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## Membrane stabilizing and thrombolytic activities of methanolic extract of *Trichosanthes dioica* Roxb. Shoot

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### Abstract

Pointed gourd (*Trichosanthes dioica*) is a common and popular vegetable in our country. This vegetable is locally known as “Patal”. The shoot of pointed gourd (Pataler loti) is also used as a vegetable. The present study was undertaken to evaluate the inherent anti-inflammatory and thrombolytic activity of methanolic extract of the shoots of *Trichosanthes dioica* (METDS). *In vitro* anti-inflammatory activity was uncovered by using human erythrocyte membrane stabilization and egg albumin denaturation method. *In vitro* thrombolytic activity was investigated and compared with streptokinase. In hypotonic solution-induced haemolysis and heat-induced haemolysis, METDS protects 66.39%, 71.59% RBC from lysis. METDS dose dependently showed a potent anti-inflammatory and anti-arthritis activity. In addition, thrombolytic activity was found to be 28.46±4.16%, 9.67±6.37 and 65.96±4.25% for METDS, normal saline and streptokinase (a positive control) respectively. Therefore, it can be concluded that the shoots of *Trichosanthes dioica* possess promising thrombolytic, anti-inflammatory that may be a potential agent for prevention of inflammation.

**Keywords:** *Curcuma zedoaria*, rhizome, anti-inflammatory, thrombolytic, anti-arthritis.

### 1. Introduction

Inflammation, a complex localized response of vascular tissue to foreign substances characterized by redness, warmth, swelling and pain, is a normal protective response to tissue injury [1-2]. But prolonged inflammation is harmful for health as it produces various diseases like rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart diseases etc. Some non-steroidal drugs are used for the management of inflammatory condition which may cause gastric bleeding, ulceration, bone marrow disturbance, kidney and liver dysfunction [3-4]. For these reason, there is a need to find alternative and natural drugs having less or no side effects to use for oxidative stress and chronic inflammatory disease.

The plant *Trichosanthes dioica*, popularly known as ‘Patal’, belongs to the family Cucurbitaceae and is an important summer cucurbit vegetable of Bangladesh. It is climber plant with cordate-oblong leaves, white flowers and oblong green fruits. Traditionally it is used for the treatment of various ailments such as bronchitis, biliousness, jaundice, liver affections (enlargement), cough and blood diseases. It is also used as antipyretic, diuretic, cardiostonic and laxative [5]. The leaf juice is rubbed over the chest in liver congestion and over the whole body in intermittent fevers [6]. *Trichosanthes dioica* possesses anti-inflammatory activity [7]; blood sugar, serum cholesterol, high density lipoprotein, phospholipids and triglyceride lowering activity [8-9].

However, anti-inflammatory and thrombolytic activities of methanol extract of shoot of this plant has not been reported elsewhere. From this viewpoint the present study was carried out the anti-inflammatory and thrombolytic activities of the shoot extract of *Trichosanthes dioica* Roxb.

### 2. Materials and methods

#### 2.1. Plant materials

Shoots of *Trichosanthes dioica* (Family: Cucurbitaceae) were selected for the present investigation. The plant samples were collected during the month of June-July, 2015 from the relevant area of Rajshahi University campus. The plants were authenticated by the authority of Botany Department, University of Rajshahi, Rajshahi.

#### 2.2. Extraction

The shade dried and powdered Shoots of *Trichosanthes dioica* (Roxb.) was successively extracted with methanol at room temperature and after filtration, filtrates were evaporated

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under reduced pressure at 40 °C using a rotary evaporator to have methanol (METDS) extracts.

### 2.3. Evaluation of *in vitro* anti-inflammatory activity

#### 2.3.1. Membrane stabilizing activity

Two methods namely hypotonic solution-induced and heat-induced human erythrocyte haemolysis were applied to illuminate the membrane stabilizing activity [10-11].

#### Hypotonic solution- induced haemolysis

To perform this test, 0.5 ml of RBC suspension and 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) were placed in a test tube which is followed by the addition of 1ml of extract/standard with various concentrations. A mixture containing all of the materials mentioned above except the sample/standard was taken as control. After successive incubation (for 10 minutes) and centrifugation (at 3000rpm for 10 min) the absorbance was taken at 540 nm. Acetyl salicylic Acid treating similarly was used as reference standard.

#### Heat- induced haemolysis

5 ml aliquots (containing isotonic buffer) of the extract were kept into two sets of centrifuge tubes. Same amount of vehicle was served as control. 30 µl of RBC suspension was mixed to each centrifuge tube. Then, incubation of one pair mixed to each centrifuge tubes was carried out at 54°C for 20 min in a water bath. The temperature of another pair was maintained at 0-5°C. The mixture of each tube was then centrifuged at 1300 rpm for 5 min. Absorbance was recorded of the supernatant at 540 nm. Acetyl salicylic Acid treating similarly was used as reference standard.

#### 2.3. Egg albumin denaturation assay

The method described by Dapurkar *et al* was followed to perform the egg albumin denaturation assay [12] in which 5ml of reaction mixture was made by taking 2 ml of sample extract with various concentrations, 0.2 ml of albumin isolated from fresh hen's egg and 2.8 ml of phosphate buffered saline (PBS, pH 6.4). Same amount of doubled-distilled water was taken as control. Incubation of each mixture was carried out at 37°C±2 for 15 min followed by heating at 70°C for 5 min. The mixtures were cooled and their absorbance was recorded at 660 nm. Acetyl salicylic Acid treating similarly was used as reference standard.

### 2.4. Determination of thrombolytic activity

The thrombolytic activity of METDS was evaluated by the method developed by Daginawala (2006) [13] using streptokinase (SK) as the standard. Commercially available lyophilized Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd.) of 15, 00,000 I.U., was collected and 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µL (30,000 I.U) was used for *in vitro* thrombolysis. Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1mL of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

## 3. Result and Discussions

### 3.1. Anti-inflammatory activity assay

Table 1 showed that METDS significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat induced haemolysis when compared to the standard acetyl salicylic acid. In hypotonic solution and heat induced conditions, the METDS inhibited 60.19% and 67.17% haemolysis of RBCs, respectively as compared to 74.93% and 95.96% inhibited by acetyl salicylic acid, respectively (500 µg/ml). The erythrocyte membrane resembles to lysosomal membrane. Compounds with membrane stabilizing properties can interfere with the release of phospholipases that trigger the formation of inflammatory mediators [14]. Hypotonic solution induced hemolysis assay showed that the extract effectively protect the membrane of RBC. The extract also inhibited the heat induced hemolysis of RBCs.

It has been reported that protein denaturation is one of the causes of rheumatic arthritis. Several anti-inflammatory drugs have been reported to inhibit thermally induced protein denaturation at a dose dependent manner [14]. In Egg Albumin denaturation method METDS showed 33.67%, 41.35%, 48.66%, 56.00% and 63.37% inhibition of Egg Albumin denaturation for concentrations of 100, 200, 300, 400 and 500 µg/ml, while at the same concentrations, standard acetyl salicylic acid showed 41.01%, 58.97%, 65.33%, 71.91% and 85.87% inhibition (Table 1). From the result of the present study, the extract had shown considerable anti-inflammatory activity.

**Table 1:** Anti-inflammatory assay of METDS

Concentration (µg/ml)	Percentage inhibition					
	Hypotonic solution induced hemolysis		Heat induced hemolysis		Protein denaturation assay	
	Metds	Asa	Metds	Asa	Metds	Asa
100	30.06	36.06	40.09	48.75	33.67	41.01
200	34.56	44.29	53.25	59.30	41.35	58.97
300	38.65	51.30	60.65	74.52	48.66	65.33
400	50.15	63.09	64.89	86.84	56.00	71.91
500	60.19	74.93	67.17	95.96	63.37	85.87

### 3.2. Thrombolytic activity

The thrombolytic activity of METDS and Normal saline were significantly different (P<0.05). At a concentration of 100µg/ml Streptokinase, METDS and Normal saline showed 65.96±4.25%, 28.46±4.16% and 9.67±6.37% thrombolytic activity, respectively (Table 2). In an attempt to discover a cardio-protective drug from natural sources, METDS were assessed for thrombolytic activity [15] and the mean difference in clot lysis percentage between normal saline and METDS

was found statistically very significant (p<0.01).

**Table 2** % of thrombolysis of METDS, Streptokinase and Normal saline

Sample	Mean of % of clot lysis
METDS	28.46±4.16
Streptokinase	65.96±4.25
Normal saline	9.67±6.37

#### 4. Conclusion

From the above results it can be concluded that the methanol extract of *Trichosanthes dioica* Roxb. shoot possess anti-inflammatory and thrombolytic activity. So this nutritive vegetable may play important role in protection against aging and inflammation. Further investigation is required to identify and characterize active compounds present in the extract.

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