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Evaluation of Anti-Arthritic Activity by Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus* WILD

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ABSTRACT
Pterocarpus indicus is a medicinal plant which is indicated for the treatment of arthritis in folklore medicine. The present study was aimed at the investigation of anti-arthritic activity in ethanolic extract of leaves and bark of Pterocarpus indicus. The anti-arthritic activity of ethanolic extract of leaves and bark of Pterocarpus indicus was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) in vitro methods. The ethanolic extract of leaves and bark of Pterocarpus indicus was subjected to in vitro study for the inhibition of protein denaturation in various concentrations ie. 20, 40, 60, 80 and 100 µg/ml separately. HRBC method was also used for the estimation of anti-arthritic activity from in various concentrations 20, 40, 60, 80 and 100 µg/ml. The percentage of inhibition for HRBC is 65% for leaves and also revealed for bark 69% and for egg albumin is 78% or leaves and for bark 82% at a concentration 100µg/ml. The ethanolic extract of leaves and bark of Pterocarpus indicus exhibited a concentration dependent inhibition of protein (albumin) denaturation. The present study is concluded that ethanolic extract of leaves and bark of Pterocarpus indicus have more potent anti-arthritic activity.
Key Words: Rheumatoid arthritis, Pterocarpus indicus, HRBC, Ethanolic Extract

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissue. RA still remains a formidable disease, being capable of producing severe crippling deformities, functional disabilities and cartilage destruction, which commonly leads to significant disability, caused by various pro inflammatory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines .The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like Cox and LOX are the potential target for chronic inflammatory conditions [1].RA is a complex process, involving synovial cell proliferation and fibrosis, pannus formation and cartilage and bone erosion. This process is mediated by an interdependent network of cytokines, prostanoids and proteolytic enzymes.Pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor (TNF-α), are central mediators in RA.

This is illustrated in patients with RA, who experience an initial cell-mediated response that leads to the presence of elevated levels of IL-1 in the synovial fluid. Furthermore, IL-1 concentrations in the plasma have been reported to correlate with disease activity. It has also been demonstrated that patients with erosive RA have higher synovial and circulating levels of IL-1 than patients without erosions. Interleukin-6 (IL-6) is an inflammatory cytokine that is characterized by pleiotropy and redundancy of action, involved in inflammation, bone metabolism, immunity, endocrine functions and in particular it is a major regulator of the synthesis of acute phase reactants by the liver.

IL-6 is produced by many different cells in the body including lymphocytes, monocyte, fibroblasts and endothelial cells. Adipose tissue is another major source of IL-6, accounting for about 30% of total circulating concentrations of IL-6 in healthy subjects. Excessive adipose tissue deposition leads to excessive production of IL-6, a high-risk factor to the RA.Body mass index (BMI) is an established risk factor for knee osteoarthritis (OA).Weight loss can help to reduce the incidence of symptomatic knee OA [2].

MATERIAL AND METHODS

Collection of Plant Material

Pterocarpus indicusstem 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 [3].

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

Inhibition of Protein Denaturation Model

2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (PBS, pH 6.4) and 2 ml distilled water were used as control solution (5 ml). 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (20, 40, 60, 80 and 100 µg/ml) were served as standard drug solution (5 ml). 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of leaves and bark of *Pterocarpus indicus* (20, 40, 60, 80 and 100 µg/ml) were taken as test solution (5 ml) [4].

All of the above solutions were adjusted to pH, 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated using the following formula-

Percentage inhibition = (Vt/Vc-1) x 100

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Human Red Blood Cell (HRBC) Membrane Stabilization Model

2 gm dextrose, 0.8 gm sodium citrate, 0.05 gm citric acid and 0.42 gm sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water. Hypotonic saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water. Isotonic saline was prepared by dissolving 0.85 gm of sodium chloride in 100 ml of distilled water. 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water. This was served as phosphate buffer (pH 7.4, 0.15 M) [5].

Preparation of Suspension (10% V/V) of Human Red Blood Cell (HRBC)

The blood was collected from healthy human volunteer who had not taken any NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution [6]. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the study [7].

Assay of Membrane Stabilizing Activity

The assay mixtures contains 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension & 0.5 ml different concentrations of extract leaves and bark of *Pterocarpus indicus*, reference sample and control were separately mixed. 1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of leaf and bark extract of *Pterocarpus indicus* of various concentrations (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) and leaves and bark of *Pterocarpus indicus* extract of various concentration (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution.

All the assay mixtures were incubated at 37°C for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100% [8-9]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula-

Percentage protection

100- [(Optical density sample/optical density control) × 100]

RESULTS AND DISCUSSION

Inhibition of Protein Denaturation Model

Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) showed inhibition of protein denaturation. The ethanolic extract of leaves and stem bark of *Pterocarpus indicus* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also showed inhibition of protein (egg albumin) denaturation. The effect of leaves extracts was found to be (78%) and for stem bark (82%) more as well as diclofenac sodium (95%) at a concentration 100 µg/ml. The results are summarized in Table 1 [10].

Human Red Blood Cell (HRBC) Membrane Stabilization Model

Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) exhibited stabilization towards HRBC membrane. The ethanolic extract of leaves and bark of *Pterocarpus indicus* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also exhibited stabilization towards HRBC membrane. The effect of stem extracts was found to be more (69%) and for leaves (65%) as well as diclofenac sodium (84%) at a concentration 100 µg/ml. The results are summarized in Table 2.

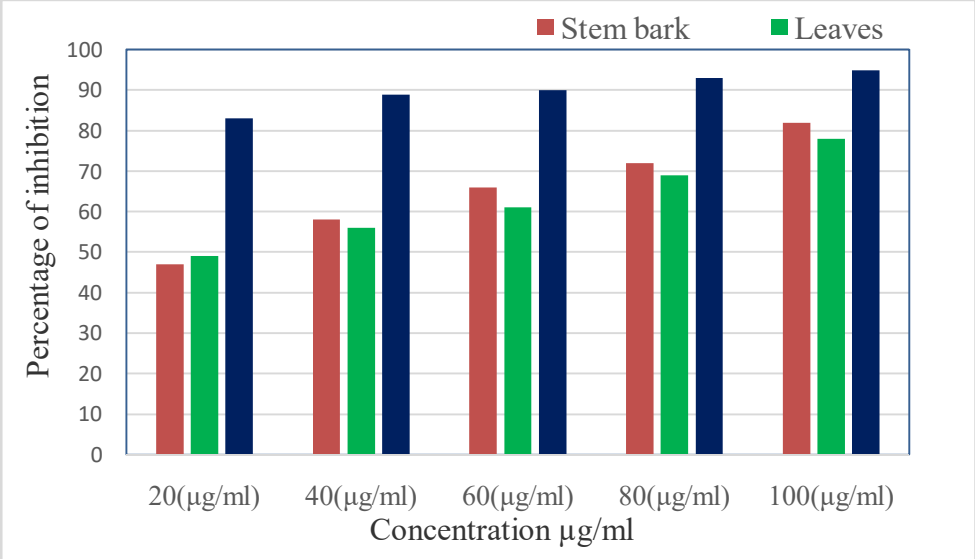
HRBC method was selected for the *in vitro* evaluation because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [11]. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced haemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [12 -16]. Rheumatoid arthritis is an autoimmune inflammatory disorder affecting almost 1-3% of the world population. The word Arthritis means inflammation of the joint ("artho" means the joint and "it is" meaning inflammation of the joint). RA occurs when our immune system attacks the tissues near joints, this is due to release of certain chemical and enzymes that begin to eat away the cartilage and bones. Arthritis is one of the major diseases, in the world which affects many people. In the modern world the normal life style of people is that they do not have a balanced diet, no proper exercise, the modern life style is people always sit in front of laptops for long time, these are the main causes which leads to arthritis. So the younger generations are also affected by this disease. Hence there are different treatments available for arthritis like NSAID'S, Steroids, etc. these treatments can relieve pain, and the disease can be controlled to a certain extent, with severe side effects. The quality control and standardization must be improved for traditional Indian system leaves of medicine. Therefore, alternative treatments are of high interest means by using medicinal plants or phytotherapy.

Table 1 *In vitro* anti-arthritic activity of the ethanolic extract of leaves and stem bark of *Pterocarpus* indicus using protein denaturation method and comparison with standard drug diclofenac sodium.

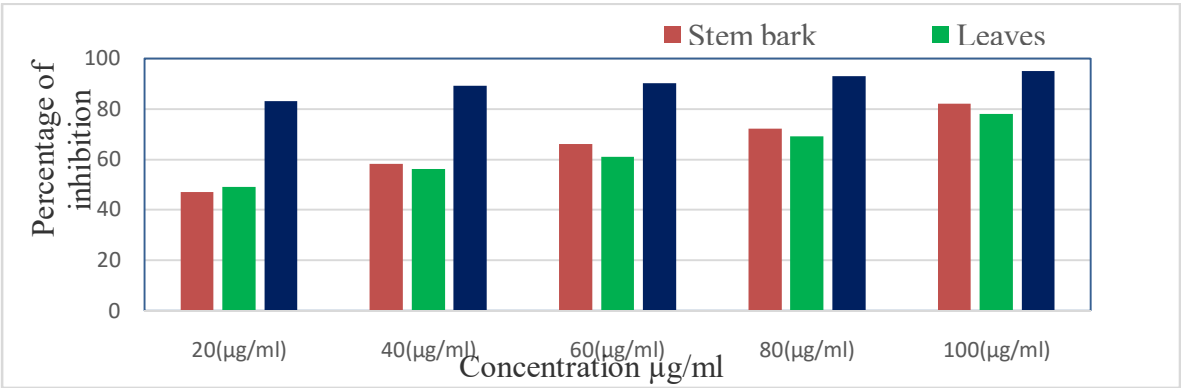
S. No	Concentration (µg/ml)	Protein Denaturation (%)		
		P. indicus Bark	*P. indicus* Leaves	Diclofenac Sodium
1	20	47	49	83
2	40	58	56	89
3	60	66	61	90
4	80	72	69	93
5	100	82	78	95

Table 2 *In vitro* anti-arthritic activity of the ethanolic extract of leaves and bark of *Pterocarpus indicus* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.

S. No	Concentration (µg/ml)	HRBC Membrane Stabilization Assay (%)		
		P. indicus Bark	*P. indicus* Leaves	Diclofenac Sodium
1	20	57	52	66
2	40	58	58	71
3	60	65	61	76
4	80	66	64	80
5	100	69	65	84



Graph 1 *In vitro* anti-arthritic activity of the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* using protein denaturation method and comparison with standard drug diclofenac sodium.



Graph 2 *In vitro* anti-arthritic activity of the ethanolic extract of leaves and bark of *Pterocarpus indicus* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.

CONCLUSION

The ethanolic extract of Leaves and bark of *Pterocarpus indicus* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also showed inhibition of protein (egg albumin) denaturation and also exhibited stabilization towards HRBC membrane. Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml. The percentage of inhibition for HRBC is 69% for bark and 65% for leaves and egg albumin is 82% for bark and 78% for leaves of *Pterocarpus indicus* at a concentration 100µg/ml.The effect of leaves extracts was found to be more as well as diclofenac sodium. However further research on detailed isolation of another active phytoconstituents possessing the therapeutic activity and clinical study for the evaluation of safety and efficacy of the drug needs to be assessed.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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