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Determination of LD_{50} of aqueous extract of *Anagallis arvensis* in Wistar rats

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Abstract
For the determination of LD_{50} of aqueous extract of *Anagallis arvensis* plant through oral route thirty Wistar rats were procured and divided into 5 groups (each contain 6 rats). Group I used as control group which was fed with *ad- libitum* normal feed and water. Single dose of aqueous extract of *Anagallis arvensis* was given orally @ 500, 1000, 2000 and 4000 mg/kg b.wt. to the rats of group IInd, IIIrd, IVth and Vth, respectively. The LD_{50} of aqueous extract of *Anagallis arvensis* plant through oral route was 1781.76 mg/kg b.wt. in Wistar rats. Histopathological examination of kidney showed focal to multifocal, moderate to severe congestion, occasional haemorrhagic cystic degeneration and necrotic changes. There was mild to moderate congestion, dilation of central vein and focal necrosis in parenchyma of liver.

Keywords: *Anagallis arvensis*, LD_{50}, aqueous extract, Wistar rats

Introduction
Anagallis arvensis (Family: Primulaceae) is a native summer or winter annual, sometimes perennial weed (Clapham *et al.* 1987) [3]. Its spread is worldwide, probably as a contaminant in crop seeds (Holm *et al.*1977) [6]. In local Marathi language *Anagallis arvensis* called Nilifuli or Dhorkakada or Ran draksh. It has been reported that *Anagallis arvensis* causes poisoning in sheep (Wahny, 1942 [10]. and Lander, 1944) [7]. *Anagallis arvensis* causes mortality in cattle, buffalo, sheep, goats, horses, poultry, rabbits and birds too (Pande *et al*. 2016) [8]. Sadekar *et al.* (1995) [9]. reported 31 deaths in a herd of 46 cattle and buffaloes in February, 1994, due to *Anagallis arvensis* poisoning in Akola region of Maharashtra state. In Karnataka state, during grazing *Anagallis arvensis* toxicity in cattle and buffaloes occurred due to accidental ingestion of the weed. It results in death of 8 animals and recovery of 4 animals after symptomatic treatment (Harish *et al.* 2006) [5].
Abbas *et al.* (2012) [1]. evaluated phytochemical potential of *Anagallis arvensis*, it contains flavonoids s, saponins, tannins, steroids, glycosides, alkaloids, anthrequinones. Quantitatively *Anagallis arvensis* contains Alkaloids 2.21±0.02%, Saponins 1.29±0.03%, Flavonoids 4.43±0.10% and Tannins 9.56±0.03%. *Anagallis arvensis* plants are nephrotoxic in nature and its LD_{50} of alcoholic extract through intraperitoneal route is 10.718 mg/kg b.wt. (Al-sultan *et al.* 2003) [2]. Therefore, present study was conducted to evaluate LD_{50} of aqueous extract of *Anagallis arvensis* in Wistar rats through oral route**.**

Material and Methods
Experimental Wistar rats
Thirty Wistar rats (15 male and 15 female) were used for present study. All the rats were procured from the Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary & Animal Sciences, Parbhani. The experiment was carried out after approval of the protocol by IAEC as per guidelines of CPCSEA.

Collection and Preparation of plant extract of *Anagallis arvensis:*
Anagallis arvensis plant was collected from nearby area of Parbhani, Maharashtra (Figure 1). After collection of whole plant, it was air dried and then, grinded by using electric grinder and powder of plant was obtained. Then aqueous extract was prepared by using hot water extraction method. Whole plant (stem, leaves, flower and fruits) powder of *Anagallis arvensis* @100gm added into 800 ml of distilled water. The solution was boiled till it becomes half.

After cooling, it was filtered with muslin cloth and whatman filter paper no.42 and final aqueous extract of *Anagallis arvensis* was obtained (Figure 2).

Fig 1: Anagallis *arvensis* plant

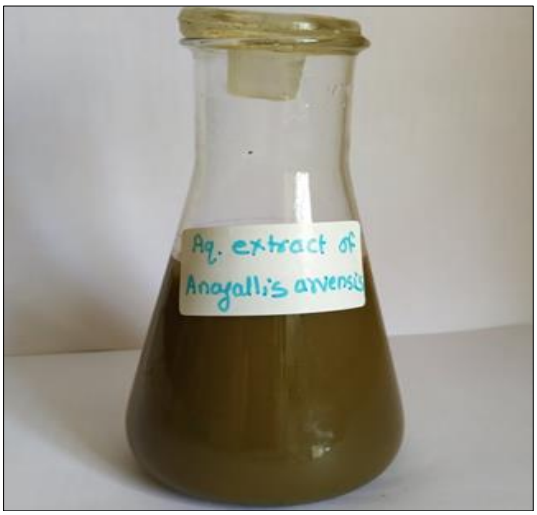

Fig 2: Aqueous extract of *Anagallis arvensis* plant

Experimental design

According to the method described by Weil (1952), for the determination of LD_{50} of *Anagallis arvensis*, Thirty Wistar rats of both sexes were divided into 5 groups, each comprised of six rats (3 Male and 3 Female). Group I used as control group and was fed with *ad- libitum* normal feed and water. Single dose of aqueous extract of *Anagallis arvensis* was given orally @ 500, 1000, 2000 and 4000 mg/kg b.wt. to the rats of group IInd, IIIrd, IVth and Vth respectively. After single dose administration, the experimental rats were observed critically to note behavioral changes and mortality for 14 days.

Pathomorphological study

Post mortem examination of died rats was carried out and kidney, liver, lung, heart, intestine were collected and stored in 10% formalin for histopathological examination. After fixation, the tissue pieces were processed as per the standard procedure (Culling, 1974) [4]. for further histopathological studies.

Results and Discussion

The experimental Wistar rats from group I and II did not show any behavioral changes throughout study period, however, the rats from group III showed dullness, depression, inappetance and increasesd thirst. Few rats showed occasional diarrhea.

The experimental rats of group IV and V were with restlessness, gastrointestinal disturbances like diarrhoea, anorexia, thirst and increased urine output just after 6-8 hr post administration. After 15 – 18 hrs of post administration, rats showed difficulty in breathing, depression, tremors and then ended by coma and death. Similar results were observed by Al-sultan *et al.* (2003) [2].

After administration of dose, within first 24 hrs mortality was noted in rats of group IVth and Vth. Three rats (2 Female and 1Male) from IVth group and all six rats from Vth group were died. After 2nd day post administration, one female rat from group IIIrd found to be dead. There after all the rats remained alive till 14th day (Table no. 1).

Table 1: Table showing details of mortality of experimental rats after administrated single dose of aqueous extract *Anagallis arvensis*

Group no.	No. of rats/group (3 Male+ 3 Female)	Dose of *Anagallis arvensis* extract (mg/kg b.wt.)	No. of rats died	% of rats died
1.	6	-	0	0
2.	6	500	0	0
3.	6	1000	1	16.66
4.	6	2000	3	50.00
5.	6	4000	6	100.00

According to Weil (1952), formula for determination of LD_{50} is

Log m = Da + d (f + 1) for n = 6 and k = 3
= log 500 + log 2 (0.83333 + 1)
= 2.69897 + 0.30103 x 1.83333
= 2.69897 + 0.55188
= 3.25085

Antilog= 1781.76

LD_{50} = 1781.76 mg/kg b.wt.

LD_{50} of aqueous extract of *Anagallis arvensis* through oral route is 1781.76 mg/kg b.wt.

Gross pathology

The gross pathological examination of died rats reveled mild to moderate congestion, focal haemorrhages, softing of kidney. Also, there was mild congestion and necrotic foci on liver. The necrosy examination of remaining experimental rats scarified on day 14th did not showed any appreciable gross pathological changes.

Histopathological changes

On histopathological assessment, kidney showed focal to multifocal, moderate to severe congestion, occasional haemorrhagic cystic degeneration and coagulative necrotic changes (Figure 3 and 4). There was mild to moderate congestion, dilation of central vein and focal necrosis in parenchyma of liver (Figure 5). Heart showed mild focal congestion and haemorrhages in rats (Figure 6). Intestine reveled severe inflammatory cell infiltration, desquamation of

epithelium, denudation of villi and acute catarrhal exudation in the lumen.

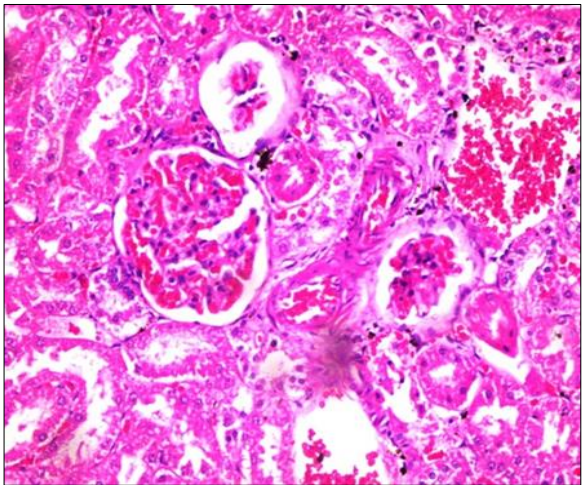

Fig 3: Note severe congestion, cellular swelling, MNC infiltration and degenerative changes in kidney (H&E 400X)

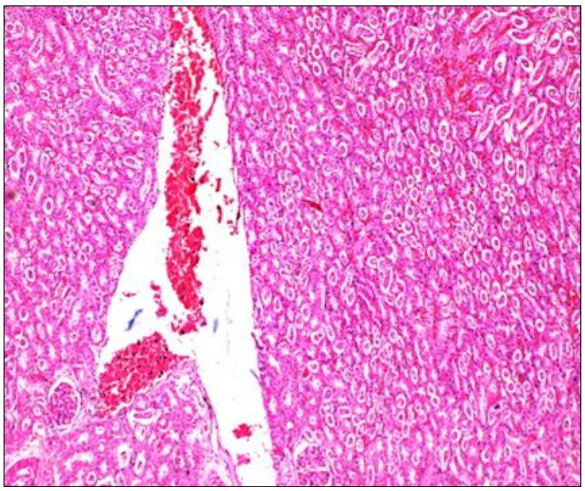

Fig 4: Note cyst with haemorrhagic content, congestion and degenerative changes in kidney of rat (H&E 100X)

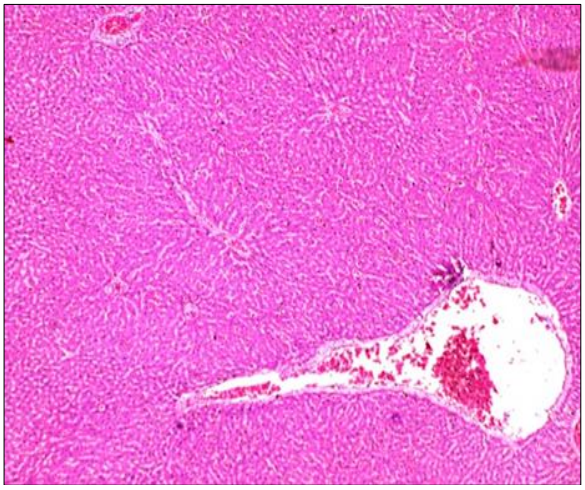

Fig 5: Microphotograph of liver with dilation of central vein and congestion (H&E 100X)

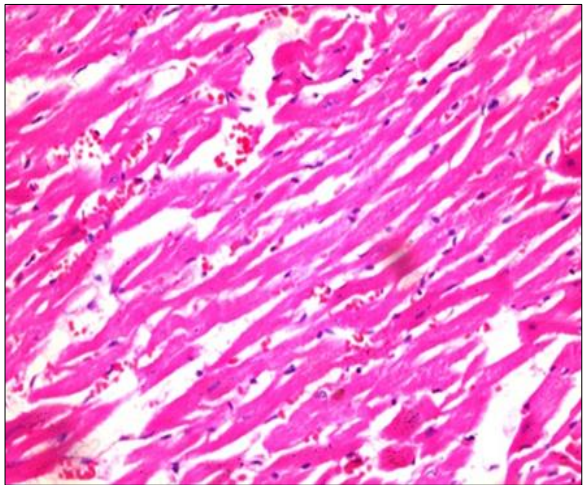

Fig 6: Microphotograph of heart showed mild haemorrhages (H&E 100X)

Conclusion

Plant was nephrotoxic in nature and LD_{50} of aqueous extract of *Anagallis arvensis* through oral route was 1781.76 mg/kg b.wt.

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