

In Vivo Analgesic Potential in Swiss Albino Mice and in Vitro Thrombolytic and Membrane Stabilizing Activities of Methanolic Extract From Suaeda Maritima Whole Plant

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ABSTRACT

Objective: Cold methanolic extract of Suaeda maritima have been considered to screen their biological properties such as analgesic, membrane stabilizing and thrombolytic activity. Although some investigations have previously been reported with this plant, the whole plant was not studied yet to date. In the present study we aimed to investigate in vivo and in vitro bioactivities of Suaeda maritima.

Material and Methods: The pharmacological analgesic activity was studied by acetic acid induced writhing method in vivo in a mouse model. Membrane stabilizing activity was assessed by inhibition of haemolysis at hypotonic solution induced condition in vitro, and thrombolytic activity was evaluated by employing Streptokinase as standard in vitro.

Results: Extract of Suaeda maritima at a dose of 500 mg/kg body weight showed most significant inhibition of writhing at a rate of 82.25% in mice. Among all the fractions, crude methanol extract (ME) showed maximum effect with a value of 61.01% inhibition of hemolysis at 10 mg/ml dose, when standard acetyl salicylic acid (0.10 mg/mL) revealed 66.37% inhibition of hemolysis. When clots were treated with 100 µl, moderate clot lysis activity observed with each of the test sample, however highest effect was found at 10 mg/ml dose (49.13%).

Conclusion: The Suaeda maritima extract showed a remarkable analgesic effect on a mouse model and membrane stabilizing and thrombolytic activities in vivo models. It may introduce a new dimension in human model in analgesic, membrane stabilizing and antithrombolytic activities.

Keywords: suaeda maritima, writhing, membrane stabilizing, thrombolytic

ÖZET

Suaeda Maritima tüm bitkisinin metanollü özütünün İsviçre albino farelerinde gösterilen in vivo analjezik potansiyeli ve özütün in vitro trombolitik ve membran üzerinde dengeleyici etkinliği

Amaç: Bu çalışmada; Suaeda maritima bitkisinin soğuk metanollü özütü araştırılıp ağrı kesici, membran üzerinde dengeleyici ve trombolitik etkinlikleri gibi biyolojik özellikleri değerlendirilmiştir. Bu bitki türü hakkında bir adet çalışma bulunsa da tüm bitkinin kullanımı bugüne dek incelenmemiştir. Çalışmamızda Suaeda maritima'nın in vivo ve in vitro biyoaktivitelerini araştırmayı amaçladık.

Gereç ve Yöntem: Farmakolojik analjezik etkinliği asetik asit tetiklediği kıvrınma yöntemi kullanılarak bir fare modelinde in vivo araştırıldı. Membran üzerinde dengeleyici etkinliği hipotonik solüsyon tetiklediği durumda sağlanan hemoliz inhibisyonuna göre incelendi. Trombolitik etkinliği ise standart olarak streptokinaz kullanarak in vitro değerlendirildi.

Bulgular: Fare modelinde Suaeda maritima özütünün en etkili bulunan dozu 500 mg/kg vücut ağırlığı kıvrınmanın %82.25 azaltma oranını sağladı. Hemoliz inhibisyonuna gelince, standart olarak kullanılan asetil salisilik asit (0.10 mg/mL) %66.37 hemoliz inhibisyonu gösterirken metanollü kaba özüt (10 mg/ml doz) tüm fraksiyonlardan en büyük etkiyi gösterdi (%66.37). Pıhtıların 100 µl ile işlendiğinde her deney örneğinde orta derecede pıhtı yıkımı bulunurken en yüksek etki 10 mg/mL doz kullanarak bulundu (%49.13).

Sonuç: Suaeda maritima özütü fare modelinde önemli bir analjezik etki ve in vitro modellerde membran üzerinde dengeleyici ve trombolitik etkinlikler gösterdi. İnsan modeline analjezik, membran dengeleyici ve trombolitik etkinliklerin açısından yeni bir boyut getirebilir.

Anahtar kelimeler: suaeda maritima, kıvrınma, membran dengelenmesi, trombolitik

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Introduction

Mangroves are utilized as a renewable resource (1). They are harvested for water-resistant durable wood that are used to build houses, pilings, boats, furniture and in the production of charcoal. Mangrove bark is the extensive source of tannins and other dyes, where leaves are subjected to livestock feed, medicine, tea, and as a substitute for tobacco for smoking (2,3). They are growing in the tropical and subtropical intertidal regions of the world with high salt tolerance potentiality (4).

Mangroves are the source of several bioactive compounds and secondary metabolites with toxicological, pharmacological and ecological importance including alkaloids, phenolics, tannins, flavonoids, steroids and terpenoids (5). Nowadays several species of them are being used as insecticides and pesticides. Use of mangroves as potent source of natural antioxidant and antimicrobial agents in various herbal medicines for the treatment of diseases like cancer, diabetes, HIV etc has been reported by various authors (6,7-10). But there are very limited reports on analgesic, thrombolytic and many other biological activities of Indian mangroves (4,6).

Suaeda maritima (L.), a herbaceous mangrove, has been used as leafy vegetable for making juice and curries feeding cattle, goats and sheep. This plant widely distributed on the landward margin of mangrove (4-6). The juice of this herb has also been reported importance for treatment of Hepatitis. By considering all of these frameworks the present mangrove species would be selected for this study. The present study was designed to evaluate the analgesic, thrombolytic and membrane stabilizing activities of whole plants of *Suaeda maritima* with a goal of evaluating the bioefficiency of the plant for its possible pharmaceutical applications.

Materials and Methods

Collection and Identification

For this present investigation, the aforementioned medicinal plants was collected from Sonadia Dheep of Cox's Bazar and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. Accession number of *Suaeda maritima* is DACB-41628.

Drying and Grinding

The every collected plant components (leaves, fruits and stem) were separated from undesirable materials or other plants or plant components. They were preserved for one week. The plant components were ground into a rough powder with the assistance of an appropriate grinder. The powder was held on an airtight condition in a cool, dark and dry place till analysis.

Cold Extraction (Methanol Extraction)

About 400 gm of high-powered material of plant was taken in an exceedingly clean, flat bell-bottomed glass and soaked in 1600 ml of 80% methanol. The glass was sealed and waited with shaking and stirring in regular intervals for 12 days. The entire mixture then underwent a rough filtration by a chunk of unpolluted, white cotton material. Then it had been filtered through Whatman paper (Bibby RE200, Sterilin Ltd., UK).

Collection of Blood Samples

Three ml of blood was collected from each healthy Bangladeshi male human volunteer (n=5) under standard condition. The collected blood was kept into a test tube containing ethylenediamine tetraacetic acid (EDTA) to prevent clotting and stored until analysis.

In Vivo Analgesic Activity Test

The analgesic activity for methanolic extract of *Suaeda maritima* was inferred in mice with the modification by Hussain et al. (12) of the method of Koster et al. (11). A

Table 1: Effects of *Suaeda maritima* and in acetic acid induced writhing method

Treatment	Groups	Dose (mg/kg)	No. of writhes (5min) (Mean ± S.E.M)	% Inhibition of writhing
Control	I	-	20.66±1.55	-
Acetic Acid	II	10	2.33±1.53**	88.75%
<i>Suaeda maritima</i>	ME	250	4.5±2.78	63.69%
	ME	500	3.66±2.75*	82.25%

Each value represents the mean ± SEM (n=5). ME 250, ME 500 = Methanolic extract of *Suaeda maritima* at 250 and 500 mg/kg body weight respectively, Probability values calculated as compared to control using one way-ANOVA followed by Dunnet's t-test; *indicates the result is significant at p<0.05, **indicates the result is more significant at p<0.01.

reflexive writhing test was conducted in mice. During this study 20 mice were randomly divided into four groups where each group contained 5 mice. Control group, acetic acid group for tissue writhing, and two study groups with different *Suaeda maritima* doses after writhing by acetic acid (Table 1). Inhibition of moving was evaluated by Associate in Nursing index of physiological state and was calculated by considering the subsequent formula (12):

$$\text{Inhibition (\%)} = ((Wc - Wt) \times 100) / Wc$$

Where, Wc is the average number of writhing reflex in the control group and Wt is the average number of writhing reflex in the treatment group.

Membrane Stabilizing Activity Test

Erythrocyte Suspension

The blood was rinsed by using isotonic solution (0.9% saline) for 3 times. The quantity of saline was measured and reconstituted as a 4/100 (v/v) suspension with isotonic solution (pH 7.4) that contained in 1 L of distilled water: NaH_2PO_4 , 2H₂O, 0.26 g; Na_2HPO_4 , 1.15 g; NaCl, 9 g (10 ml orthophosphate buffer). Thus, the finally collected suspension was the stock RBC (RBC) suspension.

Hypotonic Solution-Induced Hemolysis

The membrane stabilizing activity of the extracts was evaluated by using hypotonic solution iatrogenic human red blood cell hemolysis that is established by Sikder with minor modification (13). The experiments were dispensed with freshly ready hypotonic solution. Stock red blood cell (RBC) suspension (0.50 mL) was mixed with 5 ml of hypotonic solution (50 metric linear unit NaCl) in 10 metric linear unit phosphate buffer saline (pH seven.4) containing either the various methanolic extract (2, 4, 6, 8, 10 mg/mL) or acetyl salicylic acid (0.10 mg/mL). The acetyl salicylic acid was used as a reference commonplace. The mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000 revolutions per minute and the absorbance of the supernatant was measured at 540 nm victimisation Shimadzu actinic ray photometer (16). The proportion inhibition of either hemolysis or membrane stabilization was calculated by using the subsequent equation:

$$\% \text{ Inhibition of hemolysis} = 100 \times \{ (OD1 - OD2) / OD1 \}$$

Where, OD1 = Optical density of hypotonic- buffered saline solution alone (control) and OD2 = Optical density of test sample in hypotonic solution.

Thrombolytic Activity

The thrombolytic activity of the methanolic extract of this plant was evaluated by the modification of Ghosh et al. (15) of the method developed by Dagainawala (14). A commercially available enzyme was used and 1,500,000 I.U. lyophilised Altepage (Streptokinase) vial (Beacon pharmaceutical Ltd.) was mixed with 5 ml sterile H₂O. 100 µl containing 30,000 I.U from this solution was used for in vitro lysis. Blood was collected from 5 healthy human volunteers without a history of use of oral contraceptive pills or anticoagulant medicines. One ml of blood was transferred into the dried and weighed small centrifuge tubes and was allowed to create clots. 3 replicates of every sample were prepared as described previously (16). The coagulation activity of volunteers was measured by the method developed by Dagainawala. The centrifuge tubes containing 1 ml blood were incubated at 37°C for 45 minutes. The clot was dried and tubes having clots were weighed again to find weights of clots (clot weight = weight of tube containing clot – weight of tube alone). Then 100 µl of solution with various concentrations of crude methanolic extract was added into the tubes containing clots, and 100 µl of enzyme (SK) and 100 mg of H₂O were added to the pre-formed clot as positive and negative controls. All the tubes were then incubated at 37°C for 90 minutes. After incubation, fluid was removed and tubes were weighed again to find the amount of disintegrated clot (17). Difference of weight before and after clot lysis was calculate as proportion of clot lysis as shown below:

$$\% \text{ of clot lysis} = (\text{wt of released clot} / \text{clot wt}) \times 100$$

Statistical Analysis

All the above assays were conducted in triplicate and repeated three times for consistency of results and statistical purpose. The data were expressed as mean ± SD and analyzed by one way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software of 10 version. p<0.05 was considered as statistically significant.

Results

Investigation of Acetic Acid Induced Writhing Test

Table-1 demonstrated the results of analgesic effect measured by acetic acid induced writhing method. The highest inhibition with a rate of 82.25% was shown at a dose of 500 mg/kg body weight of *Suaeda maritima*. On the other hand, a dose of 250

Table 2: Effects of extract of different concentrations of Suaeda maritima on hypotonic solution-induced hemolysis of erythrocyte membrane

Treatment Group	Concentration	Absorbance(OD) of test sample	% inhibition of haemolysis
Control	---		
ME1	2 mg/ml	0.234±2.31	35.54%
ME2	4 mg/ml	0.221±3.1	39.19%
ME3	6 mg/ml	0.202±0.77	44.35%
ME4	8 mg/ml	0.193±1.65	51.79%
ME5	10 mg/ml	0.175±1.87	61.01%
ASA	0.10 mg/ml	0.104±2.01	71.35%

Here, ME stands for methanolic extract of Suaeda maritima and ASA stands for standard acety salicylic acid. Percent inhibition of hemolysis was calculated relative to acetyl salicylic acid and results represented in means ± standard deviation (n= 5).

Table 3: Thrombolytic potentials investigations of methanolic extract of Suaeda maritima

Treatment	% Clot lysis (Mean±SEM)
ME (2 mg/ml)	17.01±1.50*
ME (4 mg/ml)	21.48±2.31*
ME (6 mg/ml)	28.88±1.49**
ME (8 mg/ml)	36.24±0.68***
ME (10 mg/ml)	49.13±3.64**
Streptokinase	62.18±3.71**
Control	1.19±0.98

Here, ME stands for methanolic extract of Suaeda maritima and. Rate of inhibition of clot lysis was calculated relative to standard streptokinase and results represented in means ± standard deviation (n= 5); *p<0.05, **p<0.01, ***p<0.001

mg/kg body weight of Suaeda maritima also provided a significant inhibition with a rate of 63.69%.

Investigation of Membrane Stabilizing Potentials

The various extracts of Suaeda maritima as 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml were tested to show the activity against lysis of human corpuscle membrane evoked by hypotonic solution, and compared to acetyl salicylic acid (0.10 mg/mL). Values were shown in Table 2. Acetyl salicylic acid (0.10 mg/mL) provided 71.35% of inhibition of hemolysis evoked by hypotonic solution. In study groups, crude methyl alcohol extract (ME) at 10 mg/ml dose showed highest rate of inhibition with 61.01% rate.

Investigation of Thrombolytic Activity

Table 3 showed the results of the clot lysis of the plant extracts with positive management (Streptokinase) and negative management (distilled water). Enzyme (100 µl) as positive management provided 62.18% clot lysis and water (as negative control) was only provided 1.19% clot lysis. In study groups, crude methyl alcohol extract (ME) at 10 mg/ml dose showed highest rate of clot lysis with 49.13% of rate.

Discussion

The response of the plant extract at acetic acid induced writhing test is used to screen both peripheral and centrally acting analgesic activity. Tissue injury leads to the subsequent elaboration of interleukin-1 and tumor necrosis factor-TNF-α, which is responsible to initiate the synthesis and release of autacoid prostaglandin E2 and F2α by the endothelium and pericytes of brain capillaries that excite pain nerve endings (18,19). In the present study, the methanol extracts of Suaeda maritima at lower doses did not show significant inhibition on acetic acid-induced writhing response but with a higher dose of plant extract showed significant inhibition of writhing. On the other hand, the reference drug acetyl salicylic acid (250 mg/kg) produced significant protective effects towards the acetic acid-induced pain in mice. The analgesic action of Suaeda maritima can be attributed to the blockade of release of the endogenous mediators of pain i.e. the prostaglandins. It suggests that Suaeda maritima has more inhibitory action on the cyclooxygenase pathway which is actually involved in the synthesis of prostaglandin. In another study it was reported that herbs containing phyto-constituents as like as bioflavonoids, such as quercetin, which are present in our investigated plant extracts, may confer pain and inflammation falling activity by inhibiting lipoxygenase, cyclooxygenase, and phospholipase enzyme (20). May be due the presence of such chemical constituent, our present plant extract showed analgesic activity.

Present study evidenced that the methanolic extract of the plant dose-dependently protects the human erythrocyte membrane against lysis induced by hypotonic solution and heat induced condition. During inflammation, phagocytes release many lysosomal enzymes and hydrolytic components to the extracellular space, which assists a variety of disorders by inducing damages of the surrounding organelles and tissues

(21). Studies evidence that non-steroidal anti-inflammatory drugs act through stabilization of lysosomal membranes by inhibiting these lysosomal enzymes. Again, lysis of the RBC membranes accompanied by the oxidation when exposed to harmful substances such as hypotonic medium, heat, etc through lysis of hemoglobin (22). Thus mechanism of anti-inflammatory activity of the plant extract is assessed by considering their potentials in inhibition of hypotonicity and heat induced RBC membrane lysis, because human RBC membranes are considered similar to lysosomal membrane components (23). One can also assumed that the possible mode of action of the extract and standard anti-inflammatory drugs may be connected with binding to the erythrocyte membranes through consequent alteration of surface charges of cells. Some research works were able to reveal the name of some responsible chemical components present in the extracts, which are well known for their anti-inflammatory activity (24). Both in vitro and in vivo studies in experimental animals showed that the flavonoids exert stabilizing effects largely on lysosomes (25) while tannin and saponins are also capable of stabilizing the erythrocyte membrane with an ability of binding with cations and other biomolecules (26). Our present research reveals that plant methanolic extracts showed potent RBC membrane stabilization activity with a good protection against both hypotonic solution induced condition may be this due to the presence of phytoconstituent like flavonoids. Very recently phytopharmacological investigation able to create a new field to discover plant derivative drugs and renew the attention in herbal medicines, where 30% of the pharmaceuticals are prepared from plants derivatives (27). Some severe outcomes such as stroke and myocardial infraction manifested due to the failure of hemostasis and consequent formation of blood clots in the circulatory system. Fibrinolytic agents such as urokinase, tissue plasminogen activator and streptokinase used for clinical intervention for pathological development of blood clots. Many research works have been undertaken to discover antithrombotic (anticoagulant and antiplatelet) effect of plants and natural food sources in order to prevention of coronary events and stroke (28). In the present study Methanolic extract of Suaeda maritima showed significant thrombolytic activity, this effect may be possible due to phytoconstituents such as tannin, alkaloid, and saponin present in the plant extracts affecting activation of plasminogen both by fibrin-dependent and fibrin-independent mechanisms similar to Streptokinase

which causes extra production of plasmin which breaks down fibrin the major constituent of thrombi, to dissolve unwanted blood clots. Several studies support our present findings (29-31). This study displays the in vitro pharmaceutical potential of crude methanolic extract Suaeda maritima stem barks mistreatment human blood. The take a look at model used may be a recently developed low-cost and straightforward technique which may be performed with restricted facilities obtainable in countries like Bangladesh. However this method is valid, sensitive and reliable. The results show moderate pharmaceutical activity. This can be necessary finding which can have important implications in vessel health. Additionally, this finding might indicate the chance of developing novel pharmaceutical agents from the stem barks of the plant.

Conclusion

In light of the results of the present study, it can be concluded that this plant extract possesses analgesic membrane stabilizing and thrombolytic properties which led us to the inference that the plant extract may contain bioactive compounds which may aid ongoing analgesic and anti-inflammatory drug discovery from floristic resources. Hence, further studies are suggested to be undertaken to pinpoint the exact compound(s) and to better understand the mechanism of such actions scientifically. This will emphasize the isolation and characterization of active principles responsible for these activities of Suaeda maritima. So, further studies are necessary to isolate and reveal the active compound contained in the crude extracts of Suaeda maritima responsible for these activity and to establish the mechanism of action.

Contribution Categories	Name of Author
Development of study idea	M.M.R., M.S.H., M.O.R.
Methodological design of the study	M.S.H., M.M.O.R., M.A.H.
Data acquisition and process	M.M.R., M.A.H., M.S.M.
Data analysis and interpretation	N.S., M.A.S., M.S.M.
Literature review	N.S., M.A.S., M.S.M.
Manuscript writing	M.M.R., M.S.H., M.M.O.R.
Manuscript review and revision	N.S., M.A.S., M.A.H.

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