Membrane stabilization — A possible mechanism of action for the anti-inflammatory activity of a Bangladeshi medicinal plant: Erioglossum rubiginosum (Bara Harina)

Pankaj Chandra Debnath, Abhijit Das*, Amirul Islam Sajib, Md. Mahadi Hassan

Department of Pharmacy, Noakhali Science and Technology University, Noakhali 3814, Bangladesh

ABSTRACT

Aims: The present study aimed at assessing the effect of methanol extract of Erioglossum rubiginosum, a widely used shrub plant in folkloric medicine, in experimentally induced inflammation, using human red blood cell (HRBC) membrane stabilization as study method.

Methods: The methanol extract of leaves of E. rubiginosum and its pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to assays for lysis of erythrocytes and % of inhibition in hypotonic solution and heat induced lysis, using acetyl salicylic acid as standard drug, in an in vitro model.

Results: At the concentration of 1 mg/ml, methanol extract and its pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates significantly inhibited hypotonic solution induced lysis of the human red blood cell membrane with values of 0.428 ± 0.005, 0.204 ± 0.003, 0.233 ± 0.002, 0.411 ± 0.003, 0.439 ± 0.003% respectively; which were comparable to the standard drug acetyl salicylic acid (0.1 mg/ml). 0.166 ± 0.003. In case of heat induced HRBC hemolysis, the plant extracts also showed significant activity where the values of inhibitory actions were 14.46 ± 0.344, 28.23 ± 0.315, 25.18 ± 0.303, 17.09 ± 0.365, 13.44 ± 0.470% for methanol extract, pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates respectively.

Conclusion: It can be postulated from the observed results that the membrane stabilizing action and inhibition of erythrocyte lysis property of E. rubiginosum may be the possible mechanism of action of its anti-inflammatory activity. So, further studies are suggested to evaluate the anti-inflammatory and analgesic activities of the plant.

Copyright © 2013, Phcog.Net, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli and also a part of non-specific immune response that occurs in reaction to any type of bodily injury. It is also an attempt of an organism to remove the injurious stimuli and initiate the healing process. The inflammation process initiates with the activation and release of different types of mediators, such as: histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins, some plasma enzyme systems and the kinin system. These mediators, working collectively, increase vasodilation and blood vessels permeability, which further lead to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues. Some laboratory and pathological data also supported that inflammation has a role in both the initiation and the progression of atherosclerosis. 

Again, the erythrocyte membrane resembles lysosomal membrane as such; the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. Therefore, as membrane stabilizes that interfere in the release and or action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc.

There are many agents or drugs namely anti-inflammatory agents or drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) to treat the consequences of inflammation. The effect of these drugs including herbal preparation on the stabilization of erythrocyte membrane exposed to hypotonic and heat has been studied extensively. But these studies showed that, these drugs are not free from adverse effects, as they are responsible for intestinal side effects and mucosal erosions that can progress into ulcers. For these reasons many researchers have focused on medicinal plants for finding natural anti-inflammatory drugs.

Erioglossum rubiginosum (local name — Bara Harina) is a shrub or small tree, with a compact, bushy crown and up to 12 m tall. It...
belongs to the family Sapindaceae and is available in forests at low and medium altitudes throughout the Philippines, northern India to Indo-China, Thailand and also some tropical countries. This plant is extensively used as folkloric medicine; such as: roots are used as astringent, leaves and fruits are used for the treatment of fever and poulticing. As a part of our continuing studies of medicinal plants of Bangladesh the methanol extract and the fractions of leaves of *E. rubiginosum* growing in Bangladesh were screened for membrane stabilizing activity for the first time and we, here in, report the results of our preliminary investigation.

2. Materials and methods

2.1. Collection and preparation of plant material

The leaves of *E. rubiginosum* were collected from Dhaka, Bangladesh, in July 2012. The plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit. The sun dried powdered leaves (500 mg) of *E. rubiginosum* was macerated in 2.5 L of 99.8% methanol (Merck KGaA, Darmstadt, Germany). After 15 days the solution was filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 45 °C to dryness and thus a blackish—green semisolid mass of the extract was obtained. The concentrated methanolic extract was partitioned by modified Kupchan method and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.2. Collection of blood samples

Human RBCs were collected for the study. 7 ml of blood from each of the healthy Bangladeshi male human volunteers (aged 20–23 years) without a history of oral contraceptive or anticoagulant therapy and free from diseases (using a protocol approved by Institutional Ethics Committee). The collected RBCs were kept in a test tube with an anticoagulant EDTA under standard conditions of temperature 23 ± 2 °C and relative humidity 55 ± 10%.

2.3. Reagents and chemicals

All the solutions, reagents used in this study were of analytical grades. They were procured from Sigma Chemical Co. Ltd. (St. Louis, MO, USA) and E. Merck (Germany). All the solutions, reagents and buffers were prepared with glass distilled water.

2.4. Assay of membrane stabilization

2.4.1. Erythrocyte suspension

The blood was washed three times using isotonic solution (0.9% saline). The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 1 L of distilled water: NaH₂PO₄·2H₂O, 0.26 g; Na₂HPO₄, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus the suspension finally collected was the stock erythrocyte (RBC) suspension.

2.4.2. Hypotonic solution induced hemolysis

The membrane stabilizing activity of the extract was evaluated by using hypotonic solution induced human erythrocyte hemolysis, designed by with minor modification. To prepare the erythrocyte suspension whole blood (7 ml) was obtained using syringes (containing anticoagulant EDTA) from male volunteers through puncture of the anti-cubital vein. The blood was centrifuged, using centrifugal machine, for 10 min at 3000 g and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The test sample, consisted of stock erythrocyte (RBC) suspension (0.50 ml), was 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, was 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 using UV spectrometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation: % inhibition of hemolysis = 100 × \( \frac{OD1 - OD2}{OD1} \)

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

2.4.3. Heat-induced hemolysis

Aliquots (5 ml) of the isotonic buffer, containing 1.0 mg/ml of different extracts of the plant were put into two duplicate sets of centrifuge tubes.¹¹ 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. 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 using UV spectrometer. The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation: % inhibition of hemolysis = 100 × \( 1 - \frac{OD2 - OD1}{OD3 - OD1} \)

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

2.5. Statistical analysis

Data obtained were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). All values are expressed as mean ± SD for three replicates. Data were analyzed by one-way ANOVA and the

<p>| Table 1 Effect of extracts of <em>Erioglossum rubiginosum</em> on hypotonic solution induced hemolysis of erythrocyte membrane. |
|---------------------------------------------------------|-------------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th>Optical density of samples in hypotonic solution (Mean ± SD)</th>
<th>Percentage inhibition of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.39 ± 0.026</td>
<td>—</td>
</tr>
<tr>
<td>ME</td>
<td>1</td>
<td>0.428 ± 0.005***</td>
<td>27.46 ± 0.942***</td>
</tr>
<tr>
<td>PESF</td>
<td>1</td>
<td>0.204 ± 0.003*</td>
<td>60.57 ± 0.428***</td>
</tr>
<tr>
<td>CTSF</td>
<td>1</td>
<td>0.233 ± 0.002**</td>
<td>71.92 ± 0.519***</td>
</tr>
<tr>
<td>AQSF</td>
<td>1</td>
<td>0.439 ± 0.003***</td>
<td>71.92 ± 0.519***</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>1</td>
<td>0.166 ± 0.003**</td>
<td>71.92 ± 0.519***</td>
</tr>
</tbody>
</table>

Level of Significance *** – p < 0.001, ** – p < 0.01, percent inhibition of migration was calculated relative to control.
statistical significance differences were analyzed using paired t-test. $P < 0.05$ was considered statistically significant.

3. Result

The crude methanol extract of leaves of *E. rubiginosum*, as well as different partitionates derived from this extract, were subjected to assays for membrane stabilizing activities by following standard protocols and the obtained results were statistically represented in Tables 1 and 2. The results showed that the extracts (at concentration 1 mg/ml) were significantly potent on human erythrocyte, adequately protecting it against hypotonic solution and heat-induced lysed, when compared with the standard drug acetyl salicylic acid (0.10 mg/ml).

In hypotonic solution induced conditions, the samples were found to inhibit lysis of erythrocyte membrane within the range of 25.65 ± 0.519% to 65.36 ± 0.519%. Among the samples, the pet-ether soluble fraction (PESF) of leaves of *E. rubiginosum* displayed high inhibition (65.36 ± 0.519%) hemolysis of RBC as compared to 71.92 ± 0.519% demonstrated by acetyl salicylic acid, while the minimum inhibition capacity was observed for aqueous soluble fraction (AQSF) (Table 1). Besides, in heat-induced conditions, the samples were found to inhibit lysis of erythrocyte membrane within the range of 13.44 ± 0.470% to 28.23 ± 0.315%. Here, the maximum inhibitory capacity of RBC hemolysis was observed for pet-ether soluble fraction (PESF), 28.23 ± 0.315%, as compared to 30.55 ± 0.53% demonstrated by acetyl salicylic acid. In this case, the aqueous soluble fraction also revealed the minimal RBC hemolysis inhibition capacity (Table 2).

4. Discussion

It is relevant from the present study that the methanol extract and its different partitionates protected the human erythrocyte membrane against lysis induced by hypotonic solution and heat. During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes to the extracellular space, which causes damages of the surrounding organelles and tissues and also assists a variety of disorders.17 It was found that the NSAIDS act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membranes. Again, exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat etc. results in the lysis of the membranes, accompanied by oxidation and lysis of hemoglobin.18 The inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of the plant extract, because human red blood cell (HRBC) membranes are considered similar to lysosomal membrane components.19

It may be said that, the possible mode of action of the extract, its fractions and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the hemolysis of red blood cells. In some research work, it has been reported that, some chemical constituents present in the extracts may exert the same mechanism, which are well known for their anti-inflammatory activity.20 Both in *in vitro* and *in vivo* study in experimental animals, demonstrated that flavonoids exert extensive stabilizing effects on lysosomes21,22 while tannin and saponins have the ability to bind cations and other biomolecules, and are able to stabilize the erythrocyte membrane.10,23

In this research work it was found that all the extracts of the plant showed potent RBC membrane stabilization activity with good percentage protection against both hypotonic solution and heat-induced lysis. The pet-ether soluble fraction (PESF) of the extract of the plant was found be a better choice. Because, in both the test methods, that is hypotonic solution induced and heat induced heamolysis, the percentage (%) of heamolysis inhibitory action of pet-ether soluble fraction (PESF) was better than the other extracts. The heamolysis inhibitory action of the extracts may be due to synergistic effect produced by phyto-constituents present in these extracts.

5. Conclusion

On the basis of these results of the current study, it could be inferred that the extracts/fractions of *E. rubiginosum* contained principles that were capable of stabilizing human red blood cells membranes against hypotonic solution and heat induced lysis. The plant therefore could be regarded as a natural source of membrane stabilizers and could be used as an alternative remedy for the management and treatment of inflammatory related disorders and diseases. So, further studies are suggested to identify and isolate the chemical constituents responsible for the membrane stabilizing activity and also to evaluate the anti-inflammatory activity of the plant extract.

Conflicts of interest

All authors have none to declare.

Acknowledgment

The authors are thankful to all the teachers and staffs of the Department of Pharmacy, Noakhali Science and Technology University for their support and co-operation.

References


