ANTI-HISTAMINE ACTIVITY OF AQUEOUS EXTRACT AND MACROMOLECULAR FRACTION OF *Psychotria sarmentosa* LEAVES

W.M.K.M. Ratnayake*[1], U.G. Chandrika[1], A.M. Abeysekera[2], T.S. Suresh[1] and N. Salim[3]

[1]Department of Biochemistry, Faculty of Medical Sciences; [2]Department of Chemistry, and [3]Department of Botany, Faculty of Applied Sciences; University of Sri Jayewardenepura

**Corresponding author (email: kratnayake1984@gmail.com)*

Introduction

Psychotria sarmentosa Blume (named "Gonica" in Sinhala; Family: Rubiaceae) is a twinning shrub with oblanceolate or elliptic leaves growing in low elevation forests (< 700 m) in Southern and Southwestern parts of Sri Lanka. It has a long history of being used in folk medicine in Sri Lanka. Mainly, immature leaves are consumed in the form of a traditional porridge as well as a tempered vegetable salad. Indigenous healers prescribe an aqueous extract of leaves for individuals who have been physically assaulted, indicating that it may possess potent analgesic and/or anti-inflammatory activity. Previous studies have shown that aqueous extract of *P. sarmentosa* has significant anti-inflammatory activity on carrageenan induced rat paw oedema model which is widely used for determining the acute phase of the inflammation characterized with involvement of different chemical mediators such as histamine, serotonin and prostaglandins. Only a very few scientific studies have been carried out to investigate mechanisms of anti-inflammatory activity of leaves of *P. sarmentosa*. Hence, it is worth conducting an investigation which is focused to evaluate the mechanisms of anti-inflammatory potential. The general objective of the present study was to determine the anti-histamine effect of *P. sarmentosa* and its ethanol insoluble macromolecular precipitate.

Methodology

Plant material

Fresh *P. sarmentosa*, stems with leaves were purchased from local market and authenticated by Dr. D.S.A. Wijesundara, Director General, Department of National Botanic Gardens, Peradeniya, Sri Lanka.

Preparation of plant extracts

Aqueous extract of *P. sarmentosa* was made by grinding 100.0 g of fresh leaves with 200.0 mL of water in a mortar and pestle. The filtered extract was boiled and freeze dried. The macromolecular fraction was precipitated from the aqueous extract by slowly adding five volumes of absolute ethanol while stirring. The precipitate was obtained by centrifugation at 3000 rpm for ten minutes and it was washed several times with absolute ethanol. Then it was dried in a desiccator. The required amounts of each sample were dissolved in distilled water for oral administration to rats.

Ethical clearance

The protocol for animal experiment was approved by the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka (No. 35/15). International guidelines and recommendations of Federation of European Laboratory Animal Science Associations (FELASA) were followed for handling of

animals. Assay was carried out at the Animal House of University of Sri Jayewardenepura, Sri Lanka.

Animals

Healthy adult male, Wistar rats weighing 150-200 g were purchased from Medical Research Institute, Colombo 8, Sri Lanka. Rats were housed under standard conditions with a natural light-dark cycle and fed with standard diet and water *ad libitum*. The animals were acclimatized for at least one week to the laboratory conditions prior to the experiment.

Assay for anti-histamine activity

Twenty-four Wistar rats were randomly selected and grouped as positive control, negative control and two test groups (n=6/group). Fur on left lateral side of the back of these rats was shaved and after 24 h, the test groups received 100 mg of freeze dried aqueous extract (FDAE) per kg of body weight and ethanol insoluble macromolecular precipitate (EIMP) per kg of body weight. The positive and the negative control groups received chlorpheniramine (0.67 mg/kg) and 1.0 mL distilled water respectively. After 1 h histamine dihydrochloride in saline (50 µL of 200 µg/mL) was injected subcutaneously into the skin where the fur has been removed and 2 min. later the area of the wheal formed was measured. Anti-histamine activity was calculated compared to the respective controls by using following formula;

$$\% \text{ Inhibition of wheal formation} = [(A_{N.C} - A)/A_{N.C}] \times 100$$

Where, $A_{N.C}$ is the area of the wheal of negative control and A, is the area of the wheal of test. Data analysis was carried out using one-way analysis variance (ANOVA). Results with $p<0.05$ were considered as statistically significant.

Results and Discussion

The results of the area of the wheal formed in each group are presented in Table 1. The FDAE and EIMP significantly inhibited wheal formation on the skin of the rat after injection of histamine when compared to the control group. But, there was no significant difference between two groups which were received 100 mg/kg of FDAE and EIMP.

Table 01. Area of the wheal formation in anti-histamine assay on Wistar rat

Group	Area of wheal ± SEM (cm^2)
Control	1.86 ± 0.12
FDAE (100 mg/kg)	1.20 ± 0.04*
EIMP (100 mg/kg)	1.01 ± 0.04*
Chlorpheniramine (0.67 mg/kg)	0.81 ± 0.05*

Values are expressed as mean ± SEM (n=6); p<0.001, compared to control group.

The percentage of inhibition of wheal formation in each group is showed in Figure 1. The FDAE and EIMP significantly inhibited wheal formation by 35.5% and 45.7% respectively ($p<0.001$) when compared to the control group. This anti-histamine activity was comparable to that of the reference drug, chlorpheniramine which caused an inhibition of 56.4% ($P<0.001$).

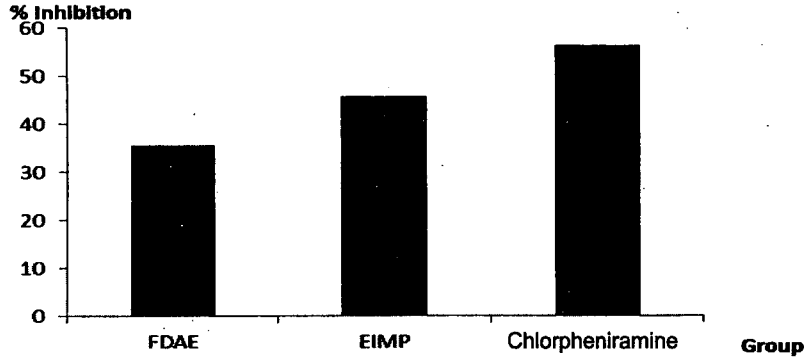

Figure 1. Anti-histamine activity of *P. sarmentosa* on wheal formation test

Inflammation is the biological response of the body to local injury and infection, which is characterized by redness, oedema, fever, pain and loss of function. Previous studies have shown that FDAE and EIMP significantly inhibited the inflammation on carrageenan-induced paw oedema model (Ratnayake *et al.*, 2015). The inflammatory condition induced by carrageenan involves step-wise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase (Ravi *et al.*, 2009). These chemical substances increase the vascular permeability, there by promoting accumulation of fluid in tissues that accounts for the oedema. Hence, the potent anti-histamine effect of FDAE and EIMP would have contributed to its anti-inflammatory activity.

Conclusions and Recommendations

The present study on aqueous extract of *P. sarmentosa* leaves and its' macromolecular fraction has demonstrated that it has significant anti-histamine properties and it justifies the traditional use of this plant in the treatment of various types of inflammation.

References

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