

## Membrane stabilizing activity of *Russelia equisetiformis*, Schlecht & Chan

\*Awe, E.O.<sup>1</sup>, Makinde. J.M.<sup>2</sup>, Adeloye, O.A.,<sup>3</sup> Banjoko, S.O.<sup>4</sup>

<sup>1</sup>Department of Pharmacology & Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.

<sup>2</sup>Department of Pharmacology and Therapeutics College of Medicine, University of Ibadan, Ibadan, Nigeria

<sup>3</sup>Department of chemistry, Obafemi Awolowo University, Ile-ife, Nigeria.

<sup>4</sup>Department Chemical Pathology, Obafemi Awolowo University, Ile-ife, Nigeria

\* Corresponding author

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### ABSTRACT

Present study deals with membrane stabilizing activity of aqueous-ethanol extract of *Russelia equisetiformis* Schlecht & Chan (Family- *Scrophulariaceae*) (AERE) *in vivo* and *in vitro* experimental conditions. AERE (25-100 mg/ml) produced significant changes ( $P < 0.05$ ) of membrane stabilization of red blood cells (RBC) exposed to heat stress-induced haemolysis in a concentration-dependent manner, compared with the control without extract. AERE produced no significant changes ( $P > 0.05$ ) of packed cell volume (PCV) and white blood cells (WBC) in rats orally administered AERE, at the dose levels of 25, 50 and 100 mg/kg compared with the pretreatment values. Thus we can say that aqueous-ethanol extract of *R. equisetiformis* possesses the membrane stabilizing activity.

**Keywords:** *Russelia equisetiformis*, Membrane stabilization, Flavonoids, Triterpenes.

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### INTRODUCTION

Phytochemical analysis of *Russelia equisetiformis* Schlecht & Chan (*Scrophulariaceae*), shows it contains triterpenes of lupane-type and flavonoids (Burns, et al., 2000; Awe, et al., 2008). Studies on some medicinal plant extracts, have reported significant decrease in PCV, HB and WBC, when administered orally to Wistar rats for five days and this decrease was attributed to the haemolysis of the blood cells, as a result of cell membrane destabilization by the extracts (Wu Tianshung, et al., 1997;

Plotnikov, et al., 1998). Various medicinal substances such as butadion, escinol, esculetin, glyvenol, rutin and flavonoids preparations are capable of stabilizing the erythrocyte membrane when subjected to hypotonic haemolysis (Chaika and Khadzhai, 1977; Anna, et al., 2006; Manivannana and Sukumar, 2007). Triterpenes isolated from *Burseraceae* and *Asteraceae* families have anti-pruritus and anti-inflammatory activities, and these actions were suggested to be related to a membrane stabilizing action of mast cell and neutrophils membranes (Bettina, et al., 2003; Oliviera, et al., 2004).

Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes (Sessa and Weisman, 1968; Litman, et al., 1976; Horie, et al., 1979; Oyedapo and Famurewa, 1995). Drugs like anesthetics, tranquilizers and non-steroidal antiinflammatories stabilize erythrocytes against hypotonic-induced stress haemolysis therefore prevent the release of haemoglobin as a result of their membrane stabilizing activity (Seeman, 1972). This membrane stabilizing activity of RBC membrane exhibited by some drugs, serves as a useful in vitro method for assessing the anti-inflammatory activity of various compounds (Naibi, et al., 1985). Anti-inflammatory and analgesic activities of *R. equisetiformis* have been reported (Awe, et al., 2005). Here, we report the membrane stabilizing activity of *Russelia equisetiformis*.

## MATERIALS AND METHODS

**Plant material:** Plant sample was collected in the month of October, 2005 from Bodija in the Southwestern part of Nigeria, and was authenticated by Dr. S.O Oni, a taxonomist in the herbarium of the Forest Research Institute, Ibadan, Nigeria (FRIN), where voucher specimen was deposited with number 106998. The plant was air-dried at laboratory temperature 25-27°C and reduced to powdery form using an electric blender.  
**Animals:** Twenty Wistar albino rats weighing between 219-230 g of mixed sex used for this study were bred and housed in the pre-clinical animal house, College of Medicine, University of Ibadan, Ibadan, Nigeria. The animals were kept and maintained under laboratory conditions of temperature, humidity and light; and were allowed free access to food (standard pellet diet) and drinking water *ad libitum*

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

**Extraction:** The powdered material (400 g) was extracted with 500 ml of 50% aqueous-ethanol in the cold for 72 h. The ethanol extract was evaporated to dryness using rotary evaporator under reduced pressure at 40°C and a yield of 85.3 g (21.3 %) was obtained. Phytochemical screening of the extract was done as described by Trease and Evans, 1989.

**Effect of extract on blood cells:** Twenty rats were divided into four groups of five animals each. Group 1 which served as control received 20 % Tween 80 (vehicle) at 10 ml/kg while groups 2, 3, 4, received extract orally using oral cannular at the doses of 25, 50, 100 mg/kg respectively for 7 days. The doses chosen were based on the median lethal dose (LD<sub>50</sub>) obtained, 2,250 mg/kg for the plant's extract. Blood samples (2 ml) were collected each from rats of all groups, seven days after treatment into heparinized

tubes for the analysis of PCV, Hb and WBC as described by Jain, 1986. Because the plant's extract used in this study was dissolved in 20% Tween80, at the beginning of experiment, 20% Tween80 (10 mg/kg)-treated rats were used as control.

**Effect of the Extract on Membrane Stabilization:** This was carried out using the method of Oyedapo and Famurewa, 1995 as modified by Olajide, et al., 2000. Blood sample (2 ml) was collected from another normal rat. The blood was centrifuged and the supernatant carefully removed with a sterile pipette. The packed cells were re-suspended in an equal volume of isotonic saline and centrifuged again. The process was repeated four times until the supernatants were clear. A 10% red blood cell (RBC) suspension was then prepared with normal saline and kept at 4°C undisturbed before use. A reaction mixture (4.5 ml) consisting of 2ml hypotonic saline (0.25%, w/v NaOH and 0.15M sodium phosphate buffer, P<sup>H</sup> 7.4) and varying concentration of the extract (25, 50 and 100 mg/ml) in normal saline to make volume 4.0 ml. Then 0.5 ml of 10% HRBC in normal saline was added. Two controls were prepared, one with 1.0 ml of isotonic saline without red blood cells, (control 1) and the other one with 1 ml of extract solution without red blood cells (control2). The mixtures were incubated at 56°C for 30 minutes. The tubes were cooled under running water for 20 minutes, and mixtures were centrifuged. The absorbance of supernatants was read at 540nm wave length using spectrophotometer. The percentage membrane-stabilizing activity was determined using equation of Sadique, et al., 1989.

$$\% \text{ stabilizing activity} = 100 - \frac{\text{Extract absorbance value} - \text{Control absorbance2}}{\text{Control 1 absorbance value}} \times 100$$

**Statistical Analysis:** Values are expressed as mean + SEM. Statistical significance was determined using two-way ANOVA test. Values with P<0.05 were considered significant.

## RESULTS

The extract has no significant changes (P>0.05) in PCV, HC and WBC values determined at the dose levels of 25, 50 and 100 mg/kg compared with the pre-treatment values (Figure-1A, B and C).

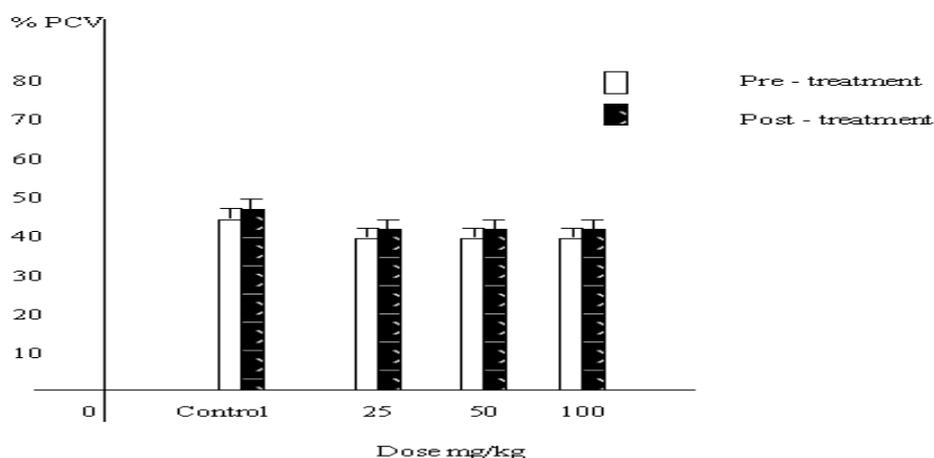


Figure-1A: Showing effect of AERE on PCV.

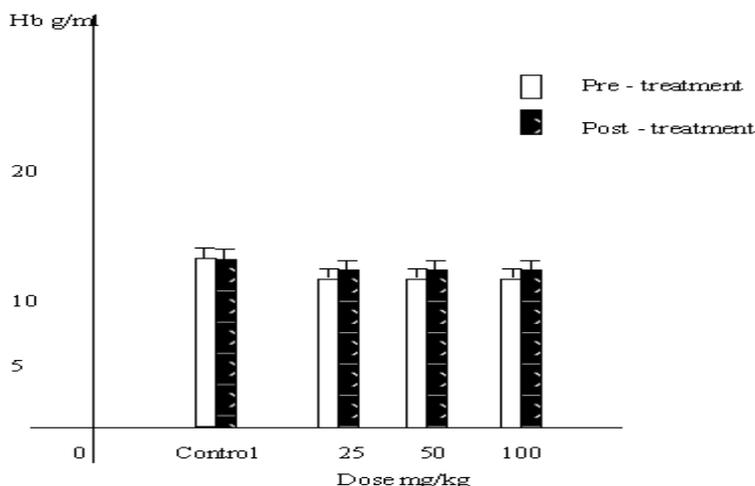


Figure-1B: Showing effect of AERE on Hb.

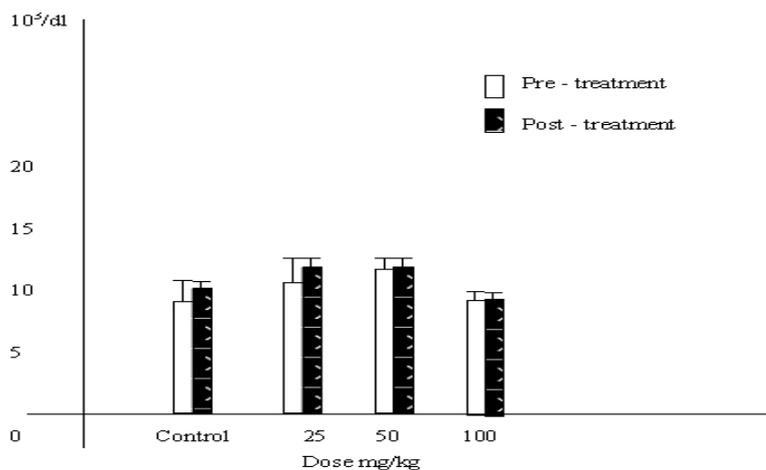


Figure-1C: Showing effect of AERE on WBC.

- Data are expressed as  $\pm$ SEM (n=5) for each group.
- No significant difference ( $P > 0.05$ ), between the pre-treatment and post-treatment values using two-way ANOVA statistical analysis

In the *in vitro* experiment carried out using rat blood, the membrane stabilizing activity of the extract increased significantly ( $P < 0.05$ ) in a concentration-dependent manner (25-100 mg/ml) compared with indomethacin (5 mg/ml) (Table-1, Figure-2).

**Table-1: Effect of *R. equisetiformis* crude extracts on red blood cells membranes**

Extract	Concentration mg/ml	%Membrane Stability*
<i>R. equisetiformis</i>	25	10.7 $\pm$ 1.62
<i>R. equisetiformis</i>	50	47.6 $\pm$ 3.60
<i>R. equisetiformis</i>	100	60.30 $\pm$ 4.56
Indomethacin	5	62.8 $\pm$ 4.75

- \*Results are average of triplicate experiments.

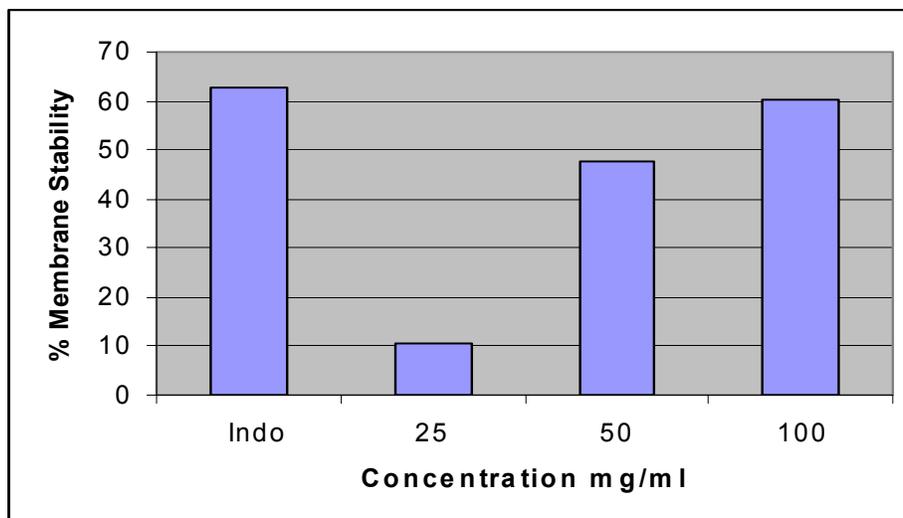


Figure- 4: Effect of extract of *R.equisetiformis* on Red blood cell membrane.

## DISCUSSION

In present study, no destruction or lytic effect of orally administered plant's extract were observed on any blood cells in rats, which could have occurred as a result of cell membrane destabilization (Hess and Milong, 1972). This probably indicates, the membrane stabilizing action of the extract *in vivo*. The study, also demonstrated capability of the extract, to stabilize red blood cell membrane, which is an indication of the extract's ability to prevent rupture, or haemolysis in hypotonic-stress induced condition. The exact mechanism of action, responsible for the membrane stabilizing activity of the plant extract, could not be established in this study. We were also not able identify with certainty, the chemical constituent (s) of AERE that might be responsible for the observed membrane stabilizing action. However, a number of investigators have shown that, flavonoids, triterpenoids, and a host of other secondary plant metabolites, exhibited analgesic, anti-inflammatory effects as a result of their membrane stabilizing action in various experimental animal models ( Bettina, et al., 2003; Jorge, et al., 2004; David, 2007; Chandra and Balaji, 2008). It has also been reported that, there is production of free radicals, such as lipid peroxide and superoxide in various conditions, such as heat-induced stress haemolysis, due to cell membrane destabilization (Agarwal and Rangari, 2003). Flavonoids, triterpenoids and other phenolic compounds are good scavengers of free radicals due to their antioxidant properties (Smith, et al., 1992; Repetto and Liesuy, 2002; Miliiauskas, et al., 2004. Kumar, et al., 2008). Since studied plant extract has been reported to contain triterpenes and flavonoids (Burns, et al., 2000; Awe, et al., 2008), it is not unreasonable, therefore, to speculate that flavonoids, triterpenoids, and other chemical components are responsible for the observed membrane stabilizing action, and probably as a result of their antioxidant activity, thereby acting as free radicals scavengers.

## CONCLUSION

The experimental evidence obtained in the present laboratory animal study indicates that, the aqueous-ethanol extract of *R. equisetiformis* possesses membrane stabilizing property. *R. equisetiformis* extract could serve as a useful supplementary therapy in hemolytic disease, and also in **free radical-mediated oxidative cell injury conditions**.

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