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Study of The Effect of Sub-Lethal Dimethoate on Ovary of the Fresh Water Indian Cat Fish

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ABSTRACT - In the present papere, maturable oocytes of Heteropneustis fossilis exposed to LH and commercial formulation of four OPI, viz, malathion, cypermethrin, birlane and gardona at various concentrations in vitro to explore the possibility of any direct effect of these insecticides on LH-induced oocytes maturation. Lead is reported to form nuclear inclusion bodies in the oocytes of fish (Katti and Sathyanesan, 1987) but no such report available on organophosphorus insecticides. In the present study, an attempt has been made to study this in H. fossilis.

KETWORDS: OPI, Gonadosomatic index, Indian Cat Fish

I. INTRODUCTION

In an earlier study (Haider and Upadhyaya, 1985), they have observed th loss of stage II and III oocytes accompanied by a significant fall in gonadosomatic index, cessation of vitellogenesis, and inactivation of steriodogenis in gravid catfish, Clarius batrachus following 12 weeks chronic exposure to sublethal concentration of commercial formulations of four OPI. Since the maintenance of stage III oocytes, vitellogenesis and steroidogenesis depends on the pituitary gonadotropic stimulation, its absence was though to be the possible reason for these effects induced by OPI. Mani and Saxena (1985) also opined that low degree of recrudenscence in the ovaries of fenitrothion (an OPI) treated Channa punctatus may be attributed to low titers of gonadotropins. However, the possibility of direct action of these OPI on affected organs cannot be ruled out, since it is known that OPI affect several enzymes other than acetylcholinesterase (Corbett, 1974; Eto, 1974; Cremlyn, 1978). Hence, to find out whether there exists any direct effect of these OPI on the ovaries, in the present investigation I have considered the oocyte maturation as the parameter. In vertevrates, including fishes, meiotic activity in primary oocyte is arrested at the diplotene stage of first prophase which is followed by enormous enlargement of the nucleus called germinal vesicle (GV) and cytoplasmic growth. At the end of cytoplasmic growth phase i.e., vitellogenesis, the meiotic activity of primary oocyte has to be resumed in order to render the oocytes fertilizable. Resumption of the meiotic activity is known as oocyte maturation. In fishes gonadotropin acts on follicular layers of oocytes to produce the maturation (Masui and Clarke, 1979). In M. vittatus I have observed that luteinizing hormone (LH) is the most potent in vitro inducer of oocyte maturation among all the mammalian pituitary hormones.

In the present experiment, maturable oocytes of Heteropneustis fossilis exposed to LH and commercial formulation of four OPI, viz, malathion, cypermethrin, birlane and gardona at various concentrations in vitro to explore the possibility of any direct effect of these insecticides on LH-induced oocytes maturation. Lead is reported to form nuclear inclusion bodies in the oocytes of fish (Katti and Sathyanesan, 1987) but no such report available on organophosphorus insecticides. In the present study, an attempt has been made to study this in H. fossilis.

II. MATERIALS AND METHODS

Present investigation has been carried out to study the effect of sub-lethal Dimethoate on 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 jar 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 Dimethoate (50% E.C) is procured from local market and a pilot experiment was done to find out the LC50 value of Dimethoate by probit analysis (Finney, 1964) and LC50 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 Dimethoate and the Experimental Group is treated with pesticide Dimethoate 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

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MICROTOMY is being used for the histological study purpose of ovary of the fish Heteropneustes fossilis.

III. RESULTS AND DISCUSSION

3.1. Results of Dimethoate Treated Testes

3.1. (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 testes in control groups were normal and systematically arranged.

3.1. (b) Experimental Groups

After 10 days of interval, histopathological examination of the testes of *Heteropneustes fossilis* clearly shows that the parenchymal architecture of the testes 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.

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 testes tissues. Acute and extensive necrosis of testes 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.

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

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.

Different investigators and authors noticed toxical changes in the testes of catfish after exposure to organophosphate and allied group of pesticides. Desai et al. (1984) reported histological changes in the testes 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 testes and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis and lipidosis in the testes. 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 testes was evident by increased volume of sinusoidal space.

The hazardous effect of the pyrethroid insecticide, fenvalerate on the histology and histochemistry of the testes 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 testes 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 testes which mainly included architectural changes in the testes, 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.

3.2 Acetylcholine Esterase Activity 1n Brain and Ovary of The Test Animal

Organophosphate pesticides are competitive inhibitors of acetylecholineesterase (AChE), the key enzyme in the transmission of nerve impulse. AChE is readily phosphorylated by the organophosphate pesticides at the active site serine (Aldrige and Reiner, 1972; Taylor, 1990) the selectivity of action of organophosphates is that it causes inhibition of AChE and accumulation of acetylecholine at the synapse (Loskowsky and Dettbam, 1975) over stimulating the postsynaptic cells (Pope *et al*., 1995). Reports also demonstrated that the organophosphate pesticide agents can bind to the acetylecholine receptors and this direct interaction is responsible for the manifestation of stress (Pope *et al*., 1995).

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Therefore, the AChE activity in different tissues (Brain and Ovary) in experimental animal *Heteropneustes fossilis* has been used in the present investigation as the neurophysiological marker or early signs of organophosphate neurotoxicity. Results of previous studies have suggested that even the undetectable quantity of organophosphate pesticides will affect the enzymatic activity. Several authors have reported that enzymes of the same tissue of different species show difference in the sensitive to various organophosphate insecticides (Pan and Dutta, 1998; Monserret and Bianchini, 1998). In the present work all the groups treated with Dimethoate and dichlorvos organophosphate pesticides revealed significant ($P>0.01$) inhibition of AChE activity in the brain as well as ovary of exposed fish. The inhibition of enzyme was more significant at higher doses of pesticides to fish in both the cases. The time, dose and species related differences in enzyme susceptibility to organophosphate pesticides can primarily be attributed to dissimilar enzyme amount and inhibitor affinity degree to cholinesterase receptor. Although 50% or more depletion is supposed to be life threatening, available investigatrion shows that some fish are capable to tolerate over 90% inhibition in AChE activity (Day and Scott, 1990). More than 90% depletion was also reported by Balint *et al.* (1995); Pan and Dutta, (1998) in fish exposed to various insecticides. The highest reduction in the present study 72% which is considerably low. Oruce and Usta, (2007) reported that *Heteropneustes fossilis* showed to be more resistant to diazinon, this may be because of its low rate of bioactivation and relatively high activity of detoxicating enzymes (Keizer *et al*., 1991).

Rath and Misra, (1981) and Ansari and Kumar, (1984) reported that inhibition of acetylecholine activity has relation with age of fish, concentration of pesticide and time of exposure. Their findings extended a considerable support to our observations.

Therefore, the present study demonstrates that both the organophosphate pesticides (dimethoate and dichlorvos) are potent inhibitors of brain and ovarian AChE activity, but under identical dose the rate of enzyme inhibition was different for different pesticides.

IV. CONCLUSIONS

Very little information is available on the reversibility of the adverse effects of pesticides after withdrawal of treatment. The majority of studies show the effects of a single dose of pesticides. The use of graded doses of pesticides could help in identifying in the minimum pesticide dose that will affect fish reproduction and other targets. Adequate field studies have not been carried out in India. There is practically no basic information on the reproductive status of fishes collected from pesticide polluted natural water ecosystems. The effects of pesticides on fishes discussed herein are based on experimental laboratory conditions. Most Indian workers have carried out their studies in static systems in which fishes are exposed to pesticide solutions, which are replaced with the same concentration at definite intervals, while in nature fishes usually live in flow-through water bodies. Moreover, a good amount of pesticides undergo degradation, absorption, and transformation depending on the physio-chemical and biological factors of the static or natural water ecosystem. These laboratory data therefore have to be validated with field data. Studies on the effects of pesticides on reproduction have also ignored the impact of changing environmental factors such as photoperiod, temperature, salinity, pH, nutritional status, etc., since these factors not only synchronize and influence reproduction , but also influence the metabolism of pesticides and thereby the overall nature and degree of their impact.

Hence, on the basis of this study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of these organophosphate pesticides on fish species.

REFERENCES

[1] Abdus, S. B., Badrun N. and Quamrun, N. (2001). Effects of sumithion on the Histological changes of spotted Murrel, Channa punctatus (Bloch). Pak. J. Bio. Sci. 4(10): 1288-1290.

[2] Adhikari, S., Sarkar, B., Chattopadhyay, A., Chattopadhyay, D.N., Sarkar, S. K. and Ayyappan, S. (2006). Effect of cypermethrin on breeding performances of a fresh water fish, Labeo rohita (Hamilton). Chemistry and Ecology vol. 22(3): 211-218.

[3] APVMA (2008): Australian pesticides and veterinary Medicines Authority Dichlorvos: Environmental Assessment APVMA. P.O. Box E240 KINGSTON Act 2604 Australia pp: 1-71.

[4] Auta, J. and Ogueji, E. O. (2006). Sublethal Effect of Dimethoate on growth and Food utilization of Oreochromis niloticus (Trewavas) Med. J.Fish-Int. 1(4-2): 163-166.

[5] Boyd, C. E (1964). Insecticides cause mosquito fish to abort. progr.fish.cult. 26:138.

[6] Caio, R. D. A., Ian, P. G. A., Patricia, F. C., Luiz, B. C. and Ramilson, S. B. (2007). Effect of dicholorovos on the acetylcholinesterase from tambaqui, Colorsoma macropomum brain. Environ. Toxicol. 22(2): 194-202.

[7] Campagna, A. F., Eler, M. N., Espindola, E. L. G., Senhorini, J.A., Rego, R. F. and Silva, L. O. L. (2006). Dimethoate 40% organophosphorus pesticide toxicity in Prochilodus lineatus eggs and larvae.

 DOI : 10.35291/2454-9150.2020.0081

[8] Chandra, S. and Ram, R.N. (2004). First ovarian maturity and recovery response in common carp, Cyprinus carpio after exposure to carbofuran. J. Environ. Biol. 25: 239-249.

[9] Chciko, G. M., Podgornaya, V. A., Zhelnin, Y. Y. (2000). Acetylcholinesterase and butrylcholinesterase activities in brain and plasma of fresh water teleosts: cross-species and cross family differences. Comp Biochem physiol C. toxicol pharmacol. 127(3): 233-42.

[10] Chindall, Ac; Sikoki; F.D. Vincent-Akpu, IJEOMA (2004). Chirashree, C., Arun K. R., Samir B. and Shelly B. (1992). Non lethal concentrations of pesticides impair ovarian function in the fresh water perch, Anabas testudinenus. Environmental biology of fishes. 36: 319-324, 1993.

[11] Claudia, A. D. B., Mana, D. F. M. V. and Erica P. G.C. (2003). Ovary maturation stages and Oocyte features in three species of the Neotropical fish Aemiodus (Muller, 1842) Brazilian archives of Biology and technology vol. 46: on pp 433-441.

[12] Cowman, D. and Mazanti, L. (2000). Ecotonicology of "new generation" pesticides to amphibians. In. C. Bishop, G. Linder and D. sparling (Eds). Ecotoxicology of Amphibrans, Reptiles. pp. 233-268. Pensacola, FL: SETAC.

[13] Frumin, G. T., chuiko, G. M., Pavlov, D. F. and Menzykova, V. (2009). New rapid method to evaluate the median effect concentrations of xenobiotics in hydrobiouts. Hum Exp toxicol. 21: 20.

[14] Ghosh, S. K. and Ponniah, A.G. (2001). Freshwater Fish Habitat Science and Management in India. Aquat. Ecosyst. Health Manage. 4(4): 367-380.

[15] Graham, R. S. and Katherine A. S. (2003). The effects of Environmental pollutants on complex fish behaviour. Integrating behavioural and physiological indicators of toxicity. Aquat toxicol 10: 64(4): 451-60.

[16] Gupta, R. C. (2006). Toxicology of organophosphate and carbamate compounds Amsterdam Boston Elsevier Academic Press 763.

[17] Hanson, R., DoDoo, D. K., Essumang, D. K. (2007). Uptake and toxicity of some pesticides on three freshwater fishes, Oreochromis niloticus, Clarias gariepirnus and Chrysicthys nigrodigitatus. Toxicological and Environmental chemistry. 89(1): 111-123

[18] Hiran, M. D. and Dane, A. (2000). Effects of Endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. Environ toxicol chem. 21(7): 1469-74.

[19] Kumar, S. and Singh, M. (2000). Toxocity of dinethoate to a fresh water teleost, Catta catta. J. Exp. 2001. India. 3(1): 83-88.

[20] Ligia, M. S., Benjamim, E. and Metry, B. (2008). Effects of endosulfan sublethal concentrations on carp, Cyprinus carpio, (Linnaeus, 1758). Morphometric, hystologic, ultrastructural analyses and Cholinesterase activity evaluation. Braz. J. Vet. Res. Anim. Sci. Sao Paulo, V. 45, n. 2, p. 87-94.

[21] Martine, B., Christelle, E., Xavier C. and View, S. (2004). The use of Zebra fish mutants to identify secondary target Effects of acetyleholinesterase inhibitors. Toxsci. 77, No. 2,325-333.

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