Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

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Impact of Ethanolic Extract of *Dissotis rotundifolia* Leaves on Wistar Rat Erythrocyte Membrane

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Magna Scientia Advanced Biology and Pharmacy, 2022, 06(01), 074–078

Publication history: Received on 25 April 2022; revised on 18 June 2022; accepted on 20 June 2022

Article DOI: https://doi.org/10.30574/msabp.2022.6.1.0069

Abstract

Medicinal plants have been shown to affect the stability of the erythrocyte membrane. *Dissotis rotundifolia* leaves have been used in Nigeria to treat certain diseased conditions not minding the effect on the erythrocyte membrane. The aimed of this work is to investigate the impact ethanolic extract of *Dissotis rotundifolia* leaves have on wistar rat erythrocyte membrane. Twenty-five adult *wistar* albino rats were randomly distributed into five groups administered 0 (control), 50, 100, 150 and 200 mg/100 g body weight. Erythrocytes osmotic fragility was determined by measuring the release of haemoglobin from blood added to tubes containing serially diluted phosphate buffered saline (PBS, pH 7.4). The results of mean corpuscular fragility (MCF) showed non-significant ($p > 0.05$) decrease for 50 and 100 mg/100 g body weight doses, while 150 and 200 mg/100 g body weight doses showed non-significant ($p > 0.05$) increase. The erythrocytes were stabilized by 50, 100 and 150 mg/100 g body weight, while 200 mg/100 g body weight destabilized the erythrocyte membrane. Also, 200 mg/100 g body weight dose showed significant ($p \leq 0.05$) increase in malondialdehyde (MDA) levels. At relatively high doses, *Dissotis rotundifolia* may induce oxidative stress causing significant levels of MDA production resulting in alteration of the chemical and structural composition of the membrane. In conclusion *Dissotis rotundifolia* at low doses may be useful in maintaining the erythrocyte by stabilizing the membrane, whereas high doses destabilize the membrane and compromise membrane integrity.

Keywords: Erythrocytes; Membrane; Fragility; *Dissotis rotundifolia*; Lipid Peroxidation

1. Introduction

Erythrocyte fragility refers to the tendency of erythrocyte to haemolyse under stressful conditions. It refers to the degree of haemolysis that occurs when the erythrocyte is subjected to osmotic stress in hypotonic medium. [1]. Erythrocyte fragility is affected by the composition and integrity of the membrane, and the cell surface-area to volume ratio [2]. Drugs [3, 4] and medicinal plants [5, 6, 7, 8] have been reported as some of the external factors that can influence erythrocyte osmotic fragility [9].

Medicinal plants reported to stabilize the erythrocyte membrane include *Anacardium occidentale*, *Psidium guajava* and *Terminalia catappa* [5], *Carica papaya* [10] and *Solanum aethiopicum* [11]. These plants act by decreasing membrane fragility. *Allium cepa* and *Allium sativa* on the other hand, increased erythrocyte membrane fragility in rats due to membrane damage [12] hence destabilizing the erythrocyte membrane and compromising its integrity.

Oxidative stress is one factor that compromises erythrocyte membrane integrity [13, 14]. Lipid peroxidation, a process whereby oxidants attack lipid containing carbon-carbon double bonds with insertion of oxygen, resulting in lipid peroxyl readicals and hydroperoxides [15] caused by oxidative stress also compromise the integrity of the erythrocyte membrane.

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This study is aimed at investigating the impact of ethanolic extract of *Dissotis rotundifolia* leaves on wistar rat erythrocyte membrane stability.

2. Material and Methods

2.1. Collection and Extraction of Plant Materials

Dissotic rotundifolia leaves were obtained and shade dried prior to extraction. The dried leaves were pulverized to obtain fine powder. Six hundred (600) grams of dried powder soaked with 1.5 liters of ethanol was allowed to stand for 72 hrs. The ethanol solvent was evaporated in water bath at 50 °C to obtain the dry leave extract.

2.2. Experimental Animals and Design

Twenty-five adult *wistar* albino rats with average weight of 200 g were randomly distributed into five groups. They were allowed to acclimatize for two weeks and were given standard poultry diet *ad libitum*.

The five groups (five rats each) were administered the extract as follows: group 1 served as control (0 *mg/100 g*), group 2 – 5 were given 50, 100, 150 and 200 *mg/100 g*. The extract was administered orally by intubation. The duration of this experiment was 21 days.

2.3. Collection and Preparation of Sample

Blood samples were collected by cardiac puncture. Erythrocytes were washed as described by Tsakiris *et al*., [16]. Within 2 hours of collection, portions of 1.0 cm^3 of the samples were introduced into centrifuge tubes that contain 3.0 ml of buffer solution pH = 7.4. Erythrocytes were separated from plasma by centrifuging for 10 min at 1200 g, Erythrocytes were washed three times by 3 similar centrifugations with same buffer. Washed erythrocytes were re-suspended in 1.0 cm^3 of same buffer solution. The test was done with the washed intact erythrocytes

2.4. Haemoglobin Estimation

Haemoglobin stock was diluted (1.0 g/dl – 10.0 g/dl) and a calibration curve prepared. The concentration of haemoglobin was estimated by incubating 50ul of the erythrocytes with 4.95 ml of Drabkins reagent at ambient temperature for 10 mins and optical density read at 540nm against Drabkins reagent as blank.

2.5. Determination of Osmotic Fragility

Benford and Kenned, [17] method was used for erythrocytes osmotic fragility determination. Twenty (20) microliters of blood was added 5 ml of phosphate buffered saline (pH 7.4) of serial concentrations (0 - 0.85%) saline. The mixtures were allowed to stand for 1 hour at ambient temperature. Then the mixtures were centrifuged for 5 minutes at 1580 g. The supernatants were carefully collected and haemoglobin content determined at 540 nm using distilled water as blank. Percentage haemolysis in each buffered saline concentration was evaluated taking the concentration with the highest absorbance as 100% haemolysis.

2.6. Stability Evaluation

Mean corpuscular fragility (MCF) was extrapolated from the osmotic fragility curve. The relative capacity of the drugs to stabilize or destabilize the erythrocyte was calculated using the relationship below expressed by Parpart *et al*., [18].

$$RS\ (\%) = \frac{MCF_{control} - MCF_{test}}{MCF_{control}} \times 100$$

RS = Relative Stability of erythrocyte membrane

2.7. Lipid Peroxidation Assay

An aliquot of 0.4 ml of the sample was mixed with 1.6cm^3 of Tris-KCl buffer, 0.5 ml of 30% TCA. and 0.5 ml of 0.75% TBA. The mixture was placed in a water bath at 80^0C for 45minutes. the mixture was cooled in ice bath and centrifuged at 3000 g. The absorbance of the clear supernatant was read at 532nm against a reference blank of distilled water. The malondialdehyde level was calculated using the relationship expressed Adam-Vizi and Seregi, [19].

$$\text{MDA (units/gHb)} = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532nm} \times \text{volume of sample} \times \text{gHb}}$$

3. Results and Discussion

The study investigated the impact of ethanolic leave extract of *Dissotis rotundifolia* on wistar rat erythrocytes membrane fragility and stability.

Osmotic fragility curve for the rat erythrocytes exposed to different doses of *Dissotis rotudifolia* extract is presented in figure 1. The haemolysis pattern of rat erythrocyte suspended in increasing concentration of saline conforms to published report by Chikezie and Uwakwe, [5], Saad and Habib, [6], Swem *et al*., [7] and Duchnowicz *et al*., [20]. Osmotic fragility is affected by certain factors including the chemical composition of the membrane and its viscoelasticity [21]. The increase in osmotic fragility of rat erythrocytes in the presence of *Dissotis rotudifolia* was not significant ($p \geq 0.05$) at all the doses studied. Also the level of haemolysis was not significant ($p \geq 0.05$) when compared to the control.

The Mean corpuscular fragility values of the erythrocytes of the rats administered with *Dissotis rotundifolia* extract are shown in table 1. Mean corpuscular fragility index is the concentration of PBS solution that caused 50% haemolysis of erythrocyte [22]. Compared to the control there was a non-significant ($p \geq 0.05$) decrease for 50 and 100 mg/100 g b.wt doses, while 150 and 200 mg/100 g b.wt doses showed non-significant ($p \geq 0.05$) increase. The non-significant ($p \geq 0.05$) increase observed 150 and 200 mg/100 g b.wt doses however, may be as a result of mild chemical and structural changes in the erythrocyte membrane induced by *Dissotis rotundifolia* extract. These changes may have increased the permeability of the rat erythrocyte membrane. Increase in erythrocyte membrane fragility as reported by Suhail *et al*., [23] may be due to decreased fluidity of the membrane and the ability of the membrane to withstand osmotic changes. Also increased erythrocyte membrane fragility is possible due to oxidative stress and lipid content alteration [24].

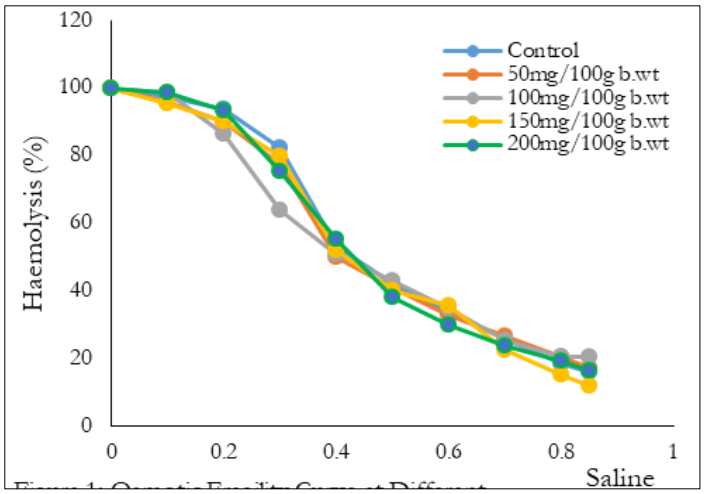

Figure 1: Osmotic Fragility Curve of %Haemolysis versus Saline Concentration at Different Concentrations of *Dissotis rotundufolia*

The relative stability of erythrocytes in the rats administered with ethanolic leave extract of *Dissotis rotundifolia* given in table 1 showed that only the erythrocyte membrane of the rats administered with 200 mg/100 g b.wt dose were destabilized. All other doses (50, 100 and 150 mg/100 g b.wt) administered stabilized the erythrocyte membrane and therefore are not likely to compromise the membrane integrity.

Lipid peroxidation assay results also given in table 1 showed that 50, 100 and 150 mg/100 g b.wt doses showed non-significant ($p \geq 0.05$) increase in MDA levels. However, the rats administered with 200 mg/100 g b.wt showed significant ($p < 0.05$) increase in MDA levels. MDA is a convenient biomarker for lipid peroxidation because of its easy reaction with TBA [25]. The destabilization of the erythrocytes membrane of rat administered with 200 mg/100 g b.wt is expected since lipid peroxidation occurred in the erythrocyte membrane of rats given the same dose as seen in the significant ($p < 0.05$) increase in MDA levels.

Dissotis rotundifolia is seen in the present study to induces oxidative stress in rats administered with high doses, which in turn cause lipid peroxidation of the erythrocyte membrane. The oxidative stress as reported by Ahur *et al*., [12] and Adamu *et al*., [13] compromise the integrity of the erythrocyte membrane. At low doses (50, 100 and 150 mg/100 g b.wt), studied *Dissotis rotundifolia* stabilized the erythrocyte membrane and is therefore, not likely to compromise its integrity. The investigations in this study did not elucidate the mechanism of action and the active agents in *Dissotis rotundifolia* extracts that are responsible for its erythrocyte membrane stabilizing/destabilizing effect. However, the results showed that there may be some level of modification on the erythrocyte membrane leading to its destabilization.

Table 1 Mean Corpuscular Fragility (MCF) index, MDA levels and Relative Stability of Rat Erythrocyte Membrane Administered *Dissotis rotundufolia* Leave Extract

Dosage (mg/100g b.wt)	**MCF (g/100 ml)**	**Relative Stability (%)**	**MDA (units/gHb)**
Control (0)	0.421±0.02a	-	0.017±0.002a
50	0.400±0.10 a	4.99S	0.017±0.003a
100	0.405±0.01a	3.80S	0.019±0.001a
150	0.415±0.03a	2.86 S	0.020±0.001a
200	0.430±0.09a	-2.14 D	0.045±0.003b

D = destabilized, S = stabilized. Values are MEAN ± +SD of triplicate determinations. Means with different superscript letters in the same column are statistically different at 95 % confidence limit ($p < 0.05$).

4. Conclusion

The present *in vivo* studies revealed the fact that ethanolic leave extract of *Dissotis rotundifolia* destabilizes the erythrocyte membrane of wistar rats at relatively high doses. Results in the present investigation also indicated that *Dissotis rotundifolia* at low doses may be useful in maintaining the erythrocyte integrity by stabilizing the membrane. However, further investigations are needed to ascertain the active agents in *Dissotis rotundifolia* and their mechanism of action as agents of membrane destabilization.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the support given by Rosemary Yafogho during the experimental process. We also acknowledge Mrs. Ann Tarilayefa Wodu-Ebizimor for typing the article.

Disclosure of conflict of interest

Authors declare no conflict of interest.

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