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Preliminary *in vitro* assessment of anti-inflammatory property of *Mikania scandens* flower extract

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ABSTRACT

The present experiment was conducted to assess the anti-inflammatory property of hydroalcoholic extract of the flowers from *Mikania scandens* against the denaturation of protein *in vitro*. The test extract at different concentrations was incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to evaluate 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 the test extract. The effect of diclofenac sodium was found to be less when compared with the test extract. Form the present findings it can be concluded that the *M. scandens* flower possessed marked anti-inflammatory effect against the denaturation of protein *in vitro*.

Key words: Anti-inflammatory, *Mikania scandens*, flower, protein denaturation.

INTRODUCTION

Inflammation is a pathophysiological 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 most 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] Recently, we have also reported the *in vitro* anti-inflammatory effects of its aerial parts and root. [9] The present experiment was conducted to assess the anti-inflammatory property of *M. scandens* flower against the denaturation of protein *in vitro.*

MATERIALS AND METHODS

Plant material

The flowers of *Mikania scandens* (L.) Willd. (Asteraceae), were collected during December, 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 present study.

Preparation of extract (FMS)

The powdered plant material was first defatted by using petroleum ether (60°-80°C). The defatted plant material (22 g) was extracted with 50% aqueous ethanol (180 ml) by boiling under reflux for 80 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (FMS, yield: 27.86 %). The dry extract was kept in a refrigerator until use. FMS was subjected to preliminary phytochemical studies as per reported methods. [10]

Anti-inflammatory bioassay *in vitro*

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 extract (FMS) 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. [9, 11] 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 the 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 extract of *M. scandens* flower. 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.* [12, 13] 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 FMS was evaluated against the denaturation of egg albumin. The results are summarized in Table 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the test extract throughout the concentration range of 31.25 to 1000 µg/ml. Diclofenac sodium (at the concentration range of 78.125 to 2500 µg/ml) was used as the reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 2); however, the effect of diclofenac sodium was found to be less as compared with that of FMS. This was further confirmed by comparing their IC_{50} values (Table 3).

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 extract and the reference drug diclofenac sodium. [14]

Preliminary phytochemical analysis revealed the presence of alkaloids, saponins, polyphenols, reducing sugars and carbohydrates in FMS. Polyphenols are well known natural products known to possess several notable biological properties. [15] In the present study, the *in vitro* anti-inflammatory effect of FMS may be due to its polyphenols contents. The effect may be due to synergistic effect rather than single constituent.

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. [16] Therefore, form the findings of the present preliminary experiment it can be concluded that the flower 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 inflammatory diseases.

Table: 1. Influence of FMS against protein denaturation

Concentration (µg/ml)	% Inhibition
Control	-
31.25	6.15
62.5	42.92
125	84.61
250	134.61
500	464.61
1000	1262.30

Table: 2. Influence of diclofenac sodium against 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: 3. IC_{50} values of FMS and diclofenac sodium against protein denaturation

Treatments	IC_{50} values (µg/ml)
FMS	73.44
Diclofenac sodium	625

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