International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.4, pp 2060-2063, Oct-Dec 2011

Anti-Inflammatory activity of *Thespesia populnea* fruits by Membrane Stabilization

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Abstract: In the present study, the ethyl alcohol and aqueous extract of *Thespesia populnea* were investigated for anti-inflammatory activity by HRBC method. The preventation of hypo tonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity.These extracts show biphasic effects. Their activities were compared with standard drug diclofenac.
Key words *Thespesia populnea*, anti-inflammatory, phytoconstituents.

INTRODUCTION

The inflammatory responses involve a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue break down and repair which are aimed at host defense and usually activated in most disease conditions[1].Now a day's much interest has arisen in the search of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agents without too many side effects. So present study was undertaken to establish scientific evidence for anti-inflammatory activity of fruit extracts of Thespesia populnea

Thespesia populnea (Linn.) So land ex Correa (Family – Malvaceae) is very popular as a medicinal plant as mentioned in the ancient text of ethnic medicine. This plant is distributed mainly along the coastal regions throughout India, often planted as avenue tree. It grows to a maximum height of 18 meters. Fruits are globose or oblong brown capsules covered with minute peltate scales, pubescent, channelled along the back[2]. The bark is so often fibrous and fissured in nature with grey to brown in colours. The leaves are simple, alternate, long petiolate, cordate, entire, acuminate, prominent nerves 5 – 7 with peltate scales on one or both surfaces. The flowers are yellow with purple base, slowly changing to purple on withering[3].

This plant is astringent, cooling and antidiahrroeal. The bark and fruits posses more curative properties. The bark is astringent and is prescribed in the Philippines for the treatment of dysentery in the form of a decoction [4].

It is used in folk medicine as a poultice for external applications for the treatment of scabies, psoriasis and other skin ailments. The poultice prepared from fruits, flowers and leaves are also found to be useful in rheumatoid arthritis [5].

Earlier the plant has been studied for its antibacterial, antiviral[6], wound healing, anticancer[7], antisteroidogenic activity [8] and for dermatitis [9]. Aqueous extracts of fruits of this plant are reported for its wound healing activity[10].

A popular marketed hepatoprotective Ayurvedic preparation is "Kamilari" consisting of extracts of Thespesia populnea flowers, Elettaria cardamom, Zingiber officinalis, Glycyrrhiza glabra, Piper longum and Honey [11].

In the current study, in vitro experiments were conducted to determine the possible anti-inflammatory effect of ethyl alcohol and aqueous extract of fruit of Thespesia populnea (Linn)

MATERIALS AND METHODS

Plant Material: The leaves of *Thespesia populnea* were collected from Kottayam district in Kerala, India in October 2006. The same were authenticated by Mr.K G Srekanth,Senior Research Officer,Pharmacognosy Unit, Govt Ayurveda Research Institute,Poojapura,Thiruvananthapuram, Kerala. A voucher specimen PC-03/2006 was submitted at Academy Of Pharmaceutical Science, Pariyaram Medical College, Kannur for future reference. Dried fruits were ground to coarse powder, passed through sieve no 24 and stored in air tight container and used for further extraction.

Ethyl alcohol extract The shade dried powdered fruits (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The ethyl alcohol extract was concentrated in vacuum to a syrupy consistency. The percentage yield of extract was found to be 4.12 %.

Aqueous extract -The aqueous extract was prepared using fresh powder by maceration process. 100gm of the powdered drug were taken in a 2000ml conical flask with 500ml of distilled water and 10ml chloroform was added as preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc is discarded and airtight container in its filtrate dried under hot air oven at 45^0C to semisolid mass which was stored in a refrigerator below 10^0C. The percentage yield of extract was found to be 6.19 %.

METHODS

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity[12] (Gandhisan etal. 1991). Blood was collected from healthy volunteer who had not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2%dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%.pH 7.2) and a 10 %(v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in the table 2), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixtures were incubated at 37^0C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water as 100%.

Table 1: Phytochemical screening of plant material *Thespesia Populnea*

Phytochemical constituents	Ethyl Alcohol Extract	Aqueous Extract
Carbohydrates	+	+
Steroids	+	-
Alkaloids	+	+
Saponins	-	-
Terpenoides	+	-
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+

(+): Present (-): Absent

Table 2: In vitro anti inflammatory activity of ethyl alcohol and aqueous extract of *Thespesia populnea*

Treatment	Conc. (mcg/ml)	Absorbance (540nm)	% inhibition
Control	--------	0.49±0.018	----------
Ethyl alcohol	1000 500 250	0.19±0.05 0.22±0.08 0.24±0.001	61.2 55.1 51.0
Aqueous	1000 500 250	0.20±.02 0.23±0.004 0.25±0.002	59.1 53.0 49.9
Diclofenac	50	0.13±0.05	73.4

Values are expressed as mean ± SEM.n=6 in each groups

RESULTS AND DISCUSSION

The ethyl alcohol and aqueous extracts of *Thespesia populnea* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Phytochemical investigation reveals that ethyl alcohol extract contains carbohydrates, steroids,alkaloids,terpenoids, flavanoids,tannins, polyphenols while aqueous extract contains carbohydrates, alkaloids, flavanoids,tannins, poly phenols . Both extracts showed significant anti-inflammatory activity in a concentration depended manner. Ethyl alcohol extract at a concentration of 1000 mcg/ml showed 61% protection of HRBC in hypotonic solution compared with standard diclofinac which showed 73% protection.

The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG). Non-steroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (Cyclooxygenase) or by stabilizing the lysosomal membrane. The extracts exhibited membrane stabilization effects by inhibiting hypo tonicity induced lyses of erythrocyte membrane[13]. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further tissue inflammation and damage up on extra cellular release. Some of the NSAIDs are known to exhibit membrane stabilization due to osmotic loss of intracellular electrolyte and fluid components[14]. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components.

The study also provides a strong evidence for the use of the fruits of Thespesia populnea in folkloric treatment as anti-inflammatory agent. The activity may be due to the presence of one or more phytochemical constuients.

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