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Effect of aqueous extract of *Anagallis arvensis* and its amelioration by *Tribulus terrestris* in Wistar rats: Haemato biochemical study

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Abstract

The subacute toxicopathological study was conducted to note toxic effect of *Anagallis arvensis* and its amelioration by *Tribulus terrestris* treatment in Wistar rats. The study was conducted for 28 days and evaluated through haemato biochemical changes at 0, 14th and 28th day of trial. The toxicated rats showed significant reduction in Hb, PCV, TEC at 14th as well as 28th day and Neutrophil at 28th only whereas, there was significant increase mean value of TLC, and blood clotting time at 14th and 28th day and Lymphocyte at 28th day only. There was significant incline in AST, BUN Creatinine and Phosphorus level with decline in total protein and calcium level in toxicated rats. The mean ALT and blood glucose levels were numerically increased than control group in toxicated rats. The mean values of haematological and biochemical parameters in rats of treatment group remained comparable with control group indicating its beneficial effect.

Keywords: *Anagallis arvensis*, *Tribulus terrestris*, subacute toxicopathological, haemato biochemical

Introduction

Livestock is very important in agriculture sector of India. Animals mainly graze on green plants, grasses, crops etc. During grazing, animal accidentally ate toxic plants along with edible parts. It may results into death of animal, decrease in milk production, weight loss due to gastro-intestinal disturbances, abortion, decreased efficiency of animal and other effects on the animal. It ultimately affects farmer's socioeconomic status.

Scarlet pimpernel i.e. *Anagallis arvensis* is a native summer or winter annual, sometimes perennial weed^[1]. It's spread is worldwide, probably as a contaminant in crop seeds, also, it occurs in cultivated fields, gardens, lawns, meadows, pastures and waste places^[2]. In local Marathi language *Anagallis arvensis* called *Nilifuli* or *Dhorkakada* or *Ran draksh*. In many reports it has been reported that *Anagallis arvensis* cause mortality in cattle, buffalo, sheep, goats, horses, poultry, rabbits and birds too^[3]. Mature *Anagallis arvensis* plant contains more oxalate (15.06%) than the immature plants (12.68%). More than 10% oxalate in plant cause death of animal^[4].

Anagallis arvensis toxicity in cattle and buffaloes due to accidental ingestion of the weed in Karnataka state results into death of 8 animals^[5]. In Uruguay outbreak of *Anagallis arvensis* poisoning in cattle showed morbidity varied between 3.2% to 53.2% and lethality between 42.6% to 100% and in sheep it showed morbidity was 2.8 to 42.9% and lethality was within 81.3 to 100%^[6]. *Anagallis arvensis* plants are reported to be nephrotoxic in nature. Nephro-protective and anti-nephrotoxic properties of *Tribulus terrestris* has been proved against many nephrotoxic chemicals.

Considering these facts, present study was conducted to evaluate subacute toxic of *Anagallis arvensis* and its amelioration by *Tribulus terrestris* in Wistar rats.

Material and Methods

Experimental Wistar rats

Forty Wistar rats (20 male and 20 female) 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 IAEC as per guidelines of CPCSEA.

Collection of *Anagallis arvensis* and fruit of *Tribulus terrestris*

Anagallis arvensis plant was collected from nearby area of Parbhani, Maharashtra. Fruit of *Tribulus terrestris* was purchased from local market Parbhani, it was grinded and powdered used for present study @1% of total feed intake of rat daily through feed.

Preparations of plant extract of *Anagallis arvensis*

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 *Anagallis arvensis* @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 *Anagallis arvensis* was obtained.

Experimental design

The forty Wistar rats were divided into 4 different groups, each group comprised of 05 male and 05 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 *Anagallis arvensis* @ 178.17 mg/kg of b.wt. Group III used as a plant control, in which *Tribulus terrestris* fruit powder was fed @ 1% of feed. Group IV used as treatment, in which rats were toxicated with *Anagallis arvensis* @ 178.17 mg/kg of b.wt. and treated with *Tribulus terrestris* fruit powder @ 1% of feed daily for 28 days.

Haemato-biochemical parameters

The blood samples from all the rats of experimental groups were subjected for haematological studies such as Hb, PCV, TEC, TLC, DLC & blood clotting time and for the biochemical studies such as ALT, AST, Serum total protein, Blood glucose, Blood urea nitrogen, creatinine, calcium and phosphorus by using standard diagnostic reagent kits at 0, 14th and 28th day of study.

Statistical Analysis

Data generated for various parameters were statistically

analyzed by applying (CRD) Completely Randomized Design [7].

Results and Discussion

Haematological parameters

Table no. 1 showed the mean value of Haematological parameters of experimental rats at 14th and 28th day of study interval.

Mean values of blood parameters in rats of group I (control group) and III (plant control) were within the normal physiological limits and it did not differ significantly throughout the experimental trial.

The mean values of Haemoglobin, Packed Cell Volume and Total Erythrocyte Count were reduced in rats of group II than control group. The reduction of haemoglobin concentration, PCV and TEC levels in rats toxicated by *Anagallis arvensis* plant was due to harmful effects of saponin [8]. Saponin induces haemolysis and eryptosis as well as cell membrane scrambling. This effect was partially due to entry of extracellular Ca²⁺ and ceramide formation [9]. Al-snafi [10] reported that *Anagallis arvensis* plant have cytotoxic effect, it also reduces cell survival and induces cell damage.

The mean value of Hb, PCV and TEC in rats of group IV improved significantly than rats of group II and found to be statistically comparable with the mean value in rats of group I and III.

Tribulus terrestris plant improves haemoglobin concentration, PCV and TEC level in rats of group IV. It occurred due to *Tribulus terrestris* fruit extract which possess antioxidant, apoptosis inhibitory and vasodilator properties [11, 12]. The antioxidant activity of *Tribulus terrestris* could be due to its flavonoid content [13].

Total leukocyte count, Lymphocyte count and Blood clotting time in rats of group II were increased significantly whereas, Neutrophil count was decreased than control group value. The increase in mean TLC value might be due to body response to oxalate toxemia and metabolic disturbances caused by uraemia condition [14]. The mean values of these parameters in group IV improved and remained statistically comparable with mean value of control group indicating beneficial effect of *Tribulus terrestris* in present study.

Table 1: Mean value of Haematological parameters of experimental rats at 14th and 28th day of study interval

Parameters	Days	Group I	Group II	Group III	Group IV	CD	statistics
Haemoglobin (Gm/dl)	14 day	13.56 ^a ±0.25	11.35 ^b ±0.30	13.67 ^a ±0.43	12.82 ^a ±0.39	1.017	S
	28 day	13.72 ^a ±0.30	10.87 ^b ±0.31	13.61 ^a ±0.39	13.01 ^a ±0.32	0.959	S
PCV(%)	14 day	40.1 ^a ±0.76	33.2 ^b ±0.86	40.4 ^a ±0.89	38.4 ^a ±0.83	2.415	S
	28 day	40 ^a ±0.88	31.8 ^b ±0.97	40.3 ^a ±1.17	39.4 ^a ±0.94	2.868	S
TEC (million/cumm)	14 day	6.67 ^a ±0.23	5.23 ^b ±0.23	6.54 ^a ±0.23	6.10 ^a ±0.32	0.751	S
	28 day	6.81 ^a ±0.17	4.96 ^b ±0.16	6.79 ^a ±0.20	6.53 ^a ±0.20	0.542	S
TLC (thousand/cumm)	14 day	8.63±0.28	10.12±0.28	8.37±0.72	9.10±0.58	-	NS
	28 day	8.44 ^b ±0.27	11.55 ^a ±0.54	8.77 ^b ±0.24	8.52 ^b ±0.49	1.179	S
Neutrophil (%)	14 day	27.4±0.47	26.2±0.32	27.1±0.43	26.8±0.46	-	NS
	28 day	27 ^a ±0.51	24.7 ^b ±0.26	26.8 ^a ±0.38	26.5 ^a ±0.40	1.153	S
Lymphocyte (%)	14 day	65.6±0.6	66.4±0.47	65.3±0.66	65.7±0.57	-	NS
	28 day	65.7 ^b ±0.49	68 ^a ±0.76	65.9 ^b ±0.64	66.2 ^b ±0.51	1.754	S
Blood clotting time (sec)	14 day	56.8 ^c ±1.16	70.4 ^a ±0.84	57.2 ^c ±0.94	60.9 ^b ±1.04	2.886	S
	28 day	56 ^b ±0.96	79.1 ^a ±0.86	56.5 ^b ±0.84	57.2 ^b ±0.86	2.543	S

* Means bearing similar superscripts in column and rows do not differ significantly ($P < 0.05$)

Biochemical parameters

Table no. 2 showed the mean value of biochemical parameters of experimental rats at 14th and 28th day of study interval.

Mean values of biochemical parameters in rats of group I (control group) and III (plant control) were within the normal

limits and remained comparable among themselves throughout the trial.

There was increase in the mean serum calcium level and decrease in mean serum phosphorus level in rats of group II. After ingestion of plant, oxalate combines with calcium and

then it converts into insoluble salt i.e. calcium oxalate salt and same could results into hypocalcaemia condition [15]. Whereas, group IV values remained comparable with mean values of control group. These values obtained in rats of group IV indicated ameliorative effect of *Tribulus terrestris* fruit powder against *Anagallis arvensis* induced toxicity. Aggarwal *et al.* [16]. reported that *Tribulus terrestris* extract have a potential to inhibit nucleation and growth of the calcium oxalate crystals and also have cytoprotective action. In present study, there was numerical elevation in mean ALT level and significantly increased in mean AST level at 28th day of interval in rats of group II. In group IV rats, ALT and AST values remained similar to respective control group values. These obtained values in rats of group IV showed hepatoprotective action of *Tribulus terrestris* fruit powder. There was significant decline in serum total protein level and numerical rise in blood glucose level in rats of group II as

compared to respective control group value. The mean value of serum total protein in rats of group IV improved significantly than rats of group II and it remained comparable with the mean value of control group. The mean blood glucose level value in rats of group IV stayed similar with the mean control group value.

There was significant incline in mean values of BUN and creatinine in rats of group II at 14th as well 28th day of study period. Increased level of BUN and serum creatinine occurs due to renal insufficiency caused by excess feeding of oxalate containing plants [17]. However, BUN and creatinine level in rats of group IV were decreased than rats of group II. These values obtained in rats of group IV might have resulted due to nephroprotective action of *Tribulus terrestris* fruit powder. Hepato-renal protective effects of *Tribulus terrestris* results due to presence of antioxidant substances like total flavonoids and polyphenols compounds [18].

Table 2: Mean value of Biochemical parameters of experimental rats at 14th and 28th day of study interval

Parameters	Days	Group I	Group II	Group III	Group IV	CD	statistics
Calcium (mg/dl)	14 day	11.39 ^a ±0.90	7.79 ^b ±0.47	10.79 ^a ±0.72	9.29 ^{ab} ±0.99	2.30	S
	28 day	12.06 ^a ±0.64	6.75 ^b ±0.61	11.11 ^a ±0.86	10.99 ^a ±0.94	2.24	S
Phosphorus (mg/dl)	14 day	6.07 ^b ±0.64	10.35 ^a ±0.62	6.38 ^b ±0.34	5.90 ^b ±0.38	1.489	S
	28 day	6.37 ^b ±0.37	11.21 ^a ±0.72	6.16 ^b ±0.29	6.69 ^b ±0.39	1.362	S
Blood Glucose (mg/dl)	14 day	83.41±1.18	85.22±0.94	82.65±1.15	84.14±1.06	-	NS
	28 day	82.56±1.07	85.69±1.07	83.39±1.10	83.10±1.02	-	NS
Total protein (gm/dl)	14 day	5.40 ^a ±0.37	3.09 ^b ±0.48	5.63 ^a ±0.31	4.04 ^b ±0.36	1.123	S
	28 day	5.57 ^a ±0.29	2.99 ^b ±0.31	5.07 ^a ±0.43	4.99 ^a ±0.28	0.973	S
AST (IU/L)	14 day	61.98±1.24	66.42±1.52	62.51±1.05	63.35±0.89	-	NS
	28 day	62.48 ^b ±1.25	69.13 ^a ±1.33	63.42 ^b ±1.13	64.32 ^b ±0.91	3.361	S
ALT (IU/L)	14 day	32.12±1.15	34.13±1.1	32.24±0.79	33.16±0.87	-	NS
	28 day	33.08±1.35	35.31±0.68	32.44±1.07	34.21±0.7	-	NS
Blood Urea Nitrogen (mg/dl)	14 day	21.63 ^b ±0.93	46.82 ^a ±1.23	20.82 ^b ±0.88	22.85 ^b ±0.74	2.786	S
	28 day	20.75 ^b ±1.02	53.57 ^a ±0.86	21.49 ^b ±1.25	22.62 ^b ±0.68	2.81	S
Creatinine (mg/dl)	14 day	0.70 ^c ±0.06	4.37 ^a ±0.28	0.68 ^c ±0.06	2.83 ^b ±0.14	0.477	S
	28 day	0.69 ^b ±0.04	4.61 ^a ±0.24	0.73 ^b ±0.08	0.94 ^b ±0.10	0.406	S

* Means bearing similar superscripts in column and rows do not differ significantly (P< 0.05)

Conclusion

The study concluded that *Anagallis arvensis* could induce nephrotoxicity, hepatotoxicity in Wistar rats when administered daily @ 178.17mg/dl. Also, administration of *Tribulus terrestris* fruit powder daily @1% of feed produced ameliorative effect against *Anagallis arvensis* induced toxicity in Wistar rats.

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