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Oral toxicity study of ethanolic extracts from aerial parts of *Ipomoea pes-caprae (l.)* R.br on wistar albino rats

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Abstract

Ipomoea pes-caprae (L.) R. Br also called a beach plant belongs to the family Convolvulaceae is used traditionally in many ailments including inflammatory conditions such as Rheumatoid arthritis, Ankylosing spondylitis, Osteoarthritis, Gout etc., and also in conditions such as Pain, Ulcer, Cancer and Wounds. This study was undertaken to assess the acute oral toxicity and repeated dose 28-day oral toxicity profile of aerial parts of *Ipomoea pes-caprae*. Parameters like body weight changes, signs of toxicity, mortality, reversible/ irreversible changes were observed and recorded during acute oral toxicity study. Haematological parameters and histopathology of liver, heart, kidney, brain and lung were observed and were recorded during repeated dose 28-day oral toxicity study. Ethanolic extracts of leaves and stems of *Ipomoea pes-caprae* showed no signs of toxicity, no mortality and no significant changes in body weight during acute oral toxicity study. Both extracts did not show any significant changes in the haematological parameters and histopathological study of liver, heart, kidney, brain and lung showed no significant changes in the normal architecture during repeated dose 28-day oral toxicity study. Also, there were no signs and symptoms of toxicity, no mortality, no alteration in behaviour, no body weight changes and no significant changes in food and water intake were observed during the 28-day oral toxicity study.

Keywords: *Ipomoea pes-caprae*, OECD 423, OECD 407, acute oral toxicity, repeated dose 28-day oral toxicity.

1. INTRODUCTION

Ipomoea pes-caprae is a prostrate perennial, often covering large areas; stems long-trailing often several metres in length, rooting at the nodes, glabrous. It has pink petals with a darker centre [1]. It has a biological activity like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulinogenic and hypoglycemic [2]. It is also used in the inhibition of platelet aggregation, diarrhoea, vomiting, and piles. The *in-vivo* & *in-vitro* anti-inflammatory has been reported [3]. The anti-nociceptive activities of IP have already been proved [4]. Our study aims to assess the safety of ethanolic extracts of the leaf (EELIP) and stem (EESIP).

2. MATERIALS AND METHODS

2.1 Plant Material

The whole plant of *Ipomoea pes-caprae* was collected from coastal areas of the district, Tamil Nadu and authenticated by Dr.P.Jayaraman (Botanist), Director PARC, West Tambaram, Chennai. The leaves and stems were segregated, dried, powdered and were extracted separately with ethanol using soxhlet apparatus for 48 hrs. The solvent was distilled at a lower temperature under reduced pressure and concentrated on a water bath to get the crude extract which is stored in a desiccator for future use.

2.2 Experimentation

The animal studies were carried out with the institutional animal ethical committee clearance (Ref:(IAEC/I/02/CLBMCP/2012 dated 28.08.2012)). Because of ascertaining the oral toxic characteristics of our extract, acute oral toxicity study was conducted.

2.2.1 Acute oral toxicity test

Female Wistar albino rats weighing 200-250 gms and female Swiss albino mice weighing 20-25gms were selected for these studies and they were maintained in standard laboratory condition. Rats fasted overnight and mice were fasted 4 hours before the study but had free access to water. The acute toxicity class method is a stepwise procedure with 3 animals of single-sex per step. Depending on the mortality and morbidity status of the animals, an average of 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for an acceptable data-based scientific conclusion.

This method is based on biometric evaluation with fixed doses (5, 50, 300, 2000 mg/kg p.o) and results allow the substance to be ranked and classified according to Globally Harmonised System (GHS) for the classification of extracts which cause acute toxicity.

When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. The starting dose levels of ethanol extracts and their fractions of *Canthium Parviflorum* and *Merremia emarginata.* was 2000mg / kg body weight per oral. Dose-volume was administered 0.1 ml / 100 gm. After oral administration, the animals were observed at an hourly basis for 24 hours to assess the general behaviour and mortality. They were further observed for 14 days for toxic symptoms and mortality [5]. The flow chart in the figure depicts the procedure adopted for this method (Figure 1).

Grouping:

Group I- treated with 1 % CMC P.O.
Group II- treated with 2000 mg/kg EELIP P.O.
Group III- treated with 2000 mg/kg EESIP P.O.
Animals were weighed before starting and ending of acute toxicity studies.

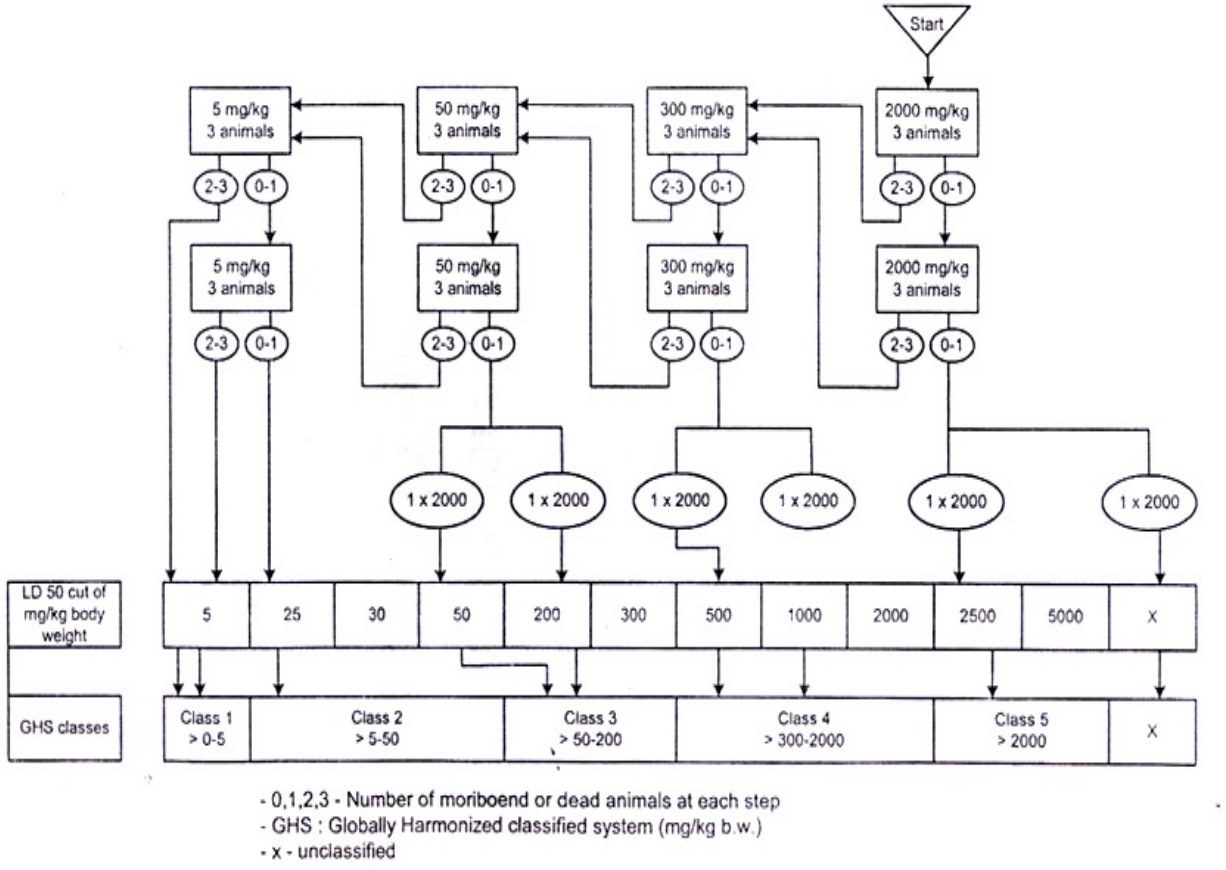

Figure 1: Acute oral toxicity study (Acute toxic class method) OECD guidelines 423

2.2.2 Repeated dose 28-day oral toxicity study

a) The rationale of the study:

In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute toxicity testing. This TG is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure for 28 days.

b) Procedure:

6 Adult Wistar albino rats (3 male and 3 female) weighing 200-250 gms were used for sub-acute toxicity (OECD 407(3rd version, Oct 2008)). These animals were maintained in polypropylene cages under identical animal house conditions and provided with standard pellet and water *ad libitum*. Generally, at least three test groups and a control group should be used, but if from an assessment of other data, no effects would be expected at a dose of 1000mg/kg bw/d, a limit test may be performed (OECD 407(3rd version, Oct 2008)). Sub-acute toxicity studies were carried out for the ethanolic extracts of leaves and stems of *Ipomoea pes-caprae* at the dose of 1000 mg/kg for 28 days p.o. Animals were observed for signs and symptoms of toxicity, alteration of behaviour, changes in food and water intake, body weight changes and mortality. Blood samples were collected 24hr after the last dose of ethanolic extracts and fractions for haematological studies. A portion of vital organs like liver, brain, kidney, heart and lungs were dissected out and kept in 10% formalin for histopathological studies. Haematological parameters like RBC, Hb, Total WBC and differential WBC were also determined, after collecting the blood through retro-orbital puncture [5].

Grouping:

Group I- treated with 1 % CMC p.o.

Group II- treated with 1000 mg/kg EELIP p.o.

Group III- treated with 1000 mg/kg EESIP p.o.

2.3 Statistical analysis:

The statistical analysis was carried out by one-way ANOVA followed by Dunnett's *posthoc* test using GraphPad Prism (Version 8.4.2). All the values were expressed as mean ± SEM. p values less than 0.05 was considered as statistically significant.

3. RESULTS

3.1 Acute oral toxicity study:

EELIP and EESIP showed no notable signs of toxicity, no mortality, no Reversible/ Irreversible toxic signs and no significant changes in body weight, during the acute toxicity study. Hence the LD_{50} and globally harmonized classified system of EELIP and EESIP were found to be > 2000 mg/kg body wt (Class V) (Table: 1).

Table 1: Acute Oral Toxicity Study (Acute Toxic Class Method- OECD Guideline 423)

S. No	Group	Dose	Weight of Animals (gm)		Signs of toxicity	Mortality	Reversible / Irreversible	Duration of observation
			Before test	After test				
1.	Group I- Normal	1 % CMC	224 ± 3	220 ± 2	No signs of toxicity	No mortality observed	Nil	14 days
2.	Group II- EELIP	2000 mg/kg	217 ± 5	214 ± 4	No signs of toxicity	No mortality observed	Nil	14 days
3.	Group III- EESIP	2000 mg/kg	225 ± 3	223 ± 4	No signs of toxicity	No mortality observed	Nil	14 days
p value = 0.0611, non-significant								

Table 2: Repeated dose 28-day oral toxicity study.

Group	Dose	Weight of Animals (gm)		Signs and symptoms of toxicity	Mortality	Alteration of behaviour, changes in food and water intake	Duration of observation
		Before test	After test				
Group I- Normal	1 % CMC	219 ±3	220 ± 4	No signs of toxicity	No mortality observed	No significant changes observed	28 days
Group II- EELIP	1000 mg/kg	225 ± 5	224 ± 4	No signs of toxicity	No mortality observed	No significant changes observed	28 days
Group III- EESIP	1000 mg/kg	221 ± 4	223 ± 3	No signs of toxicity	No mortality observed	No significant changes observed	28 days
P value = 0.0611, Non-Significant							

Table 3: Effect of EELIP & EESIP on Haematological parameters (Repeated dose 28-day oral toxicity study)

Groups	Dose	Hb%	RBC × 10^6/mm^3	WBC 10^3/mm^3	ESR	PCV	% Neutrophil	% Eosinophil	% Lymphocytes	% Monocytes
Control	1 % CMC	12.35±1.45	4.57 ±0.78	7.95±1.46	3.56±0.37	42.32±2.57	23.57±1.65	3.67±0.75	68.96±3.47	1.98±0.47
EELIP	1000 mg/kg	12.57±1.24	4.36±0.65	8.12±1.23	3.74±0.65	47.35±2.78	22.08±1.35	4.12±0.89	72.67±3.69	2.22±0.79
EESIP	1000 mg/kg	11.25±1.09	3.89±0.55	7.42±1.05	2.98±0.44	40.35±2.26	21.67±0.99	3.45±0.48	70.35±3.24	2.10±0.88
P value = 0.9904, Non-Significant										

3.2 Repeated dose oral toxicity study:

EELIP and EESIP showed no signs of toxicity, no mortality, no Reversible/ Irreversible alteration of behaviour and no significant changes in body weight, during the study period (Table: 2).

Haematological parameters

The evaluation of haematological parameters like Hb, RBC, WBC, ESR, PCV, Neutrophil, Eosinophil, Lymphocytes and monocytes showed no significant changes on both EELIP and EESIP treated animals at the dose of 100mg/kg b.wt when compared to control (Table: 3).

Histopathology

Control animals showed normal architecture of all the vital organs like liver, kidney, heart, lung and brain. Both EELIP and EESIP at the repeated dose of 1000 mg/kg showed normal liver with central vein wand cords of hepatocytes (Figure 2) followed by congested glomeruli with mild tubular epithelial damage in the kidney (Figure 3) alongside with mild change in a cardiac fibre of heart (Figure 4) and mild dilation of alveoli emphysematous in the lung (Figure 5) and showed normal brain tissues with astrocytes and nerve fibres (Figure 6). Therefore, the histopathological study of liver, heart, kidney, brain and lung showed no significant changes in the normal architecture during repeated dose 28-day oral toxicity study.

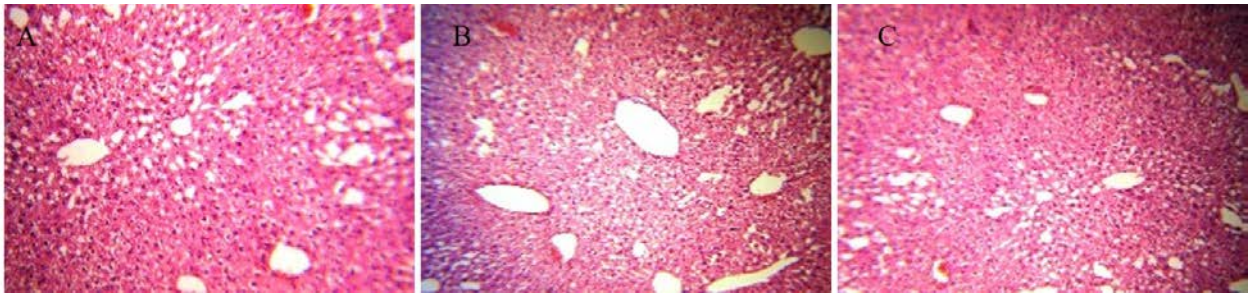

Figure 2: Histopathological results of the liver after repeated dose toxicity study by H&E staining. (A) Control (B) EELIP 1000 mg/kg (C) EESIP 1000 mg/kg.

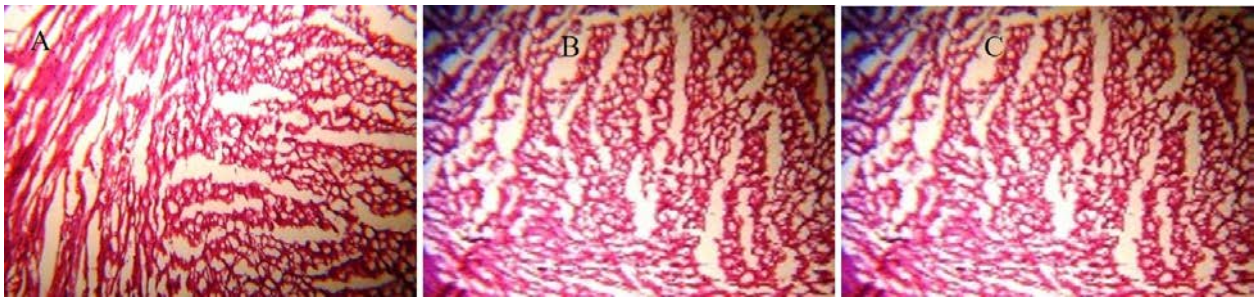

Figure 3: Histopathological results of heart after repeated dose toxicity study by H&E staining. (A) Control (B) EELIP 1000 mg/kg (C) EESIP 1000 mg/kg.

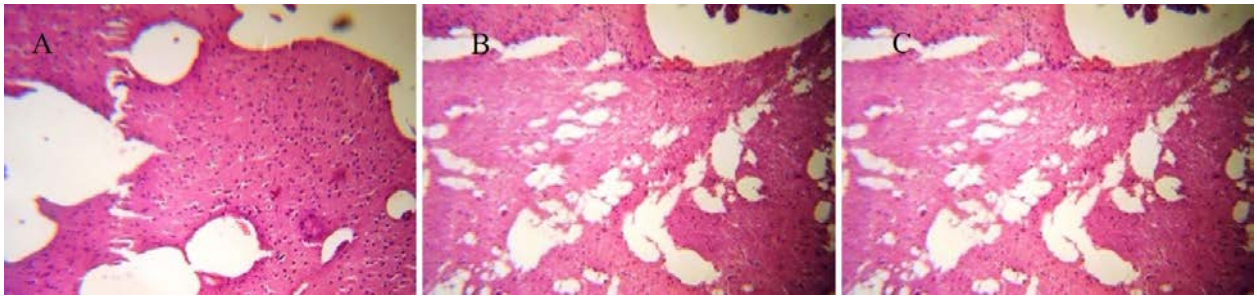

Figure 4: Histopathological results of the kidney after repeated dose toxicity study by H&E staining. (A) Control (B) EELIP 1000 mg/kg (C) EESIP 1000 mg/kg.

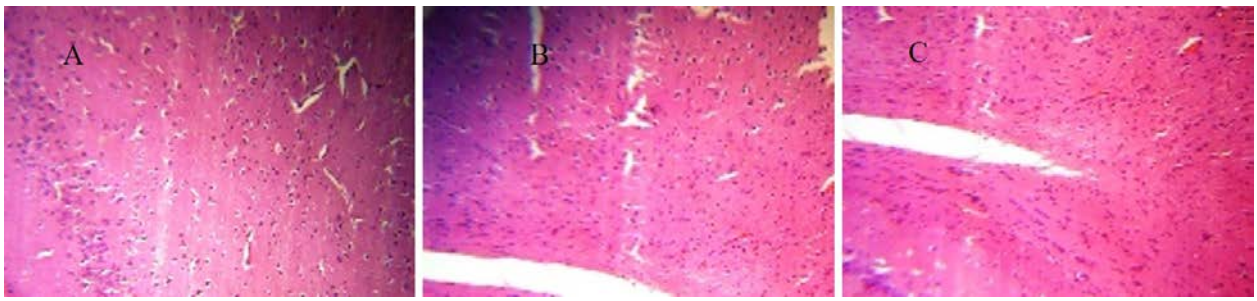

Figure 5: Histopathological results of the brain after repeated dose toxicity study by H&E staining. (A) Control (B) EELIP 1000 mg/kg (C) EESIP 1000 mg/kg.

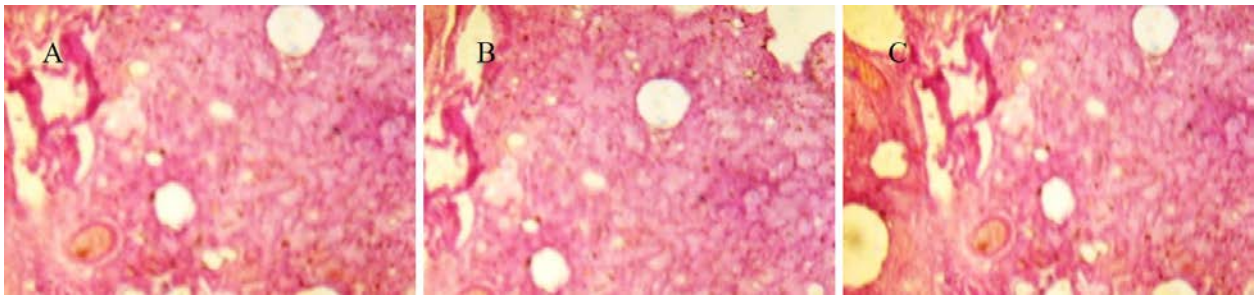

Figure 6: Histopathological results of the lung after repeated dose toxicity study by H&E staining. (A) Control (B) EELIP 1000 mg/kg (C) EESIP 1000 mg/kg.

4. DISCUSSION

Medicinal plants and their use in traditional medicine plays a vital role in primary healthcare throughout the world [6]. *Ipomoea pes-caprae* is one of such plant with a wide medicinal potential. But there is very limited or no proper scientific evidence about its safety during consumption in any form. The acute dermal toxicity of both leaves and stem were previously studied by the method detailed as per OECD 434 and found to be safe [7].

In the current study, primarily the rats were treated with ethanolic extracts of both leaves and stem at a dose of 2000 mg/kg as per OECD 423. From the results, it was noticed that both the extracts have a margin of safety and produced no alteration in behaviour and mortality. Further investigation was done by repeated dose toxicity studies using OECD 407 guidelines. The haematological parameters showed no significant difference when compared to the control group which clearly shows its wide margin of safety [8].

The histopathological results showed no or minimal morphological changes without producing any toxic effects. The results were similar to that of the work of [9]. thus, IP could be safe and can be used for the treatment of various disorders soon.

5. CONCLUSION

The results of acute oral toxicity study and repeated dose 28-day oral toxicity study of ethanolic extracts of leaves and stems of *Ipomoea pes-caprae* indicate their non-toxic nature at the test dose level tested. The LD_{50} of both leaf and stem extracts of *Ipomoea pes-caprae* was found to be >2000 mg/kg by acute oral toxicity study. Also, 1000 mg/kg of leaf and stem extracts of *Ipomoea pes-caprae* was found to be safe on repeated dose 28-day oral toxicity study which clearly shows its margin of safety. Hence this study forms a basis to select the oral dose level of *Ipomoea pes-caprae* to further evaluate its efficacy on various ailments including rheumatoid arthritis and other immune disorders.

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