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IN VITRO MEMBRANE STABILIZING ACTIVITY OF LEAF EXTRACTS OF *PAEDERIA FOETIDA* (L.)

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ABSTRACT

In this present study, the leaf extracts of *Paederia foetida* were subjected to evaluation of the membrane stabilizing activity by using human erythrocyte and the results were compared with standard anti-inflammatory drug, acetyl salicylic acid (ASA). *In vitro* membrane stabilizing activity for hypotonic solution induced haemolysis, the ethanol extract inhibited 76.92% haemolysis of RBCs as compared to 85.42% produced by acetyl salicylic acid (ASA) and during heat induced condition different organic soluble materials of *Paederia foetida* demonstrated 76.68%, 77.19% and 74.83% inhibition of haemolysis of RBCs respectively whereas ASA inhibited 78.04%.

KEYWORDS: *Paederia foetida*, membrane stabilizing activity, human erythrocyte, acetyl salicylic acid.

INTRODUCTION

Paederia foetida is an important Ayurvedic medicinal herb. *Paederia* is from the Greek word *paederos* meaning opals, for some of the species have translucent drupes. *Foetida* means stinking. Its Sanskrit name is Gandha Prasirini. The word meaning in sanskrit is – it spreads bad smell. It is a unique feature of this herb. Gandha means smell, prasarini means spreading. Hindi name- Gandhaprasarani or Pasaran; English name- Chinese Flower Plant, Bengali name- Gandhabhaduliya, Gandhabhadule, Gandal. It was indicated for the treatment of gout, diarrhea, piles, dysentery, calculi, stomachic, emetic, ulcers and different type of inflammations. It has also been reported for anti-nociceptive[1] antiviral,[2] anti-

diarrheal,[3] anti-tussive[4] and anti-inflammatory.[5] *Paederia foetida* belonging to family Rubiaceae is one of 30 species in the genus *Paederia*. The origin of this plant is considered to be Eastern and southern Asian. It is usually found in different parts of Bangladesh and also in India like Assam, Bihar and Orissa. It possesses perennial twining vine from woody rootstock; stems to 7 m (23 ft) or more, climbing, or prostrate and rooting at the nodes. Leaves are opposite (rarely in whorls of 3), with conspicuous stipules. Petioles are commonly to 6 cm (2.4 in) long; blades entire, oval to linear-lanceolate, 2-11 cm (1-4.3 in) long, hairy or glabrous, often lobed at base. The leaves and stems have disagreeable odor, especially when crushed. Flowers are small, grayish pink or lilac, in broad or long, "leafy" curving clusters, terminal or at leaf axils. Fruits are shiny brown, nearly globose capsule, to 0.7 cm (0.3 in) wide, with 2 black roundish seeds, often dotted with white raphides.[6] Major chemical constituents like asperuloside, scandoside, paederoside and a-and b paederine etc. are present in this plant.[7] As a part of our continuing studies on medicinal plants of Bangladesh the organic soluble materials of the leaf extracts of *Paederia foetida* were evaluated for *in-vitro* membrane stabilizing activity for the first time.[8-15]

MATERIALS AND METHODS

Collection, identification and processing of plant sample

The leaves of *Paederia foetida* was collected from Botanical garden, Curzon Hall at the University of Dhaka in June 2014 and was taxnomically identified with the help of the National Herbarium of Bangladesh, Mirpur-1, Dhaka (DACB; Accession Number- 39585). Leaf was sun dried for seven days. The dried leaf were then ground in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction procedure

The powdered plant parts (22 gm) were successively extracted in a soxhlet extractor at elevated temperature using 250 ml of distilled Methanol (40-60)°C which was followed by ethanol, and chloroform. After extraction all extracts kept in refrigerator 4°C for future investigation with their necessary markings for identification.

Blood sample

Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 5ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Membrane stabilizing activity

The membrane stabilization by hypotonic solution and heat-induced haemolysis method was used to assess anti-inflammatory activity of the plant extracts by following standard protocol.[16] Since the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane.[17] The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant 3.1% Na-citrate). The blood was centrifuged and blood cells were washed three times with solution (154mM NaCl) in 10mM sodium phosphate buffer (pH 7.4) through centrifugation for 10min at 3000g.[18-19]

Hypotonic solution-induced haemolysis

The test sample consisted of stock erythrocyte (RBC) suspension (0.5mL) mixed with 5mL of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0mg/mL) or acetyl salicylic acid (ASA) (0.1mg/mL). The control sample consisted of 0.5mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10min at room temperature, centrifuged for 10min at 3000g and the absorbance of the supernatant was measured at 540nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

% inhibition of haemolysis = $100 \times (OD_1-OD_2)/OD_1$

Where, OD_1= optical density of hypotonic-buffered saline solution alone (control)

OD_2= optical density of test sample in hypotonic solution

Heat-induced haemolysis

Isotonic buffer containing aliquots (5ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 56°C for 30min in a water bath, while the other pair was maintained at (0-5) °C in an ice bath. The reaction mixture was centrifuged for 5min at 2500g and the absorbance of the supernatant was measured at 560 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

% Inhibition of hemolysis = $100 \times [1- (OD_1-OD_2)/ (OD_3-OD_1)]$

Where, OD_1= optical density of unheated test sample

OD_2= optical density of heated test sample

OD_3= optical density of heated control sample

RESULTS AND DISCUSSIONS

Membrane stabilizing activity

In the study of membrane stabilizing activity, the leaf extracts of *Paederia foetida* at concentration of 1.0mg/mL were tested against the lysis of human erythrocyte membrane induced by hypotonic solution as well as heat, and compared with the standard acetyl salicylic acid (ASA) (table 1). For hypotonic solution induced haemolysis, at a concentration of 1.0mg/ mL, the ethanol extract inhibited 76.92% haemolysis of RBCs as compared to 85.42% produced by acetyl salicylic acid (0.10mg/mL. The methanol and chloroform soluble extractives also revealed good inhibition of haemolysis of RBCs. On the other hand, during heat induced condition different organic soluble materials of *Paederia foetida* demonstrated 76.68%, 77.19% and 74.83% inhibition of haemolysis of RBCs, respectively whereas ASA inhibited 78.04%. To confirm the membrane stabilizing activity of *Paederia foetida* of the above mentioned model, experiments were performed on the erythrocyte membrane. A possible explanation of the stabilizing activity of different extractives due to an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of the membrane or shrinkage of the cell and an interaction with membrane proteins. The present investigation suggests that the membrane stabilizing activity of *Paederia foetida* may be playing a significant role in its anti-inflammatory activity.[19]

Table 1: Effect of extractives of *Paederia foetida* on hypotonic solution and heat induced haemolysis of erythrocyte

Samples	Concentration (mg/mL)	% Inhibition of Haemolysis	
		Hypotonic solution	Heat induced
Control	50mM	_	_
Acetyl salicylic acid (ASA)	0.1	85.42±0.025	78.04±0.033
Methanol extract	1	72.47±0.018	76.68±0.041
Ethanol extract	1	76.92±0.023	77.19±0.065
Chloroform extract	1	63.97±0.004	74.83±0.052

Values are mean ± SEM (n=6)

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