Research Article

Study on anti-inflammatory potential of crude aqueous leaf extract of *Pterocarpus marsupium*

K. Janani, R. Gayathri*, V. Vishnu Priya

ABSTRACT

Aim: This study aims to analyze the anti-inflammatory property of crude aqueous leaf extract of *Pterocarpus marsupium* (PM). **Introduction:** PM belong to the Fabaceae family. The useful parts of this plant are leaves, heartwood, flowers, and gum. It is odorless, astringent in taste, and sticks in the teeth coloring saliva red in color. PM is widely used in Ayurveda and Rasayana for the management of various metabolic disorders. All active principles of PM are thermostable. **Materials and Methods:** The collected leaves of PM are well dried, powdered to prepare the crude aqueous leaf extract, and then subjected to albumin denaturation assay and human red blood cell (HRBC) membrane stabilization method to analyze the anti-inflammatory activity of PM. **Results:** By doing albumin denaturation assay, maximum inhibition of 78% was observed at 0.4 mg/ml. Diclofenac, a standard anti-inflammation drug, showed the maximum inhibition of 80% at the concentration of 0.4 mg/ml. Followed by HRBC membrane stabilization method, PM exhibited effective anti-inflammatory activity by inhibiting hemolysis to a greater extent. Maximum inhibition of 82% was noted at 0.5 mg/ml while diclofenac showed maximum inhibition of 87% at 0.5 mg/ml. **Conclusion:** Inflammation is the body's natural defense mechanism. At times, it becomes negative as it prolongs the treatment time. From this study, it is evident that PM possess anti-inflammatory properties.

KEY WORDS: Anti-inflammatory activity, Crude aqueous extract, *Pterocarpus marsupium*

INTRODUCTION

From the advent of humankind, medicinal plants have been extensively exploited for curing various diseases. *Pterocarpus marsupium* (PM) is one such typical medicinal plant being used across the world.[1,2] PM belong to the Fabaceae family. The useful parts of this plant are leaves, heartwood, flowers, and gum. It is odorless, astringent in taste, and sticks in the teeth coloring saliva red in color. PM is widely used in traditional medication for the management of various metabolic disorders. All active principles of PM are thermostable. In Nepal, the wooden tumbler prepared from the heartwood of PM is used for drinking water as a traditional remedy for human diseases.[3] Heartwood extract of this plant is proven to possess polyphenolic compounds (such as flavonoids, diphenylpropane derivatives, and sesquiterpenes), which exhibits strong antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer activities and is used for treating diabetes, jaundice, ulcer, gastritis, etc.[4] As a versatile plant, Pterocarpus marsupium has been proved to have broad spectrum of pharmacological actions. This plant has been conventionally used in Ayurveda, Unani, Rasayana, as well as Homeopathic systems of medicine more than 2000 years.[5-7] In India, it can be seen, particularly in the regions of Western Ghats, Karnataka, Kerala, in Gujarat, Madhya Pradesh, Bihar, and Orissa. PM Roxb.-Fabaceae (PM), being indigenous to India, is called Indian Kino Tree or Malabar Tree. Due to the exploitation of the tree for its timber and medicinal bark, its population is decreasing in the wild, and thus, it has been mentioned in the red data book.[8-10] As every part of the tree has been used as domestic remedy against wide range of human ailments from antiquity, Pterocarpus marsupium has become a cynosure of modern medicine.[11-13]

Inflammation is a part of the complex biological process of vascular tissues, in response to harmful

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stimuli.[14-16] Many studies have revealed that pathogens, damaged cells, or irritants trigger the event of inflammation. It is a protective mechanism, through which our body detects and eliminates the stimuli, followed by healing of damaged tissues, organs, and system.[17] An inflammatory response involves macrophages, neutrophils that secrete several anti-inflammatory mediators responsible for the initiation, progression, persistence, regulation, and eventual resolution of the acute state of inflammation (short period). Reports have shown that if resolution does not happen in the acute phase; then, it will turn into a chronic phase. Such chronic inflammation (long period) acts as a burden for healthcare professionals in proceeding the treatments. For instance, chronic inflammation is known to play a role in the development of obesity-associated diabetes secondary to insulin resistance.[18] Inflammation generally occurs when infectious microbes such as bacteria, viruses, or fungi enter the body, resides in certain tissues, and/or circulates in the blood.

In this study, the anti-inflammatory property of PM is evaluated using aqueous extract prepared from the leaves of the plant.

MATERIALS AND METHODS

Collection of Plant Material

PM was purchased in a fresh condition from the herbal health care center, Chennai. Then, the leaves of the plant were plucked off from the plant and then washed up to 3–4 times using distilled water [Figure 1]. The collected leaves were dried in shade nearly 1 week. The well-dried leaves were powdered finely using a blender and stored in an airtight container. Powdered leaves of PM were loaded into Soxhlet extractor and subjected to extraction with ethanol.

Preparation of Leaf Extract

Powdered leaves of PM were put into Soxhlet extractor and subjected to extraction with ethanol. Following extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

Figure 1: Powdered leaves of *Pterocarpus marsupium*

In Vitro Anti-inflammatory Activity of PM by Albumin Denaturation Inhibition Assay

The anti-inflammatory activity of PM was studied using inhibition of albumin denaturation technique which was studied according to the method of Prakash and Dass (2010). The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples, the turbidity was measured at 660 nm (UV–Visible Spectrophotometer Model 371, Elico India Ltd.). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control–Abs Sample) × 100/Abs control.

In Vitro Anti-inflammatory Activity of PM by Human Red Blood Cell (HRBC) Membrane Stabilization Method

A 1.0 mL of test sample of different concentrations (0.1–0.5 mg/ml) in 1 ml of 0.2 M phosphate buffer and 0.5 mL of 10% HRBC suspension, 0.5 ml of 0.25% hyposaline were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared by distilled water instead of hyposaline to produce 100% hemolysis without plant extracts. The percentage of membrane stabilization or protection was calculated using the following formula:

$$\text{Percentage of protection} = \frac{\text{1 - OD of Test Sample}}{\text{OD of Control sample}} \times 100$$

Statistical Analysis

The data were be subjected to statistical analysis using one-way analysis of variance and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $P < 0.05$.

RESULTS AND DISCUSSION

The anti-inflammatory property of leaf aqueous extract based on heat-induced protein denaturation and HRBC stabilization method was performed. Protein denaturation is a process, in which proteins tend to lose their tertiary and secondary structure by applying external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins are deprived of their biological function when denatured. *In vitro*

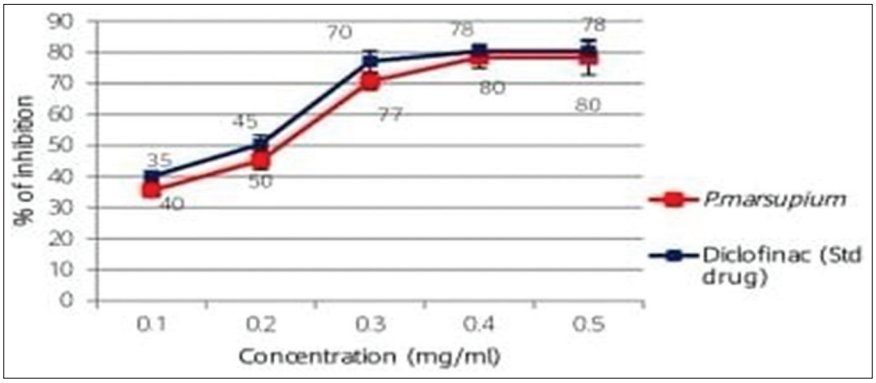

Figure 2: Albumin denaturation inhibition (% inhibition of protein by denaturation). Each line represents mean ± standard error of mean of three independent observations. Significance at $P < 0.05$

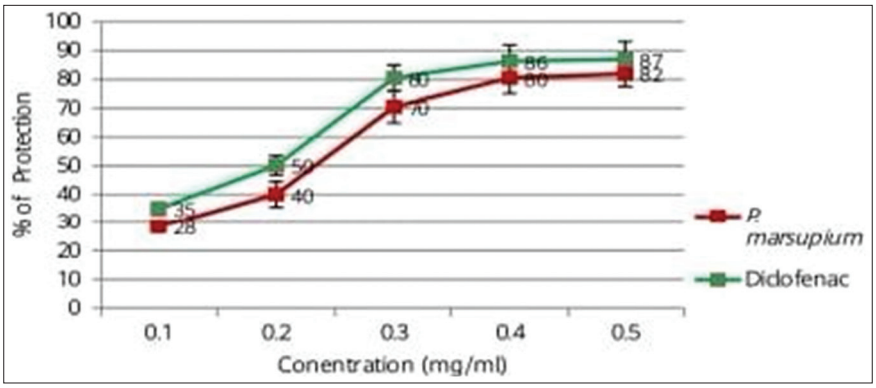

Figure 3: Human red blood cell membrane stabilization. Each line represents mean ± standard error of mean of three independent observations. Significance at $P < 0.05$

stabilization of HRBC membrane by hypotonicity induced membrane lysis is used to estimate the anti-inflammatory property.

By doing albumin denaturation assay, it showed enhanced activity of inhibiting heat-induced albumin denaturation. Maximum inhibition of 78% was observed at 0.4 mg/ml [Figure 2]. Diclofenac, a standard anti-inflammation drug, showed the maximum inhibition of 80% at the concentration of 0.4 mg/ml.

Followed by HRBC membrane stabilization method, PM exhibited effective anti-inflammatory activity by inhibiting hemolysis to a greater extent. Maximum inhibition of 82% was noted at 0.5 mg/ml while diclofenac showed maximum inhibition of 87% at 0.5 mg/ml [Figure 3].

Chronic inflammation can eventually cause several diseases and conditions, including cancer and rheumatoid arthritis. Although a lot of commercial medications such as aspirin, diclofenac, ibuprofen, indomethacin, and naproxen provides relief from inflammation, they might cause severe health issues such as peptic ulcer, allergies, and renal failures on due course of time. From this study, it is evident that PM possess anti-inflammatory properties and can serve as a natural alternative source with no/minimum side effects comparatively. Thus, this study shows that PM can be used as an anti-inflammatory agent.

CONCLUSION

In this study, the anti-inflammatory property of the leaf extract of PM is confirmed. Inflammation is the body's natural defense mechanism. At times, it becomes negative as it prolongs the treatment time. Thus, the crude aqueous leaf extract PM with anti-inflammatory potential acts as a boon to treat patients without any adverse consequences like that of synthetic steroidal and nonsteroidal anti-inflammatory drugs.

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