Original Article

Toxic Effects of *Datura Metel* Aqueous Leaf Extract on Common Carp – *Cyprinu Scarpio,* Based on the Histology of Gills and Intestine

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ABSTRACT

Background: To solve the problems caused by using synthetic pesticides in agricultural fields and aquaculture ponds studies are being carried out on the feasibility of using biopesticides or plant extracts, which are less toxic. Extensive research is being carried out by researchers globally to manufacture pesticides using plant extracts. The present study evaluated the effect of *Datura metel* plant's aqueous leaf extract on histological changes in gill and intestine of the common carp *Cyprinus carpio*.

Methods: Twenty-four hour LC50 value was determined by standard methods and was calculated as 100 ppm. The fishes were exposed to sublethal concentration (1/10th of 24 h LC 50 value i.e., 10 ppm) for a period of 14 d. After 7 and 14 d, the gill and intestine were dissected out from both the exposed and control groups and were processed and sectioned at 4 µm and stained with H & E stain. The slides were then observed under 40 x magnification.

Results: The exposed gills showed vacuolation, bending of secondary gill lamellae, and cell masses between the secondary gill lamellae thickened to such an extent that interlamellar spaces were completely occluded. Intestines demonstrated narrowing of lumen, vacuolation, inflammation and rupture of epithelium. The pathological changes were more severe after 14 days of exposure in both the tissues.

Conclusion: Aqueous leaf extract of *D. metel* can be used as an agrochemical. Because of its toxicity its usage should be monitored well.

Keywords: *Cyprinus Carpio, Datura Metel,* Gills, Intestines, Pesticides.

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INTRODUCTION

A large number of pesticides having chlorinated hydrocarbons are being extensively used in integrated farming and agricultural fields to get protection against insects, weeds and diseases. Enormous use of pesticides in aquaculture and agriculture fields is now becoming a global problem [1]. All types of pesticides and herbicides sprayed on the fields ultimately reach non-target organisms through air, water and food chain [2]. Keeping in view the problems caused by the synthetic pesticides to the non-target organisms, biologically active compounds of plants are being used to produce biopesticides [3].

Solanaceae is the largest family in the plant kingdom and more than 3000 species are known, including several species with potent secondary substances of pharmaceutical and pesticidal importance. Such plants possess numerous bioactive compounds including steroids, alkaloids and flavonoids [4, 5]. Hence, new active substances solanaceae plants are always discovered in different regions [4, 6]. Datura species are widely distributed in different parts of the world including Mexico, U.S, Caribbean Islands, India, China and Africa [7-9]. *Datura* species are rich in alkaloids (e.g., hyoscyamine, hyoscine, atropine, and scopolamine), saponins, flavonoid, phenols, essential oils and cardiac glycosides [10, 11] and its insecticidal activity has been documented in different parts of the world [12, 13].

The main principle behind using fishes as bioindicator is due to its sensitiveness towards water quality and thus is being used extensively to assess the water quality of aquatic ecosystems [14-16].

The common carp, *Cyprinus carpio* (Linn.) is the most extensively farmed species of fish in the world. This fish is very much preferred for cultivation in ponds because of its

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excellent growth rate, omnivorous habit, breeding in confined waters, hardy nature and easy adaptability to artificial feeds. The possibility of rearing carp in flooded rice fields has been contemplated for many years, and superior rice fields were obtained from field in which carps was reared.

The present study evaluated the effect of *D. metel* plant's aqueous leaf extract on histological changes in gill and intestine of the common carp *C. carpio*.

MATERIALS AND METHODS

Fish

Common carps (*C. carpio*) ranging 10± 0.5 cm in length from and 8± 0.25 gr in weight were collected from Kolsagar reservoir, Mahaboobnagar, Telangana, India. The fish were stocked in 500 litre tanks containing dechlorinated tap water and were acclimatised for 15 d. They were fed twice daily with commercially available pellets. The water was renewed every 24 h daily.

Preparation of Aqueous Leaf Extract

Mature leaves of *D. metel* plant, were collected from Osmania University Campus, Hyderabad, Telangana, India. The leaves were thoroughly washed and dried in shade for 10 d and then pulverised to fine powder in an electric blender. Aqueous leaf extract was prepared by dissolving 50 gr of powdered leaves in 1 lit of distilled water (5%) and kept at room temperature for 24 h. After 24 h, the mixture was filtered and the extract was used immediately in the experiment.

Determination of 24hr LC50 and Sub-Lethal Toxicity Testing

The fish were divided into 11 groups; each group 10 fish in glass aquaria containing 15 litres of dechlorinated tap water. Twenty-four hours before the commencement of LC50 testing, we stopped feeding the fish. The concentrations of *D. metel* aqueous leaf extract in the aquaria was 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 175 ppm, 200 ppm, 225 ppm and 250 ppm respectively and the 11^{th} group served as the control. The aquaria were observed for 24 h for clinical signs like skin pigmentation, swimming pattern, response to stimuli and mortality. The 24 h LC50 value was recorded and tested by probit analysis as described earlier [17].

We considered $1/10^{th}$ value of the resulting 24 h LC50 value as sublethal concentration i.e., 10 ppm. Ten groups of fish were exposed to the sublethal concentration for a period of 14 d. Throughout the exposure period the fish were fed twice daily with commercial pellets and the water was renewed every 24 h. One group of the fish did not receive *Datura* and served as the control group. After 7 and 14 days the fishes from both the exposed and control group were dissected and the gill and intestine were carefully removed and washed in 0.9% saline and fixed in 10% formalin for 24 h. The tissues were then dehydrated in graded series of ethanol, embedded in paraffin and sectioned at 4 µm and were stained with H & E stain. The slides were observed under light microscope at 40x magnification and were then photographed with Olympus digital camera attached to the microscope.

Ethical Consideration

The Ethics Committee was informed about the research work and the use of the fishes. As such, there was no ethical committee disapproval because the research work included the use of fishes, cultured in artificial ponds.

RESULTS

The 24 h LC50 value was 100 ppm and $1/10^{th}$ of the 24 h LC50 value was taken as the sub-lethal concentration i.e., 10 ppm.

The gills of the control fish is shown in Figure 1. Below operculum, there are four branchial arches. Each branchial arch consists of two hemibranches consisting of two rows of tapered and flattened gill filaments. On the upper and lower surfaces of each gill filament, there are a series of flattened leaf like structures, called secondary gill lamellae, which form the respiratory surface. Epithelial wall of each secondary gill lamellae is held apart and supported by pillar cells. The gills of the fish exposed to aqueous leaf extract for 7 days showed curling of the tips of the secondary gill lamellae, slight bending of the secondary gill lamellae, shortening of secondary gill lamellae and vacuolation of secondary gill lamellae (Figure 2). The gills of the fish exposed to the leaf extract for 14 days demonstrated curling and

bending of secondary gill lamellae, cell masses between the secondary gill lamellae thickened to such an extent that interlamellar spaces were completely occluded, giving the gill filaments a compact appearance.

The intestine of the control fish are demonstrated in Figure 4. The outermost serosal covering consists of a single layer of epithelial cells, subserosal smooth muscle fibres arranged in distinctive pattern: the outer being longitudinal and inner circular, submucosa consists of connective tissue fibres, nerves and blood vessels, muscularis mucosa with two layers of muscles (outer longitudinal and inner circular), and gastric mucosa epithelial coat forming inner layer of columnar prismatic cells with basically located nuclei. The entire mucosa is folded into finger like processes called villi. The intestine of fish exposed to the aqueous leaf extract for 7 days showed narrowing of intestinal lumen, vacuolation of intestinal villi, flattened and fused villi, tissue disintegration and inflammation at the base of the villi (Figure 5). The intestine of fish exposed to aqueous leaf extract for 14 d illustrated narrowing of intestinal lumen, shortening of villi, rupture and disintegration of epithelium and inflammation throughout the villi (Figure 6).

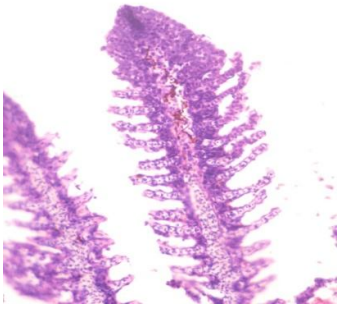

Figure 1. Gills of control carps at 40x, H&E.

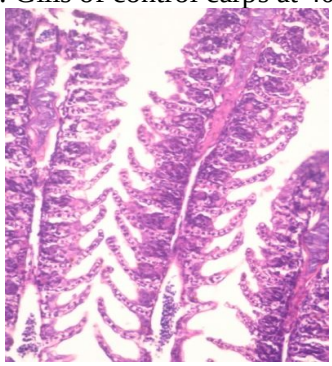

Figure 2. Gill of carps exposed to *Datura metel* aqueous leaf extract after 7 days at, 40x H&E.

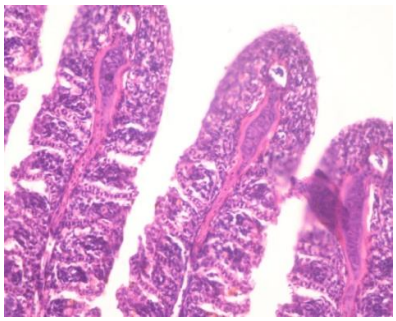

Figure 3. gill of carps exposed to *Datura metel* aqueous leaf extract after 14 days at 40x, H&E.

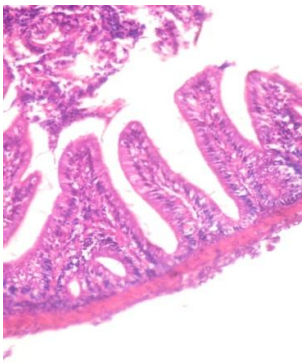

Figure 4. Intestine of control, carps at 40x, H&E.

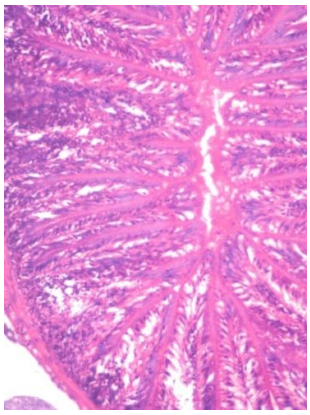

Figure 5. Intestine of carps exposed to *Datura metel* aqueous leaf extract after 7 days, 40x H&E.

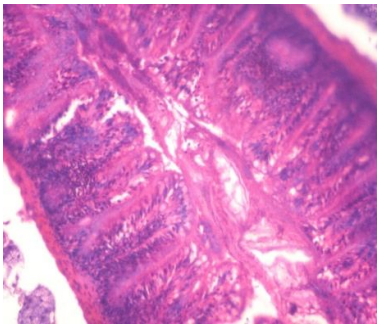

Figure 6. intestine of carps exposed to *Datura metel* aqueous leaf extract after 14 days at 40x, H&E.

DISCUSSIONS

In the present study, exposure to aqueous leaf extract of *D. metel* resulted in clear toxic effects on gills and intestines of common carp *C. carpio*, which was more severe at 14 days. Pesticides play an important in removing weed fishes and other pests from aquaculture ponds. Several plants have phytotoxic compounds, which have pesticidal properties. This kind of study has been reported by several researchers [18-21]. The extract of *Ipomeaa quatica* can poison the fish leading to pathological alterations in their tissues and organs [22].

The pesticidal potential and phytotoxic properties of plant extracts have been reported by several researchers [18-21]. The extract of *I. aquatica* can poison the fish leading to pathological alterations in their tissues and organs [22].

Gills are lifeline for fishes as they have an important role in carrying out various processes like respiration, osmoregulation and excretion. As gills are in continuous contact with water, so are very sensitive to water quality. Therefore, this organ is considered the primary target for the contaminants [23]. Damage to the gills will affect oxygen exchange and tissue respiration, culminating in organ and tissue hypoxia. We observed gill damage both at seven and 14 d of exposure and it was more severe in the 14 d. Similar damage in the form of curling of secondary gill lamellae and lamellar fusion have been reported in pesticide exposed fish by several workers [24- 26]. Similarly, fusion of secondary gill lamellae, epithelial hyperplasia and enhanced mucous production have been reported in *Labeo rohita* after exposure to phenol [27].

The intestine of the exposed fish showed many changes and the pathological changes were more severe after 14 d of exposure when compared to the 7 d exposure. Degenerative changes at the tips of villi, vacuolation and loss of structural integrity of mucosal folds in the intestine of *C. carpio* exposed to atrazine is reported [28]. The pathological alterations in the intestine of our studied fish were in agreement with those observed by previous reports [29, 30]. The present results are also in agreement with observations concerning the effects of different pesticides on fish intestine [31]; terbuthujazine [32]; hexachlorocyclohexane [33]; cyphenothrin [34]; thiodan [35]; deltamethrin [36]; aldrin and heptachlor [37] and fenvalerate [38].

CONCLUSION

The present study showed that aqueous leaf extract of *D. metel* is toxic to *C. carpio* and affects the structure and function of its respiratory system and intestine at sub-lethal concentrations causing considerable deterioration in fish health. Therefore, it is concluded that the use of *D. metel* aqueous leaf extracts can be used as a biological control in eradicating predators and unwanted organisms in the pond by the farmer instead of using agrochemicals, although ,because of its toxicity, its usage should be monitored well.

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