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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF BORRERIA HISPIDA LINN. ROOT EXTRACTS IN EXPERIMENTAL MODELS

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ABSTRACT

The present study was designed to evaluate the anti-inflammatory (carrageenan induced rat paw edema test) and analgesic (acetic acid induced writhing in mice method) activities of methanol and ethyl acetate extracts of roots of *Borreria hispida* Linn. The maximum percentage inhibition produced by methanol extract at 200 and 400mg/kg body weight was 60.72 and 78.53% respectively at 3rd hr. Ethyl acetate extract showed maximum anti-inflammatory effect at 400mg/kg dose and the percentage inhibition of paw edema was found to be 55.07%. However both the extracts exhibited dose-dependent anti-inflammatory activity. In the acetic acid –induced writhing in rats, methanol and ethyl acetate extracts showed a significant reduction in pain in mice. The maximum percentage protection in writhing induced by acetic acid was 74.07% and 62.81% by methanol and ethyl acetate extracts respectively at 400mg/kg body weight. However, standard (Ibuprofen 50mg/kg body weight) showed highly significant inhibition at the same time.

Key words: *Borreria hispida*, Anti-inflammatory, Analgesic, Carrageenan, Edema, Writhing.

INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries (1). Most of the anti-inflammatory drugs now available are potential inhibitors of cyclo-oxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins.

Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required (2). Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and

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cardiovascular disease (3). Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions (4). However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes (5, 6, 7). Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates (8, 9). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research on plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (10).

Borreria hispida (Rubiaceae) is being used in various health care systems for the treatment of various disorders including life threatening diseases. The *Borreria hispida*, commonly known as "GATHIYU OR SHANKHLO" is perennial herb, easily available and grown as a hedge plant along home gardens throughout the India. Ethno botanically, *Borreria hispida* (Rubiaceae) has been used as therapeutic agent in the treatment of various pathological conditions. It is used as an antieczemic, anti bacterial and also used in cardiovascular disorder etc. (11, 12). It is used as fodder and also consumed as vegetable in times of scarcity. Herb is rich in calcium and phosphorous. Extract of leaves is given for hemorrhoids and gall stones, seeds are used as demulcent and used in diarrhea, dysentery (13). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Search for anti-inflammatory, Hepatoprotective, antidiabetic, analgesic and antioxidant molecules Available online on www.ijprd.com

from plant origin is the on-going project in our liberties. Thus with an interest the present investigation was carried out to evaluate the anti-inflammatory as well as antimicrobial potential of *Borreria hispida* in experimental models (albino rats).

MATERIALS AND METHODS

Plant material

The plant *Borreria hispida* Linn. was collected at Chintapalli, near Visakhapatnam, Andhra Pradesh, India in the month of June. It was authenticated by Dr.M.Venkaiah, Associate Professor, Department of Botany, Andhra University. The specimen (Voucher no. PR/GVSK-06/2012) was kept in the herbarium of the Pharmacognosy Division of Andhra University, Visakhapatnam.

Preparation of extract

The freshly collected roots of *Borreria hispida* L. were dried under shade and powdered. The powdered material was sieved to separate the coarsely powdered material and the fine powder. The coarsely powdered material was extracted with methanol continuously using soxhlet apparatus and the obtained extract was concentrated to a small volume under vacuum (50°C) and then dried in a vacuum desiccators. The fine powder was macerated with 2Lof methanol and the final liquid extract was concentrated and evaporated under reduced pressure until a soft mass is obtained. The dried methanol extract was suspended in water and fractioned with ethyl acetate and chloroform. These fractions were then subjected to qualitative phytochemical analysis.

Chemicals

All the chemicals used in this study are of analytical grade. Carrageenan and Dimethyl sulphoxide were purchased from Sai chemicals near Rama talkies, Visakhapatnam. Ibuprofen was used as the reference standard for evaluating anti-inflammatory activity where as Chloramphenicol and Nystatin served as the reference standards for antibacterial and antifungal activities respectively.

Animals

Healthy adult Wister albino rats of either sex weighing between 200-250gm were obtained from

M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. They were housed in standard polypropylene cages at a constant temperature $25\pm 2^{\circ}\text{C}$ in a 12 hour light and dark cycle provided with standard diet with water ad libitum throughout the experiments. They were fed with standard laboratory diet supplied by M/s. Rayans Biotechnologies Pvt. Ltd., Hyderabad, A.P, India. All experimental protocols were approved by the institutional animal ethical committee (approval no) Reg.no:516/01A/CPCSEA under the regulation of committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi.

EXPERIMENTAL DESIGN

The methanol extract of the plant were assessed for anti-inflammatory and analgesic activities by using Carrageenan induced paw edema in rats and acetic acid induced writhing in mice respectively.

Anti-inflammatory activity: Carrageenan induced paw edema in rats

Carrageenan-induced paw edema model was used for evaluating potential of methanol and ethyl acetate extracts of the plant on inflammation. In this method, acute inflammation was produced by the administration of 0.1 ml of 1% (w/v) of Carrageenan (Sigma Co.) in the sub plantar region of left hind paw of rat. The standard drug and extract (50mg/kg orally) were administered 30 min before the Carrageenan injection. In this study Ibuprofen was taken as standard anti-inflammatory agent (14). The experimental protocol comprises as follows:

- Group I - Control (normal saline 0.5ml / kg)
- Group II – Ibuprofen (50mg/kg)
- Groups III, IV, V- served as test groups treated with 100, 200 and 400mg/kg of MEBH respectively.
- Groups VI, VII, VIII - served as test groups treated with 100, 200 and 400mg/kg of EAEBH respectively.

The volume of the paw was measured immediately and also at the end of 2hours and 4 hours after the administration of Carrageenan using

plethysmometer (15). The percentage inhibition of inflammation was calculated by following formula: (16)

$$\% \text{Inhibition} = \frac{\text{Increase in paw volume (control)} - \text{Increase in paw volume (test)}}{\text{Increase in paw volume (control)}} \times 100$$

Analgesic Activity: Acetic acid-induced writhing in mice

Acetic acid-induced writhing model was used for evaluating the potential of methanol (MEBH) and ethyl acetate (EAEBH) extracts of the plant on pain. In this method, pain was produced by the administration of 1% v/v of acetic acid (1mL/100g body weight of rats). The rats were placed in separate boxes under observation immediately after acetic acid injection and number of writhing responses such as abdominal constrictions, trunk twisting and hind limb stretching's were counted over a period of 20 min. (17)The experimental protocol comprises as follows:

- Group I- served as control group and was treated with 2%w/v gum acacia solution.
- Group II- was treated with Diclofenac (25mg/kg, orally).
- Groups III, IV, V- served as test groups treated with 100, 200 and 400mg/kg of MEBH respectively.
- Groups VI, VII, VIII - served as test groups treated with 100, 200 and 400mg/kg of EAEBH respectively.

Statistical analysis

Statistical analysis was done using Graph pad prism version 5.0. All the values were expressed as mean \pm SEM. The data was analyzed using Dunett t-test. In all tests, the criterion for statistical significance was $p < 0.01$.

RESULTS

Effect of the *Borreria hispida* root extracts on Carrageenan-induced Paw Oedema (acute model):

The rats treated with oral administration of the methanol and ethyl acetate extracts of *Borreria hispida* roots significantly reduced acute paw oedema volume as compared to control. The percentage inhibition of paw oedema was

increased with time and gave maximum effect at 3rd and 4th hours, then declined. The maximum percentage inhibition produced by methanol extract at 200 and 400mg/kg body weight was 60.72 and 78.53% respectively at 3rd hr. Ethyl acetate extract showed maximum anti-inflammatory effect at 400mg/kg dose and the percentage inhibition of paw oedema was found to be 55.07%. However both the extracts exhibited dose-dependent anti-inflammatory activity. However, the standard showed highly significant inhibition at the same time. Results were shown in Tables 1, 2 and 3.

Effect of the *Borreria hispida* root extracts on acetic-acid induced writhing responses:

In the acetic acid –induced writhing in mice, methanol and ethyl acetate extracts showed a significant reduction in pain in mice. The maximum percentage protection in writhing induced by acetic acid was 74.07% and 62.81% by methanol and ethyl acetate extracts respectively at 400mg/kg body weight. However, standard showed highly significant inhibition at the same time. Results were expressed in Tables 4 & 5.

Table 1. Anti - inflammatory effect of MEBH roots on Carrageenan induced paw oedema in rats

Groups	Treatment	Volume of mercury displaced in ml at various time intervals in hours.						
		0min	30min	60min	120min	180min	240min	300min
I	Vehicle (control)	0.31± 0.25	0.39± 0.26	0.49± 0.057	0.64± 0.057	0.69± 0.057	0.67± 0.057	0.64± 0.058
II	Ibuprofen (50mg/kg)	0.19± 0.005***	0.26± 0.05***	0.31± 0.005***	0.36± 0.02***	0.27± 0.003***	0.26± 0.003***	0.21± 0.04***
III	MEBH 100mg/kg	0.32± 0.01**	0.338± 0.02**	0.40± 0.02**	0.43± 0.05**	0.38± 0.03**	0.37± 0.03**	0.35± 0.04**
IV	MEBH 200mg/kg	0.28± 0.01***	0.31± 0.02***	0.326± 0.02***	0.35± 0.05***	0.27± 0.03***	0.24± 0.03***	0.23± 0.04***
V	MEBH 400mg/kg	0.23± 0.01***	0.28± 0.01***	0.27± 0.02***	0.36± 0.05***	0.15± 0.03***	0.16± 0.03***	0.16± 0.04***

All the values are expressed as Mean ± SEM; n=6, ***P<0.001, **P.0.01 are significant when compared with control

Table 2. Anti - inflammatory effect of EAEBH roots on Carrageenan induced paw oedema in rats

Groups	Treatment	Volume of mercury displaced in ml at various time intervals in hours.						
		0min	30min	60min	120min	180min	240min	300min
I	Vehicle (control)	0.31± 0.25	0.39± 0.26	0.49± 0.06	0.64± 0.057	0.69± 0.07	0.67± 0.057	0.64± 0.058
II	Ibuprofen (50mg/kg)	0.19± 0.005***	0.26± 0.05***	0.31± 0.005***	0.36± 0.02***	0.27± 0.003***	0.26± 0.003***	0.21± 0.04***
VI	EAEBH 100mg/kg	0.31± 0.01	0.37± 0.03	0.45± 0.02	0.55± 0.05	0.47± 0.05	0.48± 0.03	0.45± 0.03
VII	EAEBH 200mg/kg	0.27± 0.08***	0.34± 0.02***	0.40± 0.03***	0.39± 0.05***	0.37± 0.02***	0.36± 0.06***	0.36± 0.04***
VIII	EAEBH 400mg/kg	0.20± 0.01***	0.31± 0.01***	0.36± 0.02***	0.34± 0.05***	0.31± 0.03***	0.30± 0.03***	0.29± 0.07***

All the values are expressed as Mean ± SEM; n=6, ***P<0.001, **P.0.01 are significant when compared with control

Table 3: Comparison of the effect of Methanol and Ethyl acetate extracts of Borreria hispida roots on %inhibition of paw oedema at 2nd, 3rd and 4th hours of treatment

Treatment	% Inhibition of Paw oedema at 3 rd and 4 th hours of treatment					
	MEBH roots			EAEBH roots		
	120min	180min	240min	120min	180min	240min
Vehicle	-	-		-	-	
Ibuprofen (50mg/kg)	43.7%	60.8%	61.19%	43.7%	60.8%	61.19%
Extract (100mg/kg)	32.8%	44.64%	44.77%	14.06%	31.88%	28.33%
Extract (200mg/kg)	45.3%	60.72%	64.17%	39.06%	46.37%	46.26%
Extract (400mg/kg)	56.37%	78.53%	76.12%	46.8%	55.07%	55.2%

Table 4: Analgesic effect of MEBH roots on acetic-acid induced writhing responses

Groups	Treatment	Number of writhing responses	% Protection/inhibition of writhings'
I	Vehicle (control)	66±0.22	-
II	Ibuprofen(50mg/kg)	7±0.09	89.3%
III	MEBH 100mg/kg	31.03±0.19	52.98%
IV	MEBH 200mg/kg	25.87±0.32	60.8%
V	MEBH 400mg/kg	17.11±0.02	74.07%

All the values are expressed as Mean ± SEM; n=6

Table 5: Analgesic effect of EAEBH roots on acetic-acid induced writhing responses

Groups	Treatment	Number of writhing responses	% Protection/inhibition of writhing
I	Vehicle (control)	66±0.22	-
II	Ibuprofen(50mg/kg)	7±0.09	89.3%
VI	EAEBH 100mg/kg	37.86±0.19	42.63%
VII	EAEBH 200mg/kg	32.07±0.32	51.4%
VIII	EAEBH 400mg/kg	24.54±0.02	62.81%

All the values are expressed as Mean ± SEM; n=6

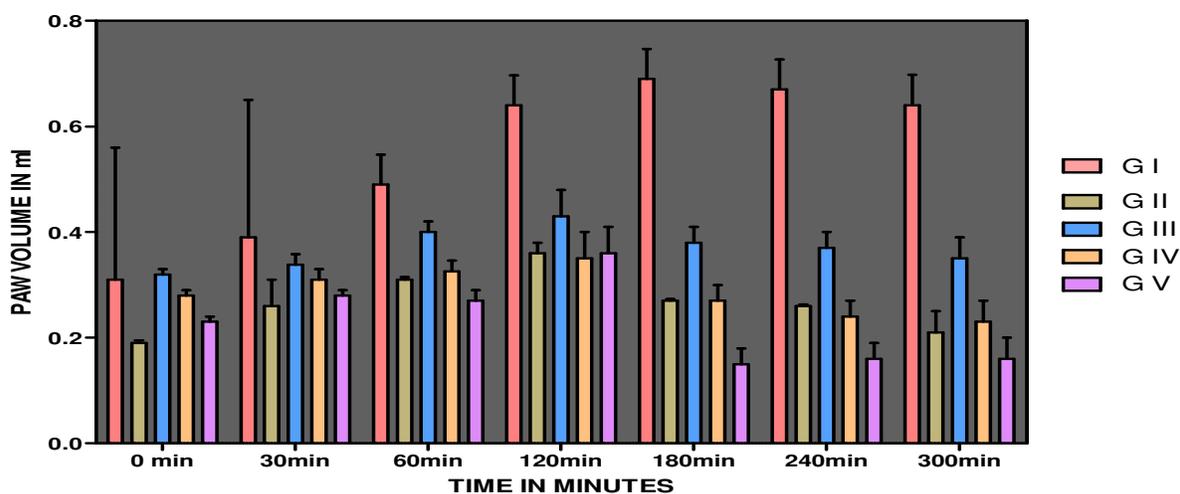
Fig 1: Histogram showing the effect of MEBH roots on Carrageenan induced paw oedema in rats

Fig 2: Histogram showing the effect of EAEBH roots on Carrageenan induced paw oedema in rats

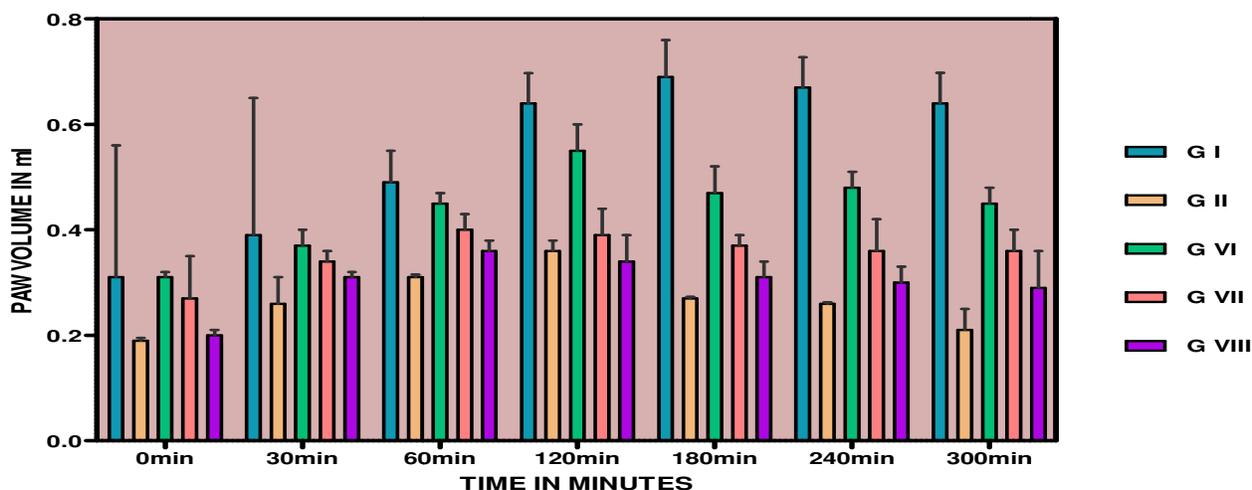


Fig 3: Comparison of the analgesic effect of Methanol and Ethyl acetate extracts of Borreria hispida roots

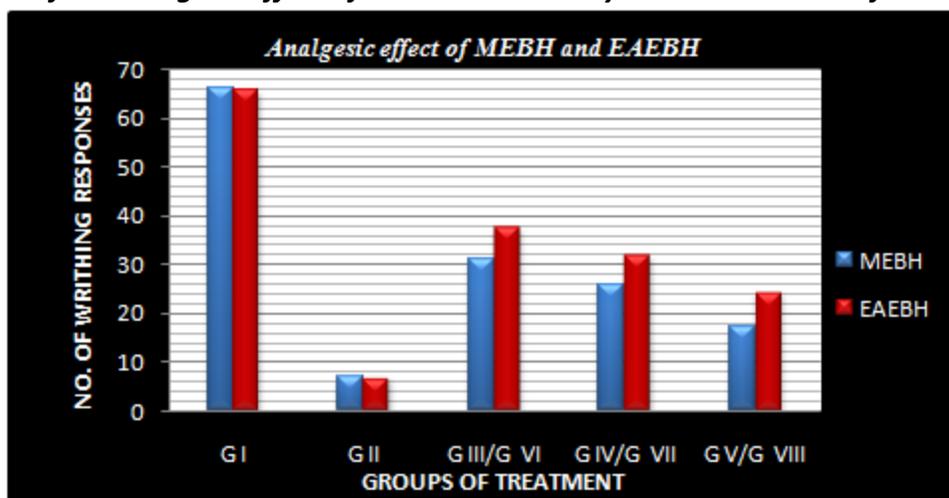


Image 1: Carrageenan induced paw edema in experimental rats; (A) shows Inflammation at 1st hr of Carrageenan injection; (B) shows Inflammation at 2nd hr of Carrageenan injection; (C) Shows Inflammation at 4th hr of Carrageenan injection.



Image 2: Reduction of Carrageenan induced paw inflammation at 2nd hr of treatment with 400mg/kg MEBH**Image 3: Acetic acid induced writhing responses in experimental rats.****DISCUSSION**

The extract significantly ($P < 0.001$) reduced the number of abdominal writhings induced by acetic acid in rats. Abdominal constrictions, trunk twisting and hind limb stretching's induced by acetic acid are used to screen the peripheral analgesic effect (18). The results support the hypothesis of participation in the inhibition of prostaglandin synthesis since the nociceptive mechanism of abdominal writhing induced by acetic acid involves the process or release of arachidonic acid metabolites via cyclo-oxygenase (COX) and prostaglandin biosynthesis. The effect of the extracts on acetic acid-induced abdominal writhing suggested that they might inhibit or modify responses to pain mediated by nociceptors peripherally (19). In conclusion, the results of the present study indicated that both the extracts

of *Borreria hispida* might contain constituents capable of relieving or modifying responses to pain caused by chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms.

The result of the present investigation revealed that the roots of *Borreria hispida* possesses a moderate anti-inflammatory effect that was evidenced by the significant reduction in paw oedema method. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) which is commonly used to induce acute inflammation and is believed to be biphasic (20). The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, and prostaglandin (21). It has been reported that the second phase of the oedema is sensitive to most clinically effective

anti-inflammatory drugs, which has frequently to access the anti-oedematous effect of natural products (22). Prostaglandins play a major role in the development of the second phase of the reaction, which is measured at around 3 hour's time (23). The carrageenan-induced paw oedema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effects of non-steroidal anti-inflammatory agents against which primarily inhibits the enzyme COX involved in prostaglandin synthesis. Based on the results, it can be inferred that the inhibitory effect of extracts on carrageenan-induced inflammation in rats may be due to the inhibition of enzyme cyclo-oxygenase. But lipo-oxygenase inhibitors also possess significant anti-inflammatory action against carrageenan-induced paw oedema.

CONCLUSION

Carrageenan-induced paw oedema is the standard experimental model of acute inflammation. The results of the present study suggest that the methanol and ethyl acetate extracts of roots of *Borreria hispida* Linn at doses of 200 and 400mg/kg body weight significantly suppressed carrageenan-induced paw oedema in rats and also demonstrated significant analgesic activity in acetic-acid induced writhing in mice. On the preliminary Phytochemical screening, methanol and ethyl acetate extracts of *Borreria hispida* found to contain Flavonoids and triterpenoids. Flavonoids are known to target prostaglandins which are involved in the acute inflammation and pain perception. The significant analgesic, anti-inflammatory and wound healing action may be attributed to the phytoconstituents present in it. The present study offered a scientific proof to the traditional use of *Borreria hispida*. However, further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities.

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