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Comparative in vitro evaluation of anti-inflammatory effects of aerial parts and roots from *Mikania scandens*

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ABSTRACT

The present study was conducted to evaluate and compare the anti-inflammatory effects of hydroalcoholic extracts of aerial parts and roots from *Mikania scandens* against the denaturation of protein in vitro. The test extracts at different concentrations were incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present results exhibited a concentration dependent inhibition of protein (albumin) denaturation by both the test extracts. The root extract was found to be more active than aerial parts extract. Form the present findings it can be concluded that both the aerial parts and roots from M. scandens possessed marked anti-inflammatory effect against the denaturation of protein in vitro.

Key words: Anti-inflammatory, *Mikania scandens*, aerial parts, roots, protein denaturation

INTRODUCTION

Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. [1] The commonly used drug for management of inflammatory conditions are non steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric

irritation leading to formation of gastric ulcers. [2, 3] Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Mikania scandens (L.) Willd. (Asteraceae), known as climbing hemp weed in English, is a twining herbaceous climbing vine with long-petioled, opposite leaves and small homogamous flower-heads, grown as a common weed throughout the plains of India and Bangladesh. Traditionally, the plant has been used for some medicinal purposes in the Indian subcontinent. Aqueous leaf extracts of this plant have been used in folk medicine to treat stomach ulcers. The plant is thought to be efficacious in the treatment of gastric problems. Traditionally its leaf juice is applied to the affected area of body in treatment of wounds and bruises. The plant is regarded as a rich source of vitamin A and C and also contains vitamin B, mikanin, friedelin, efifriedinol, and some sesquiterpene dilactones including mikanolide, dihydromikanolide, deoxymikanolide, and scandenolide. Three deterpenic acids known as kaurenic acid, butyryloxykaurenic acid, and benzoyloxykaurenic acid, stigmasterol and betasitosterin have also been isolated from this plant. [4-6]

It has come to the author's notice that the rural people of Hooghly, Bardhaman, and Medinipur districts of West Bengal state of India use the young leaves of this plant in management of insect bites and stings. Previous researchers have reported analgesic and in vitro antioxidant activities of M. scandens leaf. [7] In our previous study, we have reported some important neuropharmacological properties of M. scandens aerial parts. [8] The present study was conducted to evaluate and compare the anti-inflammatory effects of M. scandens aerial parts and roots against the denaturation of protein in vitro.

MATERIALS AND METHODS

Plant material

The entire plant of M. scandens was collected during June-July, 2011 from Gotan region of Bardhaman district of West Bengal, India. The species was authenticated by Dr. P. Lakshminarasimhan, Scientist D, at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India, and a voucher specimen

(CNH/44/2011/Tech.II/476) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology, Delhi Road, Sugandha, Hooghly 712102, India. Just after collection, the plant material was washed thoroughly with running tap water and shade dried at room temperature (24-26 ºC) and ground mechanically into a coarse powder.

Drugs and chemicals

Diclofenac sodium was obtained from Organic Chemical Industries Pvt. Ltd., Kolkata 70001, West Bengal, India. Double distilled water from all-glass still was used throughout the study.

Preparation of aerial parts extract (HAMS)

The powdered plant material was first defatted by using petroleum ether (60°-80°C). The defatted plant material (45 g) was extracted with 50% aqueous ethanol (400 ml) by boiling under reflux for 90 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (HAMS, yield: 11.78%). The dry extract was kept in a refrigerator until use. HAMS was subjected to preliminary phytochemical studies as per reported methods. [9]

Preparation of root extract (RMS)

The powdered plant material (50 g) was extracted with 50% aqueous ethanol (400 ml) by boiling under reflux for 90 minutes. The extract was filtered and evaporated to dryness in vacuo to yield the dry extract (RMS, yield: 8.35%). The dry extract was kept in a vacuum desiccator until use. RMS was also subjected to preliminary phytochemical studies as per reported methods. [9]

In vitro anti-inflammatory bioassay

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extracts (HAMS and RMS) so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in a BOD incubator (Labline Technologies) for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/ml) was used as reference drug and treated similarly for determination of absorbance. The

percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times [V_t / V_c - 1]$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined form dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

There are certain problems associated with use of animals in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available, or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of hydroalcoholic extracts of M. scandens aerial parts and root (HAMS and RMS). Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of tissue proteins in vivo. [10, 11] Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

In the present study the in vitro anti-inflammatory effect of HAMS and RMS was evaluated against the denaturation of egg albumin. The results are summarized in Tables 1 and 2. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by both the test extracts throughout the concentration range of 31.25 to 1000 µg/ml. The RMS was found to be more active than HAMS. Diclofenac sodium (at the concentration range of 78 to 2500 µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 3); however, the effect of diclofenac sodium was found to be less as compared with HAMS and RMS. This was further confirmed by comparing their IC_{50} values (Table 4).

The increments in absorbance of test sample with respect to control indicated stabilization of protein i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extracts and the reference drug diclofenac sodium. [12]

Preliminary phytochemical analysis revealed the presence of flavonoids, steroids, alkaloids, tannins, saponins, and carbohydrates in HAMS; whereas alkaloids, saponins, phenolics and carbohydrates in RMS. Polyphenols are well known natural products known to possess several notable biological properties. [13] In the present study, the in vitro anti-inflammatory effect of HAMS and RMS may be due to their polyphenols contents. The effect may be due to synergistic effect rather than single constituent.

Table: 1. Effect of HAMS on protein denaturation

Concentration (µg/ml)	% Inhibition
Control	-
31.25	20
62.5	40
125	80
250	120
500	200
1000	280

Table: 2. Effect of RMS on protein denaturation.

Concentration (µg/ml)	% Inhibition
Control	-
31.25	20
62.5	40
125	100
250	180
500	360
1000	1000

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH. [14] Therefore, form the findings of the present preliminary study it can be concluded that both the aerial parts and roots of *Mikania scandens* had marked anti-inflammatory effect against the denaturation of protein in vitro. It is suggested that anti-inflammatory effect of this plant should be further evaluated in other experimental models in pursuit of newer phytotherapeutics against inflammation.

Table: 3. Effect of diclofenac sodium on protein denaturation.

Concentration (µg/ml)	% Inhibition
Control	-
78.125	12.5
156.25	12.5
312.5	25
625	50
1250	212.5
2500	812.5

Table: 4. IC_{50} values of HAMS, RMS and diclofenac sodium against protein denaturation.

Treatments	IC_{50} values (µg/ml)
HAMS	78.125
RMS	71.81
Diclofenac sodium	625

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