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EFFECTS OF CYPERMETHRIN ON HISTOPATHOLOGY OF GONADS OF A FRESH WATER FISH *Anabas testudineus* (Bloch.)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author AKJ designed the study, performed statistical analysis, wrote the protocol and wrote the first draft manuscript. Author Anupama managed the literature searched and performed the histopathological examinations. Both authors read and approved the manuscript.

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ABSTRACT

Pyrethroid pesticides are very commonly used for pest control in agriculture fields and may reach the aquatic system through irrigation and rain. As a result many non-target organisms including fishes of the freshwater ecosystems are adversely affected. Pyrethroid insecticide cypermethrin was used for this investigation to study the histopathological alterations in testes & ovary of a fresh water fish *Anabas testudineus*. The acute toxicity of cypermethrin to the test fish was determined for 24, 48, 72 and 96 hours. The LC_{50} values by regression equation determined were 0.15 ppm, 0.20 ppm, 0.25 ppm and 0.30 ppm respectively. The sublethal concentration determined was 0.106 ppm. The fishes to this exposure period reflects significant changes in their gonads in comparison to the control fish. In the testes, Intra-lobular edema, breakage of germinal epithelium and extensive vacuolization were reported while in the ovary large intra follicular spaces and rupture of nuclear membrane in stage II oocytes were observed. In the present study, it was concluded that cypermethrin badly affect the reproductive process in the fish.

Keywords: *Anabas testudineus*; cypermethrin; LC_{50}; Oocytes; testis.

1. INTRODUCTION

Pesticides are often considered as a quick easy and inexpensive solution for controlling the pests. However, its use comes at significant cost. Pesticides have contaminated almost every part of our environment and their residues are found in almost all parts of our environment. Pesticides can be circulated into different ecosystems by different agents [1]. It has been reported that only about one percent of

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applied pesticides land on the target and the rest contaminates the environment [2]. The pesticides which are liberated into the aquatic environment have a detritus effect on fish and subsequently on human beings [3].

Fishes are important component of food chain so, any effect of toxicant may have adverse impact on the nutritive values of fish and on the human being due to their consumption [4]. Review of available literature on fish and pesticidal impacts indicate that the sublethal doses of most of the pesticides causes varying extent of histopathological injuries to the different organs of fishes [5,6,7]. It has been proved by various workers that histopathological investigations are sensitive tool to detect the direct effects of chemical toxicants within the various organs of fish in laboratory [8,9]. Hence, in the present study cypermethrin, a fourth generation synthetic pyrethroid was used to investigate the histopathological alterations in testes and ovary of a fresh water fish *Anabas testudineus*.

2. MATERIALS AND METHODS

The adult living species of *Anabas testudineus* were collected at a local fish pond and were brought to the laboratory in wide mouthed large earthen posts half filled with natural water and covered with a piece of mosquito net. Every effort was taken to give less stress to the fishes during transportation. The fishes were washed with 0.1% $KMnO_4$ solution to remove dermal infections if any. Healthy fishes of average length 12-16 cm and weight 80-100 gm were transferred one by one with the help of small hand net to 40 litre rectangular glass aquarium and acclimatized in the laboratory conditions for a fortnight. Running tap water was used in all the experiments and no aeration was done. They were not given food for the first three days of acclimation & after that fed with chopped goat liver once a day. Bioassay was conducted for the determination of LC_{50} values cypermethrin for 24, 48, 72 and 96 hours following the methods of APHA, AWWA and WPCF [10]. The LC_{50} values for these periods were determined 0.15 ppm, 0.20 ppm, 0.25 ppm and 0.30 ppm respectively. The sublethal dose determined was 0.106 ppm by the formula of Hart et al. At the end of exposure period (day 30) the control and exposed fishes were first individually weighed and then dissected in ringer's saline and the testes and ovary were taken out, fixed in aqueous Bouin's and 10% neutral formalin fixatives for 24 hours and in Carnoy's fixative for four hours. After fixation the tissues fixed in the former two fixatives were thoroughly washed in running tap water and dehydrated in graded alcoholic series, cleansed in benzene and processed for embedding in the paraffin wax. Serial sections of each of the test tissues were cut at 5 μ and stained with Haemotoxylin – Eosin. Selected slides were subjected to routine histological examination and photomicrography.

3. RESULTS AND DISCUSSION

3.1 Testes

In the control fish, testes showed cells of different spermatogenic stages in testicular lobules. The spermatogonia were spherical in shape and larger than primary spermatocytes. Secondary spermatocytes were smaller than both spermatogonia and primary spermatocytes. The germinal epithelium was distinct (Fig. 1). At the end of exposure period of 30 days to a sublethal concentration of 0.106 ppm of cypermethrin numerous degenerative changes were observed in the testes. The cypermethrin exposed testes exhibited intra-lobular edema, lesions, breakage of germinal epithelium at some places increased thickness of lobular wall and loss of interstitial cells (Figs. 2 & 3).

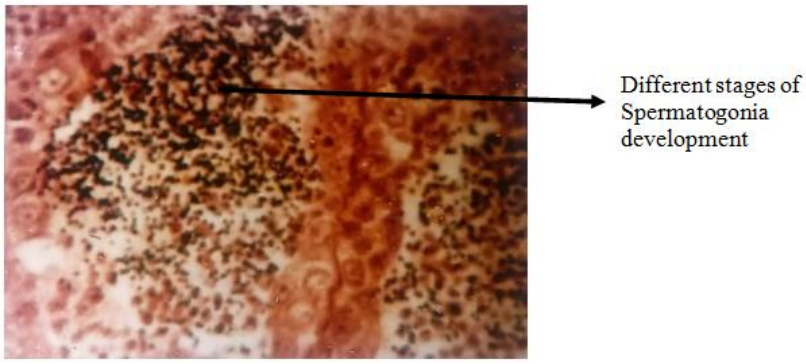

Fig. 1. Histology of testis of control fish showing spermatogonia in various stages of development (H & E X 600)

Fig. 2. Histology of cyptermethrin treated testis showing vacuolization (H & E X 1500)

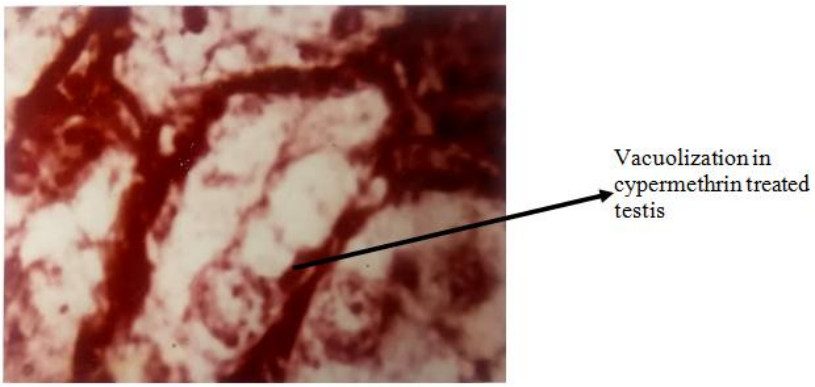

Fig. 3. Cyptermethrin treated testis showing breakedown of germinal epithelium (H & E X 600)

The present findings are in conformity with the findings of some notable works. Cadmium toxicity to gonads of *Garra mullya* [11]. Changed architecture of lobules, necrosis, atrophy of interstitial cells and damage to germinal epithelium were reported in the testes of *Puntius ticto* exposed to cadmium acetate [12]. Similarly in the testes of *Colisa fasciatus* pycnotic nuclei, reduction in leydig's cells was reported under arsenic exposure [13].

3.2 Ovary

The ovary of control fish showed different stages of development of eggs. The germinal epithelium was conspicuous and rounded nuclei were observed in the cells. Stage I, II, III, IV and V oocytes were observed out of which stage IV oocytes were larger in size. Stage V oocytes represent fully grown mature eggs packed with yolk mass (Fig. 4). On the other hand

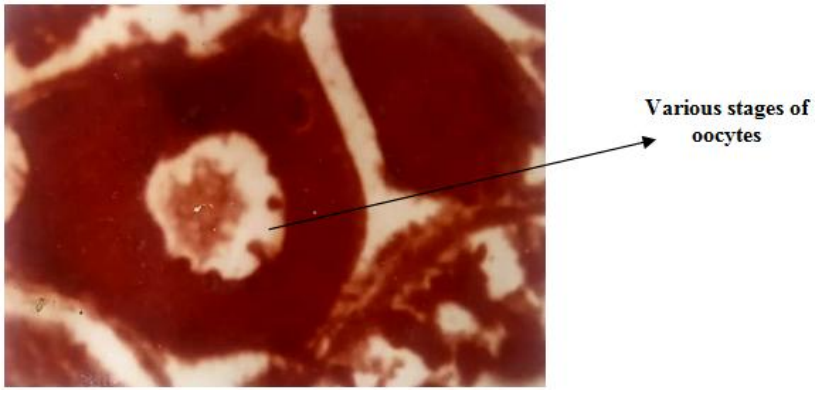

Fig. 4. Various stages of oocytes in the ovary of control fish (H & E X 1500)

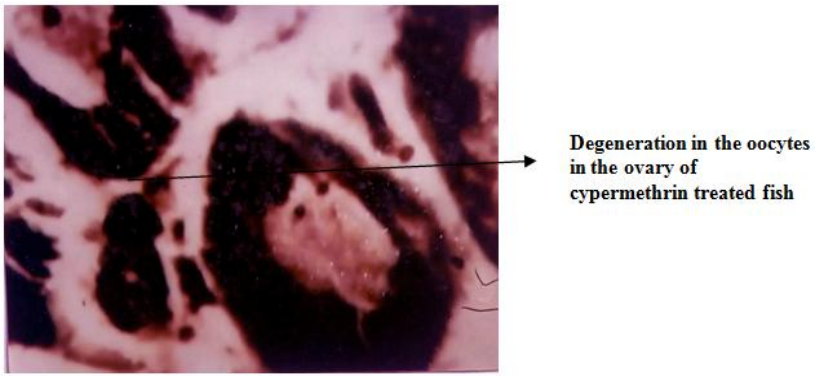

Fig. 5. Ovary of cyptermethrin treated fish showing degeneration in the oocytes (H & E X 1500)

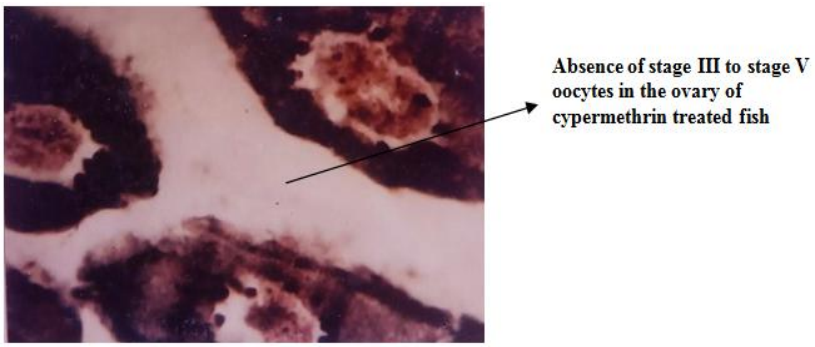

Fig. 6. Ovary of cyptermethrin treated fish showing absence of stage III to stage V oocytes (H & E X 600)

Cypermethrin exposed ovary showed large intra-follicular spaces and rupture of nuclear membrane in stage II oocytes. Absence of stage III & V oocytes reflects complete absence of vitellogenesis under pesticide stress (Figs. 5 & 6). Similar pathological changes have been reported by some workers on various fishes exposed to a number of pollutants. Effects of sublethal poisoning of zinc, copper and lead were reported in the gonads of teleost *Puntius conchonius* [14]. Very similar results were reported in the gonads of a freshwater fish *Clarias batrachus* under phorate exposure [15] and in *Anabas testudineus* under Nickel chloride exposure [16].

4. CONCLUSION

Reproduction is essential for the survival of fish population. Any change in the environmental parameters of fish can affect its reproductive success. Present investigation conclude that the cypermethrin has wide range of adverse effects on histopathology of reproductive organs of fish even in very low dose and hence, disorders may occur in the reproductive process. So the use of pesticide near the aquatic environment should be avoided.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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