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Toxicological Impact of Profenofos on the Testis of Fresh Water Air-breathing Fish *Channa gachua(Bloch)*

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Abstract—*Pesticides of various categories are frequently used against a number of pests in the field to increase the crop production, though these chemicals are highly toxic to other species in the environment. Pesticides are extensively used to curtail pest menace of agricultural field but they enter into hydrobiological system and ultimately affect non-target organism like fish. Pesticides are the major source of water pollution, as it eradicates the economically important species either indirectly through breaking the biological chains or directly producing toxic stress and chemical changes.*
The present study is aimed to assess the histopathological impact of lethal (0.3 ppm) and sub lethal (0.06 ppm) concentrations of profenofos in the testis of an air breathing fresh water teleost Channa gachua. The histopathology revealed cytotoxic damage, general inflammatory response abnormalities are quite prominent, large number of both inter and intra-tubular vacuoles was maximum. Gross condensation of spermatogenic cells, which is evident by clump formations and appearance of inflammatory lesions are also quite prominent ,vacuolation in tubular epithelium increases, Inflammatory cells are seen in the testicular tissue, distortion of seminiferous epithelium is quite prominent, shrinkage of interstitial cells and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue.

Keywords*: Profenofos, histopathology, Channa gachua, Testis*

1. INTRODUCTION

These guidelines include complete descriptions of the fonts, spacing, and related information for producing your proceedings manuscripts. The global race for development has put stress on all the systems of society including agriculture. The growing population and subsequent increase in need of food is sorted out by use of modern agriculture techniques. The dark side of this development lies in excessive use of organic fertilizers, pesticides and herbicides, which continually contaminates the water sources and are indicator of pollution. The pollution has become a major challenge and threat to the very existence of mankind on the earth. Pollution of the aquatic environment is a serious problem growing day by day. Increasing number and the amount of agricultural, industrial and commercial chemicals discharged into the aquatic environment leads to various deleterious effects on the aquatic organisms. Aquatic organisms including fish accumulate pollutants directly from contaminated water and indirectly via the food chain. All water pollution affects organisms that live in these water bodies. It occurs when pollutants are discharged directly or indirectly without adequate treatment to remove the harmful constituents (Agarwal et al., 2010). Thus, pesticide contamination of surface water has been documented worldwide and constitute a major issue that gives rise to concerns at local, regional, national and global scales (Huber et al., 2000; Cerejeira et al., 2003).

Increasing population is the most important reason for rapid degradation of environment. The ever-increasing population necessitated more food, infrastructure leading to the expansion of the agriculture, industries and urbanization. In the agriculture expansion pesticides are stress used in agriculture operations to curtail pest menace (Echier et al., 1978, Sampath et al., 1991) also extend toxic effect on non- target species like fish as well as human beings. The prevalence of pesticides in the environment has become a matter of great concern as they are not processed in natural condition within a reasonable time.

Increasing attention has been given during past decades to the protection of the aquatic environment against pollution both nationally and internationally. To indicate the degree of pollution, the presence or absence of fish has been widely used as biological indicator in the fresh water. The chief source of contamination are industrial waste discharge, agriculture, household waste disposal and fuel combustion (Woodling et al., 2001, Patra et al., 2005, and Suarup et al., 2006, Saxena and Garg 2011).

The workers have collected data on the concentration of pollutants, which are lethal to the fish either in short or long term exposure, such data can provide very necessary information apart from identifying a boundary limit above which fish is likely to be killed. For example, they can indicate the relation between the effects of environment factors such as

water hardness, temperature, pH value, dissolved oxygen and toxicity of pollutants. The magnitude of pesticide pollution was studied in the Indian fishes by various workers (Dalela et al., 1979; Dubale and Shah; Pandey and Shukla; 1980; Rashatwar and llyas, 1984; Sadhu and Mukopadhya, 1985; Ghosh and Chaterjee,1989; Medda et al., 1995, Bhattacharya et al., 1997; Munshi et al., 1999, Rakesh et al., 2009, Naveed et al., 2010). Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism.

Pesticide, which have been used in present investigation is profenofos, which is extensively used in agriculture. This pesticide interfere and interact with various biochemical and physiological activities of fish. This has been found to be highly toxic not only to fishes but also to those animals, which constitute the food of the fishes. Profenofos a well known oranophosphate pesticide is a potential toxciant polluting the aquatic system which has been in agricultural uses for controlling pests.

Channa gachua commonly known as “Chenga” is one of the important fresh water fish in tropical region of India belonging to the group of air breathing teleosts. It lives in Stagnant water where abundant growth of water hyacinth occurs and survives out of water for considerably long period if the skin remains moist. This is due to possession, in addition to four pair of gills, a pair of suprabranchial chambers and dendritics plates which together constitute the air breathing organ of the fish. Although the fish possesses a pair of accessory respiration organs but it depends more on gills (55%) for respiration (Singh, et al., 1982). They come to water surface to obtain air and passes it down into chamber.

This fish contains high amount of carbohydrate fat, protein and essential amino acids etc. having therapeutic use. Histopathological studies thus give us useful data concerning tissue damage prior to external manifestation. In the present study, an attempt has been made to determine the histopathological impact of profenofos on the testis of an air breathing fresh water fish Channa gachua.

2. MATERIALS AND METHOD

Healthy live fish Channa gachua were collected from local Gandak river, district Muzaffarpur and brought to the laboratory in polythene bags half filled with natural water. Fishes were washed with 0.1% KMNO4 to remove dermal infection. Healthy fish (average length 18- 20 cm and weight 10-15gm) were selected and transferred to the glass aquaria for stocking and acclimation to different test conditions- light, temperature, and physio – chemical properties of water for 15 days. Fishes were fed with chopped chicken everyday and the feeding was stopped a day before the initiation of experiment (static acute –bioassay) and no food was supplied during the period of this test only in chronic exposure period they were given food. The feeding schedule was strictly maintained in order to avoid any starvation.

Physio-chemical characterization of water :- PH 7.0, temperature 270c, Salinity 107 mg/l, Cl 44.3mg/l, CO2 8.0mg/l, DO 6.3mg/l, hardness 114mg/l, CaCo3 56mg/l and Specific gravity 1.00372.

1ml of Profenofos was dissolved in 1litre of distilled water and used as the stock solution for different concentrations of Profenofos. It was stored in a clean standard flask at room temperature in the laboratory.

Bioassays were conducted for the determination of Lc50 values of Profenofos for 24, 48, 72, and 96 hours following the method of APHA (1998).For acute bioassay there were four test concentration in addition to one control. The fish were held in normal water for at least one day before the start of test. For carry out an experimentation, five glass jars filled with 10 litres of tap water were taken. One glass jar was not exposed to pesticide and served as control group. Four glass jars were exposed to different concentrations of Profenofos pesticide and served as treated group .Healthy and well acclimatized 10 fishes were transferred one by one with the help of small hand net from the acclimatizing glass aquaria to the both Control and treated glass jars. The fish of the control group was not exposed to Profenofos .Fishes of treated group were exposed to different concentrations of Profenofos (0.2, 0.4, 0.6 and 0.8 ppm) and their percent survival at different time interval (24, 48,72,and 96 hours) were recorded as a measure of acute toxicity test and further statistical analysis. Fishes that failed to respond even to strong stimuli were considered dead and remove immediately. Mortality was observed up to 96 hours. The Lc50 value for 96 hours was determined by probit analysis method (Finney 1971) and was found to be 0.3 ppm and sub lethal concentrations, one-fifth of 96 hours Lc50 value (0.06 ppm).

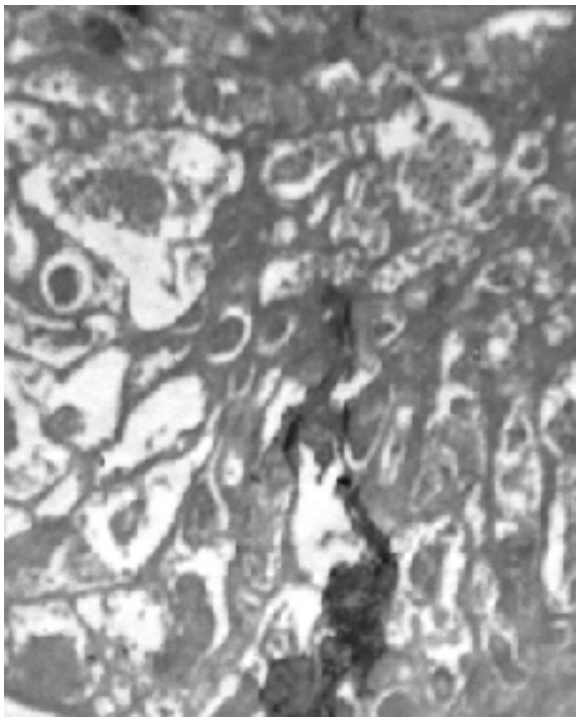
For studying the histopathology of the testis, 10 fishes were exposed to sub-lethal concentrations of Profenofos (0.06 ppm) up to 4 days and 15 days. Control group were also maintained separately. After 4 days and 15 days fishes from both the control and treated group fishes were dissected and testis tissues were removed. The testis of treated and control fish were isolated and fixed in aqueous bouin’s fixative for 24 hours. Testis tissue was thoroughly washed in running tap water till yellow colour of picric acid went off. The testis tissue of both group were dehydrated in different grades of alcohol, cleaned in xylene and paraffin blocks were prepared. Paraffin sections were cut at 6 micron thickness with the help of rotatory microtome machine. Ribbon of sections was taken on slides for stretching on stretching machine. Sections were stained with hematoxyline and eosine. After that it was mounted in DPX. Stained section of testis tissues were

observed under microscope and microphotographs were taken for the histopathological examination

3. RESULTS

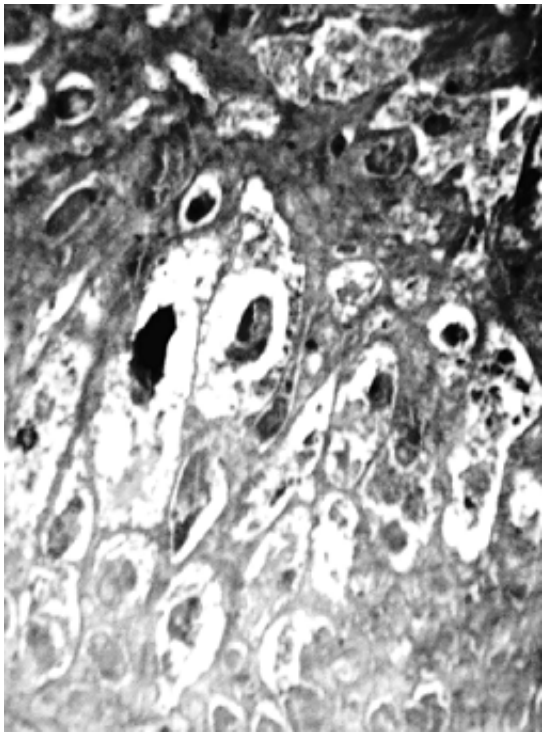
3.1 Control Testis

Testis is enclosed in a peripheral connective tissue sheath. The innermost layer of this sheath tunica propria, projects into the lumen of testis forming the seminiferous tubules. These tubules are lined internally with tubular or seminiferous or spermatogonic epithelium which gives rise to spermatocytes. The spermatocytes are later transformed into next developmental stage of spermatids and then to spermatozoa. Masses of spermatozoa can be seen lodged in seminiferous lobules, located at the blind ends of seminiferous tubules. This lobular part can be distinguished into somatic cells and germ cells. The central portion of the testis is made up of glandular tissue consisting of large and spherical interstitial glandular cells, fibroblasts, blood and lymph vessels (Fig.1).



3.2 Effect of profenofos

After 4 days exposure of profenofos with sublethal concentrations (0.06 ppm) extensive cytotoxic damage, general inflammatory response abnormalities are quite prominent, large number of both inter and intra-tubular vacuoles was maximum. Gross condensation of spermatogenic cells, which is evident by clump formations and appearance of inflammatory lesions are also quite prominent ,vacuolation in tubular epithelium increases, Inflammatory cells are seen in the testicular tissue. (Fig. - 2)



After 15 days of exposure distortion of seminiferous epithelium is quite prominent, shrinkage of interstitial cells and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue.(Fig. - 3)

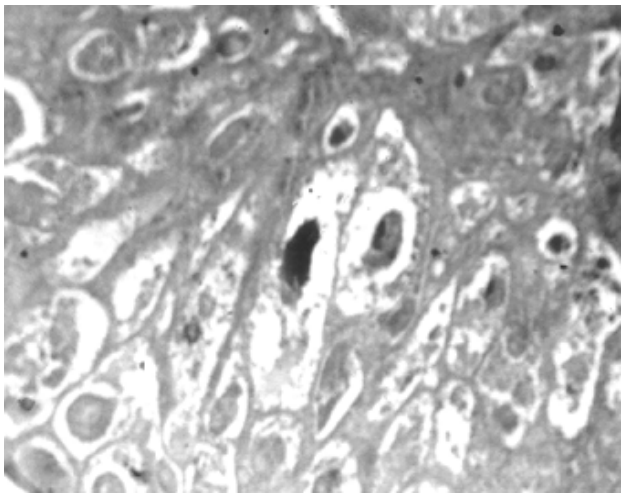


Fig. 1: T.S of testis of control fish Channa gachua:-blood vessels, connective tissue, interstitial glandular cells, seminiferous tubules, spermatocytes, seminiferous epithelium are normal. H & E x 100

BV – blood vessels

CT – connective tissue

IGC – interstitial glandular cells

ST – seminiferous tubules

S – spermatocytes

SE -- seminiferous epithelium

FIG. 2 :- T.S of Testis of Channa gachua exposed to profenofos with sublethal concentration (0.06 ppm) for 4 days showing condensation of spermatocytes,vacuolation of tubular cells. H & E x 100

CS – Condensation of spermatocytes

V – vacuolation of tubular cells

FIG. 3 :- T.S of Testis of Channa gachua exposed to profenofos with sublethal concentration (0.06 ppm) for 15 days showing distortion of seminiferous epithelium, shrinkage of interstitial cells. H & E x 100

DS – Distortion of seminiferous epithelium

SI – Shrinkage of interstitial cells

4. DISCUSSION

Histopathological examination of testis proved to be the most sensitive and significant indicators of chronic pesticides poisoning in Channa gachua. Result revealed that Profenofos induced discernible micro structural alternations in the testis of the Channa gachua. The deleterious effects were more pronounced in the profenofos stress. The morphological changes in testis gave better understanding of the mode of action of the tested pesticides. The changes in the microstructure of testis in present investigation are not characteristic only of profenofos, but a number of other pesticides are also known to produce the similar histopathological changes in testis of a fish. Testis of Channa gachua shows significant changes when exposed to pesticide profenofos for different exposure periods. Extensive cytotoxic damage, general inflammatory response and other histological abnormalities are quite prominent. Profenofos intoxication results in distortion of the tubular epithelium and condensation of tubular cells. Exposure dependent and concentration mediated increase in the number of inflammatory cells is evident of intoxication. maximum number of inflammatory cells can be seen vacuolation and inflammatory response, distortion of seminiferous epithelium is quite prominent. Further, exposure of profenofos, even with lesser concentration (results in condensation of spermatocytes besides inflammation and inter-tubular vacuolation. Further histological damage can be visualized in the form of shrinkage of interstitial cells, condensation of spermatocytes and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal et al., 1985; Ruby et al., 1986, 1987). Short term static bioassays conducted in our laboratory clearly indicate that exposure of profenofos is responsible for histoanatomical damage of fish testis in terms of condensation of spermatogonic cells, vacuolation of tubular cells and distortion of seminiferous cells along with inflammatory lesions. Further, appearance of these symptoms even after shortest exposure of profenofos for 24 hr is really alarming and is quite suggestive of the fact that fish testis are quite susceptible to this pollutant which is also evident by 29.13% decrease in GSI. Shrinkage of interstitial cells and vacuolation of tubular cells which has resulted in peculiar starry sky appearance of the testicular tissue after longer duration of exposures (72 and 96 hr) even with low doses is a strong evidence for testicular atrophy. These studies, thus, reveal that histo-anatomical damage of testis is exposure dependent and dose mediated. These findings are also in agreement with our earlier biochemical data generated by testicular tissue in response to LAS exposure (Trivedi et al., 2001). Although, only scanty information is available regarding profenofos induced testicular impairment in fishes, present findings are well comparable with histo-pathological anomalies in testis of other animals and fishes under the impact of various environmental pollutants. The degenerative changes in seminiferous tubules, enlarged interstitium and haemorrhage in intertubular area in albino rats exposed to pesticides have been reported (Dutta and Dikshith (1973); Nigam et al. (1979) and Baronia and Sahai (1993). In the present study tubules and spermatogenic cells, have been observed in profenofos exposed fish. These changes may culminate in the partial or total arrest of spermatogenesis. Katti and Sathyanesan (1985) observed exposure dependent and concentration-mediated changes in testis of C. batrachus treated with lead. Hilderbrand et al. (1973), Sankar and Mondal (1973) and Gunn and Gould (1975) have reported similar observations in lead treated rats. Present study, thus suggests that the extent of damage of fish testis not only depends on the concentration of the toxicant but also on the time of exposure. A continuous decrease in Gonadosomatic index (GSI) with the increase of the dose of profenofos shows that this surfactant chemical causes cellular damage, checks the growth and maturation of sex cells. Finally it can be concluded that exposure to profenfos, can result in decreased fertility potential in Channa gachua.

5. CONCLUSION

In the present investigation histopathological impact of profenofos on the testis of fresh water fish Channa gachua shows the extensive cytotoxic damage, general inflammatory response abnormalities are quite prominent, large number of both inter and intra-tubular vacuoles was maximum. Gross condensation of spermatogenic cells, which is evident by clump formations and appearance of inflammatory lesions are also quite prominent ,vacuolation in tubular epithelium increases, Inflammatory cells are seen in the testicular tissue, distortion of seminiferous epithelium is quite prominent, shrinkage of interstitial cells and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue.

(Fig. - 3)

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