PRELIMINARY STUDIES ON ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *BALIOSPERMUM MONTANUM.*

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Summary

The methanolic extract of root of *Baliospermum montanum* was screened for anti-inflammatory and analgesic activity. Anti-inflammatory activity was studied in wister rats using carrageenan induced paw edema model and analgesic was studied in albino mice using Hot plate and writhing model at the doses of 100.200,300 mg/kg. The extract produced dose dependent and significant inhibition for paw edema, hot plate and writhings and was compared with standard drugs.

Keywords: Anti-inflammtory, Analgesic, Hot Plate, Writhing.

Introduction

Baliospermum montanum plant is locally known as Danti, and its roots have long been used as Ayurvedic remedy for jaundice[1]. It is distributed throughout India, Burma, and Malaya[2]. In India,it is distributed from Kashmir eastwards to Arunachal Pradesh, up to an elevation of 1,000 m and southwards into peninsular India, ascending to an altitude of 1,800 m in the hills of Kerala[3]. Almost all the parts of Danti are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as purgative, anthelmintic, diuretic, diaphoretic, rubefacient, febrifuge and tonic[4]. They are also reported to be useful in dropsy, constipation, jaundice, leprosy and skin diseases. The leaves are found to be good for asthma and bronchitis[5]. The tribals of Madhya Pradesh and Karimnagar district, Andhra Pradesh, India using leaves of danti for the treatment of asthma[6 ,7], and in headache[8].Decoction of stem is used to get relief from toothache[9, 10]. The roots of the plant are practiced as laxative[10, 11], in dropsy,jaundice, anasarca[6, 12], in rheumatism, anemia[13], and also in the treatment of jaundice, skin diseases, helminthic infections, leucoderma and piles.

Materials and Methods

Plant Material

The roots of Baliospermum montanum were collected from Warngal, Andhara Pradesh, India. It was authenticated by Prof. V. Raju, Dept of Botany, Kakatiya University, Warangal, India.

Preparation of extract

The roots were cleaned, shade dried and coarsely powdered. The coarse powder of roots was then exhaustively extracted by maceration process using methanol as solvent. After extraction, the methanolic extract was concentrated by air-dried and it is preserved in a vacuum desiccator. The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Drugs and chemicals

The drugs and chemicals used were carrageenan and acetic acid (SD fine chemicals Limited, Mumbai), gum acacia and diclofenac sodium (Dr. Reddy's Labs, Hyderabad), Pentazocine (Pure Pharma Ltd., Mumbai) and methanol (Merck, Mumbai).

Animals

Wister rats (150-200 g) and albino mice (25-30g) of either sex were used for assessing the biological activity studies. The animals were housed in polypropylene cages and maintained under standard husbandry conditions and had free access to food and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Analgesic activity

Hot- Plate Test:

The hot plate test was used to measure analgesic activity by the method described by Turner[14] was used. The mice were first treared with different doses of B. montanum (100,200 &300 mg/kg, p.o) after 1hr of extract administration. They were placed on a hot plate maintained at 55± 1.0^0 C. A cut of period of 15 sec was considered as maximal latency to avoid injury to the paws. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reation time. Pentazocine (5 mg/kg s.c.) was used as a reference drug.

Writhing Test:

Abdominal construction induced by intraperitoneal injection of acetic acid was carried out according to the procedures described previously[15]. The leaf extract of B. montanum (100,200 &300 mg/kg, p.o). Diclofenac sodium 20 mg/kg was used as reference standard.. The extract and reference drug were administered orally 30 min before the administration of 0.7% acetic acid in a volume of 10mg/kg i.p. Control animals received 2% of gum acacia under the same experimental condition. Immediately after injection of the acetic acid, each animal was isolated in an individual cage and the normal of construction was cumulatively counted for a period of 20 min, beginning 3 min after acetic acid injection. The number of writhing and stretching was recorded and the % was calculated using the following ratio:

% of protection= (Control mean-Treated mean)/Control mean*100

Anti-inflammatory activity

Carrageenan-induced paw edema method

Anti-inflammatory activity was evaluated by the carrageenean-induced paw oedema method [16]. Animals were divided into five groups of albino Wistar rats of either sex weighing 150–200 g, 6 animals each, were orally dosed with the vehicle as control(2% gum acacia), and the methanolic extract two dose levels (100, 200 & 300 mg/kg). Diclofenac sodium, 20 mg /kg, was administered as standard drug for comparison.

Carrageenan (0.1 ml of a 1% suspension in saline) was injected sub plantar region of the right hind paw of each rat. The vehicle, drug and extract were administered 30 min prior to the injection of Carrageenan.Paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately at 30 min, 1, 2 and 3 h after carrageenean injection.

A significant reduction in the paw volume compared to vehicle treated control animals was considered a inflammatory response.

% Inhibition= $[(V_T - V_0)$ control $-(V_T - V_0)$ treated groups$] / (V_T - V_0)$ control *100

V_0 = paw volume of the rat before administration of Carrageenan

V_T = paw volume of the rat after administration of Carrageenan at different time intervals

Statistical Analysis

All the results were expressed as Mean ± SEM. Data was analyzed using one-way ANOVA followed by Dunnett's t-test. P-values < 0.05 were considered as statistically significant.

Results and Discussion

Hot Plate Method:

Three doses of extract of *B. montanum* increased the reaction time in dose dependent manner to the thermal stimulus (table-1). The highest nociception of thermal stimulus

was exhibited at a higher dose (300 mg/kg) of methanolic extract B. montanum, which is comparable to that of Pentazocine. . The enhanced analgesic effect of methanolic extract of B. montanum in the hot plate test might be due to the inhibition of prostaglandin synthesis.

Acetic acid writhing test:

Analgesic activity of *B. montanum* was assessed by acetic acid induced writhing reflex and the results are shown in the table-2. The various doses of methanolic extract have shown significant inhibition in a dose dependent manner and which is compared with Standard drug.

Anti-Inflammatory activity:

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility[17]. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and mediated by brabykinin, leucotriens, polymorph nuclear cells [18]. The results of the carrageenan induced oedema were shown in (Table - 3). From results, it was observed that the methanolic extract of *B. montanum* in doses of 100,200 and 300 mg/kg, p.o produced dose-dependent inhibition of paw edema volume. For the control group, 30 min after the injection of the phlogistic agent, carrageenan produced a localized oedema in the rat paw. The swelling increased progressively at 4^{th} hr to a maximum volume of 1.77 ± 0.05 and remained obvious 24 hr after injection. The test extract and the standard drugs produced a significant inhibition of paw edema as compared to the control.

Conclusions

The data obtained in this study demonstrated that the methanolic extract of B. montanum have a significant analgesic and anti inflammatory activity. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

Table 1: Effect of methanolic extract from B. montanum on the hot plate test in mice

S. No	Group	Dose (mg/kg)	Reaction time after administration of control/ standard/ extract in sec			
			0 min	60 min	120 min	240 min
1.	Control		5.13 ± 0.51	5.51 ± 0.36	5.67 ± 0.24	5.33 ± 0.49
2.	Pentazocine	10	4.28 ± 0.28	12.12 ± 0.28[a]	14.16 ± 1.32[a]	12.81 ± 0.96[a]
3.	B. montanum	100	4.81 ± 0.24	9.29 ± 0.70[a]	12.17 ± 1.53[a]	11.55 ± 1.12[a]
4.	B. montanum	200	4.32 ± 0.36	11.64 ± 1.19[a]	13.38 ± 0.89[a]	12.06 ± 0.88[a]
5.	B. montanum	300	4.48 ± 0.31	12.68± 1.91[a]	13.89 ± 1.19[a]	13.10 ± 0.91[a]

Values are in Mean ± SEM; (n =6), a= $p < 0.001$ Vs Control.

Table 2: Effect of methanolic extract from B. montanum on acetic acid induced writhing test in mice

S. No	Group	Dose (mg/kg)	No. of writhes	% inhibition
1.	Control		74.32 ± 8.21	-----
2.	Diclofenac	10	30.69 ± 0.61	58.74[b]
3	. B. montanum	100	63.01 ± 6.12	15.29[a]
4	B. montanum	200	52.41 ± 4.61	29.40[a]
5.	B. montanum	300	43.42 ± 2.24	42.89[b]

Values are in mean ± SEM; (n =6), a= $p < 0.05$, b= $p < 0.001$ Vs Control.

Table 3: Effect of methanolic extract from B. montanum on the paw edema test in rats

S. No	Group	Dose (mg/kg)	Paw edema volume after			
			1 hr	2 hr	3 hr	4 hr
1.	Control		1.35 ± 0.12	1.41 ± 0.10	1.64 ± 0.21	1.77 ± 0.05
2.	Diclofenac Sodium	20	0.96 ± 0.03[b]	0.90 ± 0.03[b]	0.88 ± 0.02[b]	0.87 ± 0.01[b]
3	B. montanum	100	1.21 ± 0.02[a]	1.17 ± 0.02[b]	0.96 ± 0.02[b]	0.92 ± 0.01[b]
4	B. montanum	200	1.17 ± 0.01[a]	0.99 ± 0.01[b]	0.94 ± 0.02[b]	0.98 ± 0.02[b]
5.	B. montanum	300	0.99 ± 0.02[b]	0.96 ± 0.01[b]	0.90 ± 0.01[b]	0.88 ± 0.01[b]

Values are in mean ± SEM; (n =6), a= $p<0.05$, b=$p<0.0001$ Vs Control.

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