The Science Probe *Vol. 1 No. 1 (May 2012)* *Page No- 1-13* *ISSN:*

A Study on the Effect of Organophosphorus Pesticide Malathion on Hepato-Renal and Reproductive Organs of *Heteropneustes fossilis* (Bloch)

Sanjoy Deka[1] and Rita Mahanta[2]

[1]Research Scholar, Dept. of Zoology, Gauhati University

email-sanjumon78@rediffmail.com.

[2]Associate Professor, Dept. of Zoology, Cotton College

ritamahanta@yahoo.co.in

ABSTRACT

Organophosphorus pesticides are used in the agricultural field due to their rapid biodegradability and non-persistent nature to control the pest but their broad spectrum of harmful effects extends far beyond the pest. It is proved by the facts that are experimentally studied by numbers of investigators with the application of varying concentration of pesticides on the living organisms around the world. Main objective of this paper is to carryout an empirical study to investigate the effect of sub-lethal Malathion on liver, kidney and ovary of the freshwater catfish "*Heteropneustes fossilis.*". LC_{50} value of Malathion was calculated by probit analysis (Finney, 1964) and LC_{50} for 96 hours is found to be 0.98 ppm. Sub-lethal concentration of 0.2 ppm is prepared by using standard technique (APHA, 1985). For this study, Control Group was being freed from the treatment of Malathion where as experimental group was treated with sub-lethal Malathion concentration of 0.2 ppm. Histological tissues were collected from both control group and experimental group at three different time interval up to one month and the technique of MICROTOMY is being used for the histopathological study of the tissues.

KEY WORDS: Catfish, Liver, Kidney, Ovary, Malathion, LC_{50}, Microtomy, Histopathology

INTRODUCTION

Pesticides, the biologically active chemicals are used to a great extent for pest control but their spectrum of activity often extends far beyond the pest. Malathion is reported to have a low toxicity to mammals and relatively high toxicity to fish (Mount and Stephen, 1967).[1] Malathion can reach the aquatic environment by the means of direct application, spray, drift, aerial spraying, and washing, from the atmosphere by precipitation, erosion and runoff from agricultural land, in factory effluents and in sewage (Edwards, 1973).[2] Frank *et al.* (1990) reported contamination of farm wells by various pesticides including Malathion.[3]

The magnitude of pesticide pollution was studied in the Indian fishes by various workers (Dalela *et al*., 1979; Dubale and Shah, 1984; Pandey and Shukla, 1980; Rashatwar and Ilyas, 1984; Sadhu and Mukhopadhyay, 1985; Shukla and Pandey, 1985, Ghosh and Chatterjee, 1989; Medda *et al.*, 1995, Bhattacharya *et al*., 1997; Munshi *et al.*, 1999, Rakesh *et al*., 2009, Naveed *et al.*,2010,).

Malathion, an organophosphate pesticide is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Like other pesticides, lethal and sub-lethal treatment of Malathion exerts various toxic effects on fish. Malaoxon, a primary metabolite of Malathion is 60 times more toxic than Malathion and it seriously and chronically poison the occupants living in the environment. Detectable liver injury in the fish *Heteropneustes fossilis* exposed to 30 ppm of rogor have been observed by Gadhia (1989).

Sakakushi (1972) reported liver damage and hepatic necrosis in fish under Malathion stress.

Dutta et al. (1994) also reported notable microscopic changes in ovigerous lamellae, oocytes at different stages of development and the nucleus of the immature oocyte of the catfish *Heteropneustes fossilis.*

Pugazhvendan *et al. (*2009) exposed *Ophicoephalus punctatus* for 7 days to Malathion at a concentration of 10, 12, 14,16,18,20 µ1/1 and 100ppm under laboratory conditions and reported severe histological changes in brain, liver, ovary and tissues of the fish.

Organophosphorus pesticides have broad spectrum of harmful effects in the living world. It is proved by the facts that are experimentally studied by numbers of investigators with the application of varying concentration of pesticides on the living organisms around the world. To rescue the living world from the harmful effects of these chemical substances as well as to find some alternative ways to control the pests, it is very much necessary to carry out an impressive research works on diversity of organisms by applying varying concentration of pesticides.

However, the reports on the effects of low dose of Malathion in Indian cat fishes are still scanty. In the light of above information and ideas, present investigation is aimed to study the "Effect of sub-lethal Malathion on liver, kidney and ovary of the catfish "*Heteropneustes fossili."*.

[1] Quoted in Chelliah R. Richmonds and Hiran M. Dutta. 1992. Effect of Malathion on Brain Acetylcholinesterase Activity of Bluegill Sunfish *Lepomis macrochirus.Bull. Environ. Contam. Toxicol.*49:431-435

[2] i*bidem*

[3] *ibid*

MATERIALS AND METHODS

Present investigation has been carried out to study the effect of sub-lethal Malathion on liver kidney and ovary of the freshwater Indian cat fish *Heteropneustes fossilis.* Healthy and sexually mature specimen of *Heteropneustes fossilis* of equal size group (12 ± 3 cm) and average weight (12 to 15 gm) are procured from the local market and the fishes were kept in glass aquarium containing 80 litres of fresh water in the laboratory at about water temperature 25 ± 3º c. They are acclimatized for 15 days in the experimental water in laboratory condition before the commencement of the experiment. The water of the aquarium was changed daily and fishes are fed daily with commercial fish food. Fishes are starved for 24 hours prior to the experiment and are not fed during the period of experiment (Dalela *et al.*, 1979).

The organophosphorus pesticide Malathion (50% E.C) is procured from local market and a pilot experiment was done to find out the LC_{50} value of Malathion by probit analysis (Finney, 1964) and LC_{50} for 96 hours is found to be 0.98 ppm. Sub-lethal concentration of 0.2 ppm is prepared by using standard technique (APHA, 1985). 0.2 ppm is the 50th part of LC_{50} respectively.

In this experiment, the specimens were kept in two experimental groups. **Control Group** is being freed from the treatment of Malathion and the **Experimental Group** is treated with pesticide Malathion of sub-lethal concentration of 0.2 ppm. Histological tissues were collected from both the group at three different time interval (10 days, 20 days and 30 days) up to one month for the study. The technique of **MICROTOMY** is being used for the histological study purpose of liver, kidney and ovary of the fish *Heteropneustes fossilis.*

RESULTS

1. Results of Malathion Treated Liver

1. (a) Control Group

The parenchyma cells like hepatocytes, billiary epithelial tissues, nuclei and non parenchyma tissues like bile ducts, hepatopancreas, arteries and veins of the liver in control groups were normal and systematically arranged (Fig. 1)**.**

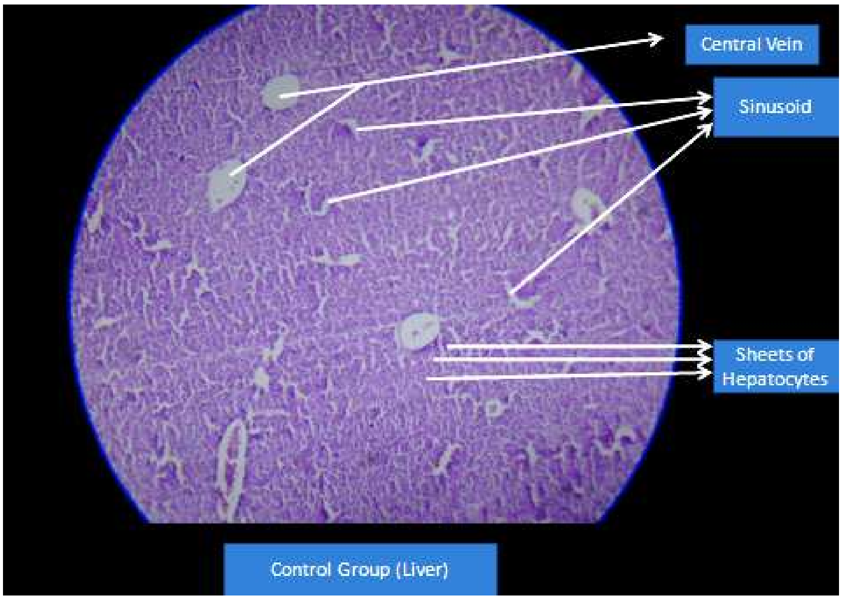

Fig 1: Showing the parenchyma and non parenchyma tissues of the liver in control groups were normal and systematically arranged.

1. (b) Experimental Groups

After **10 days** of interval, histopathological examination of the liver of *Heteropneustes fossilis* clearly shows that the parenchymal architecture of the liver is disturbed and hepatocyte show dissociation, the hepatocyte appears swollen and cytoplasm appears granular. The hepatocyte nuclei become pycnotic. During these 10 days of exposure, patchy degeneration and isolated degenerated elements around the parenchyma cells were observed with progressive increase of fibro connective tissue. As a result, signs of congestion were noticed at the sinusoid (Fig. 2).

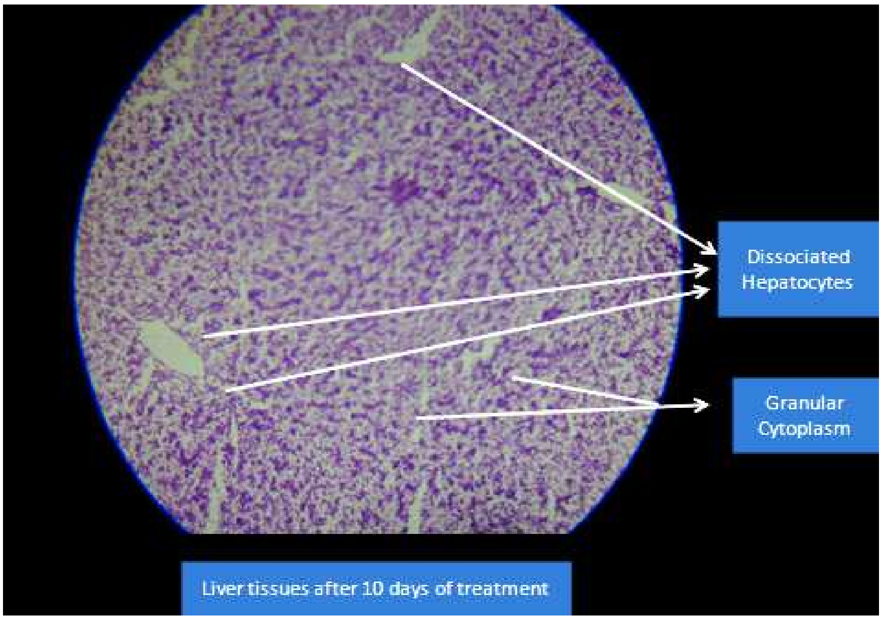

Fig 2: Showing the disturbed parenchymal architecture of the liver after 10 days of exposure to 02 ppm Malathion.

In long-term (**20 days)** treatment, the effect became more prominent with appearance of apoptotic cells. Blood capillary endothelium ruptured and blood was spilled into the liver tissues (Fig. 3).

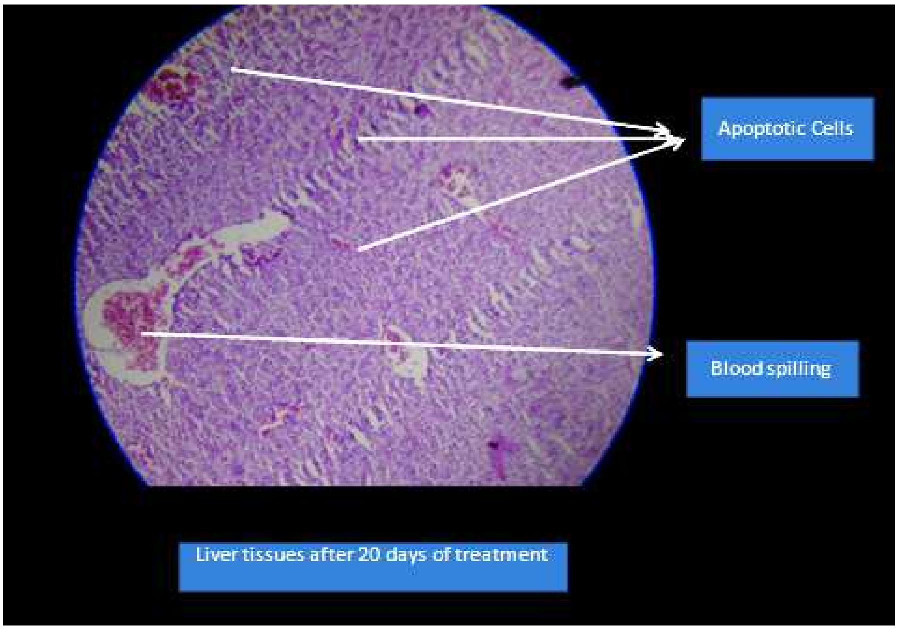

Fig 3: Showing apoptotic cells ruptured blood capillary endothelium after 20days of exposure to 0.2 ppm Malathion.

Acute and extensive necrosis of liver cells was observed particularly focal necrosis a common feature in catfish. The density of the connective tissue increased markedly leading to more congestion. The size was variable with concentration and was usually located in the vicinity of hepatic arteries and bile ducts.

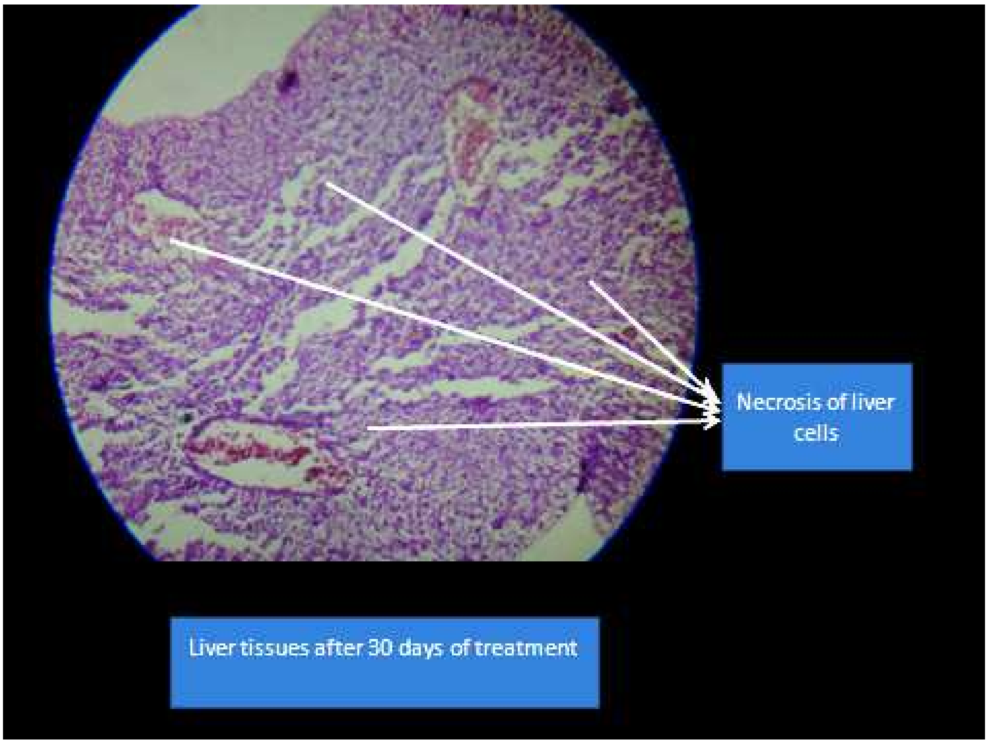

Fig 4: Showing acute and extensive necrosis of liver cells after 30days of exposure to 0.2 ppm Malathion.

At prolonged exposure after **30 days** of observation, the congestion impeded blood circulation resulting in tissue ischemic. Acute and extensive necrosis of liver cells was observed particularly focal necrosis a common feature in catfish (Fig. 4).

Hepatic tissue of treated specimens showed varied degree of hepatic cirrhosis as evident in the density of fibrous connective tissue within and around the hepatic parenchyma. Changes that occurred is also reflected in the treatment and consisted of damage to the biliary columnar epithelial cells which are separated from the connective tissue.

Discussion

Different investigators and authors noticed toxical changes in the liver of catfish after exposure to organophosphate and allied group of pesticides. Desai *et al.* (1984) reported histological changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos and found that at the initial stage of intoxication, necrosis and vacuolization of hepatocytes were recorded, while fatty degeneration was observed later on.

Elezaby *et al*. (2001) studied the effect of Malathion on the fish *Oreochromis niloticus* and has observed that Malathion induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis and lipidosis in the liver.

Shukla *et al.* (2005), noticed in his observation that when the catfish *Clarias batrachus* is exposed to the increased concentration (0.16/mL) of the organophosphate pesticide Nuvan, the hepatocytes exhibited reduction in their size and peripheral accumulation of cytoplasm. The nuclei of the hepatocytes lost their rounded appearance and the cell boundaries became obliterated at places after 20 days of pesticide exposure. The hemorrhage in liver was evident by increased volume of sinusoidal space.

The hazardous effect of the pyrethroid insecticide, fenvalerate on the histology and histochemistry of the liver of the catfish (*Clarias gariepinus*) after exposure to 1/10LC for 5 and 10 days was investigated by S.A.Sakr *et al.* (2005). The results showed that the histopathological changes induced in the liver were mainly represented by cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration, necrosis and fatty infiltrations.

In our present study we have recorded histopathological changes due to Malathion toxicity in the liver which mainly included architectural changes in the liver, hepatocytes swelling, dissociation of hepatocytes, hepatocytes showing pycnotic nuclei, broken sinusoidal endothelium, ruptured blood vessels with haemorrhage and vacuoles in the haepatocytes. At the dose of 0.2 ppm, severe necrotic hepatocytes, pyknosis, hypertrophy, haemorrhage and vacuolation were observed for the fishes in the experimental group. Fishes injected with experimental dose (0.2 ppm) showed areas with disrupted parenchymal architecture and necrosis. The hepatocyte nuclei began to condense and cytoplasm of these cells was highly vacuolated.

2. Results of Malathion Treated Kidney

2. (a) Control Group

Kidney tubules and haematopoietic cells were normal and systematically arranged in the control treatment for all the control fish species. Control group showing compact renal mass and renal tubules.

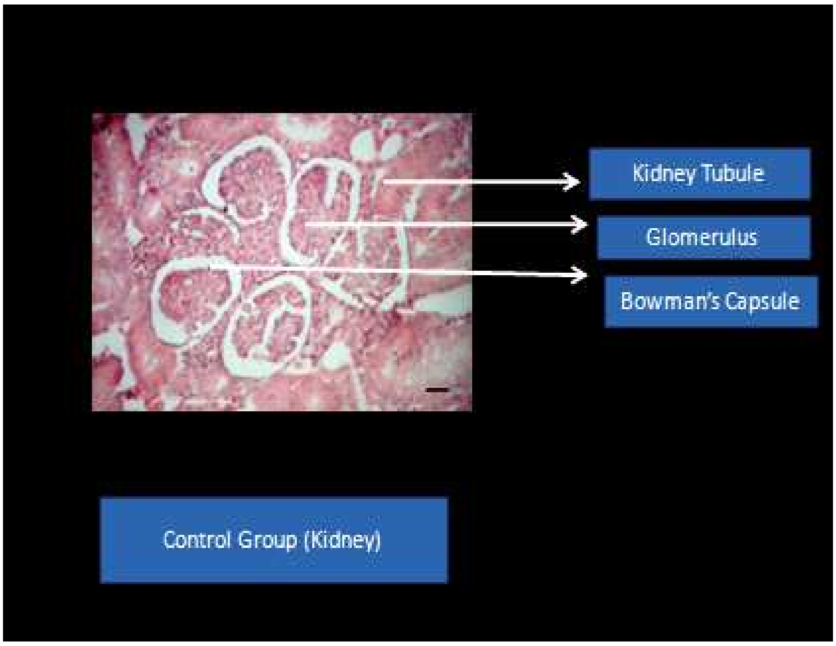

Fig 5: Showing normal and systematically arranged kidney tubules and haematopoietic cells

The normal renal cortex contains glomeruli, other vessels, tubules and interstitium. Normally, all the glomerular capillaries having the same thickness, which is very thin (almost wispy). With normal cellularity, cell nuclei are not clustered or overlapping. In the cortex but not the medulla, the tubules are almost back to back, i.e. the tubular basement membranes almost touching. There is very little interstitium in the cortex. Intrarenal arteries have very little intima, i.e. there is little or no space between the endothelium and the muscularis (Fig. 5).

2. (b) Experimental group

Lesions in the kidney tissues of fish exposed to Malathion were characterized by degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen.

Experimental group (0.2 ppm:**10 days**) showing disintegrated renal tubules and ruptured blood vessel and pyknosis; showing broken blood capillary and blood entering into the tissue; cells (lymphoid tissues) show necrosis and many cells show apoptosis (Fig. 6).

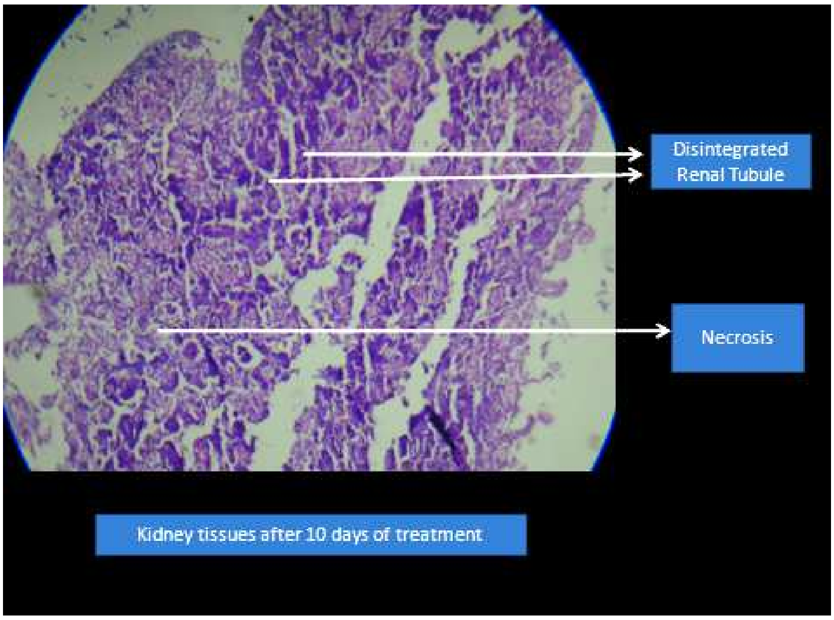

Fig 6: Showing disintegrated renal tubules and necrosis of cells after 10days of exposure to 0.2 ppm Malathion.

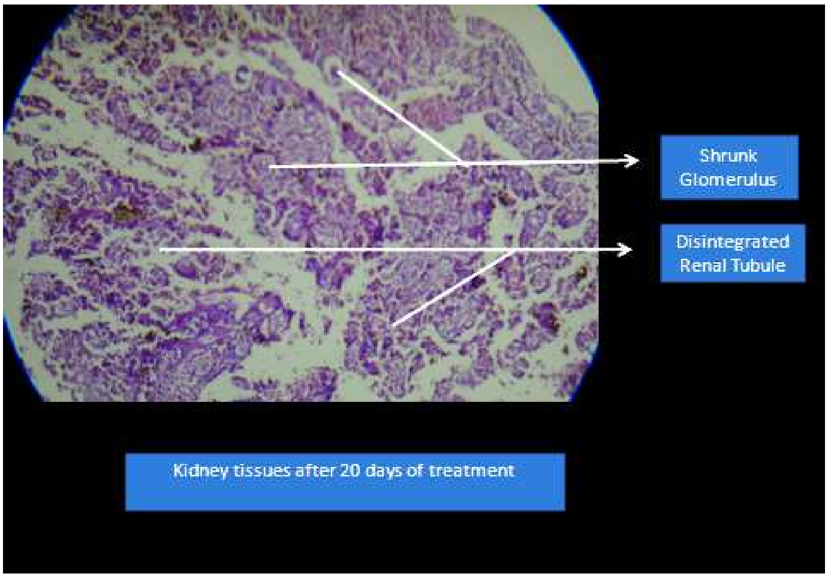

Fig 7: Showing large space between tubules and tissue, shrunk glomerulus with large Bowman's space, Enlarged lumen and Glomerulus, disintegrated renal tubules after 20days of exposure to 0.2 ppm Malathion.

Experimental group (0.2 ppm: **20 days**) showing large space between tubules and tissue, shrunk glomerulus with large Bowman's space, enlarged lumen and glomerulus, disintegrated renal tubules (Fig. 7).

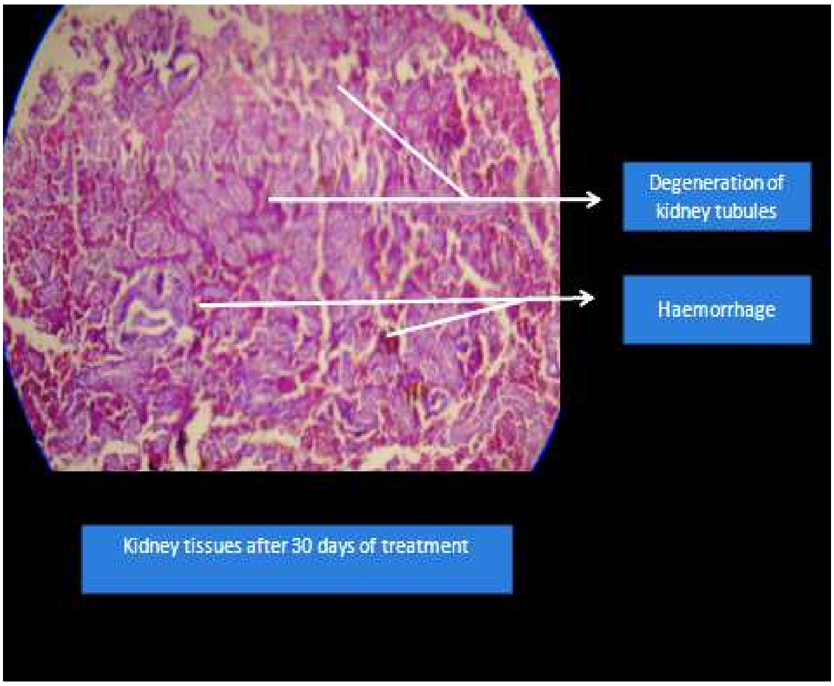

Fig 8: Showing degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage after 30days of exposure to 0.2 ppm Malathion.

In **30-day-**treated kidney, degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage were also recorded at the dose of 0.2 ppm for the fish species (Fig. 8).

Discussion

According to Selected toxicity information from HSDB, one of the National Library of Medicine's databases, following the admin of (3) h-o,o,s-trimethyl phosphorothioate (oos-tmp), an impurity of Malathion, to rats, showing significant radioactivity to liver, kidneys & ileum (Imamura t *et al.*, 1984).

Thus in our present investigation we have recorded degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage. That means if the process of exposure of the Malathion will continue then ultimately the whole architectural change will be noticed. This will finally hamper the normal anatomy and functioning of the kidney.

3. Results of the Malathion Treated Ovary

3. (a) Controlled group

The ovary consists of a series of ovarian lamellae, radially oriented towards the lumen and containing oocytes at different stages of development, in agreement with the maturation stage of the ovary.

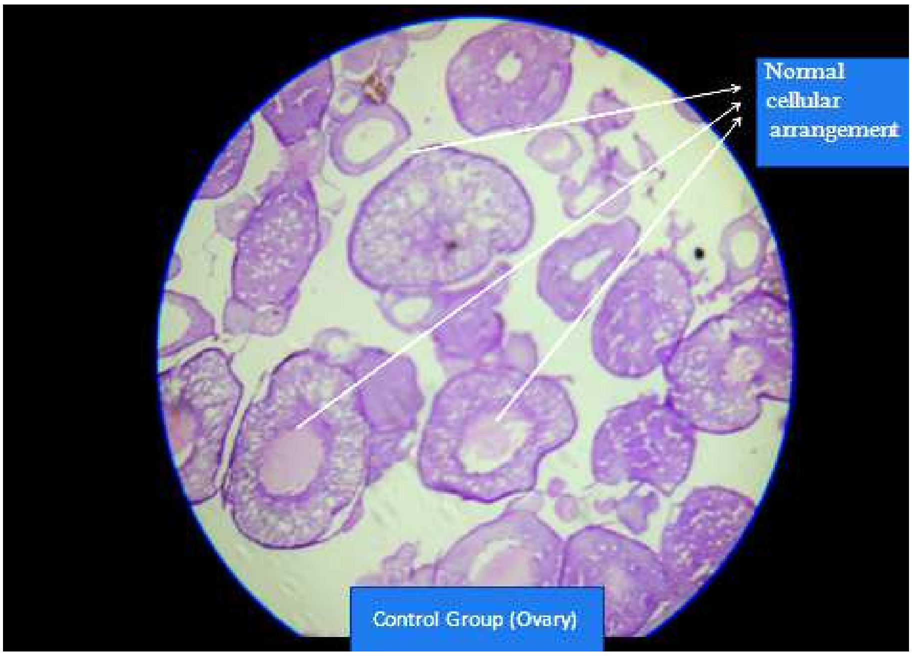

Fig. 9: Showing the Control Group (Ovary) of the Fish.

The control group also revealed well developed stage IV oocytes with properly distributed provitelline nucleoli. Control group showing mature oocytes, nucleus, and nucleolus in the oocyte. Oocyte filled with yolk granules (Fig. 9).

3. (b) Experimental group

After 10days of exposure to Malathion, primary follicles began to show adhesion and as well as cytoplasmic retraction in oocyte occured. Cytoplasmic degeneration and the number of atretic oocytes increased. Damages to the oocyte started to occur. Cytoplasmic retraction and clumping was more visible in oocyte. Partial destruction of the ovigerous lamellae and vitellogenic membrane occurred. So In 10 days-treated ovary,overall deformed tissue was observed in the Section of the ovary of *Heteropneustes fossilis* (Fig. 10).

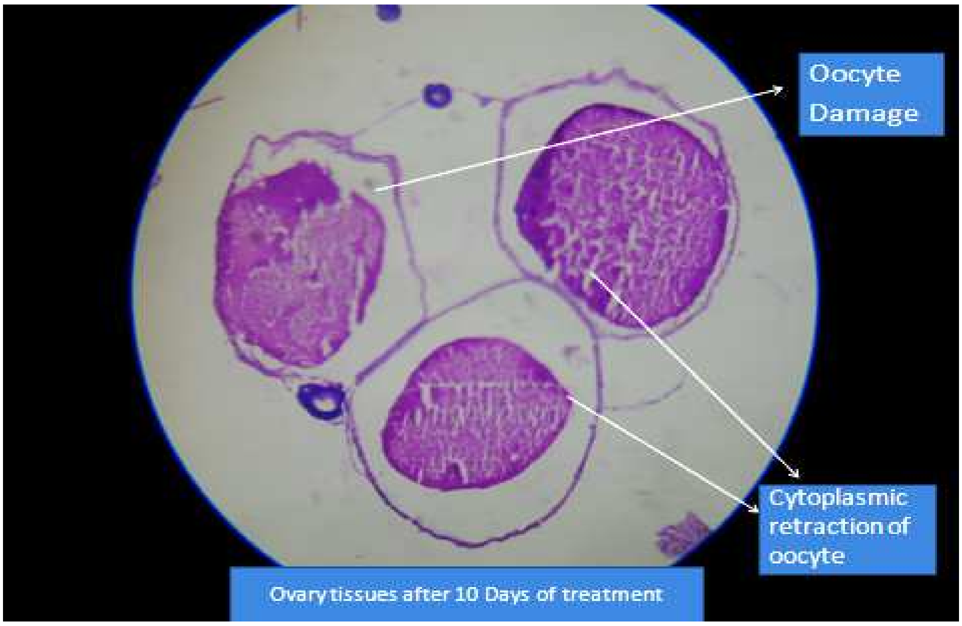

Fig. 10: Showing the damaged and retracted Oocyte after 10days of exposure to 0.2 ppm Malathion.

At 20 days interval continued destruction of follicles was observed (Fig. 11).

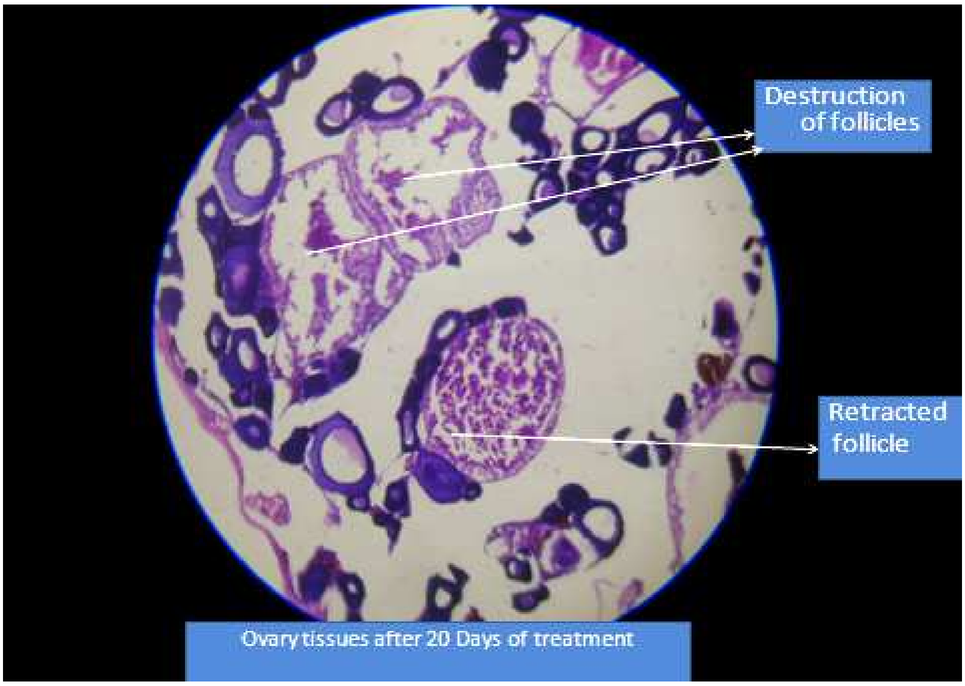

Fig. 11: Showing the Destructed Follicles after 20days of exposure to 0.2 ppm Malathion.

Severe damage of the ovigerous lamellae, increased intrafollicular spaces, vacuolated cytoplasm, extrusion of karyoplasm and necrosis in the cytoplasm were most evident following 30 days of exposure, (Fig.12). The ovarian wall became frayed and broken.

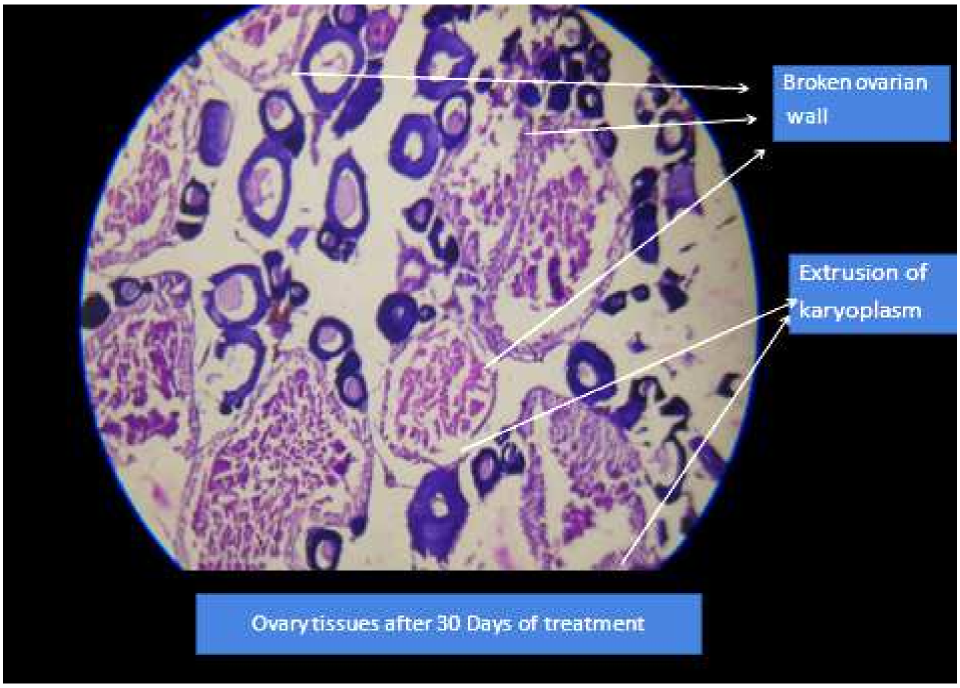

Fig. 12: Showing severe damage of the ovary after 30days of exposure to 0.2 ppm Malathion.

Additionally, a marked increase of atretic follicles, shrinkage, and embedded nucleoli into the surrounding cytoplasm in oocyte were observed .This study revealed that oocytes at their different stages of maturation get affected differently at various exposures. Based on observations of the ovarian tissue compositional and structural changes following given exposure times, it becomes evident that consistent sub-lethal doses of Malathion can bring a considerable change in the overall histological structures of the fish *Heteropneustes fossilis*.

However, the developing oocytes had less yolk, follicle cells were deformed and necrosis was also observed at some places.

Discussion

According to Ramachandra Mohan M (2000), lower dosage of Malathion brings about a reduction in the ovarian weight and retard the growth of the pre-vitellogenic oocytes. A higher dosage of Malathion on the other hand results in the degeneration of the immature oocytes and rupture of follicular epithelium. The findings suggest that the histopathological changes in the ovary might be a reflection of the disturbance in the endocrine/hormonal imbalance.

During an investigation by Chatterjee *et al.* (1997), where *Heteropneustes fossilis* were exposed to sub-lethal doses (0.5, 1, and 2 mg/L) of carbofuran (CF) for 30 days at 25 ± 1°C, it was observed that CF altered both the area and the percentage occurrence of the various types of primary oocytes in the ovary compared to that of the control fish. The degeneration of follicular walls, connective tissues and vacuolization in the ooplasm of the stage II and III oocytes were observed in CF-treated fish (0.5–2 mg/L). It appears that CF at sub- lethal concentrations inhibits oocyte maturational processes in catfish.

H. M. Dutta *et al.* (1994), observed effects of a sub-lethal (1.2 mg 1^{-1}) organophosphate, Malathion, in the ovary of breathing catfish, *Heteropneustes fossilis*. He noticed microscopic changes that occured on ovigerous lamellae. He observed Clumping of cytoplasm, Degeneration in the follicular cells, shrinkage of nuclear material, increased atretic oocytes, along with ruptured follicular epithelium.

Freshwater murrel, *Channa orientalis* was exposed to sub-lethal concentrations of two organophosphorus pesticides namely Nuvan (0.27 mg.1-1) and Dimecron (0.55 mg.1-1) for a period of 30 and 45 days. It was observed that both the Nuvan and Dimecron decreased gonadosomatic index, reduced diameter of different stages of oocytes and number of later stages of oocyte development and significant increase in the percentage of atretic follicles in ovaries of exposed animals (Saksena DN *et al*., 1999).

Hiran M Dutta *et al*. (2003), studied the effects of the insecticide, diazinon (an organophosphorous compound), on the ovaries of bluegill (*Lepomis macrohirus*). He noticed adhesion of primary follicles, cytoplasmic retraction in oocyte II, cytoplasmic degeneration, increased atretic oocytes, damages to the oocyte IV, Partial destruction of the ovigerous lamellae and vitellogenic membrane destruction of follicles, Severe damage of the ovigerous lamellae, increased intrafollicular spaces, vacuolated cytoplasm, extrusion of karyoplasm and necrosis in the cytoplasm .

With these regards, in our present study we found that oocytes at their different stages of maturation get affected differently. Based on observations of the ovarian tissue compositional and structural changes following given exposure times, it becomes evident that consistent sub-lethal doses of Malathion can and will alter normal microscopic anatomy of the fish ovary.

REFERENCES

1. APHA, 1985: Standard methods for the examination of water and wastewater. APHA
2. Bhattacharya S, Mondal S. 1997. Disruption of endocrine functions in fish by environmental contaminants. In: International Congress of Comparative Endocrinology, ed. Advances in comparative endocrinology: *Proceedings of the 13th International Congress of Comparative Endocrinology,Yokohama*, November 16-21, 1997. Bologna: Monduzzi Editore, International Proceedings Division, 1729-1732.
3. Borthakur M. K., Mahanta R., Goswami U. C. 2006. Cytochrome p450, Arylhydrocarbon Hydroxylase and Xanthine Oxidase Activity during Sublethal Malathion Exposure in Freshwater Catfish *Heteropneustes fossilis***.** *Journal of the Inland Fisheries Society of India.* 38(1) : 9-14.
4. Chatterjee S., Dutta A. B. and Ghosh R.1997. The influence of technical grade carbofuran (CF) on the egg maturational processes of catfish, *Heteropneustes fossilis* (Bloch). *Arch Environ Contam Toxicol***.** 32(4):426-30**.** Retrieved on 9th June 2011 from http://www.ncbi.nlm.nih.gov/pubmed/9175511.
5. Chaudhury D.K., Sadhu A.K. and Mukhopadhyay P.K. 1984. Toxicity of two organophosphorus insecticides Malathion Phosphamidon to the fish *Channa striatus. Environ. Ecol*., 2 (2): 143-148.
6. Dalela R.C., Bhatnagar M.C.,Tyagi A.K., &Verma S.R.1979. Histological damage of gills in *Channa gachua* after acute and subacute exposure to endosulfan and Rogor. *Mikroskopie (Vienna),* 35: 301-307.
7. Desai A.K., Joshi U.M., Ambadka P.M., 1984. Histological observations on the liver of *Tilapia mossambica* after exposure to monocrotophos, an organophosphorus insecticide. *Toxicol. Lett*. 21: 325-331.
8. Dubale, M.S. and Shah, P. 1984. Toxic effect of Malathion on the Kidney of a Freshwater teleost *Channa gachua*. *Comp. Physiol.Ecol*., 9(3): 238-244.
9. Dutta H. M., Meijer H. J. M. 2003. Sub-lethal effects of diazinon on the structure of the testis of bluegill, *Lepomis macrochirus*: a microscopic analysis. *Environ Pollut.* 125 (3):355-360.
10. Dutta H. M. , Nath A. , Adhikari S. , Roy P. K. , Singh N. K. and Munshi J. S. D.1994. Sublethal Malathion induced changes in the ovary of an air- breathing fish, *Heteropneustes fossilis*: a histological study. *Hydrobiologia,* 294 (3): 215-218.
11. Dutta HM, Maxwell LB.2003. Histological examination of sublethal effects of diazinon on ovary of bluegill, *Lepomis macrochirus*..*Environ Pollut*.. 121(1):95-102.
12. Dutta, H.M., J.S.D. Munshi, P.K. Roy, N.K. Singh, and C.R. Richmonds. 1992. Variation in toxicity of Malathion to air and water-breathing teleosts. *Bulletin Environmental Contamination and Toxicology* 49:279-284.
13. Edwards C. A. 1973. Pesticides residues in soil and water. *Environmental Pollution by Pesticides*, Plenum Press, London (1973).
14. Elezaby, M.M., El-Serafy S., Heckmann R., Kh Sharf Eldeen, M.M. Seddek. 2001. Effect of some toxicants on the fresh water fish *Oreochromis niloticus*. *J. Egypt Ger. Soc. Zool*. 36: 407-434.
15. Finney, D.J. 1964: Probit analysis. Cambridge University Press, London 333-360.

16. Gadhia, M. 1989. Histopathological lesions induced by rogor in the liver of a siluroid. *J.Inland.Fish.Soc.India*.21 (1): 55-57.

17. Imamura T, Hasegawa l. 1984. Role of metabolic activation, covalent binding, and glutathione depletion in pulmonary toxicity produced by an impurity of Malathion. *Toxicol Appl Pharmacol* . 72 (3): 476-83.

18. Medda, C., Bhattacharyya, B., Sarkar, S. K., Ganguly, S., Basu, T. K. 1995. Effect of rotenone on activity of some enzymes on freshwater fish. *Bull. Environ. Contam.Toxicol*, 16(1):55-60.

19. Munshi JD, Dutta HM, Singh NK, Roy PK, Adhikari S, Dogra JV, Ali MM. 1999. Effect of Malathion, an organophosphorus pesticide, on the serum proteins of Heteropneustes fossilis (BLOCH). *J Environ Pathol Toxicol Oncol*. 18(1):79-83

20. Naveed A, Venkateshwarlu P. and Janaiah C. 2010. Impact of Sublethal Concentration of Triazophos on Regulation of Protein Metabolism in the fish *Channa punctatus* (Bloch). *African Journal of Biotechnology*. 9(45): 7753-7758.

21. Pandey R. K., Singh R. N., Singh S., Singh N. N. and Das V. K. 2009. Acute toxicity bioassay of dimethoate on freshwater airbreathing catfish, Heteropneustes fossilis (Bloch). *Journal of Environmental Biolog.* 30(3): 437-440.

22. Pugazhvendan S.R., Narendiran N. J., Kumaran R.G., Kumaran S. and Alagappan K.M. 2009. Effect of Malathion Toxicity in the Freshwater Fish Ophiocephalus punctatus-A Histological and Histochemical Study. *World Journal of Fish and Marine Sciences* 1 (3): 218-224.

23. Ramachandra M. M. 2000. Malathion induced changes in the ovary of freshwater fish, Glossogobins giuris (Ham). *Polln Res*, 19(1): 73-75.

24. Rashatwar and Ilyas, 1984. Effect of Phosphamidon in a Freshwater Teleost Fish *Nemachelius denisonii* (Day)- Histopathological and Biochemical Studies. *J.Environ.Biol*. 5(1):1-8.

25. Ryan DL, Fukuto TR. 1984: *Pest Biochem Physiol* 21 (3): 349-57.

26. Sadhu, A.K., and Mukhopadhyay P.K.1985. Comparative Effect of Two Pesticides Malathion and Carbofuran on Testes of *Clarias batrachus* (Linn.). *J.Environ.Biol*. 6(3):217-222.

27. Sakakushi, H., 1972: *Bull. Tap. Soc.Sci.Fish*., 38: 555.

28. 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.

29. Shukla L. and Pandey A. K. 1980. 1986. Restitution of thyroid activity in the DDT exposed *Sarotherodon massambicus*: A histological and histochemical profile. *Water, Air, & Soil Pollution*, 27: 225-236.

30. Shukla S., Dass S. K., Saksena D. N. 2005. Effect of sublethal exposure of an organophosphorus pesticide, Nuvan on liver of catfish, Clarias batrachus. *Nature Env Polln Techno*, 4(3) :447–452.

--------------------××××××-------------------