly through a number of aquatic pollutants. Fish occupy a prominent position in the field of toxicity studies. They are continued to be an extremely reliable component of an aquatic ecosystem, serve as best bioindicators of water pollution and are the inhabitants that cannot escape from the detrimental effects of these pollutants (Neelima *et al.,* 2016).

It is believed that the fish possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species (Al-akel *et al.,* 2010; Ahmad, 2011). Therefore, it is important to examine the toxic effects of xenobiotics on fish since they constitute an important link in food chain and their contamination by pesticides imbalance the aquatic system.

Biomarkers and bioindicators has been extensively used as proxies to determine responses at individual level to stressors, with biomarkers being more species-specific and with higher variability of responses compared to bioindicators (Schulz and Martins-Junior, 2001; Solé *et al.,* 2010; Linde-Arias *et al.,* 2008 and Barrilli *et al.,* 2015). The integrated use of biomarkers and bioindicators is suggested as an evaluation tool, since they are effective means to determine the impact of pollution in the aquatic environment (Reynolds *et al.,* 2003).

Biochemical markers are measurable responses to the exposure of an organism to xenobiotics as well as very suitable biosensors of aquatic pollutants. Aquatic pollution can easily be detected through biomarkers, as enzymes provide one of the most reliable means of assessing the degree of exposure of animals to pollutants. Some of the biochemical alteration occurring in the body gives the first indication of the stress in the organism and hence effect on the part of the pollution (Rathod *et al.,* 2009). The biochemical studies are good parameters which help to see the effects of toxicants on metabolism of fish (Kajare *et al.,* 2000, Banaee *et al.,* 2008).

Keeping in view, the present study was undertaken to investigate the effects of 4-nonylphenol on the biochemical parameters of African cat fish, *Clarias gariepinus* (Burchell, 1822).

MATERIALS AND METHODS

For the present study, the fresh water African catfish, *Clarias gariepinus* (Burchell, 1822), were selected because of its availability in local market and its convenient size. It could be safely transported and maintained easily under laboratory conditions because of its air breathing habit, its hardy nature, moreover suit the experimental work.

All the fishes used during the present study were brought from the local market. The body weight of fish ranged between 250-350 gm and their length varied between 30-37cm. The fishs were maintained in glass aquaria containing 30lit of tap water,under normal conditions of light and temperature. The fishes were fed with minced goat liver every alternate day and water changed at an interval of one day. The fishs were acclimatised for one weak by keeping 6 fishes, 3 male and 3 female in one aquarium prior to their use in the experiment.

For the present study, the chemical 4-nonylphenol was purchased from Hi-Media where benzene used as a solvent (33.3mg 4-nonylphenol dissolved in 1ml of benzene). The four aquaria were taken, filled with 30 lit tap water and in each aquarium 3 male and 3 female fishes were kept .The fishes without exposure of any toxicant in one aquarium were treated as control

group and other aquaria exposed to toxicant were treated as experimentalgroup and labelled them accordingly. The fishes from the experimental group exposed to three sub lethal concentrations (70, 100 and 130ug/lit) of 4-nonylphenol for 10 days.

For biochemical estimation of protein and cholesterol` the fishes from both control and experimental group were anesthetized with the 2-phenoxyethanol. After anesthetization, blood were collected in EDTAtube by cutting caudal fin and centrifuged to collect plasma. For protein estimation Lowry's method were used whereas for the estimation of cholesterol Zakes method used.

OBSERVATIONS AND RESULTS

1. Effects of 4-NP on protein concentration

The concentration of protein in plasma of fish, *Clarias gariepinus* (Burchell, 1822) from experimental group showed the decrease in the protein concentration in both male and female as compared to the control group (Fig. 1 and 2). The protein concentration of male fish from control group showed 17.67 ± 0.42 mg/dl whereas the protein concentration of male fish exposed to 70µg/lit, 100µg/lit and 130µg/lit 4-NP showed 15 ± 1.29 mg/dl, 14.5 ± 1.06 mg/dl and 12.33 ± 0.8 mg/dl respectively. (Table 1).

Table 1: Showing the protein concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

Group	Concentration of Protein (mg/dl) in Male
Control	17.67 ± 0.42
70 µg /lit 4-NP	15 ± 1.29
100 µg /lit 4-NP	14.5 ± 1.06
130 µg /lit 4-NP	12.33 ± 0.8

(Values are expressed in Mean ±SE)

Table 2: Showing the protein concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

Group	Concentration of protein (mg/dl) in Female
Control	19.67 ± 0.8
70 µg /lit 4-NP	16.67 ± 0.67
100 µg /lit 4-NP	15.33 ± 0.61
130 µg /lit 4-NP	13.33 ± 0.8

(Values are expressed in Mean ±SE)

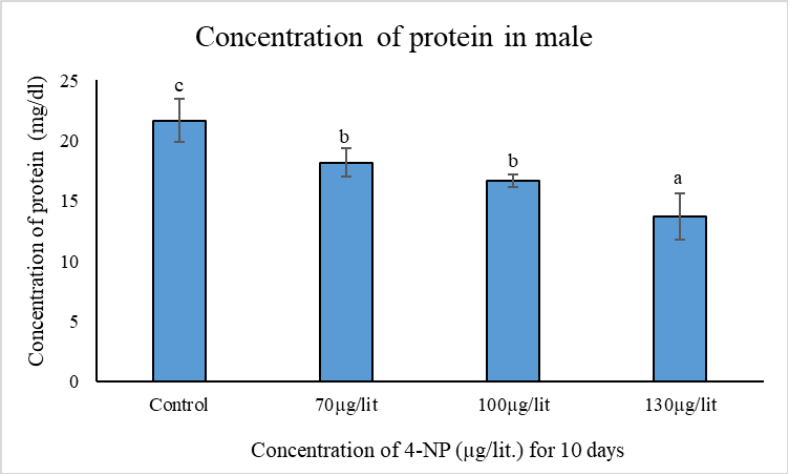

Fig.1: Showing the protein concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

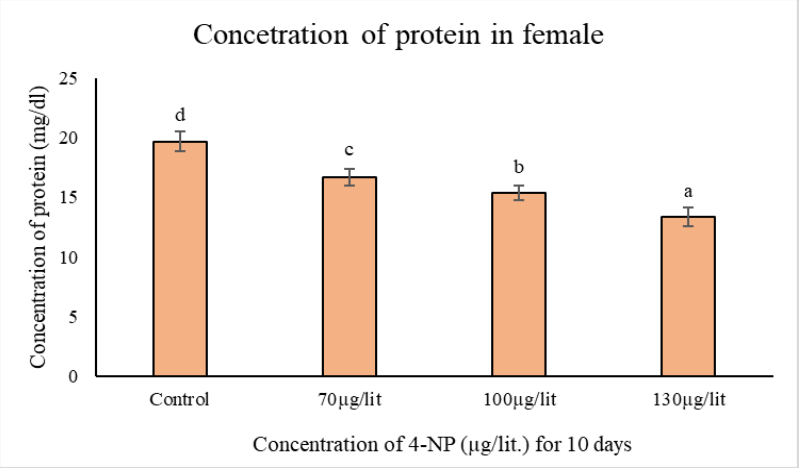

Fig. 2: Showing the protein concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

Similarly, the protein concentration of female fish from control group showed 19.67± 0.8 mg/dl whereas the protein concentration of female fish exposed to 70µg/lit, 100µg/lit and to130µg/lit 4-NP showed 16.67 ± 0.67 mg/dl, 15.33 ± 0.61 mg/dl and 13.33 ± 0.8mg/dl respectively (Table 2).

2. Effects of 4-NP on cholesterol concentration

The concentration of cholesterol in plasma of fish, *Clarias gariepinus* (Burchell, 1822) from experimental group showed the decrease in the cholesterol concentration in both male and female as compared to the control group (Fig. 3 and 4).

The cholesterol concentration of male fish from control group showed 277.18 ± 9.78 mg/dl whereas the cholesterol concentration of male fish exposed to 70µg/lit, 100µg/lit and 130µg/lit 4-NP showed 186.9 ± 7.59 mg/dl, 148.27 ± 4.57 mg/dl and 146 ± 11.05 mg/dl respectively (Table 3).

Similarly, the cholesterol concentration of female fish from control group showed 307.63 ± 7.37 mg/dl whereas the cholesterol concentration of female fish exposed to 70µg/lit, 100µg/lit and 130µg/lit 4-NP showed 293.17 ± 7.69 mg/dl, 267.75 ± 5.14 mg/dl and 220.58 ± 13.81 mg/dl respectively (Table 4).

Table 3: Showing the cholesterol concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

Group	Concentration of Cholesterol (mg/dl) in Male
Control	277.18 ± 9.78
70 µg /lit 4-NP	186.9 ± 7.59
100 µg /lit 4-NP	148.27 ± 4.57
130 µg /lit 4-NP	146 ± 11.05

(Values are expressed in Mean ±SE)

Table 4: Showing the cholesterol concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

Group	Concentration of Cholesterol (mg/dl) in Female
Control	307.63 ± 7.37
70 µg /lit 4-NP	293.17 ± 7.69
100 µg /lit 4-NP	267.75 ± 5.14
130 µg /lit 4-NP	220.58 ± 13.81

(Values are expressed in Mean ±SE)

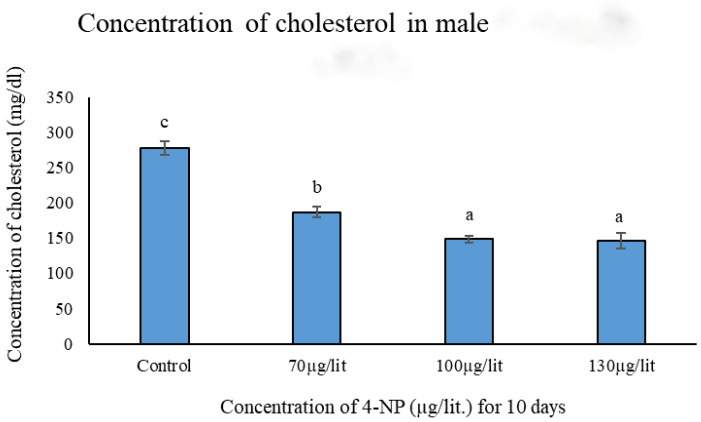

Fig. 3: Showing the cholesterol concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

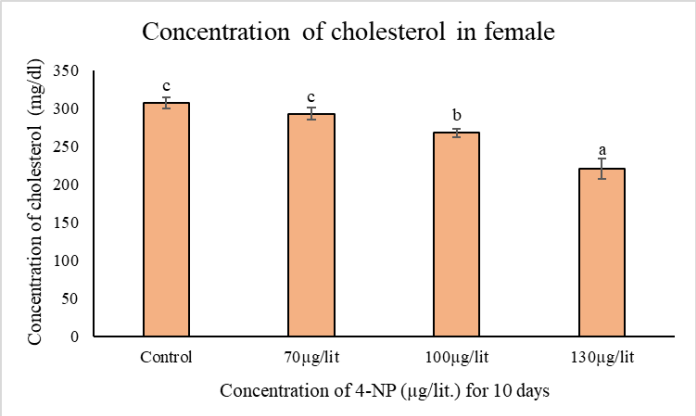

Fig.4: Showing the cholesterol concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of 4-NP for 10 days.

DISCUSSION

Proteins are the macromolecules in the biological system and are derived from high molecular weight polypeptides made up from amino acids. They constitute about one fifth of an animal body on wet weight basis. The concentration of proteins in serum or plasma is a result of dynamic equilibrium between the rate of its biosynthesis and degradation process. Proteins play an important role as energy precursors for fish and other organisms under stress conditions. Changes in each of these blood components have been employed as useful general indicators of stress in teleosts (Das *et al.*, 2004).

In the present study, it has been found that there was decrease in the level of protein in the group of fishes exposed to different concentrations of 4-NP for 10 days as compared to the control group. Similar results were reported by Senthil Kumaran *et al.*, 2011in *Clarias gariepinus,* exposed to nonylphenol and octylphenol, Senthil Kumar and Sivasubramanian (2015) in freshwater fish *Catla catla* exposed to Ammonium sulphate, Khalid Abdullah Al-Ghanim, (2012) in *Oreochromis niloticus* after exposure of sub-lethal concentrations of malathion, Begum, (2005) in *Clarias batrachus* during exposure of cypermethrin, Olalekan (2014) in *Clarias gariepinus* exposed to Cypermethrin, Velisek *et al.,* (2012) in common carp *Cyprinus carpio* (L.) exposed to simazine, Ramesh and Saravanan, (2008) in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos, Dogan and Can, (2011) in *Oncorhynchus mykiss* exposed to dimethoate, Jaroli and Sharma (2005) in Liver of *Channa punctatus* exposed to organophosphate, Oruç and Usta, (2007) in rainbow trout, and in Cyprinus carpio exposed to diazinon and Saravanan *et al.,* (2012) in an Indian major carp, *Cirrhinus mrigala* exposed to Ibuprofen.

The decreased protein levels might be associated with damage of cells caused by 4-NP. The decrease in protein content under stress induced by 4-NP could also be attributed to the use of amino acids in various catabolic reactions and the 4-NP may either act by activating or inhibiting enzyme activities in the cell or destruction of the cell organelles with liberation of particular enzymes is one of the reasons to alter the expression of total proteins (Balakrishnan and Sendhilvadivu, 2018). During stress conditions organisms need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein and lipids to meet the increased energy demand (Singh *et al*., 2010, Lavanya *et al.,* 2011).

Under conditions of stress many organisms will mobilize proteins as an energy source *via* the oxidation of amino acids. Decreased protein level may be associated with stress mediated mobilization of proteins to fulfill an increased element for energy by the fish to cope with environmental condition exposed by the toxicant (Jenkins *et al.,* 2003). The reduction in total protein content may be due to increased proteolysis and possible utilization of their product for metabolic purposes as reported by Ravinder *et al.,* (1988).

Another reason behind the hypoprotinemia observed in the group of fishes exposed to 4-NP is oxidative stress influenced by excess reactive oxygen species (ROS) produced are known to damage proteins. The depletion of total protein content was observed in this investigation can be correlated to this fact that the 4-NPmay induced oxidative stress are known to damage proteins (Zhang X *et al.,* 2005, Osman *et al.,* 2010). Depletion of proteins might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery might be due to the blocking of protein synthesis, protein denaturation, or interruption in the amino acid synthesis (Vasantharaja *et al.,* 2012, Kori-Siakpere *et al.,* 2006, David *et al.* 2004 and Singh *et al.,* 2010).

Serum cholesterol is a term that includes the total level of cholesterol found in the blood stream. Measuring the level of total cholesterol includes identifying all types or classes of cholesterol that found in the system. Intestinal cholesterol absorption plays a major role in maintaining total body cholesterol homeostasis.

In the present study it has been found that there was decrease in the level of cholesterol in the group of fishes exposed to different concentrations of 4-NP for 10 days as compared to the control group. The similar findings also reported by the Logaswamy and Remia, 2009, Shakoori *et al.,* (1996), Jee *et al.,* 2005, Jipsa *et al.,* 2014, Lakshman *et al.,* 1988 and Riaz, (2018).

The reduction in the cholesterol level depends on its utilization in the manufacture of cortisol arising from stress created by the toxins (Madrid, 2012). The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietry cholesterol as reported by Kanagaraj *et al.* (1993). Shakoori *et al.,* in 1996, reported that the cholesterol decrease may be due to utilization of fatty deposits instead of glucose for energy purpose.

Decreased cholesterol levels in the present study after 10 days exposure of 4-NP may be attributed to stress-mediated mobilization of these compounds to fulfill an increased demand for energy by the fish to cope with detrimental conditions imposed by the toxicant (Jee*et al.,* 2005). 4-NP may inhibite the absolute rates of both fatty acid and cholesterol synthesis in the liver and fatty acid synthesis in the adipose tissue which leads to decrease in the level of cholesterol as found in the present study.

CONCLUSION

The present study concludes that the 4-NP has marked effects on the biochemical parameters of the fish which ultimately causes the disturbance in the normal physiological processes and homeostasis.

Acknowledgment

Authors are thankful to Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for proving finanacial support.

Conflict of Interest

The author declares that there is no conflict of interest.

REFERENCES

Ahmad Z (2011) Toxicity bioassay and haematological changes induced by diazinon in common carp, *Cyprinus carpio. Afr. J. Biotechnol.,* 10: 13852-13859.

Al-Akel AS, Alkahem-Al-Balawi HF, Al-Misned F, Mahboob S, Ahmad Z, Suliman EM (2010) Effects of dietary copper

exposure on accumulation, growth, and haematological parameters in *Cyprinus carpio*. *Toxicol. Environ. Chem.,* 92: 1865-1878.

Balakrishnan N and Sendhilvadivu (2018) Effect of BPA on protein, lipid profile in serum of albino rats and impact of vitamin E and thyronorm in BPA induced albino rats. *International Journal of Science and Research,* 7 (1): 907:911.

Banaee M, Mirvaghefi AR, Ahmadi K and Banaee S (2008) Determination of LC50 and investigation of acute toxicity effects of diazinon on haematology and serology indices of common carp (*Cyprinus carpio*). *J. Mar. Sci. Tech. Res.,* 3(2): 1-10.

Barrilli, GHC, Rocha O, Negreiros NF and Verani JR (2015) Influence of environmental quality of the tributaries of the Monjolinho River on the relative condition factor (Kn) of the local ichthyofauna. *Biota Neotropica*, 15 (1): e20140107.

Begum G (2005) In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. *Pesticide Biochemistry and Physiology,* 82 185–196.

Das P, Ayyappan S, Jena J and Das B (2004) Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacult. Res*., 35: 134–143.

David M, Mushigeri SB, Shivakumari R and Philip GH (2004) Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alteration in protein metabolic profile. *Chemosphere*, 56:347- 352.

Dogan D and Can C (2011) Hematological, biochemical, and behavioral responses of *Oncorhynchus mykiss* to dimethoate. *Fish Physiol Biochem,* 37:951–958.

Food and Agriculture Organization (Food and Agriculture Organization (FAO). (1989) Evaluation of certain food additives and contaminants. 33rd report of joint FAO/WHO Expert Committee on food addives. Technical report series, 776.

Havelková M, Randák T, Blahová J, Nská IS, and Svobodová Z (2008) Biochemical markers for the assessment of aquatic environment contamination. *Interdisc. Toxicol,* 1(2): 169–181.

Holt SJ. (1967) The contribution of freshwater fish production to human nutrition and well-being. In: The biological basis of freshwater fish production (Ed: S.D.Gerking). Blackwell scientific publication, Oxford, 455 – 457.

Jaroli DP and Sharma BL (2005) Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus*. *Asian J. Exp. Sci.,* 19(1): 121-129.

Jee JH, Masroor F and Kang JC (2005) Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research*, 36: 898-905.

Jenkins F, Smith J, Rajanna B, Shameem U, Umadevi K, Sandhya V and Madhavi R (2003) Effect of sublethal concentration of endosulfan on hematological and serum biochemical parameters in the carp, *Cyprinus carpio. Bull. Environ. Contam. Toxicol.* 70: 993-947.

Jipsa JR, Kalavathi R, Dhanya PY and Logaswamy S (2014) Studies on the impact of a Cypermethrin insecticide on oxygen consumption and certain biochemical constituents of a fish *Tilapia mossambica*. *International Journal of Fisheries and Aquatic Studies*, 1(5): 93-97.

Kajare A, Singh S and Shrivastava K (2000) Malathion induced biochemical changes in kidney of freshwater fish, *Clarias batrachus. Ecotoxicol. Env. Monit.,* 10: 11-14.

Kanagaraj MK, Ramesh M, Sivakumari K and Manavalaramanujam R (1993) Impact of acid pollution on the serum haemolymph cholesterol of the crab, *Paratelphusa hydrodromous. J. Ecotoxicol. Environ. Monit.,* 3(2): 099-102.

Khalid Abdullah Al-Ghanim (2012) Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of *Oreochromis niloticus*. *Scientific Research and Essays*. 7(16): 1674-1680.

Kori- Siakpere O, Ake JEG and Avworo UM (2006) Sub lethal effects of some selected haematological parameters of Heteroclarias (a hybrid of *Heterobranchus bidorsailis* and *Clarias gariepinus*). *Int. J. Zool. Res*., 2:77-83.

Lakshman R, Campbell BS, Chirlel SJ and Ekarohita N (1988) Effects of 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) on de novo fatty acid and cholesterol synthesis in the rat. *Lipids*, 23(9):904-906.

Lavanya S, Ramesh M, Kavitha C and Malarvizhi A (2011) Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere,* 82:977–985.

Linde-Arias AR, Inacio AF, Albuquerque C, Freire MM and Moreira JC (2008a) Biomarkers in an invasive fish species, *Oreochromis niloticus*, to assess the effects of pollution in a highly degraded Brazilian River. *The Science of the Total Environment*, 399(1-3):186-192.

Linde-Arias AR, Inacio AF, Novo LA, Albuquerque S and Moreira, JC (2008b) Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul. *Environmental Pollution*, 156 (3): 974-979.

Logaswamy S and Remia KM (2009) Impact of Cypermethrin and Ekalux on respiratory and some biochemical activities of a fresh water fish *Tilapia mossambica*. *Current biotica*. 3 (1): 65-73.

Madrid MLA (2012) Hepatic Effects from Sub-acute Exposure to Insecticides in Adult Male Wistar Rats. Insecticides Advances in Integrated Pest Management. Chapter 12.

Mehreen R (2018) Toxic effects of insecticides malathion and cypermethrin on biochemical, hematological and hormonal parameters in blood of male rabbit (*Oryctolagus cuniculus*). Thesis. Department of Zoology Islamia College Peshawar.

Neelima P, Rao KG, Rao SG, Rao GN and Rao JCS (2016) Biomarkers of Cypermethrin (Synthetic Pyrethroid) Toxicity - Biochemical alterations in Cyprinus carpio, a

freshwater edible fish. *Int. J. Biol. Med. Res*.7 (2):5574-5581.

Nikam SM, Shejule KB and Patil RB (2011) Study of acute toxicity of Metasystox on the freshwater fish, Nemacheilus botia, from Kedrai dam in Maharashtra, India. Biology and *Medicine,* 3 (4): 13-17.

Olalekan A (2014) Biochemical Response of *Clarias gariepinus* to Cypermethrin. *Journal of Environmental & Analytical Toxicology*, 5(1):1-4.

Oruc EO and Usta D (2007) Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ Toxicol Pharmacol,* 23:48–55.

Osman KA, Al-Humaid AM, Al-Rehiayani SM and Al-Redhaiman KN (2010) Monitoring of pesticide residues in vegetables marketed in Al-Qasim region, Saudi Arabia. *Ecotoxicol. Environ. Saf.,* 73: 1433-1439.

Ramesh M and Saravanan M (2008) Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *International Journal of Integrative Biology,* 3(1), 80:83.

Rathod DS, Lokhunde MV and Shembekar VS (2009) Toxic impact of dimethoate on the biochemical composition of vital tissues of fish, *Arias dussumieri*. *Shodh Samik. Muyan,* 2: 147-149.

Ravinder V and Suryanarayana N (1988) Decis induced biochemical alterations in a fresh water catfish, *Clarias batrachus. Indian J. Comp. Animal Physiol*., 6: 5-12.

Reynolds WJ, Feist SW, Jones GJ, Lyons BP, Sheahan DA and Stentiford GD (2003) Comparison of biomarker and pathological responses in flounder (*Platichthys flesus* L.) induced by ingested polycyclic aromatic hydrocarbon (PAH) contamination. *Chemosphere*, 52(7):1135-1145.

Saravanan M, Usha Devi K, Malarvizhi A and Ramesh M (2012) Effects of Ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp, *Cirrhinus mrigala. Environmental Toxicology and Pharmacology*, 34:14–22.

Schulz U.H. and Martins-Jujnior H (2001) *Astyanax fasciatus* as bioindicator of water pollution of rio dos Sinos, RS, Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 61(4):615-622.

Senthil Kumar K and Sivasubramanian S (2015) Biochemical investigation on fertilizer ammonium sulphate exposure to freshwater fish *Catla catla*. *World Journal of Science and Research*, 1(2): 49-52.

Senthil Kumaran S, Chokkalingam K, Mathan R and Tamara G (2011) Toxicity studies of nonylphenol and octylphenol: hormonal, hematological and biochemical effects in *Clarias gariepinus. J. Appl. Toxicol*., 31: 752–761.

Shakoori AR, Mughal AL and Iqbal M (1996) Effect of sublethal doses of fenvalarate (A synthetic pyrethroid) administered continuously for 4 weeks on the blood, liver, muscles of freshwater fish, *Etenopharyngodon idella. Bull. Environ. Contam. Toxicol.,* 57: 487 – 499.

Singh KS, Singh SKS and Yadav RP (2010) Toxicological and biochemical alteration of cypermethrin (synthetic Pyrethroids) against freshwater teleost *Colisa fasciatus* at different Seasons. *World J. Zool.,* 5(7): 25-32.

Solé M, Antó M, Baena M, Carrasson M, Cartes JE and Maynou F (2010) Hepatic biomarkers of xenobiotic metabolism in eighteen marine fish from NW Mediterranean shelf and slope waters in relation to some of their biological and ecological variables. *Marine Environmental Research*, 70(2):181-188.

Vasantharaja C, Pugazhendy K, Venkatesan S, Meenambal M, Prabakaran S and Jayachandran K (2012) Acute toxicity of cypermethrin and its impact on biochemical alteration in the fresh water fish *Cirrhinus mrigala* (Hamilton) and protective effect of *Cardiospermum helicacabum* (Linn). *International Journal of Pharmaceutical & Biological Archives,* 3(1):146-152.

Velisek J, Stara A, Machova J and Svobodova Z (2012) Effects of long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio* L.). *Ecotoxicology and Environmental Safety*, 76:79–86.

Zhang X, Chen CH, Confino E, Barnes R, Milad M and Kazer RR (2005) Increased endometrial thickness is associated with improved treatment outcome for selected patients undergoing in vitro fertilization embryo transfer. *Fertility and Sterility*, 83: 336– 340.