

Research Article



Effect of Ethanol Extract of *Adenopus breviflorus* (Benth) on Erythrocyte Osmotic Fragility in Male Wistar Rats

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ABSTRACT

This study aims at investigating the effect of Ethanol Extract of *Adenopus breviflorus* (EEAB) on erythrocyte osmotic fragility in male rats. Eight hundred grams of air-dried pulverized whole fruits of *Adenopus breviflorus* were cold macerated in 70% ethanol and concentrated using water – bath. Twenty eight male rats (150 – 200g) were divided into control (distilled water) and EEAB – treated (62.5mg/kg, 125mg/kg, 250 mg/kg) groups (7 per group). The animals were orally treated on daily basis for 30 days. Erythrocyte osmotic fragility was determined by spectrophotometry method. Data were analyzed using descriptive statistics and ANOVA at $p=0.05$. The results showed that treatment of rats for 30 days with EEAB (62.5mg/kg, 125 mg/kg) produced no significant ($p>0.05$) change in erythrocyte osmotic fragility at all the NaCl concentrations (0.1% - 0.9%) relative to the control, while EEAB (250 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentration of 0.7% relative to the control. These findings probably indicate that EEAB could be useful in the treatment of hemolysis.

Keywords: *Adenopusbreviflorus*, Osmotic fragility, Erythrocytes, NaCl concentrations, Rats.

INTRODUCTION

A *denopus breviflorus* (Roberty) belongs to the family of Cucurbitaceae which are mostly prostrate or climbing herbaceous annuals. It is commonly called Wild colocynth in English language, "Ogbenwa" in Ibo language and "Tagiri" by the Yoruba language speaking people of Nigeria¹.

The plant is used medicinally as a purgative in Tanganyika and as a vermifuge in Nigeria¹. A decoction from the plant is said to be used in Nigeria for headache¹. The plant is used for money-making charms by the Yoruba herbalists of South -West Nigeria². It is used in West Africa for a wide range of gastrointestinal disorders and measles in man. It is also used as an anticonvulsant, sedative and pain killer³.

Pharmacologically, it is used as an anti- implantation agent⁴, abortifacient⁵, broad spectrum antibacterial agent⁶ as well as an anti-oxidant and anti-ulcerogenic agent⁷.

Since the ethanol extract of this plant has been reported to have a lot of beneficial potentialities on the hematological profile and blood chemistry of male rats⁸, this study therefore aims to investigate the effect of ethanol extract of this plant on erythrocyte osmotic fragility in male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Adult male albino mice weighing between 150 g - 200 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, AfeBabalola University

were used. They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

All experiments were carried out in compliance with the recommendations of AfeBabalola University Ethics Committee on guiding principles on care and use of animals.

Plant Material

Fresh samples of *Adenopus breviflorus* fruit were bought in Bodija Market, Ibadan, and were authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where a voucher specimen (No. FHI.108336) was deposited in their Herbarium.

Preparation of Crude Ethanol Extract

Large quantity (7.5 kg) of fresh specimens of the whole fruit of *Adenopus breviflorus* were washed free of debris and pulverized using mortar and pestle and air-dried for eight weeks.

The resultant dried specimens (800 g) were macerated and extracted with 70% ethanol (1:2 wt./vol.) for 72 hours at room temperature (26 – 28°C).

The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm).

The 70% ethanol was later evaporated using water-bath to give a percentage yield of 8.6% of the starting sample.

The dried sample was reconstituted in distilled water to make up test solutions of known concentration.



Experimental Design

Twenty-eight male albino rats were randomly divided into four groups, with each consisting of seven animals. The four groups were subjected to the following oral treatments once a day for thirty (30) days and the dosages of EEAB used in this study were in accordance with those reported by²:

Group I: received 0.5 ml/100 g of distilled water as control group

Group II: received 62.5 mg/kg of EEAB

Group III: received 125 mg/kg of EEAB

Group IV: received 250 mg/kg of EEAB

Collection of Blood Samples

Twenty four hours after the last dosing of all groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for erythrocyte osmotic fragility study.

Determination of Erythrocyte Osmotic Fragility

The erythrocyte osmotic fragility of rats was evaluated using the method of⁹.

One percent (%) sodium chloride (NaCl) solution was buffered with phosphate solution, Na_2HPO_4 (1.3 mg/mg) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.24 mg/mg). Lower dilutions of NaCl solution (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8% and 0.9%) were prepared in test tubes and a tenth test tube contained only distilled water (0.0%). The pH of the distilled water (7.0) and those of the NaCl solutions (7.4) were measured using a pH meter (Digital pH meter, Labtech). Five millimeters of each concentration of NaCl was put in a test tube (9 in all) and 5 ml distilled water (0.0%) was put in the tenth tube. To each test tube was pipetted 0.02 ml of blood using a micropipette.

The contents were thoroughly mixed and allowed to stand for thirty minutes at room temperature (28 – 29°C). The test tubes were then centrifuge at 3,000 rpm for ten minutes.

The Optical Density (O.D.) of each supernatant solution (a measure of the degree of hemolysis) was measured with a spectrophotometer (SM23A) at a wavelength of 540 nm using a tube of distilled water as blank.

The degree of hemolysis in the distilled water test tube was taken as 100% and the others were read in relation to it.

Percentage hemolysis

$$= \frac{\text{O. D. of Test Solution}}{\text{O. D. of Standard Solution}} \times 100$$

A cumulative erythrocyte osmotic fragiligram was obtained by plotting the mean percentage hemolysis for the four groups of rats against the concentrations of the NaCl solution.

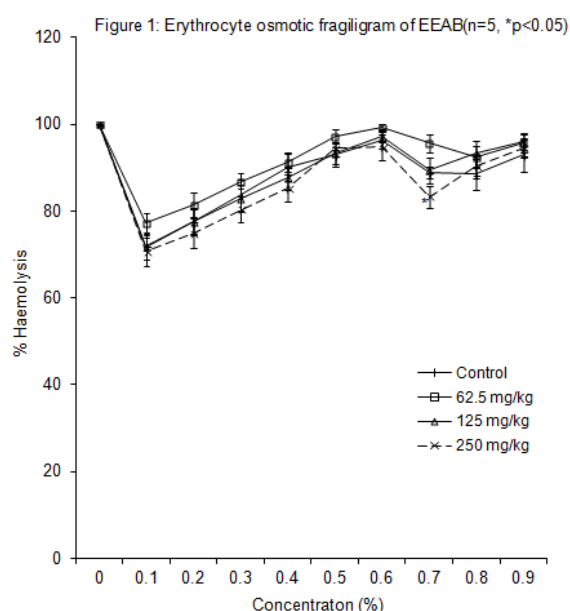
Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The effect of varying doses of EEAB on erythrocyte osmotic fragility after treatment of rats for 30 days is shown in Figure 1.

Treatment of rats for 30 days with EEAB (62.5mg/kg, 125 mg/kg) produced no significant ($p < 0.05$) change in erythrocyte osmotic fragility at all the NaCl concentrations (0.1% - 0.9%) relative to the control, while EEAB (250 mg/kg) caused significant ($p < 0.05$) reduction in erythrocyte osmotic fragility at NaCl concentration of 0.7% relative to the control.



DISCUSSION

The osmotic fragility assay is a classical, rapid, useful and easy technique that has permitted to obtain relevant information about the interactions of natural and synthetic drugs with cellular membrane¹⁰. It has also been reported that some drugs are capable of inducing alterations on the shape and physiology of the red cells¹¹.

The results have shown that EEAB produced significant and insignificant reductions in erythrocytes osmotic fragility at various NaCl concentrations after thirty days of treatments. This probably indicates that the extract stabilize or strengthen the membrane integrity of red blood cells since it has been reported that erythrocytes osmotic fragility is used as a measure of the tensile strength of the red cell membrane¹². The reduction in erythrocyte osmotic fragility produced by the extract probably indicates its usefulness in the treatment of hemolysis since it has been reported that osmotic fragility

refers to the degree or proportion of hemolysis that occurs when a sample of red blood cells are subjected to osmotic stress by being placed in a hypotonic solution¹³.

The reduction in erythrocyte osmotic fragility induced by the extract could indicate an enhancement of red blood cells viability since it has been reported that the osmotic fragility of mammalian red blood cells is indicative of their viability¹⁴. The reduction in erythrocyte osmotic fragility caused by the extract probably indicates that the extract could be useful in the treatment of hereditary spherocytosis and hypernatremia since erythrocyte osmotic fragility is often performed to aid with the diagnosis of diseases associated with RBC membrane abnormalities and some diseases linked to increased osmotic fragility induce hereditary spherocytosis and hypernatremia.

However, the decrease in erythrocyte osmotic fragility produced by the extract probably indicate an induction of chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia and polycythemia vera by the extract since some diseases linked to decreased osmotic fragility include chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia and polycythemia vera. Similar results were reported by¹⁵ in Saffron and Cinnamon treated human beings.

In conclusion, this study has shown that ethanol extract of *Adenopus breviflorus* could probably be useful in the treatment of hemolysis.

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