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Assessment of Acute Dermal Toxicity 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 (IP) is a fabulous plant which was traditionally used in various inflammatory conditions such as Rheumatoid arthritis, Alkylosing spondylitis, Osteoarthritis, Gout etc and also in conditions such as Pain, Ulcer, Cancer and Wounds. The dermal toxicity of Ethanolic extracts from aerial parts of *Ipomoea Pes-caprae* (L.) R.Br was ascertained by carrying out acute dermal toxicity study (OECD 434). Acute dermal toxicity was carried out by single topical application of EELIP and EESIP (Ethanolic extracts of Leaves and Stems of *Ipomoea Pes-caprae* (L.) R.Br) at the dose of 2000 mg/kg. The parameters like body weight changes, signs of toxicity, mortality were recorded during the 14 day study. No significant changes in body weight were observed during acute toxicity studies. No signs of toxicity and mortality were observed during both the acute dermal toxicity studies. The LD₅₀ of both leaf and stem extracts were found to be >2000 mg/kg by acute dermal toxicity study.

Keywords: *Ipomoea Pes-caprae*, Rheumatoid arthritis, wounds, acute dermal toxicity

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INTRODUCTION

Virtually all cultures worldwide have relied historically, or continue to rely on medicinal plants for primary health care. Approximately one-third of all traditional medicines are for treatment of wounds or skin disorders, compared to only 1-3% of modern drugs. The use of such medicinal plant extracts for the treatment of skin disorders arguably has been based largely on historical/anecdotal evidence, since there has been relatively little data available in the scientific literature, particularly with regard to the efficacy of plant extracts in controlled clinical trials. Adverse effects of plants on skin reviewed include: irritant contact dermatitis caused mechanically (spines, irritant hairs) or by irritant chemicals in plant sap; phytophotodermatitis resulting from skin contamination by plants containing furocoumarins, and subsequent exposure to UV light; and immediate (type I) or delayed hypersensitivity contact reactions mediated by the immune system in individuals sensitized to plants or plant products (e.g. peanut allergy, poison ivy (*Toxicodendron*) poisoning) [1]. *Ipomoea* is the largest genus in the flowering plant family Convolvulaceae, with over 500 species. *Ipomoea pes-caprae* is a valuable medicinal plant, distributed in the tropics and subtropics regions and used in folk and tribal medicines. It is a pan tropical, trailing vine that routinely colonizes on sand dunes. It grows just above the high tide line along coastal beaches, forming large mats that assist in stabilizing sands. This is an evergreen perennial with a large, thick root that can be 10 ft long and 2 inch in diameter. The entire plant is glabrous and somewhat fleshy. The stem runs along the ground rooting at the nodes with only the flowers being erect [2-3]. Traditionally *Ipomoea pes-caprae* is used in different ways like; the juice from the succulent leaves has been used as a first aid to treat jelly fish stings. Some Indians use it in ritual baths to alleviate evil spells. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties [4]. The preliminary phytochemical analysis were previously carried out and revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols, terpenoids and glycosides both in EELIP and EESIP [5]. The *in vivo* & *in vitro* anti-inflammatory activities of have also been reported [6]. The compounds responsible for the anti-inflammatory and anti-nociceptive actions have also been isolated. 2-hydroxy-4,4,7-trimethyl-1(4H)-naphthalenone, (-)-mellein, eugenol and 4-vinyl-guaiacol were the Compounds inhibiting prostaglandin synthesis isolated from IP [7]. Compounds such as glochidone, betulinic acid, alpha- and beta-amyrin acetate, isoquercitrin isolated from IP were found to be responsible for its anti-nociceptive properties [8]. IP was found to also possess anti-haemolytic, antispasmodic, anti-histamine, anti-cancer, antioxidant, antispasmodic, anticancer, antihistaminic, insulogenic and hypoglycemic activities [9,10]. So according to the folkloric and scientific claims of IP, it can topically act as analgesic, anti-inflammatory, anti-ulcerogenic and also be used to treat wounds.

Before evaluating its topical effectiveness, the dermal safety of IP has to be established. But there exists on scientific evidence about the dermal safety of IP, the present study focuses on establishing the acute dermal safety of ethanolic extract from leaves and stems of IP on wistar albino rats.



MATERIALS AND METHODS

Plant Material:

Whole plant of IP were collected from coastal areas of 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 lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

Preparation of Ethanolic extract of IP:

Fresh leaves of IP were washed in running water. After shade drying at room temperature, 1 kg of dried leaf was coarsely powdered and it was sieved using sieve number 60. Extraction process was carried out by ethanol using soxlet extraction apparatus after air drying coarse powder. The extract thus obtained was allowed to stand at room temperature for 24 hrs. A semi-solid mass was obtained after it was filtered and concentrated by rotary vacuum pump. The percentage yields of leaf and stem were found to be 2.41% and 4.15% respectively.

Experimentation:

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

Animal Species Used:

Nulliparous and non-pregnant female wistar albino rats between 8 and 12 weeks old and with weight falling within an interval of $\pm 20\%$ of the average body weight were used for the study.

Housing and Feeding Conditions:

The temperature of the experimental animal room was $22\pm 3^{\circ}\text{C}$. The relative humidity was between 50-60%. The sequence of 12 hours light and 12 hours dark cycle was maintained through artificial lighting. For feeding, conventional laboratory diets were used with an unlimited supply of drinking water.

Preparation of Animals:

The animals were acclimatised to the laboratory conditions for at least five days prior to the start of the study. Animals are randomly selected for use in the study and marked to provide individual identification. Approximately 24 hours before the study, fur on the dorsal area of the

trunk of the test animals was removed by shaving. Care was taken to avoid abrading the skin, which could alter its permeability. Approximately 10% of the body surface area was cleared for the application of the test substance.

Administration of Doses:

The semi-solid mass of leaf and stem extracts of IP were made into ointment using vaseline as vehicle. The test substance was applied uniformly as thin film over an area which is approximately 10 % of the total body surface area. Test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape in a manner that the animals cannot ingest the test substance throughout a 24-hour exposure period. Care is taken that the dressing did not restrain the movement of the animal.

Acute Dermal Toxicity (14- day Dermal Toxicity Study):

Acute dermal toxicity comprises of a sighting study and a main study.

Sighting Study (Limit Test):

Rationale of the Study:

The limit test can be used in situations where there is sufficient information on non-toxicity of the test material used. All the constituents identified and isolated from IP so far were found to non-toxic. Based on the non-toxic indications in the constituents of IP a limit test of 2000 mg/kg was done straightaway.

Procedure:

Three groups of animals each containing 1 wistar albino rat was used. The animal in the Group 1 was applied with 2000 mg/kg of ointment base (Vaseline). The EELIP ointment and EESIP ointment at the dose of 2000 mg/kg was applied topically as a thin layer on the 10% surface area of Group 2 and Group 3 animals. Animals were observed immediately after dosing for at least once during the first 30minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Observations include signs of toxicity like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern.

Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Body weights of the test animals were also noted.

Main Study (Limit Test):**Rationale of the Study:**

If topical application of 2000 mg/kg EELIP during the sighting study was found to be non-toxic, a main study was carried out.

Procedure:

In this study 3 groups of animals containing 4 each were used. The 3 animal groups were dosed in the same manner as described in the sighting study and observations were also noted in the same way [11].

Motor Activity:

Motor activity was measured by an open field of dimensions 50×50×60 cm whose floor was divided into 12×12 cm squares by black lines. The number head dippings, rearings and line crossings were scored during 20 min time. After each measurement the open field was cleaned with a damp cloth [12].

Statistical Analysis:

The statistical analyses were carried out by one way anova followed by Dunnet's test using Graph pad prism. Results were expressed as Mean ± Standard error. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Table 1: Toxicity effects of EELIP and EESIP (Acute dermal toxicity study- Sighting study)

S. No	Group/ Animal	Dose	Signs of Toxicity (Reversible / Irreversible)	Mortality	Duration of Observation
1.	Group 1	2000 mg/kg of Ointment base	No signs of toxicity	No mortality observed	14 days
2.	Group 2	2000 mg/kg of EELIP ointment	No signs of toxicity	No mortality observed	14 days
3.	Group 3	2000 mg/kg of EESIP	No signs of toxicity	No mortality observed	14 days

Table 2: Toxicity effects of EELIP and EESIP (Acute dermal toxicity study- Main study)

S. No	Group/ Animal	Dose	Signs of Toxicity (Reversible / Irreversible)	Mortality	Duration of observation
1.	Group 1	2000 mg/kg of ointment base	No signs of toxicity	No mortality observed	14 days
2.	Group 2	2000 mg/kg of EELIP ointment	No signs of toxicity	No mortality observed	14 days
3.	Group 3	2000 mg/kg of EESIP ointment	No signs of toxicity	No mortality observed	14 days

The extract treated and control animals showed no signs of toxicity or mortality (Tab. 2 & 3).

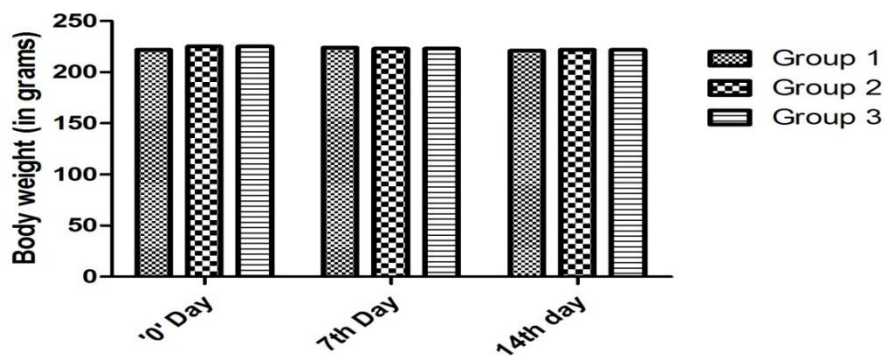


Figure 1: Effect of EELIP and EESIP on body weight (Acute dermal toxicity study- Sighting study)

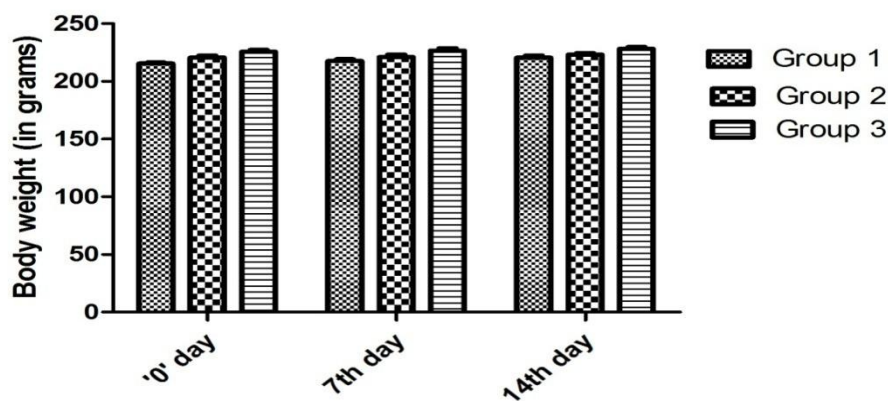


Figure 2: Effect of EELIP and EESIP on body weight (Acute dermal toxicity study- Main study)

There were no significant changes in body weight in control as well as extract treated groups (Fig. 1 & 2).

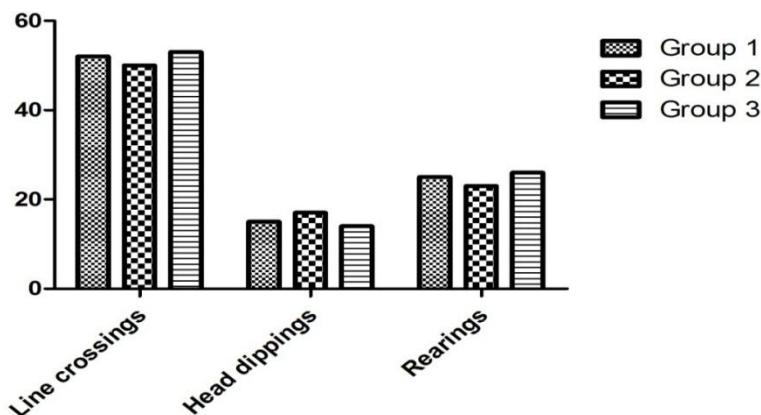


Figure 3: Effect of EELIP and EESIP on open field exploratory activity (Acute dermal toxicity study- Sighting Study)

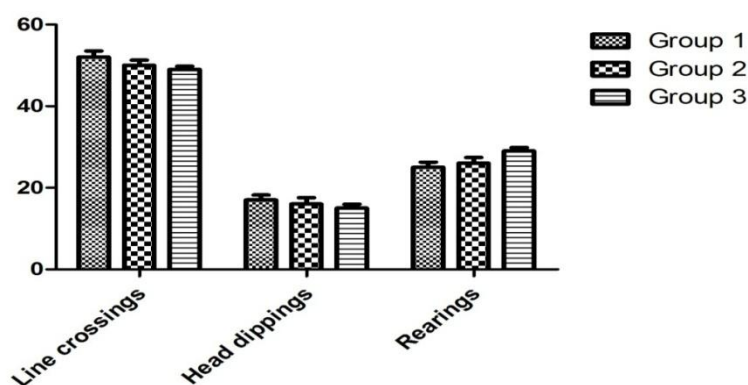


Figure 4: Effect of EELIP and EESIP on open field exploratory activity (Acute dermal toxicity study- Main Study)

The open fields exploratory activities such as head dippings, line crossings and rearing behavior did not get significantly altered in extract treated groups as compared to control animals (Fig. 3 & 4).

CONCLUSION

The results of acute dermal toxicity of the ethanolic extracts from leaves and stem of IP indicate their non-toxic nature at the tested dose level. The LD₅₀ of both leaf and stem extracts were found to be >2000 mg/kg by acute dermal toxicity study. Hence this study forms a basis to select the topical dose level of IP, to further evaluate its topical analgesic, anti-inflammatory, anti-ulcerogenic and wound healing properties.

REFERENCES

- [1] Mantle D, Gok MA and Lennard TW. Adverse Drug React Toxicol Rev 2001; 20(2):89-103.
- [2] Devall MS. J Coastal Research 1992; 8(2): 442-456.

- [3] Jirawongse V, Pharadai T and Tantivatana P. J National Research Council of Thailand 1979; 9: 17-24.
- [4] KirtikarBasu. A text book of Indian Medicinal Plant Vol. III, II edition, 2006; 1726.
- [5] N Deepak Venkataraman, W Clement Atlee, T Purushoth Prabhu, G Surya, R Kannan, I Sheik Nasar. Research J of Pharm., Biological and Chemical Sciences 2013; 4(2): 1560.
- [6] U Pongprayoon L, Bohlin P, Soonthornsaratune and S. Wasuwat. Phytotherapy Research 2006; 5(2): 63-66.
- [7] Pongprayoon U, Baeckström P, Jacobsson U, Lindström M and Bohlin L. Planta Med 1991;57(6): 515-8.
- [8] Krogh R, Kroth R, Berti C, Madeira AO, Souza MM, Cechinel-Filho V, Delle-Monache F and Yunes RA. Pharmazie 1999;54(6): 464-6.
- [9] AshishManigaunha N, Ganesh and M D Kharya. Int J Phytomedicine 2010;2: 18-21.
- [10] Umamaheshwari G, T Ramanathan, and R Shanmugapriya. Int J Pharm Tech Research 2012;4(2):848-85.
- [11] OECD draft guideline 434 for testing of chemicals: Acute Dermal Toxicity – Fixed Dose Procedure, 14 May 2004 (1st Version).
- [12] Saitoh AM, Yamada M, Yamada S, Kobayashi S, Hirose N, Honda K and Kamei. J Psychopharmacology 2006; 188: 10-21.