Research Article

THROMBOLYTIC AND MEMBRANE STABILIZING PROPERTIES OF METHANOLIC EXTRACTS OF *AVICENNIA MARINA* LEAVES & BARKS

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Article Received on: 23/04/16 Revised on: 23/05/16 Approved for publication: 18/06/16

DOI: 10.7897/2230-8407.07659

ABSTRACT

The present study was commenced to judge ethnomedicinal worth of the plant. We tried to analyze in vitro thrombolytic and membrane stabilising activities of methanolic extracts of *Avicennia marina*. Crude methanolic extracts of *A. marina* leaves & barks at numerous conc. were used for in vitro thrombolytic and membrane stabilising activities. Just in case of thrombolytic study, it absolutely was dose dependently accumulated, wherever 10 mg/ml concentration most importantly showed 13.22% lysis of clot ($p < 0.001$) by in vitro clot lysis assay technique. Streptokinase was used as standard & water was treated as negative management. Crude methanolic extracts of *A. marina* dose dependently increased in membrane stabilising study, whereas 10 mg/ml concentration most importantly showed 10.11% & 22.23% inhibition of haemolysis severally by both hypotonic solution and heat induced haemolysis of RBC membrane. Acetyl salicylic acid was used as standard in membrane stabilising study.

Key words: *Avicennia marina*, thrombolytic, membrane stabilizing activities.

INTRODUCTION

Nature continuously performs as an excellent supply of rescue for individual by providing completely different remedies from its plants, animals, and different sources to care all ailments of the world¹. Our ancient system of medication and cognitive content generally uses the complete medicinal plant or a section of it for the treatment of all kinds of diseases successfully². The plant drugs shows comparatively non-toxic secure and nearly free from adverse effects³. Medicative plants represent the key constituents of native medicines. Nowadays there is associate gaining interest by the scientists to outline the secrets of those ancient favored medicines⁴.

Thrombosis is a fatal illness that is defined by the formation of blood clots (thrombus) within the vascular system owing to the imbalance of physiological condition system of physiological procedures that results in vascular blockade and whereas regaining it causes fatal consequences, like cerebral or infarction and even death⁵. Thromboembolic disorders like respiratory organ emboli, deep vein occlusion, strokes and heart attacks etc. are the foremost causes of morbidity and mortality in developed countries⁶. Thrombolytic therapy applies medication referred to as thrombolytic agents, like alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots are widely used throughout the world for the treatment of thromboembolic diseases though streptokinase and urokinase are the primary selections in Indian regions because of the simple reach and lower value as compared to completely different thrombolytic medication^{7, 8}. But the weak

substrate specificity of those first-generation medications (streptokinase and urokinase) normally ends up in some major aspect effects like general disintegration, and hemorrhage⁹. One of the foremost vital pathological havoc is inflammation. It is a region of non-specific immune reaction that happens in response to any kind of bodily damage, is a complicated biological response of vascular tissues to deleterious stimuli^{10, 11}. Membrane stabilising agents are helpful to scale back the hyper-excitability of nerves¹².

A. marina ordinarily called grey mangrove or white mangrove which is accessible in Bangladesh, Indonesia, Malaysia, Africa, Brazil, and Central America. The purpose of the current study was to research the methanolic extracts of *A. marina* for thrombolytic, membrane stabilizing & antimicrobial activities.

MATERIALS AND METHODS

Collection of plant materials: The leaves and barks of *A. marina* were purveyed from Sonadia Deep, Cox's Bazar, Bangladesh on April 2015. It is locally called Tellamada, Morichabaen. Its accession number is 38311, which was confirmed by the National Herbarium Institute, Mirpur, Dhaka, Bangladesh.

Preparation of extraction: After consolidation of leaves and barks of *A. marina* were thoroughly cleaned up with water. Then the collected plant materials were excised, desiccated, and floured. About 500g of the floured materials were doused in 1.5 litre of methanol at room temperature for two weeks. Then the

solution was refined treating filter cloth and Whatman's filter paper and condensed with a rotary evaporator. It exhibited a brown granular. The brown granular was christened as crude methanolic extract.

THROMBOLYTIC ACTIVITY

Standard drug: Streptokinase (SK), Commercially procurable lyophilized Altepare (Streptokinase) vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was accumulated and 5 ml sterile distilled water was assembled and mingled duly. This suspension was employed as a standard from which 100 μ l (30,000 I.U.) was used for *in vitro* thrombolysis¹³.

Preparation of test sample: Five separated test solutions were used to assess the thrombolytic activity of the plant extracts. The plant extracts were fused in methanol and jolting forcefully on a vortex mixer to make different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The suspension was remained overnight and blended to carry away the soluble supernatant, which was filtered through a 0.22 micron syringe filter. In this way test samples were equipped for thrombolytic screening.

Thrombolytic potential: Thrombolytic activities of *A. marina* were performed by in vitro clot lysis assay technique¹⁴. 5 ml of venous blood were drawn from healthy volunteers without a memoir of oral contraceptive or anticoagulant therapy, which were delivered in five totally different preweighed sterile micro centrifuge tubes (0.5 ml/tube) and incubated at 37 °C for 45 minutes. After clot appearance, the serum was entirely superseded without disorganizing the clot and each tube having clot was again weighed to ascertain the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Each micro-centrifuge tube containing clot was rightly named and 100 μ l of the test samples from each concentration (2, 4, 6, 8 and 10 mg/ml respectively) were put to the tubes accordingly¹⁴. As a positive control, 100 μ l of streptokinase (SK) and as a negative control, 100 μ l of distilled water were individually totalized to the control tubes. All the tubes were then incubated at 37 °C for 90 minutes and investigated for clot lysis. After incubation, the fluid was withdrawn and tubes were again weighed to overlook the distinction in weight after clot disruption. Dissimilation derived in weight received before and after clot lysis was revealed as percentage of clot lysis as present below:

$$\% \text{ of clot lysis} = \frac{\text{weight of released clot}}{\text{weight of clot before treatment}} \times 100$$

MEMBRANE STABILIZING ACTIVITY

Preparation of test sample: Methanolic extracts of *A. marina* with different concentration (2, 4, 6, 8, & 10 mg/ml respectively) were made as the test samples for membrane stabilizing study.

Drug: Standard Acetyl Salicylic Acid (ASA) or Aspirin was used as standard drug for disparities with methanolic extracts of *A. marina*.

Red Blood Cells (RBC) Collection: 5 ml of whole blood was collected from healthy human volunteers in a test tube containing an anticoagulant (EDTA 2.2 mg/ml of blood) under standard conditions of temperature 23 $\pm$ 2°C and relative humidity 55 $\pm$ 10%.

Preparation of Phosphate Buffer Solution: A buffer is an aqueous solution that has a very stable pH. A pH of about 7.4

with buffer strength of 10 mM was found using 0.0352% monosodium phosphate dehydrate and 0.1099% disodium phosphate anhydrate. The buffer was made by adding 0.352 gm monosodium phosphate dehydrate and 1.099 gm disodium phosphate anhydrate to 1000 mL water.

Preparation of Isotonic Solution: A solution that has a concentration of electrolytes, nonelectrolytes or a summation of the two that will assert equivalent osmotic pressure as that solution with which it is being compared. Either 0.16M sodium chloride (NaCl) solution (approximately 0.95% salt in water) or 0.3M non-electrolyte solution is nearly isotonic with human red blood cells. For the propagation of 500 ml isotonic solution of 154 mM strength, 4.5045 gm NaCl was added and mixed.

Preparation of Hypotonic Solution: A solution of lower osmotic pressure than that of a reference solution or of an isotonic solution is called hypotonic solution. For the preparation of 500 ml hypotonic solution, having strength of 50 mM, 1.4625 gm NaCl was added and mixed.

Erythrocyte Suspension: For the preparation of erythrocyte suspension, the collected RBC was centrifuged, supernatant was removed and the blood cells were washed three times with isotonic solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifuge action for 10 min at 3000 rpm using the same volume as supernatant. Finally it was resuspended in the same volume of this isotonic buffer solution.

Effect on Haemolysis: In vitro membrane stabilizing screening of *A. marina* was performed by the following two methods^{14, 15}.

Hypotonic Solution- Induced Haemolysis: 0.5 ml of this erythrocyte suspension was combined with 5 ml hypotonic solution (50 mM NaCl) holding either the various concentration of crude methanolic extracts (2, 4, 6, 8 & 10 mg/ml respectively) or Acetyl Salicylic Acid (0.10 mg/ml). The Acetyl Salicylic Acid was used as a reference standard. 0.5 ml of RBCs was mixed with hypotonic buffered saline alone to form the control sample. The mixture was incubated for 10 minute at room temperature, then centrifuged for 10 min at 3000 rpm and ultimately the optical density of supernatant was assessed at 540 nm.

The percentage inhibition of haemolysis was calculated by the following equation:

$$\% \text{ inhibition of haemolysis} = \frac{(\text{OD control} - \text{OD test sample})}{\text{OD control}} \times 100$$

Heat induced haemolysis

Aliquots (5 ml) of the isotonic buffer, containing completely different concentration of the crude methanolic extracts of *A. marina* were placed into two duplicate sets of centrifuge tubes¹⁴. The vehicle, within the same quantity, was added to different tube as control. Erythrocyte suspension (30 μ L) was added to every tube and mixed gently by inversion. One combine of the tubes was incubated at 54 °C for 20 min in an exceedingly water bath. The other pair was preserved 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 gauged at 540 nm using UV spectrometer. The percentage prohibition or provocation of hemolysis in tests and was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = \frac{(\text{OD2} - \text{OD1})}{(\text{OD3} - \text{OD1})} \times 100$$

Where, OD₁ =test sample unheated, OD₂ = test sample heated and, OD₃ = control sample heated.

Statistical analysis: The results are expressed as mean $\pm$ SD. Statistical comparisons were made using Dunnett's test. Significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Thrombolytic activity: The effects of *A. marina* on in-vitro clot lysis are presented in Table-1. From table-1, it is conspicuous that the proportion of clot lysis was 45.49% when 100 μ l of streptokinase (30,000 I.U.) was used as a positive management, whereas just in case of water (negative control) the percentage of clot lysis was negligible (6.03%). The percentage of clot lysis was dose dependently multiplied whereas 10mg/ml concentration additionally considerably showed 13.22% lysis of clot ($p < 0.01$).

Table 1: Effects of different concentration of methanolic extracts of *A. marina* and the standard on in vitro clot lysis

Treatment	W1	W2	W3	% of clot lysis (mean $\pm$ SD)	P - value
ME (2mg/ml)	0.8298	0.1640	0.1565	4.57 $\pm$ 0.046	< 0.49
ME (4mg/ml)	0.8155	0.1889	0.1769	6.35 $\pm$ 0.067	< 0.86
ME (6mg/ml)	0.8380	0.1903	0.1725	9.35 $\pm$ 0.084	< 0.12
ME (8mg/ml)	0.8196	0.1789	0.1588	11.23 $\pm$ 0.037	< 0.03
ME(10mg/ml)	0.8316	0.1716	0.1489	13.22 $\pm$ 0.052	< 0.01
Blank (water) and Streptokinase					
Treatment	W1	W2	W3	% of lysis	P - Value
SK	0.8275	0.3833	0.2089	45.49 $\pm$ 0.068	< 0.001
Blank	0.8167	0.3263	0.3065	6.03 $\pm$ 0.077	

Level of Significance ***= $P < 0.001$, **= $P < 0.01$, = $P < 0.05$

W1 = Weight of vial alone; W2 = Weight of clot containing vial; W3 = Weight of clot containing vial after clot disruption; SK =Streptokinase

Figure 1: Effects of crude methanolic extracts of *A. marina* leaves & barks on in-vitro clot lysis. Here, ME= methanolic extract, SK= streptokinase

MEMBRANE STABILIZING ACTIVITIES

The membrane stabilizing activities of the Crude methanolic extracts of *A. marina* are showed in Table 2 & 3. The crude methanolic extracts of *A. marina* dose dependently enhanced in membrane stabilizing study, whereas 10mg/ml concentration most importantly presented 10.11% & 27.76% inhibition of

haemolysis severally by hypotonic solution and heat induced haemolysis of red blood cell membrane. Acetyl salicylic acid was used as standard in membrane stabilization. ASA (0.10 mg/mL) revealed 69.98% & 71.97% inhibition of haemolysis respectively evoked by hypotonic solution and heat induced haemolysis correspondingly.

Table 2: Effects of crude methanolic extracts of *A. marina* on hypotonic solution-induced haemolysis of erythrocyte membrane

Treatment	Concentration	Optical density of samples in hypotonic solution (Mean $\pm$ SD)	% inhibition of haemolysis
Control		3.698 $\pm$ 0.0053	
ME	2 mg/ml	3.620 $\pm$ 0.0037	2.10 $\pm$ 0.049
ME	4 mg/ml	3.570 $\pm$ 0.004	3.46 $\pm$ 0.036
ME	6 mg/ml	3.505 $\pm$ 0.0019*	5.21 $\pm$ 0.044
ME	8 mg/ml	3.399 $\pm$ 0.0027*	8.08 $\pm$ 0.029
ME	10 mg/ml	3.324 $\pm$ 0.019**	10.11 $\pm$ 0.037
Acetyl salicylic acid	0.10 mg/ml	1.110 $\pm$ 0.0045***	69.98 $\pm$ 0.017

Table 3: Effects of crude methanolic extracts of *A. marina* on heat induced haemolysis of erythrocyte membrane

Treatment	Concentration	OD of sample $\pm$ SD		% inhibition of Haemolysis
		Heated Solution	Unheated Solution	
Control	- - -	1.093 $\pm$ 0.029		
ME	2 mg/ml	0.879 $\pm$ 0.127	0.856 $\pm$ 0.021	10.74 $\pm$ 0.067%
ME	4 mg/ml	0.780 $\pm$ 0.004	0.733 $\pm$ 0.117	15.01 $\pm$ 0.056%
ME	6 mg/ml	0.715 $\pm$ 0.114	0.627 $\pm$ 0.032	23.28 $\pm$ 0.047%
ME	8 mg/ml	0.587 $\pm$ 0.096	0.456 $\pm$ 0.142*	25.88 $\pm$ 0.077%
ME	10 mg/ml	0.405 $\pm$ 0.078	0.227 $\pm$ 0.091**	27.76 $\pm$ 0.091%
Acetyl Salicylic Acid	0.10mg/ml	0.672 $\pm$ 0.065	0.369 $\pm$ 0.039***	71.97 $\pm$ 2.68%

Figure 2: Effects of different conc. of *A. marina* on hypotonic solution induced haemolysis of erythrocyte membrane Figure 3: Effects of different conc. of *A. marina* on heat induced haemolysis of erythrocyte membrane.

CONCLUSION

This study corroborated that the crude methanolic extracts of *A. marina* leaves & barks at higher concentration showed moderate in-vitro thrombolytic & membrane stabilizing activities.

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Cite this article as:

S. M. Naim Uddin, Md. Shalahuddin Millat, Israt Jahan Ira, Md. Mizanur Rahman Moghal, Muhammad Rashedul Islam. Thrombolytic and membrane stabilizing properties of methanolic extracts of *Avicennia marina* leaves & barks. Int. Res. J. Pharm. 2016;7(6):34-38 <http://dx.doi.org/10.7897/2230-8407.07659>

Source of support: Nil, Conflict of interest: None Declared

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