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Antiinflammatory activity of fractionated extracts of *Achyranthes aspera* Linn leaves

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ABSTRACT

The present work has been designed to evaluate the potency of anti-inflammatory activity of different fractions of ethanolic extract of *Achyranthes aspera* leaves. Carrageenan induced rat paw oedema method was used for screening. Ethanolic, ethyl acetate and hexane fraction was screened among which ethyl acetate fraction was found to be most potent one with percentage inhibition of 50, 74, 84, 86% at 1st to 4th hour respectively. By this experiment it seems that leaves of *Achyranthes aspera* can be used for the treatment of acute inflammation.

Keywords: Antiinflammatory activity, *Achyranthes aspera*, Carrageenan induced rat paw oedema.

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INTRODUCTION

Traditional and folk remedies have provided us with important drugs in the treatment of many diseases and are being increasingly subjected to scientific study. The family of anti-inflammatory drugs is no exception. Salicylates had their origin in the 'willow bark' of folk medicine. Paracetamol, Cortisone, gold salts, and Phenylbutazone made their way into clinical medicine serendipitously (Gross, 1973). Inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis and other connective tissue diseases are a major cause of morbidity. Anti-inflammatory agents have been traditionally evaluated by studying their effect on inflammation produced in animals by injecting foreign or noxious agents (Ghosh MN., 1984). Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation (Reynold, 1993; Roberts & Marrow, 2001). Thus there is still a need to develop newer and safer anti-inflammatory drugs. *Achyranthes aspera* is most widely found plant belonging to the family Amaranthaceae. It is also known as Apamarga, Latjira, Uttaranee and Safed aghedo in various regional languages. It is widely distributed throughout India and other countries in Wastelands, road sides and open filed. The whole plant, the roots and the seeds possess the medicinal properties against many ailments. Ethanopharmacological studies depicted its use in dropsy, skin eruptions, colic, as a diuretic, astringent and purgative (Bhatnagar et al., 1973; Raj et al., 1978) as an antidote for snake bite (Selvanayagum et al., 1995). The inflorescence is used in cough. The seeds are employed as an emetic, purgative, and cathartic, in gonorrhoea, whooping cough, as an anti-asthmatic and for insect bite (Raj et al., 1978; Reddy et al., 1989). Different fractions of ethanolic extract of *A.aspera* were screened for its anti-inflammatory activity by carrageenan induced rat paw oedema method.

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MATERIALS AND METHOD

Animals

Twelve week-old healthy Wistar rats (150–200 g) of either sex procured from inbred facility of Srinivas College of Pharmacy, Mangalore were used for this study. They are maintained under standard conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity $60 \pm 5\%$ and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libidum. The institutional animal ethics committee (IAEC) approved the protocol to carry out the experiment.

Plant material

The plant leaves were collected in the monsoon season along the hilly areas, waste lands and road sides from Mangalore District, Karnataka state, India. The taxonomic authentication of the plant was done by Dr. Ummanabad Srinivasa, Professor, Department of Pharmacognosy and Phytochemistry, Srinivas college of Pharmacy, Mangalore. A voucher specimen USMO-3/2011 is preserved in our research laboratory for future reference. The collected leaves were shade dried, coarsely powdered and stored in a closed container for further use.

Preparation of extract

Leaves of *A.aspera* (100gm) were extracted by 70% ethanol by Soxhlet apparatus. It was then concentrated and dried in hot air oven. Dried extract was fractionated using different solvents in increasing order of polarity i.e. hexane, ethyl acetate and chloroform and ethanol. Upon these chloroform fraction was not used for the experiment due to very low percentage yield. All the extracts were dried and stored in desiccator until further use. For experimental method the dried extracts were suspended in 1% gum tragacanth in normal saline and used for anti inflammatory activity.

Drugs and chemicals

Gum tragacanth (HIMEDIA), carrageenan (HIMEDIA), Indomethacin, ethanol, hexane, chloroform and ethyl acetate were procured from different suppliers.

Assessment of anti-inflammatory activity

The anti-inflammatory test was carried out using a carrageenan induced rat hind paw oedema as a model for acute inflammation. Animals were divided into five groups each comprising six animals. One group kept as control and treated with 1% gum tragacanth suspension at 2ml/kg dose. Another group was kept as standard and treated with indomethacin (20mg/kg). Three groups were used as test and treated with various extracts (hexane, ethyl acetate and ethanolic) at dose of 400mg/kg by intraperitoneal route.

Oedema was induced by giving sub planter injection of 0.1 ml of 1% carrageenan into left hind paw prior to injection of test extracts and standard. The increase in the paw volume was measured from 0th hour to 4th hour. The plethysmograph apparatus was used for the measurement of rat paw volume. The percentage

of edema inhibition was calculated. Anti-inflammatory activity was expressed as "mean increase in paw volume \pm SEM" in terms of ml and percentage inhibition in paw volume by different extracts.

RESULT AND DISCUSSION

Various fractions of ethanolic extract of *A.aspera* caused marked inhibition in inflammation as shown in **table 1**. Upon three fractions ethyl acetate fraction caused most significant effect 50, 74, 84, 86% inhibition at 1st to 4th hour respectively. Other like ethanolic fraction showed 40, 30, 26 and 44% while hexane fraction showed 34, 32, 36 and 37% inhibition. Inflammation continues to be an area of a great interest for research, due to the non-availability of safer and more effective anti-inflammatory agents. The present study revealed some of the pharmacological basis for the ethnomedicinal use of *A.aspera* in the treatment of inflammation. The ethyl acetate fraction of ethanolic extract of *A.aspera* showed a good anti-inflammatory activity against acute inflammation, suppressing the rat paw oedema both at the early and later phases. The early phase of oedema, beginning from 1 h after the administration of the irritant, is due to the release of histamine and serotonin, while the later phase, occurring from 3 to 5 h after the administration of the irritant is induced by bradykinin, protease, prostaglandin and lysosome (Wallace, 2002; Harriot et al., 2004). Extracts of *A.aspera* had shown to suppress the release of various mediators in both early and late phase of inflammation. More over ethanolic fraction also decreased the release of mediators in early phase but effect on chronic inflammation was not satisfactory.

Table:1 Anti-inflammatory activity of *Achyranthes aspera* leaves.

Drug	Dose	Δ paw volume(ml)			
		1 st hour	2 nd hour	3 rd hour	4 th hour
Control(1% tragacanth)	2ml/kg	0.30 \pm 0.01	0.50 \pm 0.02	0.62 \pm 0.02	0.57 \pm 0.01
Indomethacin	20mg/kg	0.12 \pm 0.02 60%	0.24 \pm 0.02 52%	0.26 \pm 0.01 58%	0.21 \pm 0.02 63%
Ethanolic fraction	400mg/kg	0.18 \pm 0.02 40%	0.35 \pm 0.02 30%	0.46 \pm 0.03 26%	0.32 \pm 0.01 44%
Ethyl acetate fraction	400mg/kg	0.15 \pm 0.01 50%	0.13 \pm 0.02 74%	0.10 \pm 0.01 84%	0.08 \pm 0.01 86%
Hexane fraction	400mg/kg	0.20 \pm 0.01 34%	0.34 \pm 0.01 32%	0.40 \pm 0.01 36%	0.36 \pm 0.02 37%

All the values are expressed in mean \pm SEM

CONCLUSION

It was concluded that the various fractions of ethanolic extract of leaves of *A.aspera* possess anti-inflammatory activity. Amongst all, ethyl acetate fraction is having most potent activity. Further study is requiring for isolation and identification of active compounds and to confirm exact mechanism.

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