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Research Article

HISTOPATHOLOGICAL ALTERATIONS IN THE GILLS OF FRESH WATER FISH *CYPRINUS CARPIO* EXPOSED TO ACUTE LETHAL TOXICITY OF PHORATE

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Abstract- The effect of acute lethal toxicity of phorate (ALTP) on the histopathology of gills of the fresh water fish *Cyprinus carpio* (*C. carpio*) was investigated in the present study. Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the Probit method of Finney and the LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration for acute toxicity study. Fish were exposed to ALTP (LC_{50}/96 hours-0.71 ppm/l) for one day and 4 days and the differential acute toxicity tests were carried out under laboratory conditions. On exposure for a period of 1 day to the acute toxicity of phorate (ATP) no significant pathological changes were observed in the gills of the fish except indications of the initiation of degenerative changes. Further, on exposure for a period of 4 days to ATP, the pathological changes observed in the gills of the fish *C. carpio* were significant structural degeneration of primary gill lamellae, secondary gill lamellae and pillar cells along with epithelial lifting and desquamation. There was hypertrophy in the gill lamellae, erosion of surface epithelial cells and loss of lamellar structures in the gill due to atrophy of the gill lamellae. In the exposed fish, respiratory distress and abnormal behaviour were observed. On exposure to ATP, though initially caused a mild damage to the gill of the fish at day first, but further exposure to ATP for 4 days; it caused an irreversible damage.

Keywords- Acute lethal, Phorate, *Cyprinus carpio*, Pillar cells, Hypertrophy, Necrosis, Atrophy.

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Introduction

The inclusion of various pollutants like pesticides, in the aquatic ecosystem and their deposition in the biotic system is known to cause several structural and functional changes in the aquatic organisms like fish. Pesticides are unusual among environmental pollutants, in that as they are used deliberately for the purpose of killing harmful insects and pests. Due to the indiscriminate use of pesticides, the aquatic environment is currently under threat as these are disturbing the biodiversity of aquatic ecosystem [1]. The pesticides play an important role in the production and preservation of food and other commercial crops by keeping a check on many species of harmful pests. Heavy dependence modern agriculture on chemical substances like pesticides is posing a serious threat to the ecological balance of the aquatic environment.

Organophosphates (OPs) are the widely used group of pesticides in the world because of their less persistence and rapid degradation in the environment. Exposure of aquatic ecosystems to OP insecticides is difficult to assess because of their short persistence and low solubility in water. As they are highly toxic to aquatic organisms like fresh water fishes, monitoring of these insecticides is important. Fish are the non-target organisms, which are generally exposed to multiple concentrations of pesticides under field conditions. Since OPs inhibit AChE activity, they are powerful nerve poisons and are highly toxic to the non-target aquatic organisms like fish [2, 3]. Several investigators worked on the toxicity of OP pesticides in fish [4-8]. The level of toxicity of pesticides may differ from one type to the other and from one species to the other [9, 10]. The differences are related to the chemical nature of the toxicant, the interaction of the chemical with biological systems, the resistance capacity of the animal, detoxification mechanisms involved, the purity of the pesticide and the additives or emulsifiers present in the commercial grade formulations.

Among fish species, different metabolic pathways may result in different patterns of biotransformation of pesticides, leading to more or less toxic metabolites [11]. Due to the exposure to pesticides, the extent of damage to the animals like fish depends on breathing rate, length, weight, corporal surface and body weight ratio [12, 13] of the animal. The level of toxicity of a toxicant like pesticide can be measured in terms of its concentration or dose, which kills a known number of populations of a given species within a definite period of time. The evaluation of toxicity of a test chemical is a sensitive phenomenon, which can be influenced by several factors such as size [14], weight [9], nutritional status [15], its developmental stage [16], species specificity [17-19], the time of exposure and temperature [20], chronobiology of the animal [21], increase in animal density [22] and sex of the animal [23, 24]. Every pesticide may vary greatly in its toxicity and persistence [25].

Phorate is an Organophosphorous insecticide (OPI) which is widely used throughout the world and also in India as a broad spectrum insecticide on numerous crops including paddy and groundnut. It used to control rootworms, sucking and chewing insects, leafhoppers and leaf miners [26, 27] in the root and field crops and pine forests. It is an important OPI to which the fresh water fishes are frequently exposed due to the indiscriminate use of it by the farmers. It is highly toxic and extremely fast-acting on bird species, freshwater fish, and aquatic invertebrates [28]. Thus, the objective of this study was to investigate the acute lethal toxic effects of phorate on the histopathological changes in the gills of common carp *C. carpio*.

Materials and Methods

Test Species

The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fish, having great commercial value. The animals were starved for 24 hours prior to each estimation to avoid any influence of differential feeding.

Test Chemical

Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an OPI which is widely used throughout the world and also in India including Andhra Pradesh as a broad spectrum insecticide on numerous crops. Commercial names of phorate are Thimet, Rampart, Granutox, Agrimet etc and its molecular formula is $C_7 H_{17} O_2 PS_3$.

Procurement and maintenance of fish

Fingerlings of *C. carpio* fish were brought from the department of fisheries, Anantapur, Andhra Pradesh and released into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. Then the fish were separated into the batch of having the size of 10 ± 2 gm and were maintained in static water without any flow [29]. Water was renewed every day to provide fresh water, rich in oxygen. During experimentation, water was aerated once a day to prevent hypoxic conditions, if any [30]. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of animals, size, sex etc [31], precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it water from the same source has been used for maintenance of fish. The size of the animals selected was also maintained strictly throughout the investigation.

Acute toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the Probit method of Finney [32]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity of phorate.

Experimental Design

60 fishes were divided into 3 groups comprising of 20 fishes each. The group I was considered as normal control, group II and III were experimental groups. Group II was exposed to ATP (LC_{50} of Phorate= 0.71 ppm/l) for 1 day and group III for 4 days. Then the fish were sacrificed and gills were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

Histopathology

The histological sections of the gills of acute toxicity exposed fish were taken by adopting the procedure as described by Humason [33]. The tissues were isolated from control and the phorate treated fish and rinsed with physiological saline solution (0.9% NaCl) to remove blood, mucus and debris adhering to the tissues. They were fixed in Bouin's fluid for 24 hours and the fixative was removed by washing through running tap water overnight. The tissues were processed for dehydration using ethyl alcohol as the dehydrating agent and were passed through a graded series of alcohols, cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with hematoxylin [34] and counter stained with eosin (dissolved in 95% alcohol). Then the sections were mounted in canada balsam after dehydration and cleaning and photomicrographs were taken using the magnus photomicrographing equipment.

Results and Discussion

Results

The structure of the gill of normal control *C. carpio* fish is composed of primary gill lamellae and secondary gill lamellae with well marked inter lamellar spaces. Primary gill lamellae consisted of multilayered epithelium, vascular system and cartilaginous skeletal structure filament. Numerous secondary lamellae were lined up along both sides of primary lamella. Secondary gill lamella was constituted of epithelial cells supported by pillar cells [Fig-I].

Histopathological study in gills

On exposure for a period of 1 day to ATP, no significant pathological changes were observed in the gills of the fish *C. carpio* except indications of the initiation of degenerative changes [Fig-Ia]. Further, on exposure for a period of 4 days to ATP, the pathological changes observed in the gills of the fish *C. carpio* were structural degeneration of primary gill lamellae, secondary gill lamellae and pillar cells along with epithelial lifting and desquamation. There was hypertrophy in the gill lamellae, erosion of surface epithelial cells and loss of lamellar structures in the gill due to atrophy of the gill lamellae [Fig-Ib].

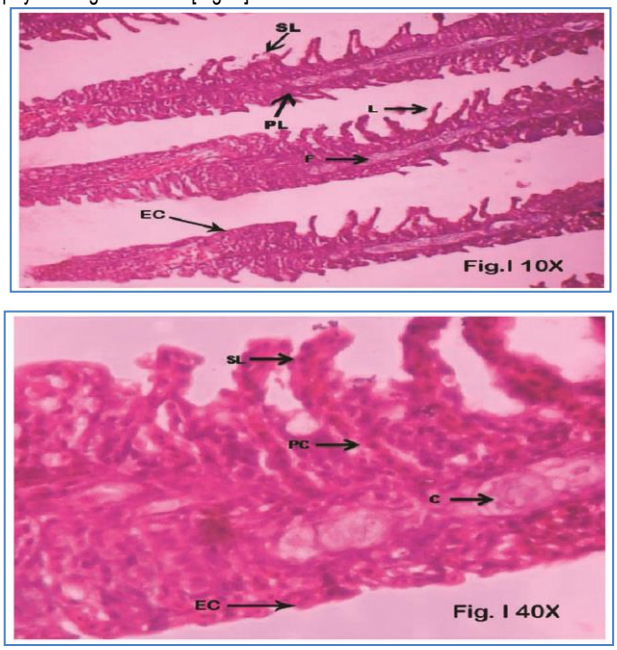


Fig-I The normal architecture of the control fish gill tissue showing primary lamellae (PL), secondary lamellae (SL), epithelial cells (EC), lamella (L), filament (F), chondrocytes (C) and pillar cells (PC) with well marked inter lamellar spaces with lower magnification (10X) and higher magnification (40X).

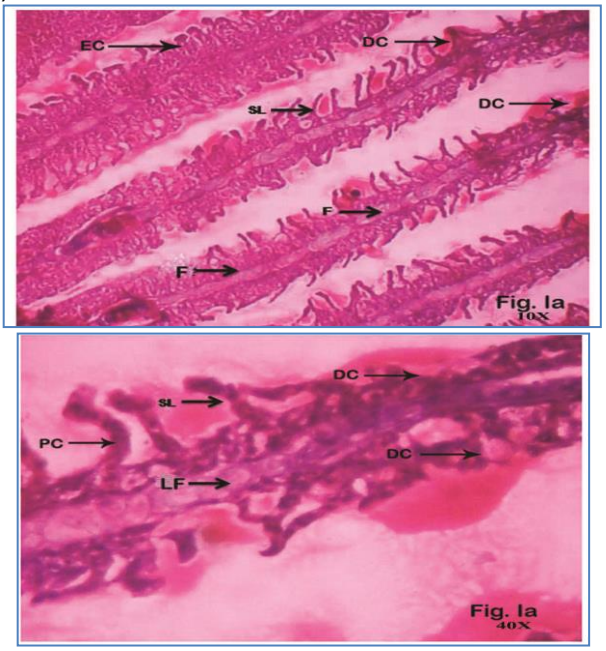
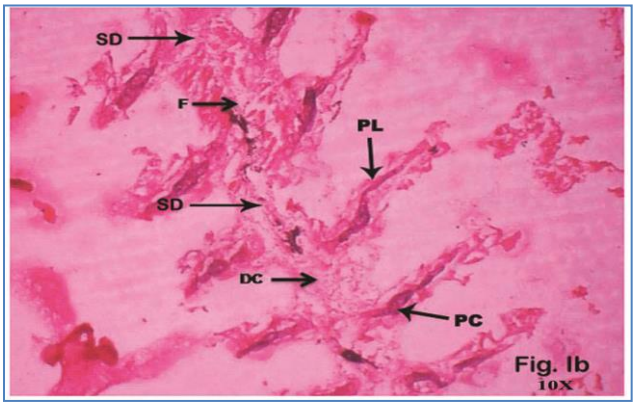


Figure Ia. The Gill of the fish exposed to ATP for 1 day showing primary lamellae (PL), secondary lamellae (SL), epithelial cells (EC), lamellar filament (LF) and pillar cells (PC) with mild degenerative changes in normal cytoarchitecture with lower magnification (10X) and higher magnification (40X).



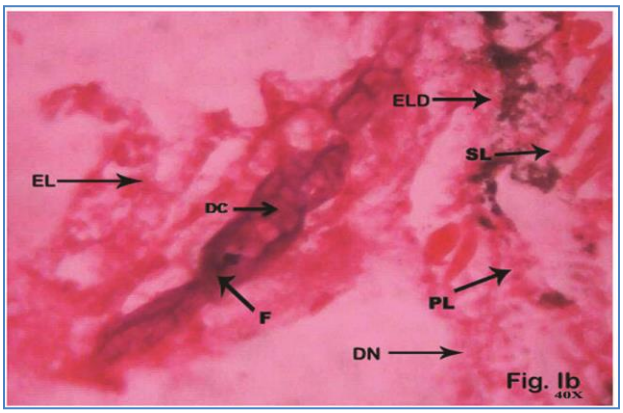


Figure Ib. The gill of the fish exposed to ATP for 4 days showing structural degeneration (SD) in primary gill lamellae (PL), secondary gill lamellae (SL), filament (F) and pillar cells (PC) along with the degenerative changes (DC) such as epithelial lifting (EL), desquamation (DN) epithelial lifting and desquamation (ELD) with lower magnification (10X) and higher magnification (40X).

Discussion

Histopathological studies in fish are valuable tools for the assessment if toxic effects of pesticides to the aquatic organisms and also for monitoring water pollution. In the present study, it is clearly indicated that the phorate has induced pronounced pathological changes in the gills of the fish *C. carpio* exposed to ATP [Fig-Ia and Ib]. Various histopathological responses during the acute toxicity of pesticides could bring a relationship between the level of accumulation of the pesticide and to the various physiological and biochemical activities of the animal. In the present study on exposure to ATP, relative to controls, severe degenerative changes were observed in the gills of the fish, *C. carpio*. The frequency of pathological changes, increased with increasing the exposure time of the fish, *C. carpio*. These histopathological responses of the fish *C. carpio* exposed to ATP in the present study reveal the degree of damage caused by this pesticide to the gills of the fish. The extent of damage is progressive over the period of exposure [35] to the ATP suggest that the histopathological responses depend on the concentrations of pesticides and the length of the period of fish exposure to pesticides.

Since the gills of fish like *C. carpio* are the primary route for the entry of pesticides, they are generally considered as good indicators of water quality [36] and being models for studies of toxic impact [37-39] of pesticides fishes. In the present study, the observed pathological changes in the gills of *C. carpio* were hyperplasia and hypertrophy in the epithelial cells and their lifting, disorganization and aneurysm in lamellae, rupture of epithelium and pillar cells and necrosis due to acute phorate toxicosis. Similar types of pathological changes were observed by many researchers on exposure to different pesticides and extracts. Ayoola [35] observed cellular infiltration, irregular lamellar epithelium, epithelial lifting, Oedama, thickening of the epithelium in primary lamellae and fusion of secondary lamellae in nile tilapia (*Oreochromis niloticus*) exposed to glyphosate.

At different concentrations of glyphosate for 96 h exposure, the frequency of pathological changes, increased with increasing the exposure time. Ayoola and Ajani [40] observed congestion, severe gill damage and infiltration and swell in the tip of the gill filament in *Clarias gariepinus* after exposing to lethal concentrations of cypermethin.

Halis Boran et al [41] studied the histopathological changes induced by acute toxicity (96 h LC_{50}) of maneb and carbaryl in gill, liver, kidney tissues of rainbow trout, *Oncorhynchus mykiss* and observed edema in gill lamellae, epithelial separation from lamellae, fusion of lamellae, epithelial cell swelling and necrosis in maneb and carbaryl exposed fish. Ayoola Simeon Oluwatoyin [42] studied histopathology in nile tilapia, *Oreochromis niloticus* exposed to lethal concentrations (96 h LC_{50}) of aqueous and ethanolic extracts of *Ipomoea aquatica* leaf and observed cell lesion in filament, malignancy, degeneration of cells, necrosis and inflammation in the gills. The damage occurred to the secondary gill lamellae with light precipitation of mucous in the fish at day 1 of exposure to ATP indicate that most of the pesticides affect the organ systems during the initial period of exposure. These changes may be a part of defense mechanism of the fish. The secondary gill lamellar changes in the gill of the fish might have occurred due to the failure of gaseous changes across the respiratory epithelium on exposure to the ATP. It can be speculated that pathological changes like fusion of lamellae, epithelial hyperplasia, degeneration and epithelial lifting may increase the contact of toxicants with the gill, which leads to the impairment of respiration and health in fish.

As the gills are important organs for osmoregulation, respiration and excretion in fishes, the cellular damage in the gills of the fish *C. carpio* induced by phorate toxicity might also impaired the osmoregulatory function of the fish. Aneurism was observed at day 4 on exposure to ATP. It occurs due to the damage of pillar cells and blood vessels, which leads to release of large quantities of blood that push the lamellar epithelium outward. Structural degeneration of primary gill lamellae, secondary gill lamellae and pillar cells along with epithelial lifting and desquamation are the direct deleterious effects induced by phorate exposure. Thus, the histological changes that were taken place in the present study, at the initial period of exposure in the gills of the fish on exposure to ATP at day 1 might be a part of defense mechanism of the fish. On prolonged exposure for 4 days, due to further accumulation of phorate in the gills of the fish, it caused destruction in the organ structures. The degree of destruction in the tissues of the fish gill appeared to be linearly proportional to the period of exposure [43, 44].

Conclusion

On exposure to ATP, though initially it caused a mild damage to the gills of the fish at day 1, but further exposure to ATP for 4 days; it caused irreversible damage to the giils of the fish *C. carpio*. Thus the changes induced by ATP in the structure and morphology of the gills of the fish are not only dependent on the concentration of the pesticide, but also on the length of the exposure period. Frequency and magnitude of tissue damage depends not only on the concentration of pesticides but also on the duration of the exposure period of fishes to pesticides like phorate and other orgnaophosphates.

Conflict of Interest: None declared.

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