An Elsevier Indexed Journal ISSN-2230-7346

**Journal of Global Trends in Pharmaceutical Sciences**

Research Article

# *IN VITRO* EVALUATION OF ANTHELMINTHIC AND ANTI - INFLAMMATORY ACTIVITIES OF *PONGAMMIA PINNATA* BARK

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**ARTICLE INFO**

***Key words:***

Anthelminthic activity,
Anti-Inflammatory activity,
*In vitro* HRBC membrane stabilization,
Protein denaturation,
*Pongamia pinnata.*

**ABSTRACT**

The aim of the present study is to investigate the anthelminthic and *in vitro* anti-inflammatory activities of ethanolic bark extract of *Pongamia pinnata. Pherethima posthuma* is used as test worm for anthelminthic activity. The time of paralysis and time of death were studied and activity was compared with albendazole as reference standard for anthelminthic activity. In vitro anti-inflammatory activity was evaluated by using HRBC membrane stabilization method and Protein denaturation method using Diclofenac Sodium as reference standard for *in vitro* anti-inflammatory activity. The results of the present study have established that plant has significant anthelminthic and anti- inflammatory activities.

## INTRODUCTION

The medicinal value of plants has been documented in almost all ancient civilization. The plants are the natural sources of drugs; most of the present drugs are derived from the plants. Each part of the plant like leaves, stem, flowers, fruits, bark, roots and seeds has various medicinal values. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives. They have profound therapeutic benefits and more affordable treatment and would overcome the resistance produced by the pathogens [1]. Inflammation is the response of living tissues to injury or infection during which lysosomal enzymes are released which produces many disorders which results in tissue injury by damaging the macromolecules and lipid peroxidation of membranes.

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Stabilisation of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release [2]. Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminths produce serious problems in human and other animals [3]. *Pongammia pinnata* is a medium sized glabrous, perennial tree grows in the regions of South Eastern Asia and Australia [4]. In the traditional system of medicines such as ayurveda and unani the *Pongamia pinnata* plant is used as anti-inflammatory, anti plasmodial, anti nociceptive, anti lipid peroxidative, anti diarrhoeal, anti ulcer, anti hyperammonic and antioxidant [5].

## MATERIALS AND METHODS:

***Plant material:*** The stem bark of plant *Pongamia pinnata* was collected from the surampalem local area of East Godavari District, Andhra Pradesh. The plant was identified and authenticated by T.Raghuram, Taxonomist, Maharani College, Peddapuram

***Preparation of Extract:*** The freshly collected bark of plant was cleaned from dirt, dried under shade and then coarsely powdered manually .The powder was macerated in ethanol for a period of 7 days and then subjected to hot percolation for 8 hrs .Then the solution was filtered, concentrated and dried.

***Chemicals and Instrument:*** All the chemicals used were of analytical grade.The instruments used were UV-Visible spectrophotometer (ELICO-SL210), Centrifuge, Shimadzu electronic balance.

***In vitro anti-inflammatory activity:***

***a) Chemicals and reagents:*** Diclofenac sodium, Citric acid, Dextrose, Sodium chloride, Tri-Sodium citrate, Bovine Serum albumin,Phosphate buffer, 1N Hydrochloric acid.

***b) HRBC membrane stabilization method:*** The principle concerned in this method is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. Blood was collected (2mL) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% Dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10% v/v suspension was prepared with normal saline. Different concentrations of extract 200 and 500 mg/mL were prepared using distilled water and to each concentration 1mL of phosphate buffer, 2mL hyposaline and 0.5mL HRBC suspension were added. Diclofenac sodium ( 100 mg/ml) was used as reference standard and control were separately mixed with 1mL of phosphate buffer, 2mL hyposaline solution and 0.5mL of 10% HRBC suspension was added to the reaction mixture. The above mixture were incubated at 37 $^{0}$ for 30 min and centrifuged at 3000rpm for 20min. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm [6].

The percentage of HRBC membrane stabilization or protection was calculated by using the formula

$$\%\ \textbf{Membrane Stabilisation} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

***c) Inhibition of protein denaturation method***: Test solution (0.5mL) consists of 0.45 mL of bovine serum albumin ( 5% aqueous solution) and 0.05 mL of *Pongammia pinnata* bark extracts ( 200 and 500 mg/mL).Control solution consists of (0.5mL) consists of 0.45 mL of bovine serum albumin ( 5% aqueous solution) and 0.05 mLof distilled water. Standard solution consists of (0.5mL) consists of 0.45 mL of bovine serum albumin ( 5% aqueous solution) and 0.05 mL of 100 mg/mL of Diclofenac Sodium.All of the above solutions were adjusted to pH 7.3 using a small amount of 1N Hcl. The samples were incubated at 37°C for 15 minutes and heated at 57°C for 3 minutes. After cooling the above solutions the absorbance was measured using UV-Visible spectrophotometer at416nm [7].

The percentage inhibition of protein denaturation was calculated as

$$\%\ \text{inhibition of protein denaturation} = 100 - \frac{\text{Optical density of test solution} - \text{optical density of control}}{\text{optical density of control}} \times 100$$

***Anthelminthic activity:***

***a) Worm collection:*** The Anthelminthic assay was performed on healthy adult earth worms *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings [8]. Because of easy availability earth worms have been used widely for the evaluation of anthelminthic compounds in vitro.All the earth worms were of approximately equal size (6cm). They were collected from local moist place of Aditya nagar gardens situated in Surampalem, washed with saline and kept in water.

***b) Evaluation of Anthelminthic Activity:***

The Anthelminthic activity was carried out as described by Ajaiyoeba EO.et.al, 2001, with minor modifications.The Indian earthworm of nearly equal size, six in each group was taken for the experiment. The Anthelminthic activity was performed by using extract concentrations (i.e. 250 & 500 mg/ml). 50ml of each concentration was prepared and six worms were placed in it. Time for paralysis was noted when no movement of earth worm could be observed, except when the worms were shaken vigorously Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when sprinkled with warm water ($50^{0}$C).The Anthelminthic activity of ethanolic extract of *Pongamia pinnata* is compared with the standard drug Albendazole (20mg/mL) [9-10].

## RESULTS AND DISCUSSION:

The results for *in vitro* anti-inflammatory activity were shown in Table 1&2 and represented in Fig. 1&2, and anthelminthic activty was shown in Table 3 and represented in Fig. 3&4.

***In vitro Anti-inflammatory activity:***

Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also protective attempt by the organism to remove the injurious stimuli and initiate the healing process [11].

**Table 1**: **HRBC-membrane stabilisation method:**

| Concentration(mg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 200 | 0.16±0.09 | 48 |
| 500 | 0.07±0.02 | 75 |
| Diclofenac Sodium-100(Standard) | 0.09±0.06 | 71 |
| control | 031±0.02 | --- |

**Table 2: Protein denaturation method:**

| Concentration(mg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 200 | 0.20±0.02 | 47 |
| 500 | 0.11±0.07 | 71 |
| Standard Diclofenac Sodium-100 | 0.12±0.01 | 68 |
| control | 0.38±0.05 | --- |

**Table 3: Anthelminthic activity of ethanolic bark extract of *Pongamia pinnata***

| Group | Concenration (mg/ml) | Paralysis time (Min) | Death time (Min) |
|---|---|---|---|
| Ethanolic extract of pongama pinnata bark | 250 | 30.33±0.577 | 35.33±0.881 |
| | 500 | 25.95±0.741 | 29.72±0.070 |
| Standard Albendazole | 20 | 20.21±0.381 | 23.31±0.810 |
| Distilled water | - | - | - |

**Fig. 1: HRBC Membrane Stabilisation Method**

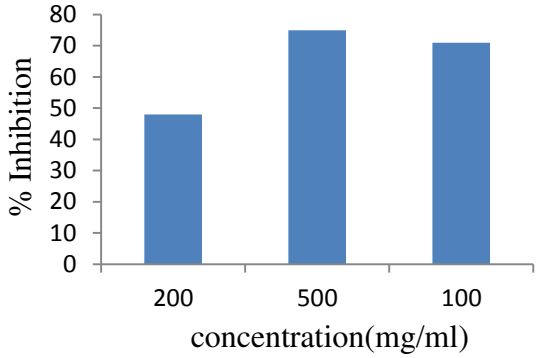



**Fig. 2: Inhibition of protein denaturation**

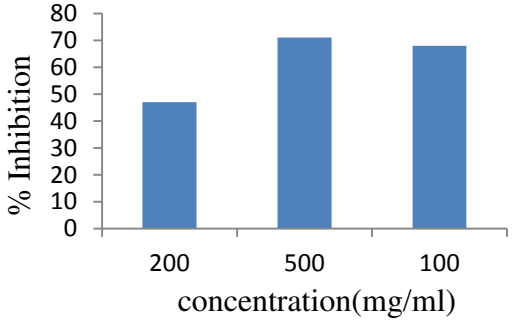


**Fig. 3: Paralysis Time**

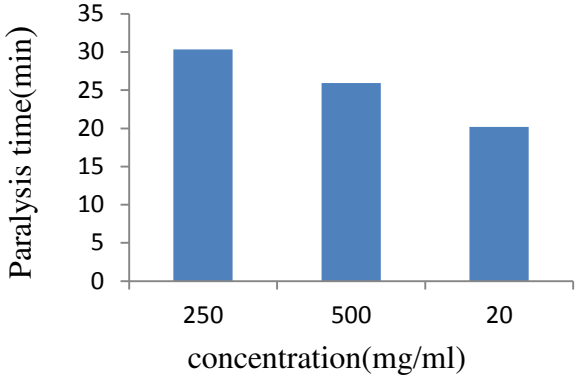
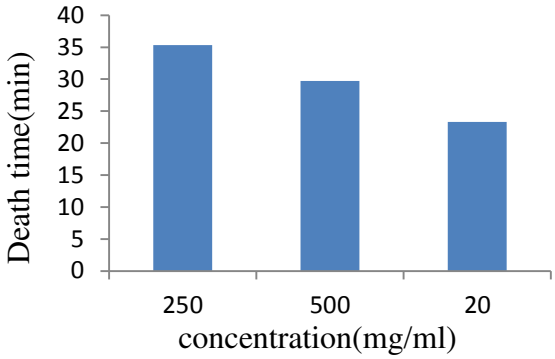



**Fig. 4: Death Time:**




The results of *in vitro* anti-Inflammatory activity by HRBC membrane stabilization and inhibition of protein denaturation method are shown in **Table 1 & 2**, **Fig.1 &2**.The extract exhibits concentration dependant activity. HRBC membrane stabilization has been used as a method to study the in vitro anti- inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane [12] and its stabilization implies that the extract may well stabilize the lysosomal membrane. Stabilisation of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which causes further tissue inflammation and damage upon extracellular release. Some of the NSAIDS are known to have membrane stabilization properties which may be contributing to the anti-inflammatory properties. Denaturation of proteins is responsible for the cause of inflammation in conditions like rheumatoid arthritis hence by prevention of protein denaturation may also help in preventing inflammatory conditions. NSAIDS acts in similar way in preventing inflammation [13].The stem bark extract of *Pongamia pinnata* exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane and it also inhibits the denaturation of proteins and its effect were compared with the standard drug. Recent studies have provoked that many flavonoids and related polyphenols contributed significantly to the anti-inflammatory activities of many plants [14].

***Anthelminthic activity:***

Parasitic helminthes affect human being and animals by causing considerable hard ship and stunted growth. Most diseases caused by helminthes are of chronic and debilitating in nature. The devolpment of anthelminthic resistance until high cost of conventional anthelminthic drugs lead to the evaluation of medicinal plants as an alternative source of Anthelminthics[15]. *Pongamia pinnata* bark extract shows significant anthelminthic activity as evident from **Table 3**, **Fig.4&5** at a concentration 250 and 500 mg/mL against *Pherethima posthuma*. The activity was found to be increased with dose.

Tannins, the secondary metabolite, occur in several plants is responsible for anthelminthic property [16].Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or binds to the glycoprotein on the cuticle of parasite and cause death [17].

The results of the present study suggest that the extract of *Pongamia pinnata* could be used in con-

trol of anthelminthic infections, ascariasis etc; as the worms used in study are in resemblance with the intestinal parasitic worms. Preliminary phytochemical screening of the *Pongamia pinnata* bark has shown the presence of alkaloids, tannins, flavonoids, triterpenes and reducing sugars [18].Hence the presence of tannins and flavonoids present in the extract may contribute for anthelminthic and anti-inflammatory activity.

**CONCLUSION:**

The results obtained revealed that the extract has shown significant anthelminthic and anti-inflammatory activities and the results were compared with the standard reference drug. Further research investigations may be carried out to isolate the actual phytoconstituents responsible for anti- inflammatory and anthelminthic activities.

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