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Phytochemical Investigation and Anti-Inflammatory Activity of *Bauhinia vahlii*

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Abstract
The aim of the present investigation is to evaluate the anti-inflammatory property of the leaf exacts of Bauhinia vahlii. Bauhinia vahlii of the family Fabaceae is a vigorous climbing shrub able to grow into the tops of the trees in the forest is found from Sikkim and Nepal across India and Punjab, Pakistan. Ayurveda: Stem bark is used for dysentery and diarrhoea and as an astringent. Leaf is used for malaria and headache. The present study aimed at the evaluation of anti-inflammatory property of the aqueous and alcoholic extracts of the leaves by carrageenan induced paw oedema methods. The followed method showed significant ($P<0.05$) anti-inflammatory property of the different extracts tested. The alcoholic extract at a concentration of 300 mg/mL showed potent activity on comparing with the standard drug diclofenac sodium.

Keywords
Bauhinia vahlii, Anti-inflammatory activity, Ethanol extract, Acetone extract, Carrageenan

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INTRODUCTION
Plants are natural and traditional sources of medication in large parts of the world. Herbs have been used since ancient times by physicians and also by layman to treat a great variety of human diseases. A wide variety of herbs singly and in mixture have been extensively investigated in basic biological sciences to evaluate their chief as well as supplementary, complementary and synergistic action in health and diseases1. Herbal products are receiving increasing public interest, and herbal treatment is now the most popular alternative therapy2. The World Health Organization (WHO) estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care3, 4. Inflammation is a reaction of living tissues towards injury, and it comprises systemic and local responses5. In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently compiled an inventory of medicinal plants listing over 20000 species. The family Fabaceae consists of several important medicinal

plants with wide range of pharmacological, biological activities and interesting phyto chemical constituents. The main action of anti-inflammatory agents is the inhibition of Cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins. The plant Bauhinia vahlii or Camel foot creeper belongs to the family Fabaceae is a deciduous tree of 10 m high is globally distributed across India, Nepal and Bhutan up to an altitude of 1500 m. Some ethnic communities give stem bark extraction with cow milk (3:2) as cure of glandular inflammation. The present study aimed to evaluate the anti-inflammatory activity of Bauhinia vahlii by carrageenan induced paw oedema method.

MATERIAL AND METHODS

Preparation of extracts

Fresh leaves of Bauhinia vahlii were collected from local area of Gunupur district Odisha and were authenticated by botanist. The leaves were dried in shade and powdered to a coarse form. It was then successively extracted with petroleum ether, ethanol and acetone using continuous hot soxhlet extract as each of their increasing order of polarity. The extracts were concentrated under reduced pressure and preserved at low temperature.

Preliminary Phytochemical screening

Phytochemical[6] analysis of PEBV, EEBV and AEBV were performed using standard procedures to identify the constituents present in them.

Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals. Reference standard diclofenac sodium was obtained as gift sample from MRL labs Chennai. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

Animals

Adult Wister albino rats (80 g -120 g) of either sex were used for the *in vivo* evaluation. They were housed under standard laboratory conditions and were fed with standard animal feed and water ad libitum. The experimental protocol was approved by institutional animal ethical committee. In this study a total of 8 groups of six rats each.

Acute toxicity test

Acute toxicity study was performed as per OECD guidelines 423.[7] (Acute toxicity class method).

In vivo Anti-inflammatory activity

Among the three extracts only ethanol and acetone extract were taken for anti-inflammatory activity screening due to presence of maximum phyto constituents as per preliminary phytochemical screening.

Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw[8]. The extracts at different concentrations were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

Group 1: Vehicle control (2% Tween 80).
Group2: Standard(Diclofenac5mg/kgp.o.).
Group 3, 4 and 5: EEBV (200, 300 and 400 mg/kg, p.o.), respectively.
Group 6, 7 and 8: AEBV (200, 300 and 400 mg/kg, p.o.), respectively.
Group 9, 10 and 11: AECFC (100, 200 and 400 mg/kg, p.o.), respectively.

Percentage inhibition of paw volume between treated and control group was calculated by the following formula,

$$\% \text{ of Inhibition} = \frac{V_C - V_T}{V_C} \times 100$$

Where, V_T and V_C are the mean increase in paw volume in treated and control groups, respectively.

Statistical analysis

Statistical analysis was done using one-way analysis of variance followed by Dunnets test. *P*values greater than 0.05 were considered as significant.

Table 1: Preliminary phytochemical screening

Sr. No.	Test	PEBV	EEBV	AEBV
1		-	+	+
2		-	-	+
3		-	-	+
4		-	-	+
5		-	+	-
6		+	+	+
7		+	+	-
8		+	+	-
9		-	+	-
10		+	+	+
11		-	+	-

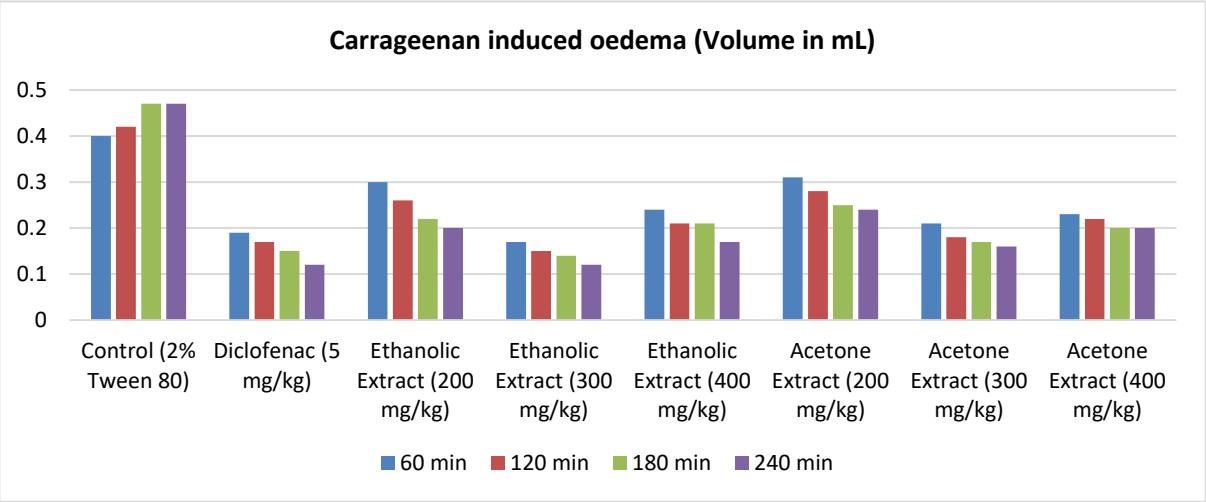

Table 2: Anti-inflammatory activity of *Bauhinia vahlii*on carrageenan induced oedema

Test (dose mg/kg)	Carrageenan induced oedema (Volume in Ml)				% of inhibition at 240 min
	60 min	120 min	180 min	240 min	
Control (2% Tween 80)	0.40± 0.21	0.42± 0.093	0.47± 0.16	0.47± 0.21	-
Diclofenac (5 mg/kg)	0.19± 0.33*	0.17± 0.078*	0.15± 0.28*	0.12± 0.66*	74.46
Ethanolic Extract (200 mg/kg)	0.30± 0.33*	0.26± 0.28*	0.22± 0.064*	0.20± 0.023*	57.44
Ethanolic Extract (300 mg/kg)	0.17± 0.45*[a]	0.15± 0.52*[a]	0.14± 0.21*[a]	0.12± 0.71*[a]	74.46
Ethanolic Extract (400 mg/kg)	0.24± 0.71*[a]	0.21± 0.091*[a]	0.21± 0.16*[a]	0.17± 0.33*[a]	63.38
Acetone Extract (200 mg/kg)	0.31± 0.45*	0.28± 0.66*	0.25± 0.023*	0.24± 0.16*	48.93
Acetone Extract (300 mg/kg)	0.21± 0.33*[a]	0.18± 0.45*[a]	0.17± 0.33*[a]	0.16± 0.055*[a]	65.95
Acetone Extract (400 mg/kg)	0.23± 0.71*[a]	0.22± 0.37*[a]	0.20± 0.18*[a]	0.20± 0.24*	57.44

Values are expressed in mean ± SEM (n=6); *- P<0.05 with control; a- P<0.05 with standard.

RESULTS

Acute toxicity studies

The extracts of Bauhinia vahlii did not show any sign of toxicity up to 2 000 mg/kg body weight and hence it was considered to be safe.

In vivo anti-inflammatory activity

The extracts of Bauhinia vahlii at different concentrations showed significant reduction in the paw volume of rats. The ethanolic extract at concentration of 300 mg/mL showed potent activity compared with the reference standard Diclofenac sodium. The results were tabulated in Table 2.

DISCUSSION

Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedima in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the like[9-11]. Leaf extract of Bauhinia vahlii showed significant anti-inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids[12] present in the extract. The present result indicates the efficacy of Bauhinia vahlii as an effective therapeutic agent in the treatment of acute inflammations. The result of present study authentifies the folk lore information on the anti-inflammatory property of the leaf extract of Bauhinia vahlii. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to identification of the possible mechanism of its anti-inflammatory property.

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