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Research article

Anti-inflammatory activity of *Pupalia lappacea* L.Juss

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ABSTRACT

Pupalia lappacea (L) Juss is an erect shrub used in folklore medicine to treat bone fractures and in inflammatory conditions. Methanolic extract of aerial parts shown is claimed in traditional medicine that the leaves of the plant are used in the treatment of inflammation. In the present study, the methanolic extract of *Pupalia lappacea* was screened for its anti-inflammatory activity using carrageenan induced rat paw edema egg white induced paw oedema models. The methanolic extract at the dose of 200 mg/kg p.o exhibited significant anti-inflammatory activity in carrageenan induced paw edema model ($p < 0.01$). In egg white induced model, methanolic extract at the dose of 200 mg/kg inhibited paw oedema significantly ($p < 0.01$) indicating that both test samples inhibit the increase in number of fibroblasts and synthesis of collagen and mucopolysaccharides during prostaglandin formation during the inflammation. These experimental results have established a pharmacological evidence for the folklore claim of the drug to be used as an anti inflammatory agent. HPTLC analysis of the extract shows the presence of gallic acid 1.24 μ g/ml, ferulic acid 2.00 μ g/ml, chlorogenic acid 46.25 μ g/ml and rutin 7.02 μ g/ml of the extract which were responsible for the claimed anti-inflammatory action in the animal models studied.

Keywords: *Pupalia lappacea*, Methanolic extract, Aerial parts, Diclofenac sodium, Carrageenan, Egg white, HPTLC, Rutin, Gallic acid, Ferulic acid and Chlorogenic acid.

INTRODUCTION

Herbal medicines are becoming more and more popular now a days. Among the entire flora 35,000 to 70,000 species have been used for medicinal purposes. This is the highest proportion of medicinal plants known for their medicinal purposes in any country of the world for the existing flora of that respective country. *Pupalia lappacea* belongs to the family Amaranthaceae is commonly known as Forest Burr or Creeping cock's comb. It is an erect or straggling under shrub found in the hedges of fields, fruit orchards, dry scrub forests and waste places of Kashmir to Kauman at an altitude of 300-1050 m and in all states of India¹. The leaf paste of *Pupalia lappacea* with edible oil (Sesamum or Carthamus) is an

effective and inexpensive treatment of bone fracture for human beings as well as cattle. Stem is used as tooth brush, for treating toothache. Poultice of the fresh leaves is used in treatment of boils, new and chronic wounds. A decoction of the black powder of the plant is drunk to cure piles and for enema, fever and malaria².

In this present work the methanolic extract of *Pupalia lappacea* was preclinically evaluated for anti-inflammatory activity by *in vivo* carrageenan and egg white induced paw edema method and by *in vitro* heat induced haemolytic method and protein inhibition method.

MATERIALS AND METHODS

Collection

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The aerial parts were purchased from Dr.Madhava Chetty, Sri Venkateswara University, Tirupathi and was air dried until free from moisture. Then they were subjected to size reduction to get coarse powder of desired particle size. The powdered drug was subjected to extraction with petroleum ether and methanol in a Soxhlet extractor, temperature was maintained on an electric heating mantel with thermostat control. The extracts were then concentrated to 3/4th of their original mass using rotary vapour apparatus. The concentrated extract were then transferred to a china dish and evaporated on a thermostat controlled water bath till it formed a thick paste. The thick mass was vacuum dried in a dessicator till it is free from moisture.

Phytochemical test

Phytochemical tests on the extract was performed using standard procedures³.

HPTLC analysis

Chromatography was performed on silica gel F254 HPTLC pre-coated plates. Samples were applied on the plates as band of 7mm width using a Camag Linomat V sample applicator at the distance of 14mm from the edge of the plates. The mobile phase was constituted of ethyl acetate-acetic acid-formic acid-water 100:11:11:27 (V/V/V/V)^{4, 5}. After development, plates were dried and derivatised in NP-PEG reagent. The finger prints were evaluated at 366 nm in fluorescence mode with WinCats and VideoScan software. Extracts Peaks, Rf values, Peak height and Peak area were given in Table - . The HPTLC chromatogram was given as Fig – 1.

Animals

Normal healthy male wistar albino rats (180-240g) were housed under standard environmental conditions at temperature (25±2° C) and light and dark (12: 12 h). They were fed with standard pellet diet and water ad libitum. Toxicity and Pharmacological studies were carried out with approval of (Reg.No.GNIP (TKR)/CPCSEA /IAEC /2013/06) IAEC members for the title, method selected animal species and parameters to be evaluated.

Toxicity study

The acute toxicity study was performed to assess the acute toxic effects and to determine the minimum lethal dose of the plant extract as per the

guideline OECD 423 acute toxic class oral method. The methanolic extract of *Pupalia lappacea* was administered orally to different groups of overnight fasted mice at the dose 5, 50, 300 and 2000mg/kg of body weight. After the administration of the extracts, animals were observed continuously for 24hrs for any toxic manifestation. Further the animals were kept under investigation upto a period of one week.⁶

Pharmacological screening

Anti-inflammatory activity –

Carrageenan induced paw oedema

For carrageenan – induced paw edema model, healthy rats (100-190gm, n=6) of either sex were grouped into three groups containing six animals per group. The animals were fasted over night prior to the start of the experiment, and water ad libitum. First group control (negative control) received normal saline solution (10ml/kg) and second group received test drug *Pupalia lappacea* methanolic extract (200mg/kg) and third group received standard drug diclofenac sodium (4.5mg/kg) 1ml dissolved in normal saline orally with the help of an oral catheter respectively. After 1h, the rats were challenged with 0.2ml of 1% W/V solution of carrageenan into the sub plantar side of the right hand paw. The paw was marked with ink at the level of lateral malleous and immersed in mercury up to the mark. A plethysmometer was used for the measurement of rat paw edema volume⁷.

The paw volume was measured immediately after injection (0 h) and then every hour till 3 hour after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. The average paw swelling in the group of drug treated rats was compared with untreated control rats and the percent of inhibition of the edema formation was determined using formula:

Percentage inhibition = $100 \times (1 - V_t/V_c)$, Where, 'Vc' represents edema volume in control, 'Vt' represents edema volume in the group treated with test drug.

Fresh egg white induced paw edema

Swiss albino rats were divided into three groups. Each consists of 6 animals, First group served as negative control. The second group received diclofenac sodium (4.5mg/kg). The third group received methanolic extract of *Pupalia lappacea* (200mg/kg). Edema was induced by administration of 0.75ml of undiluted fresh egg white in the sub-

plantar region 3. The paw volume was measured at 0 min – 60 min after the injection of undiluted fresh egg white using plethysmograph⁸. Then percentage inhibition of edema is calculated by using formula:

Percentage inhibition of edema = [(mean of control – mean of test) / mean of control] X100

RESULTS

The percentage yield of the methanolic extract was 20% W/V of methanol. The phytochemical evaluation showed the presence of alkaloids, terpenoids, flavonoids, carbohydrates, tannins, phenolic compounds, saponins and phytosterols. Toxicity study was done upto 2000mg/kg and the animals were monitored for 24 hrs to 72 hours. The animals didn't show any toxic symptoms and the abnormal behaviour after extract administration. From this the extract was found to be safe even up to 2000mg/kg and the LD₅₀ was calculated as 200mg/kg as the therapeutic dose 1/10 LD₅₀=ED₅₀. *In vivo* anti-inflammatory activity was screened at the dose of 200mg/kg. The methanolic extract at the dose of 200 mg/kg, p.o exhibited significant anti-inflammatory effect in carrageenan induced paw edema model (p<0.01) Table - 2. It was a well known fact that carrageenan induced paw edema model was commonly employed as an experimental model for evaluating anti-inflammatory activity of natural products the extract inhibited the inflammatory mediators and prostaglandin synthesis there by exhibited anti-inflammatory action. Also, the extract inhibited the paw edema formation and few volumes in egg white induced few edema model. The inflammatory is physiological characteristic of vascular tissue damage which was inhibited by the extract significantly (p<0.01) in egg white induced paw inflammation Table – 3.

DISCUSSION

In spite of the tremendous development in the field of synthetic drug during recent era. They are found

to have some are other side effects where as plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy plants against inflammation so as to explants them as herbal anti-inflammatory agents.

It is well known that carrageenan induced paw oedema is characterised by biphasic events which involves of different inflammatory mediators. In the first phase, chemical mediators such as histamine and serotonin play a role, while in second phase (3 – 4 hour after carrageenan injection) kinin and prostaglandins are involved. The results revealed that administration of methanolic extract inhibited the oedema starting from the first hour and during all phases of inflammation which probably inhibition of different aspects in chemical mediators of inflammation. The anti-inflammatory effect may be due to their content of tannins, flavonoids, alkaloids, saponins and carbohydrates. Diclofenac sodium is a cyclo-oxygenase inhibitor and can be said to the inhibit the cyclooxygenase enzyme but lipooxygenase inhibitors also posses significant antiinflammatory activity against carrageenan induced paw edema. So inhibition of carrageenan induced paw oedema by the crude extract could also be due to its inhibitory activity on the lipooxygenase activity^{9,10}.

Exudation which is a consequence of vascular permeability at various times after injury. Chemically induced permeability can causes an immediate reaction and its inhibition suggests that the extract may effectively suppress the exudative phase of acute inflammation induced by undiluted fresh egg white. The results also shows the effect of extracted compound on a oedematous response to fresh egg white paw oedema provoking an inhibitory effect equal to that of standard. From the above studies, it was quite apparent that the methanolic extract of *Pupalia lappacea* possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicine.

Table - 1HPTLC estimation methanolic extract of *Pupalia lappacea*

Flavonoids	Rf value	Peak height	Peak area	Concentration µgm/ml
Gallic acid	0.06	38.672	343.048	1.24
Ferulic acid	0.08	42.469	1436.64	2.00
Chlorogenic acid	0.78	837.28	837.28	46.25
Rutin	0.92	946.229	946.229	7.02

Figure – 1 HPTLC Chromatogram of methanolic extract of *Pupalia lappacea*

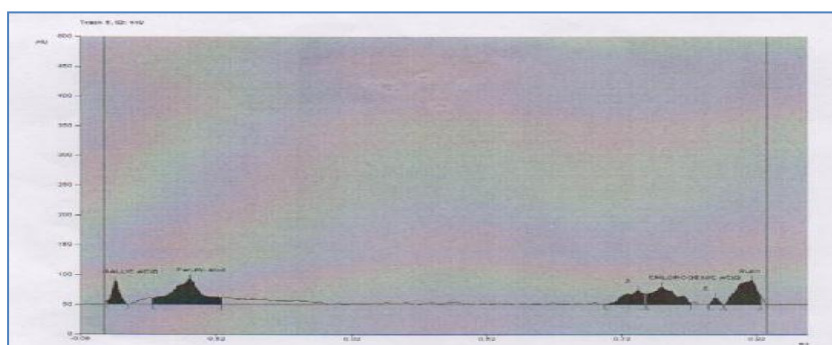


Table –2 Anti-inflammatory activity of *Pupalia lappacea* methanolic extract by carrageenan induced

Treatment	0 min	30 min	60 min	120 min	240 min
Control	0.48 ±0.01	0.31 ±0.01	0.36±0.02	0.36±0.02	0.38 ±0.01
PL extract (200mg/kg)	1.05±0.02	0.75±0.02	0.36±0.02*	0.150±0.02**	0.05±0.00**
Diclofenac sodium (4.5mg/kg)	0.068±0.02	0.51±0.01*	0.26±0.08**	0.02±0.02**	0.01±0.02**

paw oedema method

n=6, values are expressed in mean ±SEM, *P<0.05, **P<0.01, Assessed using One way ANOVA followed by Tukey’s multiple comparison test.

Figure –2 Anti-inflammatory activity of *Pupalia lappacea* methanolic extract by carrageenan induced paw oedema method

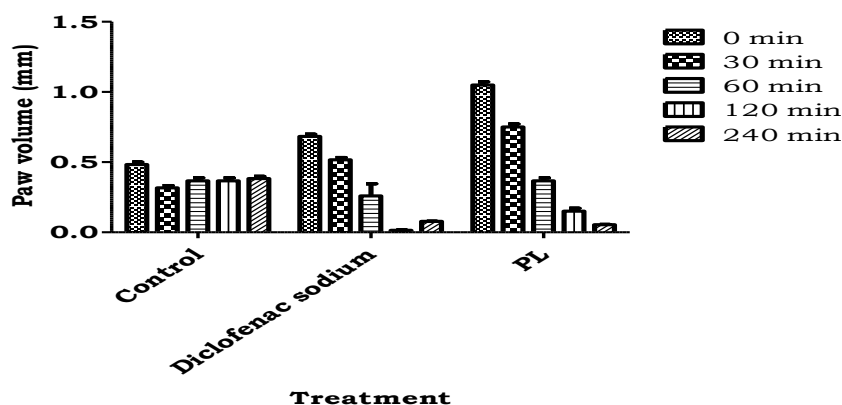
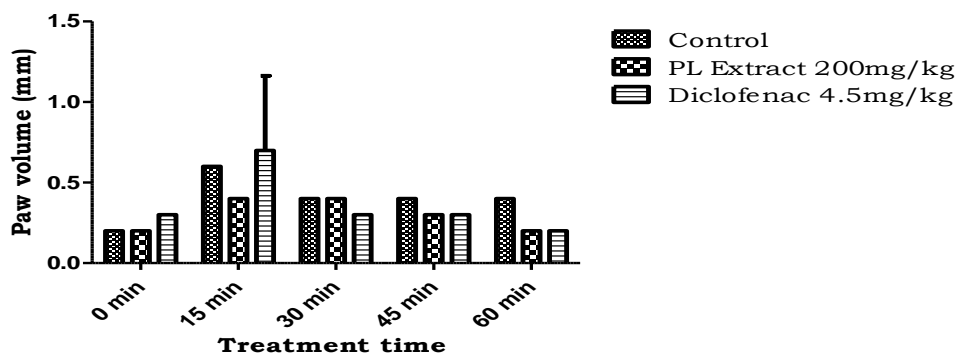


Table – 3 Anti-inflammatory activity of *Pupalia lappacea* methanolic extract by Egg white induced paw oedema method

Treatment	0 min	15 min	30 min	45 min	60 min
Control	0.20±0.00	0.60±0.00	0.40±0.00	0.40±0.00	0.40±0.00
PL extract (200mg/kg)	0.20±0.00	0.40±0.00	0.40±0.00	0.30±0.00*	0.20±0.00*
Diclofenac sodium (4.5mg/kg)	0.30±0.00	0.70±0.00	0.30±0.00**	0.30±0.00	0.10±0.00**

n=6, Values are expressed in Mean±SEM,*p<0.01, **P<0.001, Assessed using One way ANOVA followed by Tukey’s multiple comparison test.

Figure –3 Anti-inflammatory activity of *Pupalia lappacea* methanolic extract by Egg white induced paw oedema method



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