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Anti-Inflammatory activity of *Pterocarpus indicus* Willd. Stem Bark and Leaves

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ABSTRACT
Inflammation According to WHO, the term inflammation is defined as a vital part of the immune system response to injury and infection. It is the body way of signalling the immune system to heal and repair damaged tissue, as well as defend itself against foreign invaders, such as viruses and bacteria. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reaction that may damage the cells of organisms this is the main reason for causing of inflammation. The effects of inflammation include long-term damage, dysfunction and failure of various organs. Inflammation may have the characteristic symptoms such as pain, redness, immobility, swelling, heat. The main symptoms are mouth sores, chest pain, abdominal pain, fever, rash, joint pain. Anti-inflammatory is the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signalling to the brain. They bind to sulfonylurea receptors on the ß-cell plasma membrane, causing closure to ATP sensitive potassium channels. Therefore in the present study the ethanolic extract of stem bark of Pterocarpus indicus was studied for maximum percentage Stabilization of Anti proteinase action as 70% at 100µg/ml, maximum percentage ofHeat induced haemolysis as 69% at 100µg/ml, maximum Percentage of Anti lipoxygenase activity is 57% at 100µg/ml and leaves also revealed maximum percentage Stabilization of Anti proteinase action as 71% at 100µg/ml, maximum percentage of Heat induced haemolysis as 65% at 100µg/ml, maximum Percentage of Anti lipoxygenase activity as 44% at 100µg/ml using an in vitro model. Diclofenac were used as standard Drugs. The study revealed that the different concentration of extracts exhibit potent radical scavenging activity. Therefore, it is suggested that the ethanolic extract of stem bark and leaves of Pterocarpus indicus is a potential source for natural anti-inflammation activity compounds and could have potential use in the management of Inflammation.
Key Words:*Inflammation, Pterocarpus indicus, lipoxygenase, proteinase, haemolysis*

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INTRODUCTION

Inflammation is a defence response of our body to hazardous stimuli such as allergens and/or injury to the tissues; on the other hand, uncontrolled inflammatory response is the main cause of a vast continuum of disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune diseases imposing a huge economic burden on individuals and consequently on the society[1]. When infectious microorganisms invade the body inflammation usually occurs. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration. Mostly, both the innate immune response as well as the adaptive immune response are involved in the formation of inflammation. The foremost defence mechanism against invading microorganisms and cancer cells is the innate immune system involving the activity of various cells including macrophages, mast cells and dendritic cells. The adaptive immune systems involve the activity of more specialized cells such as B and T cells who are responsible for eradicating invading pathogens and cancer cells by producing specific receptors and antibodies[2-9].

There are various medicines for controlling and suppressing inflammatory crisis; steroids, non-steroid anti-inflammatory drugs, and immune suppressant are the practical examples of these medications which are associated with adverse effects while in practice our goal is to apply minimum effective dose by the highest efficacy with the least adverse effects. Thus, we need to apply natural anti

inflammatory factors within medication therapy to achieve increased pharmacological response and the lowest degree of unwanted side effects [10].

MATERIAL AND METHODS

Collection of Plant Material

Pterocarpus indicus stem bark and leaves were collected from Senthankudi Village, Pudukkottai District, Tamil Nadu, India. The plant was identified, authenticated and confirmed by, Dr. S. John Britto, The Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu.

Preparation of Plant Extract

Pterocarpus indicus stem bark and leaves *were separat*ely washed in running water, cut into small pieces and then shade dried for a week at 35-40^{0}C, after which it was grinded to a uniform powder of 40 mesh size. The extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of ethanol using a soxhlet extractor continuously for 10 hrs. The extracts were filtered through Whatman filter paper No.42 (125mm) to remove all extractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labelled sterile bottles and kept at -40^{0}C. The filtrate obtained was used as sample solution for the further isolation [11].

Anti-Inflammatory Activity of Leaves and Stem Bark of *Pterocarpus indicus*

Anti-Proteinase Action

The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 Mm TrisHCl buffer (pH7.4) and 1 ml test sample of different concentrations (100 −500 µg/ml). The mixture was incubated at 37º C for 5 min and then 1 ml 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction.

Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated [12-13].

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Heat Induced Haemolysis

The reaction mixture (4.5 ml) consists of 2 ml of hyposaline (0.25% w\v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4) and 1 ml of test solution (50 µg\\ml, 100 µg\\ml, 300 µg\\ml, and 500 µg\ml) in isosaline, 0.5 ml of 10% HRBC in isosaline was added. 1 ml of distilled used instead of hyposaline (to produce 100% haemolysis) for test control, while product control lacked red blood cells. The solutions were incubated for 30 min at 37º C and centrifuged at 3000 rpm 20 min. Diclofenac sodium was used as the reference drug. In the suspension, the haemoglobin content was estimated using a spectrophotometer at 560 nm. Percentage membrane stabilizing activity was calculated as follows [14].

$$\% \text{ Membrane stabilization} = \frac{\text{Abs control} - \text{Abs treated}}{\text{Abs treated}} \times 100$$

Anti-lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxydase as enzyme. Test samples were dissolved in 0.25 ml of 2M borate buffer pH9.0 and added 0.25 ml of lipoxydase enzyme solution (20000 U/ml) and incubated for 5 min at 25º C. After which, 1.0 ml of linoleic acid solution (0.66Mm) added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference sample.The percent inhibition was calculated from the following equation,

% inhibition = [{Abs control- Abs sample}/ Abs control] × 100

A dose response curve was plotted to determine IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged [14].

RESULTS AND DISCUSSION

Anti-Proteinase Action

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leucocytes proteinase plays an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided proteinase inhibitors. Anti-proteinase activity at different concentration as shown in Table 1. It showed maximum inhibition of 59%-75% for bark and 49%-71% for leaves at 100 µg\ml. Diclofenac showed maximum inhibition of 40%-66% at 100µg\ml.

Anti-Lipoxygenase Activity

The establishment of new in vitro test system has stimulated the screening of plants aiming to find leads for the development of new drugs. The plant lipoxygenase pathway is in many respects equivalent to the "arachidonic acid cascades" in animals. For this reason the in vitro inhibition of lipoxygenase constitutes the good model for the screening of plant with anti-inflammatory potential. Anti-lipoxygenase activity at different concentration as shown in Table 2. It showed maximum inhibition of 34%-57% for bark and 30%-46% for leaves at 100 µg\ml. Diclofenac showed maximum inhibition of 40%-66% at 100µg\ml.The extracts inhibited lipoxygenase enzyme activity. This indicates that plant is more useful in studies of inflammation and in various related physiological studies, aging and disease such as cancer, neurological disorder etc.

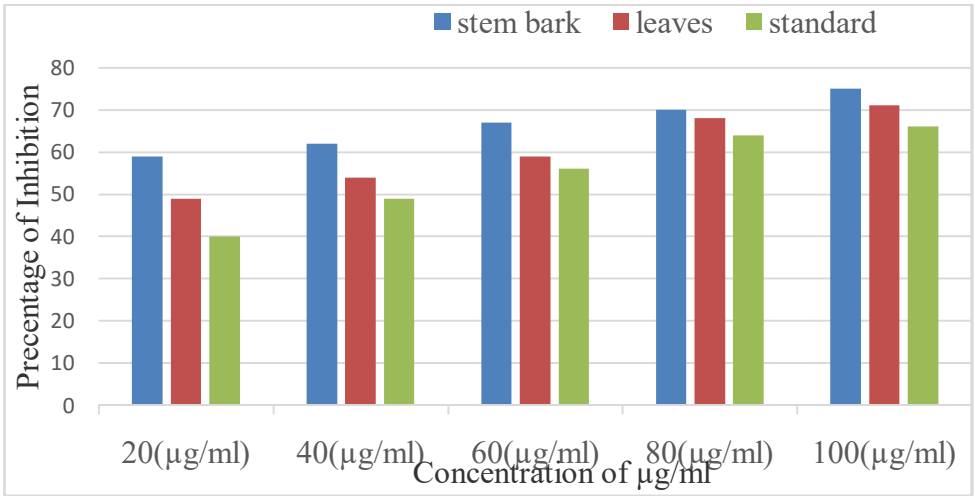
Heat Induced Haemolysis

Heat induced haemolysis activity at different concentration as shown in Table 3. It showed maximum inhibition of 57-75% for bark and 53%-70% for leaves at 100 µg\ml. Diclofenac showed maximum inhibition of 40%-66% at 100µg\ml. The extract was effect in inhibiting the heat induced haemolysis at different concentrations.

In the present study, results indicate that the ethanol extracts of *Pterocarpus indicus* possess anti-inflammatory properties. The strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols may exhibit this activity. The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. Purification of each bioactive compound is necessary and this purified form of the compound can be used which may show increased activity. This study gives on idea that the compound of the plant *Pterocarpus indicus* can be used as lead compound for designing a potent anti-inflammatory drug.

Table 1 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using anti proteinase method and comparison with standard drug diclofenac.

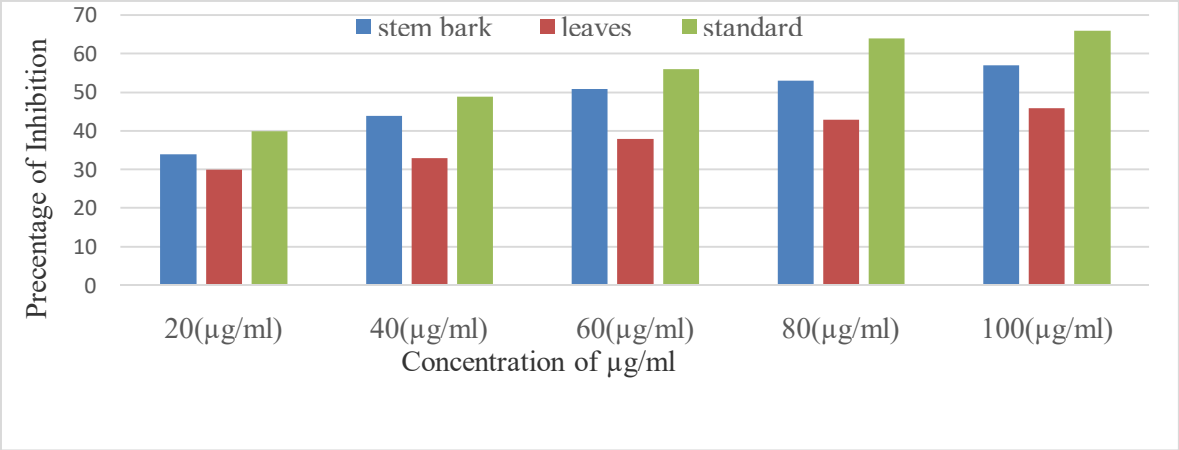
	Concentration (µg/ml)	***P. indicus* Bark**	***P. indicus* Leaves**	**Diclofenac**
1	20	59	49	40
2	40	62	54	49
3	60	67	59	56
4	80	70	68	64
5	100	75	71	66



Graph 1 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using anti proteinase method and comparison with standard drug diclofenac.

Table 2 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using Anti-Lipoxygenase Activity and comparison with standard drug diclofenac.

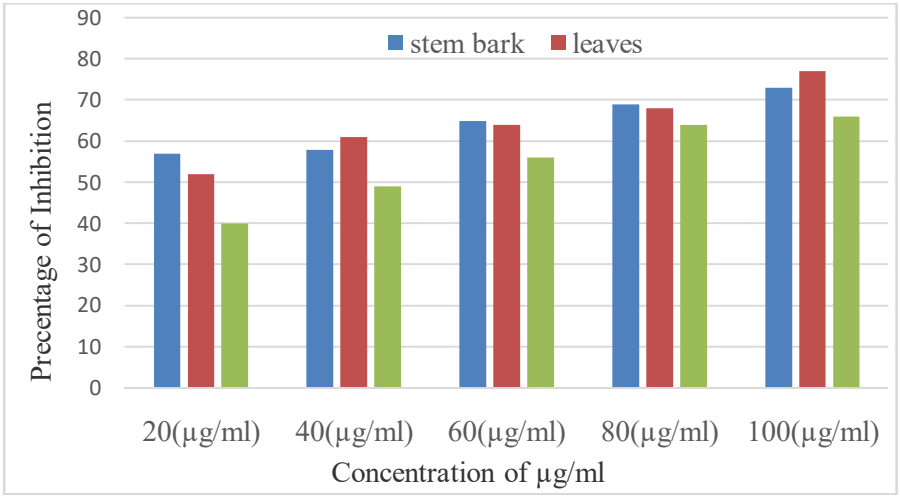
S. No	Concentration (µg/ml)	*P. indicus* Bark	*P. indicus* Leaves	Diclofenac
1	20	34	30	40
2	40	44	33	49
3	60	51	38	56
4	80	53	43	64
5	100	57	46	66



Graph 2 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using Anti-Lipoxygenase and comparison with standard drug diclofenac.

Table 3 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using Heat Induced HaemolysisActivity and comparison with standard drug diclofenac.

S. No	Concentration (µg/ml)	*P. indicus* Bark	*P. indicus* Leaves	Diclofenac
1	20	57	53	40
2	40	60	58	49
3	60	65	61	56
4	80	70	65	64
5	100	75	70	66



Graph 3 *In vitro* Anti-Inflammatory activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using Heat Induced Haemolysis and comparison with standard drug diclofenac.

CONCLUSION

Therefore, it is suggested that the ethanolic extract of stem bark and *Pterocarpus indicus* of *Pterocarpus indicus* is a potential source for natural anti-inflammatory compounds and could have potential use in the management of Inflammation.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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