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Research Article

A STUDY ON EVALUATION OF INVITRO ANTI INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF *TEPROSIAPURPUREA* (*LINN*)

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ABSTRACT

Many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The plant *Tephrosiapurpurea*(Fabaceae) has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. It is reported to contain various flavonoides, alkaloids, steroids, phenolic compounds. It has been reported as antibacterial, anticancer, antioxidant, antiulcer, hepatoprotective, immunomodulatory, antilithiatic, free radical scavenging, antileishmanial, antimicrobial and wound healing activities. In the present study areal parts of*Tephrosiapurpurea*are extracted with Methanol, the extract was screened for anti-inflammatory activity by HRBC(Human Red Blood Cell) membrane stabilization model. Methanolic extract of *Tephrosiapurpurea*showed significant activity when compared to the standard diclofenac sodium.The percentage of stabilization was found to be (45±0.36)% and (49.66±0.66)% at concentration of 500µg/ml of *T. purpurea*extract and standard respectively, the results suggest that the methanolic extract of T.*purpurea*possesantiinflammatory activity.

Keywords: In vitroAnti inflammatory activity, HRBC membrane stabilization, % stabilization.

INTRODUCTION

Plants have been used as alternative remedy for the treatment of various ailments since ancienttimes. In recent years considerable research has been progressed in the exploitation of medicinal plants, in the treatment of various stress related disorders caused by metabolism of oxygen leads to generation of free radicals. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or radical scavenging mechanism as part of their activity[1,4]. The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species (ROS) from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukine-I, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[2-3]. Most clinically important medicine belongs to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation related diseases. Though these have potent activity and long term administration is required for treatments of chronic diseases. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects

are desirable to substitute chemical therapeutics[5].

Tephrosiapurpurea commonly known asSharapunkha (Sansrit), called as Sarponkh (Hindi), Vempali (Telugu) which is not official in Ayurvedic Pharmacopoeia1. *Tephrosiapurpurea*(Linn.) Pers. (Leguminosae) is a highly branched, sub-erect, herbaceous perennial herb. The phytochemical investigations on *Tephrosiapurpurea*have revealed the presence of glycosides, carotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols[6]. It is also good source of minerals and amino acids[7]. Although the *Tephrosiapurpurea*plant has reported as antibacterial, anticancer, antioxidant, antiulcer, hepatoprotective, immunomodulatory, antilithiatic, free radical scavenging, antileishmanial, antimicrobial and wound healing activities[7-8]. In the present study methanol extract of *Teprosiapurpurea* areal parts are evaluated for anti inflammatory activity.

MATERIALS AND METHODS

Collection of Plant

The arialparts of *Tephrosiapurpurea*was collected inKothuru village surrounded to Mother Teresa Pharmacy College,Sathupally. The collected parts aredried under shade for 8 days, mechanically powdered and stored in a container.

Preparation of extract

The 50gms of powdered drug was accurately weighed, packed in thimble flask, 400ml of methanol added to 500 ml round bottom flask, the Soxhelet assembly set at 35^{o}c, extraction process continued till the color of packed material changed to colorless, then the extract was filtered,transferred into china dishes, air dried, the weight of dried residue 6gm (12%w/w), stored in container until use.

In vitro anti inflammatory activity

Fresh whole human blood 5 ml was collected and transfer to the centrifuged tubes containing Sodium citrate solution of 3.8% to prevent clotting. Thenthe tubes are centrifuged at 3000 rpm for 10 min. The volume of sediment was measure and reconstitute as 10% v/v suspension with normal saline it gives HRBC Suspension[9].

The plant extracts are made into100, 200, 300, 400, 500 µg/ml by serial dilution with normal saline and Diclofenac sodium was also made into similar concentration it was considered as standard.

The experimental procedure was continued by taking 1.0 ml of test sample of different concentrations (100,200,300,400,500 µg/ml), and 0.5 ml of 10% HRBC suspension, 1 ml of 0.2 M phosphatebuffer, 1 ml hyposaline are incubate at 37^{0}C for 30 min then centrifuged at 3,000 rpm for20 min, the hemoglobin content ofsupernatant solution was estimated by spectrophotometry at 560 nm[10]. Diclofenac sodium was used as standard and a control was prepared without extracts. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula.

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

RESULTS

The *in vitro* anti-inflammatory activity was screened by HRBC membrane stabilization method results are tabulated in table1. Methanolic extract of *Tephrosiapurpurea* showed significant activity when compared to the standard diclofenac sodium.The percentage of stabilization was found to be (45$\pm$0.36)% and (49.66$\pm$0.66)% at concentration of 500µg/ml of *T. purpurea*extract and standard respectively.

DISCUSSION

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and lack of rationale for their use when other suitable methods are available or could be investigated, hence in the present study the HRBC membrane stabilization method was selected for in vitro assessment of anti inflammatory activity. *Tephrosiapurpurea* extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the liposomal membrane[11] 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 causes further tissue inflammation and damage upon extracellular release[12].The exact mechanism of extract is not known, the hypotonicity induced hemolysis may arise from shrinkage of the cells due to osmotic loss

of intracellular electrolyte and fluid components or interaction with the membrane proteins[13].the extract may inhibits the processthat results it shown an anti inflammatory activity.

CONCLUSION

Based on the above results it is suggest that the methanolic extract of *Teprosiapurpurea*possesanti inflammatory activity screened by in vitro HRBC Membrane stabilization method. Antiinflammatory may be due to the presence of many phytoconstituents like flavonoids in the extract. However, further studies are required to identify the lead molecule in the extract and to study the mechanism of action.

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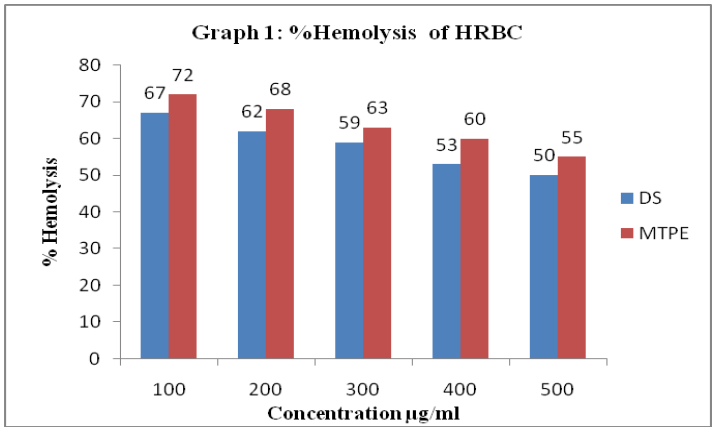
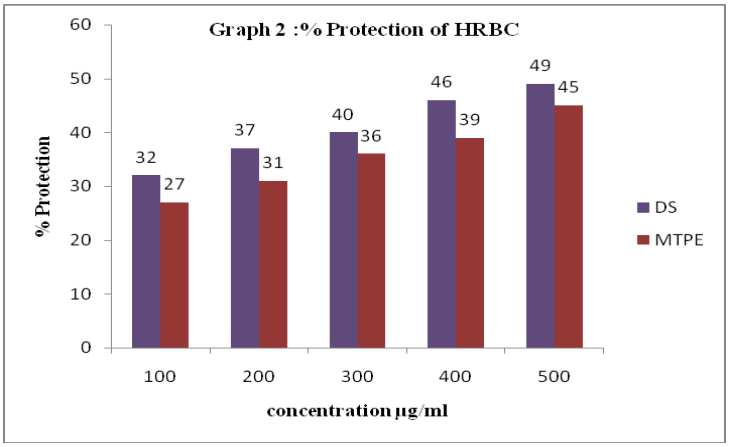
Table 1: Anti inflammatory activity of Methanolic *Tephrosiapurpurea*extract (MTPE)

S.No	Concentration (µg/ml)	% Hemolysis (Mean ± SEM)		% Protection (Mean ± SEM)	
		Diclofenac	MTPE	Diclofenac	MTPE
1	100	67.33±0.76	32.66±0.76	32.66±0.86	27.66±0.66
	200	62.33±0.66	37.66±0.66	37.66±0.35	31.66±1.27
	300	59.66±0.36	40.33±0.66	40.33±0.43	36.33±1.27
	400	63.33±0.76	36.66±0.76	36.66±0.76	39.33±0.76
	500	50.33±0.66	49.66±0.66	49.66±0.66	45.00±0.36





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