Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(6); 1010-1015

ISSN: 0975-4873

Research Article

Preliminary Findings on Effect of Aqueous Leaf Extract of *Aspilia africana* on Erythrocyte Osmotic Fragility and Na^+ K^+ ATPase Activity in the Rat

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Available Online: 9th June, 2016

ABSTRACT

Objective*: Aspilia africana* C.D Adams (Asteraceae) has been shown to enhance erythropoiesis in laboratory animals but its effect on erythrocyte membrane stability especially in stressful conditions is yet to be fully elucidated. In this study, we evaluated the effect of aqueous leaf extract of *Aspilia africana* (ALEAA) on erythrocyte osmotic fragility and Na^+ K^+ ATPase in rats. Methods: Male albino rats (150-170g) were treated with varying doses of ALEAA; 250, 500 and 750 mg/kg/d for twenty-one (21) days. The control group received no extract but distilled water. Blood samples were obtained by cardiac puncture. Osmotic fragility was determined by standard procedures while Na^+ K^+ ATPase activity and total protein concentration were determined spectrophotometrically from the erythrocyte ghost membranes prepared by osmotic lysis. Results: The haemolysis percentage change was seen to be more pronounced in 500 and 750 mg/kg doses, where a significant decrease was observed when compared to the control group between 0.5-0.2% salt solution ($p<0.05$). The total erythrocyte protein was significantly higher in 500 and 750 mg/kg than the control (5.88 ± 0.52; 5.01 ± 0.20 vs 3.59 ± 0.21 µg/mL respectively). The extract also caused 44.1% and 102% increase in the activities of total Na^+ K^+ ATPase at 500 and 750 mg/kg respectively; while, an increase of 63% and 71.4% was observed in the ouabin sensitive ATPase activity. Conclusion: It is concluded that the aqueous leaf extract of *Aspilia africana* decreased the osmotic fragility, and enhanced Na^+ K^+ ATPase activity of the red blood cell in albino rats.

Keywords: *Aspilia africana*, red cell, membrane enzyme, osmotic stress

INTRODUCTION

Plants serve as a source of nutrients and primary needs for about 80% of people in developing countries[1], where there is an increasing demand for medicinal plants that often help to save lives. One of such plants explored in folkloric medicine in Africa to cure certain illnesses and heal injuries is *Aspilia Africana*[2]. *Aspilia africana* is a common weed of field crops in West Africa found in fallow land, especially in the forest zone. It is semi woody herb occurring throughout the region of the savannah and tropical Africa on wastelands[3,4]. It is known by various names among the Nigerian populace; *Orangila* in Igbo, *Tozalin* in Hausa, *Yunyun* in Yoruba and *Edemedong* in Efik. It is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Earlier studies have demonstrated that it can be used to stop bleeding, accelerate healing of wounds, treat rheumatic pain[5]. Interestingly, the plant is used to treat different diseases in different ecological zones, perhaps due to varying chemical composition as a result of various ecological conditions of different places. For example, Kenyans used it as anti-helminthic, while in Uganda it is used to treat gonorrhea[6], and the decoction used to cure eye problem and as a lotion for the face to relieve febrile headache[7]. In South-eastern Nigeria, leaves of this plant is claimed to be effective in the treatment of stomach ache and bleeding gastric ulcers especially when taken as aqueous decoction. Scientifically, the plant has been validated for haemostatic[8], anti-inflammatory[9], anti-ulcer[10], antioxidant[11], anti-malaria[12], and antimicrobial[13] properties. Scientific reports suggest that different crude extracts of the plant contain specific bioactive constituents that could have varied effects on its biological activities[9,14]. Phytochemical analysis and subsequent quantification revealed the presence of high amount of some bioactive compounds; saponins, tannins, alkaloids, flavonoids, terpenoid and phenol, but little amount of steroids, phylobatannin and cardiac glycoside[14]. Recently, the aqueous leaf extract of *Aspilia africana* was observed to enhance erythropoiesis in laboratory animals[15,16]. Osmotic fragility index is a measure of the resistance of red blood cells to lysis by osmotic stress[17]. The test measures level of stability and functionality of plasma membrane[18] in which membrane-bound enzymes like Na^+ K^+ ATPase play a fundamental role. Na^+ K^+ ATPase is a soluble conserved trimetric pump which plays a central role in exchange and transportation of the substances across cell membrane[19]. The basic function of the Na^+ K^+-ATPase or

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sodium pump is to maintain the high Na^+ and K^+ gradient across the plasma membrane[20]. This, in turn, regulates cell volume, cytoplasmic pH, and drives a variety of secondary transport processes such as Na^+- dependent glucose and amino acid transport, muscle contraction, nerve impulses generation and propagation[19]. However, Na^+ K^+ ATPase has also been reported as one of the membrane proteins affected structurally and functionally in various disorders like sickle-cell anaemia[21], diabetes mellitus[22], hypertension[23], neurological disorders[24] etc.

Although, the haematopoietic stimulating properties of the plant have been reported, there is dearth of documented information on the effect of this plant on the stability and/or maintenance of integrity of the red cell membrane in stressful conditions. Since osmotic fragility represents a measure of the tensile strength of the red cell membrane and the Na^+ K^+ ATPase pump, the primary mechanism by which the cell prevents lysis from osmotic stress, this study was designed to evaluate the effect of *Aspilia Africana* on the erythrocyte osmotic fragility and Na^+ K^+ ATPase activity in albino rats of Wistar strain.

MATERIALS AND METHOD

Preparation of Aqueous extract of Aspilia africana

A bulk of fresh *Aspilia africana* leaves (single batch) sufficient for the study was collected from the field after authentication at the herbarium of the Department of Pharmacognosy, Igbinedion University, Okada where a voucher specimen is deposited. The leaves were air-dried at room temperature and pulverized to fine powder. The extraction was carried out using Soxhlet extractor with water as solvent. The extract was then concentrated to approximately 10% of the original volume using a rotary evaporator (BUCHI, type RE111, Rotavapor).

Experimental animals and Housing protocol

Male albino rats weighing between (150-170g) used for this study were obtained from the Central Animal Facility, College of Health Sciences, Igbinedion University, Okada. They were examined for a period of two (2) weeks and confirmed to be free from diseases and infections.

The animals were grouped into 5 cages, each of which contained 5 rats; the cages used were plastic cages with well-ventilated cover. They acclimatized to the standard laboratory conditions (12 hours day and night cycle). They were fed with standard rat diet (pelletized) and had access to drinking water *ad libitum*. The beddings were changed every 3 days to ensure proper sanitation, and the cages constructed large enough to allow considerable movement for the animals. The Central Animal Facility/Ethics Committee of College of Health Sciences, Igbinedion University approved the experimental protocols.

Experimental design and Procedures

Animal Grouping and Treatment: The animals were randomly assigned to groups as follows: Group 1: Control group, received distilled water**;** Group 2: 250 mg/kg/d aqueous extract of *Aspilia africana***;** Group 3: 500 mg/kg/d aqueous extract of *Aspilia africana***;** Group 4: 750 mg/kg/d aqueous extract of *Aspilia africana.* The extract was administered to the rats by gavage for twenty-one (21) days.

Blood collection**:** After twenty-one (21) days, the animals were sacrificed by cervical dislocation and blood quickly collected via cardiac puncture. The euthanized rat was placed on its back on a cork board and strapped with two adhesive pins each across the fore and hind legs. 40 x 0.8 mm needle was inserted in the centre line at the tip of the sternum and pushed forward at an angle of 45^0C till it punctured the heart. After 2.5 ml cardiac blood specimen was collected, the needle was quickly with-drawn and the blood was transferred into a clean EDTA- container (thoroughly mixed) ready for investigations.

Determination of Erythrocyte Osmotic Fragility: Osmotic fragility was determined by a measure of haemoglobin released from red blood cells when placed in an environment containing serial dilutions of Phosphate Buffer Saline (PBS) solution as described by Oyewale[25], with minor modifications[26]. Twenty microliters (20 µl) portion of red blood cells suspended in 1.0 ml buffer solution: pH = 7.4 (Tris HCl/140 mM NaCl/1.0 mM$MgCl_2$/10.0 mM glucose), was added to test tube containing 5.0ml of PBS solution, pH = 7.4 - {NaCl (9.0 g)/$Na_2HPO_4.2H_2O$ (1.71 g)/$NaH_2PO_4.2H_2PO_4.2H_2O$ (2.43 g) per 1 L of distilled water}, of serial concentrations in the order of 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3 and 0.2 g/100ml. The ninth test tube contained distilled water. The test tubes were allowed to stand for 30 minutes at room temperature (24°C). Subsequently, the contents of test tubes were centrifuged at 1,200 g for 10 min. The supernatant was decanted and haemoglobin content determined spectrophotometrically at 540 nm wavelength using PBS (0.9 g/100 ml) solution as blank. Haemolysis in each test tube was expressed as a percentage calculated as thus:

$$\% \text{ Haemolysis} = \frac{\textit{Optical Density of Test Solution}}{\textit{Optical Density of Standard Solution}} \textit{ X100}$$

Erythrocyte Ghost Membrane (EGM) Preparation: One ml of freshly collected whole blood was used for the EGM preparation. The whole blood was centrifuged at 3,000 rpm for 10 minutes. The plasma was removed to obtain the packed erythrocytes. The erythrocytes were then washed twice in five times volume of isotonic buffer at 4,200 rpm for 20 minutes.

The supernatant was decanted and the pelleted cells haemolysed in five times volume of hypotonic buffer and centrifuged at 4200 rpm for 20 minutes. This was then repeated four times and the supernatant decanted each time. The pink ghost was then washed five times in four times volume of washing buffer at 4200 rpm for 20 minutes each. The supernatants were decanted. The entire washing process was done in a refrigerated centrifuge at 4^0C.

Determination of Na^+ K^+ ATPase Activity: The erythrocyte total ATPase activity was determine by incubating 50 µL of ghost membrane suspension with 5 mM Tris-ATP, 25 mM KCl, 75 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 25 mM Tris-HCl, pH 7.5 in a total volume of 500 µL for 90 minutes at 37^0C in a shaking water bath (150 rpm). The reaction was stopped by adding TCA to a final concentration of 5% (wt/vol). After centrifugation for 20 minutes at 1,500g, an aliquot of the supernatant was used to measure total inorganic phosphate liberated by the

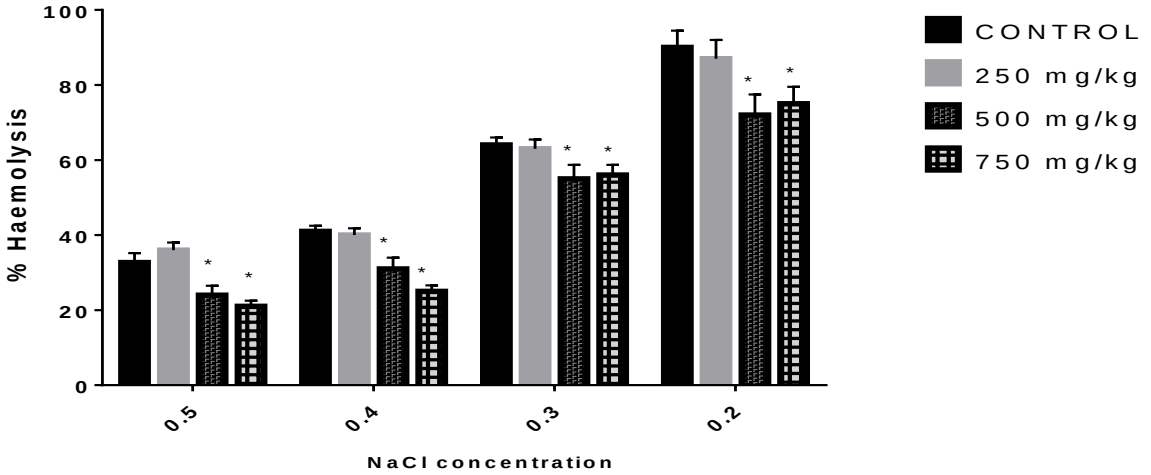

Figure 1: Effect of graded doses of aqueous leaf extract of *Aspilia africana* on red cell osmotic fragility in the rat. *Each bar represents Mean ± SEM of five experiments; *p<0.05 (c.f control).*

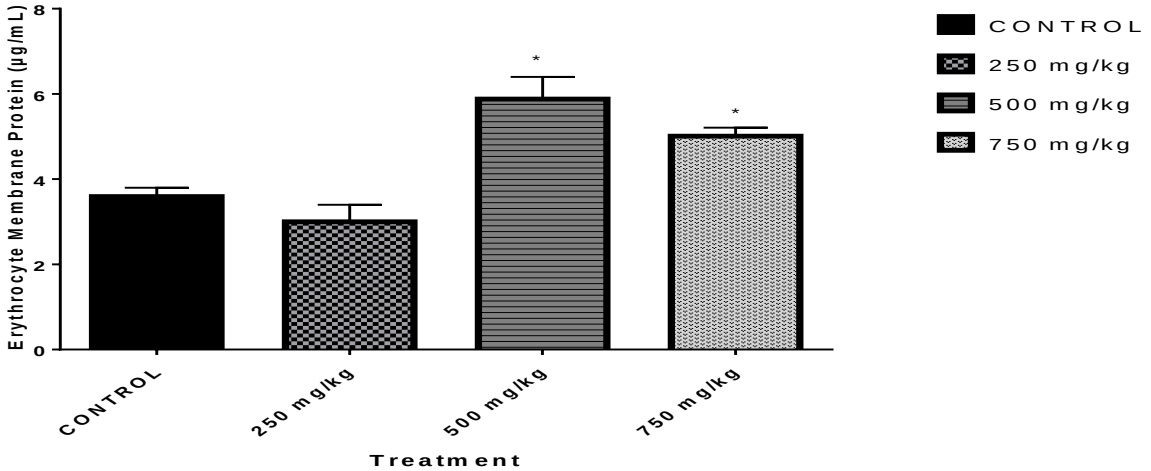

Figure 2: Effect of graded doses of aqueous leaf extract of *Aspilia africana* on erythrocyte membrane protein in the rat. *Each bar represents Mean ± SEM of five experiments; *p<0.05 (c.f control).*

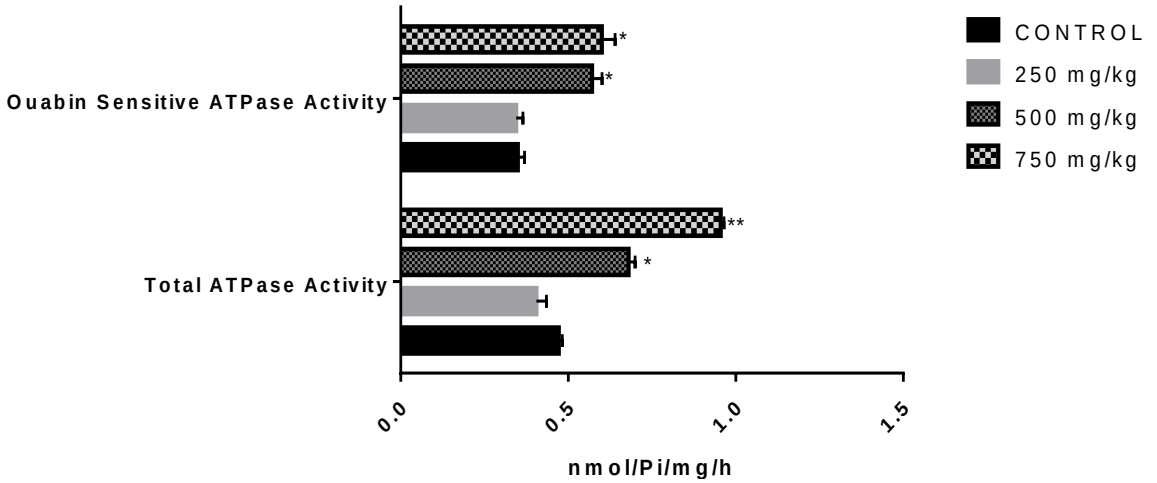

Figure 3: Effect of graded doses of aqueous leaf extract of *Aspilia africana* on Na^+-K^+ ATPase in the rat. *Each bar represents Mean ± SEM of five experiments; *p<0.05, **p<0.001 (c.f control).*

reaction of Fiske and Subbarrow[27]. This assay was repeated in the presence of 200 µM ouabin, an inhibitor of Na^+ K^+-ATPase. Total Na^+ K^+-ATPase activity was expressed as nanomole of inorgsnic phosphate liberated per milligram membrane protein per hour. The activity of Na^+ K^+-ATPase was subsequently determined by substracting total Na^+ K^+-ATPase activity in the presence

of ouabin from enzyme activity in the absence of the inhibitor drug.

Determination Of Ghost Erythrocyte Membrane Protein: This was determined according to the method of Lowry *et al.*[28] after solubilizing aliquots of ghost membrane suspension with 0.2% SDS. Bovine serum albumin (BSA) (50-300 µg) was used as standard. Absorbance was measured in spectrophotometer at 720 nm.

Statistical Analyses

Data are presented as Mean ± SEM and subjected to one way analysis of variance (ANOVA) and Tukey test using Graphpad prism version 6.0 for windows from GraphPad software, San Diego, California, USA. P values < 0.05 were regarded significant.

RESULTS

Effect of aqueous leaf extract of Aspilia africana on erythrocyte osmotic fragility: The osmotic fragility decreased as the concentration of NaCl increased in both control and treatment groups. However, percentage change was seen to be more pronounced in 500 and 750 mg/kg doses of the extract especially between 0.5-0.2 concentrations when compared with the control groups (Fig. 1).

Effect of aqueous leaf extract of *Aspilia africana* on erythrocyte membrane protein and Na^+-K^+ ATPase activity: The Mean ± SEM erythrocyte protein concentration of rats treated with 500 mg/kg and 750 mg/kg extracts were found to be significantly higher than control (5.88 ± 0.52 µg/mL; 5.01 ± 0.20 µg/mL vs 3.59 ± 0.21 µg/mL respectively) (Figure 2). Similarly, the extract caused 44.1% and 102% increase in the activities of rats erythrocytes total Na^+ K^+-ATPase at 500 mg/kg and 750 mg/kg respectively; while, an increase of 63% and 71.4% was observed in the ouabin sensitive ATPase activity (Figure 3).

DISCUSSION

The result of this study showed that varying doses of *Aspilia africana* could alter the strength or integrity of the red cell membrane in hypotonic solutions with NaCl content ranging from 0.1% - 0.9%. Osmotic fragility has always been used as a measure of tensile strength of the red cell membrane[29]. It was revealed that the osmotic fragility of the erythrocytes improved on treatment with aqueous leaf extract of *Aspilia africana* when compared to the non-treated erythrocytes *in vivo*. This may interpret that the plant has the ability of membrane stabilization even in stressful conditions. This is in agreement with other studies earlier reported on the plant[30-33]. It has been established that erythrocyte damage, which is manifest in high osmotic fragility exhibited by some medicinal plants, may be attributed to lipid peroxidation[34]. This is because it increases the susceptibility to oxidative stress thereby enhancing free radical attack on the erythrocyte membrane resulting in their damage[35]. But, interestingly, *Aspilia africana* possesses antioxidant activity which could counter any oxidative damage[36]. It is also known to possess flavonoids in large quantities[37]. which studies have shown to inhibit peroxidation and, consequently stabilizes the integrity of the red cell membrane against hypotonic lysis. Physiologically, sodium pump's involvement in diverse processes suggests that alteration of the pump activity by endogenous or xenobiotic factors may play a key role in many bodily activities, for example, modulation of cardiac contractility, control of sodium in the renal tubules, vascular contractility, neuromuscular release and processing[38]. Hence, this may underscore the link its dysfunction to a number of disorders including sickle cell disease, cardiovascular, renal, neurological, and metabolic disorders[39]. Studies have equally shown that modulation of the activity of Na^+ K^+ ATPase might prove helpful in the therapy of all these disorders[40]. A number of medicinal plants have been shown to increase the activity of Na^+ K^+ ATPase, especially in situations where there is abnormalities in cation transport like sickling[41,40]. And since the aqueous leaf extract of *Aspilia africana* has been previously reported to enhance erythropoiesis, the present work was aimed at investigating its possible effect on red cell membrane integrity through resistance to osmotic stress, and sodium pump activity. Na^+ K^+ ATPase activity was observed to be increased in erythrocytes treated with aqueous leaf extract of *Aspilia africana* compared to the non-treated erythrocytes (*in vivo*) in a dose dependent fashion. This increased activity may be attributed to the presence of high concentration of alkaloids in the aqueous leaf extract of *Aspilia africana.* Similar results have been reported in other plants with high concentration of alkaloids[40], also, the presence of high concentration of alkaloids had been reported to cause such a change[42]. Hence, it can be suggested, from the data obtained from the study, that aqueous leaf extract of *Aspilia africana* may have a magnified impact on regulating sodium-potassium pump in health and disease. From the observation of data in this study, it can be concluded that aqueous extract of *Aspilia africana* reduced the osmotic fragility of erythrocyte membrane and enhanced the activity of Na^+ K^+ ATPase. Therefore, this further emphasized the earlier works on the medicinal benefits of *Aspilia africana*, and its effects on red cell membrane stability.

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