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INVITRO ANTI INFLAMMATORY ACTIVITY OF LEAVES EXTRACT OF PAVONIA ZEYLANICA CAV.

Pitchiah Kumar M.*[1], Sivasankar[2], Senthilvel G.[3] and Lakshmipathy Prabhu. R[4]

[1]State Licensing Authority, Directorate of Indian Medicine and Homeopathy, Arumbakkam, Chennai-600106, Tamilnadu, India.

[2]Lecturer, Sairam Siddha Medical College, Chennai- 600044, Tamilnadu, India.

[3]Central Drug Inspector, Ministry of AYUSH, New Delhi-110023, India.

[4]Asst.Professor, Chettinad Hospital & Research Institute, Chennai 603103, Tamilnadu.

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***Corresponding Author**
Pitchiah Kumar M.
State Licensing Authority, Directorate of Indian Medicine and Homeopathy, Arumbakkam, Chennai-600106, Tamilnadu, India.

ABSTRACT

Pavonia zeylanica Cav. (Familiy -Malvaceae) is a valuable medicinal plant which has been used in siddha system of medicine. The leaves extract of Pavonia zeylanica Cav was assessed for its anti inflammatory activity by invito methods. Invitro activity were evaluated using bovine albumin denaturation assay and membrane stabilization activity using HRBC method. Diclofenac used as standard drug. The ethanolic extract of Pavonia zeylanica Cav leaves at various concentrations (100,200,400 and 800µg) showed significant inhibition in bovine albumin denaturation assay and showed the significant membrane stabilizing action on human red blood cell membrane. Based on results the present study shows that ethanolic extract of Pavonia zeylanica Cav can be a better alternative source of anti-inflammatory agents.

KEYWORDS: Anti-inflammatory, Pavonia zeylanica Cav, Diclofenac sodium, Bovine albumin HRBC Membrane stabilization.

INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain redness, pain, heat, swelling and associated with the increase in protein denaturation, increase to vascular permeability and membrane alteration. The cell injury caused by microbes, physical agents or chemical agents. stress can cause Inflammation of tissue. It is a defensive response that is characterized by redness, pain, heat, and swelling. Inflammation is one of the body's defense

mechanism response to a tissue to damage of tissue caused by burns due to heat, radiation, bacterial or viral invasion.[1]

Inflammation classified into two types acute and chronic. The early reaction to the body of dangerous stimuli and is achieved by the increased movement towards plasma and blood cells like leukocytes from the blood into the injured tissues called as acute inflammation.

The chronic inflammation progressed with movement of cells towards the site of inflammation and simultaneous destruction. NSAIDs and Corticosteroids are widely used to treat inflammation and inflammatory disorders. NSIADs have more adverse effects like gastritis, hypersensitivity, liver damage and Corticosteroids have secondary infections and bone deformity.

Hence need to identify new compounds with potent anti inflammatory effects and minimal side effects.

Pavonia zeylanica Cav is a small herb belongs to family o f malvaceace and distributed over Africa, South West Arabia, Pakistan, India, Sri Lanka, Mauritius. Within India Pavonia zeylanica Cav found in Rajasthan, Maharashtra, Karnataka and Tamil Nadu in wastelands. The leaves of Pavonia zeylanica Cav in serrate nature.[2] Similar names are Ceylon Swamp Mallow, Pavonia and Fragrant Pavonia. The synonym for Pavonia zeylanica Cav is Pavonia odorata in tamil Chirtamutti and various other names called as, Kurundotti, Mammatti, Sittamutti, Sevagan, Thengai poondu and telugu peramutti.The plant is used in folk medicine, siddha and ayurveda to treat inflammation, hemorrhage and dysentery activities have been reported.[3] The leaves and roots is commonly used for medicinal purpose. The phytochemistry of Pavonia zeylanica Cav leaves contain Alkaloids, Glycosides, Tanins, Flavonoids and Triterpinoids.[4]

Pavonia zeylancia picture

J.M.Garg -https://commons.wikimedia.org/w/index.php?curid=5259417

MATERIALS AND METHODS

Preparation of extracts: Pavonia zeylanica plant was collected from Tiruvannamalai forest, Tamilnadu. The plant leaves was dried and make in to a uniform powder using a blender and stored in containers with cork.

The leaves of Pavonia zeylanica were thoroughly cleaned with water and dried in the shade at room temperature, made into a uniform coarse powder and stored in a closed vessel.

The dried powder of Pavonia zeylanica leaves were extracted with 95 % v/v of ethanol in soxhlet apparatus for 5 cycles. The extract was then dried and stored in containers.

Statistical analysis: Results are expressed as Mean ± SEM. The difference between experimental groups was compared by OneWay Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test) using the soft ware SPSS.

Assessment of invitro anti-inflammatory activity

Inhibition of albumin denaturation[5&6]

The reaction mixture consisted of test and standard agents and 1% aqueous solution of bovine albumin fraction and the pH was adjusted with 1N HCl. The reaction mixture was incubated at 37ºC for 20 mins and then heated to 51ºC for 20 mins, after cooling the mixtures the turbidity was measured at 660nm.The experiment was performed in triplicate.

The inhibition of protein denaturation was calculated as follows

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

Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension[7]: The fresh whole blood was collected from the healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks earlier to the experiment. The blood samples were centrifuged at 3000 rpm for 10min and were washed thrice with normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis[8]: The reaction mixture (2ml) consisted of 1 ml of Pavonia zeylanica leaves extract in different concentrations (100-800µg/ml) and 1 ml of 10% RBCs suspension, normal saline used as control and the standard drug was Diclofenac. The

mixtures were incubated in water bath at 56 ºC for 30min. After 30 min the mixtures were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance were taken at 560 nm. The experiment was performed in triplicates.

The inhibition of Hemolysis was calculated as follows

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

Hypotonicity-induced haemolysis[9]

Pavonia zeylanica leaves extract in different concentrations (100-500µg/ml), standard and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. The control, test and standard mixtures were incubated at 37.0c for 30 minutes and centrifuged at 3000rpm. The supernatant liquid was decant and the haemoglobin content was estimated by a spectrophotometer at 560nm.

The percentage protection for hemolysis was calculated as follows

Percentage protection = 100- (OD sample/OD control) x 100

RESULTS

Inhibition of albumin denaturation

The maximum inhibition of Pavonia zeylanica leaves extract showed 81% was observed at 800 µg/ml and statistically significant compared with control normal saline($p<0.01$). The standard drug diclofenac showed the maximum inhibition 93% at the concentration of 800 µg/ml compared with control (Figure 1).

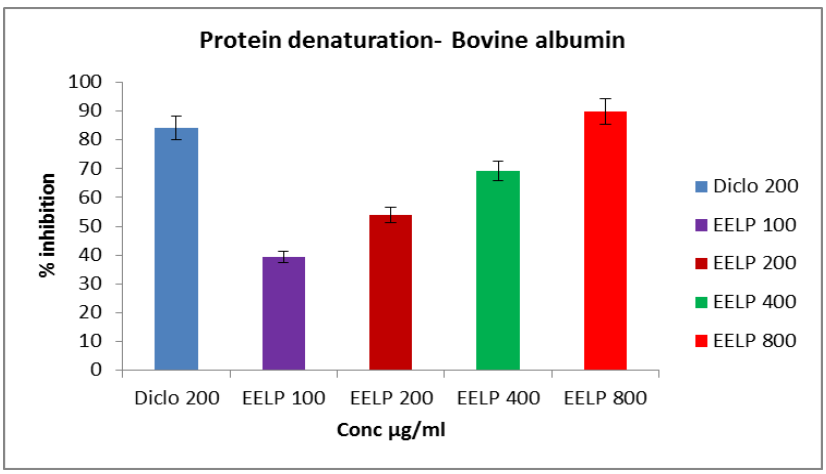

Figure. 1: Protein denaturation – Bovine albumin.

Heat Induced Hemolysis: The Pavonia zeylanica leaves extract and diclofenac were showed effective against inhibiting the heat induced haemolysis at various concentrations(100-800 µg/ml). The Pavonia zeylanica leaves extract at concentration 400 and 800µg/ml protect significantly ($p<0.05$) on heat induced hemolysis (Table 1). Diclofenac 200µg/ml offered a significant ($p<0.05$) protection against damaging effect of heat solution.

Table. 1: Effect of Pavonia zeylanica leaves extract on heat induced haemolysis of HRBCs.

Concentration	Absorbance	% of inhibition
Control	0.242	0
Diclofenac 200	0.148±0.17*	38.84
EEPL 100	0.195±0.28*	24.38
EEPL 200	0.181±0.23*	27.68
EEPL 400	0.162±0.16*	33.05
EEPL 800	0.154±0.09*	35.53

Values are mean ± SEM, n=3,* $p<0.05$ compared with control

Hypotonicity Induced Haemolysis

The Pavonia zeylanica leaves extract at concentration range of 400-800µg/ml protects significantly ($p<0.01$) the erythrocyte membrane against lysis induced by hypotonic solution. The maximum effect were observed % protection for the concentration of 800µg/ml, Diclofenac sodium (200µg/ml) showed a significant ($p<0.01$) protection against the damaging effect of hypotonic solution and showed 51% inhibition of RBC haemolysis when compared with control (Table 2).

Table. 2: Effect of Pavonia zeylanica leaves extract on hypotonicity induced haemolysis of HRBCs.

Concentation	% of protection
Control	-
Diclofenac 200	73.52±0.16*
EEPL 100	36.35±0.21*
EEPL 200	51.16±0.19*
EEPL 400	64.57±0.29*
EEPL 800	87.64±0.08*

Values are mean ± SEM, n=3,* $p<0.05$ compared with control

DISCUSSION

Inhibition of albumin denaturation: Protein denaturation is a process in which proteins lose their structure like tertiary and secondary structure due to external stimuli or compound. The

biological proteins are losing their biological function when become denatured. The denaturation of proteins is the evidence for inflammation The anti-inflammation activity was studied by effectiveness of plant extract to inhibit the process protein denaturation.

Membrane stabilization

The HRBC membrane stabilization method commonly used to study the invitro anti inflammatory activity. The lysosomal membrane stabilization involved in the process of inflammation.[10and11] The stabilization of lysosomal is important to limiting the inflammatory response by preventing the release of lysosomal constituents which causes further tissue inflammation and damage. Pavonia zeylanica leaves extract to stabilize the red blood cell membrane by inhibiting the release of lytic enzymes and active mediators of inflammation. In acute or chronic inflammation the extra cellular activity of lysosomal enzymes involved and produce various disorders. The inhibition of the lysosomal enzyme was the most vital mechanism for anti inflammatory activity.[12]

The inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a evidence of the mechanism of anti-inflammatory activity of Pavonia zeylanica leaves extract.

CONCLUSION

In the present study, results indicate that the Pavonia zeylanica leaves extract to possess anti-inflammatory properties. These activities may be due to the chemical compounds present in the leaves such alkaloids, flavonoids, tannins and phenols. The Pavonia zeylanica leaves extract acts as free radical inhibitors or scavenger and inhibited albumin denaturation and stabilized the Red Blood Cells membrane and protect the heat induced hemolysis. The presents of Stabilization of lysosomal membrane are important to limiting the inflammatory process. The anti inflammatory activity mechanism possibly presents antioxidants presents in Pavonia zeylanica leaves. The important chemical compounds like flavonoids and tanins could be responsible for anti inflammatory activity, further study will reveal the mechanism of action. From the above study it was concluded that the ethanolic extract of leaves of Pavonia zeylanica has significant inhibition of albumin denaturation, membrane stabilization and hemolysis protection property.

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