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RESEARCH ARTICLE

EVALUATION OF *IN VIVO* ANTIINFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF *ARISTOLOCHIA BRACTEATA* RETZ WHOLE PLANT IN RATS

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ABSTRACT

The present study was aimed for scientific evaluation of the antiinflammatory activity of ethanol extract of *Aristolochia bracteata* Retz whole plant by *in vivo* carrageenan induced paw edema model in albino rats. Ethanol extract showed dose dependent antiinflammatory activity. The ethanol extract has shown a significant ($p < 0.001$) percentage inhibition of paw edema 80.79% and 87.70% on 3 hour at 200 and 400 mg/kg respectively. The study was compared with standard drug indomethacin 85.11% (10mg/kg). This result provide a scientific basis for the use of the whole plant of *A.bracteata* as an antiinflammatory agent.

INTRODUCTION

Inflammation is a fundamental pathological process of an immune system towards tissue damage, tissue malfunction and infection (Ashley *et al.*, 2012). This complex reaction results from the release of local hormones like prostaglandins, histamine, serotonin and cytokines. These biochemical molecules regulate both homeostatic and pathological reactions such as inflammation, fever and pain (Miller *et al.*, 2006). Inflammation is associated with various diseases. Suppression of inflammation still continues to be a challenge to the scientists despite the availability of number of NSAID's. This is because NSAID's not only exhibit antiinflammatory and analgesic activity but also cause gastrointestinal complications ranging from dyspepsia to upper GI tract bleeding and perforation (Schenone *et al.*, 2006). Consequently there is need for development of new antiinflammatory agents with minimum side effects. In this context the value of herbal medicinal plants, herbs and spices is priceless as the treatment is 100% natural with no side effects. *Aristolochia bracteata* Retz is a shrub distributed throughout India, belonging to the family Aristolochiaceae. *A.bracteata* is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al.*, 2003). In Indigenous system of medicine, it is reported that the leaves were used for skin diseases, rheumatism and analgesic (Manikandan *et al.*, 2006).

The whole plant was used as purgative and anthelmintic, antipyretic and antiinflammatory agents (Kadam *et al.*, 2012). Along with its use against health disorders, it was felt worthwhile to screen the antiinflammatory activity of *A.bracteata* so that its further role in natural antiinflammatory agent is explored. Hence, the present study was aimed to investigate the antiinflammatory activity of *A.bracteata* ethanol extract of whole plant.

MATERIALS AND METHODS

Plant material

The whole plant of *Aristolochia bracteata* Retz was freshly collected from Kouvandampalayam, Coimbatore district, Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for antiinflammatory activity

The dried whole plant material of *A.bracteata* was powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for preliminary phytochemical screening (Brinda *et al.*, 1981) and antiinflammatory activity.

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Animals

Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±20C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*. Study was carried out as per IAFC approval No: 82 /PHARMA/SCRI, 2010.

Acute toxicity study

Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

to the rats and Vc represents difference of increased volume in the control groups.

Statistical analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a *p* values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS

The phytochemical screening of ethanol extract of whole plant of *A.bracteata* revealed the presence of alkaloid, anthraquinone, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of whole plant of *A.bracteata*. In the present study, the antiinflammatory activity of ethanol extract of whole plant of *A.bracteata* were assessed in albino rats using carrageenan induced rat paw edema model. Table 1 showed that the antiinflammatory activity of ethanol extract of whole plant of *A.bracteata* significantly inhibited the rat paw edema at 3rd hour post carrageenan were 80.79% and 87.70% for 200mg/kg and 400mg/kg respectively.

Table 1. Effect of the whole plant ethanol extracts of *A. bracteata* on the percentage inhibition of carrageenan induced paw edema

Treatment Groups	Dose mg/kg	Oedema volume (ml)				% Inhibition after 180 minutes
		0 minute	60 minutes	120 minutes	180 minutes	
Group I	Normal saline	30.28±1.65	73.92±1.68	102.26±1.65	129.56±2.14	-
Group II	200 mg/kg	35.27±1.46	42.13±1.08*	34.27±0.54***	24.88±0.84***	80.79
Group III	400 mg/kg	29.54±1.38	38.16±1.55*	30.65±0.17***	15.93±0.54***	87.70
Group IV	10 mg/kg	27.28±1.13	36.93±1.39**	26.62±0.87***	19.29±0.77***	85.11

Each Value is SEM ± 5 individual observations

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001 Compared paw oedema induced control vs drug treated rats.

Antiinflammatory activity of carrageenan induced hind paw edema

Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups were as follows. Group I - Control (normal saline), Group - II and III – Ethanol extract of *A.bracteata* whole plant (200 and 400 mg/kg, p.o.), Group IV – Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard antiinflammatory drug. After one hour of a administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min, 60min, 120min, 180min, 240min, 360min, and 480min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula;

$$\text{Percentage inhibition} = \frac{(V_c - V_t)}{V_c} \times 100$$

Where, Vt the percentage represents the percentage difference in increased paw volume after the administration of test drugs

The results were compared with indomethacin at 10mg/kg which shows paw reduction of 85.11%.

DISCUSSION

Carrageenan induced inflammation is the most sensitive and reliable model for evaluating acute phase and orally active antiinflammatory agents. The time course of increase in paw edema is represented as biphasic event. Development of carrageenan induced inflammation during 1 hour is due to the release of histamine and serotonin, (Anuradha *et al.*, 2002; Kumar *et al.*, 2014) along with trauma of injection, where as the second phase and third phase is attributed to the release of Kinin like substances and prostaglandins respectively (Nayak and Patel, 2010; Valiollah *et al.*, 2011). In the present study, ethanol extract of *A.bracteata* whole plant possessed significant antiinflammatory activity as compared to control group. This result indicates that the ethanol extract can prevent the release of inflammatory mediators like histamine, kinin and prostaglandin or may be antagonizing the activity of the mediators after their release. This antiinflammatory effect of the extract observed might be due to the presence of flavonoids and saponins in the plant. 3, 7, 11, 15 – Tetramethyl -2-hexadecan-1-01, Phytol, 9, 12, 15 – Octadecatrienoic acid, methyl ester, (Z, Z, Z) -, Vitamin E and β-Sitosterol were reported in the ethanol extract of *A.bracteata* whole plant by GC-MS analysis. These compounds may have the role in antiinflammatory effect. (Jegadeeswari *et al.*, 2012).

Conclusion

The significant *in vivo* antiinflammatory activity exhibited by the extracts of *A.bracteata* in the carrageenan induced rat paw edema model provide some scientific evidence in support of the traditional uses of this plant for treatment of various inflammatory conditions.

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