

Membrane Stabilizing and Thrombolytic Activity of *Withania somnifera* Root

Mohammad Shahriar*¹, Fariha Alam¹ and Mir Muhammad Nasir Uddin²

¹Phytochemistry Research Laboratory, University of Asia Pacific, Dhaka, Bangladesh

²Department of Pharmacy, University of Chittagong, Chittagong, Bangladesh

Address for Correspondence

Department of Pharmacy University of Asia Pacific, House no. 73, Road no. 5A, Dhanmondi, Dhaka-1209, Bangladesh

E-mail: shahriar@uap-bd.edu

ABSTRACT

In this present study, the root extracts of *Withania somnifera* were subjected to a comparative evaluation of the membrane stabilization and thrombolytic activity on human blood sample. The thrombolytic and membrane stabilizing activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK) and standard anti-inflammatory drug, acetyl salicylic acid (ASA), respectively. The Methanol extract showed the highest thrombolytic activity (68.14%). On the other hand hypotonic solution induced haemolysis, at a concentration of 1.0 mg/ mL, the methanol extract inhibited 71.90% haemolysis of RBCs as compared to 73.56% produced by acetyl salicylic acid (0.10 mg/mL). And in case of heat induced condition different organic soluble materials of *Withania somnifera* demonstrated 54.03%, 53.13% and 38.54% inhibition of hemolysis of RBCs, respectively whereas ASA inhibited 48.10%.

Keywords: *Withania somnifera*, Membrane stabilizing activity, Thrombolytic activity.

INTRODUCTION

The plants which are useful for healing diseases are called medicinal plant. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." There are more than 500 medicinal plants growing in our country¹.

Recently World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially

for their primary health care. It has been recorded that about 450 to 500 plants growing or available in Bangladesh have therapeutic values^{1,2}. In Bangladesh, people living in the remote hilly areas, such as, ethnic communities rely mostly on herbal medicines. Bangladesh, a country fertile deltaic land has a rich diversity of flora of medicinal plants scattered throughout the forests, crop fields, roadsides gardens and wastelands.

Thrombolytic therapy reduces mortality and preserves left ventricular function in patients with myocardial infarction. Streptokinase is widely used fibrinolytic drug that was used in this study as standard. All thrombolytic agents work by activating the enzyme plasminogen that clears the cross-linked fibrin mesh³. In the present study root extracts of *Withania somnifera* were used to evaluate thrombolytic activity on human blood sample.

Withania somnifera is a small and erect evergreen woody under shrub that grows up to a height of 1-m tall and belongs to the family of solanaceae locally known as Ashwagandha. This plant is capable of growing wildy not only in all the drier parts of the subtropical Bangladesh i.e. in Nator, Savar, and North-western parts of Bangladesh but also in India, Congo, South Africa, Egypt, Morocco, Jordan, Pakistan and Afghanistan. The roots are the main portions of the whole plant as they possess wide number of the therapeutic agents. The crude aqueous extract of the plant contains the phenolics and flavonoids which are said to be the potent antioxidants⁴. Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics⁵. 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.

MATERIALS AND METHOD

Plant material

The plant roots were collected during July, 2013, from Savar, Dhaka, Bangladesh. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur,

Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 35903) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The roots were sun dried for seven days. The dried plant part was then ground in coarse powder using high capacity grinding machine.

Preparation of extract

Hot solvent extraction process was used for extraction of the plant material. Soxhlet extractor was used for the extraction procedure. Plant material was extracted by the solvents- methanol, ethanol and chloroform. After extraction, was kept at petri dishes and dried at room temperature. After drying, extracts were stored in petri dishes and kept in refrigerator for further use.

Streptokinase (SK)

Commercially available lyophilized Altepase (Streptokinase) vial (Trade name-S-Kinase from Popular pharmaceutical Ltd.) of 15, 00,000 I.U., was collected and 5 ml 0.9% NaCl 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 500 ml 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 was evaluated by the method developed by Dagainawala (2006)³ with slightly modified and by 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 membrane⁶. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced mice erythrocyte haemolysis⁷.

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 (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 extract (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-

$$\% \text{ inhibition of haemolysis} = 100 \times (\text{OD}_1 - \text{OD}_2 / \text{OD}_1).$$

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 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 (30 μL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath, while the other pair was maintained at (0-5)° 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:

$$\% \text{ Inhibition of hemolysis} = 100 \times [1 - (\text{OD}_2 - \text{OD}_1 / \text{OD}_3 - \text{OD}_1)].$$

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.

Statistical analysis

Data was expressed as Mean \pm SEM (Standard error of Mean).

RESULTS AND DISCUSSION

Thrombolytic activity

As a part of discovery of cardio-protective drugs from natural sources the extractives of *Withania somnifera* were assessed for thrombolytic activity and the results are presented in table 1.

Addition of 100 μL Streptokinase (30,000 I.U.), standard to the clots along with 90 minutes of incubation at 37°C, showed 66.77% clot lysis. Clots when treated with 100 μL sterile distilled water (control) showed only negligible clot lysis (2.64%). In this study, the methanol extract of *Withania somnifera* revealed thrombolytic activity of 68.14%, whereas ethanol and chloroform extracts of *Withania somnifera* (21.15% and

17.46%) displayed moderate thrombolytic activities.

Membrane stabilizing activity

The root extracts of *Withania somnifera* at concentration of 1.0 mg/mL, were tested against lysis of human erythrocyte membrane induced by hypotonic solution as well as heat, and compared with the standard acetyl salicylic acid (ASA) (0.10 mg/ml) (table 2).

For hypotonic solution induced haemolysis, at a concentration of 1.0 mg/ mL, the methanol extract inhibited 71.90% haemolysis of RBCs as compared to 73.56% produced by acetyl salicylic acid (0.10 mg/mL). On the other hand, during heat induced condition different organic soluble materials of *Withania somnifera* demonstrated 54.03%, 53.13% and 38.54% inhibition of hemolysis of RBCs, respectively whereas ASA inhibited 48.10%.

As a part of our continuing studies on medicinal plants of Bangladesh the root extracts of *Withania somnifera* were used to evaluate membrane stabilizing and thrombolytic activity on human blood sample. The present study also reflects some of those activities as well like our previous study⁸⁻¹⁰.

CONCLUSION

The study clearly indicates that the extracts possess membrane stabilizing and thrombolytic activities. These finding justify the traditional uses of this plant in the treatment of cardiac patients. Further research is necessary for elucidating the active principles.

REFERENCES

1. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd

- ed., *Asiatic Society of Bangladesh*, Dhaka; 1998: 4-19.
2. Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal Plants of Bangladesh. *Bangladesh Council of Scientific and Industrial Research*, Dhaka; 1994: 5-11.
3. Dagainawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal*. 2006; 4(2): 1-4.
4. Mehrotra V, Shubhi M, Vandna K, Radhey S, Kshipra M, Ashwani KS, Shoma PN. Antioxidant and antimicrobial activities of aqueous extract of *Withania somnifera* against methicillin-resistant *Staphylococcus aureus*. *J Microbiol Biotech Res*. 2011; 1(1): 40-45.
5. Bhatnagar M, Jain CP, Isodia SS. Anti-ulcer activity of *Withania somnifera* in stress plus pyloric ligation induced gastric ulcer in rats. *J of Cell and Tissue Research*. 2005; 5(1): 287-292.
6. Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr J Biotechnol*. 2008; 7: 3129-3133.
7. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterap*. 1999; 70: 251-257.
8. Parvin D, Uddin MMN, Islam MS, Parvin S, Shahriar M. Phytochemical screenings, thrombolytic activity and antimicrobial properties of the leaf extracts of *Lablab purpureus*. *Ame J Res Comm*. 2013; 1(2): 49-55.
9. Uddin MMN, Mahmuduzzaman M, Md Islam MS, Parvin S, Shahriar M. Phytochemical screenings and thrombolytic activity of the leaf extracts of *Adhatoda vasica*. *The Exp j*. 2013; 7(4): 438-441.
10. Shahriar M, Islam MS, Parvin S, Hoque S. Thrombolytic activity and antimicrobial properties of *Ficus hispida*. *J Sci Res*. 2013; 5(2):393-397.

Table 1. % Clot lysis by different extracts of *Withania somnifera*

Samples	% of clot lysis
Methanol Extract	68.14 ± 3.77
Ethanol Extract	21.15 ± 0.60
Chloroform Extract	17.46 ± 2.36
Control (blank)	2.64 ± 0.22
Streptokinase (Std.)	66.77 ± 0.66

Values are expressed as mean ± S.D. (n=6)

Table 2. Effect of extractives of *Withania somnifera* on hypotonic solution & heat induced hemolysis of erythrocyte

Samples	Concentration (mg/mL)	% Inhibition of haemolysis	
		Hypotonic solution	Heat induced
Hypotonic solution (Control)	50 mM	—	—
Acetyl salicylic acid (ASA)	0.1	73.56 ± 0.02	48.10± 0.76
Methanol Extract	1	71.90± 0.99	54.03± 0.00
Ethanol Extract	1	62.43± 0.84	53.13 ± 0.94
Chloroform Extract	1	26.38± 0.47	38.54 ± 0.74

Values are expressed as mean ± S.D. (n=6)