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Research Article

ANTIINFLAMMATORY AND ANALGESIC ACTIVITY OF *BOERHAAVIA DIFFUSA* L.

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Abstract: Four different extracts viz. Pet. ether, dichloromethane, ethanol and water extracts of *Boerhaavia diffusa* was used for accessing antiinflammatory and analgesic activity at a dose of 200 mg/kg b. w. Of all the extracts tested the ethanol extract was found to be the most active against formalin induced inflammation and acetic acid induced writhing for analgesia. The results are summarized in the tables. The activity exhibited by the ethanol extract at a dose of 200 mg/kg b.w. may be due to the presence of chemical components alkaloids present in the ethanol extract.
Key**words:** *Boerhaavia diffusa,* Antiinflammatory, Analgesic, Nyctaginaceae

INTRODUCTION

Boerhaavia diffusa Linn. (Family: Nyctaginaceae) is a indigenous diffused perennial herbaceous creeping weed. It is found throughout the warmer parts of the country and also up to an altitude of 2000 m in the Himalayan region. It grows well on wastelands after the rainy season.[1] *B. diffusa grows* up to 1 m long, having spreading branches. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy, hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The leaves are ovate-oblong, round, or subcordate at the base and smooth above. The margins of the leaves are smooth, wavy or undulate. The upper surface of the leaves is green, smooth and glabrous, whereas it is pinkish white and hairy beneath. The flowers are minute, subcapitate, present 4-10 together in small bracteolate umbrellas, mainly red or rose, but the white varieties are also known. The achene fruit is detachable, ovate, oblong, pubescent, five-ribbed and glandular, anthocarpous and viscid on the ribs.[2] It has a large root system bearing rootlets. The tap root is tuberous, cylindrical to narrowly fusiform, conical or tapering, light yellow, brown or brownish grey. It is thick, fleshy and very bitter in taste. Literature survey revealed that the root of *B. diffusa* contains rotenoids known as boeravinones (A - F),[3-7] punarnavoside, a phenolic glycoside,[8] C-methyl flavone has been isolated from *B. diffusa* roots.[9] Two known lignans viz., liriodendrin and syringaresinol mono-β-D-glycoside,[10] a purine nucleoside hypoxanthine 9-Larabinose, [11] dihydroisofuroxanthone-borhavine[12], phytosterols[13,14] have been isolated from the plant. It contains about 0.04 % of alkaloids known as punarnavine and punernavoside, an antifibrinolytic agent. It also contains about 6 % of potassium nitrate, an oily substance, and ursolic acid.[15] The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids.[16] The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid.

Traditionally the roots, aerial parts of the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine. The root is used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers.[17] It cures corneal ulcers and night blindness[18], and helps to restore virility in men. People in tribal areas use it to hasten childbirth.[19] The juice of *B. diffusa* leaves serves as a lotion in ophthalmia and when administered orally as a blood purifier and to relieve muscular pain.[20]

The biological reports from the literature revealed that ethanol extract of the roots of *B. diffusa* was evaluated for immunomodulation,[21] immunosuppressive[22,23], adaptogenic[24-27] antioxidant,[28-32] cytotoxicity[33], cancer[34], serum protein and lowered serum cholesterol level,[35] antiproliferative,[36] breast cancers,[36] hepatoprotective,[37-38] anti-inflammatory [39,] diuretic,[40] kidney disorders[41] antidiabetic,[42-43] antiviral[44,] anticonvulsant[45] and toxicological studies. [46] In view of all the above findings, in our present investigation, we wish to test anti-inflammatory and analgesic potential of different solvent extracts of *B. diffusa.*

MATERIAL AND METHODS

Collection of Plant Material

The whole plant of *B. diffusa* was collected in and around Bellary district, Karnataka, India, during the month of May-June, 2010 and authenticated at Department of Botany, Smt. A.S.M. College, Bellary. The freshly collected plant material was washed with water and immediately sprayed with ethanol and dried under shade at room temperature. The dried plant material was cut into small pieces and powdered in a blender. The powdered plant material was stored in sterile containers for further use.

Extraction of plant material

The powdered plant material (100 g) was subjected for hot continuous extraction by Soxhlet apparatus using solvents of different polarity starting from non-polar to polar, petroleum ether (40-60 ^{o}C), dichloromethane and methanol). The plant material was extracted with corresponding solvents successively for about 48 h each. After complete extraction the solvents were removed using a Buchi type solvent evaporator under reduced pressure and controlled temperature. The petroleum ether extract yielded 1.2 g dichloromethane extract yielded 1.1 g, ethanol extract yielded 3 g and the water extract yielded around 7 grams, respectively of the extract. The extracts were stored in freezer at around -10°C until further use.

Phytochemical Analysis

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, anthroquinones, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and flavonoids using the method of Khandelwal, 2007[47]**.** The extracted samples were stirred with dilute HCl and filtered. This filtrate was tested carefully and used for compound analysis. In this alkaloids (Mayer's test), carbohydrates and glycosides (Molish test), saponins (chloroform and H_2SO_4 test), protein and amino acid (Millon's test), phytosterols (Libermann-Burchard's test), phenolic compound and tannin (ferric chloride test and lead acetate test) were carried out. The results are represented in table-1

Table 1: Phytochemical test for different solvent extracts of *B. diffusa*

Sl. No.	Solvent Extracts	Alk	Ste	Carb	Tan	Fla	Gly	Sap	Phe	Oils and fats
1	Petroleum ether	-	+	-	-	-	-	-	-	+
2	Dichloromethane	-	+	-	-	-	-	-	-	+
3	Ethanol	+	-	+	+	+	+	-	+	-
4	Water	-	-	+	-	-	+	-	-	-

Alk-Alkaloids, Ste- Steroids, Carb- Carbohydrates, Tan –Tannins, Fla-Flavanoids, Gly-Glycosides, Sap-Saponins, Phe-Phenolics

Anti-inflammatory Activity

Anti-inflammatory activity was studied by formalin induced rat hind paw oedema measured by plethysmograph [48,49] (mercury displacement method). Wistar strain rats of either sex weighing between 150-200 g were divided into five groups of six animals each. The extracts were suspended in 1%, Tween-80 & administered orally (200 mg/kg/b.w.) to rats 1 hour before formalin injection. Phenylbutazone (10 mg/kg b.w.) is given to standard group. Formalin was prepared as 1% v/v solution in 0.9 % w/v NaCl & inject 0.1 ml underneath the planter region of the paw. The third, fourth, fifth and sixth group of animals were treated with crude extracts of *B. diffusa* at a dose of 200 mg/kg body weight, orally.

Control group 1: Formalin + 1%, Tween-80 (10 ml /kg b.w.)
Standard group 2: Formalin + Phenylbutazone (10 mg/kg b.w.)
Test group 3: Formalin + Petroleum ether extract (200 mg/kg b.w.)
Test group 4: Formalin + Dichloromethane extract (200 mg/kg b.w.)
Test group 5: Formalin + Ethanol extract (200 mg/kg b.w.)
Test group 6: Formalin + Water extract (200 mg/kg b.w.)

The volume of paw oedema was measured in control, standard and treated groups accordingly 1, 2, 3 and 4 h after formalin injection. The percentage of inhibition of edema was calculated using formula:

% Inhibition of edema = (Vc-Vt/Vc) x100

Where Vt=Paw volume in test group animals and Vc=Paw volume in control group animals

The data were analyzed by one-way ANOVA. According to this test, there was a significant difference between the drug treated groups and control at the level of $P<0.05$. To analyze the spectrum of anti-inflammatory activity, Duncan multiple range test (DMRT) was used. The results are represented in table-2

Table 2. *In vivo* antiinflammatory activity of *B. diffusa* in albino rats.

Group	Treatment	Route	Dose mg/kg	Paw volume in ml			
				1h	2h	3h	4h
Group I	**Control (Tween-80, 1%)**	p.o.	--	0.24 ±0.005	0.24 ±0.005	0.24 ±0.005	0.24 ±0.005
Group II	**Phenylbutazone**	p.o.	10	0.20 ±0.003	0.18 ±0.003*	0.16 ±0.004*	0.10 ±0.004*
Group III	**Petroleum ether extract**	p.o.	200	0.24 ±0.005	0.23 ±0.005	0.22 ±0.005	0.22 ±0.005
Group IV	**Dichloromethane extract**	p.o.	200	0.22 ±0.003	0.23 ±0.003	0.23 ±0.003	0.23 ±0.003
Group V	**Etanol extract**	p.o.	200	0.22 ±0.003	0.18 ±0.003*	0.14 ±0.004*	0.12 ±0.004*
Group VI	**Water extract**	p.o.	200	0.24 ±0.005	0.24 ±0.005	0.24 ±0.005	0.24 ±0.005

Values are expressed in mean±SE , n=6 in each group. *P<0.05.

Analgesic Activity

The analgesic activity was performed by acetic acid induced writhing test in mice.[55,56] Six groups of four mice each (25-35 grams of b.w.) were selected and 0.6% acetic acid (dose 10 ml/kg of b.w.) was injected intraperitoneally. The numbers of writhes were counted for 15 min., after 5 min. of injection of acetic acid to each mice. This reading is taken as a control. Next day the same groups of mice were used for evaluating analgesic activity. Each group was administered orally with the suspension of four different solvent(petroleum ether, dichloromethane, ethanol and water) extracts of *B. diffusa* at a dose of 200 mg/kg b.w. 1 hour before the injection of acetic acid. After 5 min of acetic acid injection the mice were observed for the number of writhes for the duration of 15 min. The mean value for each group was calculated and compared with that of control group. Analgin was used as standard for comparison. The results are summarised in table-3.

Table 3: Results of analgesic activity of various extract of *B. diffusa*

Sl. No.	Groups	Treatment	No. of Animals	Dose (mg/kg, b.w.)	0 Min.	15 Min.	30 Min.	45 Min.	60 Min.
1	I	Control (1%, 1 ml, Tween-80)	6	-	2.697 ±0.173	2.92 ±0.132	2.90 ±0.128	2.92 ±0.132	2.92 ±0.132
2	II	Analgin Standard	6	30	3.72 ±0.112	5.54 ±0.134	6.30 ±0.110	8.20 ±0.154	9.20 ±0.140
3	III	Petroleum ether extract	6	200	2.70 ±0.172	2.92 ±0.132	2.86 ±0.135	2.92 ±0.132	2.92 ±0.132
4	IV	$CHCl_3$ extract	6	200	2.77 ±0.141	2.85 ±0.136	3.12 ±0.166*	3.40 ±0.226**	3.40 ±0.226**
5	V	Ethanol extract	6	200	2.76 ±0.140	5.22 ±0.224	7.44 ±0.210*	8.15 ±0.112	9.40 ±0.334
6	VI	Water extract	6	200	2.60 ±0.173	2.86 ±0.132	2.92 ±0.128	2.86 ±0.132	2.72 ±0.144

***P≤0.05,**P≤0.001 when compared with control**

RESULTS AND DISCUSSION

From Table no 1 the results reveal that the phytoconstituents present in the petroleum ether and dichloromethane extract is Steroids oil and fats. The ethanol extract of *B. diffusa* contains alkaloids, carbohydrates, tannins, flavonoids and glycosides. Whereas, the water extract contains carbohydrates and glycosides which are confirmed by the phytochemical tests perfomed using the respective reagents for the respective class of constituents.

The results of anti-inflammatory activity are represented in Table 2 and analgesic activity in Table 3. Antiinflammatory activity results revealed that at 1 h, phenylbutazone exhibited good anti-inflammatory activity compared to all the crude extracts of various solvents (Table 1). At 2 h, phenylbutazone and ethanol extract showed good anti-inflammatory activity compared to other groups. Similarly at 3 h, phenylbutazone, crude ethanol extract exhibited good antiinflammatory activity. Whereas, at 4 h, antiinflammatory activity was statistically different in all the test groups except the ethanol extrct where the results were are very much comparable with that of standard. It means, phenylbutazone showed highest antiinflammatory activity followed by crude ethanol extract. However, other extracts did not show much anti-inflammatory activity irrespective of the time intervals. Hence, the results of the present investigation conclude that the crude ethanol extract of *B. diffusa* is accountable for the anti-inflammatory activity.

The analgesic activity exhibited by *B. diffusa* states that only the ethanol extract exhibited good analgesic potential when compared with standard drug analgin at a dose of 200 mg/kg. Whereas, other extracts did not show any analgesic activity. The anti-inflammatory and analgesic activity exhibited by the ethanol extract of *B. diffusa* might be due to the presence of alkaloid punarnava in the ethanol extract. Further work is currently in progress to isolate the pure alkaloids from the extracts and to check their anti-inflammatory potential in our laboratory.

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