



Anti-inflammatory Activity of Whole Plant Extract of *Andrographis echinoides* Against Carrageenan Induced Paw Edema

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Accepted on: 23-02-2016; Finalized on: 31-03-2016.

ABSTRACT

This study was intended to evaluate the anti-inflammatory activity of ethanol extract of *Andrographis echinoides* whole plant in carrageenan induced paw edema in albino rats. The whole plant extract of this plant were administered orally at doses of 100, 200 and 400 mg/kg body weight and the study was compared with a standard drug indomethacin (10 mg/kg). The highest dose of *Andrographis echinoides* whole plant extract (400 mg/kg) produced a maximum inhibition of 85.44% at 3 hours after administration which was more effective. Further detailed studies are in progress for the isolation of single entity responsible for anti-inflammatory activity and development of suitable formulations.

Keywords: *Andrographis echinoides*, Inflammation, Albino rats, Indomethacin.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is the body defence reaction in order to eliminate or limit the spread of injurious agents. There are various components in an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain.

Inflammation is a part of host defence mechanism. There are several tissue factors that are known to be involved in the inflammation reactions such as release of histamines, bradykinin and prostaglandins. There are two types of inflammation and they are acute and chronic. The signs of acute inflammation are warmth, redness, pain, swelling and loss of function. The signs of chronic inflammation is long lasting pain, redness and swelling and is caused by the persistence of an irritant, which may be biological, physical or chemical in nature.¹ Eventhough most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are in search for natural anti-inflammatory drug without any diverse effect.²

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and still remain as main new source of structurally important chemical substances that lead to the development of innovative drugs.^{3,4} The use of medicinal plants for the treatment of diseases is associated with folk medicine from different parts of the world.^{5,6} Nowadays the search for new anti-inflammatory agents from the huge array of medicinal plant resources is intensifying.⁷ In fact a variety of bioactive components have been shown to modulate inflammatory responses.⁸

Andrographis echinoides (Acanthaceae) which is commonly known as 'false water willow' is an herb commonly found

throughout India. The genus *Andrographis* is native of India contains 28 species of small annual shrubs essentially distributed in tropical Asia. Some of them are medicinally important. The plants from genus *Andrographis* are used in goiter, liver diseases⁹, fever, fertility problems, bacterial¹⁰, malarial, helminthic, fungal, diarrhea and larvicidal disorders.^{11,12} Leaf juice boiled with coconut oil is used to control falling and graying of hair.¹³ The literature survey revealed that there is very little pharmacological information on this plant and thus prompted us to analyze this plant.

The present study focuses on evaluating the anti-inflammatory activity of whole plant of *Andrographis echinoides*. To our knowledge no report on the effect of this plant on experimental information. This study was therefore undertaken to evaluate the effect of ethanol extract of whole plant of *Andrographis echinoides* on anti-inflammatory activity in carrageenan induced rat paw edema.

MATERIALS AND METHODS

The whole plant of *Andrographis echinoides* were collected from Surandai, Tirunelveli District, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of Plant Extract for Anti-inflammatory Activity

The dried aerial parts of *Andrographis echinoides* were 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 antiinflammatory activity.

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±2°C) 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*.

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.¹⁴ 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 upto 2000 mg/kg body weight.

Antiinflammatory Activity

Carrageenan Induced Hind Paw Edema

Albino rats of either seven or six weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group - II, III and IV – ethanol extract of *A. echioides* (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o.), Group V – Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard antiinflammatory drug.

After one hour of the 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., 60 mins., 120 mins., 180 mins., 240 mins., 360 mins., and 480 mins. 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:

$$\text{Percentage inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where, V_t represents the percentage difference in increased paw volume after the administration of test drugs to the rats and V_c 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

In the present study, the antiinflammatory activity of ethanol extract of *A. echioides* was assayed in Albino rats using carrageenan induced paw edema (acute inflammatory) method. Table 1 shows that the antiinflammatory activity of ethanol extract of the whole plant of *A. echioides* significantly inhibited the rat paw edema at 3rd hr post carrageenan were 74.44%, 81.66% and 85.44% for 100, 200 and 400mg/kg of ethanol extract of *A. echioides* respectively. It shows that the plant extract have significant ($p < 0.001$) antiinflammatory effects and the results were compared with indomethacin (10 mg/kg) and showed percentage paw volume reduction of 85.25%.

Table 1: Effect of *A. echioides* extract on the percentage inhibition of Carrageenan induced paw edema

Treatment Groups	Edema volume (ml)					% Inhibition after 180 min
	Dose mg/kg	0 min	60 min	120 min	180 min	
Group-I	Normal saline	29.16±1.26	88.16±1.53	108.65±1.38	143.54±1.67	-
Group-II	100mg/kg	32.18±1.06	52.18±1.92*	43.85±1.15**	32.68±1.56***	74.44
Group-III	200 mg/kg	30.86±1.92	45.80±1.54**	36.18±2.18***	26.32±1.39***	81.66
Group-IV	400 mg/kg	28.15±1.03	41.16±1.88***	29.16±1.67***	20.88±0.98***	85.44
Indomethacin (Group-V)	10 mg/kg	29.96±1.18	42.88±1.54	28.22±1.13***	21.16±1.08***	85.25

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$, Compared paw edema induced control vs drug treated rats.



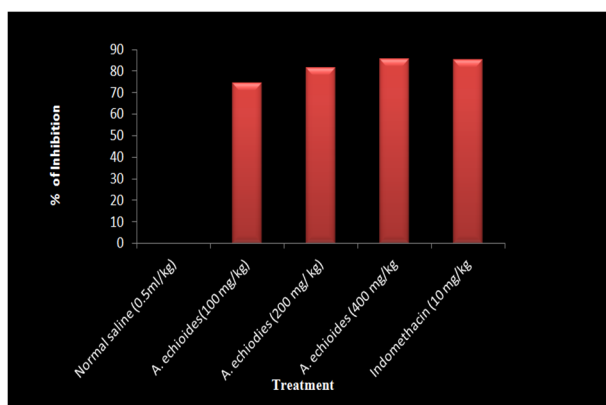


Figure 1: Effect of *A. echioides* whole plant extract on the percentage of inhibition after 180 mins.

DISCUSSION

Carrageenan-induced edema has been commonly used as an experimental model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the surrounding damaged tissues. On the other hand, the late phase is sustained by prostaglandins and mediated by bradykinin and leukotrienes, produced by tissue macrophages.^{15,16,17}

The probable mechanism of antiinflammatory action of extract may be due to its influence on the second phase of inflammation the cyclooxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan^{18,19}. There are also evidences that compounds inhibiting the carrageenan induced edema are effective in inhibiting the enzyme cyclooxygenase.

Based on these reports, the inhibitory effect of *A. echioides* extract on carrageenan induced inflammation could be mediated via this mechanism. Thus, the results of the study would support the traditional use of *A. echioides* in some painful and inflammatory conditions. It can be concluded that the ethanol extract of *A. echioides* has antiinflammatory activity against carrageenan induced paw edema in rats. This activity may be due to their content of flavonoids, steroids, saponins and carbohydrates. The ethanol extract of *A. echioides* (400 mg/kg) showed better activity profile compared to the low dose hence it can be said to possess majority of the activity. This study demonstrates the efficacy of ethanol extract of *A. echioides* as an antiinflammatory agent and also scientifically justifies the use of this plant as an antiedematous agent in folk medicine, however further studies are required to determine the constituents responsible for its antiinflammatory activity and further authenticate its mechanism of action.

Acknowledgement

The Authors wishes to thank Dr. R. Sampatharaj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur, for their assistance in animal studies.

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Source of Support: Nil, **Conflict of Interest:** None.

