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Research Article

HISTOLOGICAL RESPONSES IN A COMMON CARP *CYPRINUSCARPIO* INDUCED BY SUB LETHAL CONCENTRATION OF SYNTHETIC PYRETHROID LAMBDA CYHALOTHRIN

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ABSTRACT

One of the synthetic pyrethroid like lambdacyhalothrinis commonly used for pests like aphids, Colorado beetles, thrips, Coleoptera larvae and adults in agricultural crops such as cereals, cotton, potatoes, vegetables etc. In this study a 96hLC_{50}value of lambdacyhalothrinon *Cyprinuscarpio* by semi-static bioassay renewal method was found. One- eighth (0.2µg/L) of the 96h LC_{50} (1.62µg/L) were selected as sub lethal concentration. Upper and lower confidence limits at 95% for LC_{50}were 1.783 and 1.435 respectively. Sexually mature common carp (*Cyprinuscarpio*) of both sexes were exposed to 1/8th(0.2µg/L) of $LC_{50}$for 1, 5, 10, 15 and 20 days and gonad histomorphology was assessed. Testicular changes in the treated fish include progressive increase in the gaps in the interstitium between lobules and atrophy of the germinal epithelium. The changes pronounced as the days of exposure increased. The ovary also showed significant changes as the days of exposure increased. The changes include broken ovarian follicle, cytolasmic clumping and interfollicular spaces, wrinkled oocytes and atreticoocytes.

Keywords: *Cyprinuscarpio*, Pyrethroid, Toxicity, Lambda cyhalothrin and Histoarchitecture.

INTRODUCTION

Pesticide usage is a critical concern which may have an adverse effect on the delicate ecosystem. The transport of pesticides to delicate ecosystem therefore creates a need to fully understand the effects in the resident biota. In many areas of the world these sensitive ecosystems are at a risk because of non - point source runoff of pesticides from agricultural and urban sources to aquatic ecosystems affecting aquatic biota (Austin,1999; Srivastava et al.,2008).Pesticides are among the most hazardous chemicals not only to human beings but also other organisms. Pesticides are extensively used to protect agricultural crops from the damage caused by pests.The excess usage of pesticides may cause pollution to the nearby ecosystem through wind and runoff; ultimately it leads adverse effects on non-target organisms.

Pyrethroids are synthetic chemical analogues of pyrethrinswhich are naturally occurring insecticidal compounds produced in the flowers of *Chrysanthemum (Chrysanthemumcineraria folium)*. Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, homes and gardens (Amwey and Weston 2005; Oros and Werner 2005).

Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures (Polat et al., 2002). Lambda-cyhalothrin is a mixture ofisomers of cyhalothrin. Lambda cyhalothrin, interrupt the functioning of the nervous system in an organism. By interrupting the nervous system of insects, lambdacyhalothrin may cause paralysis or death. It is highly toxic to many fish and aquatic invertebrate species. The toxic

effect of synthetic pyrethroid lambda cyhalothrinwas found on the functioning of endocrine glands in freshwater catfish, *Clariasbatrachus* (Saravanan et al., 2007).

Histopathology is an effective tool to visualize the stress-induced structural changes in cells and tissues, and has been widely used as biomarkers in the evaluation of various stressors (microbial pathogens, toxic compounds, nutritional and adverse environmental conditions), both in the laboratory (Wester and Canton, 1991; Thophon et al., 2003; Marchand et al., 2009) and field studies (Hinton et al., 1992; Teh et al., 1997).The toxicity in fish blocks the

Sodium channels of nerve filaments which initiate histological change (Kumaraguru et al. 1982).Fish is one of the bioindicator species that play an important role in monitoring water pollution as it responds with great sensitivity to changes in the aquatic environment due to its sensitive to enzyme and hormone disruptors. Chronic exposure to low levels of pesticides may have a more significant effect on fish population than acute poisoning. Doses of pesticides that are nothigh enough to kill fish are associated with subtle change sin behavior and physiology that impair both survival and reproduction (Kegley et al.,1999).

MATERIALS AND METHODS

Procurement of fish and chemical supply

Healthy and active *C.carpio* were procured from State Fisheries Department, Dharwad, Karnataka. They were stored in cement tanks which werecleanedwith0.01% potassium permanganate to avoid infection to the fish. The fishes were fed with commercial dry feed pellets. The carp were acclimatized to laboratory conditions for 20d.

The technical gradelambda cyhalothrin (purity 90%) was procured from Rallies India Enterprises, Mumbai, India. Stock solutions were prepared accordingly using organic solvent acetone. The physico-chemical characteristics of water were analyzed following the methods mentioned in APHA. (Anonymous 2005)

Experimental procedure for acute toxicity

Experiments were performed according to the Organization for Economic Cooperation and Development (OECD) standard method to determine the LC50 of *C.carpio*. Fishes were exposed in batches of 10 in 20L of test medium to varying concentrations suchas1, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4µg /Loftechnical grade lambdacyhalothrin with six replicates for LC_{50} calculation. One blank and one control containing the solubility agent (acetone) were run in addition to test series. The survival rate was noted for a period of 96h. The concentration at which around 50% survival/mortality occurred after 96h was taken as the median lethal concentration. The 96h LC_{50} of lambdacyhalothrinon *C. carpio*was foundtobe1.6µg/L.TheLC_{50}with95%confidence limits for lambda cyhalothrin were determined for 96h by probitanalysis Finney(1971).

Study periods and toxicant concentrations

Sub lethal concentrations of one-eighth(0.2µg/L) of the 96h LC_{50}were selected for sub acute studies. Fishes were exposed to sub lethal concentration of technical lambdacyhalothrin for1, 5, 10, 15 and 20 days. After treatment, both the experimental and control fish were sacrificed at the of 1, 5, 10, 15 and 20 days. Tissues like testis and ovaries were removed and transferred in aqueous Bouin's fluid. After fixation for 24-30 hours, tissues were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in the paraffin. Tissues embedded in paraffin were cut at 6µm using a semi-automated microtome. Then the sections were stained with Haematoxylin and Eosin. The sections were viewed and photographed by using an Olympus phase contrast microscope (Olympus BX51, Tokyo, Japan) with an attached camera (ProgResc 3,Jenoptic-Germany).

RESULTS AND DISCUSSION

The physicochemical characteristics of water were analyzed and found as followstemperature24±1°C,pH 8±0.2 at 24°C, dissolved oxygen 8.2±0.5mg/L, total hardness 126.4±3mg as $CaCO_3$/L, total alkalinity 210.5±4.5mg as $CaCO_3$/L. The 96h $LC_{50}$of lambda cyhalothrin for the freshwater fish *C.carpio*was found to be 1.6µg/L.

Mature testis of a control carp characterized by the presence of spermatogenic stages like spermatogonia(Sg),spermatocytes(Sc),spermatids(St),spermatozoa(Sz) and sertoli cells(Se)Fig.1(A). Testis of a male carp exposed to sub lethal concentration (0.2µg/L) cyhalothrin for one day showing apparently normal histoarchitecture as in control. Fig.1(B). Presence of mild changes like little gap in the interstitium between lobules were observed in testis exposed tosub lethal concentration (0.2µg/L) cyhalothrin for 5days Fig 1(C). The diameter of the lobules become diminished, the lumen contains less spermatozoa and atrophy of the germinal epithelium can be seen in the testis of carp exposed to cyhalothrin for 10 and 15 days Fig.1(D) and (E).There was also progressive increase in the gaps in the interstitium between lobules and extensive loss

of the germinal epithelium in testis exposed to cyhalothrin for 20 days Fig.1(F).

The control ovary of cyprinuscarpio showed different stages of oocytes like perinuclear oocytes(PN),cortical alveolar oocytes(CA) and early vitellogenic oocytes(EV). The vitelline membrane and follicular epithelium were prominent Fig.2(A).Ovary of a female carp exposed to sub lethal concentration (0.2µg/L) cyhalothrin for one day showing apparently normal histoarchitecture as in control Fig.2(B).Mild cytoplasmic clumping and Inter-follicular spacewas clearly observed in some oocytes of the ovary exposed to sub lethal concentration (0.2µg/L) cyhalothrin for 5days Fig.2(C).Thecytomorphological structure of ovarian follicles got deformed and elongated, losing their typical configurationwere observed in ovary exposed to sub lethal concentration (0.2µg/L) cyhalothrin for 10 days Fig.2(D). Changes like cytoplasmic clumping, interfollicular space and atretic oocytesseen in the ovary of female carp exposed to cyhalothrin for 15and 20 days. Fig.1(E and F).

Muller and Beilschmidt (1990) reported 96h $LC_{50}$of lambda cyhalothrin to fish such as blue gill and lake trout with less than 1.0µg/ L. We can infer from our results that lambda cyhalothrin is highly toxic to freshwater fish *C. carpio* and comparison of the different LC_{50} values clearly depicts that the acute toxicity of lambda cyhalothrin varies with the fish species.

The experiment was conducted in the month of December where in seminiferous tubules of the control testis was developed with a concomitant increase in testicular size to attain maturation stage .During this month the lumen of the lobules were almost completely filled with spermatozoa. In the present study, significant alterations were observed in the gonadal tissue of fish treated with different exposure periods. The histological response in the treated group showed the increase in gaps in the interstitium between lobules. Similar changes were observed in the histo-morphology of carp testis after 28 days of 4-TBP. The reductionin spaces in the interstitium and the lumen was disturbed along with disintegration of lumen wall.One-tenth dose of LC50 of 4-*tert*-butylphenol causes endocrine disruption and metabolic changes in *Cyprinuscarpio(*Barseet al, 2006). Dutta and Dikshith (1973), Nigam et al. (1979),reported degenerative changes in seminiferous tubules, enlarged interstitium and hemorrhage in intertubular area in albino rats exposed to pesticides.These changes may culminate in a partial or total arrest of spermatogenesis. Extensivetesticular degeneration may lead to generalized loss of germinal epithelium which was observed in the fishexposed to more days of pesticide. The same findings were observed in the histological analysis of the testis after exposure to Pb at a concentration of 1 mg L^{-1} in which the germinal epithelium of the seminiferous tubules has been severely affected. Mayank et al(2015).Reduction in the germ cells were noticed as the days of exposure increased. The similar observation agrees with the findings in theCd affected testis morphology of the *Gymnotuscarapo*fish species (Vergilio et al 2015).Dutta and Meijer (2003)noted histopathological changes on thestructure of testes after exposure to sublethalconcentrations ofdiazinon on bluegill (*L. macrochirus).* Histological alterations observed in this study are nearly similar to those found in *Mozambique tilaia(Oreochromismossambicus*)(Mlambo et al.,2009) and black goby(*Gobius nigar*)(Louiz et al.,2009). Spermatogenesis is a complex process of cellular transformation that produces mature male gametes for sexual reproduction. Normal sperms are necessary to produce fertile offspring and thus for the evolution of a species.

The different pollutants such as industrial and agricultural wastes, pesticides and heavy metals have histopathological effects on the reproductive tissues of fish gonads (Johnson et al .1991,Lye et al .,1998;Pedlar et al .,2002; Hanna et al.,2005),these effects may disturb the development of germ cells and may reduce the ability of the fish to reproduce (Inbamani and Seenivasan,1998; Kumar and Pant,1984; Mehanna, 2005). The histopathological changes in fish tissues used as a biological indicator for pollution with pesticides with special reference to insecticides. Malathion (1.2 mg/l –catfish) induced changes in ovigerous lamella, clumping of cytoplasm, Degeneration in the follicular cells, Shrinkage of nuclear materials, increased atretic oocytes, and ruptured follicular epithelium. (Sanjoy et al , 2012). In this present investigation, the ovary showed significant changes as the days of exposure increased. The changes include broken ovarian follicle, cytoplasmic clumping and interfollicular spaces, atretic oocytes and wrinkled oocytes. Similar observations were also made by Kulshrestha et al., 1984.But in our findings significant changes were clearly observed in fish which were exposed for longer duration. Therefore, it is clearly shown that lambda cyhalothrinposes athreat to fish population dynamics by reducing fertility.

CONCLUSION

Present study reported the 96 h LC_{50} of analytical grade lambda cyhalothrinchemical in C. *carpio*as1.6ug/L. Extensive testicular degeneration may lead to localized or generalized loss of the germinal epithelium and

progressive increase in gaps in the interstitium between lobules. The ovary also showed significant changes as the days of exposure increased. The changes include broken ovarian follicle, cytoplasmic clumping and interfollicular spaces, wrinkled oocytes and atreticoocytes. There were nuclear and cytoplasmic degeneration of variable magnitude. Severity of degenerative change was more pronounced with prolonged duration. These results shows that cyhalothrin is capable of inducing histopathological alterations in testis and ovary which may cause reproductive dysfunction in the species.

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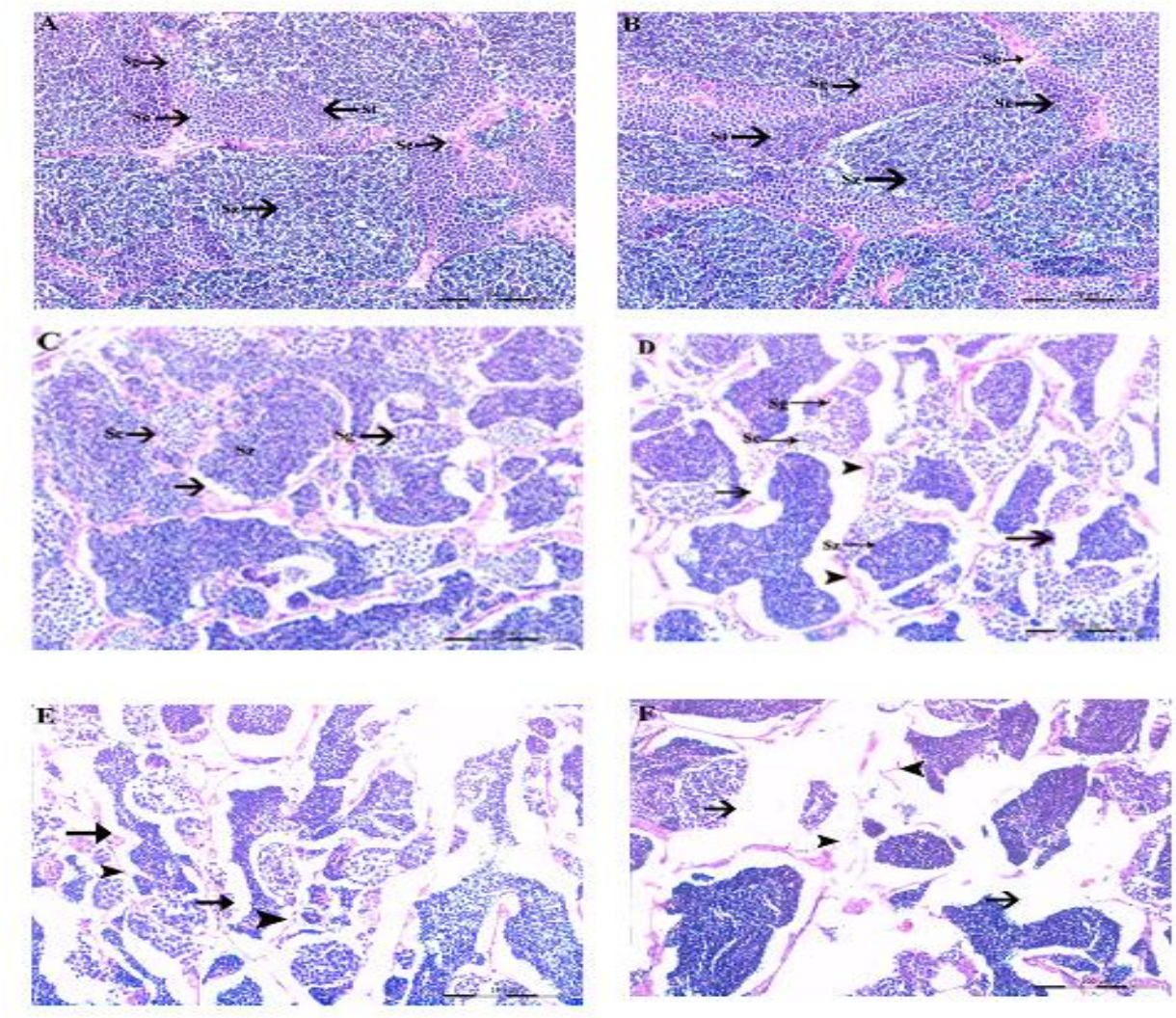

Fig. 1.Histopathological changes in testis of male common carp.(A) Mature testis of a control carp characterized by the presence of spermatogenic stages like spermatogonia (Sg),spermatocytes(Sc),spermatids(St) ,spermatozoa(Sz) and sertoli cells(Se).(B) Testis of a male carp exposed to sub lethal concentration (0.2µg/L) cyhalothrin for 1 day showing apparently normal histoarchitecture as in control.(C) Testis exposed for 5 days. Presence of mild changes like little gap in the interstitium between lobules (arrows).Occurrence of spermatogonia(Sg),spermaocytes(Sc) and spermatozoa(Sz) in the lumen . (D) Testis exposed for 10 days. The diameter of the lobules become diminished, the lumen contains less spermatozoa (arrows).Atrophy of the germinal epithelium (arrow heads). (E)Testis of a male carp exposed for 15 days. The interstitium become narrow with less spermatozoa (arrows), atrophy of the germinal epithilium(arrow heads).(F) Testis exposed for 20 days. Arrows indicate the progressive increase in the gaps in the interstitium between lobules. Arrow heads indicate extensive loss of the germinal epithelium. Bar is 100µm.HE stained

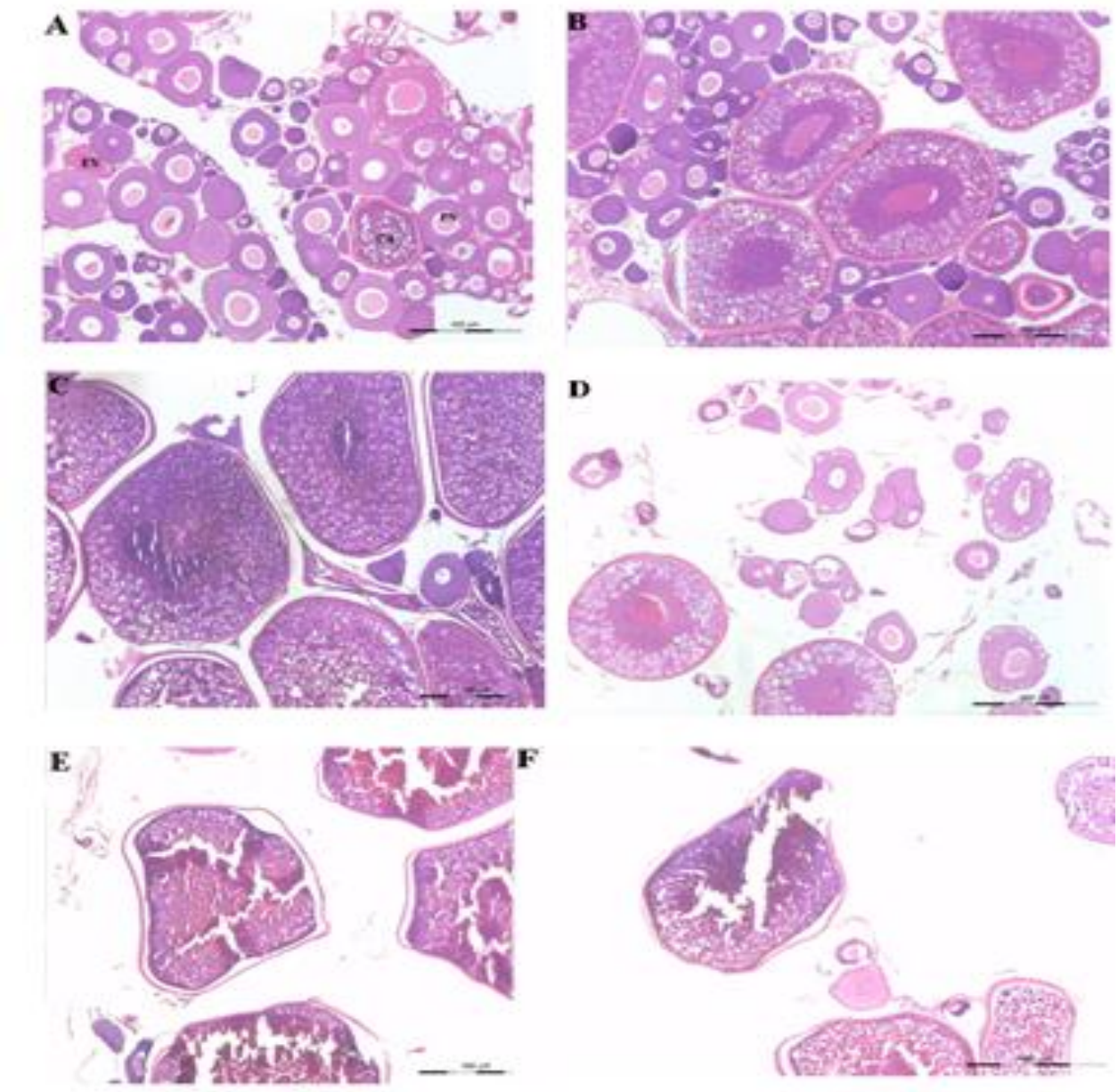

Fig. 2.Histopathological changes in Ovary of a common carp.(A) Ovary of a control carp characterized by the presence of perinuclear stage ooytes(PN),corticaL alveolar oocytes(CA) and early vitellogenic oocytes(EV). (B)Ovary of a female carp exposed to sub lethal concentration (0.2µg/L) cyhalothrin for 1 day showing apparently normal histoarchitecture as in control. (C) Ovary of a female carp exposed for 5 days. Presence of mild changes like cytoplasmic clumping and Inter-follicular space was clearly observed in some oocytes of the ovary. Fig.(D) Ovary of a female carp exposed for 10 days. Changes like wrinkled oocytes and ooplasm fracture are observed (E)Ovary of a female carp exposed for 15 days. Changes like cytoplasmic clumping; interfollicular space and atretic oocytes are seen. (F) Ovary of a female carp exposed for 20 days. Bar is 400µm.HE stained.

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