PROPHYLACTIC AND THERAPEUTIC CHRONIC ANTI-INFLAMMATORY EFFECT OF *Psychotria sarmentosa* LEAVES ON ADJUVANT-INDUCED ARTHRITIS RAT MODEL

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Introduction

Chronic inflammatory diseases remain one of the world's major health problems. Hence, there is a great deal of interest in the field of medical research for the inflammatory response. As a result of adverse effects of existing allopathic anti-inflammatory drugs, there is an attraction for investigations of the efficacy of plant based drugs used in the traditional medicine.

Psychotria sarmentosa Blume (named "Gonica" in Sinhala ; Family: Rubiaceae) 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. Our previous studies have shown that aqueous extract of leaves of this plant has significant anti-inflammatory activity on carrageenan induced rat paw oedema model which is widely used for determining the acute phase of the inflammation, as well as on formaldehyde induced paw oedema model which is a model for sub-chronic inflammation [1]. All of these scientific findings contribute to solicit the anti-inflammatory activity of this plant. Hence, in the present study an attempt has been made to evaluate its' prophylactic and therapeutic action on chronic inflammation on adjuvant-induced arthritis rat model.

Material and Methods

Plant material

Fresh *P. sarmentosa*, stems with leaves were purchased from a local market and authenticated by Dr. D. S. A. Wijesundara, Director General, Royal Botanical 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 required amount of sample was 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. 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 150-250 g were purchased from the Medical Research Institute of 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.

Preventive and therapeutic assays for adjuvant-induced arthritis

Wistar rats were randomly selected and grouped (n=6/ group) for each assay. Group I of each served as healthy control animals and all other groups were induced with arthritis by a single intra-dermal injection of 0.1 mL of suspension of Freund's Complete Adjuvant (FCA) containing 0.05%w/v *Mycobacterium butyricum* in to a footpad of the left hind paw [2]. Among the arthritis induced animals negative and positive control groups were orally administered, 1.0 mL of water and 20 mg of celecoxib / kg b. w respectively. The test group received 100 mg / kg of b.w of the freeze dried aqueous extract of *P. sarmentosa* leaves (FAPL). For the study on the preventive effect, adjuvant was injected in the day 0 and animals were orally treated as above daily from day 0 to day 19. For the therapeutic effect study, adjuvant was injected in the day 0 and animals were orally treated from day 14 to day 28. The body weight, thickness of hind paw pad and thickness of ankle joint were periodically measured for every 3-4 days.

All the results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one way analysis of variance test (ANOVA) to determine the significance of the difference between the control and test groups. *p*- values < 0.05 were considered as statistically significant.

Results & Discussion

A significant enhancement in thickness of hind paw pads and ankle joints was observed in all the adjuvant injected all groups as compared to healthy control rats in both experiments. In adjuvant injected rat group, swelling and redness developed in the injected hind paw and ankle joint and it reached maximum intensity on day 3 (first swelling phase). Thereafter, swelling slowly subsided until the tenth day and then began to swell again (second swelling phase). Oral administration of FAPL as well as the celecoxib from the day of adjuvant injection (day O) significantly ($p < 0.05$) suppressed the swelling phases. As shown in Fig.1 and Fig.2, the maximum percentage of inhibition of oedema formations at hind paw and ankle joint were found to be 79.7% and 72.0% respectively at 19^{th} day for the group that received FAPL, while it was 81.2% and 70 % for celecoxib group.

In the therapeutic assay, there was no significant difference in oedema formation in all adjuvant injected rats until 14th day ($p > 0.05$). The oral administration of FAPL and celecoxib which was the positive control, were started from day 14 and there were significant ($P<0.05$) reductions of oedema formation in both group as compared with negative control group which received distilled water. As indicated in the Fig. 3 and Fig. 4, the maximum percentage inhibition of oedema formations at hind paw and ankle joint were found to be 66.7% and 76.6% respectively at the 28th day for the group that received FAPL, while it was 57.3% and 70.2 % for celecoxib group.

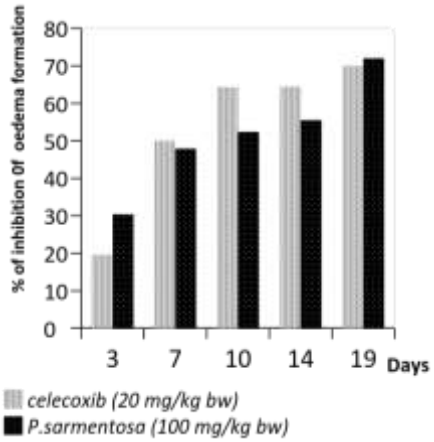

Figure1. The percentage inhibition effect on hind paw oedema formation in adjuvant induced arthritis rat model for prophylactic action

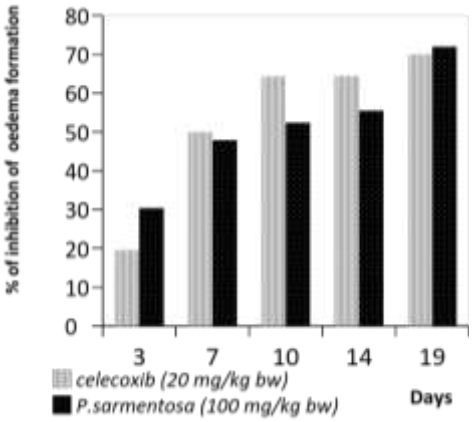

Figure2. The percentage inhibition effect on oedema formation at ankle joint in adjuvant induced arthritis rat model for prophylactic action

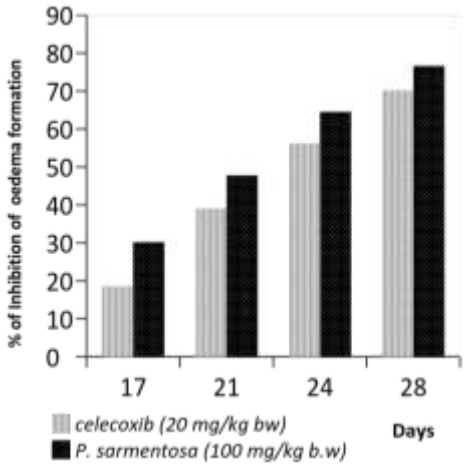

Figure3. The percentage inhibition effect on hind paw oedema formation in adjuvant induced arthritis rat model for therapeutic action

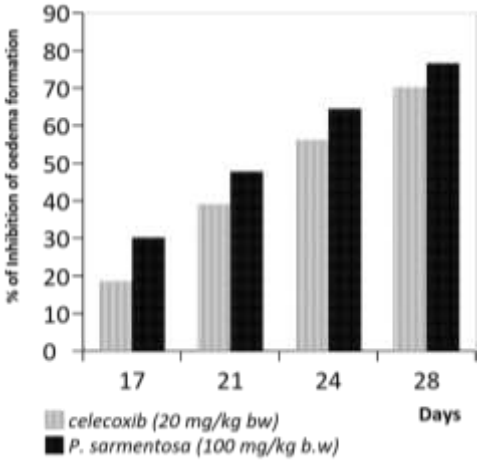

Figure4. The percentage inhibition effect on oedema formation at ankle joint in adjuvant induced arthritis rat model for therapeutic action

In both experiments (prophylactic and therapeutic), FCA injected rats showed a marked reduction in body weight gain as compared with the healthy, non-injected rat group. This reduction is significantly ($p < 0.05$) low in FAPL and celecoxib groups as compared with negative control group which received distilled water.

Rat adjuvant induced arthritis is a commonly used animal model for preclinical studies of non-steroidal anti-inflammatory drugs and it is suggested as the most convenient model for studying drugs affecting human arthritis which generates a chronic inflammatory response. In this study aqueous extract of *P. sarmentosa* leaves was able to suppress the symptoms on joint inflammation. Further, it also proved effective in preventing the disease formation.

Conclusions and Recommendations

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

Acknowledgement

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References

[1] W.M.K.M. Ratnayake *et al.*, *"Anti-inflammatory activity of aqueous extract of Psychotria sarmentosa on acute and chronic inflammatory animal (Wistar rat) models",* (In) Proceedings of 3rd Annual Secession of Sri Lanka Association for Laboratory Animal Science (SLALAS),2016, p. 22.

[2] S. G. Suke *et al.,* "Anti-arthritic and anti-inflammatory activity of combined pioglitazone and prednisolone on adjuvant-induced arthritis", European Journal of Pharmacology,2013, 718, 57-62.