

Research Article



Effects of Ethanol Extract of *Jatropha gossypifolia* (Pohl) on Erythrocyte Osmotic Fragility in Male Wistar Rats

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ABSTRACT

This study aims at investigating the effect of *Jatropha gossypifolia* (EEJG) on erythrocyte osmotic fragility in male rats. Six hundred grams of air – dried *Jatropha gossypifolia* leaves were cold macerated in 70% ethanol and concentrated using water – bath. Twenty male rats (80-150 g) were divided into control (distilled water) and EEJG – treated (62.5mg/kg, 125mg/kg, 250 mg/kg) groups (5 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 of 30 days with all the doses of EEJG (62.5mg/kg, 125mg/kg, 250 mg/kg) produced no significant ($p > 0.05$) changes in erythrocytes osmotic fragility at all the NaCl concentrations (0.1% - 0.9%) relative to the control. These findings probably indicate that EEJG has no effect on erythrocyte osmotic fragility of male rats.

Keywords: *Jatropha gossypifolia*, Osmotic fragility, Erythrocytes, NaCl concentrations, Rats.

INTRODUCTION

Jatropha gossypifolia (Pohl) belongs to the family of Euphorbiaceae, which occur preferentially in tropical and subtropical environment¹. It is commonly called Bellyache bush in English language, "Faux manioc" in French language and "Lapalapa pupa" by the Yoruba language speaking people of Nigeria.

The plant is used medicinally as an anti-inflammatory, anti-hemorrhagic, analgesic, anti-anemic and antimicrobial agents^{2,3}.

Pharmacologically, it is used as an antihypertensive⁴, antineoplastic⁵, wound healing⁶, contraceptive in female rodents⁷ and antioxidant agent⁸.

Since the ethanol extract of this plant has been reported to have overwhelming beneficial potentialities on the hematological profile and blood chemistry of male rats⁹, 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 rats weighing between 80 g – 150 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 *Jatropha gossypifolia* plants were collected from the Botanical Garden of the University of Ibadan, and were identified in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan where a voucher specimen (No. FHI.110178) was deposited in their Herbarium.

Preparation of Ethanol Extract of *Jatropha gossypifolia* (EEJG)

Large quantity (1.5 kg) of fresh specimens of the leaves of *Jatropha gossypifolia* were washed free of debris and air-dried. The dried leaves were pulverized using laboratory mortar and pestle.

Weighted portion (600 g) of the pulverized specimen was macerated with 70% ethanol (1:2 wt./vol.) for 72 hours at room temperature. 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 10.96% of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

Acute Toxicity Test

The method described by¹⁰ was used to determine the LD₅₀, which is the index of acute toxicity. Male Swiss mice (20-25 g) were used.

This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10 mg/kg, 100 mg/kg



and 1000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for twenty-four hours for mortality and general behavior.

From the results of the above step, seven different doses (2000 mg/kg, 3000 mg/kg, 4000 mg/kg, 5000 mg/kg, 6000 mg/kg, 7000 mg/kg, 8000 mg/kg) were chosen and administered orally to eight groups of animals of one mouse per group respectively. The treated animals were monitored for twenty-four hours. The LD₅₀ was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

The dosages of EEJG administered in this study were obtained from the result of the acute toxicity test.

Experimental Design

Twenty male rats (80 – 150 g) were randomly divided into four groups, with each consisting of five animals. The four groups were subjected to the following oral treatments once a day for thirty (30) days:

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

Group II: received 62.5 mg/kg of EEJG

Group III: received 125 mg/kg of EEJG

Group IV: received 250 mg/kg of EEJG

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 of ¹¹.

One percent (%) sodium chloride (NaCl) solution was buffered with phosphate solution, Na₂HPO₄ (1.3 mg/mg) and NaH₂PO₄.2H₂O (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.

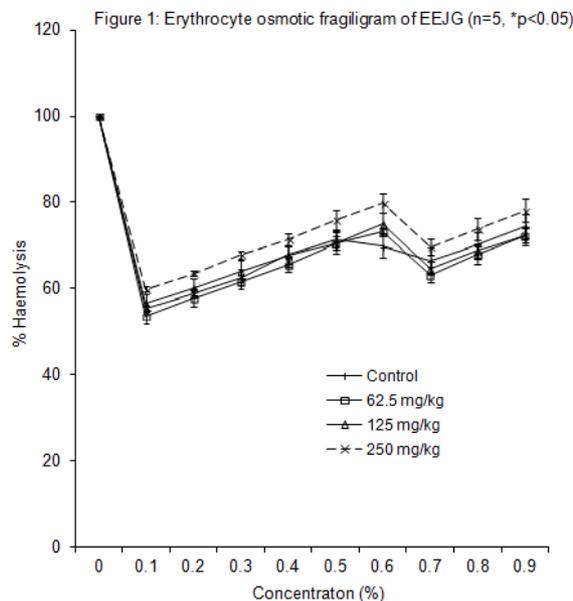
Statistical Analysis

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 EEJG on erythrocyte osmotic fragility after treatment of rats for 30 days is shown in Figure 1.

Treatment of rats for 30 days with all the doses of EEJG (62.5mg/kg, 125mg/kg, 250 mg/kg) produced no significant (p>0.05) changes in erythrocyte osmotic fragility at all the NaCl concentrations (0.1% - 0.9%) 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 membrane¹². It has also been reported that some drugs are capable of inducing alterations on the shape and physiology of the red cells¹³.

The results have shown that the extract caused no significant change in erythrocyte osmotic fragility at

various NaCl concentrations after thirty days of treatment with rats. This probably indicates that the extract has no effect on the stability or strength of red cell membrane integrity since it has been reported that erythrocytes osmotic fragility is used as a measure of the tensile strength of the red cell membrane¹⁴. The insignificant change in erythrocyte osmotic fragility produced by the extract probably indicates its ineffectiveness 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 solution¹⁵.

The insignificant change in erythrocyte osmotic fragility induced by the extract probably indicates that it has no effect on red blood cells viability since it has been reported that the osmotic fragility of mammalian red blood cells is indicative of their viability¹⁶. The insignificant change in erythrocyte osmotic fragility caused by the extract probably indicates that the extract is ineffective 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 by¹⁷ in whole blood incubated with *Lantana camara in vitro*.

In conclusion, this study has shown that the ethanol extract of *Jatropha gossypifolia* probably has no significant effect on erythrocyte osmotic fragility in male rats, *vis-à-vis*, it is probably ineffective in the treatment of hemolysis.

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