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Anti-inflammatory activity of alcoholic extract of *Adenema hyssopifolium* G.Don in acute and chronic experimental models in albino rats

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ABSTRACT

Objective: To evaluate the anti-inflammatory effect of alcoholic extract of *Adenema hyssopifolium.*

Methodology and results: Carrageen induced rat paw edema method (acute), Turpentine oil induced granuloma pouch (sub acute) and formalin induced rat hind paw edema (chronic) models in rats was adopted for the study. The alcohol extract (using 90%) at a concentration of 300 and 600 mg kg^{-1} p.o., and its ethyl acetate fractions at 25 and 50 mg kg^{-1} p.o. showed a significant dose dependent anti-inflammatory activity in carrageen induced rat hind paw edema as well as formalin induced rat hind paw edema chronic model in rats. In the chronic model, *Adenema hyssopifolium* alcoholic extract HAE 600 mg kg^{-1}), ethyl acetate fraction (50 mg kg^{-1}) and the standard drug showed 55.5, 63.4 and 68.0 % ($p < 0.001$) inhibitions, respectively.

Conclusion and application of findings: The alcohol extract of *A. hyssopifolium* exhibited anti-inflammatory activity in all the tested models. The findings justify current utilization of alcoholic extract of *Adenema hyssopifolium.*

Key words: *Adenema hyssopifolium,* inflammation, ethnobotanical use

INTRODUCTION

Adenema hyssopifolium G.Don (Gentianaceae) is a slender perennial herb of great medicinal value in India (Kiritikar & Basu,1935). It is widely used as a substitute for *Swertia chirayita* Ham.Ex Wall as febrifuge and is also used in folk medicine for treatment of Diabetes mellitus in Western and Southern India (Gupta *et al.,* 1962). Ethnomedical studies in North Gujarat (India) have revealed that the hot aqueous extract of *Adenema hyssopifolium* is used by the tribal inhabitants to treat diabetes, fever, stomachache, dyspepsia and malaria (Goyal *et al.,* 2004). The iridoid glycoside swertiamarin was reported to produce central nervous system depressant activity (Ghosal *et al.,* 1976). Chandhuri *et al.* (1975) reported the presence of monoterpene alkaloid gentiocrucine [mixture of

three compounds (I, II and III)] and Erythrocentaurin (Ghosal *et al.*,1974). Betuline, a triterpene sapogenin was isolated from *Enicostemma littorale* by earlier workers (Rai & Takar, 1966; Desai *et al.,* 1966). Seven flavonoids were isolated from alcoholic extract of *Enicostemma littorale*- and their structures were identified as Apigenin, genkwanin, isovitexin, swertisin, 5 – o−β− D – glucoside (Ghosal & Jaiswal, 1980). Methanolic extract of *A.hyssopifolium* evaluated against Dalton's ascitic lymphoma in Swiss albino mice showed inhibition of tumour cell growth (Kavimani *et al.,* 2000). In India, the entire plant in powdered form has been used in traditional treatment of tumors, and is referred to in the authoritative Book of Siddha system of Medicine "Siddha Vaidhya Pathartha Guna Vilakam" (Kannusamy, 1931).

No scientific report has been published on the anti-inflammatory activity of *A. hyssopifolium,* though the plant has been used traditionally for inflammation cases. The present study investigated the effect of the alcoholic extract and its fractions in acute, sub acute and chronic models in rats.

MATERIALS AND METHODS

Plant materials processing and extraction: The entire plant of *A. hyssopifolium* was collected during September 2004 from Madurai, Tamilnadu, India. The plant was identified and authenticated by Dr. D. Stephen, Taxonomist, American College, Madurai, India. A voucher specimen was preserved in our laboratory for future reference (R.A. No.11/04). The entire plant was washed with water, air dried for 10 days under controlled temperature (25 ± 2°C), powdered and passed through a # 40 mesh sieve and stored in an air tight container for further use. Coarsely powdered dried entire plant (1.3 kg) was successively extracted in petroleum ether (bp 40-60°C) and in cold alcohol (90%) for 72 h at room temperature. The extracts were filtered and the solvents evaporated to dryness under reduced pressure in an Eyela rotary evaporator (Japan) at 40 to 45°C. The petroleum ether extract contained alkanes and alkanols and was not processed further. The yield of the alcoholic extract was 14.72 % w/w. Ethyl acetate soluble fraction was obtained by dissolving 20 g of alcoholic extract in 40 ml of alcohol (90%) and 40 ml of ethyl acetate. Then it was extracted with each of 4 X 40 ml of ethyl acetate. Ethyl acetate fractions were then mixed in a porcelain dish and evaporated to dryness on a water bath. The yield of ethyl acetate soluble fraction was 10.2 % w/w. The alcohol extract and its ethyl acetate fractions were tested and confirmed for presence of flavones (shinode's test) and iridoid glycosides (Trim & Hill, 1952).

Animals: Swiss mice of both sex (20-25 g) and albino adult Wister rats of both sex 150-200 g) were obtained from the animal house of Pharmacology Department, A.K College of Pharmacy Tamilnadu, India and were acclimatized to laboratory conditions for 1 week before the start of the study. The study protocol was approved by the institutional animal ethics committee for the purpose of control and supervision of animals, New Delhi. The 80 mice were housed in clean polypropylene cages and were fed with standard pellet diet and water *ad libitum*

Acute toxicity study: Acute toxicity study was performed as per OECD-423 guidelines (Ecobichon, 1997; Periyanayagam *et al.,* 2006). Mice of either sex were divided into seven groups of ten animals each. The control group received 5 ml kg^{-1} of 0.5 % w/v carboxymethylcellulose orally. The other groups received 100, 200, 400, 800, 1000, 2000 and 3000 mg kg^{-1} of alcohol (90%) extracts in 0.5 % w/v sodium carboxymethylcellulose, respectively. Immediately after dosing, the animals were observed continuously for the first 4 h for behavior, and then kept under observation up to 72 h post treatment for any toxic symptoms and mortality.

Anti-inflammatory activity

Carrageenan -induced rat paw edema: The rats were divided into six groups, each consisting of six animals. Edema was induced by subplantar injection of 0.1 ml of freshly prepared 1% w/v carrageenan suspension into the right hind paw of each rat. The paw volume was measured at 0 and 3 h after the injection of carrageenan by using a plethysmometer (Winter *et al.,* 1962). The alcohol extracts of *A. hyssopifolium* at 300 and 600 mg kg^{-1} and its fractions 25 and 50 mg kg^{-1} were administered orally to the six groups of rats. The first and second groups received 5 ml kg^{-1} of 0.5 % w/v carboxy methylcellulose as vehicle control and 10 mg $kg^{-1}$of diclofenac sodium as drug control, respectively,

for comparative pharmacological assessment. Drug pretreatment was given 1 h before the injection of carrageenan.

Turpentine oil-induced granuloma pouch in rat: Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injection of 2 ml of air, followed by injection of 0.5 ml of turpentine oil into it (Selye, 1953; Robert *et al.,* 1957). Control, standard and extracts were administered orally 1 h prior to turpentine oil injection and continued for seven consecutive days. On the eighth day the pouch was opened under anesthesia, the amount of exudates was taken out with a syringe, the volume was measured and compared with those of the control and standard group of animals.

Formalin induced rat hind paw edema: About 0.1 ml of 2 % v/v formalin was injected into the sub plantar area of the right hind paw of ether anaesthetized rat (Chau, 1989). All drugs were given orally 1 h prior to formalin injection and continued for seven consecutive days. The degree of inflammation was measured plethysmometrically on the 1st and 7th day.

Statistical analysis: The statistical significance of differences between the groups was assessed by means of variance followed by Dunnett's tests. Values are expressed as mean ± S.E.M. and p values less than 0.001 were considered significant.

RESULTS

Acute toxicity study: Alcoholic extract *of A. hyssopifolium* did not cause any mortality upto a dose of 3000 mg kg^{-1}. Hence 1/10th and 1/5th of this dose, 300 $mgkg^{-1}$ and 600 $mgkg^{-1}$ were used for anti-inflammatory activity studies.

Anti-inflammatory activity: The results showed that the alcohol extract of *A. hyssopifolium* at 300 and 600 mg kg^{-1} p.o, and its ethyl acetate fractions at 25 and 50 mg kg^{-1} p.o., exhibited significant anti-inflammatory activity in all the experimental models. The alcohol extract at 600 mg kg^{-1} exhibited maximum inhibition (61.10%) of paw edema and its ethyl acetate fractions at 25 and 50 mg kg^{-1} concentrations showed 38.8 and 66% inhibition in carrageenan induced rat paw edema, while diclofenac sodium showed 74.7% inhibition of edema volume after 4 h of treatment in rats (Table 1).

Table 1: Effect of alcoholic extract of *Adenema hyssopifolium* in carrageenan induced rat hind paw edema

Treatment	Dose $mgkg^{-1}$ p.o	Paw volume increase after 3 h (ml)	Percentage of inhibition
Control	--	1.03 ± 0.05	--
Diclofenac sodium	10	0.26 ± 0.04*	74.7
Alcoholic extract	300	0.65 ± 0.05*	36.8
Alcoholic extract	600	0.40 ± 0.04*	61.1
Ethyl acetate fraction	25	0.63 ± 0.04*	38.8
Ethyl acetate fraction	50	0.35 ± 0.12*	66.0

Each value is the Mean ± S.E.M (n=6). Statistical differences from the control were determined by ANOVA followed by Dunnett's test; *p < 0.001 vs control

The results of the turpentine oil induced granuloma pouch method (Table 2) showed 51.60 % edema suppression at a dose of 600 mg kg^{-1} of alcohol extract. The ethyl acetate fractions at a dose of 25 and 50 mg kg^{-1} showed 47.5 and 63.7% inhibition of edema, respectively. The standard drug diclofenac sodium showed 71.7 % inhibition. With the formalin induced rat paw edema method, the alcohol extract at 600 mg kg^{-1} exhibited maximum inhibition of 55.5%. The ethyl acetate fractions at 25 and 50 mg kg^{-1} doses exhibited maximum inhibition of 44.4 and 63.4%, respectively. The standard drug diclofenac sodium showed 68.0% inhibition at a dose of 10 mg kg^{-1} (Table 3).

DISCUSSION

This study was carried out to establish the scientific basis for the traditional use of *A. hyssopifolium* for the treatment of inflammatory disorders. Thus the effect of alcoholic extract and its ethyl acetate fractions were tested on different anti-inflammatory models (acute, sub acute and chronic). There has been no previous scientific information of this plant regarding acute toxicity. In our acute toxicity test, neither death nor

symptoms associated with toxicity such as convulsion, ataxia, diarrhea occurred during the 72 h observation period. These results indicate the effectiveness and relative safety of the alcoholic extract for the treatment of conditions associated with inflammation.

Histamine and serotonin are the important inflammatory mediators that increase vascular permeability (Rang & Dale, 1999; Linari *et al.,* 2002). Alcoholic extract of *A. hyssopifolium* and its ethyl acetate fractions inhibited the paw edema induced by histamine and serotonin at the third hour after injection. This activity is due to the presence of flavonoids and swertiamarin in *A. hyssopifolium* (Desai *et al.,* 1966; Rai & Takar, 1966; Ghosal &Jaiswal, 1980). Granuloma pouch technique was modified (Robert & Nezamis, 1957) using turpentine oil as irritant.

Ethyl acetate fraction of *A. hyssopifolium* has shown potential inhibitory action on exudates formation. Inhibition of formalin-induced pedal edema in rats is one of the most suitable test procedures for anti-inflammatory agents (Greenwald, 1991). This model has been employed to evaluate the transudative and proliferative components of chronic inflammation. Both the alcoholic extract of *A. hyssopifolium* (600 mg kg^{-1}) and its ethyl acteate fractions (25 and 50 mg kg^{-1}) inhibited inflammatory response of formalin. These findings justify the usefulness of alcoholic extract and its ethyl acetate fractions in the treatment of inflammation.

Table 2: Effect of alcoholic extract of *Adenema hyssopifolium* in turpentine oil induced granuloma pouch

Treatment	Dose mgkg^{-1} p.o	Volume of exudates (ml)	Percentage of inhibition
Control	--	2.48 ± 0.04	--
Diclofenac sodium	10	0.7 ± 0.05*	71.7
Alcoholic extract	300	1.71 ± 0.05*	31.0
Alcoholic extract	600	1.20 ± 0.05*	51.6
Ethyl acetate fraction	25	1.3 ± 0.05*	47.5
Ethyl acetate fraction	50	0.9 ± 0.09*	63.7

Each value is the Mean ± S.E.M (n=6). Statistical differences from the control were determined by ANOVA followed by Dunnett's test; *p < 0.001 vs control

Table 3: Effect of alcoholic extract of *Adenema hyssopifolium* in Formalin induced rat hind paw oedema

Treatment	Dose mgkg^{-1} p.o	Paw volume increase on 7^{th} day (ml)	Percentage of inhibition
Control	--	1.25 ± 0.08	--
Diclofenac sodium	10	0.40 ± 0.03*	68.0
Alcoholic extract	300	0.82 ± 0.03*	34.9
Alcoholic extract	600	0.56 ± 0.04*	55.5
Ethyl acetate fraction	25	0.70 ± 0.06*	44.4
Ethyl acetate fraction	50	0.46 ± 0.02*	63.4

Each value is the Mean ± S.E.M (n=6). Statistical differences from the control were determined by ANOVA followed by Dunnett's test; *p < 0.001 vs control

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