



Ovarian Impairment in Fish, *Channa punctata* (Bloch) on Exposure to Mercuric Chloride, Caldan 50 SP and their Mixture

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Abstract

Extensive and arbitrary use of pesticides in agriculture and heavy metals in industries as catalysts have caused impending hazards to aquatic organisms. Fishes occupying higher positions in trophic level of the aquatic food chain suffers the most due to the industrial discharge and agrochemicals. Reproductive system is one of the most important systems which gets affected to a great extent and in turn affect the reproductive processes and fecundity of animals. Present investigation deals with both synergistic and individual effects of mercuric chloride (MC), an industrial effluent and Caldan 50SP (C50SP), on the histological profile of ovary of a fresh water food fish *Channa punctata* (Bloch) after 15, 30 and 60 days of exposure periods. Notable histopathological findings include deshaped ovum, ruptured ovarian epithelium, stromal hemorrhage and vacuolization in the ovaries. The extent of ovarian damage was exposure dependent under the impact of individual toxicants, however their synergistic effects pronounced more severe damage even when individual toxicants were in lesser concentration and exposure time was also comparatively less. The results obtained will be helpful to understand the combined effect of MC and C50SP on the ovary of fishes and these findings are quite suggestive of reproductive impairments leading to delayed ovarian maturity and adversely affecting process of ovulation and thus, fish production.

Keywords: Caldan 50SP (C50SP), Fish, Gonads, Mercuric Chloride (HgCl₂), Synergistic Effect

1. Introduction

Water pollution has become inevitable owing to over-industrialization, rapid civilization, green revolution and over population. Indiscriminate and extensive use of pesticides in agriculture and heavy metals in industries as catalysts and electrolytes indirectly cause potential hazards to aquatic animals^{1,2}. The agricultural run-off and water from the fields that drains into the rivers containing pesticides and industrial effluents having heavy metals are now recognized as environmental pollutants. They have considerable toxic effects on aquatic fauna including fishes. Among metals, one of the ubiquitous water pollutants is mercury. Environmental contamination due to mercury is wide spread as a result of industrial activities. It is

used as a fungicide in agriculture, in the manufacture of chlorine and sodium hydroxide, as a slime control agent in the pulp and paper industry, in the production of plastics and electrical apparatus and in mining and smelting operations³. Its toxicity was known as early as 16th century⁴. It has also been found genotoxic. It induces formation of micronuclei and also causes chromosomal aberrations in fish *Channa punctata*⁵⁻⁷.

Although pesticides play a crucial role in modern agriculture but they fall among the hazardous chemicals released by man into the environment⁸⁻¹⁰. Ideally a pesticide should be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not so, the controversy of use and abuse of pesticides has surfaced at large scale. The rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played havoc with humans and other life

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forms. C50SP is one of the most frequently used pesticides to control insect pests in agricultural fields. It is an insecticide which is derived from the naturally occurring insecticidal substance isolated from the marine segmented worms *Lumbrinereis heteropoda* and *Lumbrinereis brevicirra*. It is used against a relatively broad spectrum of insects, e.g. Lepidoptera, Coleoptera, Diptera and Hemiptera. Synthetic pyrethroid, devicyprin (cypermethrin 25%) induced gonadal impairment in a freshwater food fish, *Channa punctatus*(Bloch) have been reported by¹¹.

Multichemical exposure is the rule rather than an exception. Chemical mixtures in the environment can be and generally are complex, consisting of parent compounds, reaction or transformation products along with other residues. Exposure of biota to combinations of pollutants may sometimes result in unexpected consequences, i.e. significantly lower or greater toxic response than a simple summation of the response induced by the mixture components taken individually. Mixture of pollutants in the environment can influence the toxicity of each other which could be altogether different and more pronounced from their individual toxicological effects^{12,13}. This is called synergistic effect or synergy. Ammonia combines with copper to form very stable complex cations of cuprammonium $[\text{Cu}(\text{NH}_3)_4]^{2+}$ and it enhances the copper toxicity. James and Sampath, (1995) reported that the individual effect of copper is more toxic than ammonia and two of the combinations are more toxic than the individual effect of copper and ammonia¹⁴.

Trivedi and Rai (2007) have reported the impact of mercuric chloride, Caldan 50SP and their mixture on histological architecture of the kidney of *Channa punctata* (Bloch)¹⁵. They also investigated the effect of sub-lethal concentrations of mercuric chloride, Caldan 50SP, both individually as well as in combination by exposing the fish *Channa punctata* for 15, 30 and 60 days, on the hepatocellular profile of the fish. They reported several histopathological changes viz. inflammation, swelling, necrosis, vacuolization and hypertrophy in hepatocytes in the liver of fish *Channa punctata*^{13,16}. Potential ecotoxicological hazard of mercuric chloride, Caldan 50SP and their synergistic effect, on gills of a fresh water fish, *Channa punctata* as a test animal has also been reported Rai et al., 2018¹⁷. In present study, an effort has been made to evaluate the toxicity of Mercuric Chloride and Caldan 50SP, individually and in combination (synergistic effect), in the fish model, *Channa punctata*.

2. Materials and Methods

In the present investigation adult and healthy specimens of *Channa punctata* measuring 13.90 ± 0.394 cm in length and 29.80 ± 0.789 grams in weight were collected from local fresh water resources of Lucknow and brought to laboratory in wide mouthed plastic buckets, in natural water, avoiding stress and mechanical injuries as far as possible. Fish were transferred to large glass aquaria ($100 \times 40 \times 40$ cm) and thoroughly washed with tap water thrice and were then given prophylactic treatment to remove external infections due to bacteria, protozoa, monogenetic trematodes and arthropods. For this purpose, a wash of 1 mgL^{-1} solution of KMnO_4 for 1 hour was given to fish. Fishes were acclimatized for 10 days containing aged for proper gaseous exchange, aerators were used tap water. During this period fish were fed with minced goat liver and artificial fish food Tokyo. Every effort was made to maintain optimal condition during acclimatization.

96 h LC_{50} of the test chemicals calculated by “Trimmed Spearman-Kärber method”¹⁸. Ten fishes were exposed to sub-lethal concentration of Mercuric Chloride ($1/10^{\text{th}}$ 96 hr. LC_{50} ; 0.081 mg/l), Caldan 50SP ($1/10^{\text{th}}$ of 96 hr. LC_{50} ; 0.153 mg/l) and $1/20^{\text{th}}$ of each of their concentration together (synergistic effect) for 15, 30 and 60 days of exposure periods respectively along with a parallel control experiment. Physico-chemical characteristics of water were analyzed at the commencement and termination of each experiment, using standard procedures¹⁹.

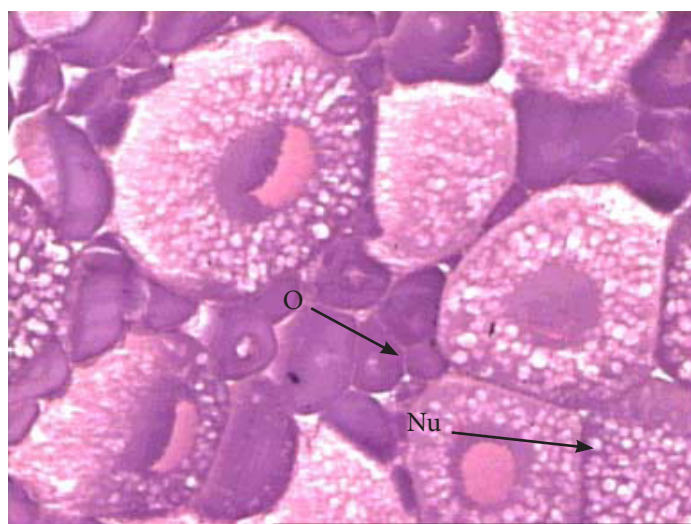
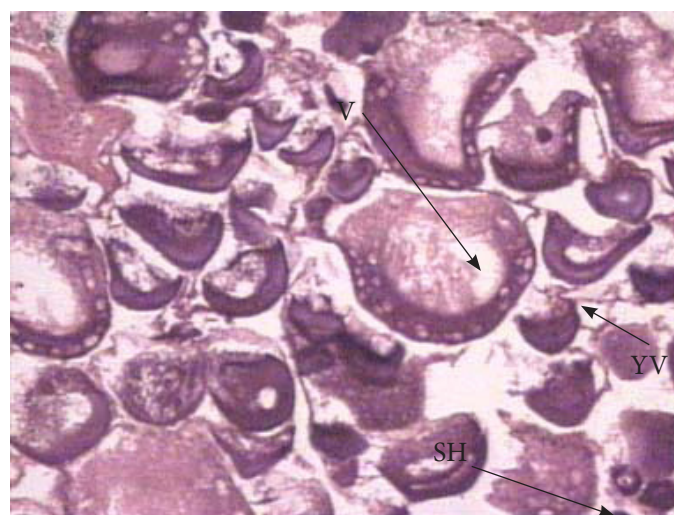
After the termination of each experiment, fishes were dissected in Ringer’s solution. Ovaries were then taken out and processed for microtomy. Significant findings were recorded by NIKON-HFX-DX trinocular microscope and NIKON-FX-35-DX camera with automatic time recorder. Changes in exposed sections of ovaries were compared with that of control sections.

3. Results

The histoanatomy of ovaries of fish exposed to sublethal concentration of Mercuric Chloride ($1/10^{\text{th}}$ 96 hr. LC_{50} ; 0.081 mg/l), Caldan 50SP ($1/10^{\text{th}}$ of 96 hr. LC_{50} ; 0.153 mg/l) and $1/20^{\text{th}}$ of each of their concentration together (synergistic effect) for 15, 30 and 60 days of exposure periods showed varied histopathological findings (Table 1).

Table 1. Individual and synergistic effects of C50SP and Mercuric Chloride on the ovary of *Channa punctata*

Exposure periods	Histology of Normal Ovary (Control)	Histology of Ovary Exposed to		
		MC	C50SP	MC+C50SP
15	The wall of ovary consists of 3 layers, peritoneum, tunica albuginea and germinal epithelium. The germ cells originate from germinal epithelium. A ripe ovum is large in size, yellowish in color and translucent. It is full of yolk globules, vesicles and nucleus. (Figure 1)	Yolk vesicles arranged at the periphery of oocytes, stromal haemorrhage and vacuoles more prominent. (Figure 2)	Yolk vesicles and globules arranged in the periphery of ovum and vacuolization in cytoplasm. (Figure 4)	Necrotic and degenerative ovum along with stromal haemorrhage. Ovum swells with large sized yolk vesicles. (Figure 6)
30		Heavy stromal haemorrhage, necrosis in ovigerous lamellae and oocyte swelling.	Rupturing of ovum, narrowing of ovum lumen, degeneration of ovarian epithelium.	Stromal haemorrhage and ruptured follicular epithelium.
60		Disruption of ovarian follicle, reduction in number of mature ovum and 2 ^o oocytes, degeneration of follicles and follicular epithelial cells, hypertrophy and hyperplasia. (Figure 3)	Vacuolization in cytoplasm, deshaped ovum with mild necrosis and stromal haemorrhage. (Figure 5)	Degeneration of ovum, severely damaged follicular epithelium and distorted architecture of ovaries. (Figure 7)

**Figure 1.** Section of the ovary of *C. punctata* from control showing normal Oocytes (O) and nucleus (Nu), (H and E × 100).**Figure 2.** Section of ovary of *C. punctata* exposed to 96 h LC₅₀ 1/10 of Mercuric Chloride for 15 days showing Yolk Vesicles (YV) at the periphery of Oocytes, Vacuolization (V) and Stromal Haemorrhage (SH), (H and E × 100).

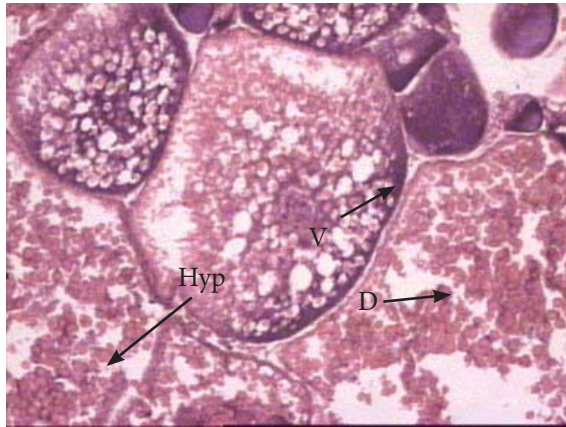


Figure 3. Section of ovary of *C. punctata* exposed to 96 h LC₅₀ 1/10 of Mercuric Chloride for 60 days showing Oocytes of abnormal size having large number of Vacuoles (V), degeneration of follicles (D) and hyperplasia (Hyp), (H and E x 100).

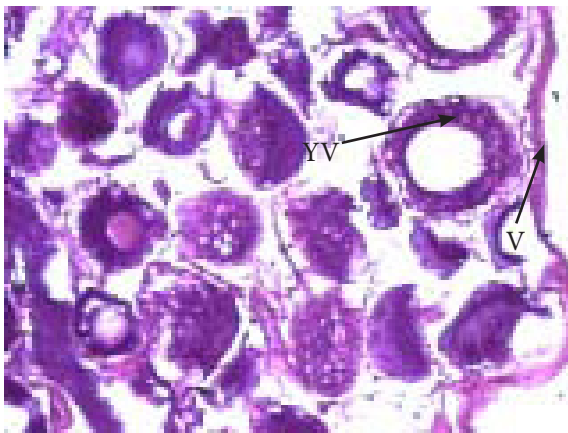


Figure 4. Section of ovary of *C. punctata* exposed to 96 h LC₅₀ 1/10 of Caldan 50SP for 15 days showing vacuolization (V) and Yolk Vesicles (YV) arranged at the periphery of the ovum (H and E x 100).

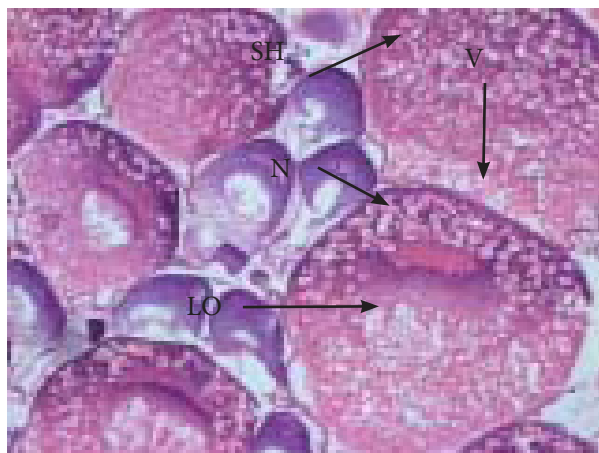


Figure 5. Section of ovary of *C. punctata* exposed to 96 h LC₅₀ 1/10 of Caldan 50SP for 60 days showing vacuolization in cytoplasm (V), almost desheathed large ovum (LO), Necrosis (N) and Stromal Haemorrhage (SH), (H and E x 100).

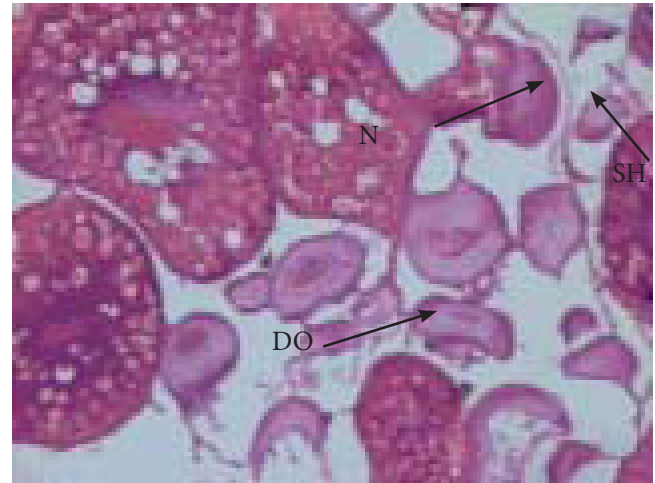


Figure 6. Section of ovary of *C. punctata* exposed to mixture of Mercuric Chloride and Caldan 50SP (96 h LC₅₀ x 1/20 of each) for 15 days showing Necrosis (N) and Degenerative Ovum (DO) along with Stromal Haemorrhage (SH), (H and E x 100).

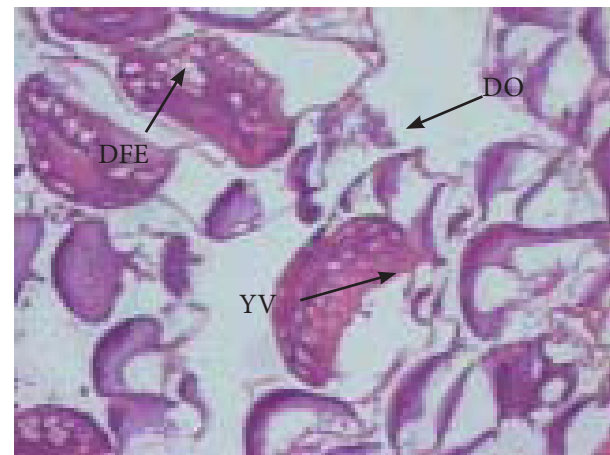


Figure 7. Section of ovary of *C. punctata* exposed to mixture of Mercuric Chloride and Caldan 50SP (96 h LC₅₀ x 1/20 of each) for 60 days showing Degenerating Ovum (DO), Yolk Vesicles (YV) and Damaged Follicular Epithelium (DFE), (H and E x 100).

4. Discussion

Fishes occupy the utmost position in the trophic level of aquatic ecosystem, thus suffer most due to the industrial discharge and pesticides which impinge in different systems. Reproductive system is one of the most important systems which to a great extent is affected by these toxicants. It is now evident that toxic substances such as heavy metals and pesticides introduced in the environment affect the reproductive processes and fecundity of animals^{20,21}.

In the present investigation, fish *C. punctata*, exposed to sublethal concentration of Mercuric Chloride registered gross histopathological changes in their ovaries and the normal configuration of the ovary was almost lost. Degenerative

changes in both ovary and testis with reduced gonadosomatic index on exposure to mercurial fungicide Emisan (MeEHgCl) in catfish *Clarias batrachus* was also reported by Das and Sengupta, 1993²². Almost similar changes were recorded by Shukla and Pandey (1984) in fish *Colisa fasciatus* exposed to 14.0 mg/l of arsenic for 15 and 30 days of exposure period²³. They observed marked degenerative changes after 30 days of exposure period. These changes included prominent follicular spaces, reduction in the development of 2nd and 3rd stage of oocyte, reduced number and diameter of nucleoli and atretic follicles. Histopathological changes in the gonads of fishes after pesticide exposure has been reported by many workers^{24,25}. The treatment of fresh water Indian cat fish *Clarias batrachus* (Linn.) with Butachlor 2 chloro-N-(2-6 diphenyl) acetamide at a dose of 1.5 µl/L for 5, 10 and 15 days led to significant decline in the number of healthy follicles and increase in the number of atretic follicles leading to altered follicular kinetics²⁶.

Exposure to C50SP also caused noticeable histopathological changes in the ovaries of *C. punctata* but the degree of changes was less pronounced as compared to Mercuric Chloride treated fish and the fish treated with mixture of toxicants. Similar changes were reported by Hazarika and Das (1998) in the air-breathing fish *H. fossilis* (Bloch) on exposure to BHC²⁷. They observed vacuolization in cytoplasm of germinal cells, partial disruption of ovarian follicles and reduction in number of matured ovum and secondary oocytes. In fishes, pesticides seem to retard gonadotropin secretions (Singh and Singh, 1981), thereby causing inhibition of gonadal growth. Zutshi (2005), observed appreciable reduction in size, with spermatids and sperms in degenerating condition and necrosis of interstitial cells after fenthion treatment in fish, *Glossogobius giuris*^{28,29}. Zutshi and Murthy (2001) reported extensive cytotoxic damage in the testis of gobiid fish *Glossogobius giuris* after fenthion exposure³⁰. They observed that cytoplasm was reduced to granular material with fragmented endoplasmic reticulum and which was devoid of mitochondria and lipid droplets. The spermatozoa of treated fish showed atrophy in the head and tail region.

Exposure of fish to the mixture of toxicants (MC+C50SP) showed profound histopathological changes in the gonads as compared to the fish treated with single toxicant. Trivedi and Rai (2007) have reported the synergistic effect of Mercuric Chloride and Caldan 50SP on histological architecture of the kidney of *Channa punctata* (Bloch)¹⁵. They also investigated the effect of sub-lethal concentrations of mercuric chloride, Caldan 50SP, both individually as well as in combination by exposing the fish *Channa punctata* for 15, 30 and 60 days, on the hepatocellular profile of the fish. They reported several histopathological changes viz. inflammation, swelling, necrosis,

vacuolization and hypertrophy in hepatocytes in the liver of fish *Channa punctata*¹⁶. Potential ecotoxicological hazard of mercuric chloride, Caldan 50SP and their synergistic effect, on gills of a fresh water fish, *Channa punctata* as a test animal has also been reported by Rai et al., 2018¹⁷. Inadequate literature is available supporting such histopathological changes due to metal and pesticides mixture exposure to the fish and other animals. But it is quite suggestive that these alterations may lead to the suppression of ovarian activity resulting in the impairment of reproductive potential of the fish.

5. Conclusion

Present study concludes that metal and pesticide exposure alter the normal architecture of ovarian tissue of *C. punctata*. The toxicity of Mercuric Chloride and Caldan 50SP is dose and time dependent. Although the exact mechanism of such alterations may be yet to be known, it can be established that oxidative stress is one of the prime causes for such aforementioned histo-pathological insults in the fishes. Definitely, there is an urgent need for proper monitoring and regulation of concentration of pesticides and heavy metals and their residues in aquatic habitats not only to safeguard fish biodiversity but consumer's health as well.

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