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ANTI-INFLAMMATORY ACTIVITY OF *ALSTONIA SCHOLARIS* IN ALBINO RATS

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ABSTRACT: Evaluation of anti-inflammatory activities along with the phytochemical screening of hot methanolic extract of *A. scholaris* stem bark (ASE) in albino rats was undertaken. The preliminary phytochemical screening of the plant revealed the presence of tannins, alkaloids, saponins, phystosterols, phenolic compounds, glycoside and flavonoids. Oral LD50 of ASE by limit test was found to be above 2000 mg/kg. Two dose level of 200 (1/10 LD50) and 400 mg/kg (1/5 LD50) were selected for studying the anti -inflammatory activity of ASE using the carrageenan - induced acute paw oedema model in rats. The extract showed significant ($p < 0.01$) dose dependent reduction in rat paw oedema. The percentages of inhibition of oedema were 42.55 and 53.19 with 200 and 400 mg/kg, p.o. doses of ASE, respectively, as compared to control. The anti-inflammatory action of ASE can be attributed to its flavonoid contents, which are known to act through inhibition of prostaglandin biosynthesis.

Key Words: *A. scholaris*, Albino rats, Oral LD50, Acute paw oedema, Anti-inflammatory, Flavonoid.

INTRODUCTION

Alstonia scholaris Linn. R. br. (Chhatiyan) is widely found in India in Sub-Himalayan region from the Yamuna eastward, abundantly found in West Bengal and South India (Nandkarni 1976). Its bark, known as "Dita Bark" is traditionally used as stimulant, carminative, stomachic, bitter tonic, astringent, aphrodisiac, expectorant and febrifuge. It is also useful in chronic diarrhoea, catarrhal fever, malarial fever, leprosy, skin diseases, pruritis, tumors, chronic and foul ulcers, asthma, bronchitis, agalactia and debility (Nandkarni 1976, Kirtikar 1980). The effect of ethanolic extract of leaves of *Alstonia scholaris* was evaluated in experimental models of pain and inflammation (Arulmozhi *et al.* 2007). There was a significant inhibition in carrageenan induced paw edema by the extract. The present study aims to explore the anti inflammatory activities of hot methanolic *A. scholaris* stem bark extract (ASE).

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Table 1: Effect of hot methanolic extract of *A. scholaris* stem bark on carrageenan-induced rat-paw oedema (Mean with SE of 6 replicates)

Group	Treatment	Mean Rat paw Vol. (ml) ± SE		Mean Rat paw Oedema Vol. (ml) ± SE (B-A)	Decrease in Oedema (%)
		0h (A)	3h(B)		
I	Normal saline + carrageenan	0.73±0.02	1.20±0.03	0.47±0.19	--
II	Phenylbutazone (100 mg/kg) + carrageenan	0.73±0.05	0.91±0.05	0.13**±0.05	72.34
III	ASE (200mg/kg) + carrageenan	0.83±0.02	1.10±0.00	0.27**±0.10	42.55
IV	ASE (400 mg/kg) + carrageenan	0.82±0.03	1.03±0.03	0.22**±0.08	53.19

****P< 0.01 compared to group I**

MATERIALS AND METHODS

Alstonia scholaris-sample preparation

Stem bark of *Alstonia scholaris* was procured in bulk in the month of February-March from dairy farm unit of College of Veterinary Science & Animal Husbandry, Anjora, Durg, India and was botanically identified. The stem bark was properly cleaned, shade dried and ground into a fine powder. Hot methanolic extract of the bark powder was prepared in the soxhlet's apparatus. The soluble part was concentrated over a boiling water bath and was stored in air tight containers and used when required. The crude extract was suspended in physiological saline for administration to albino rats.

Animals

Twenty four young weaned Wistar rats (100 - 150g) were obtained from a registered laboratory animal breeder. The animals were grouped and housed in polyacrylic cages and maintained in an air conditioned Lab. Animal House attached to the Department of

Pharmacology & Toxicology. All animals were fed with standard laboratory animal diet with free access to clean drinking water. The animals were acclimatized to the laboratory conditions for 10 days before commencement of experiment. All the experimental protocol were approved by the Institutional Animal Ethical Committee (IAEC), College of Veterinary Science & Animsl Husbandry(Anjora), Durg (CG), India and were in accordance to the guidelines of the CPCSEA, Ministry of` Forests and Environment, Government of India.

Acute oral toxicity study

Limit test was performed as per OECD guideline for testing of chemicals (OECD 1998) to evaluate the acute oral toxicity of ASE in female albino rats with the upper limit dose of 2000 mg/kg. The mortality, behaviour and signs and symptoms of toxicity, if any, were recorded for a period of 14 days of post administration.

Phytochemical screening

ASE was qualitatively tested for the presence of phytochemical constituents such as tannins, alkaloids, saponins, phytosterols, phenolic compounds, glycosides and flavonoids. They were identified by characteristic colour changes using standard procedures (Raaman 2006).

Anti-inflammatory activity

This test was performed, using rat-paw oedema model induced by phlogistic agent carrageenan, according to the method described by Winter *et al.*(1962). Twenty four male albino rats weighing between 120 and 150g kept off-fed for 16 h (but having free access to drinking water), were selected for the study. The rats were randomly assigned to four groups, each containing six animals (Table1). The Group I animals served as control which received normal saline orally. The Group II rats were orally administered with the reference anti-inflammatory drug, phenylbutazone at 100 mg/kg p.o. The Groups III and IV animals received the test extract orally at 200 and 400 mg/kg, p.o. respectively. The above treatments were given to all the rats of different groups, 1h before giving the injection of the phlogistic agent into the foot. The phlogistic agent, carrageenan, prepared as 1% suspension in sterile normal saline was injected (0.1ml) into the planter aponeurosis of right hind paw of each rat with the help of a 26 gauze needle. The volume of the injected-paw of each rat was measured immediately after carrageenan injection (0 h) and subsequently after 3 h. Increase in foot volume at 3 h after phlogistic agent was adopted as a measure of the effect and the difference in paw volume (at 3 h minus 0 h) was adopted as a measure of the inflammatory effect (oedema). The paw volume was measured by water displacement method using plethysmometer (Bhat *et al.* 1977). The percentage of inhibition of oedema was calculated from the difference in oedema volume between treated and control groups.

STATISTICAL ANALYSIS

Data were expressed as mean ± SE. The results were analysed by one-way ANOVA followed by Dunnett's t test.

RESULTS AND DISCUSSION

In acute oral toxicity study no death was recorded, and therefore acute oral LD50 of ASE in rats was considered to be more than 2000 mg/kg. The anti-inflammatory activity of ASE

was assessed by measuring the reduction in carrageenan-induced paw oedema in rats. The mean oedema volume of rats of Group I (control), II (phenylbutazone) and III and IV (ASE 200 and 400 mg/kg, p.o.) were 0.47±0.19 ml, 0.13±0.05 ml, 0.27± 0.10 ml and 0.22±0.08 ml, respectively. The results showed that ASE (200 and 400 mg/kg, p.o.) produced significant ($P< 0.01$) inhibition of carrageenan-induced acute paw oedema in rats compared to control. The percentages of inhibition of oedema were 42.55 and 53.19 at 200 and 400 mg/kg doses of ASE, respectively compared to untreated control. Phenylbutazone (100 mg/kg), however, inhibited the oedema volume by 72.34%. The above result demonstrates that ASE has good anti-inflammatory activity against carrageenan - induced acute paw-oedema in rats (Table 1).

Carrageenan-induced hind paw oedema is the standard experimental model of acute inflammation. The injection of carrageenan into the hind feet of the rats causes local oedema and this process forms the basis of commonly used test for non-steroidal anti-inflammatory drugs (NSAIDs). The early vascular changes and the oedematous phase following disruption of permeability as induced by carrageenan were effectively antagonized by ASE. Carrageenan-induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins; whereas, the second phase is related to the release of prostaglandins and slow reacting substances, which peak at 3 h (Vinegar *et al.* 1969). The anti-inflammatory activity of ASE could be because of its antagonistic action of these mediator(s).

Phytochemical screening of ASE revealed the presence of alkaloids, saponins, phytosterols, phenolic compounds, tannins, flavonoids and glycosides. Interestingly, compounds like flavonoids, have been shown to possess anti-inflammatory activity (Kim *et al.* 2004), and the claim made by Attaway and Zaborsky (1993) that compounds with anti-inflammatory activity also possess anti-nociceptive activity seems to support our present findings on ASE. Flavonoids are potent inhibitors of nitric oxide synthase type 2 that are involved in the synthesis of NO (Olszanecki *et al.* 2002) via indirect blockade of the cyclo-oxygenase and/or lipoxygenase pathways (Robak *et al.* 1998), and of the protein kinase C and L -arginine/NO pathways (Meotti *et al.* 2005) that are known to take part in a series of molecular events leading to anti-nociceptive (Machelska *et al.* 1997) and anti-inflammatory (Kim *et al.* 2004) activities. Further, Middleton *et al.*(2000) reported on the flavonoid's potential to inhibit phospholipase A_2 and phospholipase C, which are important enzymes in a cascade of inflammatory process. Therefore, the anti-inflammatory activity of ASE, might be also associated with the similar inhibitory effects of flavonoids in the cascade of inflammatory processes.

CONCLUSION

The promising anti-inflammatory activity of ASE warrents further studies to establish its clinical usefulness in the alleviation of inflammation and painful conditions in man and animals.

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