Research Article

Effects of Aqueous and Methanol Extracts of *Portulaca oleracea* on Erythrocyte Osmotic Fragility in Male Wistar Rats

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ABSTRACT

Portulaca oleracea is a fleshy annual herb which is distributed throughout the temperate and tropical areas of the world. The crude extracts of this plant have been reported to have some beneficial effects on hematological profile and blood chemistry of rats. This study investigates the effects of Aqueous and Methanol Extracts of *Portulca oleracea* (AEPO, MEPO) on erythrocyte osmotic fragility in male rats. Thirty-five male rats (150 - 200 g) were divided into control (distilled water), AEPO (25, 50, 75 mg/kg) and MEPO (25, 50, 75 mg/kg) treated groups. The animals were orally treated on daily basis for thirty days. Erythrocyte osmotic fragility was determined by spectrophotometry method. The results showed that there were significant ($p<0.05$) reductions in erythrocyte osmotic fragility of rats treated for 15 days with AEPO (25 mg/kg, 50 mg/kg, 50 mg/kg) at NaCl concentrations of 0.1 % - 0.6 % relative to the control. Treatment of rats for 30 days with MEPO (75 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.2 % and 0.3 % relative to the control. These findings in animal model probably indicate that AEPO and MEPO could be useful in the treatment of hemolysis.

Keywords: *Portulaca oleracea,* Osmotic fragility, Erythrocytes, NaCl concentrations, Rats.

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is a warm - climate annual herb and has cosmopolitan distribution. It is commonly called Purslane in English language and "Esan omode" or "Papasan" by the Yoruba tribe of South - West Nigeria[1].

It is used medicinally in Ghana for heart – palpitations[2]. The plant is used as a diuretic in Nigeria[3]. A tisane of the plant is drunk in Trinidad as a vermifuge[4].

Pharmacologically, its' aqueous and methanol extracts have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations[5]. Its extracts have been reported to cause reduction in locomotor activity and an increase in the onset time of pentylenetetrazole (PTZ) – induced convulsion in rats[6]. Its extracts have also been reported to have beneficial effects on the hematological functions and blood chemistry of rats[7].

Since this plant extracts have been reported to have some beneficial effects on the hematological functions and blood chemistry of rats[7], this study therefore aims to investigate the effects of aqueous and methanol extracts of this plant on erythrocyte osmotic fragility in male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Adult male rats weighing between 150 g and 200 g bred in the Pre-clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola 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 Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh specimens of *Portulaca oleraceae* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where it was identified and assigned a voucher specimen number FHI 108334.

Preparation of the Extracts

Large quantity (2 kg) of the fresh specimens of *Portulaca oleracea* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air - dried for six weeks and then pulverized using laboratory mortar and pestle and was later divided into two samples A and B.

(i) Aqueous Extract of *Portulaca oleracea* (AEPO)

Weighted Portion (400.0 g) of sample A was macerated and extracted with distilled water (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 distilled water was later evaporated using steam-bath to give a

percentage yield of 11.8 % of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

(ii) Methanol Extract of *Portulaca olearacea* (MEPO)

Weighted portion (350.0 g) of sample B was macerated and extracted with 70 % methanol (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 % methanol was later evaporated using steam-bath to give a percentage yield of 10.2 % of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

Ten grams of AEPO and MEPO were dissolved in 100 ml of distilled water to give a concentration of 0.1 g/ml. The dosages of AEPO and MEPO administered in these studies were in accordance with those reported by[8].

Animal Grouping and Extracts Administration

Thirty-five male rats weighing between 150 g-200 g were randomly divided into seven groups, with each group consisting of five animals.

The seven groups of rats were subjected to the following oral treatments once a day for thirty days and blood samples were collected for analysis at the middle and end of the experiment:

Group I received 25 mg/kg of AEPO

Group II received 50 mg/kg of AEPO

Group III received 75 mg/kg of AEPO

Group IV received 25 mg/kg of MEPO

Group V received 50 mg/kg of MEPO

Group VI received 75 mg/kg of MEPO

Group VII received 0.5 ml of distilled water as the control group.

Collection of Blood Samples

Twenty four hours after the last dosing of all groups (days 16 and 31), 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 of[9].

One percent (%) sodium chloride (NaCl) solution was buffered with phosphate solution, Na_2HPO_4 (1.3 mg/mg) and NaH_2PO_4. $2H_2O$ (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.

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

Cumulative erythrocyte osmotic flagiligrams were obtained by plotting the mean percentage hemolysis for the seven 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 effects of varying doses of AEPO and MEPO on erythrocyte osmotic fragility after treatment of rats for 15 and 30 days are shown in Figures 1, 2, 3 and 4.

Treatment of rats for 15 days with AEPO (25 mg/kg and 50 mg/kg) caused significant ($p<0.05$) reductions in erythrocyte osmotic fragility at NaCl concentrations of 0.1 % - 0.6 % relative to the control. Treatment of rats for 15 days with AEPO (75 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.2 %, 0.4 %, 0.5 % and 0.6 % relative to the control. Treatment of rats for 30 days with AEPO (25 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.1 %, 0.3 %, 0.6 %, 0.7 % and 0.9 % relative to the control, while AEPO (50 mg/kg) caused significant ($p<0.05$) reduction in erythrocytes osmotic fragility at NaCl concentrations of 0.3 % - 0.6 % relative to the control. Also, AEPO (75 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.5 % relative to the control.

Treatment of rats for 15 days with MEPO (25 mg/kg) caused no significant ($p>0.05$) change in erythrocytes osmotic fragility at NaCl concentrations of 0.1 % - 0.9 % relative to the control, while MEPO (50 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic

fragility at NaCl concentrations of 0.8 % and 0.9 % relative to the control. Also, MEPO (75 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.2 %, 0.5 % and 0.6 %. Treatment of rats for 30 days with MEPO (25 mg/kg) produced significant ($p<0.05$) decrease in erythrocyte osmotic fragility at NaCl concentrations of 0.4 %, 0.5 %, 0.6 % and 0.9 % relative to the control, while MEPO (50 mg/kg) produced no significant ($p>0.05$) change in erythrocyte osmotic fragility at NaCl concentrations of 0.1 % - 0.9 % relative to the control. However, MEPO (75 mg/kg) caused significant ($p<0.05$) reduction in erythrocyte osmotic fragility at NaCl concentrations of 0.2 % and 0.3 % relative to the control.

Figure 1: Erythrocyte osmotic fragiligram of AEPO on day 15 (n=5, *p<0.05)

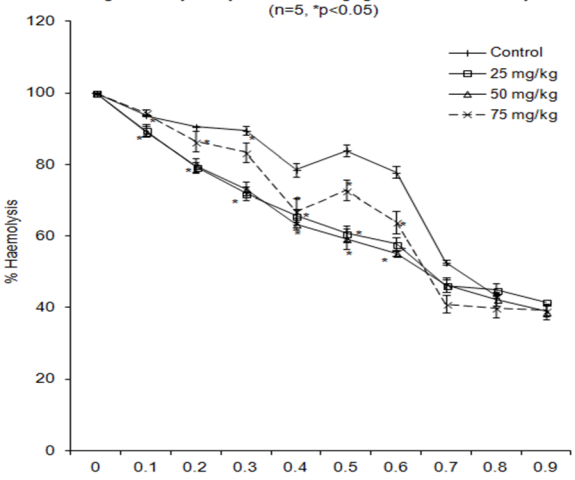


Figure 2: Erythrocyte osmotic fragiligram of AEPO on day 30 (n=5, *p<0.05)

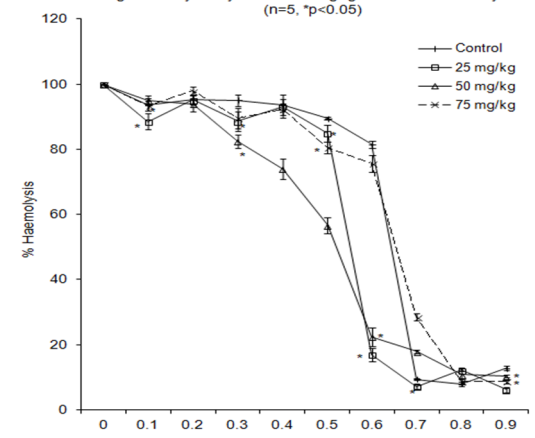


Figure 3: Erythrocyte osmotic fragiligram of MEPO on day 15 (n=5, *p<0.05)

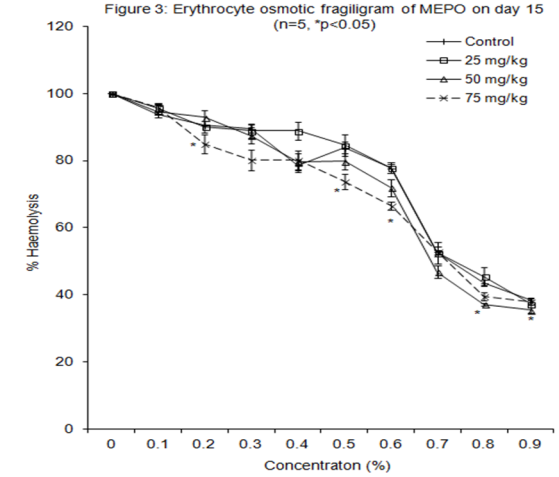
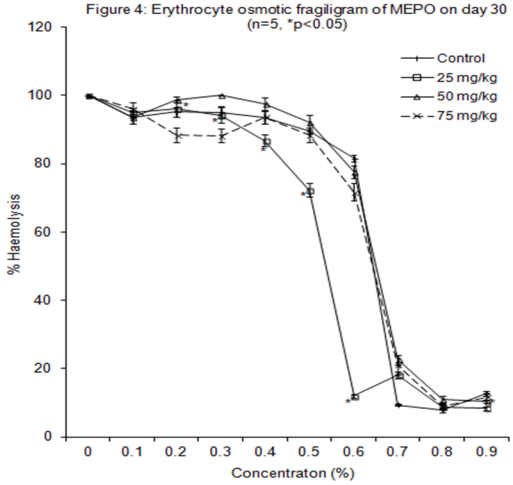


Figure 4: Erythrocyte osmotic fragiligram of MEPO on day 30 (n=5, *p<0.05)



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 membrane[10]. It has also been reported that some drugs are capable of inducing alterations on the shape and physiology of the red cells[11].

The results have shown that AEPO and MEPO caused significant reductions in erythrocyte osmotic fragility at various NaCl concentrations after fifteen and thirty days of treatments. This probably indicates that the extracts stabilize or strengthen the membrane integrity of red blood cells since it has been reported that erythrocyte osmotic fragility is used as a measure of the tensile strength of the red cell membrane[12]. The significant reductions in erythrocyte osmotic fragility produced by the extracts probably indicates their 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 solution[13]. Similar results were reported by[14] in Saffron and Cinnamon treated human beings.

The significant reductions in erythrocyte osmotic fragility induced by the extracts could probably be responsible for the use of this plant in the treatments of hematuria in West Africa[15] and hemoptysis in Southern Africa[16]. The significant reductions in erythrocyte osmotic fragility induced by the extracts could also indicate enhancement of red blood cells' viability since it has been reported that the osmotic fragility of mammalian red blood cells is indicative of their viability[17]. The significant reductions in erythrocyte osmotic fragility caused by the extracts probably indicate that the extracts 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. Contrary result was reported by Khanna[10] using homologous recombination in mouse embryonic stem cells.

However, the significant decrease in erythrocyte osmotic fragility produced by the extracts probably indicate induction of chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia and polycythemia vera by the extracts since some diseases linked to decreased osmotic fragility include chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia and polycythemia vera.

In conclusion, this study has shown that the significant reductions in erythrocyte osmotic fragility induced by the extracts of this plant could probably be responsible for the use of this plant in the treatments of hematuria in West Africa[15] and hemoptysis in Southern Africa[16]; its aqueous and methanol extracts could also probably be useful in the treatments of hemolysis as well as having some hematological deleterious effects.

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