

## Histopathological study of neemax induced kidney of *Rasbora daniconius*

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### Abstract

Neem Indian lilac, *Azadiracta Indica* is renewable resource of various useful product. Today it is being used in a variety of products, such as in the field of Agriculture, Medicine, toileteries, cosmetics livestock, production and health etc. Acute toxicity of neemseed powder(Neemax) was studied using fresh water fish *Rasbora daniconius*. Excess amount of Neemax showed acute toxicity on the various organ of fish liver, gills and kidney.

**Key Words:-** Neemax ,Kidney,Azadiracta

### Introduction

Neem (*Azadiracta indica*) native of Indian subcontinent is a highly esteemed tree for the people in the region for centuries its derivatives have found used in agriculture, etc. Although uses of neem tree in India as a wonder drug is traced as far back as 4500 years ago. Fresh water fish *Rasbora daniconius* is native of Indian subcontinent, it is fresh water fish. The kidney of *Rasbora* is a glomerulus kidney and confirms in many respects to the general teleostean pattern. Nephron comprising of-

- (i) Renal corpuscles with glomerules
- (ii) Ciliated neck segment
- (iii) Initial proximal segment
- (iv) Second proximal segment
- (v) Distal segment and collecting tubules.

Ali and Salih (1982) reported haemorrhagic erosions and degeneration of liver, kidney etc. in sheep by using (*Azadiracta indica*). Bhide *et al.* (1958) reported degeneration of kidney of puppies Kanungo (1996) include degeneration changes in kidney of poultry birds. Until last two decades histopathological studies were limited to light microscopic techniques but by using electron microscopes workers are able to study histopathology of these organs (Leino *et al.*, 1990; Hemelrat *et al.*, 1990; Fisher-Schesl *et al.*, 1991; Sateesh, 1997; Rao, 1998; Nayak, 2001) have investigated pathological lesion in kidney at ultrastructural level.

## Material And Method

The fish *Rasbora daniconius* were collected from unpolluted upstream river Betwa (Vidisha) the fish were acclimatized in collapsible plastic pool for one week under laboratory conditions with normal feeding. The neem seed powder Neemax manufactured by Ecomax Agro System Ltd. Mumbai was used for the study. Stock solution was prepared by soaking desirable quantity of Neemax in water for three hours then it was filtered through cotton cloth and filtrate. Series of concentration were prepared by adding stock solution in ten litres of water (APHA 1985). Fresh stock solution was prepared for every set of bioassay test and experiments for ultrastructural study. The acute toxicity of Neemax was tested by 96 hours static bioassay tests. Fish of similar size were selected for one set of experiments. Dead fish were removed from aquaria immediately. Mortality were recorded after 24, 48, 72 and 96 hrs duration. Screening test were conducted prior conducting full scale toxicity tests. The surface ultrastructure of fish of control and Neemax exposed fish was studied by scanning Electron microscopy. Ultra structural study was undertaken by Transmission Electron Microscopy to observe cellular and sub cellular alterations in kidney of Neemax exposed fish.

## Results and Discussion

Pathological lesions were studied in the renal tubule of fish after one week exposure of neem seed powder. Numerous mitochondria were observed in both initial proximal segment and II proximal segment (fig. 1,2). But in mitochondria crystal were not clearly observed, at some places empty spaces were seen in the cytoplasm of kidney tubules indicating beginning of degenerating process (fig. 1,2). In a cell of secondary proximal tubule concentric lamellae of RER were seen around electron dense material and increased intensity of SER was observed.

The initial proximal segment have been characterised by the presence of microvilli in the increasing surface area of tubular cells for re-absorption.

After exposure of neem number of mitochondria was found increased in some cells of renal tubule along with proliferation of RER the present study reveals that exposure of toxicants such as neemax causes several damage to the kidney of fish and such damage is almost irreparable.

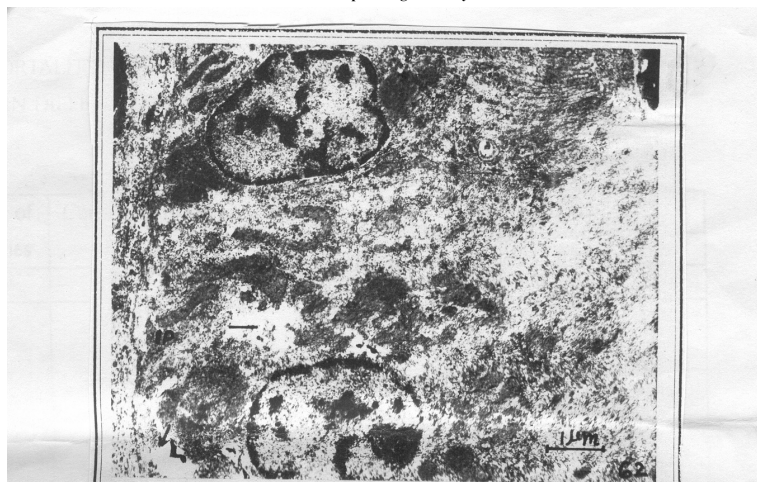


Fig. 1 TEM of initial proximal segment of nephron of one week neem exposed fish showing empty spaces indicating degeneration (Arrows).



Fig. 2 TEM of second proximal segment (SP) of nephron of one week neem treated fish showing concentric lamellar formation of RER (Arrow).

**Table 1: Mortality and percentage mortality of *Rasbora daniconius* in different concentration of Neem seed Powder-Nemas after different exposure durations temperature  $30 \pm 2^{\circ}\text{C}$** 

No. of Fishes	Concentrations mg/lts.	Exposure durations			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
30	600	Nil	Nil	Nil	Nil
30	700	Nil	Nil	Nil	6 (20 %)
30	800	6 (20 %)	6 (20 %)	6 (20 %)	12 (40%)
30	1000	12 (40%)	12 (40%)	12 (40%)	12 (40%)
30	1200	12 (40%)	18 (60 %)	18 (60%)	18 (60%)
30	1300	12 (40%)	18 (60%)	18 (60%)	24 (80%)
30	1400	18 (60%)	24 (80%)	24 (80%)	30 (100%)
30	1500	24 (80 %)	24 (80%)	30 (100%)	30 (100%)
30	2000	30 (100%)	30 (100%)	30 (100%)	30 (100%)

**Table 2: Acute toxicity 96 Hrs.  $\text{LC}_{50}$  of neem based products to fish species with mean values and calculated values of Azadirachtin content**

Test Chemical	Toxicity 96 hrs. mg/lts $\text{LC}_{50}$ mean S.E.	Calculated Azadirachtin content mg/l	No. of test fish species	Change of $\text{LC}_{50}$ (%) 24 to 96 hrs.
AZA Azadiractin (49% AZA 51%ONC)	>4	1.96	1	0
AZT, Azatin TMEC (3% AZA-27, ONC, 70% AZT-A)	>4 $\pm$ 0.4	0.12	3	39
NEX, Neem Extract (23% AZA, 77% ONC)	7 $\pm$ 3	1.61	3	34
MAR, MargosanO (0.3% AZA-12%, ONC 87.71% MARB, 72.6%)	33 $\pm$ 3	0.09	3	41
PHE, Pherotech 4.6%, AZA 15% ONC, 70.4%PHE-C)	72 $\pm$ 6	3.312	3	22

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