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Evaluation of antinociceptive and antipyretic effect of *Pupalia lappacea* Juss

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ABSTRACT

Pupalia lappacea Juss (Family: Amaranthaceae) is claimed to be useful in treatment of bone fracture, wounds, boils, cough, toothache, fever and malaria. The study was aimed to evaluate the 80% aqueous ethanolic extract of aerial parts of *P. lappacea* for antinociceptive and antipyretic activities to verify the traditional claim. The extract was orally administered at doses of 200, 400 and 600 mg/kg. The extract has significantly ($P<0.01$) reduced the nociception induced by acetic acid. The effect produced was in dose dependent manner. The antinociceptive effect was not reversed by pretreatment with naloxone in acetic acid induced writhing test. In hot plate method, the extract has significantly increased the latency time of jump. The naloxone has partially antagonised the antinociception of extract in hot plate test indicating *P. lappacea* has morphinomimetic properties. In the study of the CNS-depressant effects, the extract was found to produce significant reduction in head pokes and locomotion in mice by using hole board and locomotor activity test respectively. The extract has significantly reduced the rectal temperature in yeast induced pyrexia in rats at 600 mg/kg. The activity produced by extract was in dose dependent manner. Phytochemical investigation of ethanolic extract of *P. lappacea* revealed the presence of steroids and/or triterpenoids, flavonoids and phenolic compounds which may be responsible for antinociceptive and antipyretic activity of *P. lappacea*.

***Key Words*:** Ethanolic extract, naloxone, morphine, diazepam, actophotometer, brewer's yeast.

INTRODUCTION

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 India (Anonymous, 1950). The leaf paste of *P. lappacea* with edible oil (Sesamum or Carthamus) is an effective and inexpensive treatment of bone fracture for human beings as well as cattle (Rao and Reddy, 1999). Stem is used as tooth brush, for treating toothache (Reddy *et al*., 2009). 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 malaria (Ndjonka *et al*., 2010). Phytochemical analysis of foliage afforded 8 compounds, namely; 1- docosanol, stearic acid, stigamasterol, β-sitosterol, saropeptate (N-benzoyl-L-Phyenylalaninol acetate), β-sitosterol-3-0-D–glucopyranoside, Stigmasterol-3-0-β-D-glucopyra-noside and 20 – hydroxylecdysone (Felix and Domingo, 2008).

To the best of our knowledge no pharmacological work was carried out on aerial parts of *P. lappacea.* An attempt was made to verify the traditional claim and evaluate the antinociceptive and antipyretic properties of aerial parts of *P. lappacea* by preparing 80% ethyl alcohol extract.

MATERIALS AND METHODS

Plant material

The whole plant of *Pupalia lappacea* was collected from the Osmania University campus, Hyderabad, Andhra Pradesh, India and identification was done by Dr. G. Bhadrianarayana, Head and Taxonomist, Department of Botany, Osmania University, Hyderabad, Andhra Pradesh, India.

Extraction

The dried powder of *P. lappacea* aerial parts (1000 g) was extracted with 80% aqueous ethyl alcohol (5lt)

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Table 1: Effect of ethanolic extract of *P. lappacea* on acetic acid induced abdominal writhing in mice.

Group	Treatment (mg/kg)	Writhings	% Inhibition
I	Control	122.0 ± 3.54	-
II	Ethanolic extract 200	106.7 ± 3.10	13.11
III	Ethanolic extract 400	82.50 ± 6.47[b]	32.4
IV	Ethanolic extract 600	43.00 ± 4.28 [b]	64.75
V	Aspirin 100	30.50 ± 2.74[b]	75.0
VI	Ethanolic extract 400 + Naloxone 5	86.33 ± 2.39[b]	29.5
VII	Aspirin 100 + Naloxone 5	38.17 ± 8.92[b]	68.71

Values are Mean ± SEM; n=6; [a]*P*<0.05, [b]*P*<0.01 and [c]*P*<0.001, when compared with control

at room temperature by cold maceration for 7 days. The solvent was removed under reduced pressure by rotary flash vacuum evaporator, yielding 18.5 g of extract. Preliminary phytochemical analysis was performed for the presence of alkaloids, terpenoids, steroids and their glycosides, phenols, coumarins and flavanoids using standard procedures (Kokate, 1994; Harborne, 2007).

Chemicals

All the chemicals used were of analytical grade. The standard Aspirin (USV Ltd, Mumbai), Morphine (BDH Industries Ltd, Mumbai), Diazepam (Piramal Healthcare Ltd, Mumbai), Naloxone (Samarth Pharma Ltd, Mumbai), Brewer's yeast (Skybird, Hyderabad) and Paracetamol (Cipla Ltd., Mumbai) were procured.

Test animals

Male Swiss Albino mice, 8-10 weeks old (25-30g) were used to assess the antinociceptive activity of *P. lappacea* extract. Male Wister rats (150-180 g) were used for antipyretic activity of the plant extract. Animals were procured from Mahavir enterprises (Hyderabad, Andhra Pradesh, India). The animals were housed in individual polypropylene cages under standard laboratory conditions of light, temperature (22 ± 1°C) and relative humidity for at least one week before the beginning of experiment and to adjust to the new environment. The experimentation was carried out according to the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee of G. Pulla Reddy College of Pharmacy, Hyderabad, India, approved all the procedures for investigation (IAEC Reference number: GPRCP/IAEC-2/-PCG-01).

Acute toxicity studies

To determine the acute toxicity, a single oral administration of the ethanolic extract of *P. lappacea* in different doses (500, 1000 and 2000 mg/kg) were administered to different groups of mice (Ghosh, 1984). Control group received the vehicle (CMC). The animals were observed continuously for 72 h following drug administration for death and abnormality in behavioural changes.

Pharmacological screening

Antinociceptive activity

Acetic acid- induced Writhing test

The acetic acid induced writhing test was carried out to assess antinociceptive activity in pre-screened mice (Siegmund *et al*., 1957; Koster *et al*., 1959). The pre-screened fasted animals were divided into seven groups of six animals each. Group I control received 0.5% w/v carboxyl methyl cellulose (CMC) suspension orally. Group II-IV animals received ethanolic extract 200, 400 and 600 mg/kg orally as a fine CMC suspension respectively. Group V served as positive control and received aspirin (100 mg/kg, *p.o*). To study the involvement of opioid receptors, a separate group of mice were pre-treated with non-selective opioid receptor antagonist, naloxone (5 mg/kg, *i.p*) which was administered 15 mins before oral administration of the extract (400 mg/kg) and acetyl salicylic acid (100 mg/kg) to group VI & VII animals respectively. After 30 min of extract/drug administration, all the animals were given an intraperitoneal injection of 0.6% acetic acid (volume of injection 0.1 ml/10 g) and the number of writhes produced in these animals was recorded for 30 min.

Hot-plate method

The test was carried out using Eddy's hot plate (Dolphin, Mumbai) consists of electrically heated surface and was maintained at 55±0.2°C (Eddy and Leimbach, 1953; Hosseinzabeh *et al*., 2002). Pre-screened fasted animals were divided into seven groups of ten mice each. Group I control animals received 0.5% w/v aqueous suspension of CMC. Groups II-IV animals were treated with ethanolic extract at a dose of 200, 400 and 600 mg/kg respectively as a fine suspension of CMC orally. Group V positive control received morphine sulphate (5

Table 2: Effect of ethanolic extract of *P. lappacea* on hot plate test.

Group	Treatment (mg/kg, *p.o*)	Latency time (seconds)						
		Pre-treatment	After treatment					
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
I	Control	6.31±0.54	5.42±0.82	4.69±0.92	7.77±0.46	7.22±0.65	3.64±0.57	7.61±0.48
II	Ethanolic ext- 200	3.63±0.56	3.23±0.48	7.28±0.86[a]	7.47±0.77	5.49±0.50	5.13±0.33	9.04±0.46
III	Ethanolic ext- 400	6.03±0.83	5.66±0.84	7.53±0.48[a]	8.48±0.34	5.51±0.53	7.47±0.77[b]	8.04±0.29
IV	Ethanolic ext- 600	5.34±0.12	3.7±0.19	7.53±0.69[a]	9.67±0.45[a]	6.6±0.67	9.87±0.58[b]	9.45±1.70
V	Morphine 5	4.49±0.62	11.53±0.89[b]	13.50±0.62[b]	18.06±0.69[b]	19.10±0.35[b]	17.06±0.42[b]	15.47±0.56[b]
VI	Ethanolic ext- 400 + Naloxone 5	4.20±0.72	7.00±0.47	7.60±0.27[a]	7.26±0.17	9.19±1.125	3.23±0.56	6.26±0.60
VII	Morphine 5 + Naloxone 5	4.30±0.40	4.85±0.40	6.96±0.42	7.14±0.18	8.93±0.41	4.13±0.55	3.91±0.62[b]

Values are Mean ± SEM; n=10; [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$, when compared with control

mg/kg, *p.o*). Group VI was treated with ethanolic extract (400 mg/kg, *p.o*) and naloxone (5 mg/kg, *i.p*). Group VII was treated with morphine (5 mg/kg, *p.o*) and naloxone (5 mg/kg, *i.p)*. Naloxone was injected intraperitoneally 15 min before administration of extract/standard. All the animals were pretreated with corresponding extracts/drugs 30 min before the experiment. The latency period was recorded before and after 30, 60, 90, 120, 150 and 180 min of extract and drug administration. The latency period of 20 sec was defined as complete analysis and measurement was terminated to avoid injury.

Depressant activity on CNS

Hole board method

Hole board method is used for the evaluation of behaviour in mice such as curiosity or exploration (Bossier and Simon, 1964). Poking the nose into a hole is a typical behaviour of the mouse that indicates a certain degree of curiosity. The time taken for first head dip was measured. Animals were divided into six groups of six animals each. The initial reading was obtained by placing each animal individually in the centre of the board of 5 min. Group I positive control received 0.5% w/v CMC suspension. Group II-IV received ethanolic extract of *P. lappacea* at a dose of 200, 400 & 600 mg/kg respectively as a fine suspension of CMC orally. Group V and VI was treated with diazepam (2 mg/kg, *p.o*) and morphine (10 mg/kg, *p.o*) respectively. 30 min after oral administration of test and standard substances each animal was placed carefully in the centre of the board and number of head pokes for 5 min were recorded.

Locomotor Activity

The locomotor activity was measured using Actophotometer (INCO Photoactometer, Ambala city) (Kulkarni, 1999; Yadav *et al*., 2008). The basal activity score was obtained by placing each animal individually in actophotometer for 10 min. After determining the basal activity score initially, the animals were divided into six groups of six animals each. Group I control received 0.5% w/v CMC suspension. Group II-IV received ethanolic extract of *P. lappacea* at a dose of 200, 400 & 600 mg/kg respectively as a fine suspension of CMC orally. Group V and VI was treated with diazepam (2 mg/kg, *p.o*) and morphine (10 mg/kg, *p.o*) respectively. 30 min after oral administration of test and standard substances, each animal was placed carefully in the centre of the actophotometer for recording the score for 10 mins.

Antipyretic activity

Brewer's yeast-induced pyrexia

The subcutaneous injection of 15% Brewer's yeast suspension in saline (10 ml/kg) was used to produces fever in rats (Loux *et al.*, 1972). By insertion of a digital telethermometer (Dolphin, Mumbai) probe to a depth of 2 cm into the rectum of the rats, initial rectal temperatures were recorded. Immediately after administration of yeast, food was withdrawn and 18 h post challenge, the rise in rectal temperature was recorded. Only animals which developed satisfactory pyrexia (1ºC or more increase in rectal temperature) were used. Fever induced animals were divided into five groups of six animals in each group. Group I served as pyrexia control received 0.5% w/v CMC suspension. Group II-IV animals were treated with ethanolic extract *P. lappacea* at an

Table 3: Effect of ethanolic extract of *P. lappacea* on hole board test in mice.

Group	Treatments (mg/kg, p.o)	Head pokes/dips (minutes)				
		1 min	2 min	3 min	4 min	5 min
I	Control	7.66 ± 0.84	9.00 ± 1.31	12.00 ± 1.21	10.83 ± 0.79	8.66 ± 0.42
II	Ethanolic extract 200	6.50 ± 0.99	5.16 ± 1.13^{b}	6.50 ± 0.99^{b}	7.16 ± 0.94^{b}	5.66 ± 1.68
III	Ethanolic extract 400	5.33 ± 0.42	5.33 ± 0.49^{a}	4.33 ± 0.98^{b}	5.50 ± 0.61^{b}	5.66 ± 1.25
IV	Ethanolic extract 600	2.33 ± 0.49^{b}	4.66 ± 0.55^{b}	3.16 ± 0.47^{b}	4.16 ± 0.70^{b}	2.83 ± 0.47^{b}
V	Morphine 10	2.00 ± 0.25^{b}	1.33 ± 0.33^{b}	1.66 ± 0.33^{b}	2.00 ± 0.36^{b}	1.33 ± 0.21^{b}
VI	Diazepam 2	2.33 ± 0.47^{b}	2.00 ± 0.25^{b}	2.33 ± 0.49^{b}	2.66 ± 0.42^{b}	2.16 ± 0.30^{b}

Values are Mean ± SEM; n=6; $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$, when compared with control

oral dose of 200, 400 and 600 mg/kg as a fine suspension of 0.5% w/v CMC respectively. Group V served as positive control and received paracetamol (100 mg/kg, *p.o*). Rectal temperatures of all the rats were recorded using telethermometer just prior to and at 1, 2, 3, 4 & 5h after administration of extract/drug.

Statistical analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparison. All data were expressed as mean ± S.E.M. The results obtained were compared with the control group; $P<0.05$, 0.01 & 0.001 were considered to be statistically significant.

RESULTS

The preliminary phytochemical screening of the ethanolic extract of aerial parts of *Pupalia lappacea* revealed the presence of steroids/triterpenoids, phenolic compounds, flavonoids and carbohydrates. In toxicity studies, ethanolic extracts of *P. lappacea* did not exhibit any mortality and abnormal behavioural changes up to the dose level of 2000 mg/kg b.w. in mice. Further the pharmacological studies were carried out at an oral dose of 200, 400 and 600 mg/kg.

Antinociceptive activity

The antinociceptive activity of *P. lappacea* extract was evaluated using acetic acid-induced writhing test and hot plate test. Acetic acid-induced writhing test, the peripheral analgesic method was used to investigate the analgesic activity. Table 1 illustrates the results of acetic acid-induced writhing effect of *P. lappacea* extract. The extract exhibited activity in dose dependent manner. The extract has significantly ($P<0.01$) reduced number of writhes at an oral dose of 600 mg/kg with percentage inhibition of 64.75 which is comparable with aspirin (100 mg/kg) standard with percentage inhibition of 75.0. The administration of naloxone along with *P. lappacea* (400 mg/kg) and aspirin has not altered the significant antinociceptive effects. These results indicate that the analgesic effect of extract was not antagonised by naloxone, as the extract is not mediated via the opioid receptors.

The effects of ethanolic extract of *P. lappacea* on hot plate test are shown in Table 2. Oral administration of ethanolic extract of *P. lappacea* at different dose levels resulted in significant prolongation of the latency time in the hot plate test. The ethanolic extract at a dose of 600 mg/kg has demonstrated superior antinociceptive activity compared to extract doses of 200 and 400 mg/kg. The ethanolic extract (600 mg/kg) has significantly increased the latency time and has produced maximum latency time at 90 min. On the other hand the standard morphine (5 mg/kg), significantly ($P<0.01$) increased the latency response of mice with maximum effects obtained at 120 min after treatment. Though the *P. lappacea* extract has produced significant antinociceptive effects, but effect of extract are not comparable with morphine during any course of time. The administration of opioid receptor antagonist naloxone has partially reversed the antinociceptive effects of ethanolic extract of *P. lappacea* (400 mg/kg). However, the analgesic effect produced by morphine (group VII) is significantly reversed and these effects were observed throughout the experiment.

Depressant activity on CNS

The results of hole board test on ethanolic extract of *P. lappacea* is illustrated in Table 3. Among all the three doses, ethanolic extract at dose of 600 mg/kg has significantly reduced the number of head pokes

Table 4: Effect of ethanolic extract of *P. lappacea* on locomotor activity.

Group	Treatments (mg/kg)	Locomotor activity (scores) in 10 mins		% Reduction in activity
		Before treatment	After treatment	
I	Control	821.0 ± 17.41	717.0 ± 18.97	-
II	Ethanolic extract 200	801.0 ± 27.20	638 ± 14.88	11.15
III	Ethanolic extract 400	849.2 ± 25.87	462.5 ± 37.20[b]	35.5
IV	Ethanolic extract 600	735.5 ± 26.83	448.8 ± 47.07[b]	37.4
V	Diazepam 2	718.7 ± 40.01	259.7 ± 17.45 [b]	63.8
VI	Morphine 10	631.2 ± 29.35[b]	291.5 ± 42.08[b]	59.2

Values are Mean ± SEM; n=6; [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$, when compared with control

or head dips from the starting of the experiment and the activity is comparable with diazepam (2 mg/kg) and morphine (10 mg/kg). The number of head pokes or head dips produced by ethanolic extract of *P. lappacea* (600 mg/kg) at 5th min was 2.83 ± 0.47 where as diazepam and morphine has produced 2.16 ± 0.30 and 1.33 ± 0.21 respectively.

Table 4 illustrate the results of locomotor activity. A significant decrease ($P<0.01$) in the locomotor activity was observed at different doses of ethanolic extract of *P. lappacea*. The percentage reduction in locomotor activity of extract at dose of 600 mg/kg was found to be 37.4. The standard drugs diazepam and morphine has showed percentage reduction in activity as 63.8 and 59.2 respectively.

Antipyretic activity

The results of antipyretic activity of *Pupalia lappacea* are illustrated in Table 5. The basal body temperature of rats was elevated by 1.5ºC with the subcutaneous administration of 15% Brewer's yeast. There was a progressive reduction in the rectal temperature of rats after oral administration of extract and standard drug paracetamol (100 mg/kg). The reduction in the rectal temperature after treatments was highly significant at 3h. The reduction in temperature caused by the extract was dose dependent. The animals receiving 600 mg/kg of ethanolic extract demonstrated maximum reduction in temperature at 5h.

DISCUSSION

The present results indicate that ethanolic extracts of aerial parts of *P. lappacea* have both central (hot plate test) and peripheral (writhing test) antinociceptive activity. In the peripheral analgesic screening, acetic acid induced writhing test was used to evaluate the antinociceptive effect of the extract. Acetic acid induced writhing test is mainly associated with release of prostanoids like PGE_2 and $PGF_{2\alpha}$ (Deraedt *et al*., 1980). The ethanolic extract of *P. lappacea* at 600 mg/kg has showed significant reduction in the number of writhes and the activity was dose dependent. In addition, pretreatment with non-selective opioid receptor antagonist naloxone has not antagonised the analgesic effect of the extract (400 mg/kg).

In the central analgesic screening, hot plate method was used to verify the antinociceptive effect of the ethanolic extract of *P. lappacea*. The extract at all doses showed increase in the latency period in a dose dependent manner. Pretreatment with the non-selective opioid receptor antagonist naloxone, significantly decreased the latency period of the morphine whereas it has partially antagonised the effect of ethanolic extract (400 mg/kg). These results suggest that analgesic effect of the extract of *P. lappacea* might involve the opioid receptors.

The results of antinociceptive study led us to perform further investigations of *P. lappacea* extract

Table 5: Effect of ethanolic extract of *P. lappacea* on Brewer's yeast-induced pyrexia.

Group	Treatment (mg/kg)	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
I	Control	38.57±0.042	38.60±0.603	38.77±0.105	38.47±0.187	38.27±0.152	38.33±0.210
II	Ethanolic ext- 200	38.80±0.063	38.53±0.021	38.30±0.036[b]	37.93±0.111[b]	37.70±0.063[b]	37.77±0.084[b]
III	Ethanolic ext- 400	38.63±0.021	38.30±0.036	38.17± 0.021[b]	37.77±0.055[b]	37.63±0.021[b]	37.53±0.021[b]
IV	Ethanolic ext- 600	38.67±0.147	38.23±0.117	37.80±0.0966[b]	37.73±0.021[b]	37.40±0.036[b]	37.33±0.021[b]
V	Paracetamol 100	38.70±0.073	37.67±0.055	37.47±0.021[b]	37.43±0.042[b]	37.37±0.021[b]	37.23±0.021[b]

Values are Mean ± SEM; n=6; [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$, when compared with control

on CNS. The hole board and locomotor tests were carried out on mice. In hole board method, the extract showed reduction in number of head pokes or head dips in dose dependent manner. In locomotor activity, a significant decrease (P<0.01) in activity was observed at a dose of 600 mg/kg of extract. Therefore, the extract appear to have morphinomimetic properties, which explain the depressant effects on CNS, although, the activity mechanism underlying these effects is unknown.

Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α β and TNF-α) which increase the synthesis of PGE_2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Saper and Breder, 1994). Antipyretic effect of ethanolic extract of *P. lappacea* at all dose levels has reduced the yeast elevated rectal temperature in a dose dependent manner and the effect was comparable to that of standard paracetamol (100 mg/kg). These results suggest that antipyretic effect might be due to inhibition of PGE_2 synthesis.

CONCLUSION

The phytochemical screening of the ethanolic extract of aerial parts of *Pupalia lappacea* revealed the presence of steroids and/or triterpenoids, flavonoids and phenolic compounds which may be responsible for antinociceptive and antipyretic activity (Nisar *et al*., 2008; Kumar *et al*., 2010; Ramachandran *et al*., 2011). Thus, this present study supports the claimed uses of this plant in folk medicine.

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