WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 10, 684-693 Research Article ISSN 2278 – 4357

STUDIES ON THE HEPATOPATHOLOGICAL EFFECT OF BENZENE HEXACHLORIDE ON A TELEOST FISH, *CYPRINUS CARPIO COMMUNIS*

Dr. Rakesh Singh[1], Dr. Ajit Hundet[2]* and Barun K. Prabhat[3]

[1]M V M Bhopal.

[2]GOV. D B Girls PG College, Raipur C.G.).

[3]C V Raman University, Kota Bilaspur, (C.G.).

Article Received on
24 July 2017,

Revised on 14 August 2017,
Accepted on 04 Sept. 2017,

DOI: 10.20959/wjpps201710-10165

***Corresponding Author**
Dr. Ajit Hundet
GOV. D B Girls PG College, Raipur C.G.)

ABSTRACT

We investigated the histopathological changes in the structure of liver cells in response to three and five part per million concentration of benzene hexachloride (BHC) under light microscopy with a view to analyze the damages caused because of extensive use of the pesticide. We found that in response to 3-and 5-ppm BHC intoxication, liver showed gangrene formation, vacuolization, sponginess, complete separation of blood vessels from hepatic cells, atrophy of hepatic nucleus in addition to shrinking of pancreatic tissue. Our studies indicate that the organochlorine pesticide, BHC, is very detrimental for the aquatic system as it produced pronounced alterations in the organ.

KEYWORDS: BHC, shrinking, sponginess, liver, gangrene, vacuolization.

INTRODUCTION

Pesticides are used at large scale in in husbandry, forestry, public health and in so many practices. Organochlorine (OC) pesticides are notorious for their high toxicity, persistence in the environment and ability to enter the food chain.[1] These COPs are both long-standing in watery systems and unaffected to degradation by the action of light[2] [3] reported the concentration of different pesticides such as endosulfon (15.21 ng/L) and γ-BHC (15.54 ng/L) in water samples and gamma-BHC (21.23 µg/kg) and parathion (19.14 µg/kg) in sediment samples in the liver of *H. fossilis* in the river of Kali. A detailed review on such literatures which are available on fish and intoxicants suggests that sublethal doses of most of

the pesticides cause histopathological injuries up to different extent in unlike organs, such as liver, pancreas, kidney, intestine, etc. of fishes. The extent to which alteration is produced is related to dose, duration of exposure and the type of pesticides used.[4] [5] [6] [7] [8] [9] It has been found that most of the pollutants induce mortality, genotoxicity and histopathological as well as histochemical alterations and thereby impair respiration, metabolism and many enzyme activities in the affected fishes. These chemicals have a tendency to accumulate in minute concentration in fish foods and biomagnify in fish flesh. Hence, they may have adverse effect on the well-being of humankind. Therefore, it necessitates the studying of the pesticidal effects on fish, as fishes constitute a greater part of food of not only human population but also other predatory birds as well as other animals over the entire world to gauge the ill effect of the pesticide. Hence the present experiment was designed to the carry out studying the effects of one of the such chlorinated hydrocarbons on fish liver which is metabolic center carrying the detoxification processes.

MATERIAL AND METHODS

Samples of alive fish *C. carpio* were obtained from the fish farm "Patra and Bhadhada" located in Bhopal. Succeeding treating with 0.1% potassium permanganate solution for a quarter of an hour to throw way any dermal infections, the fishes were acclimatized in aquaria, made of glass, for about 7 days. Healthy fishes measuring about 8 to 20 centimeters and weighing approximately 100 to 125 grams were selected for the experiment. Sets of 10 fishes were transferred to three differently maintained glass aquaria. Out of these aquaria, one contained dechlorinated water and rest of the two contained 3- and 5- ppm concentration of benzene hexachloride (BHC). 5-fishes were sacrificed from each group after a gap of ten days twice. These fishes were provided standard fish food daily during the tenure of experimentation. Liver of the sacrificed fishes were taken out, cut into small pieces and kept first in normal saline to remove blood then fixed into desired fixatives followed by dehydration, block preparation, sectioning (6 to 8 micron thick section), staining and examining under light microscope for histopathological changes.

RESULT

The 6-8-micron thin section of the control liver exhibited polyhedral cells arranged in a group of 4 to 6 cells having central nucleus with nucleoli. The liver cells are arranged in lobules that lack definite pattern of arrangement. Phagocytes are found in connective tissues. Few blood spaces of different sizes appear here and there throughout. Ducts of Hepatic cells are much

larger than those of the biliary. The pancreatic cells, which are in diffused condition among the hepatic cells, are clustered around the branches of hepatic portal vein (HPV) leaving a gap between hepatic and pancreatic cells. The pancreatic mass consists of two — exocrine and endocrine — cells. Exocrine cells are larger and elongated while endocrine cells are rounded and smaller. Endocrine cells are disseminated about hepatic portal vein and in between exocrine cells which are organized into acini (figure 1 &2).

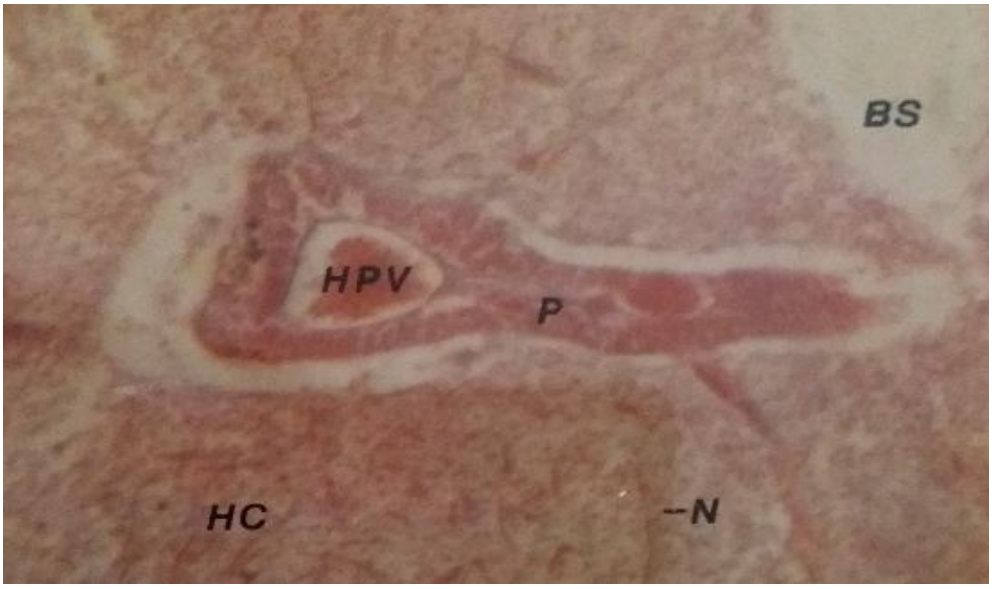

Figure 1 Pictomicrograph of transverse **section of the liver of control Cyprinus carpio (Mallory triple Atain) X-100.**

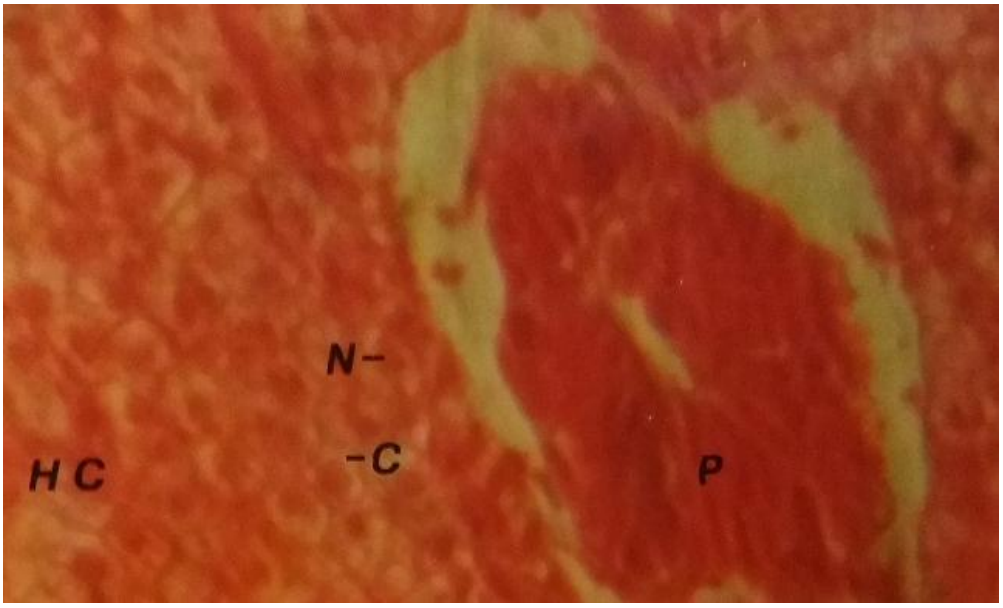

Figure 2 Pictomicrograph of transverse section of the liver of control C. carpio (H & E staining) X-100.

BHC Intoxication

In fishes treated with 3 ppm BHC for 10 days, small spaces appeared in between hepatic cords, nucleus displaced slightly, large connective tissues and cell membrane of the hepatic cells broke and complete separation of blood vessels from hepatic cells besides reduction of pancreatic tissue were found. Thereby leaving a very narrow opening of the hepatic portal vein (Fig.3). Twenty days exposure caused formation of so many small spaces between hepatic cords, connective tissues destructed completely and atrophy of nucleus in hepatic cells. No further changes were seen in the cytoplasm of the hepatic cells. Pancreatic tissues shrank obliterating the differentiation between the exocrine and endocrine pancreas.

The tissues treated with 5 ppm BHC for 10 days showed a large number of vacuoles in between hepatic cells, liver converted into spongy mass, heavy necrosis in the hepatic cells, outer wall of the blood vessels reduced in thickness while in some cases it broke. Large spaces appeared around the blood vessels and the pancreatic mass. Pancreatic mass appeared in shrunken condition. No further variation was seen after twenty days exposure except vacuolation in and around hepatic cells increased. (Figure 4).

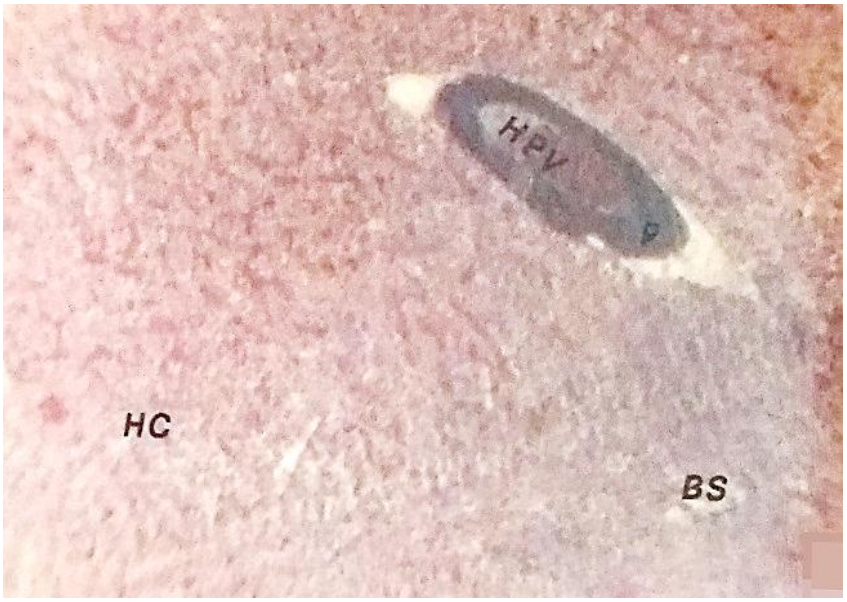

Figure 3 Pictomicrograph of transverse section of the liver of C carpio after 10 days exposure to 3-ppm BHC (Mallory Triple Stain) X-100.

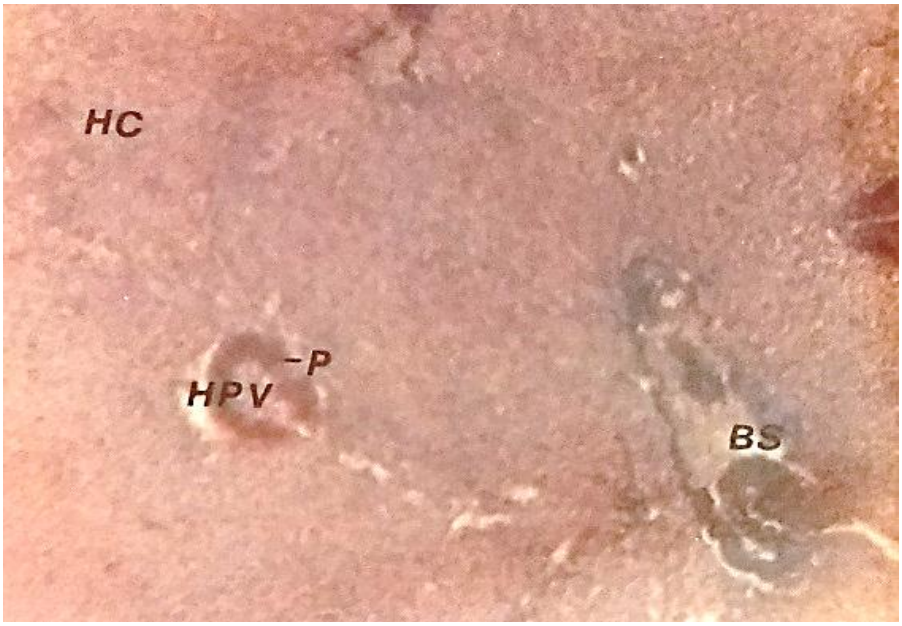

Figure 4 Pictomicrograph of transverse section of the liver of C carpio afterr 20 days exposure to 5-ppm BHC (Mallory triple Stain) X-100.

DISCUSSION

Our investigation describes the alterations in the histopathological structure of the liver cells in a bony fish named, *Cyprinus carpio,* following treatment with benzenehexachloride (BHC). We observed that the pancreatic tissues were distributed in between cells, which boosts the observation of[10] in *Labeo calbasu,*[11] in *Channa gachua* and[12] in *Glossogobius giuris*. We have observed the polyhedral hepatic cells having granular cytoplasm and arranged in hepatic cords, liver mass navigated by hepatic ducts and pancreas by hepatic portal vein and inconspicuous liver cords. These findings upkeep the outcomes of[13] in *Mystus tengra,*[11] in *Channa gachua* and[14] in *Channa punctatus* who also witnessed the pancreatic cells in acinus having exocrine and endocrine cells with acinar lumen traversed by the branches of hepatic portal vein. Such arrangements of pancreatic cells are also observed in the present analysis.[15] found eccentric nucleus showing displacement in its position besides mortification and vacuolations in hepatic cells of *Labeo rohita* following Heptachlor treatment. Necrosis (localized death of living cells) and vacuolization were also reported by[16] in *Molliensa sphenops,*[17] in *Channa punctatus* and[11] in *Channa gachua*. Analogous results are also obtained in the present investigations in the liver of *Cyprinus carpio* in response to 3- and -5 ppm BHC exposure after ten and twenty days. Our observations are in conformity with result of[18] who witnessed the presence of binucleated liver cells that are scattered in the tissue as a result of Endrin intoxication in *Clarias batrachus*.[19] reported

vacuolation, necrosis and liquification of liver cells in *Colisa fasciatus* after Lindane intoxication,[20] reported wide spread vacuoli-zation, localized necrosis and deformation of hepatic cells in the liver of *Clarias batrachus* after the exposure of Alloxan.[21] have also reported grume, hepatic vacuolations and nuclear atrophy and complete degeneration of pancreatic cells in *C. carpio* in response to Endosulfan toxicity.[22] noticed destruction of hepatocytes and islet cells after the exposure to Thiodan in the liver of *Gymonocorymbus tarzetzi*, while Agallol resulted in the degeneration of hepatocyte nucleus and islet cells in addition to local localized death of live hepatic cells.[23] reported vacuolation and slight change in hepatic cells with low concentration of Aldrin in the liver of *Cyprinus carpio*, while in higher concentration they observed degeneration of liver cells. They observed deformation in the shape of hepatic cells. Similar observations were made by[24] and[25] in the liver of *Channa punctatus* and *Clarias batrachus* respectively after pesticidal exposures.[26] studied the lethal effects of the insecticide, Thiometon on *Labeo rohita* and *Heteropneustes fossilis*. He observed histopathological lesions at 2.02 ppm exposure in *Labeo rohita*. In liver, the damage was greater in the peripheral region than in the central part. The lesions in the hepatic cells showed degeneration, rupture, vacuolation and nuclear extrusion. Some hepatic cells became hollow due to complete degeneration. In some of the cells cytoplasm and nuclei were extruded from hepatic cells. The nuclei showed vacuolation, degeneration and distortion. In *Heteropneustes fossilis*, lesions were produced after 15 and 30 ppm intoxication. Polygonal hepatic cells became spherical and showed hypertrophy, cytoplasmic degeneration and vacuolation. Intercellular spaces were enlarged. Similar observations were reported by[27], [28] reported enlargement of nuclei and necrosis of hepatocytes in the liver of *Channa gachua* after Endosulfan intoxication. In longer exposure of 30 days, necrosis and hypertrophy was increased and vacuolation appeared in the hepatic cells. Similar changes were observed in the liver mass *Glossogobius giuris* after Carbofuran intoxication by.[12]

Similar effects have been reported by[29] due to Tannic acid and by[30] due to Malathion exposure.[31] reported liver cord disarray and severe destruction of hepatocytes resulting in accumu-lation of pycnotic nuclei. Similar results were reported by[32] in the liver of *Tilapia mossambica* exposed to Heptachlor. They have also noticed damage in the liver mass that increased with the increase of exposure timings. In most of the cases congestion, swelling and pyknosis of hepatocytic nuclei with cytoplasmic vacuolation as well as clumping of blood cells.[33] have also showed abnormal fatty degeneration in liver, necrosis, and congestion at a concentration of BHC of 1.00mg/L.

CONCLUSION

As liver is the principal organ that detoxify harmful chemicals and it under goes such a tremendous alterations such as death of hepatic cells, vacuole formation, destruction of pancreas, etc. in response to the exposure of the pesticide which will not only reduces the quality of fish but also affect other organism and of course human being is no exception. Therefore, the use of the pesticide should be diminished and other sources, which are less harmfeul, or harmless should be explored.

ACKNOWLEDGEMENT

No financial support was available for conducting this experiment.

ABBREVIATIONS

HC – Hepatic Cell

BS – Blood Space

HPV – Hepatic Portal Vein

P – Pancreas

N – Nucleus

C - Cytoplasm

REFERENCES

1. J. W. Ntow, "Pesticide Residues in Volta Lake, Ghana. Lakes & Reservoirs," *Research and Management.* 2005; 10: 243-218.
2. M. Shokrzadeh, S. Saeedi Saravi and Y. Y. Zehtab, "Lindane Residues in Cultivated Cucumber and in the Most Consumed Fish in Caspian Sea (Iran).," *Toxicol Ind Health,* 2009; 25: 517-527.
3. P. K. Maurya and D. S. Malik , "Accumulation and distribution of organochlorine and organophosphorus pesticide residues in water, sediments and fishes, Heteropneustis fossilis and Puntius ticto from Kali River, India," *Journal of Toxicology and Environmental Health Sciences,* 2016; 8(5): 30-40.
4. S. K. Kulshrestha and N. Arora , "Fish and pesticides-histopathological parameters," *Intl. J. Acad. Ichthyol.* 1984; 5: 1-9.
5. A. Bhagade, "Impact of bioaccumulation of few PCBs and PAHs in freshwater fish Rasbora daniconius and Puntius ticto.," 2012.
6. R. C. A. Oliviera, Y. Vollaire, A. Sanchez-Chardi and H. Roche, "Bioaccumulation and the effect of organochlorine pesticides, PAH and heavy metals in the eel (Anguilla

anguilla) at the Camargue Nature Reserve, France," *Aquatic Toxicology*. 2005; 74(1): 53-69.

7. A. Esam, "Histopathological liver alterations in juvenile rabbit fish (Siganus canaliculatus) exposed to light Arabian crude oil, dispersed oil and dispersant.," *Ecotoxicology & Environmental Safety*. 2012; 15: 171-179.
8. M. P. C. Marina and B. M. Claudia, "Histopathology of gills, kidney and liver of Neotropical fish caged in an urban stream.," *Neotropical Ichthyology*. 2007; 5(3): 327-336.
9. P. Mario and A. S. Maria, "Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (Anguilla anguilla L.).," *Ecotoxicology & Environmental Safety*. 2002; 53(3): 331-347.
10. O. P. Jain, Morphohistology and histochemistry of the digestive glands in certain fresh water teleosts. Bhopal: Bhopal university, 1980.
11. S. Kasotia, *Pesticidal efects on gastrointestinal tract in fresh water teleost: Channa gachua*, Bhopal, 1988.
12. U. K. Mishra, Acute toxicity of certain pesticides to a fresh water teleost Glossogobius guiris. Ph.D. Thesis, Bhopal Univ. 1988.
13. S. Kaur, H. S. Toor and K. Kaur, "Histopathological changes in the digestive tract and liver of Mystus tengra exposed to Carbaryl and Endrin," in *All Indis Seminar om Icthyology*, Meerut, 1977.
14. B. S. Jha, "Histopathological changes under lead exposure in the liver of a fresh water fish, Channa punctatus (Bloch.)," *Him. J. Env. Zool.* 1990; 116-120.
15. S. K. Konar, "Some effects of sublethal levels of Heptachlor on rohu, Labeo rohita (Ham.).," *J. Inland Fish Soc. Ind.* 1970; 2: 51-54.
16. G. Chakravarty and S. K. Konar, "Chronic effects of sublethal levels of pesticides on fish," *Proc. Natl. Acad. Sci. india.* 1974; 44: 241-246.
17. K. V. Sastry and S. K. Sharma, "Endrin toxicity in liver of Channa punctatus (Bloch.)," *I. JEE*. 1978; 16: 372-373.
18. M. C. Bhattacharya, S. Mukherjee and S. Bhattacharya, "Toxic effects of endrin on hepatopancreas of fish Clarias batrachus," *Ind. J. Exp.* 1975; 13: 185-186.
19. S. R. Verma, S. P. Gupta and M. P. Tyagi, "Studies on the toxicity of lindane on Colisa fasciatus. TLM measurements and histopathological changes in certain tisssues. Gagenboar," *Morph. Jahrb. Leipzig.* 1975; 121: 38-54.

20. K. A. Goel and Agarwal, "Histopathological effects of long term Alloxan treatment on liver and kidney in fresh water teleost, Clarias batrachus.," in *All India Seminar on ichthyology*, Meerut, 1977.
21. A. Hundet and B. K. Prabhat, "Histopathological alterations in hepatopancreas of a carp fish C. carpio due to endosulfan toxicity," *Cibtech Journal of Zoology. ISSN: 2319–3883 (Online);* 2014; 3(1): 7-11, January-April.
22. C. K. Amminikutty and M. S. Rege, "Effects of acute and chronic exposure to pesticides Thiodon E.C. 35 and Agallol 3 on the liver of Widow tetra Gymonocorymbus tarzetzi (Boulenga).," *Ind. J. Exp. Biol.* 1977; 15: 197-200.
23. V. Ratnakar and C. Awasthy, "Toxicity of Aldrin to Cyprinus carpio.," *Proc. Symp. Environ. Biol.* 1979; 377-382.
24. K. Sastry and S. Sharma, "Toxic effects of endrin on liver and kidney of a fresh water teleost fish.," *Proc. Symp. Environ. Biol.* 1979; 337-342.
25. P. k. Mandal and A. K. Kulshrestha, "Histopathological changes induced by the sublethal Sumithion in Clarias batrachus.," *Ind. J. Exp. Biol.* 1980; 18(5): 547-552.
26. S. K. Konar, "Lethal effects of the insecticide Thiometon on the carp (Labeo rohita) and cat fish (Heteropneustes fossilis).," *Proc. Nat. Acad. Sci. India.* 1983; 53B(3): 178-182.
27. N. Qureshi, Q. Shammi and R. Sharma, "Histopathology of Malathion on liver of a cat fish Clarias batrachus (Linn.).," *Ind. J. Zool.* 1983; 11(1): 67-70.
28. M. C. Bhatnagar, A. K. Bana and R. C. Dalela, "Histopathological alterations in the liver of Channa gachua Ham.) exposed to endosulfan.," *Acad. of Environ. Bio. (Environ. & Pesticide Toxicity).* 1987.
29. S. Viswaranjan, S. Beena and A. Palavesam, "Effects of Tannic acid on the protein, carbohydrate and lipid levels in tissue of the fish Oreochromis mosambicus.," *Environ. & Ecol.* 1988; 6(2): 289-292.
30. U. K. Mishra and O. P. Jain, "Malathion toxicity on the intestine and liver of Glossogobius guiris (Ham.).," *Proc. Nat. Symp. Past, Present & Future of Bhopal Lakes.* 1988; 189-193.
31. R. Bakthavathsalam and C. Rajaretnam, "Effects of Lindane and Atropine sulphate on the digestive tissues of Anabas testudineus (Bloch.).," *Ind. J. Environ. Hlth.* 1990; 32(3): 284-288.
32. P. Rao, K. Vijay Joseph and K. Jayantha Rao, "Histopathological and biochemical changes in the liver of a fresh water fish exposed to Heptachlor.," *J. Nat. Con.* 1990; 2(2): 133-137.

33. S. Satyanarayan, J. P. Kotangale, S. Satyanarayan and S. Verma, "Histopathological changes due to some chlorinated hydrocarbon pesticides in the tissues to cyprinus carpio.," *IOSR Journal of Pharmacy*. 2012; 2(6): 60-66.
34. S. Satyanarayan, J. P. Kotangale, A. Satyanarayan and S. Verma, "Histopathological changes due to some chlorinated hydrocarbon pesticides in the tissues to cyprinus carpio.," *IOSR Journal of Pharmacy e-ISSN: 2250-3013*. 2012; 2(6): 60-66.