Punjab Univ. J. Zool., Vol. 31 (2), pp. 229-236, 2016

ISSN 1016-1597 (Print)
ISSN 2313-8556 (online)

Original Article

Histological and biochemical study of liver of silver carp (*Hypophthalmichthys molitrix*) after acute exposure to pyrethoride (deltamethrin)

Asma Karim[1], Wajid Ali[1*], Noreen Ahmad[1], Muhammad Irfan[2], Hafiz Abdullah Shakir[3]

[1]*Department of Zoology Government College of Science,Wahdat Road, Lahore,Pakistan*
[2]*Department of Biotechnology, University of Sargodha, Sargodha Pakistan*
[3]*Department of Zoology, University of the Punjab new campus Lahore Pakistan.*

(**Article history:** Received: August 08, 2016; Revised: December 18, 2016)

Abstract
Deltamethrin is a broad spectrum synthetic pyrethroid pesticide. It is considered less toxic as compared to organo-chlorine and organo-phosphate pesticides. Deltamethrin was selected to study the biochemical and histological alterations in liver of silver carp. The LC_{50} 1.6 µg/L was determined. Fish (n=6) were dividing into 4 groups A (control) B, C and D (experimental). Experimental groups B, C and D were exposed to 25%, 50% and 75% of LC_{50} respectively for acute exposure. The blood and liver tissue sampling was done after 96hrs for histology and biochemical analysis [alanine amino transferase (ALT), and aspartate amino-transferase (AST)]. Liver histology revealed that necrosis, nuclear pycnosis, hypertrophy of hepatocytes, vacuolization, nuclear atrophy and congestion of blood vessels were observed as compare to control group. This result was also supported by the significant increase in levels of hepatic enzymes AST and ALT in blood plasma of exposed fish as compared to control fish. These results suggested that deltamethrin is highly toxic for aquatic organism like silver carp causing malfunctioning of liver.
Key words, Deltamethrin, acute exposure, silver carp, liver histology, liver enzymes

To cite this article: KARIM, A., ALI, W., AHMAD, N., IRFAN, M. AND SHAKIR, H.A., 2016. Histological and biochemical study of liver of silver carp (*Hypophthalmichthys molitrix*) after acute exposure to pyrethoride (deltamethrin). *Punjab Univ. J. Zool.,* **31**(2): 229-236.

INTRODUCTION

With increasing population, urbanization and industrialization, water pollution by industrial, agricultural run-off and municipal sources has become a great concern for the safety of humanity (Yousfzai *et al.,* 2011). From last few decades use of pesticides has increased exponentially all over the world. Excess use of pesticides has contaminated the ecosystems with toxicant. Different monitoring programs and scientific study has been found that pesticides are present in different concentrations in ground water, crop samples and agricultural streams (Mahboob *et al.,* 2015; Sharma *et al.,* 2015; Yadav *et al.,* 2015). Environment and surface waters were impure with different insecticides had been reported in different study (Arjmandi *et al.,* 2010; Rahiminezhhad *et al.,* 2009). The effect of pesticides on aquatic animals' health was reported by Banaee and Ahmadi (2011). Pyrethroids severely eluted with water, adsorbed on sediments and soil. There is slight affinity for bioaccumulation in organisms (Haya, 1989).Their toxicity is in the parts per billion for non-target organisms (Bradbury and Coats., 1989). Fish during their life cycle may be exposed to a wide range of pesticides like other aquatic organisms. When pesticides like pollutants enter into the organs of fishes may considerably alter certain biochemical and physiological processes (Banaee *et al.,* 2011). Through skin, gills or gut, different pesticides can be accumulated in fish. (Schlenk, 2005; Banaee.,*et al* 2013). Fishes are highly sensitive to pesticide among the aquatic organism due to neurotoxic effects of pyrethroids pesticides. Pyrethroids cause 1000 times' greater toxicity to fish as compare to birds and mammals. (Bradbury and Coats, 1989). Due to slow riddance of these compounds and slow metabolism, fish is considered highly sensitivity

114-PUJZ-61022280/16/0229-0236
Copyright 2016, Dept. Zool., P.U., Lahore, Pakistan
*Corresponding author: wajid_wajid1@ yahool.com

to pyrethroids (David *et al*., 2003). Fishes are observed to be poor in enzymes that hydrolyze these pesticides, unlike most organisms, in which pyrethroids are readily metabolized and have a short life (Aydin *et* al., 2005). Second significant factor is hyper sensitivity of the piscine nervous system to these insecticides. Fish brain is more vulnerable to pyrethroids than birds and mammals (Moore and Waring., 2001). The third important factor is way of exposure. Pyrethroids are absorbed directly through gills into the circulation (Aydin *et al.,* 2005). Deltamethrin (Pyrethroids) is extensively used to control pest (Bradbury and Coats, 1989). Deltamethrin (pyrethorid) is being broadly used due to its low toxicity and environmental persistence as an alternate for organophosphates and organo chlorines. Sediment samples taken from the lake and fish species confirmed the presence of deltamethrin (Balint *et al*., 1995).

Hypophthalmicthys molitrix is one of prime cultured freshwater fish in Pakistan. It was taken as experimental organism to check out the toxicity of deltamethrine. Fish stress can be observed by using histopathological analysis as a biomarker (Van der Oost, *et al.,* 2003). In field studies as well as in laboratory, histopathological alteration have been extensively used as biomarkers to the assessment of the health of fish exposed to pollutants. The liver is main organ which play key role in biotransformation process and detoxification (Van der Oost *et al*., 2003). Liver is one of the organs most affected by pollutants in the water (Rodrigues and Fanta, 1998).

To detect the deformities in the liver and other tissues blood biochemical parameters are used as indicative tool (Banaee *et al*., 2011). Main function of liver is protection of the body from potentially harmful substance absorbed from the intestinal tract, skin and gills as well as toxic metabolic products. In the heart, liver, brain, skeletal muscle, kidney, spleen, pancreas, erythrocyte and gills cells alanine aminotransferase (ALT) and aspartate amino transferase (AST) are preasent (Banaee *et al*.,2011). The present study was conducted to assess histopathological and biochemical changes in liver after deltamethrin exposure.

MATERIAL AND METHOD

Experimental animal

Live silver carp (*Hypophthalmichthys molitrix*) were purchase from Himalayan fish hatchery Muredke to test the toxicity of pesticide as experimental organism. The average weight and length of fish specimen were 159.33±5.38 g, 9.95±0.138 cm, respectively. In laboratory conditions, fish were acclimatized with uninterrupted air supply for duration of two weak. During this period, dead fish were removed instantaneously.

Pesticides

Pyrethroid deltamethrin was used as toxicant. Fishes were divided into four groups A (control group) B, C and D (experimental group). Following LC_{50} value, three sub-lethal concentrations of deltamethrin were prepared. Three doses used for the experimental groups B, C and D; group B= 0.4 µg/ L (25% of LC_{50}), group C =0.8 µg/ L (50% of LC_{50}) and group D= 1.2 µg/L (75% of LC_{50}).

Hematological and histological analyses

After 96 hours fish blood was drawn directly in sterilized syringes through cardiac puncture for biochemical studies. Blood (2 ml) was taken in serum vacationers (devoid of any clotting factor) to separate the serum. At 4000rpm blood was centrifuged for twenty minutes. Then separated serum was stored at -20˚C till further analysis. Ready to use kits (CHEM HOUSE) were used for quantitative determination of ALT and AST.

For histological studies, the small piece of liver tissue was fixed in 10% formalin after rinsing with saline solution. The processing of fixed liver was carried out following standard protocol (Mumford, 2007).

Statistical analysis

The data were statistically evaluated by ONE WAY ANOVA using SPSS 13 Statistical program. The data were reported in mean±SEM. The *** showed highly significance (P<0.001), ** high significant (0.01) and * significant (P<0.05)

RESULTS

Histopathology of liver

Liver histology of control (A) and experimental group (B= 0.4 µg/L, C =0.8 µg/L and D= 1.2 µg/L) was performed to check the toxicity of pyrethoride (deltamethrine) on silver carp. Histology of control group (A) liver showed normal hepatic tissue, hepatocytes, blood vessels and bile duct. After acute exposure histopathology of group B (0.4 µg/ L of

deltamethrine) liver showed hypertrophy, occurrence of melano macrophages, cell damage, cluster of tissues and vacuolization as compare to group A (Fig. 1). Histopathological analysis of group C (0.8 µg/L deltamethrine) showed important alterations in liver, including vacuolization, nucleus atrophy, degenerated cells, cluster of hepatocytes and necrosis as compare to group A (Fig. 2). Histopathological analysis of group D (1.2 µg/L deltamethrine) showed important alterations in liver, including bile duct obstruction degenerated hepatocytes ,necrosis ,hepatocytes separated by sinusoid containing erythrocytes, cluster of cells ,vacuolization, nucleus atrophy and damaged cells as compared to group A (Fig. 3).

Biochemical analysis

To detect the toxic effect of pesticides aspartate amino-transferase (AST) and alanine amino transferase (ALT) were choosen as a investigative tool to finds the changes in liver of control (A) and experimental group (B= 0.4 µg/L, C =0.8 µg/L and D= 1.2 µg/L). In the present experiment, AST and ALT increased significantly (P<0.001) during the acute exposure of deltamethrin as compared to the control values with increse in doses. AST value in control group A was 217±6.27, when fish were exposed to deltamethrin, AST values increased significantly (P<0.000) in Group B (785.4±6.04), Group C (397.1±5.41) and Group D (335.4±7.11) in acute exposure respectively (Table I). ALT value in control group was 54±3.56, when fish exposed to deltamethrin, ALT values increased significantly (P<0.000) in group B (109.4±4.30), group C (101.4±4.28) and group D (94.4±5.18) in acute exposure respectively (Table I).

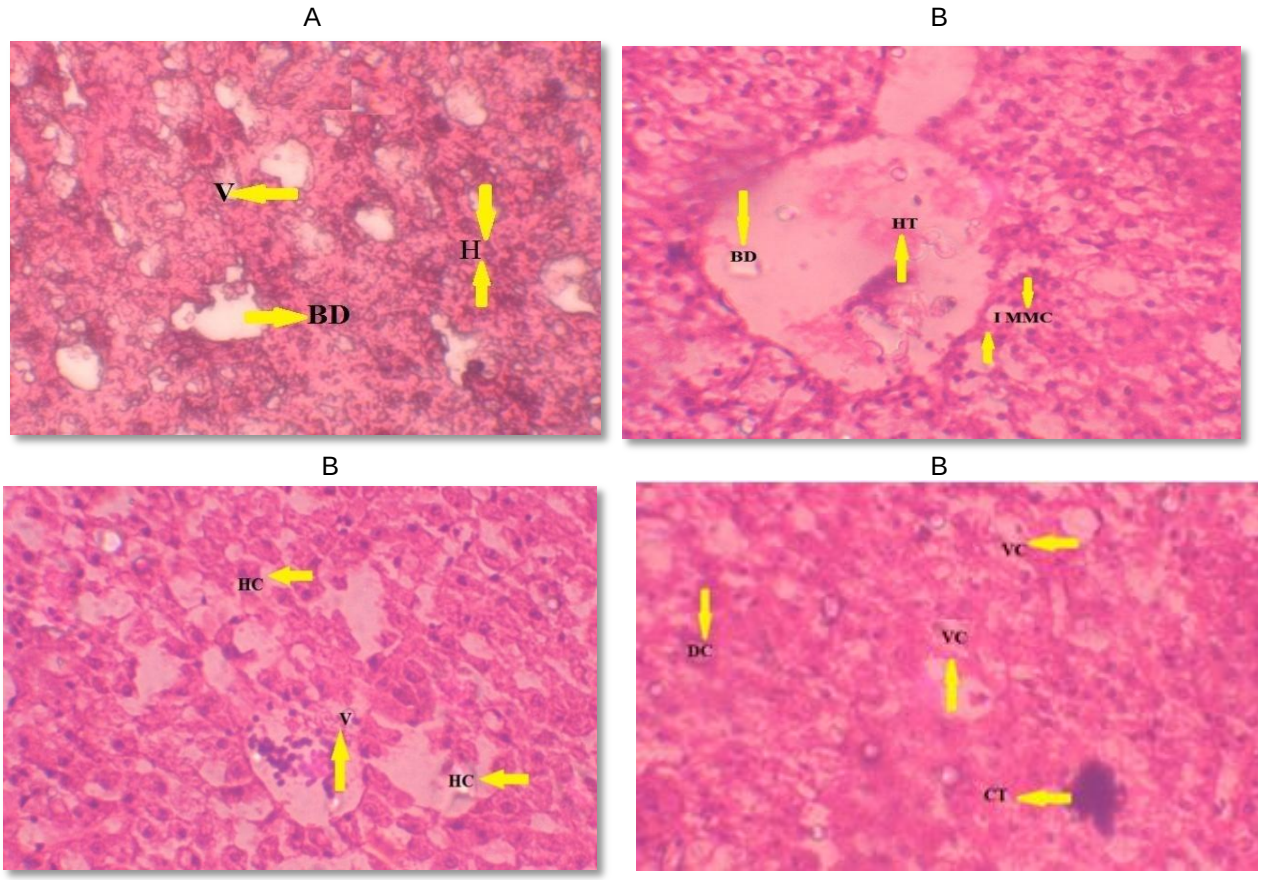

Figure 1: Photomicrograph of liver of *silver carp* A: control group showing vessels (V), bile duct (BD), hepatocytes (H). B: after acute exposure liver showing hypertrophy (HT), incidence of melanomacrophages (IMMC), hypertrophy of hepatocytes (HC), vacuolization (VC), damage cells (DC), cluster of tissues (CT).*(H & E 400X).*

Table I: Mean±SEM of ALT and AST levels among different groups.

Sr. No.	Parameters	Units	Control	Experimental Group		
			Group A	Group B	Group C	Group D
1.	ALT	U/L	54±3.56	109.4±4.30***	101.4±4.28***	94.4±5.18***
2.	AST	U/L	217±6.27	785.4±6.04***	397.1±5.41***	335.4±7.11***

*** shows high significant difference P< 0.001 (one way ANOVA)
Abbreviations: ALT: alanine aminotransferase and AST: aspartate amino transferase

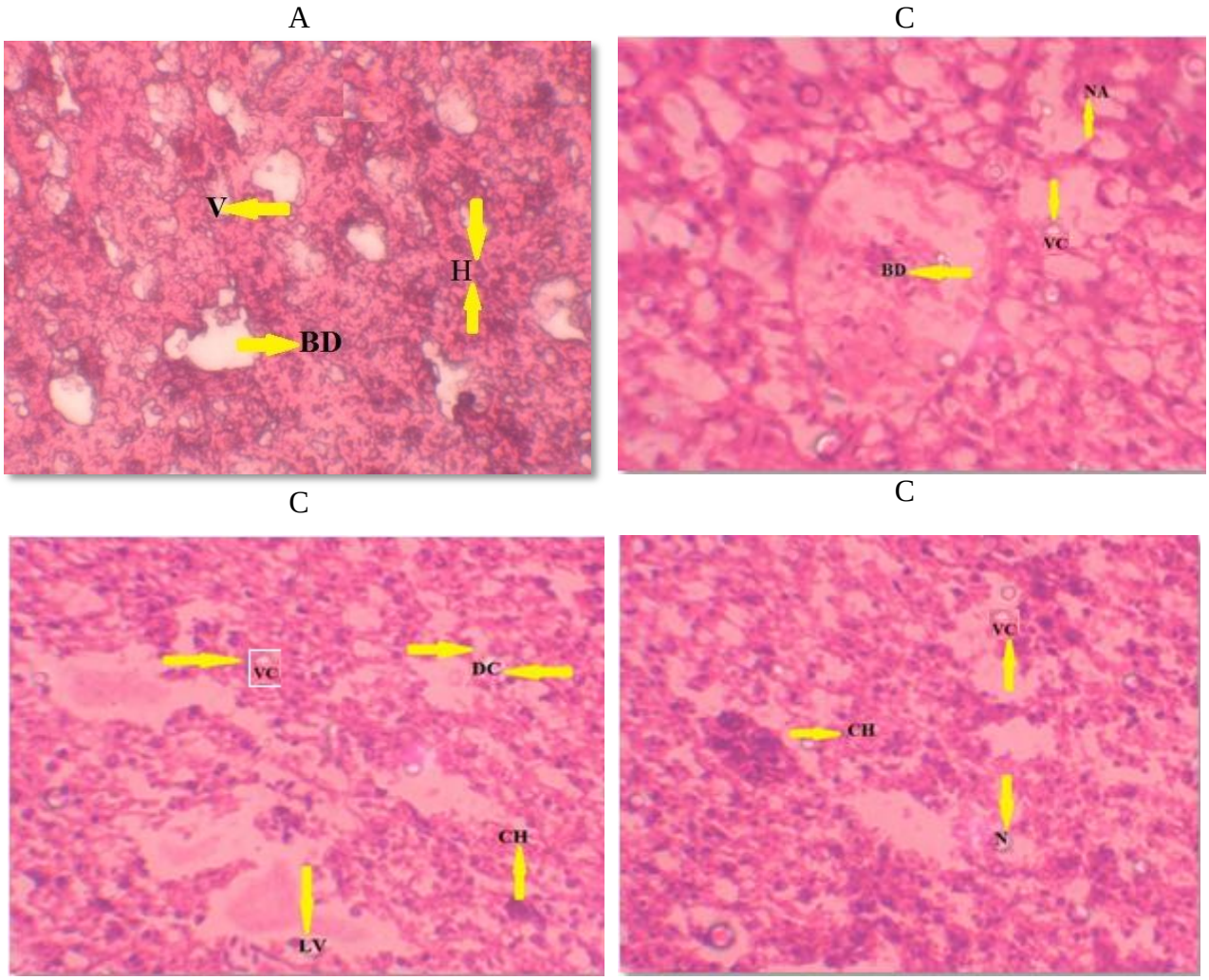

Figure 2: Photomicrograph of liver of *Hypophthalmicthyesmolitrix* (A) section of control group showing vessels (V), bile duct (BD), hepatocytes (H). (C) After acute exposure liver of *Hypophthalmicthyesmolitrix*showing Vacuolization (VC),Nucleus atrophy (NA), Large vacuolization (LV), Degenerated cells (DC),Cluster of hepatocytes (CH) necrosis (N) cluster of hepatocytes (CH).*(H & E 400X).*

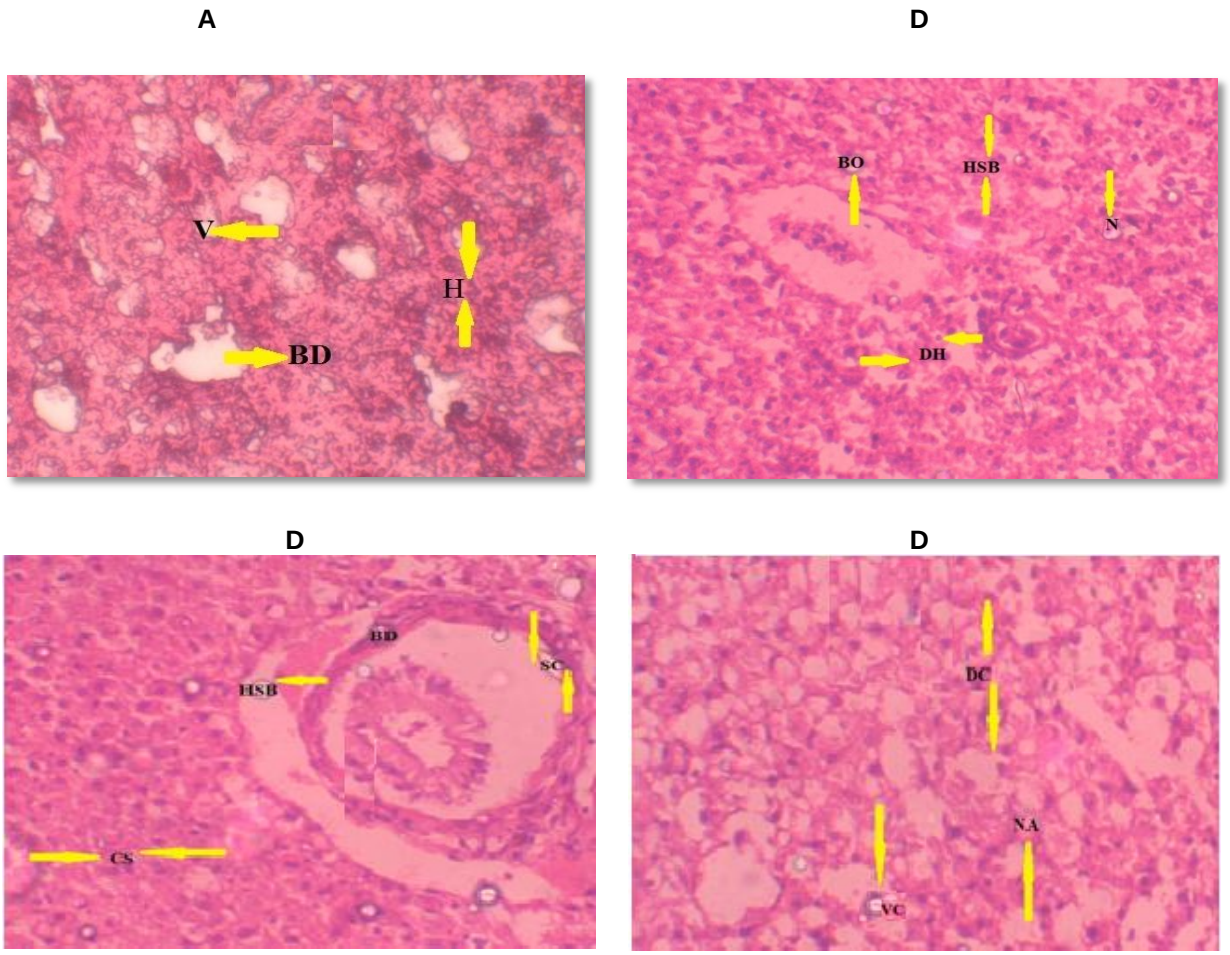

Figure 3. Photomicrograph of liver of *Hypophthalmicthyes molitrix* (A) section of control group showing vessels (V), bile duct (BD), hepatocytes (H). (D) after acute exposure liver of *Hypophthalmicthyes molitrix* showing bile duct obstruction (BO), degenerated hepatocytes (DH), necrosis (N), hepatocytes separated by sinusoid containing erythrocytes (HSB), bile duct (BD), cluster of cells (CS), separation of cells (SC), vacuolization (VC), nucleus atrophy(NA), damaged cells (DC). *(H & E 400X).*

DISCUSSION

In the aquatic environment non-target organisms especially fish have threat from toxic effect of pollutant such as pesticides (Elia *et al*., 2002). For the rapid detection of toxic effects of toxicants on organs histopathology is used (Johnson *et al*., 1993). In fish organs number of lesions are produced when it is exposed to water pollutants (Bucke *et al*., 1996).Liver in fish is surrounded by connective tissues called capsule, inside it there is a central vein and parenchyma which consist of hepatocytes arranged as two rows leaving capillaries between them called sinusoids lining with incomplete epithelial cells. Increased concentrations of toxic compounds cause structural harms. Due to which liver does not perform its regulatory mechanism (Brusl *et al.,* 1996). The risky effect on the liver histology of the catfish (*Clariasgariepinus*) was examined after exposure to fenvalerate pyrethroids, 1/10LC for 5 and 10 days (Sakr *et al*., 2005). The results demonstrate that the histological alteration in the liver were mainly of the inflammatory leucocytes infiltration, blood vessel congestion, necrosis and hepatocytes vacuolization. Nuclear pycnosis, increase of kupffer cells, fatty degeneration, necrosis, Hypertrophy of hepatocytes ,thinning of

sinusoids, and circulatory disturbances has been reported in *C. punctatus,* ,*C. carpio and G. affinis* exposed to deltamethrin (Cengiz and Unlu, 2006). Liver tissue of *C. carpio and O. niloticus* had also similar histological changes after exposure to sub-lethal concentrations of cyfluthrin and carbaryl respectively (Matos *et al.,* 2007; Sepici-Dinçel *et al*., 2009). Pesticide toxicity alters metabolic conversion process in the hepatocytes endoplasmatic reticulum, disturbing the structure of the liver and leads to necrosis of hepatocytes. Several authors observed similar findings in different fish species (Narra *et al*., 2015; Dogan and Can, 2011; Monteiro *et al*., 2009). Bifenthrin causes degeneration of hepatocytes, cytoplasmic vacuolization and pycnotic nuclei. Alanine amino-transferase (ALT) and aspartate amino-transferase (AST) are found in the liver, gills, skeletal muscle, erythrocyte, kidney, pancreas, spleen, hearts and brain (Banaee *et al.,* 2011). These enzymes are leaked into plasma when the cells are damage. (Keizer *et al*., 1995) Increased in permeability of hepatocyte membrane was observed due to lipid per oxidation. This is due to increased in intracellular level of ROS. Increased permeability of hepatocyte membrane cause seepage of liver enzymes including ALT and AST into plasma.

Increased activities of ALT and AST were observed in plasma of *Channa punctatus* exposed to organophosphorus pesticides (Agrahari *et al*., 2007). In another study increased levels of AST and ALT were observed when common carp was exposed to diazinon (Banaee *et al*., 2012). ALT and AST concentration remain high if the cellular damage is persistent (Banaee *et al*., 2011).These results are similar to our findings in which increased in level of AST and ALT in blood of Silver carp were observed due to liver damage after deltamethrin exposure.

REFERENCES

AGRAHARI, S., PANDEY, K.C. AND GOPAL, K., 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, Channapunctatus (Bloch). *Pesticide Biochemistry and Physiology,* **88**(3): 268-272

ARJMANDI, R., TAVAKOL, M. AND SHAYEGHI, M., 2010. Determination of organophosphorus insecticide residues in the rice paddies. *International Journal Environmental Science Technology*, **7**(1): 175-182.

AYDIN, R., KÖPRÜCÜ, K., DÖRÜCÜ, M., KÖPRÜCÜSS, P.M., 2005. Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. *AquacultInt*., **13**: 451–8.

BALINT, T., FERENCZY, J., KATAI F., *ET AL.,* 1997. Similarities and differences between the massive eel (*Anguilla anguilla L*.) devastations that occurred in Lake Balaton in 1991 and 1995. *Ecotoxicoogyl Environmental Saftey.,* **37**: 17–23.

BANAEE, M. AND AHMADI, K., 2011. Sub-lethal toxicity impacts of endosulfan on some biochemical parameters of the freshwater crayfish (*Astacus leptodactylus*). *Research Journal of Environmental Sciences.,* **5**(11): 827-835.

BANAEE, M., MIRVAGHEFEI, A. R., MAJAZIAMIRI, B., RAFEI, G.R. AND NEMATDOST, B., 2011. Hematological and Histopathological study of experimental diazinon poisoning in common carp fish (*Cyprinus carpio*). *Iranian Journal of Natural Resources,* **64**(1): 1-14.

BANAEE, M., MIRVAGHEFEI, A.R., MAJAZIAMIRI, B. AND RAFEI, G.R., 2012. Biochemical, blood and histopathological study of experimental diazinon poisoning in common carp fish (*Cyprinus carpio*). *Iranian Journal of Natural Resources.,* **65**(2): 119-133.

BANAEE, M., SUREDA, A., MIRVAGEFEI, A. R. AND AHMADI, K., 2013. Biochemical and histological changes in the liver tissue of Rainbow trout *(Oncorhynchus mykiss)* exposed to sub-lethal concentrations of diazinon. *Fish Physiolgy Biochemistery.* **39**(3): 489-501.

BRADBURY, S.P. AND COATS, J.R., 1989. Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. *Environmental Toxicology Chemistry,* **8**: 373–80.

BRUSL, E.J., GONZ`ALEZ, I. AND ANADON, G., 1996. The structure and function of fish liver. In: *Fish Morphology* (Eds. J.S.D. Munshi and H.M. Dutta), *Science Publishers Inc., New York.*

BUCKE, D., VETHAAK, D., LANG, T. AND MELLERGAARD, S., 1996. Common diseases and parasites of fish in the north atlantic: training guide for identification. *International Council for the Exploration of the Sea Techniques in Marine. Environmental Sciences, Copenhagen.*

CENGIZ, E.I. AND UNLU, E., 2006. Sub-lethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquito fish, Gambusiaaffinis: A microscopic study. *Environmental Toxicology and Pharmacology*. **2**: 246-253.

DAVID, M., SHIVAKUMAR, H.B., SHIVAKUMAR, R., MUSHIGERI, S.B. AND GANTI, B.H., 2003. Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the freshwater fish, *Tilapia mossambica. Indian Journal of Environmental Toxicology*. **13**: 99–102.

DOGAN, D. AND CAN, C., 2011. Endocrine disruption and altered biochemical indices in male *Oncorhynchus mykiss* in response to dimethoate Pest. *Biochemical Physiology*, **99**: 157–161.

ELIA, A.C. WALLER, W.T. AND NORTON, S.J., 2002. Biochemical responses of bluegill sunfish *Lepomis macrochirus,* Rafinesque) to atrazine induced oxidative stress. *Bulletin Environmental Contamination Toxicoogy,* **168**: 809–816.

HAYA, K., 1989. Toxicity of pyrethroid insecticide to fish. *Environ mental Toxicology Chemistry,* **8**: 381–91.

JOHNSON, L.L., STEHR, C.M., OLSON, O.P., MYERS, M.S., PIERCE, S.M., WIGREN,C. A., MCCAIN, B.B. AND VARANASI, U., 1993. Chemical contaminants and hepatic lesions in winter flounder *(Pleuronectes americanus)* from the Northeast Coast of the United States. *Environmental Science Technoogyl.,* **27**: 2759–71.

KEIZERJ, D., AGOSTINO, G., NAGEL, R., VOLPE, T., GNEMID, P. AND VITTOZZI, L., 1995. Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data Insecticides - *Development of Safer and More Effective Technologies* 134.

MAHBOOB, S., NIAZI, F., ALGHANIM, K., SULTANA, S., AL-MISNED, F. AND AHMED, Z., 2015. Health risks associated with pesticide residues in water, sediments and the muscle tissues of *Catla catla* at Head Balloki on the River Ravi. *Environmental Monitoring Assessment,* **187**: 81.

MATOS, P., FONTAINHAS-FERNANDES, A., PEIXOTO, F., CARROLA, J. AND ROCHA, E., 2007. Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. *Pesticide Biochemistry and Physiology*. **89**: 73-80.

MONTEIRO, D., RANTIN, F. AND KALININ, A., 2009. The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxã, *Bryconcephalus* Günther, 1869) exposed to organophosphate insecticide Folisuper 600 BR® methyl parathion: *Computinol Biochemical Physiology*. **149**: 40–49.

MOORE, A. AND WARING, C.P., 2001. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). *Aquatic Toxicology*., **52**: 112.

MUMFORD, S., 2007. Submitting samples and tissue processing. In: *Fish histology and histopathology* (Eds. S. Mumford, J. Heidel, C. Smith, J. Morrison, B. MacConnell, V. Blazer). USFWS-NCTC.

NARRA, MADHUSUDAN, R., RAJENDER, K., RUDRA, R., RAO, JV. AND BEGUM, G., 2015. The role of vitamin C as antioxidant in protection biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. *Chemosphere,* **132**: 172–178.

RAHIMINEZHHAD, M.; SHAHTAHERI, S.J.; GANJALIM, R.; RAHIMIFOROUSHANI, A.G.F., 2009: Moleculary imprinted soild phase extraction for trace analysis of diazinon in drinking water. *Iranian Journal of Environmental Health Science and Engineering* **6**: 97-106.

RODRIGUES, E.L. AND FANTA, E., 1998. Liver histopathology of the fish Brachydaniorerio after acute exposure to sublethal levels of the organophosphate Dimetoato 500. *Revista Brasileira de Zoologia*, **15:** 441-450.

SAKR, S.A. AND JAMAL, S.M.A.L., 2005. Fenvalerate Induced Histopathological and histochemical changes in the liver of the catfish *Clarias Gariepinus. Journal of Applied Sciences Research,* **1**(3): 263-267.

SCHLENK, D., 2005. Pesticide biotransformation in fish In: *Biochemistry and molecular biology of fishes,* (Eds. T.P. Mommsen, T.W. Moon), Elsevier B.V., **6**: 171-190.

SEPICI-DINÇEL, A., BENLI, A.Ç.K., SELVI, M., SARIKAYA, R., SAHIN, D., ÖZKUL, I. A. AND ERKOÇ, F., 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: Biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety,* **72:** 1433-1439.

SHARMA, R., PESHIN, R., SHANKAR, U., KAUL, V., AND SHARMA, S., 2015. Impact evaluation indicators of an integrated pest management program in vegetable crops in the subtropical region of Jammu and Kashmir, India. *Crop Protection,* **67**: 191–199.

VAN DEROOSTS, R., BEYER, J. AND VERMEULENN, P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology Pharmacology,* **13**: 57-149.

YADAV, I.C., DEVI, L., SYED, H., CHENG, Z., Li, J., ZHANG, G. AND JONES, K., 2015. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries, a comprehensive review of India. *Science of Total Environmental*, **511**: 123–137.

YOUSAFZA, A.M. AND SHAKOORI, A.R., 2011. Hepatic cypermethrin on tilapia (*Oreochromis niloticus*) responses of a freshwater fish against aquatic Larvae. *Bulletin of Environmental Contamination and Toxicology,* **74**: 880-885.