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EVALUATION OF ACUTE AND SUBACUTE TOXICITY OF ALCOHOLIC EXTRACT OF THE ROOTS OF *TRAGIA CANNABINA* IN RATS

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ABSTRACT

The toxicity profile of alcoholic extract of roots of *Tragia cannabina* Family: Euphorbiaceae was studied in wistar rats. The multiple oral administration of extract at single dose of 100, 500, 1000, 2000 and 3000 mg/kg body weight for 24 hrs. did not produce sign of toxicity, behavioral appearance, change on a gross appearance. The sun-acute toxicity was determined by administration of graded dosage (125, 250, and 500 mg/kg body weight; orally) of the extract daily for 28 days and the effect on body weight, organ weight and serum biochemical parameters were estimate. Body weight of dose and control rat increase throughout the duration of treatment. Our data demonstrate significantly no difference in serum concentration of aspartae amino transferase, alanine amino transferase, alkaline phosphate, protein, urea, creatinin were measured. The results showed that subacute treatment did not shows any change in corporal weight and hematological parameters. However, a change in liver weight but not in hepatic enzymes was observed. This suggests that the liver function is not altered by *Tragia cannabina* in this study. Some changes in the glucose content were observed. It may be concluded that alcoholic extract of *Tragia cannabina* do not produce significant toxic effect in rats during acute and sub-acute treatment in rats.

INTRODUCTION: *Tragia cannabina* (Euphorbiaceae) is widely used in natural medicine in south India. This plant is used for the treatment of eczema, fevers, wheezing, including liver disorder, diabetes and viral infections [1]. Despite of the popular use of this plant, by the rural communities to treat several diseases, the objective of the present study was to obtain data on the safety of the crude extract.

The present study was aimed to evaluate the acute and subacute oral toxicity of the alcohol (95%) extract of roots in mice and rats. The changes in selected biochemical and haemotological parameters were also determined.

MATERIAL AND METHODS:

Plant Material: Fresh roots of *Tragia cannabina* were collected near the Yercaud hills, Tamilnadu, India. The sample was authenticated by Botanical survey of India, Coimbatore, India and a voucher specimen was preserved at Annamalai University, India.

Preparation of the Extract: The collected plant material was dried at room temperature (30 ± 3°C), pulverized and finely sieved. The powder obtained (750g) was extracted by hot continuous extraction (Soxhlet), in 2000ml of ethanol (95%) for 72 hours. The extract was distillation and concentrated in an air

circulating oven at 54°C until total dryness. The experiment was repeated twice and 60g of alcohol extract obtained was stored at 5°C. The alcoholic (95%) extract of 750gm of sample gave a yield of 7.69% w/w.

Experimental Animals: Wistar albino rats (170-190gm) and Wistar albino mice (25-30gm) were obtained. All the animals were kept under standard environmental condition (25 ±2°C). The animals had free access to water and standard diet. Rats and mice were deprived of food but not water (16-18) h prior to administration of the extract. The principles of laboratory animals' care were followed and the department's ethical committee approves the use of the animals and the study design.

Acute Toxicity: The toxicity study as carried out using Wister albino mice (25-30 g). Animal were kept in a standard environmental condition (25 ±2°C) with a 12 h light-dark cycle and food and water were freely available. The animals were divided into one control group and five treated groups, each group consisting of six animals. The control group received saline and each treated group received the alcoholic extract in a dose of 100, 500, 1000, 2000, and 3000 mg/kg by gavage. The animals were observed continuously for 2 h, 4 h, and then they were observed each hour during 24 h after administering the extract to observe any changes in general behavior or other physiological activities.

Sub Acute Toxicity: Four groups of 6 rats received by intra gastric gavages the plant extract at the dose of 125 mg/kg, 250 mg/kg 500 mg/kg body weight and distilled water (control) every 24 h for 28 days. During the period of administration, the animals were weighted, food and water intake were monitored. At the end of the experiment all the rats were fasted for 12hrs , blood was collected from the orbital sinus under ether anesthesia for biochemical and hematological analysis. After the blood collection, the animals were sacrificed by cervical displacement and selected organ (liver, heart, lung, spleen, kidney) were removed for macroscopic analysis.

Biochemical Estimations: Blood collected into non heparinized tubes were then centrifuged at 3000 rpm for 10min. The separated serum was analyzed to evaluate the biochemical parameters by using diagnostic kits (Span Diagnostics Ltd., India). Asparatate aminotransferase (AST) and Alanine aminotransfesase (ALT) and creatinine by standard enzymatic assay methods [2] Alkaline phosphatase (ALP) was analyzed by the method of Bessey *et al*., (1946) Lowry *et al*., (1954) [3, 4] , and Plasma glucose and protein contents were determined using enzymatic spectroscopic methods [5] and cholesterol by the method zlatkis *et al*., (1952) [6]**.**

Haematological Assay: Blood sample collected in the heparinized tubes were used to investigate white blood cells, Red blood cell, haemoglobin & clotting time by usual standardized laboratory methods.

Statistical Analysis: Values were expressed as mean ±SEM. The statistical analyses of variance were done by ONE WAY ANOVA forward the Dunnett's test using software version. $P < 0.05$ was considered as the level statistical significance.

RESULTS AND DISCUSSION: Oral administration of the alcohol extract of *Tragia cannabina* doses from 100 to 3000 mg/kg did not produce significant changes in behaviors, breathing, cutaneous effects, sensory, nervous systems, responses and gastrointestinal effects in mice. These effects are observed during the experimental period. At 24 h, death of mice was observed at 2600 and 3000 mg/kg. The LD_{50} value obtained was 2600 mg/kg of body weight according to Ghosh (1984) [7] and Klassen *et al.,* (1995) method [8].

In the sub acute toxicity, the alcoholic extract of *Tragia cannabina* at dose of 125 mg/kg, 250 mg /kg, and 500 mg/kg given per os every 24 h for 28 days did not death of the animals. No sign of observable toxicity was detected during the experimental period. Rats treated with various doses of hydro-Ethanolic extract of *Tragia cannabina* had a progressive weight gained. This increase in weight is significantly different ($p < 0.05$) from that of control **(Table 1).**

The progressive increase in body weight at doses of 125, 250 and 500 mg/kg of rats during 28 days of administration of alcoholic extract of *Tragia cannabina* may indicate the improvement of the nutritional state of the animal. The growth response effect could be as the result of increased food intake. The hematological status is summarized in **Table 2.**

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF *TRAGIA CANNABINA* EXTRACT ON BODY AND ORGANS WEIGHT.

Dose (mg/kg)	Control	*T. canabina* 125mg/kgs	*T. canabina* 250mg/kgs	*T. canabina* 500mg/kgs
Body (g)	183.33 ± 1.58	185.17 ± 1.30	186.17 ±1.19	187.33 ±0.81
Liver (g)	6.61 ± 0.02	6.84 ± 0.06	6.81 ± 0.11	7.23 ± 0.08*
Heart (g)	0.76 ± 0.03	0.78 ± 0.01	0.72 ± 0.02	0.79 ±0.02
Lung (g)	1.85 ± 0.02	1.86 ± 0.01	1.89 ± 0.01	1.90±0.00
Spleen (g)	0.84 ± 0.02	0.85 ± 0.01	0.86 ±0.01	0.88±0.01
Kidney (g)	0.63 ± 0.02	0.65 ± 0.01	0.67 ±0.01	0.66±0.01

Mean values of 6 Animals ± SEM; *p < 0.05; control group received saline. No significant difference was observed in any parameter, except in liver (500 mg/kg body weight)

TABLE 2: HAEMATOLOGICAL PARAMETERS AFTER 28 DAYS TREATMENT WITH THE *TRAGIA CANNABINA* EXTRACT

Parameter	Control	*T. canabina* 125mg/kgs	*T. canabina* 250mg/kgs	*T. canabina* 500mg/kgs
Hb gm%	12.45±0.30	12.78 ±0.18	12.60 ±0.28	12.85 ±0.33
RBC10^6/Cu.mm	3.60±0.13	3.20 ±0.85	3.33 ±0.14	3.83±0.10
Total WBC 10^3/Cu. mm	7.050±0.76	7.16 ±0.61	7.216 ±0.65	7.266±0.49
Clotting Time (in Sec.)	111.16±0.90	111.8 ±0.65	112.17±1.04	112.50 ±1.08

Mean values of 6 Animals ± SEM; *p < 0.05; control group received saline. No significant difference was observed in any parameter

After of days of oral administration of alcohol extract *Tragia cannabina* was also assessed. No significant variation (p> 0.05) for RBC, WBC, HB and clotting time were observed. The results showed that the values for the RBC and WBC were slightly increased in treated groups of animals compares to the control. The small transient of values observed in blood hematology did not show any dose responsiveness. Thus, the alcohol extract of *Tragia cannabina* failed to alter the hematological parameters at significant (p>0.05) extent. The observed increase in the haemoglobin levels might be due to the increased absorption of iron. Also the increase in the haemoglobin level coupled with increase in the WBC count emphasized the beneficial effect of the extract to the general well being of the animals. Different biochemical parameters of *Tragia cannabina* is summarized in **Table 3.**

TABLE 3: EFFECT OF *TRAGIA CANNABINA* EXTRACTS ON DIFFERENT BIOCHEMICAL PARAMETERS

Dose (mg/kg)	Control	*T. canabina* 125mg/kgs	*T. canabina* 250mg/kgs	*T. canabina* 500mg/kgs
Cholesterol (mg %)	77.00 ± 2.12	79.83 ± 1.99	82.83 ± 1.89	83.00 ± 0.89
Glucose (mg %)	73.88 ± 2.50	70.23 ± 1.51	67.16 ± 1.45	65.50 ± 1.25*
Creatinine (mg %)	0.933 ± 0.04	0.867 ± 0.05	0.983 ± 0.04	0.917 ± 0.04
Urea (mg %)	49.00 ± 0.81	47.33 ± 1.08	52.50 ± 1.50	48.50 ± 1.60
Protein (gm %)	6.633 ± 0.14	6.600 ± 0.07	6.833 ± 0.10	6.805 ± 0.16
ALT (U/L)	185.00 ± 2.70	183.83 ± 1.60	180.17 ± 0.83	179.00 ± 0.81
AST (U/L)	202.00 ± 2.26	199.00 ± 2.57	197.00 ± 1.74	195.17 ± 1.19
ALP (U/L)	370.50 ± 1.12	373.20 ± 2.14	371.45 ± 1.25	375.35 ± 2.54
GGPT (U/L)	260.50 ± 2.23	263.66 ± 4.27	265.33 ± 2.10	267.50 ± 2.70

Mean values of 6 Animals ± SEM; *p < 0.05; control group received saline. No significant difference was observed in any parameter, except in glucose (500 mg/kg body weight). ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphate

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most widely used markers for measuring hepatocellular injury [9]. Other parameters such as alkaline phosphatase (ALP) and γ-glutamyl transaminase (GGT) are also useful in diagnosing hepatobiliary diseases [10].

In general, liver damage can be divided into direct destruction of hepatocytes or impairment of bile flow. In the early stage of liver damage, cytoplasmic enzymes in hepatocytes may leak from cells into blood whose membrane permeability has been increased [11]. Liver damage often leads to fat accumulation in hepatocytes [12]. However, based on our results, the increase in the relative liver weight and associated with the decrease in serum liver function tests such as ALP and AST was not in agreement with the general liver damage action by hepatotoxin [13]. This observation could indicate that liver function is protected by oral administration of *Tragia Cannabina*.

Effects of the extract on the biochemical parameters clearly showed that there was a remarkable decrease in the plasma glucose level especially at higher doses in

the treated rats compared with the control. Anti-hyperglycemic activity was already reported in literature [14].

Serum urea and creatinin were examined as indicators for kidney function tests [15] while lipid metabolism profiles were mainly represented by serum cholesterol [16]. Based on the results obtained after analyzing serum urea, creatinin, and total cholesterol, it has demonstrated that repeated administration of hydro-alcoholic extract of *Tragia cannabina* had no direct adverse effect on kidney function and also lipid metabolism in normal young rats.

CONCLUSION: According to our study, it may conclude that *Tragia cannabina* seems to be destitute of toxic effects, which could be compromise the medicinal use of this plant in folk medicine. However, further studies are necessary, such as histological and morphological experiments, to confirm this evidence.

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