Uttar Pradesh J. Zool. 26(1) : 09-15, 2006 ISSN 0256-971X

HISTOMORPHOLOGICAL CHANGES IN STOMACH AND LIVER OF *NEMACHEILUS EVEZARDI* (DAY) ON ACUTE EXPOSURE OF AQUEOUS EXTRACT OF LEAVES OF *CESTRUM NOCTURNUM* (LINN.)

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Investigations were undertaken to evaluate the toxic effect of aqueous extract of the leaves of *Cestrum nocturnum* (Linn.) to a freshwater fish *Nemacheilus evezardi* (Day). The results reveal that the fish is very sensitive to the steroid saponins and alkaloids present in the leaves of this ornamental plant. Present findings are correlated with the changes noted in the organism after exposure to digitonin, a potent ichthyotoxin of plant origin. Deleterious effect was seen as histomorphological features of stomach showed ruptured epithelial cells, vacuolization in lamina propria, reduced length of mucosa and shrinkage due to crumpled mucosal folds as compared with effects observed in control group of animals. This extract at the same dose and duration of exposure causes changes in histomorphology of liver cells, as revealed from microscopic examination of tissues. Concomitantly, deviation from normal structure of liver was also recorded, this included ruptured cell membrane, hypertrophy, vacuolization and degeneration. Alterations in histological features of mucosal layers of stomach and architecture of hepatocytes are almost parallel with digitonin toxicated organisms. These results conclusively established deleterious effects of the toxic principles *i.e.* steroids, saponins and alkaloids of *C. nocturnum* on fish, which cause toxicity and ultimate death of animals.

Key words : Ichthyotoxicity, *Cestrum nocturnum*, *Nemachelius everzardi*, stomach histology, liver histology and aquatoxicity.

INTRODUCTION

Several plants belonging to different families having a number of compounds (Saponins, tannins, alkaloids, di-and triterpenoids, glycosides etc.) having strong piscicidal activity are used to control predatory fish, disease causing insects such as mosquito larvae and harmful freshwater snails (Mahajan, 1994; Jhingran, 1995; Chaiyvareesaji *et al.*, 1997; Waliszewski *et al.*, 1999; Singh & Tiwari, 2000). The present study reports excellent piscicidal activity of four local indigenous plants such as *Balanites roxburghii*, *Cestrum nocturnum*, *Sphaeranthus indicus* and *Tephrosia purpuria* on fish *Nemacheilus sinuatus* (Patole & Mahajan, 2003). *C. nocturnum*, though reported for its toxicity in terrestrial grazing animals (Durand, 1999). Mahajan *et al.* (1989) have reported its aquatic toxicity for the first time. The toxicity of crude extract of the *C. nocturnum* to notorious fishes *Nemancheilus sinuatus*, *Lebistus reticulatus* and *Punctatus sarana* has been established (Mahajan *et al.*, 1989; Mahajan, 1994 & 1998). Application of synthetic pesticides is one of the methods used to increase aquaculture production. But due to long term persistence and slow degradability in water and body of fish, they adversely affect the production and quality of fish (Pillai *et al.*, 1980; Jain, 2000) and contaminate the aquatic environment. Air breathing predatory fish cause special problems because they are carnivorous, and survive in moist burrow even when the ponds are drained (Jhingran, 1983). To solve this problem, studies have been carried out on the possibility of using local plants as piscicides (Mahajan, 1994; Mahajan & Patole, 2004). Because the toxic effect of the plant products is degraded easily within 7-12 days (Chakroff, 1976; Mahajan *et al.*, 1989), they are safe for users. Patole & Mahajan (2003) have reported the effect of aqueous extract of this plant on the rate of oxygen consumption in fish *N.*

evezardi. Its effect on gill histomorphological changes has been studied by Patole & Mahajan (2004), in order to establish mode of action of the test material. More recently, Tiwari & Singh (2004) proposed that Oleandrian, extracted from *Nerium indicum* (Lal Kaner) may be useful substitute for synthetic pesticide in killing predatory and weed fishes from fish and shrimp culture ponds. The present work includes the extension of previous report on histology of stomach and liver changes after intoxication in fish *N. everzardi* at the same dose and duration of treatment.

MATERIALS AND METHODS

The collection and acclimatization of experimental fish *N. everzardi* is described earlier by Mahajan *et al.* (1989). The procurement and preparation of test doses of potential piscicidal plant is described in previous report (Mahajan *et al.*, 1991). LC_{50} of an aqueous extract of the leaves of the *C. nocturnum* was carried out by a biostatic assay method as described earlier (Mahajan *et al.*, 1989). Five groups of ten fishes having average weight 8 ± 1g were separated in a glass aquarium of five litre capacity. A detail of experimentation is given below :

Group I : Fishes were kept as control in water for 96 hrs
Group II : Fishes were exposed at $1/4^{th}$ dose (1.5 mg) of LC_{50} value to a standard digitonin (Merck) for 96 hrs
Group III: Fishes were exposed at $3/4^{th}$ dose (4.5 mg) of LC_{50} value to standard digitonin (Merck) for 96 hrs
Group IV: Fishes were exposed at $1/4^{th}$ dose (7.0 mg) of LC_{50} value at aqueous extract of leaves of *C. nocturnum* for 96 hrs
Group V : Fishes were exposed at $3/4^{th}$ dose (21.0 mg) of LC50 value to aqueous extract of leaves of *C. nocturnum* for 96 hrs.

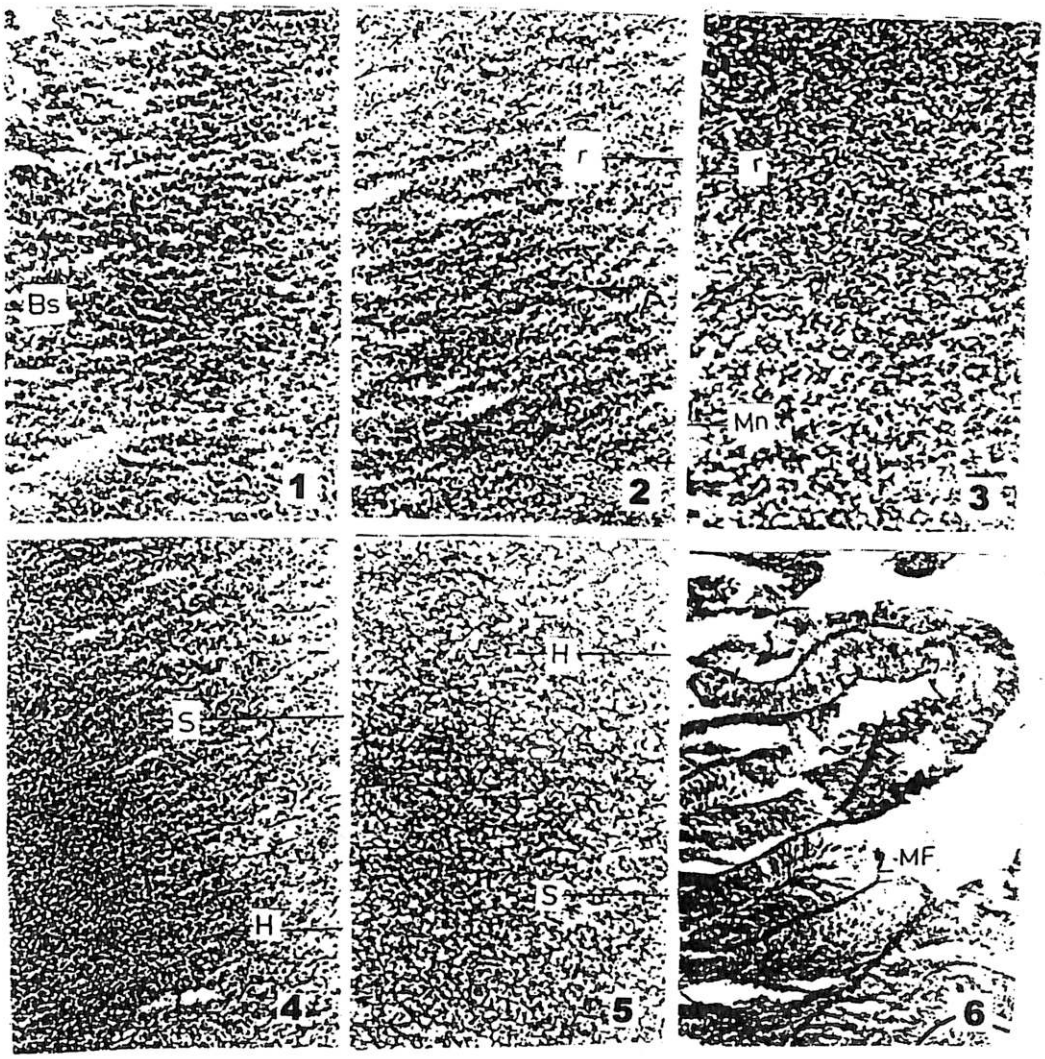
At the end of experiment, alive fishes were sacrificed, tissues like liver and stomach were fixed in Bouin's fluid, washed in running tap water for 24 hrs, until the colour of Bouin's fluid disappeared. After this, tissues were subjected to dehydration in alcohol series and cleared with xylene, then embedded in paraffin wax. A desirable piece of liver tissue was cross sectioned of about 8 micron thickness and suitable piece of stomach tissue was transverse sectioned of about 7 micron thickness. Tissue sections were stained with Harris-Hematoxyline and eosin and mounted in Destrene Plasticizer Xylene (Druvy & Willington, 1967). In this paper, the histomorphological observations on stomach and liver experimental tissues were compared with control groups of the fish *N. everzardi*.

RESULTS

Histology of liver : Histologically the liver is made up of chords of hexagonal hepatocytes which form irregular lobules separated by the connective tissues. Liver is the main organ responsible for detoxification of harmful substances which reach the liver through circulation. Thus liver is the most susceptible to toxicants entering the body of an animal.

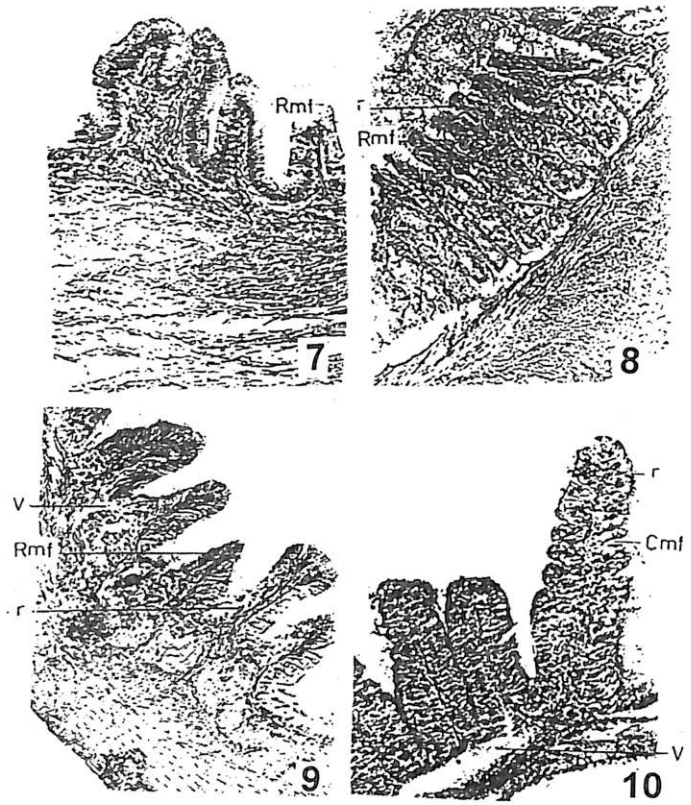
In the present investigation various observations were noted on exposure of sublethal concentrations of the test material to fish. These are as follows :

- Cross section of control fish liver showed intact hepatocytes and hepatic lobules, separated by blood sinuses (Fig. 1).
- Cross section of liver of animals exposed to one-fourth sublethal concentration of standard digitonin showed ruptured some cell membrane (Fig. 2).



Figs. 1-6 : Cross section of liver of *N. evezardi* (using sublethal conc. of digitonin for Figs. 2 & 3 whereas aqueous extract of *C. nocturnum* leaves for Figs. 4 & 5) for 96 hrs (H/E x200). **1.** Control; **2.** Exposed to $1/4^{th}$ conc (1.5 mg); **3.** Exposed to $3/4^{th}$ conc (4.5 mg); **4.** Exposed to $1/4^{th}$ conc. (7.0 mg); **5.** Exposed to $3/4^{th}$ conc (21.0 mg); **6.** T.S. of stomach control with highly folded mucosal epithelium. (Bs = Blood sinus; H = Hypertrophy; MF = Mucosal fold; Mn = Multinucleated region; r = Ruptured cell wall; S = Cell swelling).

- Cross section of liver exposed to $3/4^{th}$ sublethal concentration of standard digitonin showed ruptured cell membrane resulting in multinucleated region and necrotic effects on the cell.
- Cross section of liver of fish to $1/4^{th}$ subleathal concentrations of aqueous extract of *C. nocturnum* leaves showed hypertrophy and irregular arrangement of cells due to cell swelling (Fig. 4).
- Cross section of liver of fishes exposed to $3/4^{th}$ sublethal concentration of aqueous extract of *C. nocturnum* leaves showed hypertrophy and irregular arrangement of cell due to cell swelling.
- Some alterations noted in normal structure of liver of animals are *i.e.* ruptured cells resulting in multinucleated region, hypertrophy due to excessive cell swelling. These histomorphological changes in liver of exposed animal were found to be comparable with standard digitonin.



Figs. 7-10 : T.S. stomach of *N. evezardi* (using sublethal conc. of digitonin for Figs. 7 & 8 whereas aqueous extract of *C. nocturnum* leaves for Figs. 9 & 10) for 96 hrs (H/E x200). **7**. Exposed to $1/4^{th}$ conc (1.5 mg); **8**. Exposed to $3/4^{th}$ conc (4.5 mg); **9**. Exposed to $1/4^{th}$ conc. (7.0 mg); **10**. Exposed to $3/4^{th}$ conc (21.0 mg). (Cmf = Crumpled mucosal folds; r = Ruptured epithelial cells; Rmf = Reduced lengh of mucosal folds; V = Vacuolization)

Histology of stomach : Histologically, the stomach has four layers, serosa, muscularis mucosa, submucosa and mucosa. The thickness of these layers varies in different parts of the digestive tract. Mucosa, the inner most layer in the case of stomach is thrown into finger like projections called mucosal folds. The gastric epithelium consists of columnar epithelium. The stomach does not have direct contact with external medium. It is affected only when toxicant entes the digestive tract along with the food or water. In the present investigation various observations in transverse sections of stomach were noted on exposure of subleathal concentration of test material of fishes. These are as follows :

- Normal arrangement of highly folded mucosa with columner epithelium of the stomach of control group of animal (Fig. 6).
- One-fourth sublethal concentration of standard digitonin exposed animal showed reduced length of mucosal fold (Fig. 7). folds closer and ruptured epithelial cells (Fig. 8) in their stomach.
- Transverse section of stomach of exposed animals to one-fourth sublethal concentration of aqueous extract of the leaves of *C. nocturnum* showed ruptured epithelial membrane and lamina propria and crumpled mucosal folds (Fig. 9).

- Transverse section of stomach of exposed fishes to three-fourth sublethal concentration of aqueous extract of the leaves of the *C. nocturnum* showed reduced length of mucosal fold, ruptured epithelial cells and vacuolization in lamina propria (Fig. 10).

It is seen from the above cited observations that an exposure of fish to plant extracts, there was a departure from the normal structure of the three layers of stomach. These changes are : ruptured epithelial cells of lamina propria, reduced length of mucosal folds and folds coming together and finally crumpling of mucosal folds. All these alterations are common in all experimental groups of animals. The effects of plant extract on histomophology of stomach of fishes were found to be comparable with standard digitonin.

DISCUSSION

Liver : The impact of pesticides and insecticides are well established in aquatic and terrestrial invertebrates (Jhingran, 1983). The effects found on liver are hyperplasia of hepatocytes, rupture of cell membranes, multinucleated zone and accumulation of blood cells at places. Common changes observed by many workers are necrosis, hypertrophy and breakdown of cell membrane, degeneration and vacuolization (Chakraborty, 1974; Jauch, 1979; Ali, 1982; Jain, 2002). Mathur (1976) reported little changes in hepatic cells of *Channa punctatus* after exposure to lindane and dieldrine. These affects are due to impact of pesticides and insecticides on the liver. Necrosis of hepatic cells by aldrin, thiodon and malathion in liver of *Mollinisa sphenopus* is reported by Chakrabarty & Konar (1974), Kulshrestha & Jauhar (1984) reported distortion of hepatic cords, swollen nuclei in *Channa striatus* after treatment of sublethal dose of thiodon and sevin. Shareef *et al.* (1986) reported hypertrophy of hepatic cells, vacuolization, necrosis, breakdown of cell boundaries and binucleated hepatocytes in liver of *Barbus ticto* and *Rasbora doniconicus* after treatment of sequin. Rajnarayan & Singh (1988) reported hepatopathological changes in liver including cytoplasmolysis, nuclear picknosis and necrosis leading to complete exhaustion and disintegration of hepatocytes, when exposed to safe dose of commercial carbamate to fish *Channa punctatus* (Jain, 2000). In literature, such observations on liver by plant toxicants in fish are very scanty, although abundant data is available on liver toxicity in aquatic invertebrate (Jingran, 1983) and experimental albino rats (Satyvati *et al.*, 1987).

In the present investigation, microscopic structure of liver showed prominent symptoms such as ruptured cell wall, hypertrophy vacuolization and degeneration. They were more or less similar with the findings of above-mentioned workers, because ingredients of test material also have insecticidal properties. Change in histomorphological feature of liver may be due to plant ingredients such as flavonol glycoside and steriodal saponins in test material (Mimaki *et al.*, 2001). These observations in liver structure were compared with standard digitonin.

Stomach : Many workers have studied the toxic effects of pesticides and heavy metals effluents on digestive tract (Rao, 1999; Jain, 2000). King (1962) reported vacuolization in epithelial cells of guppies and brown trout due to dichloro diphynyl tetrachloromethane. *Pillai et al.* (1980) also observed similar changes by DDT in freshwater calm, *Indonessia coerulea* (Ica). Ali (1982) reported damage to epithelial cells of mucosa and submucosal folds due to dimecron and aldicarb treatment in *Channa gachua*. Kamble (1983) reported pyknotic nuclei and hyperplasia of mucosal polds in *Lepidocephalichthys thermalis* after treatment of benzene hexachloride and sumithion. Patole (1994) has made similar observations in fish *N. evezardi* on exposure to four indigenous plant extracts. Khilare *et al.* (1988) reported several changes those were found in the lining of digestive tract, such changes include atrophy of the mucosa and rupture of epithelial cells. Toxic effects of Copper sulphate such as necrosis in the hepatic cells, swelling of hepatic nuclei and damage in the walls of sinusoids in the liver of freshwater cat fish.

Heteropneustes fossilis (Bl.) were observed by Kothari *et al.* (1997). Recently, Patel *et al.* (2004) observed changes in the epithelial cells of stomach, hepatocytes of the liver after exposure to dimecron. Very recently, the utility of the *Nerium indicum* to remove unwanted fishes from aquaculture pond has been reported by Tiwari & Singh (2004). A few workers reported such effects of natural products on stomach of fish (Jhingran, 1983).

It is evident from the present investigation that the microscopic strucuture of stomach showed rupture epithelial cells and vacuolization in lamina propria. Reduced length of mucosal folds was prominent observation. The present studies are in concurrent with the findings of above mentioned workers. Changes induced on exposure to plant extracts may be due to plant ingredients such as steroids, glycosides and alkaloids present in them (Mimaki *et al.*, 2002). Altered stomach strucutre was compared with standard digitonin. It is, therefore, proposed that the ornamental plant *C. nocturnum* may be useful substitute for synthetic pesticides in killing predatory and weed fishes from fish and shrimp culture ponds, as it degrades within seven days of its applications. Its short term persistence in water is also an added advantage.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal and also Head of the Department of Zoology, Moolji Jaitha College, Jalgaon for their encouragement and financial assistance.

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