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Evaluation of Impact of Ethanol and Water Extract of Seeds of *Tragia involucrata Linn.* on the Urine parameters of Albino rats

Dr. Om Prakash

PGT (Zoology), +2 Project Girl's High School, Bairampur, Biraul,Darbhanga, Bihar- 848209

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ABSTRACT

Plant materials continue to play an important role in the maintenance of human health since antiquity. Over 80% of all chemical drugs originated from natural plant source. Several plants are now being used in part or as a whole to treat many diseases. One such plant is Tragia involucrata, which is a common twining annual plant,grows all over India, specially growing as a weed in cultivated field and open degraded sites.

The present investigation mainly aims to discuss the impact of ethanol and water extract of seeds of Tragia involucrata Linn. on the urine parameters of Albino rats. When the animals were treated with ethanol and water extract of the leaves of Tragia involucrata Linn. and fresh urine sample collected from individual rat for 12 hours on 29th /30th day and 59th/60th day . After collecting urine sample, commercial urine analysis strip were used to analyze glucose, bilirubin, ketone, specific gravity, pH and protein. There were no significant changes observed in the mid term (30 day) and end term (60 day) studies. However in case of protein a significant ($p<0.05$) increase in the end term study at 1000 mg/kg was observed.

After gross examination of the urine sample in which changes were seen did not show any detectable abnormalities. The result suggests ethanol and water extract of seeds of Tragia involucrata Linn. is safe when administered orally and it is safe to use as powder, paste and decoction for medicinal purpose.

1. Introduction

The use of medicinal plant as a therapy for disease condition is an age long practice. In regions with rich diversity of flora spread, it forms an important component of their natural wealth. Herbs and herbal formulations for the treatment of ailments have continued to receive increased attention because of the strong belief that these products are safe (Farnsworth and Soejarto, 1985; Said et al., 2002). This assumption to a large extent may have influenced the indiscriminate use of these formulations by many, particularly amongst the rural population. The incidence of adverse effects and sometimes life-threatening conditions allegedly emanating from these herbal medicines has been reported among various ethnic groups (Elvin-Lewis, 2001; Chan, 2003). Many plant extracts are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology. A medicinal enzyme inhibitor is often judged by its specificity (its lack of binding to other proteins) and its potency which indicates the concentration needed to inhibit the enzyme. A high specificity and potency ensure that a drug will have few side effects and thus low toxicity. This study was conducted to determine the safety profile of *Tragia involucrata* Linn. by carrying out the acute and subchronic toxicological assessment of its water and alcoholic seed extract. In the acute toxicity test, mice were administered orally with the extract up to 10 g/kg and intraperitoneally at doses of 50 – 800 mg/kg. Animals were then observed for behavioural changes, signs of toxicity, and mortality within 24 h. Surviving mice were monitored for 7 days for signs of delayed toxicity. In the subchronic toxicity test, rats were daily treated with the extract at doses of 40, 200, and 1000 mg/kg orally, for 30 days and 60 days. So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far out number those of toxicity, probably as a result of the greater demands for resources and time such exercise warrant. According to Ibarrola et al. (2000) and Mushtaq et al. (2003) pharmacological and toxicological evaluations of medicinal plants are essential for drug development.

***Tragia involucrata* :** It is found growing throughout India. It is growing as a weed of cultivated fields and open degraded sites. It is an erect, trailing or climbing, perennial evergreen herb or shrub with slender stems covered with stinging hairs. The roots possess, diaphoretic and alternate properties. They are considered useful for treating asthma, fevers, diarrhoea, excessive urination, vomiting and dermatosis. Root infusion is given to promote perspiration in fevers, and to relieve itching of the skin. A decoction is reportedly useful in the form of an infusion, they are given to treat diarrhoea and dysentry. The fresh root is given for treatment of epilepsy and snakebite. The fruits are used to treat enlarged spleen. Parts of the used are roots, leaves seeds etc. Kirtikar, K.R and Basu, B.D. (1980), Whitelaw Ainslie (1996).

Previous chemical investigation reported that the seeds of the plant contain a mixture of edible oils and a high percentage (61.7) of linoleic acid (Nasirullah, et al. 1980). Ahmed, et al. (1991) isolated a flavone and asantalene derivatives from Polygonum flaccidum.

Kar & Choudhuri et al., (2003) reported the comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats.

Dhara, et al. (2002) investigated the psychopharmacological activity of the methanol fraction of the root extract of Tragia involucrata in rodents.

Ramzi et al. (2008) reported the antimicrobial, antioxidant and cytotoxic activity of some Yemeni medicinal plants including Tragia involucrata.

2. Material and method

Plant Materials:- The fruits of selected plant T. involucrata was collected in the appropriate season. The fruits were collected and indetified by our professor.The seeds were taken out of the fruit and crushed to coarse powder. 100mg powder of the seeds of Tragia involucrata is taken in the soxhlet and extracted with 450 ml. 95% ethanol at controlled temperature. The collected extracted was concentrated under reduced pressure below 45^0c using rotary evaporator. The complete removal of the solvent from the extract was carried out in the rotary evaporator. The material thus obtained was stored at 4-5^0c until used.

Experimental Animals:- Albino rats of both sexes weighing between 120 to 150 g were used for the experiment. Animal had ad libitum access of standard laboratory diet and water, except during the previous night of experiment. The animals were grouped randomly into control and treated group containing five rats in each group. They were housed under standard environmental conditions of temperature and were allowed to free access to drinking water and pallet diet. The rats were kept in the experimental facilities for the week to allow them to be acclimated prior to dosing. Animals were put on fasting except water up to 16-18 hours prior to giving them medicine at day zero. The experimental protocol as per the WHO and OECD guideline was maintained (WHO, 2000 AND OECD, 2001).

Dose administration:- The ethanol and water extract of roots of Tragia involucrata was orally administered at concentration of 20, 40, 200 and 1000mg/kg three subsequently treated group for 30 days (midterm) and 60 days (end term study), the control group was given distilled water only.

Statistical analysis: Results were expressed as mean ± standard error of mean (SEM). Statistical significance and post hoc least-significant difference (LSD) test. The data obtained from the study were analyzed using student t-test. P value less than 0.05 were considered significantly.

Urine Analysis: Fresh urine samples collected from individual rats for 12 hours on 29^{th} /30^{th} day and 59^{th} /60^{th} were analyzed for glucose, bilirubin, ketone, specific gravity, pH, protein, urobilinogen, nitrite and leukocytes using commercial urine analysis strip.

Table A. Effect of ethanol extract of Sample No. OP/Sd/BJ5 urine parameters.

	Mid term study (30 days)				End term study (60 days)			
	Control	40mg	200mg	1000mg	Control	40mg	200mg	1000mg
Specific gravity	1.01±0.01	1.02±0.01	1.01±0.01	1.01±0.00	1.01±0.01	1.01±0.00	1.02±0.00	1.00±0.00
pH	7.60±0.40	7.00±0.22	7.50±0.80	7.50±0.01	7.60±0.40	7.90±0.24	7.60±0.24	8.10±0.20
Protein (g/L)	1.52±0.62	2.20±0.49	2.60±0.40	1.85±0.53	1.52±062	2.60±0.40	2.60±0.40	3.00±0.01*

Values are mean ± SME (n=5), *p<0.05, (significant, student t-test) vs control.

Table B. Effect of water extract of Sample No. OP/Sd/BJ5 urine parameters.

	Mid term study (30 days)				End term study (60 days)			
	Control	40mg	200mg	1000mg	Control	40mg	200mg	1000mg
Specific gravity	1.01±0.01	1.02±0.01	1.01±0.01	1.01±0.00	1.01±0.01	1.01±0.00	1.02±0.00	1.00±0.00
pH	7.60±0.40	7.00±0.22	7.50±0.80	7.50±0.01	7.60±0.40	7.90±0.24	7.60±0.24	8.10±0.20
Protein (g/L)	1.52±0.62	2.20±0.49	2.60±0.40	1.85±0.53	1.52±062	2.60±0.40	2.60±0.40	3.00±0.01*

Values are mean ± SME (n=5), *p<0.05, (significant, student t-test) vs control.

3. Results

The ethanol and water extracts of the seeds of T. involucrata did not produce any significant effect on the levels of various parameters like glucose, bilirubin, ketone, blood, urobilinogen, nitrite, leucocytes, specific gravity, pH, and protein in urine of rats in the mid-term and end-term studies, However, in case of protein a significant ($P < 0.05$) increase in the end-term study at 1000 mg/kg was observed. All the results are shown in Table No. A & B.

4. Conclusion

There were no significant effects on urine parameters like specific activity, pH, protein level etc. At five times the pharmacological dose (1000 mg/kg), extracts of T. involucrata did not produce significant effects on urine parameters in the 30 day study but elicited some significant effects in the 60 day study. In case of protein a significant ($p<0.05$) increase in the end term study at 1000 mg/kg was observed.

After gross examination of the urine sample in which changes were seen did not show any detectable abnormalities. The result suggests ethanol and water extract of seeds of *Tragia involucrata Linn.* is safe when administered orally and it is safe to use as powder, paste and decoction.

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