Improved Erythrocyte Osmotic Fragility and Packed Cell Volume following administration of *Aloe barbadensis* Juice Extract in Rats.

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Abstract

Aloe barbadensis is a popular house plant that has a long history of a multipurpose folk remedy. It has been documented to have anti-diabetic, antiseptic and anti-inflammatory effects. The effect of *Aloe barbadensis* juice extract on erythrocyte osmotic fragility, packed cell volume and haemoglobin concentration in Wistar rats was investigated.

Twenty rats were divided into four groups of five rats each. Group A served as Control and received distilled water *ad libitum*, while groups B, C and D were the experimental groups and received 50mg/kg, 100mg/kg and 150mg/kg body weight of extract respectively, orally for four weeks. The animals were sacrificed and their blood was collected through cardiac puncture. The erythrocyte osmotic fragility, packed cell volume and haemoglobin concentration were determined.

The results showed significant reductions ($p<0.05$) in the erythrocyte osmotic fragility in the groups that received 100mg/kg and 150mg/kg of the extract when compared to the control group and also significant increases ($p<0.05$) in the packed cell volume of rats that received 50mg/kg and 100mg/kg body weight of the extract.

The result suggests dose-dependent protective and modulatory effects of *Aloe barbadensis* on the integrity of the erythrocyte membrane and on the packed cell volume, respectively.

Keywords: osmotic fragility, packed cell volume, haemoglobin, Aloe vera

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Introduction

Erythrocyte osmotic fragility (EOF) is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients. [9] It is the susceptibility of erythrocytes to rupture when subjected to increasingly hypotonic (lower osmotic pressure) saline solution. Erythrocytes are characterized by a biconcave shape given to it by an excess of surface area in relation to its volume. When there is a decrease in the surface area to the cell volume, the osmotic fragility is increased. Thereby resistance of such a cell to hypotonic solution is decreased. EOF tends to increase in certain pathologic conditions such as sickle cell disease and hereditary spherocytosis.

The packed cell volume (PCV), sometimes called haematocrit, is the fraction of cells in a volume of blood after it has been centrifuged [16]. Haemoglobin is the red oxygen-carrying pigment in the erythrocyte [6]. It is made up of 4-heme subunits and a globin portion. The heme portion is responsible for the oxygenation because it contains the ferrous iron containing unit. Haemoglobin concentration (HC) is usually about 16g/dl in males and 14g/dl in females, but this is altered in several physiologic and pathologic conditions.

Aloe barbadensis (*A. barbadensis*) [family:Liliiaceae], belongs to a class of plants called 'xeroids' which have the ability to close their stomata completely to avoid loss of water [5]. The plant is the source of two herbal preparations: the aloe gel and aloe latex. The aloe latex is usually referred to as aloe juice- the bitter yellow exudate from the pericyclic tubules just beneath the outer skin of the leaves. The latex contains a series of glycosides known as anthraquinones, the most prominent of them being aloin A and B [17]. There are about 75 known components of aloe vera which are contained in about 1% of the plant while the rest is water. The components are obviously present in small amounts, hence their proportionate action is thought to arise from the synergistic effect of these substances [5].

Aloe vera has been shown to have anti-inflammatory, anti-viral, anti-septic and anti-diabetic effects; the possibility of these effects was traced to its phytochemical constituents. Though studies have revealed the effect of aloe barbadensis on some haematological parameters such as white blood cell count, red blood cell count [1.4.13] and serum lipids [2], only a

few documented studies have been done to show the effect of this plant in a dose dependent manner. This study therefore, seeks to establish the effect of aloe barbadensis juice extract in a dose-dependent manner on erythrocyte membrane stability, PCV and HC

Materials And Methods

Preparation of plant material:

Fresh leaves of *A. barbadensis* were obtained from Ilorin metropolis and sent for authentication at the Department of Biological Sciences, University of Ilorin, Nigeria, where the voucher specimen (FHI, 106934) was deposited. The leaves were air dried and reduced to powdered form; the powdered leaves were percolated in distilled water for 12 h and filtered; the filtrate was subsequently evaporated to dryness and yielded a 22.5 % dark green concentrate.

Animals:

Twenty albino wistar rats weighing between 150 and 200 g were used for this study. They were housed and acclimatized for two weeks in the Central Animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria. They were fed on standard rat pellet (Bendel Feeds, Nigeria) and were allowed water *ad libitum*. The animals were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12 h light: dark cycle. All experimental protocols and handling were in compliance with the NIH publication No 85-23 guidelines (NIH publication revises, 1985).

Experimental design:

The rats were randomly distributed into four groups of five rats each:

Group A- (control) normal and received distilled water

Group B- (treated) received 50mg/kg aqueous extract of *A. barbadensis*

Group C- (treated) received 100mg/kg aqueous extract of *A. barbadensis*

Group D- (treated) received 150mg/kg aqueous extract of *A. barbadensis*

The rats were treated for four weeks. At the end of the experimental period, they were sacrificed and blood was collected by cardiac puncture under light anaesthesia. The blood was transferred into EDTA bottles. EOF, PCV and HC was then determined.

Determination of erythrocyte osmotic fragility:

Sodium chloride (NaCl) solution was prepared in volume of 500ml each of the samples in concentration ranging from 0-1.0% at pH 7.4. A set of 11 test tubes, each containing 5ml of NaCl solution of concentration ranging from 0-1.0% with differences of 0.1% (Table 1) were arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labelled with corresponding NaCl concentration. Each test tube was mixed by gentle inversion to assure the equilibrium of the solution. 1ml pipette was used to transfer 0.02ml blood sample into each of the test tubes. Mixing was performed by gentle inversion of the test tubes. The test tubes allowed to stand at room temperature (25-27%) for 1 h. The contents were then re-mixed and centrifuged at 1500rpm for 15mins. The supernatant of each tube was transferred into a glass curette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophotometer at 540nm by reading the absorbance. The test tube labelled 11 (1.0% NaCl) was used as blank to spectrophotometer at 100% transmission or zero absorbance. The test tube labelled 1 (0% NaCl) was used as a control to determine 100% haemolysis. The same procedure was repeated for every blood sample of each rat used for the study. The percent haemolysis was calculated using the formula:

$$\%\text{heamolysis} = \frac{\text{optical density of sample}}{\text{optical density of control}}$$

Erythrocyte fragility curve was obtained by plotting percent haemolysis against the NaCl concentration.

Statistical analysis:

All results were expressed as mean ± SEM. Data was analyzed by one-way analysis of variance (ANOVA) and Duncan new multiple range test (DMRT). Differences in means were considered significant at $P< 0.05$. All analysis was performed using SPSS 17.

Results

Mean erythrocyte osmotic fragility of control and experimental groups are shown in table 2. The mean osmotic fragility in control group was 41.27 ± 11.4 as against experimental group B (50mg/kg of extract) 38.98 ± 12.2, group C (100mg/kg of extract) 35.34 ± 12.8 and group D (150mg/kg of extract) 25.89 ± 13.2. Experimental group B showed no statistically significant difference at $P<0.05$ when compared to control. Groups B and C showed significant decreases ($P<0.05$) in osmotic fragility when compared to control. Figure 1 shows the osmotic fragility curve of control and experimental groups

PCV of the control and experimental groups are also shown in table 2. The mean PCV in the control group (distilled water) was 37.25±0.95 as against experimental groups A (50mg/kg) 47.25±1.03, B (100mg/kg) 45.50±0.5 and C (150mg/kg) 36.00±1.22. Groups B and C showed statistically significant increases ($P<0.05$) in PCV when compared to the control group while group D showed an insignificant reduction ($P>0.05$).

HC of groups B (50mg/kg) 13.00±0.32 and C (100mg/kg) 13.73±0.08, showed insignificant increases when compared to the Control (distilled water) 12.80±0.39. There was a slight but insignificant

Table 1: effect of Aloe barbadensis aqueous extract on EOF, PCV and HC

Groups	Group A-Control	Group B	Group C	Group D
Parameters	(Normal saline)	(50mg/kg)	(100mg/kg)	(150mg/kg)
Osmotic fragility (%)	41.29±11.4	38.98±12.2	35.34±12.8**	25.89±13.2**
PCV (%)	37.25±0.95	47.25±1.03*	45.50±0.5*	36.00±1.22
HC (g/dl)	12.80±0.39	13.00±0.32	13.73±0.08	12.48±0.45

Values are expressed as mean ± SEM, * P<0.05 when compared to control, ** significantly reduced when compared to control

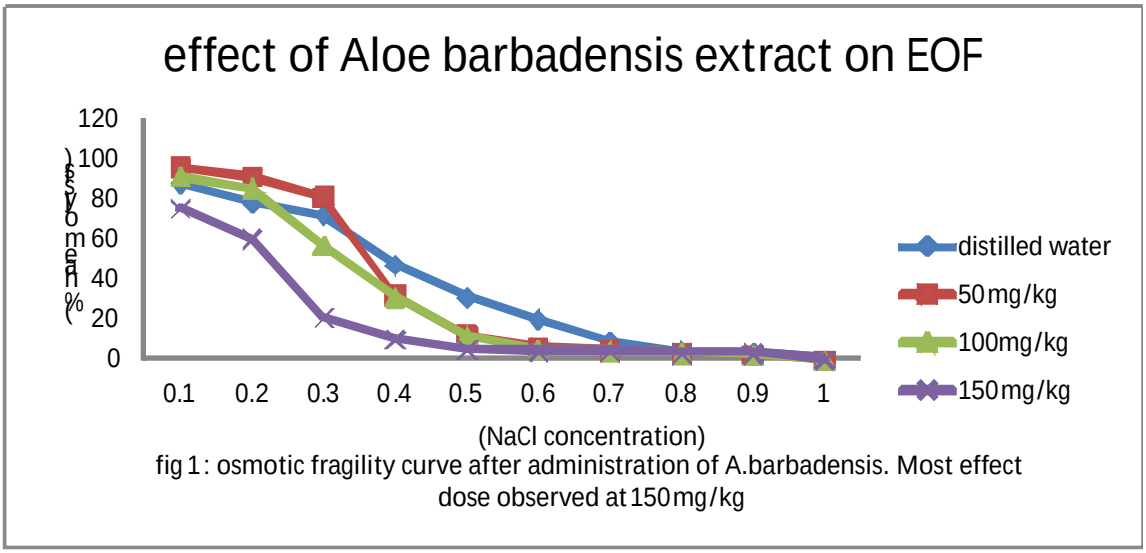

fig 1: osmotic fragility curve after administration of A.barbadensis. Most effect dose observed at 150mg/kg

reduction (P>0.05) in group D (150mg/kg). This is shown in table 2.

Discussion

A. barbadensis has been shown to have anti-inflammatory, antiviral, antiseptic and wound healing effects, even in the diabetic state, in dose-response fashion. Though various studies have been carried out on erythrocyte membrane integrity [3.9.11] to check anti-sickling effect of other extracts, and effects of several antioxidants including vitamin C & E, very little literature has been documented on the effect of *A. barbadensis* on erythrocyte membrane integrity. The present study revealed that the effect of varied concentration of A. barbadensis aqueous extract on the red blood cell membrane using the osmotic fragility test has appreciable membrane protective effects and inhibitory action on haemolysis. EOF appears to be increased in some pathologic conditions such as G6PD deficiency, sickle cell disease and spherocytosis. The significant reduction (P<0.05) in EOF obtained from group C (150mg/kg of extract) suggests that *A.barbadensis* contains substance (s) that improves and stabilizes the integral protein of the red cell membrane. Our results correspond to findings by Ugbor [21] who reported the potential effects of *A. barbadensis* in the management of sickle cell disease but contrasts to findings by Iji et al [22] who reported that there was no significant change following chronic administration of Aloe vera gel..

The significant increase in PCV in the lower doses (50mg/kg & 100mg/kg) observed from this study corresponds with findings by Abdulkadir et al [1] and Iji et al [22]. Abdulkadir et al in 2005 reported that *A. barbadensis*, along with some other medicinal plants significantly improved on the PCV. Though this observation was in contrast to studies by Alishahi et al [4] and Oguwike et al [13] who reported insignificant changes in the PCV following administration of *Aloe vera*. The changes observed suggest a probable dose dependent effect of *A. barbadensis*.

These observed reduced haemolytic and PCV elevating action of *A. barbadensis* further adds to its numerous positive effects [1. 13], especially when blood constituents are involved.

Different phytochemicals present in *A. barbadensis* could be responsible for the varied haematologic activities. The bioactive ingredients that have the therapeutic activity in plants used in traditional practice are mostly unidentified and traditional healers believe in the holistic nature of their treatment. Substances found in medicinal plants, containing the healing property of plants [18] is known as the active principle. It differs from plant to plant and examples of active principles include: anthraquinones, flavonoids,

glycosides, saponins, tannins etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases [19]. In recent years, these active principles have been extracted and used in different forms such as infusions, syrups, concoctions, decoctions, infused oils, essential oils, ointments and creams [20]. There is therefore a need for further extensive work on this plant using different dosages, different routes of administration and possible combinations in order to exploit their full potential. There is a critical need to set up a clinical experiment to isolate the main active ingredients responsible for all the medical and nutritional activities credited to *A. barbadensis*.[10] In addition, this plant should be sourced from different geographical locations. This is because the existence of different variants, soil and climatic conditions affect phytochemical constituents and hence the therapeutic actions of the same plant. [12]

It could thus be concluded that oral administration of aqueous extract of *A. barbadensis* may improve on reduced PCV and increased EOF seen in some pathologic conditions which support its traditional use though the precise mechanism(s) and site(s) of action require further elucidation.

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