An Attempt to Study Ultrastructural Changes in Kidney of *Ctenopharyngodon idellus* (Cuvier and Valenciennes) Induced by Monocrotophos Using TEM

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Abstract

TEM studies were done to study the histological effects of monocrotophos on the kidney of *Ctenopharyngodon idellus* (Cuvier and Valenciennes). Ultrastructural changes revealed change in glomerulus, increase in exposure period exhibited drastic damaging effects showing damage of the glomerulus and vacuolization of cytoplasm. The damaged mitochondria and inactivation of nuclear material was also observed.

Key words: *Ctenopharyngodon idellus*, Monocrotophos; TEM

Introduction

The problem of pollution of aquatic habitats of pesticides has attracted the attention of scientists all over the world, consequently a lot of work has been published in the past regarding the effect of pesticides on various kinds of organisms. Fishes have long been used to monitor the quality of the aquatic environment and fish histopathology is increasingly being used as an indicator of environmental stress (The et al., 1997; Stentiford et al., 2003). There are approximately 19,650-21,535 living fish species all over the world, out of which one third are freshwater and two third are marine (Cohen, 1970).

Among organic pollutants, pesticides and herbicides have important role in causing water pollution. Their increased use in agriculture leads to the contamination of water table. These deadly chemicals are carried down into ground water as well as surface waters through rain and irrigation, making it unfit for drinking purpose. Though monocrotophos, an organophosphate is banned for control of vegetable pests, but it is still being used widely because it is non-specific systemic insecticide and acaricide which is used to control a wide spectrum of chewing and sucking insects, ticks, spiders and also common mites (Burkhard and Drabek, 1994). It is widely used against cotton pests and on various crops like rice, bajra, cotton, wheat, citrus, sugarcane, sugarbeet, ornamentals, tobacco, potato, soyabeans etc.

As Grass carp, *Ctenopharyngodon idellus* is a very hardy fish which can tolerate a wide range of temperatures and is adaptable to the varying environment, it is considered to be good candidate for composite fish culture along with Indian major carps and other Indian exotic fishes. Due to its good taste, it fetches good price in the market. It is also introduced in these waterbodies which are heavily infested with macrophytes for the biological control of macrophytes.

Ultrastructural alterations observed in the proximal tubules of the kidney included nuclear degeneration, condensation and massive swelling of the mitochondria, RER fragmentation, Disorganized brush border, increased number of hydropic vacuoles upon pesticide exposure (Thophon et al., 2004). TEM studies showed the loss of plasmalemma and cell interdigitation, altered mitochondria, decrease in rough endoplasmic reticulum, free lysosomes and presence of autophagic vacuoles in the kidney of *Caquataia kraussi* and *Colossoma macropomum* after exposure of herbicide triazine (Segnini de Bravo et al., 2005). Changes in mitochondria, nuclear damage, increasing number of neutrophils, changes in melanomacrophage centres and occurrence of free macrophages were observed by Rabitto et al. (2005).

It can be concluded that histological studies can be used as an effective tool to determine the health of entire ecosystem. In the present study, an attempt to observe possible histopathological alterations in the kidney of Grass carp, *Ctenopharyngodon idellus* has been done.

Materials and Methods

Procurement of fish

The fish specimens of *Ctenopharyngodon idellus* (Cuvier and Valenciennes) used in the present study were collected from Neelamber Fish Farm, Nanoke village, District Patiala, Punjab and were acclimatized under laboratory conditions for 15 days in dechlorinated water. The tank was provided with an aerators and...
Figure 1. (a-f): Transmission electron micrographs of kidney sections of *Ctenopharyngodonidellus* (Cuvier and Valenciennes) of monocrotophos treated (15 days) fish. **Abbreviations:** BB - Brush Border, Ci – Cilia, J – Junction, M - Mitochondria, N - Nucleus, V – Vacuolisation
Figure 2. (a-f): Transmission electron micrographs of kidney sections of *Ctenopharyngodonidellus* (Cuvier and Valenciennes) of monocrotophos treated (30 days) fish. **Abbreviations:** BB – Brush Border, Ch – Chromatin Material, Ci – Cilia, DN – Damaged Nucleus, M – Mitochondria, N – Nucleus, V – Vacuolisation
filters and ahead of the start of experiment, average length and weight of fishes under investigations was calculated which varied from 13.97 cm (± 15.5) and 20.75 gm (± 7.65) respectively. To avoid the starvation, fishes were fed with fresh *Hydrilla* so that fish tissues do not show any changes.

The experiment was carried out by using the organophosphate Hilcron 36SL (Monocrotophos 36SL), manufactured by Hindustan Insecticides Ltd., having 36% W/W of monocrotophus as an active ingredient.

**Behavioural and Morphological Changes**

The exposed fishes were observed for any behavioural and morphological changes. They were observed from time to time for colour change, jerky movements, feeding habits, equilibrium etc.

**TEM Studies - Kidney**
After completion of exposure periods, fishes were sacrificed and the kidneys were removed for TEM investigations. It was washed in 10% saline for 5 minutes so as to make it free from blood. TEM studies from AIIMS, New Delhi were conducted for monocrotophos exposed fish to look at the finer details of the damage incurred on the exposure of the pesticide for different duration.

**Results**

The organic insecticides are one of the major health issues both in developing and developed countries and many of the acute poisoning cases are caused by exposure to these pesticides especially the organophosphate compounds. The contribution of these pesticides to the gradual decline of the aquatic ecosystems cannot be overlooked (Konar, 1975; Basak and Konar, 1976, 1977). The exotic varieties of fishes are exposed to sub-lethal concentration of various pesticides and therefore, it is important to study the impact of these pesticides on these fishes.

TEM studies were also done in both control and monocrotophos treated fish to look for alterations in the different cell organelles. In the control group, the epithelial cells were ovoid in shape with densely stained nucleus and randomly scattered chromatin and this was surrounded by numerous mitochondria along with rough endoplasmic reticulum. Therefore, the kidney tissue from the fish in the control group showed normal cellular structures/ architecture under transmission electron microscope.

After 15 days of exposure of monocrotophos to fish, several ultrastructural alterations were observed in the kidney of fish which include degenerative changes in the nucleus, mitochondria, and cytoplasm and in the lumen of renal tubules. Vacuolization was observed in the cytoplasm in the proximal convoluted tubule which were having numerous obliquely present microvilli giving it brush border kind of appearance and having so many cilia in the lumen (Fig. 1 a). The shrinkage of the nuclei further increased leading to the change in shape of the nucleus along with change in shape of mitochondria (Fig. 1 b, c, and e). Vacuolization was more evident in certain cells (Fig. 1 d, e, f). In 30 days monocrotophos treated fish, similar effects were observed but the damage caused was more evident both in the nuclei and the mitochondria. Degenerative changes in the nuclei were clear and the mitochondria around the nuclei seem to have drastic increase in number (Fig. 2 a, b, e). Mitochondria were also found to be damaged (Fig. 2 d). Vacuolization was also clear (Fig. 2 c, e, f). Upon further exposure of the fish to monocrotophos, no cellular structures were clear and intact, the renal tubules appeared to be grossly damaged leading to complete disarray. The nuclei shrunk drastically probably indicating the inactivation of the nuclear component (Fig. 3 a, b, c, d).

**Discussion**

The results of present TEM study on the ultrastructural changes observed in the kidney of fish exposed to monocrotophos have been correlated with the previous findings of various workers in various toxicological studies conducted with other toxicants and it has been found that the monocrotophos also induces degenerative changes as induced by other toxicants.

(Seginini de Bravo et al. 2005) studied the effect of sub-lethal doses of herbicide, 2-chloro-4, 6-his- (ethylamine-s-triazine) on the kidneys of two venezuelan cultured fish i.e. Caquetia kraussii and Colossoma macropomum. In the treated fishes, the kidney showed abundant vesicle structures, interruption and loss of basement membrane, swelling of nuclear envelope with different electron dense nuclei and mitochondria with mixed electron density matrix. Other alterations included cytoplasmic vacuolization of epithelial cells from proximal convoluted tubules which is again in line with our observations where vacuolization has found to be increasing with increased duration of the pesticide exposure.

**Conclusion**

It can be concluded that the ultrastructural changes in kidney of treated fish are in line with the previous findings of various investigators in various toxicological studies conducted with other toxicants. It has been found that the monocrotophos also induces degenerative changes as induced by other toxicants. Hence, viewing the deleterious effect of this pesticide on the grass carp, Ctenopharyngodon idellus, the indiscriminate use of monocrotophos should be prohibited through legislation.

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**References**


Research Article


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