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ASSESSMENT OF THE ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF *HOMALIUM ZEYLANICUM*

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ABSTRACT

***Ethno pharmacological relevance*:** *Homalium zeylanicum* (Flacourtaceae) has been traditionally used for treating several ailments including rheumatism, Anti-inflammatory, hepatoprotective and Anti-diabetic agent in Rayalaseema region of Andhrapradesh. However there is no scientific evidence to support its use in literature. To evaluate the analgesic and anti-inflammatory properties of the ethanolic extract of *Homalium zeylanicum* in animal models.

***Materials and methods*:** The analgesic and anti-inflammatory properties were assessed using acetic acid induced writhing test in mice, sub plantar carrageenan induced nociception, the tail-clip test and carrageenan induced oedema in rats. Three doses of the extract (100, 200, 400 mg/kg) were used for the assessment.

***Results*:** *Homalium zeylanicum* extract demonstrated strong dose-dependent analgesic and anti-inflammatory activities in all the models employed. All doses (100, 200, 400 mg/kg) produced a significant percentage inhibition The results showed that the extract (100, 200, 400 mg/kg) significantly (P<0.001) reduces the pain in male swiss albino mice by hot plate (↑23.3, ↑60.5, ↑88.4%) tail immersion (↑48.1, ↑70.4, ↑92.6 %) and acetic acid induced writhings (45.8↓, 56.5↓, 68.0↓%). Anti-inflammatory effect was tested on wistar rats using carrageenan (↓38.0, ↓45.1, ↓50.0%) as inducing agents. Tail immersion test showed a significant increase in the analgesic effect of the extract when they compared with the control. The inhibition of oedema in the carrageenan test was significant when compared to the control.

***Conclusion*:** The results indicated that *Homalium zeylanicum* showed better analgesic and anti-inflammatory properties suggesting that its traditional use in the treatment of pains and inflammatory diseases may be effective.

Keywords: *Homalium zeylanicum, A*nti-inflammatory, Analgesic, Medicinal plant, Carrageenan.

1. INTRODUCTION

Inflammation and pain is the condition results as responsive reaction of vascularised living tissue to local injury[1]. The inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischemia, antigen–antibody interactions and thermal or other physical injury. Each type of stimulus provokes a characteristic pattern of response that represents a relatively minor variation[2]. The response usually is accompanied by some familiar clinical signs such as erythema, edema, hyperalgesia and pain. A large number of NSAID's as potential analgesics and anti-inflammatory agents are used in the market.

However, on chronic usage majority of NSAIDs produces acute adverse reactions on GIT, liver and kidneys, hence necessitated Scientists across the world to search for safer herbal alternatives with analgesic and Anti-inflammatory effects[3]. Many herbal formulations were introduced into market with greater patient compliance. Homalium zeylanicum (Flacourtiaceae) is commonly known as "Liyan or Mukki". The bark and leaf of the plant is having many traditional uses in diabetes, rheumatism and wound healing activities[4]. Interesting note of this plant is that it is used since ancient in black remedies, the twigs of the plant is used in removal of evil spirits hence local name mantralamukhi[4]. In the present study was investigated for the presence of analgesic and anti-inflammatory activity.

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2. MATERIALS AND METHODS

2.1. Plant material

Ariel parts of plant of *Homalium zeylanicum was* collected during the months of January to September from Tirumala hills, Chittoor district, Andhra Pradesh, India. The plant was authenticated by Dr.Madhava chetty, Taxonomist, S.V. University, Tirupathi, India. The voucher specimen was numbered and deposited in our Research lab P.R.R.M. College of pharmacy. Collected

Leaves were thoroughly washed, shade-dried for 9days, powdered mechanically, sieved through (10/44) and stored in airtight containers[5].

2.2. Extraction

5000 grams of the powdered drug was accurately weighed and extracted using ethanol (95%) following soxhlation method. The extract was concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50°C, comminuted in a ball mill and preserved in air tight container, kept in desiccators prior to its studies[6].

2.3. Preliminary phytochemical investigation

A preliminary phytochemical investigation was carried out for that extract obtained from the *Homalium zeylanicum*[7] using analytical grade chemicals, solvents and reagents. The respective yields and the preliminary phytochemical investigation results were given in Table 1.

3. EXPERIMENTAL DESIGN

3.1. Analgesic activity:

Analgesic activity of ethanolic extract of *Homalium zeylanicum* (EEHZ) at doses 100,200 and 400 mg/kg, p.o was studied by three different methods. The results were given in Table 2.

3.1.2. Hot plate method

The study was carried out according to the method of Eddy[9]. Mice that showed nociceptive responses within 10 sec, when placed on a Eddy's hot plate (Techno, Lucknow, India) maintained at 55 ± 0.5 °C were selected for study. The mice so selected were then grouped into five (6 in each group) namely I, II, III, IV and V. The group I was treated with 2% v/v, aq. Tween 80, 10 ml / kg p.o which served as control and the II, III and IV groups were treated with the EEHZ 100, 200 and 400 mg/kg, p.o respectively and group V was treated with morphine 2 mg/kg s.c. After 30 minutes of the above treatment each mouse was placed gently on the hot plate maintained at 55 ± 0.5 °C and the reaction time was noted. The reaction time was taken as the time interval between the animals placed on the plate till the moment it began to lick its forepaws or jump. Four consecutive trials after a gap of 5 minutes were done and the mean value was calculated.

3.1.3. Tail Immersion Method

The study was performed according to the method of[10]. The animals were treated and grouped similarly as described in hot plate method. Each mouse was held in position in suitable restrainer with the tail extending out[11]. After 30 minutes of the above treatment each mouse 3-4 cm length of the tail was marked and immersed in the water bath thermostatically maintained at 51°C[12]. The withdrawal time (in seconds) of the tail from hot water. Four consecutive trials after a gap of 5 minutes were done and the mean values were calculated.

3.1.4. Acetic acid induced writhing test

The method described by Koster *et al*[13] was followed in this study. The animals were treated and grouped similarly as described in Tail Immersion Method. Thirty minutes after the above treatment each mouse was injected 10 ml / kg of 0.7 % aqueous acetic acid intraperitoneally. Each mouse was placed in a plastic transparent observation cage and number of abdominal constriction was cumulatively counted from 5 to 15 minutes. Results were expressed as percent inhibition of analgesia

4.1. Anti-inflammatory activity

Anti-inflammatory activity of ethanolic extract of *Homalium zeylanicum* (EEHZ) at doses 100, 200 and 400 mg/kg, p.o was studied by three different methods. The results were given in Table 3.

4.1.2. Carrageenan – induced rat paw edema

The study was conducted according to the method of Winter *et al* [14]. Male albino wistar rats weighing 100 – 250 g were housed in wire netted cages in a controlled room temperature 22 ± 1ºC, relative humidity 60 – 70 % and with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in the experiments were of analytical grade. Five groups of six animals each were used for the experiment. Group I of animals were administered with 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extact of *Homalium zeylanicum* (EEHZ) 100, 200 and 400 mg/kg, p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was treated with Indomethacin 20 mg/kg, p.o. One hour after oral administration, edema was induced by subplantar injection (left hind paw) of 0.1ml of 1% freshly prepared suspension of carragenan (Sigma Chemical Co., USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured at 3 hour after induction of inflammation using Plethysmometer. The percent inhibition of inflammation were calculated by using formula Percentage of inhibition inflammation= (A-B/A) X100 Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

4.1.3. Histamine induced rat paw edema

In this model edema was induced by subplantar injection (hind paw) of 0.05ml of 1% w/v, freshly prepared solution of histamine to all animals, which were grouped and treated similarly as followed in carrageenan induced rat paw edema method. The volume of the injected and the contra lateral paws were measured 3 h after induction of inflammation using Plethysmometer according to the method described by Winter *etal*[15].

4.1.4. Cotton wool induced granuloma test

Four groups of six animals each were used for the experiment. The rats were anaesthetized under ether anesthesia and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. Group I animals was given 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extract of (EEHZ) 100,200 and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was given with the standard drug Indomethacin (20 mg/kg, p.o). The treatment was

continued for seven consecutive days from the day of cotton pellets implantation[16]. The animals were anaesthetized again on 8th day and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37˚C for 24 h and dried at 60˚C to constant weight. The increment in the dry weight of the cotton pellets was taken as a measure of granuloma formation [17].

5. STATISTICAL ANALYSIS

All results were expressed as the mean ± SEM. The results were analyzed for statistical significance by one way ANOVA test using computerized Graph Pad In Stat version 3.05, Graph pad software Inc., San Diego, U.S.A.

Table 1: Preliminary phytochemical studies

Plant constituents	EEHZ
Alkaloids	absent
Amino acids	Present
Flavonoids	Present
Glycosides	Present
Triterpenoids	Present
Steroids	Present
Gums	Present
Tannins	Present
Saponins	Present

Table 2: Analgesic activity studies of EEHZ on male albino Wistar mice

Group	Treatment	Reaction time in seconds		Number of writhings
		Tail immersion	Hot plate	Acetic acid induced writhings
I	Control(2%Tween80)	2.7 ± 0.2	4.3±0.6	51.5 ± 3.4
II	Morphine 2mg/kg (Except in Writhing; Aspirin 20 mg/kg)	5.3 ± 0.4 (↑96.2%)	8.4 ± 0.34 (↑95.3%)	14.5 ± 2.9 (↓71.8%)
III	EEHZ 100 mg/kg	4.0 ± 0.1 (↑48.1)	5.3 ± 0.34 (↑23.3%)	27.9 ± 2.9 (↓45.8%)
IV	EEHZ 200 mg/kg	4.6 ±0.4* (↑70.4%)	6.9 ± 0.19 (↑60.5%)	22.4 ± 2.7** (↓56.5%)
V	EEHZ 400 mg/kg	5.2 ± 0.3 (↑92.6%)	8.1 ± 0.35 (↑88.4%)	16.5 ± 3.6*** (↓68.0%)

Each value represents the mean ± SEM. n = 6 number of animals in each group.
Values **P<0.01,*P<0.05. Compared to positive control.

Table 3: Anti-inflammatory activity studies of EEHZ on male albino Wistar rats

Group	Treatment	Carrageenan (Paw vol.)	Histamine (Paw vol.)	Wt.of Granuloma
I	2% Tween 80(10mg/kg)	53.9 ± 1.9	53.9 ± 1.9	81.5 ± 4.6
II	Indomethacin (20mg/kg)	25.4 ± 1.7 (↓51.7 %)	25.4 ± 1.7 (↓52.8 %)	32.6 ± 5.2 (↓60.0%)
III	EEHZ-100 mg/kg	31.7 ± 2.4 (↓38.0%)	30.7 ± 1.9* (↓42.3%)	52.3±4.3 (↓35.82%)
IV	EEHZ-200 mg/kg	28.7 ± 2.3** (↓45.1%)	27.8 ± 2.1 (↓48.1%)	39.4±3.9 (↓51.65%)
V	EEHZ-400 mg/kg	27.3 ± 1.7** (↓50.0%)	27.0 ± 1.9** (↓49.6%)	33.9±4.7** (↓58.40%)

p - Value was calculated by comparing with the control by students t-test,
*p<0.001, **p<0.05>0.02 N: 6

6. RESULTS AND DISCUSSION

Table 1 represents the results of phytochemical studies. The following phytochemical constituents are present in EEHZ. Those are Alkaloids, Aminoacids, Flavanoids, Glycosides, Triterpinoids, Gums, Tannins, Resins etc.,Table 2 represents the results of analgesic activity studies by three different methods. Several tests (acute and sub-acute) which differ with respect to stimulus quality, intensity and duration, were employed in evaluating the analgesic effect of the EEHZ to ascertain the analgesic properties of a substance using behavioural nociceptive tests [(18)]. In the hot plate method, the test drug EEHZ showed 23.3, 60.5% and 88.4% of inhibition at the doses of 100,200 and 400 mg/kg respectively, whereas the percent inhibition for morphine was 95.3. The effect of test drug EEHZ. In Tail immersion method, the test drug EEHZ showed 48.1% & 70.4% and 92.6% of inhibition at the doses of 100,200 and 400 mg/kg respectively, whereas the percent inhibition for morphine was 96.2. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The test drug EEHZ showed significant effect in various acute (phasic) pain models, namely, hot plate, Tail immersion, suggest that the effect on these pain models may act via centrally mediated pain control.

The abdominal writhing response induced by acetic acid is sensitive process to establish peripherally acting analgesics. Local peritoneal receptors are responsible for

abdominal writhing action. Intraperitoneal administration of acetic acid causes an increase in of PGE_2 and $PGF_2\alpha$ and produce analgesia by inducing capillary permeability and liberating endogenous substances like serotonin, histamine, prostaglandins, bradykinin, and substance P that sensitize pain nerve endings. It has been suggested that acetic acid stimulates the valinoid receptors and bradykinin B_2 receptors in the pathway comprising sensory afferent C-fibers [19]. Therefore, the observed activity may be due to interfering the synthesis or release of endogenous substances or desensitization of nerve fiber which carry pain sensation. In Acetic acid induced writhing assay the test drug EEHZ at the doses of 100, 200 and 400 mg/kg p.o. exhibited 45.8, 56.5 & 68.0% of inhibition respectively. The commercial drug Aspirin at the dose of 100 mg/kg p.o. exhibited 71.8% inhibition under similar experimental conditions. The results suggest that EEHZ also possess significant peripherally mediated analgesic effect. Hence it can be concluded that the EEHZ possesses analgesic properties, which are mediated via peripheral and central inhibitory mechanisms.

The results of anti-inflammatory studies for four different models were summarized in Table 3. Most of the investigators reported that inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents [20]. The sub planter injection of carrageenan (1% w/v) developed edema of high intensity and persisted for 3 h after injection in the control groups. The oral administration of EEHZ at the doses of 100, 200 and 400 mg/kg p.o. showed significant and dose dependent inhibition (38.0, 45.1 and 50% respectively). The commercial anti-inflammatory drug, Indomethacin showed 51.7% of inhibition at the dose of 20mg/kg p.o. The development of carrageenan induced oedema is bi-phasic. The first phase is attributed to the release of histamine, serotonin and kinins, whereas, the second phase is related to the release of prostaglandins[21]. The inhibitory action of the drug (EEHZ) on carrageenan induced paw edema in rats may be mediated through either any of the mediators alone or in combination. Hence EEHZ was further investigated against paw edema induced by individual agents like Histamine. The drug EEHZ also exhibited significant anti–inflammatory effect in the cotton pellet induced granuloma test (58.40% for 400mg/kg, p.o.). This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation [22-25]. It was observed that the gain in weight of the pellets was linear with the time. This linearity was continued for eight days and then leveled off.

Therefore, seven days was chosen as a convenient duration for the experiments followed by one day incubation at 37˚C. Results suggest that the EEHZ at doses of 100, 200 and 400 mg/kg p.o. significantly reduced the edema produced by several inducers and are comparable with many standard drugs suggested in each model. It has been reported by many researchers that flavanoids inhibit eicosanoids synthesis by inhibiting both cyclooxygenase and lipoygenase activities [20, 21], as well as hamper the non enzymatic peroxidation of polyunsaturated fatty acids required for the activation of these oxygenases [26]. Quercetin and other flavonoids inhibit leukotrienes synthesis and histamine, prostaglandins release, as well as acts as superoxide scavengers [27].

7. CONCLUSION

Ethanolic extract of *Homalium zeylanicum* was systematically evaluated for its analgesic and anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the EEHZ found to possess comparable efficacy with that of standard analgesics and anti-inflammatory drugs. , *Homalium zeylanicum*is a plant possessing therapeutic values certainly a nature's treasure for mankind for prevention and treatment of inflammation associated with pain.

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