

Research Article



ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACTS OF *ALANGIUM SALVIFOLIUM* AND *PAVONIA ZEYLANICA*

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ABSTRACT

The ethanolic extracts of stem and leaves of *Alangium Salvifolium* (EEAS) and *Pavonia Zeylanica* (EEPZ) were evaluated for analgesic and anti-inflammatory activity at the doses of 400 and 800mg/kg body weight. The acetic acid induced writhing and hot plate methods in mice were used to assess analgesic activity. Carrageenan induced paw edema in rats, which is an acute model used to assess anti-inflammatory activity. The extracts have inhibited paw edema in dose related manner. A dose dependent analgesic action was obtained against hot plate and writhing test indicated the analgesic activity may be involved in the inhibition of pain. Thus the ethanolic extracts of *Alangium Salvifolium* and *Pavonia Zeylanica* possess significant analgesic and anti-inflammatory activities.

Keywords: *Alangium Salvifolium*, *Pavonia Zeylanica*, Analgesic, Anti-inflammatory, Writhing, Carrageenan.

INTRODUCTION

Pain is one of the most common complaints for which patients seek advice and help from health professionals. Pain is not simple or easily defined-in fact, it is not one phenomenon, but several. Pain today is most appropriately defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. The etiology of chronic pain is seldom all psychological or all physical. Commonly, there is little association between the extent of injury and the amount of pain complains¹. Analgesics are the drugs which relieve pain without causing loss of consciousness. Analgesics can be evaluated in various ways: a) Prevention or relief of artificially induced pain in experimental animals. b) relief of experimental pain in human volunteers induced by radiant heat, ischemia induced with sphygmomanometer cuff or intraperitoneal bradykinin and c) Relief of pathological or incisional pain, post-puerperal pain, post-operative pain and pain due to malignancy.

Inflammation is the reaction of the living tissues to injury; it comprises systemic response (involving nervous and hormonal adjustments, and proliferation of the lymphoreticular system); and local response (pain, redness, warmth and swelling). The three important aspects of inflammation that render themselves readily to measurement are erythema (local vasodilation), edema (increased capillary permeability) and formation of granulation tissue. Compounds claimed to possess anti-inflammatory activity can be evaluate either by their ability to reduce one or more of these phenomena in experimentally induced inflammation or by testing their anti-inflammatory activity in experimental arthritis produced in animals².

Many Indian medicinal plants are reported to be useful in analgesic and anti-inflammatory. However, search for new analgesic and anti-inflammatory drug continue. *Alangium Salvifolium* belongs to the family Alangiaceae. It is commonly known as sage leaved alangium, stone mango, hill sack tree and ancole fruit plant in English, nalla oodaga, oodaga chettu, aankolam and urgu in Telugu. It is a deciduous shrub or tree. It is commonly distributed in most parts of Chittoor district of Andhra Pradesh like Tirupati, Talakona, Chandragiri and Aragonda. The root bark is used for snake bite, cutaneous troubles, anthelmintic, astringent, purgative, diaphoretic and colic. Leaves are used in diabetes and the fruits are used as astringent, tonic and laxative, whereas the seeds are used in hemorrhage. *Pavonia Zeylanica* belongs to the family Malvaceae. It is commonly known as karubenda, china mutharapulagam, peramuthi and chittimulli in Telugu. It is very commonly distributed in farm fields, wastelands and rare in forest fringers, throughout the Chittoor district of Andhra Pradesh. Whole plant is used as febrifuge and anthelmintic³.

An extended literature review shows that an Anti-arthritis activity of bark extracts of *Alangium Salvifolium* Wang⁴ and Anti-fertility activity of the stem bark of *Alangium Salvifolium* Wang in wistar female rats⁵ has been reported. Larvicidal efficacy of medicinal plant extracts against *Anopheles Stephensi* and *Culex quinquefasciatus*⁶ for *Pavonia Zeylanica* has been reported. However the plant is not scientifically explored for its analgesic and anti-inflammatory activity. Hence an effort has been made to screen the plants for analgesic and anti-inflammatory activity.



MATERIALS AND METHODS

Collection of Plant Material

The proposed plants material of fresh stem and leaves of *Alangium Salvifolium* and *Pavonia Zeylanica* were collected from Tirupati, Chittoor district of Andhra Pradesh, India. The species of the proposed study was identified and authenticated by Dr.K.Madhava Chetty, Asst. Professor of Dept.of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Voucher specimens were deposited at Dept. of Pharmacognosy for further reference.

Extraction and phytochemical screening

The shade dried powder of the stem and leaves of plants was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with ethanol after defatting with hexane until the completion of extraction. The extracts evaporated to dryness and kept in a desiccators till experimentation.

The extract was subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, alkaloids, flavonoids, carbohydrates, tannins and proteins^{7,8}.

Animals

Male albino mice (20-30gm) were used for the evaluation of analgesic activity and Wistar albino rats (200-250gm) were used to evaluate anti-inflammatory activity. The animals were maintained on the suitable nutritional and environmental conditions throughout the experiment as per the rules and regulations of the Institutional animal ethics committee. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional animal ethical committee (1423/PO/a/11/CPCSEA).

Toxicity Study⁹

An acute toxicity study was performed to determine LD₅₀ using different doses of the extracts according to the method described by Ghosh et al.

Evaluation of Analgesic activity

Fasted normal mice were divided into six groups of six mice in each group.

Acetic acid induced abdominal writhing test^{10, 11}

Group I: Control received 2ml/kg of 1% NaCMC.

Group II: Test animals received EEAS 400mg/kg body weight in 1% NaCMC p.o.

Group III: Test animals received EEAS 800mg/kg body weight in 1% NaCMC p.o.

Group IV: Test animals received EEPZ 400mg/kg body weight in 1% NaCMC p.o.

Group V: Test animals received EEPZ 800mg/kg body weight in 1% NaCMC p.o.

Group VI: Standard group animals received Aspirin at an oral dose of 100mg/kg.

Animals were treated with above scheduled doses 60 min before acetic acid administration. The total number of writhing after intra peritoneal administration of 0.1ml/10gm of 0.6% solution of acetic acid was recorded for 30 min, starting 5 min after the injection. For the purpose of scoring, a writhe is indicated by stretching the abdomen with simultaneous of at least one hind limb. The percentage inhibition of writhing by an analgesic is calculated according to the following formula:

$$\% \text{ inhibition} = \frac{\text{Average writhes in control group} - \text{Average writhes in test group}}{\text{Average writhes in control group}} \times 100$$

Hot Plate method^{12, 13}

Thirty six mice were weighed and the basal reaction time by observing hind paw licking or jump response (whichever appears first) in animals when placed on hot plate maintained at constant temperature (55°C) was taken. The animals were divided into six groups of six rats each.

Group I: Control received 2ml/kg of 1% NaCMC.

Group II: Test animals received EEAS 400mg/kg body weight in 1% NaCMC p.o.

Group III: Test animals received EEAS 800mg/kg body weight in 1% NaCMC p.o.

Group IV: Test animals received EEPZ 400mg/kg body weight in 1% NaCMC p.o.

Group V: Test animals received EEPZ 800mg/kg body weight in 1% NaCMC p.o.

Group VI: Standard group animals received Aspirin at an oral dose of 100mg/kg.

After administration of the above scheduled drugs, the reaction time was measured in seconds at 0 min (before drug challenge), 15, 30, 60 and 120 minutes.

Evaluation of Anti-inflammatory activity

Anti-inflammatory activity was determined in albino rats of either sex. The rats were divided into six groups of six animals each.

Carrageenan induced rat paw edema method^{14, 15}

One hour after oral administration of the extracts and standard drug, edema was induced to all the groups by subcutaneous injection of 0.1ml of 1% solution of Carrageenan in 0.9% w/v saline on the plantar surface of the left hind paw of rats.

Group I: Control received 2ml/kg of 1% NaCMC.

Group II: Test animals received EEAS 400mg/kg body weight in 1% NaCMC p.o.

Group III: Test animals received EEAS 800mg/kg body weight in 1% NaCMC p.o.



Group IV: Test animals received EEPZ 400mg/kg body weight in 1% NaCMC p.o.

Group V: Test animals received EEPZ 800mg/kg body weight in 1% NaCMC p.o.

Group VI: Standard group animals received Indomethacin 10mg/kg p.o.

The paw is marked with ink at the level of lateral malleolus and immersed in the mercury column of a Plethysmometer for measuring the paw volume. The paw volume was measured immediately after the Carrageenan injection and then at 3, 6, 12 and 24 hour. The percentage inhibition of inflammation was calculated by using the following formula:

$$\text{Percentage inhibition of inflammation} = \frac{A-B}{A} \times 100$$

Where, A and B denotes mean increase in paw volume of control and drug treated animals respectively.

Statistical analysis

Data obtained from pharmacological experiments were expressed as mean±SEM. The data were statistically analyzed by one way ANOVA followed by Dunnett's test.

RESULTS AND DISCUSSION

The preliminary phytochemical studies indicated the presence of alkaloids, flavonoids, terpenoids, tannins and carbohydrates. In acute toxicity study, the EEAS and EEPZ did not produced lethality up to the dose level of 6400mg/kg.

A variety of chemical agents have been used for producing pain. The intra peritoneal administration of noxious chemical substances to mice produces peritoneal irritation, which elicits a writhing response. Many chemical agents have been reported to produce writhing but acetic acid and phenylbenzoquinone are the two most commonly used irritants. The results of analgesic effect of EEAS and EEPZ in acetic acid induced writhing in mice were shown in Table-1. The number of writhes were significantly lower than the control group and the maximum inhibition is seen at EEPZ 800mg i.e., 54.25%. Standard drug has produced as protective effect and exhibited 74.84% of inhibition.

Table 1: Effect of EEAS and EEPZ by Acetic acid induced writhing in mice

Groups	Number of Writhing	Percentage of Inhibition
I Control	51.00±3.11	--
II EEAS 400mg	40.67±2.47*	20.27%
III EEAS 800mg	27.50±2.69**	46.08%
IV EEPZ 400mg	39.50±2.08*	22.55%
V EEPZ 800mg	23.33±1.56**	54.25%
VI Standard	12.83±2.49**	74.84%

Values are expressed as Mean±SEM (n=6). * p<0.05, ** p<0.01. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. The number of writhings of group II to VI are compared with group I.

Table 2: Analgesic effect of EEAS & EEPZ in mice by hot plate method

Groups	Reaction time (Seconds)				
	0 min	15 min	30 min	60 min	120 min
I Control	6.50±0.76	6.66±0.71	7.00±0.78	7.33±0.49	7.50±0.56
II EEAS 400mg	7.16±0.60	7.33±0.66	7.83±0.60	9.50±0.43*	9.83±0.65*
III EEAS 800mg	6.83±0.70	7.50±0.43	8.33±0.56	9.83±0.60*	12.83±0.48**
IV EEPZ 400mg	6.33±0.49	6.83±0.48	7.50±0.43	9.66±0.42*	11.00±0.57**
V EEPZ 800mg	7.00±0.73	7.83±0.70	9.50±0.62*	11.50±0.76**	13.33±0.42**
VI Standard	6.67±0.49	8.66±0.49	10.17±0.47**	12.67±0.61**	14.00±0.44**

Each value represents the mean±SEM, n=6. * p<0.05, ** p<0.01, Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Groups II to VI are compared with group I.

Table 3: Antiinflammatory effect of EEAS and EEPZ on Carrageenan Induced Rat Paw Edema

Groups	Mean paw volume (ml)					Percentage inhibition at 24 hour
	0 hour	3 hour	6 hour	12 hour	24 hour	
I Control	0.80±0.09	1.43±0.14	1.46±0.12	1.36±0.12	1.26±0.12	
II EEAS 400mg	0.83±0.10	1.16±0.09	1.13±0.06	0.96±0.09*	0.86±0.10*	31.75%
III EEAS 800mg	0.80±0.07	1.03±0.08*	1.00±0.07**	0.80±0.10**	0.46±0.04**	63.50%
IV EEPZ 400mg	0.86±0.11	1.20±0.05	1.16±0.09	1.03±0.09	0.90±0.11*	28.57%
V EEPZ 800mg	0.77±0.06	1.13±0.11	1.10±0.08*	0.86±0.08**	0.50±0.11**	60.32%
VI Standard	0.81±0.08	1.00±0.10*	0.96±0.11**	0.70±0.12**	0.33±0.06**	73.81%

Values are expressed as Mean±SEM (n=6). * p<0.05, ** p<0.01. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. The mean paw volumes of group II to VI are compared with group I.



The result of the hot plate method (Table-2) revealed that the reaction time for mice was significantly increased in a dose dependent manner. The reaction time significantly increases at a dose of EEPZ 800mg/kg i.e., 13.33 min at 120 min. The reaction time shown by the standard drug is 14 min. A cut off period of 15 sec was observed to avoid damage to the paws.

Experimentally induced paw edema is one of the most commonly used methods employed for the evaluation of anti-inflammatory agents. Many phlogistic agents have been used for the induction of edema, among them Carrageenan been found to be the most suitable agent and provides a good predictive value for anti-inflammatory potential of a novel compound. The percentage inhibition of edema values of Carrageenan induced rat paw edema is given in table-3. The inhibition was higher at a dose of EEAS 800mg i.e., 63.50%. However the standard drug has exhibited the percentage inhibition of edema was 73.81%.

CONCLUSION

The results of the present study confirm that ethanolic extract of stem and leaves of *Alangium Salvifolium* and *Pavonia Zeylanica* has an analgesic and anti-inflammatory activities.

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