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# Antiinflammatory Activity and Free Radical Scavenging Studies of *Aristolochia bracteolata* Lam.

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**Antiinflammatory activity of the ethanol extract of the shade dried leaves of *Aristolochia bracteolata* lam was studied in Wistar rats by using the carrageenan induced left hind paw edema method. Significant reduction of edema volume was observed in the drug treated group when compared with the standard and untreated control. Antioxidant investigations of the ethanol extract along with its two successive fractions using nitric oxide and 1,1-diphenyl-2 picryl hydrazyl (DPPH)-induced free radical assay methods showed good free radical scavenging activity, thereby supporting its antiinflammatory properties.**

Aristolochia (Fam. Aristolochiaceae), is a large genus of herbs or twining plants, found in the tropical and temperate regions of the world. Eight species are known to occur in India of which *Aristolochia bracteolata, Aristolochia indica and Aristolochia tagala* are of medicinal importance. They generally contain alkaloids and are reputed to be useful in the treatment of snake bites. *Aristolochia bracteolata* lam. (Syn: worm killer) is a perennial herb found in the Upper Gangetic plain, the Western peninsula, Bengal, Gujarat and in the South of India[1]. The plant is exceedingly bitter and its leaves are reported to possess anthelmintic and cathartic properties. It is also used in the treatment of syphilis, gonorrhea, boils, foul ulcers and in other skin diseases. The plant has been reported to possess aristolochic acid in addition to other chemical constituents, like ceryl alcohol and β-sitosterol[2].

The enzyme, phospholipase $A_2$, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase $A_2$ converts phospholipids in the cell membrane into arachidonic acid which is highly reactive and is rapidly metabolised by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation[3,4]. It is reported that aristolochic acid inhibits the activity of the enzyme phospholipase $A_2$[5]. A review of literature did not reveal any information on the antiinflammatory and antioxidant studies of this plant. The present study is therefore an attempt to assess the efficacy of this indigenous herb for its antiinflammatory activity in rats and to study the antioxidant properties of various fractions of the ethanolic extract in relation to this property.

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## MATERIALS AND METHODS

The leaves of *A. bracteolata,* were collected from the University Campus, Gulbarga, Karnataka, India, in the month of July 2001. It was authenticated in the department of Botany, Mahatma Gandhi Memorial College, Udupi, India. Wistar rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard chow diet (Pranav Agro Industries Ltd., Sangli, Maharashtra) and water *ad libitum.* They were housed in polypropylene cages maintained under standard condition (12 h light/12 h dark cycle; 25±3°; 35-60% humidity). All the chemicals and solvents used were of either pharmacopoeial or analytical grade. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee, and were cleared by the same

before starting the experiments (No. IAEC/KMC/09/2001, CPCSEA Regd No.94/1999.)

**Preparation of plant extracts:**

The shade dried powdered leaves (1 kg) were exhaustively extracted with 95% ethanol using a Soxhlet apparatus. The total ethanol extract was concentrated *in vacuo* to a syrupy consistency (yield 270 g). Ethanol extract along with its two successive fractions, ether and ethyl acetate were studied for antiinflammatory and antioxidant properties.

**Phytochemical screening:**

The coarse powder of the leaves of *A. bracteolata* (50 g) was subjected to successive extraction with different solvents in increasing order of polarity from petroleum ether (60-80°), benzene, chloroform, acetone, alcohol, to finally chloroform:water[6]. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

**Acute toxicity studies:**

Healthy Wistar rats of either sex, starved overnight, were divided into 6 groups (n=6) and were fed with increasing doses (1, 2, 4, and 8 g/kg, p.o) of the ethanol extract. The total ethanol extract administered orally in doses of upto 8 g/kg, did not produce any evident sign of toxicity and mortality in rats when observed upto 14 d after administration.

**Evaluation of antiinflammatory activity:**

The extract was evaluated for antiinflammatory activity by carrageenan-induced rat paw edema method[7]. The animals were divided into three groups of six animals each. First group serving as control received gum acacia solution 2%, second group served as positive control and received ibuprofen 100 mg/kg, and third test group received 400 mg/kg body weight of the ethanol extract of the leaves of *A. bracteolata*. Food was withdrawn overnight, but adequate supply of water was given to the rats before the experiments. The drugs were given orally with the help of an oral catheter. After one hour, a subplantar injection of 0.05 ml of 1% solution of carrageenan was administered in the left hind paw to all the three groups. The paw volume was measured with the help of a plethysmograph immediately after injection. The paw volume was again measured after 1, 2, and 3 h. The average paw swelling in a group of drug treated rats was compared with control group (treated with the vehicle) and the standard.

**Evaluation of free radical scavenging activity:**

The alcoholic, ether and ethyl acetate fractions of A. *bracteolata*, were studied for its antioxidant property at different concentrations ranging from a maximum of 1 mg/ml to a minimum of 0.002 mg/ml. To the ethanolic solution of 1,1-diphenyl-2-picryl hydrazine (DPPH, 0.05 mM) an equal volume of the test fraction (ethyl acetate and ether fractions) dissolved in methanol was added at various concentrations in a final volume of 1.0 ml. An equal amount of methanol was added to control. After 20 min, absorbance was recorded at 517 nm. The experiment was performed in triplicate. Percent scavenging was calculated using the formula [(Control – Test)/ Control]x100

**Scavenging of nitric oxide:**

Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations of test compound (ethyl acetate and ether fractions) dissolved in standard phosphate buffer 0.025M, pH: 7.4 and the tubes were incubated at 25° for 5 h. Control experiment without test compound but with equivalent amounts of buffer was conducted in an identical manner. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotisation of nitrite with sulphanilamide and subsequent coupling with naphtha ethylene diamine was read at 546 nm. Experiment was repeated in triplicate.

**Statistical analysis:**

Results expressed as mean±S.E., were evaluated by one way ANOVA with post hoc Scheffe's test. Values of $P < 0.05$ were considered statistically significant.

## RESULTS

Preliminary phytochemical screening revealed the presence of alkaloids, saponins, flavonoid glycosides, steroids and triterpenes, tannins and phenolic compounds. The ethanol extract of *A. bracteolata* showed significant reduction in the edema volume in comparison to the control and standard (Table 1). The extract reduced the oedema induced by carageenan by 60% on oral administration of 400 mg/kg as compared to the untreated control group. Ibuprofen at 100 mg/kg inhibited the oedema volume by 69%.

The alcoholic extract and ethyl acetate fraction of *A. bracteolata* showed promising antioxidant activity in reduction of DPPH in a concentration-dependant manner up to a concentration of 1mg/ml. The ether fraction showed good scavenging action against nitric oxide (NO) induced release

TABLE 1: ANTIINFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF *A. BRACTEOLATA* LEAVES.

| Treatment | Mean paw volume (ml) | %Edema inhibition (after 3 h) |
|---|---|---|
| Gum acacia (2%) oral | 0.37±0.05 | ——— |
| Ibuprofen 100 mg/kg oral | 0.13±0.02 | 69±4.6 |
| Ethanol extract of *A. bracteolata* 400 mg/kg oral | 0.14±0.03[a] | 60±6.7 |

Values are mean±S.E (n=6); [a]P<0.05 vs control (Carageenan-induced rat paw oedema model).

of free radicals with 0.5 and 1 mg/ml showing maximum nitric oxide inhibition of 45% (Table 2).

## DISCUSSION

There is a continuous search for indigenous drugs which can provide relief to pain and inflammation. The plant *A. bracteolata* has been used for several decades in India for the treatment of wound and inflammation. The present investigation revealed that the plant had a significant antiinflammatory activity in the acute inflammatory model, i.e, carrageenan induced paw edema method. Inflammation is a complex process characterized by the contribution of several mediators, including prostaglandins (PGs) and nitric oxide (NO)[3,4]. The enzyme cyclooxygenase-2 (COX-2) produced at the site of inflammation contributes to the inflammation process by generation of inflammatory mediators (prostaglandins) from arachidonic acid. The plant *A. bracteolata* has been reported to possess aristolochic acid which in turn inhibits the synthesis of arachidonic acid by inhibition of the enzyme phospholipase$A_2$[5].

Nitric oxide is a free radical produced in mammalian cells involved in the regulation of various physiological processes[8,9]. However, excess production of NO is associated with several diseases such as septic shock, acute and chronic inflammation and other autoimmune diseases[10,11]. Our studies have shown that the plant *A. bracteolata* acted as a free radical scavenger against nitric oxide, which may be responsible for the reversal of deleterious inflammatory effects of NO. Thus from our study it is evident that *A. bracteolata* is endowed with significant antiinflammatory activity as evidenced by its free radical scavenging action

TABLE 2: FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF THE LEAF OF *A. BRACTEOLATA*.

| Concentration of extract (mg/ml) | % Inhibition | | |
|---|---|---|---|
| | DPPH | | Nitric oxide |
| | Ethanol extract | Ethylacetate fraction | Ether fraction |
| 1.0 | 77.1 | 84.0 | 45.9 |
| 0.50 | 59.0 | 77.5 | 45.5 |
| 0.25 | 35.9 | 52.6 | 32.3 |
| 0.125 | 21.6 | 33.7 | 22.1 |
| 0.06 | 17.6 | 21.0 | 20.0 |
| 0.031 | 12.9 | 13.4 | 12.5 |
| 0.016 | 08.4 | 10.1 | 02.1 |
| 0.008 | 04.4 | 04.1 | - |
| 0.004 | 04.1 | 04.1 | 06.0 |
| 0.002 | 02.1 | 02.1 | - |

against NO and other free radicals, and the mechanism to inhibit the release of prostaglandins. Further studies involving the purification of the chemical components of the plant and the investigations in the biochemical pathways may result in the development of a potent antiinflammatory agent with a low toxicity and better therapeutic index.

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