

Anti-inflammatory activity of the inflorescence of *Typha elephantina* (cattail) in rats

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ABSTRACT

Objective: The methanolic extract of *Typha elephantina* inflorescence (TE) was studied for anti-inflammatory activity by *in vitro* and *in vivo* studies.

Methods: The *in vitro* anti-inflammatory potential of TE was evaluated by studying the effect of TE on inhibition of i) protein denaturation, ii) proteinase activity and iii) hypotonic saline induced hemolysis of RBC for membrane stabilization activity. The *in vivo* anti-inflammatory activity was evaluated in carrageenan induced paw edema and cotton pellet induced granuloma in rats. Wistar rats were orally administered TE (250 mg/kg and 500 mg/kg) and the standard drug diclofenac sodium (10 mg/kg) 1 h prior to a subcutaneous injection of carrageenan (0.1 ml of 1% w/v carrageenan) into their right hind paws to produce edema. The paw volumes were measured at various time intervals to assess the effect of drug treatment. In the granuloma model, 4 sterile cotton pellets were implanted in the ventral region in each rat. TE (250 mg/kg, 500 mg/kg and 750 mg/kg) and the standard drug diclofenac sodium (10 mg/kg) were administered orally for 8 days to the pellet implanted rats. The granuloma tissue formation was calculated from the dissected pellets and the activities of the marker enzymes AST, ALT and ALP were assayed from the serum. **Results:** TE at different concentrations significantly inhibited the heat induced protein denaturation and proteinase activity and exhibited good membrane stabilization by inhibiting the hemolysis of RBC. A significant reduction in paw edema and cotton pellet granuloma was observed with TE treatment when compared with the carrageenan treated and cotton pellet implanted control animals respectively. TE treatment significantly attenuated the AST, ALT & ALP activities elevated by the implanted cotton pellets. **Conclusion:** It may be concluded that TE possesses anti-inflammatory activity which might be due to an underlying antioxidant activity and lysosomal membrane stabilization. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Typha elephantina inflorescence;
Anti-inflammatory activity;
Carrageenan induced paw edema;
Cotton pellet induced granuloma.

INTRODUCTION

Inflammation is the body's defence reaction to injury in order to eliminate or limit the spread of injurious

agents as well as remove consequent necrosed cells and tissues. It can be evoked by a wide variety of noxious agents such as infections, antibodies or physical injuries. The main features of the inflammatory response

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are vasodilation, increased vascular permeability, cellular infiltration by chemotaxis, granuloma formation and activation of cells of the immune system as well as of complex enzymatic systems of blood plasma^[1]. The degree to which these occur is normally proportional to the severity of the injury and the extent of infection. During the different phases of inflammation, several mediators are released such as histamine, serotonin, chemotactic factors, bradykinins and prostaglandins which are responsible for vasodilation and increased capillary permeability due to alterations in the vascular endothelium, which leads to increased blood flow (hyperaemia) that causes redness (erythema) and the entry of fluid into the tissues (edema)^[2].

Cattails are wide ranging, commonly known plants seen growing by the water side, their range including nearly all the continents. Typhaceae, the cattail family, is comprised only of the genus *Typha*. A number of species of *Typha* occur in various continents, the common ones being *T. latifolia*, *T. angustifolia*, *T. glauca*, and *T. domingensis*^[3]. A commonly occurring species of cattail in India and Southeast Asia is *Typha elephantina* (Roxb.). Also known as elephant grass, it is a gregarious marshy plant found in stagnant water and by the sides of marshes and streams^[4]. The *T. elephantina* plant has been used in traditional Indian medicine as a coolant, an aphrodisiac and in the treatment of leprosy and the rootstalk has been employed in eastern Asia for the treatment of dysentery, gonorrhoea and measles. The male spikes and female spikes are used as medicated absorbent in treating wounds and ulcers^[5].

Chemical constituents like pentacosane, 1-triacontanol, β -sitosterol, cholesterol, quercetin, lanosterol and β -sitosteryl-3-O- β -D-glucopyranoside have been isolated from the *T. elephantina* plant^[6]. The β -sitosteryl-3-O- β -D-glucopyranoside has found to be effective against P 338 leukaemia. β -sitosterol and quercetin are potent antioxidants and have been reported to exhibit anti-inflammatory and wound healing activities^[6].

Traditionally, the inflorescence of the *Typha* species has been used to treat wounds and burns by patting it on to the wound or by burning the inflorescence and then applying it on the wound^[7]. This study was thus conducted to validate the traditional claims and investigate the anti-inflammatory activity of the inflorescence of *T. elephantina* using acute and chronic ex-

perimental models of inflammation.

MATERIALS AND METHODS

Plant material

The inflorescence of *Typha elephantina* was collected from the Bordi region of Maharashtra, India and authenticated at the Agharkar Research Institute, Pune. The voucher number allotted to the specimen is WP-097.

Extraction

Typha elephantina extract (TE)

The inflorescence of *Typha elephantina* was dried in a hot air oven at 60°C and then extracted in a soxhlet apparatus with 70% methanol at 60°C. The extract was collected, methanol was evaporated and the extract was further dried in a vacuum oven. The dry extract was stored in air tight containers in a refrigerator for further use.

Drugs and chemicals

Carrageenan was purchased from Sigma Chemical Co., St Louis, MO, USA. Bovine serum albumin (BSA) was purchased from Hi Media Laboratories, Mumbai, India. Diclofenac sodium was procured from Cipla Pharmaceuticals, India. Absorbent cotton wool was obtained from Jaycot Industries, Hyderabad, India. All other chemicals were obtained from local sources and were of analytical grade.

Experimental animals

Wistar albino rats (180-200g) of either sex were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25 ± 2°C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pune, India) and water *ad libitum*. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No.25/1999/CPCSEA) and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

Acute toxicity study (ALD50)

Acute toxicity studies were carried out on albino Wistar rats by the oral route at doses of 50 mg/kg, 100

mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg of TE as per OECD guideline No.402.

Preparation of test and reference drug solutions

An aqueous solution of carrageenan (1% w/v) was prepared in hot water and used for subcutaneous injection.

TE was prepared as a suspension in 1 % (w/v) aqueous sodium carboxymethyl cellulose (CMC) solution and used immediately.

Diclofenac sodium was suspended in 1% (w/v) aqueous sodium CMC solution and used immediately for oral treatment.

Anti-inflammatory activity

The effects of TE were evaluated by *in vitro* anti-inflammatory studies and by *in vivo* carrageenan induced hind paw edema and cotton pellet granuloma models in rats. Aspirin was used as standard in the *in vitro* antioxidant studies whereas diclofenac sodium was used as a standard drug in both the *in vivo* models for comparing the anti-inflammatory potential of TE.

In vitro anti-inflammatory activity

1. Inhibition of protein denaturation

The effect of TE on protein denaturation was evaluated by the method of Mizushima Y^[8]. Test solution (1ml) containing different concentrations (10 - 100 µg/ml) of TE or the standard drug aspirin (100 µg/ml) was mixed with 1ml of egg albumin solution (1mM) and then incubated at 27±1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. A control experiment without the test/standard compound was conducted in an identical manner. After cooling the turbidity of the solutions was measured spectrophotometrically at 660 nm. The percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) \times 100 / \text{Abs}_{\text{Control}}$$

IC₅₀ values were calculated as an average of triplicate analysis.

2. Proteinase inhibitory activity

The test was performed according to the modified method of Oyedepo and Femurewa^[9]. Test solution (1ml) containing different concentrations (10 - 100 µg/ml) of TE or the standard drug aspirin (100 µg/ml) was incubated with 0.06 mg trypsin and 1ml of 25 mM tris-

HCl buffer (pH 7.4) at 37°C for 5 minutes. Then, 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes and treated with 2.0 ml of 70% perchloric acid to terminate the reaction. The cloudy suspension was centrifuged at 4000 rpm for 5 min. A control experiment without the test compound was conducted in an identical manner. The absorbance of the supernatant was measured spectrophotometrically at 280 nm. The percent inhibition of proteinase activity was calculated as follows:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

IC₅₀ values were calculated as an average of triplicate analysis.

3. Membrane stabilization activity by hypotonic saline induced-hemolysis

The method described by Umapathy et al was used for this study^[10]. The reaction mixture (4.5ml) consisted of 1 ml of different concentrations (10 - 100 µg/ml) of TE or the standard drug aspirin (100 µg/ml) in normal saline, 2ml of hypotonic saline solution (0.25% NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4) and 0.5 ml of 10% rat RBC in normal saline. In the control experiment, instead of the drug only saline was added. All the centrifuge tubes containing the above reaction mixture were incubated at RT for 10 min. At the end of incubation, the tubes centrifuged at 1300 rpm for 3 min and the absorbance of the supernatants was read at 540 nm. The percent inhibition of hemolysis was calculated as follows:

$$\% \text{ inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

IC₅₀ values were calculated as an average of triplicate analysis.

In vivo acute model of inflammation

Carrageenan induced hind paw edema in rat^[11,12]

Albino Wistar rats weighing 180-200 gm were randomly allocated into four groups of 6 animals each and treated in the following way:

Group-1: Served as Carrageenan control, which received orally 1ml/kg of 1% sodium CMC solution + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-2: Served as Standard and received diclofenac sodium 10 mg/kg orally (1 hr prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan

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by subcutaneous injection.

Group-3: Received TE 250mg/kg orally (1 hr prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-4: Received TE 500mg/kg orally (1 hr prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Groups 2, 3 & 4 received their respective drugs 1 h prior to the carrageenan injection. Carrageenan solution (0.1 ml) of 1% w/v was injected subcutaneously into the plantar region of the right hind paw of the rats of all groups to produce edema. Paw edema volumes were measured using plethysmometer (IITC 520) at various time intervals like 0, 1, 2, 3, 4, 6 & 24 hr after the carrageenan injection. The paw edema inhibition of the standard and test drugs was calculated by comparing with the Toxicant control group rats in the following way:

$$\% \text{ inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c is the rat paw edema volume of the Toxicant control group; V_t is the rat paw edema volume of the drug treatment group

In vivo chronic model of inflammation

Cotton pellet induced granuloma in rat^[13]

Albino Wistar rats were randomly divided into four groups of 6 animals each and treated in the following way:

Group-1: Served as Pellet control, which received orally 1ml/kg of 1% sodium CMC solution daily for 8 days following subcutaneous implantation of cotton pellets.

Group-2: Termed as Standard and received diclofenac sodium 10mg/kg p.o. once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-3: Received TE 250 mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-4: Received TE 500 mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-5: Received TE 750 mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Four sterile cotton pellets (10 mg) were implanted

subcutaneously in the ventral region 2 on either side, in each rat under light ether anaesthesia. Groups 2, 3 and 4 received their respective drug treatments once daily for 8 days following implantation of cotton pellets. On the 9th day, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture. The cotton pellets along with granuloma tissue were removed and weighed immediately for wet weight. The pellets were dried in an oven at 60°C until a constant weight was obtained. The granuloma tissue formation and exudate formation was calculated using the following formulae:

Measure of granuloma tissue formation = constant dry weight – initial weight of pellet

Measure of exudate formation = wet weight of pellet - constant dry weight of pellet

The level of inhibition of granuloma tissue development was calculated using the expression:

$$\% \text{ inhibition of granuloma tissue formation} = \frac{W_{grC} - W_{grT}}{W_{grC}} \times 100$$

Where, W_{grC} = weight of granuloma tissue of the Toxicant control group; W_{grT} = weight of granuloma tissue of the treatment group

Blood collected by cardiac puncture was allowed to coagulate at room temperature for 30 min and serum was separated by centrifugation at 2500 rpm for 15 min. The separated serum was analyzed for the activities of the marker enzymes ALT, AST and ALP.

Marker enzyme assays

The lysosomal enzymes ALT, AST and ALP were assayed in serum using standard kits supplied from Accurex Biomedical Pvt Ltd (Mumbai, India). The results were expressed as IU/L.

RESULTS

In vitro anti-inflammatory activity

1. Protein denaturation activity

TE was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in TABLE 1. The IC_{50} value of TE was found to be 56.73 µg/ml. The correlation coefficient (R^2) for TE was calculated from the graph and was found to be 0.989. TE was compared with aspirin which was used as the standard. The IC_{50} value for aspirin was found to be 27.41 µg/ml at the correlation coefficient value

TABLE 1 : Effect of TE on inhibition of protein denaturation, proteinase activity and RBC hemolysis (membrane stabilization activity)

	Concentration ($\mu\text{g/ml}$)	% inhibition		
		Protein denaturation activity	Proteinase activity	RBC hemolysis for membrane stabilization
Test Samples	10	20.63 \pm 0.72	19.63 \pm 1.19	26.21 \pm 1.34
	20	29.68 \pm 0.78	28.19 \pm 1.50	34.61 \pm 2.40
	40	42.85 \pm 1.42	39.26 \pm 1.08	42.90 \pm 1.97
	60	53.96 \pm 2.07	49.59 \pm 0.79	53.47 \pm 1.62
	80	61.42 \pm 1.20	56.46 \pm 2.34	60.31 \pm 3.87
	100	74.12 \pm 3.23	64.97 \pm 1.83	68.29 \pm 2.89
Correlation coefficient value (r)		0.989	0.984	0.988
IC ₅₀ value		56.73 \pm 0.97 $\mu\text{g/ml}$	65.89 \pm 0.79 $\mu\text{g/ml}$	56.87 \pm 0.85 $\mu\text{g/ml}$
Aspirin	100	89.34 \pm 4.12	71.47 \pm 2.13	76.94 \pm 3.54

All values are expressed as mean \pm SEM for three determinations

(R²) of 0.99.

2. Proteinase inhibitory activity

TE exhibited significant antiproteinase activity at different concentrations as shown in TABLE 1. The IC₅₀ value of TE was found to be 65.89 $\mu\text{g/ml}$ at the correlation coefficient value (R²) of 0.984. TE was compared with aspirin which was used as standard. The IC₅₀ value for aspirin was found to be 53.98 $\mu\text{g/ml}$ at the correlation coefficient (R²) of 0.99.

3. Membrane stabilization activity

Membrane stabilization activity of TE was evaluated by determining the inhibition of hemolysis of RBC caused by addition of a hypotonic saline solution. TE (10 100 $\mu\text{g/ml}$) inhibited the hypotonicity-induced haemolysis of RBCs to varying degrees as shown in TABLE 1. The IC₅₀ value of TE was found to be 56.87 $\mu\text{g/ml}$ at the correlation coefficient (R²) of 0.988. TE was compared with aspirin which was used as the standard. IC₅₀ value for aspirin was found to be 42.48 $\mu\text{g/}$

ml at the correlation coefficient (R²) of 0.990.

In vivo acute model of inflammation

Carrageenan induced hind paw edema in rat

In the acute anti-inflammatory model i.e. carrageenan induced hind paw edema in rats, carrageenan treatment caused an increase in paw volumes. Treatment with standard diclofenac Na (10mg/kg) and TE at doses of 250mg/kg and 500 mg/kg caused a significant inhibition of paw edema every hour up to 24 hours when compared with the carrageenan treated group of rats. There was significant difference everywhere at all hours and all treatment groups. (TABLE 2)

In vivo chronic model of inflammation

Cotton Pellet granuloma formation model in rats

In this model, the TE at doses of 250 mg/kg, 500 mg/kg and 750 mg/kg were found to be effective in the exudatory and granulatory phases of inflammation. TE 250 mg/kg, 500 mg/kg and 750 mg/kg were found to

TABLE 2 : Effect of TE on Carrageenan induced hind paw edema in rats

Treatment group and dose (mg/kg)	Mean Paw Edema Volume (ml) Time interval in hours						
	0	1	2	3	4	6	24
Carrageenan control group	0.91 \pm 0.0044	1.20 \pm 0.0049	1.39 \pm 0.0049	1.66 \pm 0.0076	1.62 \pm 0.0089	1.58 \pm 0.0076	1.20 \pm 0.0095
Standard Diclofenac Na (10mg/kg)	0.89 \pm 0.0057 (2.19)	1.10 \pm 0.0068* (8.33)	1.20 \pm 0.0076* (13.66)	1.07 \pm 0.0071* (35.61)	0.99 \pm 0.0084* (39.09)	0.93 \pm 0.0087* (41.05)	0.90 \pm 0.0091* (24.93)
TE (250 mg/kg)	0.91 \pm 0.0067 (0)	1.17 \pm 0.0062* (1.66)	1.30 \pm 0.011* (6.49)	1.17 \pm 0.0081* (29.67)	1.12 \pm 0.0113* (30.76)	1.05 \pm 0.0100* (33.33)	0.96 \pm 0.0108* (20.19)
TE (500 mg/kg)	0.89 \pm 0.0073 (2.19)	1.13 \pm 0.0067* (5.83)	1.23 \pm 0.0065* (11.41)	1.11 \pm 0.0132* (33.09)	1.03 \pm 0.0174* (36.21)	0.97 \pm 0.0125* (38.20)	0.90 \pm 0.0037* (24.79)

Values are mean \pm SEM; N = 6 in each group; One-way ANOVA followed by Dunnett's test is applied for statistical analysis, P values: * < 0.01 when treatment groups were compared with the Carrageenan control group; The values in the bracket indicate % inhibition

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inhibit exudate formation by 30.39, 50 and 58.33 % respectively and inhibit granuloma formation by 21.04, 51.85 and 66.87 % respectively. (Figure 1)

The effect of TE on serum marker enzymes - AST, ALT & ALP is summarized in Figure 2. There was a marked increase in the serum enzyme activities of AST, ALT & ALP in the cotton pellet inserted control group of animals. Treatments with TE 250 mg/kg, 500 mg/kg, 750 mg/kg as well as diclofenac sodium (10 mg/kg) significantly attenuated the AST, ALT & ALP activities elevated by the implanted pellets. TE 750 mg/kg group was comparable with the reference standard diclofenac sodium in attenuating the pellet induced increase in serum marker enzyme activities.

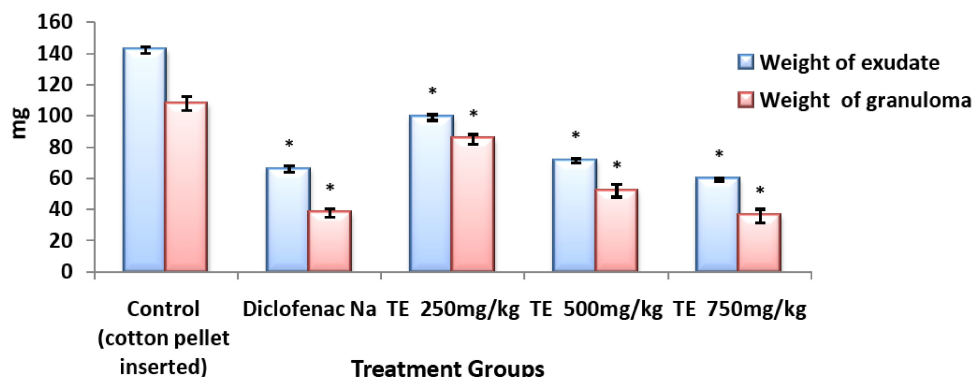
TE 750 mg/kg significantly attenuated the elevated activities of lysosomal enzymes AST, ALT and ALP by 60.15%, 73.67% and 53.39 % respectively; TE 500

mg/kg showed 40.47%, 50.77% and 30.84% attenuation, while TE 250 mg/kg showed 21.13%, 29.45% and 12.47 % attenuation of AST, ALT and ALP activities respectively when compared with the pellet implanted control group of animals (Figure 2).

DISCUSSION

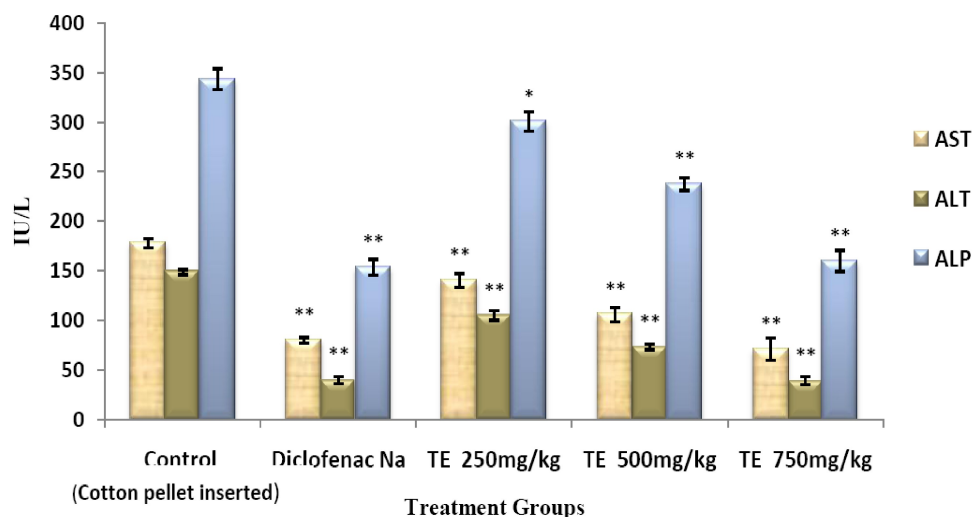
In the present work, the *in vitro* antioxidant anti-inflammatory potential of TE was evaluated by studying the effect of TE on inhibition of i) protein denaturation, ii) proteinase activity and iii) hypotonic saline induced hemolysis of RBC for membrane stabilization.

Denaturation of tissue protein is one of the well documented causes of inflammation^[10]. Membrane proteins are largely responsible for the physical properties of the cell membrane and may contribute to the regula-



Values are mean \pm SEM; N = 6 in each group; One-way ANOVA followed by Dunnett's test is applied for statistical analysis; P values: * < 0.01 when treatment groups were compared with the control (cotton pellet inserted) group

Figure 1 : Effect of TE on cotton pellet induced granuloma in rats



Values are mean \pm SEM; N = 6 in each group; One-way ANOVA followed by Dunnett's test is applied for statistical analysis; P values: * < 0.05, ** < 0.01 when treatment groups were compared with control (cotton pellet inserted) group

Figure 2 : Effect of TE on serum ALT, AST and ALP activities in cotton pellet granuloma

tion of the volume and water content of cells by controlling the movement of sodium and potassium ions through the protein channels which make up ion channels in the cell membrane. Denaturation of membrane proteins lead to leaking of lysosomal enzymes into the site of inflammation. The protein denaturation mechanism might involve alteration of electrostatic, hydrophobic, hydrogen and disulphide bonds of proteins^[8]. Agents that prevent denaturation therefore are potential candidates for anti-inflammatory drug development. TE showed significant inhibition of denaturation of protein in a concentration dependent manner.

Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules many neutral serine proteinases. It was previously reported that serine proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors^[14]. β -sitosterol present in *T. elephantina* may be responsible for this anti-proteinase activity.

Stabilization of the RBC membrane was studied to further probe the mechanism of anti inflammatory action of TE. The RBC membrane is similar to the lysosomal membrane^[15]. Hence inhibition of RBC haemolysis provides good insight into the inflammatory process especially as both events are consequent to injury. Injury to lysosome membrane usually triggers the release of phospholipase A2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators^[16]. Stabilization of the membranes of these cells prevents lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response^[17]. It is therefore expected that compounds with membrane stabilisation activity should offer significant protection of cell membrane against injurious substances. *In vitro* assessment of the effect of TE on membrane stabilization showed that it inhibited hypotonicity-induced lysis of red blood cells significantly.

Most of the models used for evaluating anti-inflammatory activity in laboratory animals involve acute inflammation produced by injection of materials such as formalin, 5-hydroxytryptamine and dextran into the hind paw of rats. The material used in the present study to produce acute inflammation is carrageenan, which is a

sulphated polysaccharide obtained from red green algae (Rhodophyceae). Carrageenan-induced rat paw oedema is a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw^[18].

Carrageenan induced rat paw edema has been described as a biphasic event in which various mediators operate in sequence to produce the inflammatory response^[19]. Histamine, serotonin and bradykinins are the first detectable mediators in the early phase of carrageenan-induced inflammation; prostaglandins (PGs) are involved in the increased vascular permeability and are detectable in the late phase of inflammation^[20]. Local and/or systemic inflammation is associated with enhanced levels of the pro-inflammatory cytokines TNF- α , IL-1, and IL-6. Kinins, once released, are able to activate B₁ and/or B₂ receptors, releasing other inflammatory mediators, such as prostaglandins (PGs), leukotrienes (LTs), histamine, nitric oxide (NO), platelet activating factor (PAF) and cytokines, among others derived mainly from leucocytes, mast cells, macrophages and endothelial cells, causing either cell influx and plasma extravasations. It has been reported that the second phase of edema is sensitive to most of the clinically effective anti-inflammatory drugs^[21]. It is this phase which has been frequently used to access the anti-edematous effect of natural products. TE showed dose dependent inhibition of paw edema in the first and the second phase. However the effect was more significant in the second phase and maximum inhibition was observed during the 3rd hour after carrageenan injection. The anti-inflammatory effect of TE may be due to inhibition of kinin release and also inhibition of prostaglandin synthesis. This activity probably will be due to its polyphenolic constituents.

The cotton pellet granuloma model has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. There are three phases in the inflammatory response in the cotton pellet induced granuloma^[22]. In the first phase imbibition of fluid containing low protein takes place at the site of cotton pellet implantation. In the second phase after 2-3 days of pellet implantation, exudation of fluid containing the protein takes place. In the 3rd phase, i.e. the proliferative phase, appearance of collagen, mucopolysaccharide synthesis, and increase in the number of fibroblasts around the cotton pellets occurs^[23]. The

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amount of newly formed connective tissue can be measured after removing and weighing the dried pellets. TE significantly decreased the final dry weight of the cotton pellets, i.e. it decreased the amount of granulomatous tissue, suggesting that it has the capability of reducing the synthesis of mucopolysaccharides and collagen and the number of fibroblasts, which are natural proliferative events of granulation in tissue formation. TE decreased the weight of granuloma tissue in a dose-dependent manner, confirming its activity in the chronic phase of inflammation.

Lysosomal enzymes used to determine the degree of inflammation, include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP), which are altered during inflammation. During inflammation there is an increase in the serum level of these enzymes. This is because the chronic phase of inflammation involves damage to the lysosomal membrane^[24]. As a result these lysosomal enzymes leak in to the blood stream. TE attenuated the granuloma elevated serum ALT, AST and ALP activities which are increased during the inflammatory process. The inhibition of lysosomal marker enzymes may be largely due to the membrane stabilizing property of TE. TE phytoconstituents, viz. β -sitosterol and quercetin are known to be potent antioxidants. It is likely that both, the antioxidant activity as well as the membrane stabilizing effect of these constituents might be contributing to the anti-inflammatory activity observed in different types of inflammation in this study.

CONCLUSION

Thus, to summarize, the methanolic extract of inflorescence of *Typha elephantina* showed significant anti-inflammatory activity probably by virtue of an underlying antioxidant activity and lysosomal membrane stabilization.

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