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Research Article

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF *EPIPRINUS MALLOTIFORMIS* LEAF METHANOLIC EXTRACT

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ABSTRACT

Objective: To evaluate the antinociceptive and anti-inflammatory properties of *Epiprinus mallotiformis* leaf methanolic extract.

Methods: *Epiprinus mallotiformis* Muell is traditionally well known medicinal plant, traditional medicinal practitioners use this plant to treat digestive problems, dysentery, external wounds, antimicrobial, laxative, vesicle calculi, ulcers, gonorrhea etc. In present investigation, the antinociceptive and anti-inflammatory effect of *Epiprinus mallotiformis* leaf methanolic extract was evaluated by dose dependant study with three different concentrations *viz.,* 100, 200 and 300 mg/kg using rats and mice. Antinociceptive activity was carried out by abdominal writhing, tail flick and hot plate methods. Where as, anti-inflammatory activity was carried out using carrageenan induced rat paw edema model.

Results: Acute toxicity studies revealed that LD_{50} for leaf methanolic extract of *E. mallotiformis* was 3000 mg/kg b.w. Antinociceptive activity by writhing method revealed that the methanolic extract at 300 mg/kg showed 73% inhibition of acetic acid induced writhing. Tail flick and hot plate method indicated that maximum possible analgesia (MPA) was observed in the animals administered with 300 mg/kg of methanolic extract. Anti-inflammatory studies revealed that *E. mallotiformis* exhibited maximum percentage-inhibition of paw volume ($P<0.01$) at 300 mg/kg of methanolic extract by 54.28 %, 56.94%, 58.11% and 61.84%, after +1 h and +4 h of carrageenan administration.

Conclusion: Results of the present investigation supported the traditional claims on *Epiprinus mallotiformis* as a potent medicinal plant. This also indicates that this plant can also be used to treat pain and inflammation conditions in human and well as animals.

Keywords: *Epiprinus mallotiformis*; Acute toxicity studies; Antinociceptive activity; Anti-inflammatory activity.

INTRODUCTION

Pain is one of the major responses of body physiological imbalance and thus affects the lifestyle of human beings. Every organ imparts pain in response to dysfunction due to potentially dangerous stimulus or injury as a defensive reaction and relieves pain by producing chemicals at the site of injury, this leads to pronounced side-effects on the physiology of the body [1]. Analgesia is the termed used to refer the reduction of pain. Many agents are available that act as analgesics, selectively arrests pain by acting either in the CNS or on peripheral pain mechanisms without significantly altering consciousness [2]. Inflammation is the response of injury in any tissue, which begins by any stimulus *viz.,* infection, physical or chemical insult, cellular damage etc [3]. This damage is reported to get initiated with the activation of transcription factors that control the expression of many inflammatory mediators including the eicosanoids, biological oxidants, cytokines, adhesion factors, digestive enzymes (proteases, hyaluronidase, collagenase and elastase). The first three mediators are therapeutic targets for the anti-inflammatory drugs. The inflammatory response changes with time and can be divided into different phases. The acute phase is characterized by the activation inflammatory genes by NF-κB along with some other transcription factors [4]. Acute inflammation is the initial response against the inflammatory stimuli, where the movement of plasma and leukocytes get elevated from blood to injury site. However, chronic inflammation is characterized by the type of cells present at the site of injury, that lead to simultaneous destruction and healing of the tissue through inflammatory process. Causes of inflammation include burns, frost bite, toxins, infection by pathogens, physical injury (blunt or penetrating), immune reactions due to hypersensitivity, ionizing radiation, foreign bodies (including splinters, dirt and debris), stress, trauma etc. [5].

Opioids or non- opioids are the important drugs that are currently used for the management of pain and non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are used for inflammatory conditions. However, these drugs are associated with side effects. Reports revealed that gastrointestinal ulceration with bleeding is observed as side effect of non-steroidal anti-inflammatory drugs (NSAIDs). Piroxicam is reported to increase the risk of bleeding in both acute and chronic therapy [6]. Opioids are one of the commonly used drugs for the management of acute postoperative pain [7].

It is not surprising that from conception to market most compounds face an uphill battle to become an approved drug. For approximately every 5,000 to 10,000 compounds that enter preclinical testing, only one is approved for marketing [8]. Drug research and development is comprehensive, expensive, time-consuming and full of risk. It is estimated that a drug from concept to market would take approximately 12 years and capitalizing costs to the point of marketing approval at a real discount rate of 11% yields a total pre-approval cost estimate of US$ 802 million [9].

Epiprinus mallotiformis Muell. belongs to the family Euphorbiaceae, it is distributed throughout Western Ghats, South Canara, Coorg, Niligiris, Kigga, evergreen and semi evergreen forest of Karnataka [10,11]. This plant is traditionally used in traditionally used to treat the diuretic, digestive problems, dysentery, external wounds, antimicrobial, laxative, remedy for vesicle calculi, ulcers, gonorrhea etc. The present study is focused on evaluation of antinociceptive and anti-inflammatory activity of *Epiprinus mallotiformis* Muell. leaf methanolic extracts.

MATERIALS AND METHODS

Plant material

The leaves sample of *Epiprinus mallotiformis* Muell. were collected from the Agumbe (13° 30′ N 75° 05′ E, 2154 Ft from mean see level), Shimoga district of Karnataka. The region comes under Malnad region, receives the maximum rain during the South West Monsoon. The samples were authenticated and herbarium was stored in the Department of Applied Botany Kuvempu University Shankaraghatta, Shimoga district of Karnataka.

Preparation of extract

Leaf material of *Epiprinus mallotiformis* was collected and shade dried for one month. Dried leaf material was porously powdered

using mechanical grinder. Porously powdered leaf material subjected for soxhlet extraction using methanol for about 72 hr. The methanolic extract was then vacuum dried using rotary flash evaporator. The dried methanolic extract was used for the evaluation of antinociceptive and anti-inflammatory activity.

Phytochemical screening

The preliminary phytochemical screening of the methanolic extract of *E. mallotiformis* was carried out in order to ascertain the presence of its phytoconstituents by utilizing standard conventional protocols [12].

Animals

Wister albino rats weighing 150-200 g and Swiss albino mice weighing 25-30 g of either sex were procured from central animal house, National College of Pharmacy, Shimoga, Karnataka, India. All the animals were kept in standard polypropylene cages and maintained under standard conditions: temperature (24±1°C), relative humidity (45-55 %) and 12:12 light: dark cycle. The animals were fed with standard rodent diet and water was given *ad libitum*. The animals were acclimatized to laboratory conditions for one week before the start of the each experiment.

Acute toxicity study

The staircase method was adopted for acute toxicity [13] of methanolic extract of *E. mallotiformis* on Wister albino rats and Swiss albino mice by fixed dose method of OECD Guideline no 425 given by committee for the purpose of control and supervision on experiments on Animals (CPCSEA) respectively [14]. 100-3000 mg/kg of methanolic extract of *E. mallotiformis* was administered by oral route to mice and rats. Animals were observed for mortality or any behavioural change for about 72 h. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC) of National College of Pharmacy, Shimoga (Ref: NCT/IAEC/CL/44/12/2012-13).

Abdominal writhing method

Antinociceptive activity of the crude methanol extract was carried out using adult Swiss albino mice of either sex weighing 20-25 g, five groups with 6 animals per group were selected for abdominal writhing method. Group I animals were treated with 0.6 % acetic acid (dose 10 mL/kg) intraperitoneally. After 5 min of injection of acetic acid, number of writhes was counted for 20 min. This reading was taken as control. Group II was administered with Diclofenac sodium (10 mg/kg) and used as standard drug for the comparison of antinociceptive activity. Group III, IV and V were administered orally with the water dissolved methanolic extract at the dose of 100, 200 and 300 mg/kg body weight, respectively [15]. After one hour incubation all the groups except group I animals were administered with acetic acid. After 5 min, each group mice were observed for the number of writhes for the duration of 20 min. The mean value for each group was calculated. A reduction in the writhing number compared to the control group was considered as evidence of antinocieption. The percentage inhibition of writhing was calculated as:

$$\% \text{ inhibition} = \left[\frac{C - T}{C}\right] x\ 100$$

Where C=mean number of writhes produced by the control group and T = mean number of writhes produced by the test groups.

Tail flick method

Swiss albino mice of either sex weighing between 20-25 g were divided into 5 groups of six mice in each group. Group I mice were treated with normal saline (10 mL/kg). Group II were administered a 10 mg/kg dose of standard drug (diclofenac sodium), III, IV and V were administered orally with the crude methanolic extract at the dose of 100, 200 and 300 mg/kg respectively. Antinociceptive effect of the test samples was determined by the tail-flick method described [16]. One to two centimetre of the tail of experimental mice was immersed in warm water kept constant at 50°C. The pain reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociceptive activity and was determined before and at 30, 60, 90, 120, 150 and 180 min after the administration of drugs [17].

Hot Plate Method

Five groups with six Swiss albino mice in each were selected for this method, which comprises both sex and weighs about 20-25 g each. Group I animals received normal saline served as control, animals of group II were administered a 10 mg/kg dose of standard drug (diclofenac sodium). While animals of Group III, IV and V were treated with 100, 200 and 300 mg/kg body weight of the crude methanolic extract of *E. mallotiformis* respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15s, was observed to avoid damage to the paw [18]. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples [19, 20].

Acute inflammatory model

Carrageenan induced paw edema in rats

Assessment of anti-inflammatory activity of *E. mallotiformis* was carried out using Wistar albino rats weighing 150-200 g, which were divided into five groups containing six animals in each group, and were used for carrageenan induced paw edema models by following the method reported by Winter [21]. All drugs were given orally to the respective groups as a suspension one hour before carrageenan injection (0.1 ml of 1% w/v suspension) [22], in the right hind paw of the rats under the plantar aponeurosis. It was injected +1h after the oral administration of the different concentrations of methanolic extract and standard drug diclofenac sodium. The inflammation was quantitated in terms of ml *i.e.,* displacement of mercury by edema using a digital plethysmometer immediately before and after carrageenan injection at +1, +2, +3, +4. The percentage inhibition of edema was calculated for each group with respect to its vehicle-treated control group.

$$\% \text{ inhibition of paw edema} = \left[1 - \frac{V_t}{V_c}\right] x\ 100$$

Where, V_c represent average increase in paw volume (average inflammation) of the control group of rats at a given time; and V_t was the average inflammation of the drug treated (i.e. Plant extracts or test drug diclofenac) rats at the same time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h following carrageenan administration. Accordingly paw edema at +1h,+2, +3, +4 was calculated.

Statistical analysis

The data of antinociceptive activity was expressed as mean ± SEM of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Turkey's t-test. The difference in values at $P<0.01$ was considered as statistically significant.

RESULTS

Phytochemical screening

Qualitative phytochemical analysis revealed the presence of flavonoids, saponins, triterpenoids, steroids and tannins in the leaf methanolic extract of *E. mallotiformis*. But alkaloids appeared to be absent.

Acute Toxicity studies

Acute toxicity studies revealed that LD_{50} for leaf methanolic extract of *E. mallotiformis* was 3000 mg/kg b.w. against both mice and rats. One tenth of this dose was considered to be safer dose for administration.

Writhing method

The number of writhes observed during 20 min period in control group was 84.93±0.31. The crude methanolic extract at the dose of

100, 200 and 300 mg/kg reduced the number of writhes to 38.84±0.16 (with 54.69% protection), 30.10±0.14 (with 64.56% protection) and 23.12±0.12 (with 73% protection), respectively. These values indicated that the responses were dose dependent. But the effect of extract at different concentrations showed slightly less potent than standard drug diclofenac sodium which showed 5.55±0.27 (with 93.47% protection) writhes. All the readings found to be significant (P<0.01) when compared to control.

Table 1: Effect of methanol leaf extract on acetic acid induced (writhing test).

S. No.	Group (n)-Treatment	Dose (mg/kg)	No of writhing (20 min)	Writhing inhibition (%)
1	Control	0.6% acetic acid	84.93±0.31	-
2	Diclofenac sodium	10	5.55±0.27**	93.47%
3	Methanol extract	100	38.84±0.16**	54.69%
4	Methanol extract	200	30.10±0.14**	64.56%
5	Methanol extract	300	23.12±0.12**	73%

Value are mean ± SE, n=6 in each group ** significant at p<0.01 and * significant at p<0.05 are compare to control.

Tail flick method

Throughout the 3 h observation, animals pre-treated with normal saline did not show significant effect on the latent period of tail-flick response. The antinociceptive effects of crude leaf extract in three different doses were evident within 0.5 hr following oral administration and the effect remained significant (P<0.01) throughout the 3 hr observation period. At 100 mg/kg dosage, the MPA was increased from (4.83±0.02) to (6.82±0.01). Likewise, at 200 mg/kg dosage, the MPA increased to (7.22±0.01). At 300 mg/kg dosage the MPA value calculated was significantly (P < 0.01) increased to (9.85±0.01). The effects of crude extract on nociceptive responses induced by noxious heat (50°C) are shown in Table 2.

Hot plate method

Throughout the 3 h observation, animals pre-treated with normal saline did not show significant effect on the latent period of licked their fore or hind paws. The antinociceptive effects of crude leaf methanolic extract in three different doses were evident within 0.5 h following oral administration and the effect remained significant (P<0.01) throughout the 3h observation period. At 100 mg/kg dosage, the MPA was increased from (3.52±0.01) to (6.82±0.01). Likewise, at 200 mg/kg dosage, the MPA increased to (6.22±0.01). At 300 mg/kg dosage the MPA value calculated was significantly (P < 0.01) increased to (8.83±0.01). The effects of crude extract on nociceptive responses induced by noxious heat (55°C) are shown in Table 3.

Table 2: Antinociceptive activity of methanol leaf extract on tail flick / tail immersion method

S. No.	Group (n)-Treatment	Dose (mg/ kg)	Basal reaction time (2nd) after					
			30 min	60 min	90 min	120 min	150min	180min
1	Control	10	3.03±0.01	2.85±0.01	3.06±0.01	3.22±0.01	3.11±0.02	3.44±0.02
2	Diclofenac sodium	10	8.24±0.01**	8.87±0.03**	10.63±0.01**	10.06±0.01**	9.54±0.03**	9.13±0.01**
3	Methanol extract	100	6.82±0.01**	6.02±0.56**	6.24±0.02**	5.85±0.02**	5.24±0.01**	4.83±0.02**
4	Methanol extract	200	7.22±0.01**	6.82±0.03**	6.05±0.02**	5.54±0.01**	5.02±0.02**	4.53±0.02**
5	Methanol extract	300	9.85±0.01**	9.51±0.02**	9.12±0.02**	8.26±0.03**	7.83±0.02**	7.53±0.01**

Value are mean ± SE, n=6 in each group, ** significant at p<0.01 and * significant at p<0.05, when compared to control group.

Table 3: Antinociceptive activity of methanol leaf extract on hot plate method

S. No.	Group (n)-Treatment	Dose (mg/ kg)	Basal reaction time (2nd) after					
			30 min	60 min	90 min	120 min	150min	180min
1	Control	NS	3.62±0.01	3.54±0.01	3.53±0.01	3.21±0.01	3.72±0.01	3.12±0.01
2	Diclofenac sodium	10	9.62±0.01**	9.84±0.01**	10.26±0.01**	10.12±0.01**	9.52±0.01**	9.22±0.01**
3	Methanol extract	100	5.82±0.01**	5.52±0.01**	5.26±0.01**	4.86±0.01**	4.02±0.01**	3.52±0.01**
4	Methanol extract	200	6.22±0.01**	5.82±0.01**	5.03±0.01**	4.52±0.01**	3.23±0.01**	3.42±0.01**
5	Methanol extract	300	8.83±0.01**	8.32±0.01**	8.12±0.01**	8.02±0.01**	7.91±0.01**	7.72±0.01**

Value are mean ± SE, n=6 in each group, ** significant at p<0.01 and * significant at p<0.05 when compared to control group.

Table 4: Effect of methanolic leaf extract on carrageenan induced rat paw edema

S. No.	Group (n) Treatment	Dose mg/kg	Mean paw volume (ml) ± SEM			
			60 min	120 min	180 min	240 min
1	Control		0.70±0.01	0.72±0.01	0.74±0.01	0.76±0.01
2	Standard	10	0.58±0.01**	0.53±0.01**	0.50±0.01**	0.40±0.01**
3	Methanolic extract	100	0.68±0.01**	0.67±0.01**	0.65±0.01**	0.60±0.01**
4	Methanolic extract	200	0.63±0.01**	0.60±0.01**	0.58±0.01**	0.55±0.01**
5	Methanolic extract	300	0.62±0.01**	0.59±0.01**	0.57±0.01**	0.53±0.01**

Value are mean ± SE, n=6 in each group ** significant at p<0.01 and * significant at p<0.05 are compare to control

Effect on carrageenan induced paw edema

Pre-treatment with *E. mallotiformis* resulted in dose-dependent reduction in carrageenan evoked hind paw edema and differed significantly (P<0.01) among the different groups of rats (Table 4). In carrageenan induced rat paw edema test, the two doses of plant extract *viz.,* 200 and 300 mg/kg showed statistically significant (P<0.01) inhibitory effect on "mean increase in paw volume" at all the time intervals as shown in Table 4. After +1 h and +4 h of carrageenan administration, maximum % inhibition of paw volume (P<0.01) by 54.28%, 56.94%, 58.11% and 61.84% was observed in animals administered with 300 mg/kg methanolic extract of *E.*

mallotiformis. However, % inhibition of paw volume of standard drug was 60.00%, 65.28%, 67.57% and 78.95% (P<0.01).

DISCUSSION

Medicinal plants formulations are widely used in several therapies including pain, inflammation and also nervous related disorders. Leaves of medicinal plants are commonly plant part as an part of prepartion of many folk and traditional herbal medicines. Flavonoids are known to primarily target the prostaglandin synthesis pathway involved in pain induction in any tissue or organ, indicating that flavonoid components of the plant extract might be responsible for antinociceptive and anti-inflammatory property of the methanolic extract of *E. mallotiformis*. In the present study, antinociceptive activity of leaf extract of *E. mallotiformis* was carried out using three different models namely tail flick method, acetic acid induced abdominal writhing and hot plate method using mice. The tail flick and hot plate method was selected to investigate central antinociceptive activity. In order to distinguish between the central and peripheral antinociceptive action, acetic acid induced abdominal writhing response in mice was conducted. The acetic acid induced writhing test is very sensitive and able to detect antinociceptive effects of extract at different dose levels that may appear in active in other methods like tail flick test [15, 23, 24]. Increased level of prostaglandins, particularly PGE-2 and PGF-2a [25] as well as lipoxygenase products [26, 27] have been found in the peritoneal fluid after intraperitoneal injection of acetic acid. The antinociceptive effect of the methanolic extract may therefore be due either to its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful messages. However, this model may not be able to indicate the mechanism of antinociceptive effect of the methanolic extract because other agents such as antihistamines [28] and myorelaxant [29] are able to reduce the pain induced by acetic acid. The centrally acting analgesics generally raise the pain threshold of mice towards heat [30]. The thermal induced nociceptive tests are more sensitive to opioid receptors and non-thermal tests are sensitive to κ-opioid receptors as they are G-protein-coupled receptors (GPCRs) [31-33].The narcotic analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal anti-inflammatory / analgesics agents (NSAIAs) inhibit only peripheral pain [34, 35,36,37]. The inhibition of pain could take place not only from the presence of opioids and/or opiodiomimetics but also from bio-active compounds and secondary metabolites that are available in the methanolic extract, this action may be due to the single phytochemical effect or due to the collaborative effect of several phytochemicals present in the leaf methanolic extract of *E. mallotiformis*. The probable mechanism of action of carrageenan-induced inflammation is bi-phasic, the first phase is attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in 2 to 3 hours [38]. The leaf methanolic extract of *E. mallotiformis* moderately inhibited the carrageenan-induced inflammation in the 3rd hour. Antinociceptive and anti-inflammatory effects have been observed in flavonoids as well as tannins [39]. Flavonoids such as quercetin are known to be effective in acute inflammation [40]. The results of this investigation revealed that the leaf methanolic extract of *E. mallotiformis* showed potential antinociceptive and anti-inflammatory effects, indicating that this study can be extrapolated further by isolating the potent phytochemical responsible for this action.

CONCLUSION

The findings of the present investigation suggests that the methanolic leaf extract of *Epiprinus mallotiformis* may contain bioactive constituents that possess antinociceptive and anti-inflammatory activities, this study also supports the ethnomedical claims on this plant in the management of pain and inflammatory conditions.

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