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TOXICITY STUDIES ON *IPOMOEA ERIOCARPA* EXTRACT IN EXPERIMENTAL ANIMALS

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ABSTRACT

Ipomoea eriocarpa R.Br. (Family: Convolvulaceae) often called annual morningglories, are summer annual or perennial broadleaf plants. The whole plant of *Ipomoea eriocarpa* is used for ulcer, fever and rheumatism. The present investigation was carried out to evaluate the safety of pet ether extract of *Ipomoea eriocarpa* (PIE) whole plant by determining its potential toxicity after acute and chronic administration in rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. General behavior adverse effects and mortality were determined for up to 14 days. In the chronic toxicity study, the PIE was administered orally at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. Biochemical and hematological parameters were determined after 6 weeks. In the acute study in rats, there was no toxicity/ death was observed at the dose of 2000mg/kg b.w. The onset of toxicity and signs of toxicity also not there. In the chronic toxicity study, no significant treatment-related changes in the levels of haematological, hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study. It suggests that the pet ether extract of *Ipomoea eriocarpa* does not appear to have significant toxicity. In view of the dose of *Ipomoea eriocarpa* consumed in traditional medicine, there is a wide margin of safety for the therapeutic use of the pet ether extract of *Ipomoea eriocarpa* whole plant.

Key words: *Ipomoea eriocarpa*, Traditional Medicine, Acute and Chronic Toxicity, Heamatological Parameters, Biochemical Parameters.

INTRODUCTION

Ipomoea eriocarpa R.Br. (Family: Convolvulaceae) often called annual morningglories, are summer annual or perennial broadleaf plants. *Ipomoea eriocarpa* R.Br. are often cultivated as ornamentals, however, under favorable conditions they can become troublesome weeds. They are also a major agricultural weed problem in the San Joaquin Valley of California, where several species of *Ipomoea* are found. Control is critical from crop emergence to harvest. Destroy seedlings while they are small, because once they have twined up stems they are difficult to control without injuring the crop. Seeds remain viable in soil for long periods. Seeds of Ipomoea species contain many types of alkaloids, including some that are neurotoxins to humans and animals when consumed. Fortunately, there is typically not enough seed in contaminated grain to cause harm to livestock. Most seedlings emerge following irrigation, but they may also appear when surface soil is too dry to allow germination of other annuals. Cotyledons (seed leaves) are butterfly shaped and more deeply notched and much larger than those of field bindweed. First true leaves are heart shaped with deep lobes at the base. Mature plants have long stems that climb and twine. Leaves are large, heart shaped and/or three lobed, and are alternate to one another along the stem. Both leaf types can occur on the same plant. The funnel-shaped flower varies in color depending on the species, from violet or blue to pink and red. Fruit are pods that release seeds through slits. Seeds germinate down to a depth of 4 inches (10 cm) or more, much deeper than most annuals. The whole plant of *Ipomoea eriocarpa* is used for ulcer, fever and rheumatism [1]. And also whole plants are used for the treatment of ulcers and sores In spite of the use of *Ipomoea eriocarpa* in traditional medicine and its

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potential for toxicity, systematic evaluation of its toxic effects is lacking. Therefore, the aim of the present study was to investigate the acute and chronic toxic effects of petroleum ether extract of *Ipomoea eriocarpa* in rodents.

MATERIALS AND METHODS

Plant material

The whole plant of *Ipomoea eriocarpa* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 9.5%w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study of *Ipomoea eriocarpa* extract in rats

The procedure was followed by using OECD 423 (Acute Toxic Class Method) [2]. The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of PIE was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of PIE and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, $1/20^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study.

Study of Chronic Toxicity of *Ipomoea eriocarpa* extract in rats

Design of Treatment

Animals were divided into 5 groups of six rats each.

Group I - Normal saline (0.9%, NaCl, 5ml/kg, p.o) once in a week for 6 weeks.

Group II- Vehicle 1% SCMC (5ml/kg, p.o) once in a week for 6 weeks.

Group III-V- Pet ether extract of *Ipomoea eriocarpa* whole plant at the dose of 100, 200 and 400 mg/kg, p.o respectively.

Animals from each group were sacrificed at the 6^{th} week, after the last dose. Different haematological and serum biochemical tests were then performed.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies and clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Methods for estimation of haematological parameters

Estimation of Hemoglobin [4], RBC count [4], WBC count [4], different leucocytic count [3], Elongation time [3] and ESR [5]were determined according to the standard procedures.

Determination of serum biochemical parameters

Blood Glucose, [6] Serum Bilirubin [7], Serum Gluconate – Oxaloacetate Transaminase (SGOT) [7], Serum Glutamate – Pyruvate Transaminase (SGPT) [7], Serum Alkaline Phosphatase (ALP) [7], Blood Cholesterol [6], Blood Urea [6] , Serum Uric Acid [6], Blood Creatinine [6] and Serum protein[6] were estimated by standard procedures.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the

groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 1).

Chronic toxicity study

The chronic oral administration of pet ether extract of *Ipomoea eriocarpa* whole plant caused no noticeable change in the general behaviour of the rats and, compared to the control group (saline and vehicle), no significant changes in body weight, food intake and utilization of food in the PIE treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

Effect of pet ether extract of *Ipomoea eriocarpa* whole plant on the haematological and biochemical parameters of rats

In the chronic toxicity study, the haematological parameters, hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) from that of the control group (Table 2) and all the values remained within normal limits throughout the experimental period. As shown in Table 3 & 4, no significant treatment-related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study.

Table 1. Acute toxicity study of pet ether extract of *Ipomoea eriocarpa* (PIE) in rats

S.No	Groups	Dose/kg b.w, p.o	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	PIE	2000 mg	165 g	170 g	No signs of Toxicity	Nil	14days
2	PIE	2000 mg	180 g	185 g	No signs of Toxicity	Nil	14days
3	PIE	2000 mg	160g	165 g	No signs of Toxicity	Nil	14days
4	PIE	2000 mg	180 g	185 g	No signs of Toxicity	Nil	14days
5	PIE	2000 mg	210 g	215 g	No signs of Toxicity	Nil	14days
6	PIE	2000 mg	205 g	210 g	No signs of Toxicity	Nil	14days

Table 2. .Effect of pet ether extract of *Ipomoea eriocarpa* (PIE) on heamotological profiles in rats

Design of treatment	Group I Saline(0.9 % W/V)	Group II Vehicle (1%SCMC)	Group III PIE	Group IV PIE	Group V PIE
Dose mg/kg	5 ml/kg,p.o	5 ml/kg,p.o	100mg/kg,p.o	200mg/kg,p.o	400mg/kg,p.o
Neutrophil (%)	24.1± 0.32	25.2 ± 0.24	35.7 ± 0.43[a]	37.5 ± 0.51[a]	39.2 ± 0.47[a]
Eosinophil (%)	1.1 ± 0.04	0.8 ± 0.25	1.5 ± 0.04[a]	0.8 ± 0.04[a]	0.8 ± 0.02[a]
Lymphocyte (%)	74.4 ± 0.27	71.5 ± 0.3	65.3 ± 1.18[a]	59.4 ± 1.12[a]	53.6 ± 1.47[a]
Monocyte (%)	3.5 ± 0.67	2.8 ± 0.44	2.5 ± 0.21[a]	2.6 ± 0.44[a]	1.9 ± 0.57[a]
Clotting time (seconds)	76.3 ± 1.57	82.2 ± 1.74	92.5 ± 1.81[a]	97.1 ± 1.69[a]	102.3 ± 1.71[a]
Haemoglobin (gm%)	14.4 ± 0.67	14.2 ± 0.51	13.7 ± 0.12[a]	12.6 ± 0.11[a]	12.3 ± 0.14[a]
RBC cells (cu.mm)×10^9(%)	8.3 ± 0.74	7.5 ± 0.5	7.7 ± 0.9[a]	6.8 ± 0.12[a]	7.7 ± 0.11[a]
WBC cells (cu.mm)×10^9 (%)	7.9 ± 0.36	7.8 ± 0.19	7.9± 1.22[a]	8.5± 1.21[a]	10.2± 1.17[a]

a- Group I & II Vs group III, IV &V. $P < 0.01$ when compared to control group

Each value represents the mean ± S.E.M six rats in each group

Table 3. Effect of pet ether extract of *Ipomoea eriocarpa* (PIE) on hepatic parameters in rats

Groups	Design of treatment	Dose Mg/kg	Glucose Mg/dl	Bilirubin Mg/dl	SGOT 1 Unit/L	SGPT 1 Unit/L	ALP 1 Unit/L	Cholestrol mg/100ml
I	Saline(0.9 % W/V)	5 ml /kg,p.o	89 ± 3.5	0.4 ± 0.001	50.8 ±0.7	32.2 ±0.7	8.3 ±0.37	60.7 ±1.9
II	Vehicle (1% SCMC)	5ml/kg,p.o	98 ± 3.3	0.6 ±0.001	57.2 ±0.4	35.3 ±1.2	8.7 ±0.33	66.6 ±1.4
III	PIE	100mg/kg,p.o	98 ± 3.5[a]	0.6 ± 0.001[a]	53.1 ±0.2[a]	36.2 ±0.6[a]	10.2 ±0.32[a]	54.2 ±1.7[a]
IV	PIE	200mg/kg,p.o	106 ± 3.4[a]	0.6 ± 0.001[a]	55.2 ±0.2[a]	37.6 ±0.2[a]	11.1 ±0.32[a]	59.1 ±1.6[a]
V	PIE	400mg/kg,p.o	107± 3.2[a]	0.7±0.001[a]	58 ± 0.6[a]	39.0±0.6[a]	12.2±0.22[a]	70.2±1.2[a]

a- Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

Table 4. Effect of pet ether extract of *Ipomoea eriocarpa* (PIE) on renal parameters in rats

Groups	Design of treatment	Dose mg/kg	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Protein gm/dl
I	Saline(0.9 % W/V)	5 ml/kg,p.o	21 ± 0.55	4.2 ± 0.7	0.9 ± 0.001	6.7 ±0.10.
II	Vehicle (1%SCMC)	5 ml/kg,p.o	22 ± 0.42	4.3 ± 0.5	1.2 ± 0.002	6.9 ±0.12
III	PIE	100mg/kg,p.o	25 ± 0.53[a]	3.8 ± 0.6[a]	1.1 ±0.001[a]	6.8 ± 0.12[a]
IV	PIE	200mg/kg,p.o	28 ± 0.52[a]	3.9 ±0.2[a]	1.2 ±0.001[a]	7.2 ± 0.31[a]
V	PIE	400mg/kg,p.o	30 ± 0.14[a]	3.6±0.6[a]	1.5±0.001[a]	7.5± 0.32[a]

a- Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

Discussion and conclusion

A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare [8,9]. Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for *Ipomoea eriocarpa*(L.). Because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems [10]. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans [11]. One should, in addition to the use of historical documentation on Alocassia macrorhiza, also have formal toxicological evaluations of this plant to optimize its safe use as a medicine. The pet ether extract of *Ipomoea eriocarpa* used in the present study offers several advantages as a form of the *Ipomoea eriocarpa* medicine [12]. But before such evaluation can be fully justified in humans, the preclinical evaluation of the safety of the *Ipomoea eriocarpa* is required.

In this study, the pet ether extract of *Ipomoea eriocarpa* was found to be non-toxic in rats when administered orally in doses up to 2000 mg mg/kg, p.o. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. Based on this animal study, may be described as being practically non-toxic.

In the six weeks chronic toxicity study, the PIE at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behaviour of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered PIE did not retard the growth of rats. After six weeks treatment, there were also no treatment related changes in the haematological parameters (i.e. hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells) between control and treated groups indicating that the PIE was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds [13] and an important index of physiological and pathological status in man and animals [14].

In addition, most of the hepatological and renal parameters (i.e. Glucose, creatinine, Bilirubin, SGOT, SGPT, ALT, urea, uric acid, protein and cholesterol,) were also unchanged by the doses of PIE 100, 200 & 400mg/kg. The lack of significant alterations in the levels of ALP,

creatinine, Bilirubin, SGOT, SGPT and cholesterol, good indicators of liver and kidney functions, respectively [15]. The transaminases (SGOT and SGPT) are well known enzymes used as biomarkers predicting possible toxicity [16]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [17]. The transaminases were not significantly increased at the doses of PIE 100, 200 & 400mg/kg. It suggests that chronic ingestion of PIE did not alter the hepatocytes and kidneys of the rats, and, furthermore the normal metabolism of the animals. The relevance of this result may be associated with the biological value of the plant *Ipomoea eriocarpa*(L.).

In conclusion, the present investigation demon strates that at doses consumed in the traditional medicine, the pet ether extract of *Ipomoea eriocarpa* may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

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