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MEMBRANE STABILIZING AND ANTI-THROMBOLYTIC ACTIVITIES OF FOUR MEDICINAL PLANTS OF BANGLADESH

ABSTRACT

Four Bangladeshi medicinal plants Withania somnifera, Terminalia arjuna, Moringa olifera and Asparagus racemosus have been investigated for their in vitro thrombolytic and membrane stabilizing. Among the four plants, the methanol extract of W. somnifera exhibited highest thrombolytic activity with clot lysis value of 36.50%. Standard streptokinase and water were used as positive and negative control which demonstrated 66.77% and 2.35% lysis of clot of human blood. The membrane stabilizing activity was assessed by using hypotonic solution and heat-induced methods and was compared with acetyl salicylic acid as standard drug.

Key words: Withania somnifera, Terminalia arjuna, Moringa olifera, Asparagus racemosus Membrane stabilizing, Thrombolytic activity.

INTRODUCTION

Withania somnifera appears to be a dense pubescent shrub that grows up to a height of 1 miter tall and belongs to the family of solanaceae. It is a popular medicinal plant of Bangladesh and locally known as ashwagandha. Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics [(1)]. A methanolic extract of the various parts of Withania somnifera had showed a potent anti-inflammatory activity. Withania somnifera is found to be a unique plant where a wider range of biological activities has been demonstrated including antagonism with several inflammatory factors and the immune modulation. Withania somnifera have been found to exhibit certain antibacterial, anti-fungal and antitumor properties [(2)].

Terminalia arjuna (Family - Combretaceae), a large tree, is found throughout the South Asian region. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. This tree is an exotic tree in Bangladesh. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. The bark of T. arjuna is anti-dysentric, antipyretic, astringent, cardiotonic, lithotriptic and tonic while the powder of the bark acts as a diuretic in cirrhosis of liver and gives relief in symptomatic hypertension [(3)]. In studies in mice, its leaves have been shown to have analgesic and anti-inflammatory properties [(4)].

Moringa oleifera (Moringaceae) is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands [(5)]. Literature revealed that the Moringa tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement [(6)]. The Moringa plant has been consumed by humans throughout the century in diverse culinary ways [(5)]. Epidemiological studies have indicated that M. oleifera leaves are a good source of nutrition and exhibit anti-tumor, antiinflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities [(7-9)].

Asparagus racemosus (Liliaceae), locally known as 'Shatamuli', is a woody climber growing to 1-2 m in height. The Bangladeshi local name 'Shatamuli' refers to its finger-like and many clustered roots. The leaves are like pine-needles, small and uniform. The inflorescence has tiny white flowers, in small spikes [(10)]. The plant is common at low altitudes in shade and in tropical climates throughout Asia, Australia and Africa. In India, the plant is called Shatavari in Hindi. Recent research indicates Shatavari enhances immune function, increases corticosteroid production, and promotes cell regeneration [(11)]. The root has long been used in Ayurveda as a tonic remedy to promote fertility and reducing menopausal symp-toms [(12)]. It is also used for dry coughs and gastric ulcers [(13)]. It is mainly known for its phytoestrogenic properties. In Ayurveda, Asparagus racemosus has been used extensively as an adaptogen to

increase the non-specific resistance of organisms against a variety of stresses [14-15]. Besides use in the treatment of diarrhoea and dysentery, the plant also has antioxidant, immune-stimulant, anti-dyspepsia and anti-tussive effects [13-18].

Previously less attention has been focused on clot lysing and membrane stabilizing activity of Withania somnifera, Terminalia arjuna, Moringa olifera and Asparagus racemosus. Thus, the aim of this study was to examine the in vitro thrombolytic and membrane stabilizing potential of these four medicinal plants of Bangladesh as a part of our continuing studies on medicinal plants of Bangladesh [19-25].

MATERIALS AND METHODS

Plant materials: The different plat parts of W. somnifera, T. arjuna, M. olifera and A. racemosus were collected from Mirpur Botanical Garden, Dhaka, Bangladesh, in July 2012. A voucher specimen for these plants has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 35903, 35904, 35905, 35906) respectively.

The sun dried and powdered plant parts (500 gm) of each plants were successively extracted in a Soxhlet extractor at elevated temperature using 200 ml of distilled Methanol (40-60)°C. All extracts were filtered individually through filter paper and poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

Streptokinase (SK): 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 sample: 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.

Thrombolytic activity: The thrombolytic activity of all extracts of the plants were evaluated by the method developed by Daginawala (2006) [26] and slightly modified by Kawsar et al. (2011) [27] using streptokinase (SK) as the standard.

Membrane stabilizing activity: 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 [28]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis [29].

To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant EDTA). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

Hypotonic solution- induced haemolysis: The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation-

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

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Where, OD_1 = optical density of hypotonic-buffered saline solution alone (control) and OD_2 = optical density of test sample in hypotonic solution.

Heat- induced haemolysis: Isotonic buffer containing aliquots (5 ml) of the different extracts were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54^{o}C for 20 min in a water bath, while the other pair was maintained at (0-5)^{o}C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

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

Where, OD_1 = optical density of unheated test sample, OD_2 = optical density of heated test sample and OD_3 = optical density of heated control sample

RESULTS AND DISCUSSION

All the extractives at 1.0 mg/mL significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat induced haemolysis, as compared to the standard acetyl salicylic acid (0.10 mg/mL) (Table 1). In hypotonic solution and heat induced conditions, the methanol extract of W. somnifera inhibted 63.95% and 45.91% haemolysis of RBCs, respectively as compared to 72.91% and 42.12% inhibited by acetyl salicylic acid, respectively (0.10 mg/mL). The methanol extract of M. olifera, A. racemosus and T. arjuna also significantly inhibited the haemolysis RBCs in both hypotonic solution and heat induced conditions.

Table 1: Effect of extractives of W. somnifera, T. arjuna, M. olifera and A. racemosus on hypotonic solution and heat-induced haemolysis of erythrocyte membrane.

Sample	Concentration (mg/mL)	Haemolysis inhibition (%)	
		Heat induced	Hypotonic solution induced
Acetyl salicylic acid	0.1	42.12±0.55	72.91±1.53
W. somnifera	1.0	45.91±3.31	63.95±3.17
T. arjuna	1.0	30.33±0.24	27.65±2.16
M. olifera	1.0	35.24±0.95	20.65±0.82
A. racemosus	1.0	32.14±1.05	18.61±0.34

Thrombolytic activity: As a part of discovery of cardio-protective drugs from natural sources the extractives of W. somnifera, T. arjuna, M. olifera and A. racemosus were assessed for thrombolytic activity and the results are presented in Table 2. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed (66.77%) lysis of clot. At the same time, distilled water was treated as negative control which exhibited negligible lysis of clot (2.35%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant. In this study, methanol extract of W. somnifera exhibited highest thrombolytic activity (36.50%). However, significant thrombolytic activity was demonstrated by the methanol extracts of T. arjuna (30.57%), A. racemosus (28.95%) and M. olifera (20.63%) respectively.

Table 2: Thrombolytic activity (in terms of % of clot lysis) of W. somnifera, T. arjuna, M. olifera and A. racemosus.

Sample	% of lysis
SK	66.77±0.26
Water	2.35±0.13
W. somnifera	36.50±2.57
T. arjuna	30.57±0.54
M. olifera	20.63±0.25
A. racemosus	28.95±1.60

SK= Streptokinase

CONCLUSION

It can be concluded that the extracts of all the above plants can be used to design anti-thrombolytic agent due to its moderate thrombolytic activity. Further work is needed to isolate the secondary metabolites and study thoroughly for more precise and accurate activities. This in vitro study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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