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PHARMACOLOGICAL SCREENING FOR ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF ERIOLAENA HOOKERIANA WT. & ARN. ROOTS

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ABSTRACT

Eriolaena hookeriana is widely distributed in Central and southern India and is available in all parts of Andhra Pradesh. In present study, the roots of *Eriolaena hookeriana* were extracted by successive solvent extraction using Methanol and Water as solvents and the extracts were screened for anti inflammatory and analgesic activities on laboratory animals. Anti inflammatory activity was measured using carrageenan induced paw edema model and analgesic activity was measured by Tail immersion method and Acetic acid-induced writhing methods. Diclofenac sodium was used as standard for both the activities. Analgesic and anti-inflammatory activities showed by both the extracts significantly at the 100 mg/kg and 250 mg/kg dose levels. AQEH extract showed significant activity when compared to that of ALEH. The root extracts of *E. hookeriana* possesses potent anti-inflammatory and moderate analgesic properties

KEY WORDS

Eriolaena hookeriana, Analgesic activity, Anti-inflammatory activity, *sterculiaceae*.

INTRODUCTION

Eriolaena hookeriana Wt & Arn, a small tree, commonly found in cleared slopes in full sun at 750-1000 m Central and South India [1]. All the plant parts are ethno botanically important. Fruits are eaten by birds, bears and monkeys. The mucilage from the bark is mixed with water and given as a cure for stomach aches, strong wood of this plant is used for agricultural implements like axes and handles. It is also having wound healing activity [2] and fresh leaves are given to cattle once in a time to increase fat in the milk [3]. *Eriolaena hookeriana Wt & Arn* plant is available in almost all the parts of Andhra Pradesh state. As it was considered as the plant available in Andhra Pradesh, there was no systematic work has been performed to reveal its therapeutic potency. Hence, in present study, an attempt was made to screen the analgesic and anti inflammatory activities of *Eriolaena hookeriana* root extracts in a systematic way using laboratory animals.

MATERIALS AND METHODS

(i) Collection of plant material:

Eriolaena hookeriana Wt & Arn plant was collected from Horsely Hills, near to Madanapally of Chittor Disrict, A.P., India and botanically identified and authenticated by Dr. K. Madhava Chetty, taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. A voucher specimen (KKK/2011/09) was deposited in Dept. of Pharmacognosy & Phytochemistry, Vaagdevi College of pharmacy, Hanamkonda, A.P, India for future reference. The root was separated carefully, washed thoroughly and shade dried. About 1 kg of root powder was obtained by mechanical grinding of the roots which was used for further studies.

(ii) *Preparation of extracts:* The extracts were prepared by continuous hot percolation method using soxhlet apparatus. Primarily, 250 gm of root powder was passed through sieve no. 40 and packed in soxhlet apparatus and extracted using methanol and water separately as solvents and the filtrate was concentrated in rotary evaporator. The extracts were calculated for their yield and stored in desiccators till further use. The extracts were designated as ALEH for methanolic and AQEH for aqueous extract respectively. And these two extracts were subjected to preliminary phytochemical screening [4-6].

(iii) Animals:

Male Albino Swiss mice, weighing 25-30 g and Male Wistar rats, weighing 150 – 200 g were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet and had free access to clean drinking water and libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee Vaagdevi College of pharmacy vide approval no CPCSEA/VCOP/2011/10/3/7.

(iv) Acute toxicity studies:

Swiss albino mice of either sex (20-25 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423. Animals were divided into three groups, three animals each. Group I received normal saline which serves as control and as there were no acute toxicity studies was performed earlier for this plant, a dose of 300 mg/kg and 2000 mg/kg was given orally to group II and III. Animals were observed for the 14 days period for any toxicity and gross behavioural changes. The

results of acute toxicity study were tabulated in Table1.

Table 1
Acute toxicity studies on E. hookeriana root extract

Groups (n=3)	Dose (mg/kg) b.w	Lethality
I	300	No
II	2000	No

(v) ***Analgesic activity***:

Analgesic activity of ALEH and AQEH was performed using two methods; they are Tail immersion method and Acetic acid-induced writhing.

a) Tail immersion method:

Male albino mice, weighing 18–25 g, were randomly divided into six groups, six animals each. Prior to the experiment, animals in all the groups were screened for sensitivity test by immersing the tail of mice gently in water maintained at 55^{o}C. The animals dislodging the tail from hot water with in 5 seconds were selected for study.

Oral administration of two extracts ALEH, AQEH of two doses 100 mg/kg, 250 mg/kg and standard Diclofenac sodium 10mg/kg as the control group in distilled water was carried. Treated animal's tail was gently immersed in hot water maintained at 55^{o}C with help of thermometer. The reaction time was recorded by using stop watch and after determination of each reaction time, the tail was carefully dried. Reaction time was noted for every 30 minutes up to 3hours [7, 8] and the results were depicted in table 2

.

Table 2
Analgesic activity of E. hookeriana by Tail immersion method

Group	Mean reaction time				
	30 min	60 min	90 min	120 min	180 min
Control	2.13 ±0.07	2.59±0.21	2.96±0.30	2.93±0.25	2.70 ± 0.54
Standard	2.93±0.07***	4.91±0.30***	6.43±0.45***	6.49±0.49***	7.58±0.44***
ALEH 100mg/kg	2.28±0.10*	2.99±0.22*	3.76±0.36**	3.52±0.38*	3.79±0.40**
ALEH 250mg/kg	2.34±0.06***	3.27±0.17***	4.77±0.36***	**4.88±0.19****	4.55±0.33***
AQEH 100mg/kg	2.30±0.05**	3.11±0.10**	3.78±0.25**	3.70±0.36**	3.75±0.52**
AQEH 250mg/kg	2.75±0.11***	3.77±0.25***	5.17±0.28***	**5.49±0.37****	**5.72±0.51****

All values were expressed as Mean ± S.D.,
****p<0.05, **p<0.01 and ***p<0.001 in response to control***.

b) ***Acetic acid-induced writhing***:

Male albino mice, weighing 18–25 g, were randomly divided into six groups, six animals each. In this method, acetic acid was administered intraperitonilly to the experimental animals to create pain sensation. The plant extracts, ALEH and AQEH in two different doses (100 and 250 mg/kg b.w) and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1ml/10 g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. The animals were placed on an observation table and observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution.

Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did

not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group Diclofenac sodium (10 mg/kg) was used as a reference substance positive control [9, 10] and the results were tabulated in table no. 3

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Table 3
Analgesic activity of E. hookeriana on acetic-acid induced writhes in mice

Group	No. of writhes	% of Inhibition
Control	37.33±3.66	
Standard	15.66±2.16***	58
ALEH 100mg/kg	31.16±3.65**	16.6
ALEH 250mg/kg	27.16±2.22***	27.3
AQEH 100mg/kg	25.15±1.16***	32.7
AQEH 250mg/kg	23.16±1.89***	38

All values were expressed as Mean ± S.D., *p<0.05, **p<0.01 and ***p<0.001.

c) Anti-inflammatory activity:

Adult male Wistar rats weighing 180-220g were divided into six groups, six animals in a each group. The animals were administered with control, standard or test extracts. After 1 hr of administration of the test and standard samples, 0.05 ml of 1% carrageenan suspension was injected into dorsal region of sub plantar surface of hind paw of rat subcutaneously with the help of 26 G needle. The initial paw volume of each rat was recorded before drug administration. The paw volumes were measured at the end of 0.5, 1, 2, 3 and 4 hrs using plethysmometer. The mean value of oedema at different hours was calculated [11, 12] and the results were tabulated in Table 4.

Percentage inhibition of oedema was calculated for each group with respect to its control group using the formula :

$$Percentage \quad reduction = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V_0 = volume of the paw of control at time't', V_t = volume of the paw of test at time't'

Table 4
Anti inflammatory activity of E. hookeriana by carrageenan induced paw oedema in rats

Group	Mean paw volume in ml				
	0 min	1hr	2 hr	3 hr	4 hr
Control	0.26±0.05	0.43± 0.05	0.58± 0.07	0.65± 0.05	0.68 ±0.04
Standard	0.15±0.05** (42.3)	0.20±0.06*** (53.4)	0.31±0.07*** (46.5)	0.36±0.05*** (44.6)	0.30±0.07*** (55.8)
ALEH 100mg/kg	0.23±0.05 (11.5)	0.35±0.05 (18.6)	0.43±0.05** (25.8)	0.50±0.08** (23)	0.48±0.07*** (29.4)
ALEH 250mg/kg	0.21±0.04 (19.2)	0.33± 0.05* (23.09)	0.41± 0.09** (29.3)	0.45±0.10*** (30.7)	0.43±0.05*** (36.7)
AQEH 100mg/kg	0.20±0.06 (23.07)	0.30±0.06** (34.4)	0.38±0.07*** (34.4)	0.41±0.07*** (36.9)	0.38±0.07*** (44.1)
AQEH 250mg/kg	0.16±0.05* (38.4)	0.25±0.05*** (41.8)	0.33±0.05*** (43.1)	0.38±0.07*** (39.7)	0.33±0.09*** (51.8)

All values were expressed as Mean ± S.D., *p<0.05, **p<0.s01 and ***p<0.001, values in parenthesis indicates the percentage inhibition of inflammation by respective group.

RESULTS AND DISCUSSION

(i) Acute toxicity study:

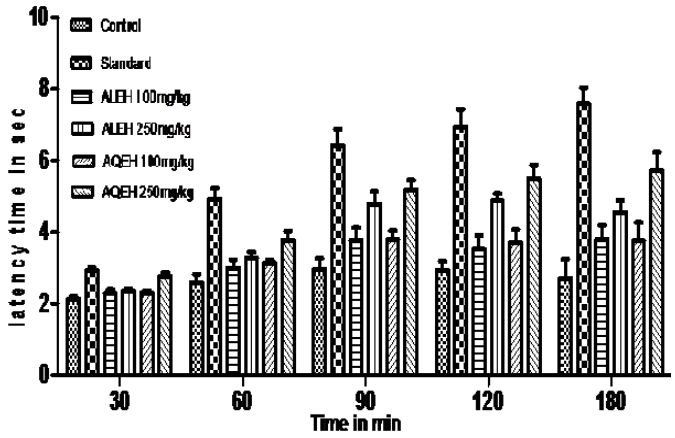
All experimental animals were observed for 14 days for any toxic signs. No mortality was observed even didn't showed any toxic signs during period of study. There is no information on extract to be tested, for animal welfare reasons the starting dose of 300 mg/kg was selected. Results were shown in the table 1 from which it was clearly understood that both AQEH and ALEH were found to be safe even at dose 2000 mg/kg (table 1). Further, there was no change in body weight and gross behavioural characters of the tested animals since LD_{50} > 2000 mg/kg, AQEH and ALEH were selected for the study at dose of 250 mg/kg and 100 mg/kg.

(ii) Analgesic activity:

Both AQEH and ALEH protected mice against both thermal and chemical induced noxious stimuli, which were evidenced from both the tail immersion (graph 1) and acetic acid-induced writhing tests (graph 2).

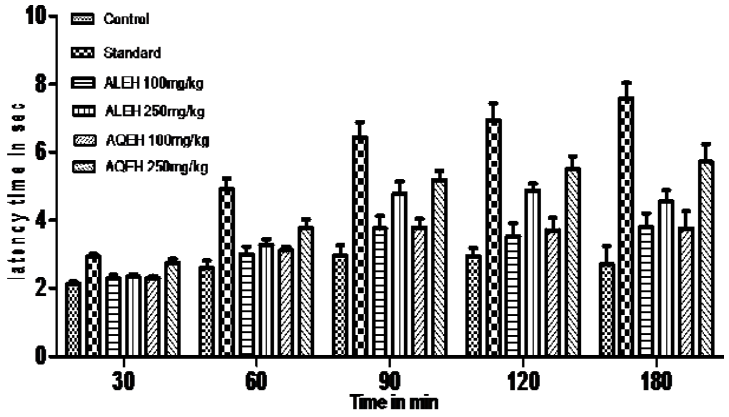
Graph 1

Analgesic activity of E. hookeriana by Tail immersion method



Variation in order of activity for AQEH in acetic acid-induced writhing and tail immersion tests indicated that the phytoconstituents (Flavonoids and phenolic compounds) present in AQEH may be responsible for central and peripheral analgesia. Acetic acid, which is used as an inducer for writhing syndromes, causes algesia by releasing of endogenous substances, which then excite the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins [13]. It is possible that *E. hookeriana* exerts an analgesic effect probably by inhibiting the synthesis of prostaglandins

Graph 2
Analgesic activity of *E. hookeriana* by writhing test



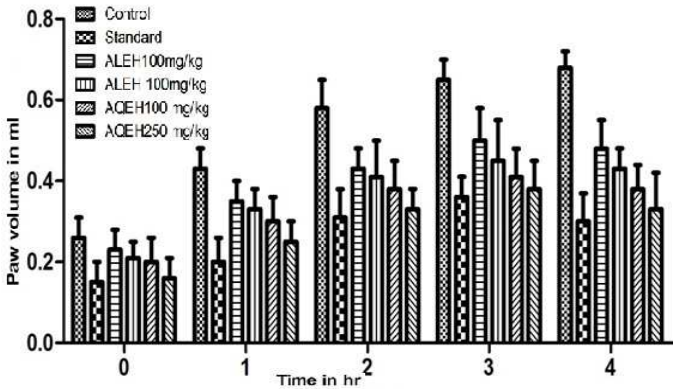
Both the extracts showed significant activity at higher doses i.e at 250mg/kg. Especially AQEH extract showed significant activity when compared to that of ALEH. It is also reported in some plants of *sterculiaceae* also posses analgesic activity. It is also reported that some plants of *sterculiaceae* showing significant reduction of COX-1 activity by the above results we can conclude that *E. hookeriana* Shows significant activity when compared to that of control.

(iii) Anti inflammatory activity:

In case anti-inflammatory the most widely used primary test to screen new anti-inflammatory agents' measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. This edema depends on the participation of kinins and polymorph nuclear leukocytes with their pro-inflammatory factors including prostaglandins. The development of edema in the paw of the rat after the injection of carrageenan has been a biphasic event. The initial phase, observed around 1 h, is attributed to the release of histamine and serotonin; the second, accelerating, phase of swelling is due to the release of prostaglandin-like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. In present study the significant activity was observed in the suppression of the first and second phases of carrageenin-induced inflammation may due to inhibition of the release of the early mediators such as histamine, serotonin and kinins. The action on the second phase may be due to an inhibition of cyclooxigenase, a prostaglandin derivative. Both the extracts ALEH AQEH showed significant reduction of inflammation in both phase in dose dependent manner (graph 3). Percentage inhibition of carrageenan induced inflammation by respective group was also calculated and shown in table 4.

Graph 3

Anti inflammatory activity of E. hookeriana by carrrageenan induced method



CONCLUSION

From the above study, it can be concluded that, *Eriolaena hookeriana Wt & Arn* root extracts were possessing analgesic and anti inflammatory activities in dose dependent manner. Presence of different phytoconstituents may be responsible for the said activities. From the above findings, it can be concluded that *Eriolaena hookeriana* possesses potent anti-inflammatory and moderate analgesic properties. Isolation and characterization of the constituents responsible for the said activities is needed.

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