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RESEARCH ARTICLE

PHARMACOGNOSY

EVALUATION OF ANTI - INFLAMMATORY ACTIVITY OF ADHATODA ZEYLANICA (MEDIC.) LEAVES EXTRACT

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ABSTRACT

Adhatoda zeylanica (Medic.) family-Acanthaceae is distributed all over the plains of India and lower Himalaya ranges, ascending to height of 1500 m. The present study is an attempt to explore the anti-inflammatory activity of ethanolic and aqueous extract of leaves of the *Adhatoda zeylanica.* Dose of 100 mg/ml and 200 mg/ml of ethanolic and aqueous extract were evaluated for their anti-inflammatory activity against carrageenan induced paw edema in rat. Both the extracts were able to show anti-inflammatory activity in dose dependant manner as compared to standard drug diclofenac sodium 100 mg/ml. The data were found statistically significant by using one way ANOVA (P< 0.001).

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KEY WORDS

Adhatoda zeylanica, Acanthaceae, Anti-inflammatory, Diclofenac sodium.

INTRODUCTION

Adhatoda zeylanica (Medic.) family-Acanthaceae is a dense shrub 1.2- 2.4 m high with many long opposite ascending branches, stem with yellowish bark, glabrous. Leaves 12.5-20 cm by 3.8-6.3 cm, elliptic – lanceolate, acuminate, minutely puberulous when young, glabrous when mature, entire, dark green above, paler beneath, base tapering. Flowers in short dense axillary pedunculate spikes 2.5 - 7.5 cm long, towards the end of the branches[1]. The plant is distributed all over the plains of India and lower Himalaya ranges, ascending to height of 1,500 m[2].

Adhatoda zeylanica contains several alkaloids but the major include pyrroloquinazoline alkaloids vasicine about 1.3% accompanied by vasicinol, vasicinone and adhatonine. Aliphatic hydroketones such as 37 – hydroxyl hexatetracont – 1 – en – 5 – one and 37 – hydroxyl hentetracontan -19- one have been reported[3].Leaves and roots are used in cough, chronic bronchitis, asthma, rheumatism[4]. *Adhatoda zeylanica* shows antispasmodic, antiseptic and insecticidal properties[5]. Fresh juice of leaves with honey relieves the irritable cough by its soothing action on the nerves and by liquefying the sputum which makes expectoration easier. Juice of the leaves is considered useful for diarrhoea and dysentery, especially in haemoptysis and in the bleeding of dysentery. The leaves are applied locally in the form of a poultice on rheumatic joints, swelling and in neuralgias. Strong decoction is a good application for scabies and other skin complaints. Both the decoction and powder form constitutes of many Ayurvedic preparations for infection of the respiratory tract[6]. *Adhatoda zeylanica* is one of the ingredients of the preparations known as Vasavaleha (Dabur), Kasamrit Herbal (Baidyanath) and Vasaka capsule (Himalaya Drug Company) [3]. However so far no systematic study on phytochemical and anti-inflammatory activity has been reported in the literature. In the present context the present study is focused to evaluate the anti-inflammatory activity of *Adhatoda zeylanica* leaves.

MATERIALS AND METHODS

Collection of leaves

The leaves of *Adhatoda zeylanica* were collected from the Herbal Garden of Jamia Hamdard, New Delhi and were authenticated by Dr. H.B. Singh, Scientist Incharge, NISCAIR, New Delhi. A voucher specimen NISCAIR/RHMD/Consult/-2007-08/855/39 is preserved for future reference.

Preparation of Extract

In the present study, the ethanolic extract of air dried leaves powdered material (500g) was prepared using soxhlet apparatus, concentrated and dried using Buchi rotavapour, it gives a brownish mass (58.56 g.) The powdered material (500g) was percolated with cold water to get the aqueous extract (30.32g). The dried ethanolic and aqueous extract were stored in a desiccators to carry out phytochemical and pharmacological studies. Each extract was subjected to qualitative chemical investigation of phytoconstituents such as alkaloids, glycosides flavonoids, tannins, carbohydrates, proteins, vitamins, coumarins, etc.

Phytochemical studies

Preliminary phytochemical screening was performed[7] .The presence of phytoconstituents such as alkaloids, carbohydrates, flavonoids, steroids and resins were confirmed.

Pharmacological screening

The animal experiments were performed according to CPCSEA guidelines and after the approval from Institutional Animal Ethics Committee (I.A.E.C.), Rajiv Academy For Pharmacy, Mathura, experiments were conducted in accordance with the standard guidelines.

Animal used

Albino rats (Wistar strain) of either sex (150-180g) were obtained from the animal house of Rajiv Academy For Pharmacy, Mathura. Animals were kept in animal caging system (four rats per cage on beds of sawdust) under the laboratory conditions (25 ± 2^0C, 12 hr. light). They were provided with animal feed pellets manufactured by Hindustan Lever (India) Ltd. Mumbai. Food was withdrawn 12hr. before the experimental work and water was provided *ad libitum*. After a 7 days of acclimatization period, animal were randomly selected for different experimental groups (6 animal/ group) and used for the *in vivo* determination of anti-inflammatory activity. During the course of the experiment the animal behavior was normal.

Drugs

Ethanolic, aqueous extracts and diclofenac sodium were prepared as suspension using 0.6% w/v sodium carboxymethyl cellulose as suspending agent.

Experimental method

Anti-inflammatory activity was evaluated using carrageenan – induced hind paw edema method [8, 9]. Carrageenan (0.1 ml of 1% w/v suspension) was injected into the sub plantar region of the right hind paw of each rat. The extracts (100 and 200 mg/ml) and diclofenac sodium (100 mg/ml) were administered orally to rats one hour before carrageenan.

Control group received an equal volume of vehicle (0.6 %w/v Sod. CMC). The dosage details are given in Table – 1. The volume of the paw was measured with a volume differential meter (Model 7140 UGO Basile) after 1, 2, 3, 4 and 5hr. of carrageenan injection. Results were determined as the percentage inhibition of edema compared to the control.

Percent inhibition of edema volume between treated and control was calculated as follows:

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc and Vt represent mean increase in paw volume in control and treated groups respectively.

Statistical analysis

All data are expressed as mean ± SEM. Statistical significance was calculated using one - way ANOVA with Dunnett's *t* test[10]. P valves < 0.001 were considered significant.

RESULT

There was significant and dose dependent anti-inflammatory activity of both the ethanolic and aqueous extracts in the acute carrageenan induced rat paw edema model.

Orally administered doses of 100 and 200 mg/ml of ethanolic extract of the leaves of *Adhatoda zeylanica* produced 33% and 40% inhibition respectively after 3 hr. The remaining aqueous extract of leaves of *A. zeylanica* produced 26% and 33% inhibition respectively after 3 hr. as compared to diclofenac sodium (Standard) (100 mg/ml) which showed 56% inhibition after 3 hr.

Orally administered doses of 100 and 200 mg/ml of ethanolic extract of the leaves of *Adhatoda zeylanica* produced 47.23% and 58% inhibition respectively after 5 hr. The remaining aqueous extract of leaves of *A. zeylanica* produced 44.4% and 52.7% inhibition respectively after 5 hr. as compared to diclofenac sodium (Standard) (100 mg/ml) which showed 69.5% inhibition after 5 hr ($P < 0.001$). Results of anti-inflammatory activity are presented in Table-1. Carrageenan induced rat paw edema throughout the observation period.

DISCUSSION

Percentage inhibition of edema volume of ethanolic, aqueous and standard drug were calculated after every hour upto 5 hr. duration. There is dose dependent inhibition of paw edema in rats as shown in Fig. 1. Prostaglandins and bradykinins were suggested to play important role in carrageenan induced edema. [11, 12]. Both steroidal and non steroidal anti inflammatory drugs can be tested by the carrageenan-induced paw inflammation test. The edema induced in the rat paw by the injection of 1% carrageenan is brought about by autocoids, histamine and 5-hydroxy tryptamine (5-HT) during the first one hour, after which kinins act, to increase the vascular permeability upto two and a half hours. The maximum inflammation is seen approximately three hours post the carrageenan injection, after which it begins to decline. Following that the prostaglandins act from two and a half hours to six hours, which results in the migration of leucocytes into the inflamed site[13]. *Adhatoda zeylanica* shows a significant inhibition of inflammation, which is comparable to the standard drug diclofenac sodium. As Phytochemical tests showed the presence of alkaloids, carbohydrates, flavonoids, steroids and resin in both the ethanolic and aqueous extract, they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exert its activity. Further study is required to know the main phytoconstituent which is responsible for the activity.

Table – 1

Anti-inflammatory activity of Adhatoda zeylanica leaves extracts on Carrageenan Induced Paw Edema in Rats

Treatment	Dose mg/ml P.O	Volume displaced in ml				
		1hr.	2hr.	3hr.	4hr.	5hr.
Control	……..	0.18 ± 0.05	0.25 ± 0.05	0.30 ± 0.08	0.33 ± 0.07	0.36 ± 0.07
Diclofenac sodium	100	0.16 ± 0.04 (11.2)	0.17 ± 0.04* (32.0)	0.13 ± 0.06* (56.0)	0.11 ± 0.04* (66.0)	0.11 ± 0.04* (69.5)
Ethanol Extract	100	0.17 ± 0.05 (5.5)	0.23 ± 0.03 (8.0)	0.20 ± 0.05* (33)	0.19 ± 0.05* (42.0)	0.19 ± 0.05* (47.2)
Ethanol Extract	200	0.16 ± 0.05 (11.12)	0.22 ± 0.05* (12.0)	0.18 ± 0.06* (40.0)	0.17 ± 0.04* (48.0)	0.15 ± 0.06* (58.3)
Aqueous Extract	100	0.17 ± 0.09 (5.5)	0.23 ± 0.08* (8.0)	0.22 ± 0.06* (26.0)	0.21 ± 0.04* (36.0)	0.20 ± 0.03* (44.0)
Aqueous Extract	200	0.17 ± 0.03 (5.5)	0.23 ± 0.04* (8.0)	0.20 ±.0.04* (33.0)	0.20 ± 0.08* (39.4)	0.17 ± 0.04* (52.7)

Results expressed as Mean ± SEM, *n* = 6 animals in each group; Values within parentheses represent the percentage inhibition. Statistical evaluation by one- way ANOVA followed by Dunnett's *t* – test; (*) Symbols represent statistical significance - P< 0.001

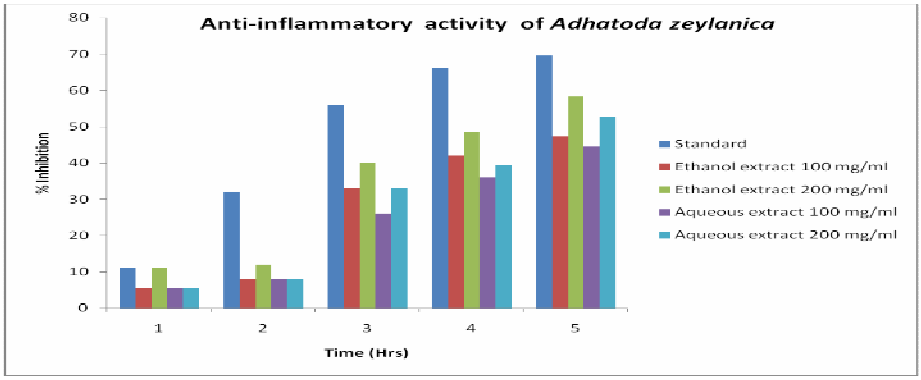

Fig1

Anti-inflammatory activity of ethanolic and aqueous extract of the leaves of Adhatoda zeylanica compared with the standard drug diclofenac sodium.

CONCLUSION

It could be concluded and confirmed that the ethanolic and aqueous extracts of leaves of plant *Adhatoda zeylanica* is having anti-inflammatory activity and exhibited activity when compared to the standard drug, which is a considerable important result. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anti-inflammatory activity.

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