PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY POTENTIAL OF DIFFERENT EXTRACTS OF *ANDROGRAPHIS ECHIOIDES* (L.) NEES STEM

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ABSTRACT

This study was designed to evaluate the anti-inflammatory effect of hexane, ethyl acetate, ethanolic and aqueous extracts of *Andrographis echioides (L.) Nees* (family: Acanthaceae) stems in rats. In our investigation, young adult male Wistar rats weighing 200–250 g were employed. The anti-inflammatory effects of A. echioides stem extracts in hexane, ethyl acetate, ethanolic, and aqueous form were investigated on rat paw oedema brought on by subplantar injections of carrageenan (0.5 ml/kg). For comparison, indomethacin (50 mg/kg p.o.) was utilised as the standard anti-inflammatory drug. The ethyl acetate and ethanolic extracts of A. echioides stems at the dosages of 200 and 400 mg/kg p.o considerably decreased the oedema in rats' paws brought on by subplantar injections of carrageenan.

However, the ethyl acetate and ethanol extracts of the plant produced relatively greater and more pronounced anti-inflammatory effect than hexane and aqueous extracts brought about in the experimental animal model used. The ethanol extract was proved to be a potent as indomethacin than remaining all extracts of stems of *A. echioides.* The results of this experimental animal study indicate that the ethanolic extract of stems of *A. echioides* possess anti-inflammatory activity, and thus lend credence to the suggested folkloric use of the plant in the management and control of inflammatory conditions in India.

Keywords: *Andrographis echioides*; Acanthaceae; Indomethacin; Anti-inflammatory activity

1. INTRODUCTION

Andrographis echiodies plant contains more number of branchlets upto 50 cm long and leaves are elongated form with approximately parallel sides to broad rounded apex and a tapering base and are sub-sessile with glandular hairs on both abaxial and adaxial surfaces. Its stem is slightly quadrangular with hairs on its surface. The plant shows raceme type of inflorescence not exceeding the leaves and is scarcely branched. The calyx of the flower is with sub equal lobes and lanceolate with glandular hairs. Corolla is white with brown tinge and it is tubular, showing the 2+3 lipped condition, which are unequal. It has stamens-2 which is exserted and straight, style slender, with capitate stigma. The capsules are ovoid, sparsely hairy, pointed above and narrowed below. The average number of the capsule per plant is 38, seed are yellow in colour and ovoid. Four seeds per capsule, 1.5mm across and glabarous [1].

Andrographis echioides, commonly known as ‘false water willow’, is a herb commonly found throughout India. The plant *Andrographis echioides* is traditionally used to treat goiter, liver diseases, fever, fertility problems, bacterial [2], malarial, helmintic, fungal, diarrhea and

larvicidal disorders [3-4]. Leaf juice boiled with coconut oil is used to control hair falling and graying [5].

An exhaustive literature review on *Andrographis echioides (L.) Nees* has revealed that this plant is used for many diseases and it was found that the anti-inflammatory activity was not carried out on the stems so for, except leaves. Therefore, the present study was under taken to search for anti-inflammatory activity of hexane, ethyl acetate, ethanol and aqueous extracts of *Andrographis echioides (L.) Nees* stems.

Around the world, musculoskeletal disorders (including osteoarthritis and rheumatoid arthritis) are the most prevalent cause of chronic impairment and even fatality [6]. The medications with antiinflammatory activity are the first choices in the pharmacological therapy of these degenerative inflammatory joint diseases among the several currently accessible therapies [7]. According to a large body of research, the cyclooxygenase (COX-1 and COX-2) and lipoxygenase (5-LOX) pathways are among the principal enzymatic pathways connected to inflammatory processes in mammalian cells, and they are particularly relevant from a clinical standpoint [8]. Non-steroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, ibuprofen, naproxen, and indomethacin) and selective COX-2 inhibitors (e.g., rofecoxib) have significant side effects despite their well-documented efficacy. For example, the former can cause internal bleeding when the stomach wall collapses [9], whereas the latter can [10-11].

For these reasons, it is currently recommended that one of the promising methods for the more effective treatment of inflammatory illnesses is the use of medicines that specifically inhibit COX-2 and 5-LOX [12]. Therefore, there is still a lot of interest in the discovery and development of new medicines from natural sources without side effects with dual COX-2/5-LOX inhibitory characteristics [13-14].

2. MATERIAL AND METHODS

Plant material:

Plant material was collected from Mangalagiri region, Guntur, A.P, during September 2017 and authenticated by Dr. P. Satyanarayana Raju, Department of Botany, University College of Sciences, Acharya Nagarjuna University, A.P, India. Voucher specimens were deposited in the herbarium of University College of Pharmaceutical Sciences (UCPS 15 dated 30 Sep 2017), Acharya Nagarjuna University, A.P, India**.**

Extraction of Plant Material:

The stems of the plant were dried in shade, pulverized and passed through a 40-mesh sieve. Dried powder (1 kg) was taken and subjected to successive extraction with hexane, ethyl acetate, ethanol and water in soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in desiccators and weighed. The yield of the hexane (AEHE), ethyl acetate (AEEA), ethanol (AEEE) and aqueous (AEAE) extracts were 3.2 g, 7.6 g, 9.3 g and 8.3 g respectively.

Phytochemical Screening:

Standard screening tests [15] were employed in screening the extracts for identifying different constituents. Conventional for detecting the presence of alkaloids, tannins, flavonoids and steroids, etc. were utilized.

Animals:

Male and female wistar albino rats weighing 200-250 g were used in the experiment. The animals were maintained under standard husbandry conditions in the animal house of the University College of Pharmaceutical Sciences, Acharya Nagarjuna University, A.P, India. The animals were kept at controlled temperature (25 ± 2 °C) in the natural light-dark cycle and had free access to feed and water. The study was approved by the Institutional animal ethical committee of Ministry Of Culture, Govt of India (516/01/a/CPCSEA).

Drugs:

Indomethacin was utilized as reference anti-inflammatory drug and carrageenan as inflammatory agent.

Acute toxicity testing:

The median lethal doses (LD_{50}) of the plant extracts were determined as described in detail by Lorke [16]. Mice fasted for 12 h were randomly divided into different groups each of which comprised of six animals. The Hexane, ethyl acetate, ethanol and aqueous extracts of *A. echiodes* at the doses of 100, 200, 400, 800 and 1600 mg/kg were separately administered orally to mice in each of the 'test' groups. Each of the mice in the 'control' group was treated with distilled water (2 ml/kg p.o). The mice in both the 'test' and 'control' groups were then allowed free access to food and water, and observed over a period of 24 h for signs of acute toxicity. The number of deaths (caused by the extracts) within this period of time were recorded.

Carrageenan-induced paw oedema in rats:

Paw oedema was induced in rats according to the method of winter [17] by the injection of a 0.1% carageenan (Sigma Chemical Company, USA) suspension in the sub plantar region of the right hind paw. Rats in the reference group received indomethacin (50 mg/kg); while rats in the control group received drug vehicle (10 ml/kg, po). The hexane, ethyl acetate, ethanol and aqueous plant extracts at the doses of 100, 200 and 400 mg/kg were administered orally to the animals 30 minutes before carrageenin injection. The details of administration of standard drug and different extracts at different doses were shown in the table 1. The hind paw volume was measured just initially before and at 1, 2, 3, 4, 5 & 6 hr after carrageenan administration using plethysmometer. The difference in left and right hind paw volume represents the degree of inflammation. Percentage inhibition of oedema was calculated by the formula [18].

$$\text{Percentage inhibition} = \left(1 - \frac{v_t}{v_c}\right) \times 100$$

Where v_t and v_c indicate mean relative changes in paw volume of the test and control respectively.

Statistical Analysis:

Results were analyzed using One way ANOVA method and expressed as Mean ± SEM. The statistical significance considered was $P < 0.05$ (confidence limit: 95%).

3. RESULTS AND DISCUSSION

Phytochemical Screening:

Phytochemical screening of hexane, ethyl acetate, ethanol and aqueous extracts revealed the presence of alkaloids, glycosides, flavonoids, triterpenoids, tannins, saponins and steroids.

Acute Toxicity:

Intraperitoneal administration of graded doses of hexane, ethyl acetate, ethanol and aqueous extracts of *A. echioides* stems in mice did not produce any significant change in the autonomic or behavioral responses during the observation period. The acute toxicity test results probably suggest that the plant is relative safe in mammals.

Effect on Carrageenin- Induced Paw oedema In Rats:

As shown in Table 1, 3 hr after injection, carrageenan-induced rat paw oedema was markedly inhibited by intraperitonial pretreatment either with extracts or Indomethacin (50 mg/kg). Ethyl acetate & ethanol extracts each at a dose of 400 mg/kg body wt produced highly significant anti-inflammatory effect and the later at the dose of 400 mg/kg body wt has shown almost as same as the effect produced by Indomethacin (Table 1).

It was reported that the development of edema after a subplantar injection of carrageenan in the animal is attributed to the release of histamine, serotonin, kinins and prostaglandins [19-20]. Oedema induced by egg albumin results from the release of histamine and serotonin [21]. The suppression of PG production, which is mediated by COX, at least shares the mechanism of action with other anti-inflammatory drugs [22]. COX has two isoforms, COX-1 and COX-2, each of which expresses itself differently in different cell types. A physiological level of PGs for healthy platelet, stomach, and renal function has been said to be provided by COX-1. Contrarily, COX-2 has been discovered to be strongly upregulated at inflammatory areas in both animals and people who have inflammatory disorders [23&24], and as a result, it is thought to be in charge of producing pro-inflammatory PG.

Table1: Percentage inhibition of Carrageenan induced paw oedema in rats by prophylactic treatment with Ibuprofen and different extracts of *Andrographis echioides*.

Animals of different groups treated with standard drug and different extracts		Percentage inhibition of the maximal paw oedema during 6h.	Percentage inhibition of total AUC paw oedema during 6h.
Andrographis echioides hexane extract (AEHE)	Group I (Drug vehicle) Group II(Ibuprofen (50 mg/kg)) Group III (AEHE-100 mg/kg) Group IV (AEHE-200 mg/kg) Group V (AEHE-400 mg/kg)	0.0 ± 0.96 78.27 ± 1.32 *** 36.51 ± 1.36^{NS} 42.37 ± 2.77^{NS} 61.43 ± 2.41 ***	0.0 ± 1.47 88.16 ± 2.08 *** 29.74 ± 2.76^{NS} 48.33 ± 2.27^{NS} 68.19 ± 1.29 ***
Andrographis echioides ethyl acetate extract (AEAE)	Group VI (AEAE -100 mg/kg) Group VII (AEAE -200 mg/kg) Group VIII (AEAE -400 mg/kg)	46.10 ± 1.64** 58.08 ± 1.75 *** 69.62 ± 2.27 ***	49.79 ± 2.14 ** 64.85 ± 1.73 ** * 77.23 ± 2.61 ***
Andrographis echioides ethanol extract (AEEE)	Group IX (AEEE -100 mg/kg) Group X (AEEE -200 mg/kg) Group X I (AEEE -400 mg/kg)	54.78 ± 1.10 ** 65.58 ± 2.38 *** 71.47 ± 1.53 ***	62.04 ± 2.66 ** 73.18 ± 1.75 *** 82.90 ± 2.42 ***
Andrographis echioides aqueous extract (AEAE)	Group XII (AEAE -100 mg/kg) Group XIII (AEAE -200 mg/kg) Group XIV (AEAE -400 mg/kg)	27.75 ± 1.24 * 47.37 ± 1.85 ** 64.31 ± 1.60***	30.15 ± 2.19 * 61.17 ± 1.94 ** 76.43 ± 2.12***

Values are mean ± S.E.M, n=6; Significance: *P<0.05, **P<0.01, ***P<0.001., NS= not significant

Results obtained in the present study provided evidence that all the extracts of *A. echioides* stems possess anti-inflammatory activity. But the AEEA and AEEE extracts at the doses of 200 and 400 mg/kg, p.o exhibited a significant anti-inflammatory activity (*P< 0.001*) than the AEHE and AEAE extracts. The paw edema volume was reduced in a dose dependent manner and it was comparable to that of Indomethacin (50 mg/kg, p.o.). The secondary metabolites alkaloids, glycosides, flavonoids, triterpenoids, tannins, saponins and steroids which are identified from plants have been discovered to possess significant anti-inflammatory effects. Safayhi and Sailer [25] as well as many other authors concluded that anti-inflammatory effect is a common property of many triterpenoids [26-29]. It is, therefore, possible that the anti-inflammatory effects observed with these extracts may be attributable to its secondary metabolites alkaloids, glycosides, flavonoids, triterpenoids, tannins, saponins and steroids.

IV CONCLUSION

These results support the traditional uses of the *A. echioides* in the treatment of different diseases and also suggest the presence of biologically active principles that are alkaloids, glycosides, flavonoids, triterpenoids, tannins, saponins and steroids might be responsible for the anti-inflammatory activity of the ethyl acetate extract of *A. echioide.* Further investigations are going on to isolate and characterize the specific biologically active principles responsible for anti-inflammatory activity from the plant extracts.

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