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## Pathological studies on subacute toxicity of *Amaranthus retroflexus* and its amelioration of *Ficus religiosa* in wistar rats

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### Abstract

**Background:** In India, mixed farming, combining crops and livestock activities have been a way of life since the dawn of civilization. Animals are mostly reared on the basis of low feed cost ratio, hence, for feeding to livestock, grazing is preferred. In some parts of Maharashtra state, there has been drought condition due to this situation; scarcity of fodder for animal is common problem. During grazing, in such situation, animal voraciously eat harmful plants, producing adverse effects on their health and ultimately on production.

**Methods:** The subacute toxicopathological study was conducted to note toxic effect of *Amaranthus retroflexus* and its amelioration by *Ficus religiosa* treatment in Wistar rats. The study was conducted for 28 days and evaluated through histopathological changes 28<sup>th</sup> day of trial.

**Result:** At the end of trial, histopathological investigation in organs such as Liver, Kidneys, Intestine, Spleen, Brain, Testes, Lungs, Adrenals and Heart of all experimental rats were attempted. On gross examination, none organ could show remarkable gross pathological change in toxicated as well as other treatment and control group except, minimal hepatomegaly and necrotic foci in female and male rats of group II. However, The experimental male and female rats of group II toxicated by administration of aqueous extract of *Amaranthus retroflexus* plant for 28 days showed histoarchitectural changes in liver, kidney, intestine, testes, adrenal and brain. In the rats of group IV which were toxicated with *Amaranthus retroflexus* and simultaneously treated with *Ficus religiosa* stem bark powder did not show any appreciable changes in liver, kidneys, heart, intestine, spleen, lungs, brain, testes and adrenals.

**Keywords:** wistar rat, toxicity, histopathological changes, nephropathy

### Introduction

Poisonous plants and weeds are widely distributed all over the world and when they get ingested accidentally, causes harmful effects *Amaranthus retroflexus* is a species of flowering plant in the family Amaranthaceae with several common names, including Red-root amaranth, Redroot pigweed, Math, Ranrajgira etc. amaranth having enriched nitrate and oxalate content. In normal circumstances this nitrate is further broken down to ammonia in the rumen and is then used by the rumen microbes to make protein. However, when large amounts of nitrate are eaten over a short period of time, the nitrite accumulates in the rumen and is absorbed in bloodstream. Nitrates are changed to nitrites in the rumen of the animal. These nitrites react so closely with the hemoglobin and convert it into methemoglobin, hence blood can no longer carry oxygen and the animal dies of asphyxiation or anoxia. Mortality seen in *Amaranthus retroflexus* ingested animals. Recent incidence of mortality of 25 cattle within one month duration was reported at Jalgaon district (Maharashtra). Nephro-protective and anti-nephrotoxic properties of *Ficus religiosa* has been proved against many nephrotoxic chemicals. Considering these facts, present study was conducted to evaluate subacute toxic of *Amaranthus retroflexus* and its amelioration by *Ficus religiosa* in Wistar rats.

### Material and Method

#### Experimental Wistar rats

Forty eight Wistar rats (24 male and 24 female) were procured from Wockhardt Research Centre, Aurangabad.

#### Collection of *Amaranthus retroflexus* and stem bark of *Ficus religiosa*

*Amaranthus retroflexus* plant and Bark of *Ficus religiosa* was collected from nearby area of Parbhani, Maharashtra. The stem bark was grinded and powdered used for present study @1%

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of total feed intake of rat daily through feed.

### Preparations of plant extract of *Amaranthus retroflexus*

After collection of whole plant, it was air dried, grinded by using electric grinder and powder. Then its aqueous extract was prepared by using hot water extraction method. Whole plant (stem, leaves, flower and fruits) powder of *Amaranthus retroflexus* @100gm added into 800 ml of distilled water. It was boiled till it becomes half of its quantity. After cooling, it was filtered with muslin cloth and Whatman filter paper no.42 and final aqueous extract of *Amaranthus retroflexus* was obtained.

### Experimental design

The forty Wistar rats were divided into 4 different groups, each group comprised of 06 male and 06 female rats as detailed below. Group I kept as a healthy control group, Group II kept as a toxic group in which rats were fed with aqueous extract of *Amaranthus retroflexus* @ 400 mg/kg of b.wt. Group III used as a plant control, in which *Ficus religiosa* bark powder was fed @ 1% of feed. Group IV used as treatment, in which rats were toxicated with of *Amaranthus retroflexus* @ 400 mg/kg of b.wt. and treated with *Ficus religiosa* bark powder @ 1% of feed daily for 28 days.

### Gross pathological studies

The experimental Wistar rats were sacrificed at the end of trial and examined by conducting systematic postmortem examination and gross lesions were recorded.

### Histopathological parameters

The pieces of suitable thickness of liver, kidneys, lungs, intestines, brain, spleen, heart, testes and adrenals will be collected to evaluate microscopic toxico-pathological alterations. The collected tissue samples were fixed and preserved in 10 percent buffer formal saline. After fixation the collected tissue pieces were processed as per the standard procedure. Paraffin embedded tissues were sectioned at 3-5 $\mu$  thickness and stained with routine Haematoxylin and Eosin method.

### Result and discussion

On gross examination, none organ could show remarkable gross pathological change in toxicated as well as other treatment and control group, except, minimal hepatomegaly and necrotic foci in 3 and 2 each female and male rats of group II.

These changes noted in liver indicated the toxicity. However, in the rats of group IV which were toxicated with *Amaranthus retroflexus* and simultaneously treated with *Ficus religiosa* stem bark powder did not show any appreciable changes indicating amelioration of induced toxication.

Also, lungs showed minimal to mild congestion and focal emphysematous changes in the rats of toxicated as well as control group indicating as an incidental change.

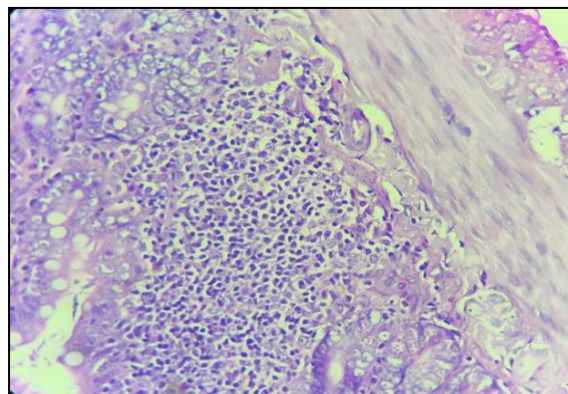
The histoarchitectural assesment of liver section in male as well as female rats of group I and III did not showed any appreciable changes, however, in experimental rats of group II liver on histopathological examination revealed minimal to mild, focal congestion, dilation of central vein and sinusoidal spaces, mild focal condensation of of nucli, focal coagulative necrotic changes in hepatocytes. Occasionally, the hepatocytes appered to be hypertrophied. Liver showed minimal to mild, focal congestion. The hepatic parenchyma

was with mononuclear cell infiltration. Most of the sections of the liver were with minimal to mild degenerative changes. Also, few sections were with presence of variable sized circumscribed vaculation in hepatocytes pushing nucli to one side and making it eccentric indicating fatty changes.

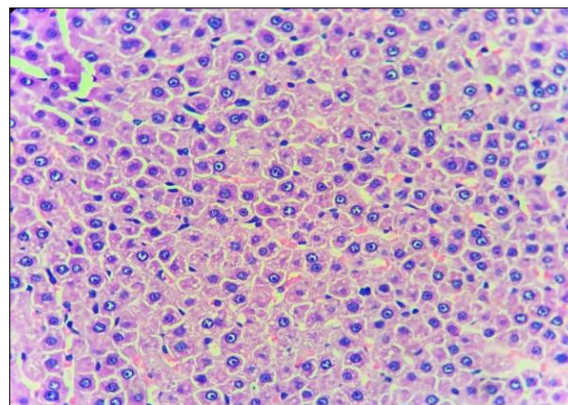
In rats of group II, the sections of kindneys on its evaluation revealed minimal to mild focal congestion, especially at corticomedullary junction, vacular degeneration, hydropic degeneration and cellular swelling. There were presence of variable sized cysts and few of them were haemorrhagic. The tubules adjacent to the cysts found to be atrophied. In addition, the sections of kidneys were with presence of hyaline casts in the lumen of exposed proximal convoluted tubules. Also, there were focal to multifocal areas of coagulative necrosis and infiltration of mononuclear cells in parenchyma of kidney.

Other histopathological changes in male as well as female rats of toxicated group were hyperplasia of epithelial cells, focal necrosis of mucosa, and denudation of villi, inflammatory cell infiltration and exfoliation of epithelium cells in lumen of intestines in male and female rats of group II. The section of testes showed depopulation of spermatogonial cells and wrinkling of basement membrane of seminiferous tubules. Male and female rats of group II showed minimal, focal congestion, vacuolation and mononuclear cell infiltration in brain and mild, focal congestion in heart, lungs and adrenals. The restoration of histoarchitectural changes noticed in male and female rats of group IV could have resulted due to treatment of *Ficus religiosa* stem bark powder.

*Amaranthus retroflexus* might have induced hepatopathy in addition to nephropathy as evidenced by increased level of liver enzymes and subsequently by histopathological changes.

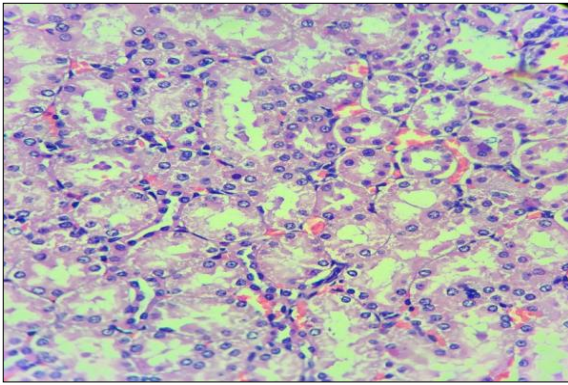


Note inflammatory cell infiltration, focal necrosis in intestinal villi of rat of group II (H&E 400X)

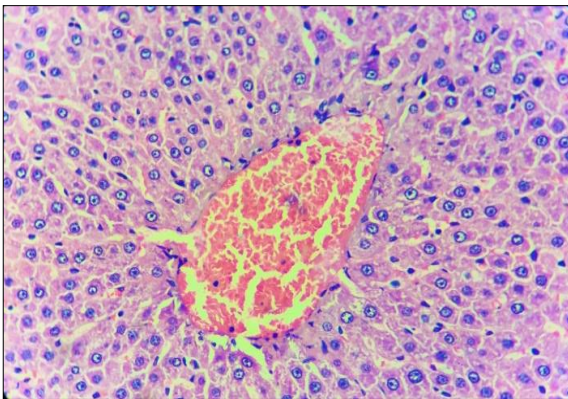


Note mild congestion, hypertrophy of hepatocytes and degenerative changes in liver in rat of grou p II (H&E, 400X)

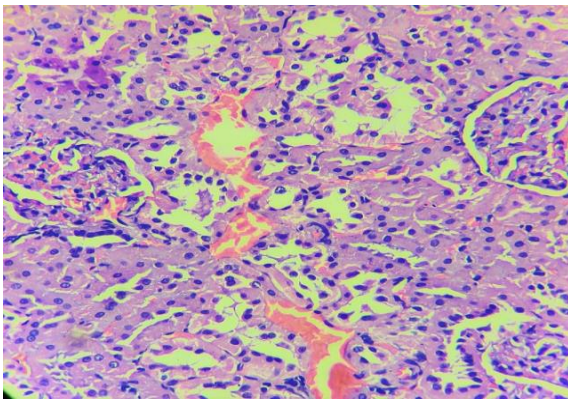




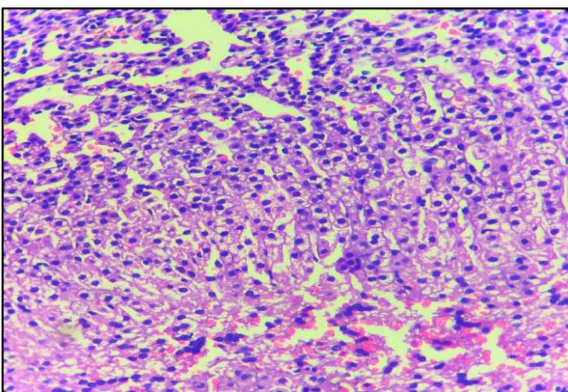
Note mild congestion and degenerative changes in kidney of rat of group II (H&E, 400X)



Microphotograph of liver with dilation of central vein and coagulative necrosis in hepatic parenchyma of rat from group II (H&E, 400X)



Note congestion, degenerative changes and MNC infiltration in kidney of rat from group II (H&E, 400X)



Note congestion and focal necrotic changes at corticomedullary junction in adrenal gland in rat of group II (H&E, 400X)

## Conclusion

The study concluded that *Amaranthus retroflexus* could induce nephrotoxicity, hepatotoxicity in male as well as female Wistar rats when administered daily @ 400mg/dl. Also, administration of *Ficus religiosa* stem bark powder daily @1% of feed produced ameliorative effect against *Amaranthus retroflexus* induced toxicity in Wistar rats.

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