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Anti-Inflammatory Potential of Ethanolic Extracts from Aerial Parts of *Ipomoea Pes-Caprae (L.) R.Br* Using Cotton Pellet Induced Granuloma Model

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ABSTRACT

Ipomoea Pes-caprae (L.) R.Br (IP) is a valuable medicinal plant, distributed in the tropics and subtropics regions and used in folk and tribal medicines. Traditionally IP is used in inflammatory conditions such as arthritis and also used to treat pain, ulcer, cancer and wounds. The acute anti-inflammatory activity of IP has been previously reported. The present study aims to discover the anti-inflammatory effect of ethanolic extracts from aerials parts of IP by sub-acute anti-inflammatory model. Completely dried leaves and stems of *I.pes-caprae* were extracted using ethanol by hot percolation method. The EELIP & EESIP (Ethanolic extract of Leaves & Stems of IP) thus obtained were subjected to preliminary phytochemical analysis and revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols and terpenoids both in leaf and stem extracts. The LD_{50} of both EELIP & EESIP were found to be >2000 mg/kg by acute oral toxicity study. Both EELIP & EESIP exhibited significant anti-inflammatory activities in dose dependent manner.

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Singh et al., 2008). A condition intermediate between chronic and acute inflammation, exhibiting some of the characteristics of each is termed as sub-acute inflammation (Dorland, 2013). Inflammation is triggered by the release of chemical mediators from the injured tissues and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include amines such as histamine, serotonin, and lipids such as prostaglandins and small peptides such as kinins (Katzung, 1988). ***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 (Devall, 2013; Jirawongse, 1979). 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 (Kirtikar & Basu, 2006).

The *invivo* (using acute anti-inflammatory model) & *invitro* anti-inflammatory activities of IP have also been reported (Pongprayoon et al., 2006). The compounds responsible for the anti-inflammatory activity 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 (Pongprayoon et al., 1991).

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IP was found to also possess anti-nociceptive, anti-haemolytic, anti-spasmodic, anti-histamine, anti-cancer, antioxidant, anticancer, antihistaminic, insulogenic and hypoglycemic activities (Ashish et al., 2010; Umamaheshwari et al., 2012). The present study aims to discover the *invivo* anti-inflammatory effect of ethanolic extract from aerials parts of IP by sub-acute inflammatory model (cotton pellet induced granuloma model).

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 leaves of Ipomoea pes-caprae

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 using 70% ethanol for 8 hours at temperature of 40˚C by soxlet 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.

Preliminary phytochemical screening

The ethanolic extracts were subjected to phytochemical chemical tests to identify the phytoconstituents using standard qualitative reagents. (Kokate, 2005; Khandelwal, 2006).

Institutional animal ethical committee clearance

The animal studies were carried out with the institutional animal ethical committee clearance (Ref:IAEC /I/02 /CLBMC / 2012 dated 28.08.2012).

Animals used

Adult male Wistar albino rats (180-250 g) were used. They were housed in standard animal cages in the Animal House section of the Department of Pharmacology, C.L.Baid Metha College of Pharmacy. They were given standard laboratory animal diet and water *ad libitum.*

Acute oral toxicity (OECD 423)

Acute toxicity study was carried out as per OECD guideline 423. Since available information suggests that mortality is unlikely at the highest starting dose level of 2000 mg/kg body weight, a limit test is conducted.

Cotton pellet induced granuloma

Wistar albino rats were divided into 6 groups each group containing 6 animals. After shaving the groin region under aseptic conditions, through a single needle incision, sterile pre-weighed cotton pellets (50 mg) soaked in 0.2 mL of distilled water containing penicillin (0.1mg) and streptomycin (0.13 mg), was implanted subcutaneously bilaterally in the groin under ketamine (15 mg/kg) anesthesia. The leaf and stem extracts (200 mg/kg and 400 mg/kg), diclofenac sodium (standard, 5 mg/kg) and control were administered orally for 9 consecutive days from the day of cotton pellet implantation (Table 1). On the 10th day the pellets were dissected out, dried at 60 ºC, and the dry weights were determined. The weight of the cotton pellet before implantation was subtracted from the weight of the dried granuloma pellets. The increment in the dry weight of the pellet was taken as a measure of granuloma formation (Winter et al., 1957).

Percentage inhibition = (Control – Treated) / Control × 100

Table. 1: Animal grouping.

S.No	Group	Treatment
1.	Group 1 (Control)	1 ml/kg of 1 % CMC P.O
2.	Group 2	200 mg/kg of EELIP P.O
3.	Group 3	400 mg/kg of EELIP P.O
4.	Group 4	200 mg/kg of EESIP P.O
5.	Group 5	400 mg/kg of EESIP P.O
6.	Group 6	5 mg/kg of Diclofenac Sodium (DS) P.O

RESULTS AND DISCUSSION

Acute oral toxicity study

The LD_{50} of both leaf and stem extracts were found to be >2000 mg/kg by acute oral toxicity study.

Preliminary phytochemical analysis

The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols and terpenoids both in leaf and stem extracts.

Effect of EELIP and EESIP on cotton pellet granuloma

The acute anti-inflammatory activity of IP and the components responsible for its anti-inflammatory activity have already been reported. The leaf and stem extracts of IP were found to exhibit significant anti-inflammatory activities in dose dependent manner. Treatment with DS 5 mg/kg and EELIP 400 mg/kg were more significant ($p<0.01$) whereas treatment with EELIP 200 mg/kg and EESIP 400 mg/kg were less significant ($p<0.05$). Treatment with EESIP 200 mg/kg was insignificant (Table 2). The percentage inhibition of EELIP at the dose of 400 mg/kg (52.84%) was comparable with the standard DS (59.98%) (Table 2). Sub-acute inflammation involves infiltration of macrophages, neutrophils and proliferation of fibroblasts (Grover, 1990). The significant anti-inflammatory effect of the extracts in cotton-pellet induced granuloma suggests its efficacy in inhibiting

the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides evident during granuloma model tissue formation (Arrigoni-Martellie, 1977).

Phytoconstituents such as Flavonoids, tannins, sterols and terpenoids revealed during phytochemical investigation were found to play a significant role in inhibition of inflammation.

Table. 2: Effect of EELIP and EESIP on cotton pellet granuloma induced rats and percentage inhibition.

S.No	Group	Weight of dry cotton pellet granuloma (mg)	Percentage inhibition
1.	Group 1 (Control)	81.21 ± 6.89	-
2.	Group 2	50.55 ± 5.45*	37.75
3.	Group 3	38.30 ± 1.01**	52.84
4.	Group 4	59.74 ± 2.47^{NS}	26.44
5.	Group 5	45.72 ± 0.71*	43.70
6.	Group 6	32.50 ± 1.91**	59.98

Values expressed as Mean ± S.E.M, (n=6); *p<0.05, **p<0.01, NS: Not Significant

CONCLUSION

The present investigation explored the potential anti-inflammatory effect of ethanolic extracts from the leaves and stems of IP on sub-acute inflammation. Flavonoids, tannins, sterols and terpenoids along with other constituents exert significant anti-inflammatory effect on sub-acute inflammatory model. The extracts further can be evaluated for their effectiveness in chronic inflammatory models.

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