

Aspilia africana Altered Haematological Parameters and Plasma Electrolytes in Rats (*Rattus norvegicus*)

Alaba Olumide Ojo^{1*}, Olajumoke Oludapo¹, Olatunbosun Olumuyiwa Onaseso¹, Lawrence Dayo Adedayo¹, Olufemi Idowu Oluranti¹, Emmanuel Olusegun Timothy¹, Bamidele Olubayode¹, Abiodun Ayoka^{1,2}

¹Department of Physiology, College of Health Sciences, Bowen University Iwo Nigeria

²Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, 220005, Ife, Nigeria

DOI: [10.36348/sjls.2020.v05i05.002](https://doi.org/10.36348/sjls.2020.v05i05.002)

| Received: 04.03.2020 | Accepted: 13.03.2020 | Published: 14.05.2020

*Corresponding author: Alaba Olumide Ojo

Abstract

Several medicinal plants have been documented for their effects on physiological processes in the body, among are their effects on haematological parameters and plasma electrolytes. Several medicinal plants have the ability to synthesize wide varieties of chemical compounds that perform important biological functions, and defend against attack from predators such as insects, fungi and herbivorous animals. They also contain several phytochemical compounds that can alter the physiological processes to varying extent in animals and human. **Objectives:** The aims of this study were to investigate the effects of ethanolic extract of *Aspilia africana* on some haematological parameters and plasma electrolytes. **Methods:** Twenty five albino male wistar rats were divided into five groups: group I(control, received 1ml/kg of distilled water), Group II(received 1ml/kg of 70% ethanol), While Groups III, IV & V were given 50mg/kg, 100mg/kg and 150mg/kg of the extract respectively. After two weeks of administration, blood samples were collected via cardiac puncture into sample bottles for haematological and plasma electrolytes analysis. **Results:** It was observed that there was significant increase in; red blood cell count, haemoglobin concentration, packed cell volume, total white blood cell count, platelet, neutrophil, eosinophil, monocyte, and lymphocyte of treated groups, mostly in group that was given 150mg/kg of the extract, while ethanol reduced haematological parameters. There were dose dependent increase in plasma sodium (88.52 ± 2.58 mEq/l), potassium (4.89 ± 0.20 mEq/l) and calcium (2.00 ± 0.15 mEq/l) compared respectively with control (Na- 71.70 ± 1.66 mEq/l, K- 3.19 ± 0.24 mEq/l and Ca- 1.41 ± 0.13 mEq/l). **Conclusion:** *Aspilia africana* showed positive haemopoietic effect and also increase plasma electrolyte concentration.

Keywords: Sodium, calcium, potassium, RBC, WBC.

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INTRODUCTION

The use of plants as medicines predates written human history. Ethnobotany is recognized as an effective way to discover future medicines. Fabricant and Farnsworth [1], reported that researchers have identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium. Angiosperms (flowering plants) were the original source of most plant medicines. Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, have medicinal properties [2, 3].

Aspilia africana is a genus of flowering plants in the daisy family. It is a semi woody herb occurring throughout the regions of the savannah and tropical Africa on wastelands [4]. It is known by various names among the Nigerian populace (*Orangila* in Igbo, *Tozalin* in Hausa and *Yunyun* in Yoruba). The plant has been reported in literature to possess antimicrobial, haemostatic, anti-inflammatory [5] and anti-fertility [6] activity, healing and anti-ulcer activity [5] in certain part of Nigeria leaves of this plant has been used in treatment of stomach ache and bleeding gastric ulcers [7]. Leaf extract and fractions of *A. africana* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerated wound healing process. *Aspilia* is thought to be used as herbal medicine by some chimpanzees [8].

Researches have shown that *Aspilia africana* has anti-ulcer effect, anti-malaria effect, anti-microbial effect and also has significant effects on the reproductive, cardiovascular systems and on wound healing. Phytochemical analysis of the plant reveals that it has a high oil and *protein* content, it is also rich in saponin, tannins, glycoside and alkaloids [4]. Adeniyi and Odufowora [9] and Iwu [10], reported the presence of four essential oils obtained by hydrodistillation of the leaves of *Aspilia africana*. However, there is dearth of information on the effect of the leaf of *Aspilia africana* on haematological parameters and plasma electrolytes.

Factors that affect blood and plasma constituents, will consequently affect different body functions. Alteration in packed cell volume, plasma electrolytes like Na⁺, K⁺, and Ca²⁺ and plasma lipid profile may alter cardiovascular function; such changes in cardiac output, blood pressure or vascular tone may predisposes to cardiovascular dysfunction [11]. Therefore the aim of this work were to examine the effects of *Aspilia africana* on haematological parameters and plasma electrolytes.

METHODOLOGY

Experimental Animals

Twenty five (25) male albino rats weighing between 150-200g were purchased from the Department of Biochemistry, Bowen University, Iwo, Osun state and were acclimatized in the Animal House, College of Health Sciences, Bowen university for four weeks under ambient condition. The rats were given feed and water ad libitum. The extract was administered for two weeks at different doses. Body weights were measured before and at the end of two weeks before collection of blood sample through cardiac puncture, the rats were anaesthetised by inhaling chloroform in a fume box. Samples were collected into lithium heparinised sample bottles and centrifuged for electrolyte analysis while EDTA sample bottles were used to collect samples for haematological analysis

Preparation of ethanolic extract of *Aspilia africana*

A bulk of fresh leaves of *A. africana* sufficient for the study was collected from the field in Bowen University during dry season. It was then authenticated in the Botany Department of the University of Ibadan. The authentication number is 22395. The leaves were air-dried and grounded into fine powder using a small electric blender.

100g of the plant powder were weighed using metler weighing balance and mixed in 714ml of 70% ethanol. The homogenate was stirred with a stirrer for about thirty minutes and then transferred into a clean conical flask. The mixture was left for 48 hours. After this period, the mixture was sieved first with a cheese cloth and then with a whatmann no. 1 filters paper into a sterile container. The filtrate which is an amber green solution was transferred into a clean beaker and placed

in a hot water bath containing water boiling at 100^oc. This was left until the whole of the ethanol evaporated leaving a paste-like extract of *Aspilia africana*. About 13g of paste was extracted and this was diluted with 100ml of distilled water giving a concentration of 0.13g/ml. It was then transferred into sterile McCartney bottles and stored in the refrigerator for daily administration to the rats.

Administration of plant extract

The rats were divided into five groups with each group consisting of five rats. Group I (control) was given 1ml/kg of distilled water, Group II was administered with 1ml/kg of 70% ethanol,(served as the second control group). Group III, Group IV and Group V were given 50mg/kg, 100mg/kg and 150mg/kg of the extract respectively, via oral gavages using a metal oropharyngeal cannula and calibrated hypodermic syringe.

Analysis of Blood Sample

Red Blood Cell Count

Red blood cell count was determined using the haemocytometer [12]. The red cell diluting pipette was used to draw blood to the 0.5 mark. The pipette was kept horizontally to prevent blood shifting. The outside of the pipette was wiped to remove any blood. Then, the blood was diluted with Haymen solution up to the 101 mark on the pipette to prevent coagulation. The clean cover slip was then fixed to the surface of the counting chamber. The tip of the pipette was brought into contact with the exposed part of the counting chamber and it was raised until the diluted blood flows under the cover slip the counting chamber was then placed on the stage of the microscope.

Using the (x40) objective lens, the cells could be seen in the squares and the corpuscles of the small 80 squares were counted.

Total White Blood Cells Count

The white bead pipette was used to draw the rat's blood to the 0.5mark. The pipette was kept horizontally to prevent blood shifting. Then, the blood was diluted using Turk's solution up to the 11 mark on the pipette. The clean cover slip was fixed to the surface of the counting chamber. The tip of the pipette was brought in contact with the exposed part of the counting chamber and it was raised until the diluted blood flows under the cover slip. The counting chamber was placed on the stage and using (x4) objective lens, the white blood cells were counted in each of the boundary squares

Differential white blood cells count

A drop of blood was applied on one end of the slide and another slide was placed at the end of the previous slide at an angle of 45^o, holding with the thumb and index finger until the blood spread across it. It was drawn slowly over the whole length of the first

slide to create a blood smear. Then, the blood was dried by being waved rapidly in the air to prevent undue shrinkage of the cells it was then stained with leishmans stain. After two minutes, an equal quantity of distilled water was added and left for ten minutes. The mixture was poured off and the film was washed off in a gentle stream of water. It was then dried with filter paper, and examined under low and high objective [12].

Determination of packed cell volume

The rat's blood to be determined is drawn up into the heparinized capillary tubes until about three-quarter of the tube. The outside of the tube was then cleaned with cotton wool to remove blood. The ends of the capillary tubes were closed with plasticine. These tubes were then fixed into the microhaematocrit centrifuge and spun for five minutes at a speed of 3000rpm. The haematocrit was then immediately read using a micro-haematocrit reader and the result expressed as percentages [12].

Estimation of Haemoglobin

Haemoglobin concentration was estimated using the Sahli acid haematin method, as described by Cheesbrough [12], 0.02 ml of blood was mixed in a dilution tube containing 0.1 mol/l hydrochloric acid (HCL) which converts the haemoglobin to acid haematin. After 10 minutes of reaction time, 0.1 mol/l HCl was added drop by drop, with mixing, until the colour of the solution (haemolysed blood) matches the colour of the permanent coloured glass comparison standard positioned alongside the dilution tube in bright diffuse day light with a sheet of white paper as background. The concentration of haemoglobin was read from the graduation at the bottom of the meniscus on the dilution tube.

Estimation of Mean Cell Haemoglobin Concentration

This is calculated by dividing the haemoglobin concentration in (g/dl) by the haematocrit value in percentage. The mean cell haemoglobin concentration is expressed as a decimal fraction.

Determination of Plasma Sodium Level

This was done using the flame photometry method. 1ml of plasma was treated with 3ml of dilute hydrochloric acid (2ml concentrated hydrochloric acid+ 1ml distilled water). The mixture was then centrifuged in a macrohaematocrit centrifuge for twenty minutes at a speed of 3000rpm. The supernatant was then decanted for analysis.

Standard solution of known concentration of (0, 10, 25, 50, 100) ppm was measured using the flame photometer and a standard curve was plotted. Then, each of the samples of the supernatant was measured and the slope obtained from the curve was used to calculate the actual concentration of sodium in each sample.

Determination of Plasma Potassium Level

It uses the same method as the determination of plasma sodium level. However, standard solution for potassium measured are at the following concentration; (0, 20, 40, 80, 100) ppm. A standard curve was obtained from the actual concentration of these standard solutions and the slope was used to calculate the actual concentration of potassium in each sample.

Determination of Plasma Calcium Level

This makes use of Atomic Absorbent spectrophotometer (AAS). For the plasma calcium to be determined, firstly, the gas supplying the spectrophotometer is switched on and so also the computer. The element calcium is selected and so also the wavelength was 422.7nm. As the nebulizer sucks the sample, the concentrations are being recorded on the computer. The results was saved and printed out.

STATISTICAL ANALYSIS

Data were presented as mean \pm SEM and analysed using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-20.0. P values < 0.05 were considered to be significant.

RESULTS

Haematological Parameters' Result

The results of the analysis of the haematological parameters; Red blood cells count, haemoglobin, packed cell volume, mean cell haemoglobin concentration, platelet, neutrophil, eosinophil, monocyte and lymphocyte are shown in Figures 1 and 2.

The red blood cell count in the experimental groups appeared to be significantly higher in groups 4 and 5 when compared to the normal control group I ($5.30 \pm 0.10 \times 10^6$ cu.mm) ($p > 0.05$) but it was insignificantly higher in group 3 when compared to the control group (Fig-1). Total white blood cells count showed a significant increase in groups 3 and 4 ($p < 0.05$) and 5 ($p < 0.01$) and showed a significant decrease in group 2 ($p < 0.05$) compared to the normal control group. ($6.38 \pm 0.30 \times 10^3$ cu.mm) (Fig-1).

The haemoglobin in the experimental group appeared to be significantly higher in groups 3,4&5 compared to the control group. Whereas, the haemoglobin in the ethanol control group appeared to be significantly lower compared to control group (13.20 ± 0.42 g/dl) ($p < 0.05$) (Fig-2). Packed cell volume in the experimental groups appeared to be significantly higher in groups 4 and 5 when compared to the control group (41.60 ± 0.93 %). Packed cell volume in the ethanol control group appeared to be insignificantly higher than in the control group) (Fig-2).

Mean cell haemoglobin concentration appeared to be significantly lower in group 5 and ethanol group compared to the control group (31.83 ± 1.47 , $p > 0.05$) (Fig-2). Platelet count in groups 3, 4 and 5 appeared to be significantly higher when compared to the normal control group. ($269.20 \pm 62.90 \times 10^3$ cu.mm) (Table-1). Neutrophil, eosinophil, monocyte and lymphocyte appeared higher in experimental group compared to the normal control group. Neutrophil

appeared significantly higher in groups 4 and 5 when compared to the normal control group ($3.76 \pm 0.27 \times 10^3$ cu.mm). Monocyte appeared significantly higher in group 5 when compared to the normal control group ($0.46 \pm 0.30 \times 10^3$ cu.mm). Lymphocyte appeared significantly lower in group treated with ethanol and significantly higher in groups 4 and 5 when compared to the control group ($2.08 \pm 0.81 \times 10^3$ cu.mm) (Table-1).

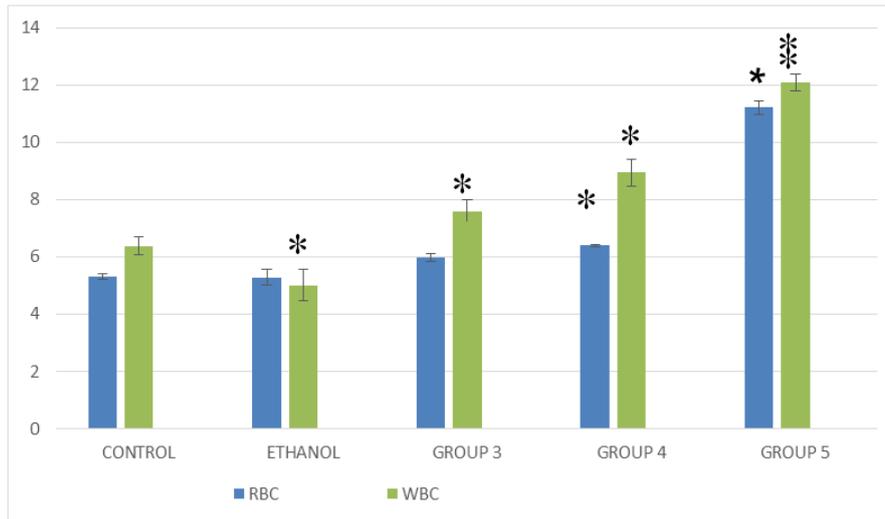


Fig-1: Bar chart shows the mean values of red blood cells and Total white blood cells with standard error of mean for all groups. ($p < 0.05$). RBC: red blood cells; WBC: white blood cells

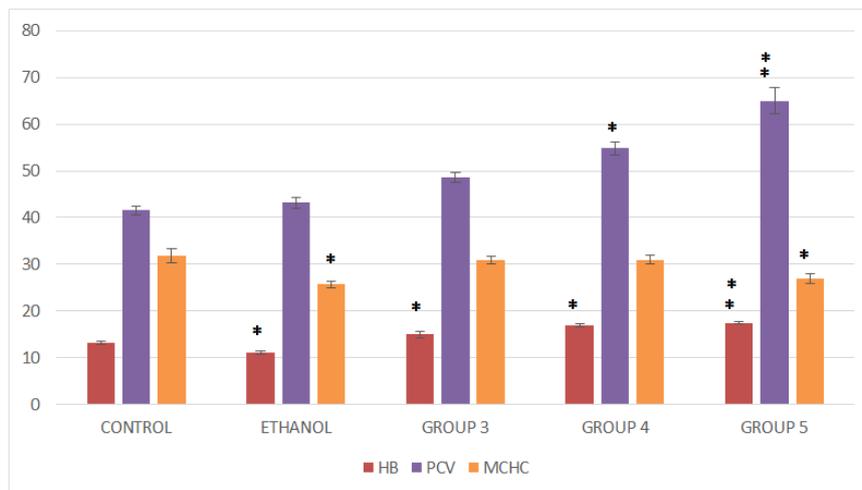


Fig-2: Bar chart shows the Mean values of Haemoglobin (HB), Packed cell volume (PCV) and mean cell haemoglobin concentration (MCHC) \pm standard error of mean

Table-1: Showing mean values of neutrophil, eosinophil, lymphocyte and monocyte and platelet \pm standard error of mean of all groups ($p < 0.05$)

	NEUTROPHIL ($\times 10^3$ cu.mm)	EOSINOPHIL ($\times 10^3$ cu.mm)	MONOCYTE ($\times 10^3$ cu.mm)	LYMPHOCYTE ($\times 10^3$ cu.mm)	PLATELET ($\times 10^4$ cu.mm)
CONTROL	3.76 ± 0.27	0.13 ± 0.03	0.46 ± 0.81	2.08 ± 0.14	26.92 ± 62.90
ETHANOL	3.16 ± 0.19	1.00 ± 0.00	0.30 ± 0.71	1.46 ± 0.19	$108.20 \pm 19.80^*$
GROUP 3	4.3 ± 0.28	0.18 ± 0.25	0.50 ± 0.10	2.66 ± 0.15	$567.80 \pm 22.29^*$
GROUP 4	$4.86 \pm 0.36^*$	0.18 ± 0.48	0.70 ± 0.11	$3.24 \pm 0.30^*$	$588.00 \pm 37.27^*$
GROUP 5	$6.68 \pm 0.17^*$	0.25 ± 0.50	$1.30 \pm 0.13^*$	$3.96 \pm 0.29^*$	$649.80 \pm 45.45^*$

Table-2: The results of the analysis of the level of plasma electrolytes

	SODIUM (mEq/L)	POTASSIUM (mEq/L)	CALCIUM (mEq/L)
CONTROL	71.70 ± 1.66	3.19 ± 0.24	1.41 ± 0.13
ETHANOL	91.73 ± 3.09*	4.04 ± 0.21*	1.67 ± 0.13
GROUP 3	45.87 ± 3.55*	2.98 ± 0.37	1.81 ± 0.38
GROUP 4	78.99 ± 5.72	4.50 ± 0.09*	1.56 ± 0.15
GROUP 5	88.52 ± 2.58*	4.89 ± 0.20*	2.00 ± 0.15*

*means P≤0.05

Plasma Electrolytes

The results of the analysis of the level of plasma electrolytes are shown on Table-2.

The sodium concentration of the treated group appeared to be higher in group 4 and 5 when compared to the normal control group. However, it was significant in group 5. Ethanol control group showed a significant increase when compared to the normal control group (71.70 ± 1.66 mEq/L) (p<0.05). Ethanol control group showed the highest increase and group a showed a decrease. The potassium concentration in the experimental groups showed an increase when compared to the control group (3.19 ± 0.24 mEq/L). The calcium concentration also showed an increase in the experimental groups when compared with the control group (1.41 ± 0.13mEq/L).

Clinical observation of the rats

The weight of the rats in all the five groups were taken before and after the administration of the plant extract. After the administration, there was an increase in weight in the group that received no extract but received 1ml of distilled water for two weeks. The group that received 1ml of ethanol showed a decrease in weight from (205.80 ± 11.76 to 180.60 ± 3.97) after the administration. The groups that received the plant extract showed a significant decrease in weight after the administration. Groups 4 and 5 showed the greatest amount of weight loss when compared to the other groups.

DISCUSSION

Haematological analysis is a useful process in the diagnosis of many diseases as well as investigation of the extent of damage to the blood [13]. Haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding [14] drugs haematopoietic activity as well as the status of the bone marrow. While unregulated alteration of the plasma electrolyte may affect the of some vital organs and systems in the body

The purpose of this research work was to determine the effects of oral administration of *A. Africana* at different concentrations on some haematological parameters and plasma electrolytes in rats. The varied level of significance noticed in the haematological parameters evaluated in this study (post-administration) between the control and the test

groups, and even within the test groups shows that there is an obvious connection between the extracts (at the various concentrations tested) and the haematological parameters and plasma electrolytes level. The outcome of this present study shows that extract of *A. africana*, particularly at 150mg/kg concentration, has significant impacts on the various haematological parameters investigated as evident in the active proliferations of blood components to varied extent as measured in the test groups, compared to the control. However, the extract of *A. africana* has a slight increasing effect on plasma electrolytes and this can be attributed to its possession of some physiochemical elements or due to certain mechanism that needs to be elucidated. The appreciable increase in the values of the haematological parameters investigated may be associated with the inherent-haematopoietic-stimulating properties possessed by the extract of *A. africana*.

Haematological parameters are particularly important for the diagnosis of anaemia in humans and most animals. The values obtained in this current study for haematocrit, haemoglobin, red blood cells and mean cell haemoglobin concentration seem to be within the normal range as reported by some researchers [15, 16], suggesting that the animals were not anaemic. Besides, the test groups had higher values for these parameters than the control, which indicates that a more efficient erythropoiesis occur following administration of the extracts. Though, Etim and Oguike [17] recorded no significant difference in the haematological parameters of non-pregnant, pregnant and lactating rabbits fed with *A. africana*.

According to the results of this experiment, groups 3, 4 and 5 showed a significant increase in red blood cells compared to the normal control group (p<0.05). Group 4 showed highest significance compared to the normal control group. This shows that the dosage of 150mg/kg has the greatest effect on haemopoiesis compared to the other dosage. At this dosage, there was an increase in red blood cell count that almost double that of the control group. This shows that the effect *A. Africana* on erythrocytes is dose dependent

Groups 3, 4 and 5 showed a significant increase in total white blood cells count compared to the normal control group. Group 5 however showed the greatest increase. This indicates that the extract of *A. africana* contains agents that stimulate an increase in

white blood cells production, which is necessary for development of immunity against infection and foreign bodies.

The haemoglobin concentration of groups 2, 3, 4 and 5 showed a significant increase when compared to that of the normal control group (13.2 ± 0.42 g/dl). Ethanol control group showed a significant decrease in haemoglobin when compared with the control group. This shows that ethanol has adverse effect on haemoglobin.

Packed cell volume in Groups 3, 4 and 5 showed a significant increase when compared to that of the normal control group (41.60 ± 0.93 %), the greatest increase was observed in group 5 that received the highest dosage (150mg/kg). The mean cell haemoglobin concentration in the ethanol group showed a significant reduction compared to the normal control group (31.83 ± 1.47 %). However, significant reduction in mean cell haemoglobin concentration was observed groups 5.

Platelet count of the ethanol group showed an insignificant reduction compared to the control group (269.20 ± 62.90). Groups 3, 4 and 5 showed a significant increase in platelet compared to the control group with group 5 showing the greatest significance.

The number of neutrophil in groups 4 and 5 were significantly higher than the normal control group ($3.76 \pm 0.27 \times 10^3$ cu.mm) with group 5 showing the greatest increase. Groups 4 and 5 also showed a significant increase in the number of lymphocytes showed a significant increase when compared to the normal control group ($2.08 \pm 0.14 \times 10^3$ cu.mm). The result of this study corroborated the observation of Taziebu *et al.*, [18] who reported significant increase in RBC, WBC, and platelet of rats given 500mg/kg of *A. Africana* extract for 28days, though toxicity was reported at higher dose. Also, Ajeigbe et al., [19] reported similar observation which corroborate the findings of this study

There was a significant increase in potassium concentration in group 4 and 5 compared to the normal control group (3.19 ± 0.24 mEq/L). However, there was an insignificant increase in the ethanol control group compared to the control group. The increase in plasma potassium levels may be attributed to the presence of potassium as a physiochemical element in *A. Africana*. Physiochemical analysis of *A. africana* shows the presence of potassium at a concentration of 13,800.37 ppm [20].

There was a significant increase in sodium concentration of group 2 and 5 when compared to the control group (71.70 ± 3.71 mEq/L). However, the greatest increase was with the ethanol group (I. e group 2). This indicates the effect of ethanol in increased plasma and renal sodium retention. Ethanol however

has a great diuretic effect. There was a significant reduction in sodium concentration in group 3 when compared to the control group. Though there is no previous study to corroborate this observation, it can be deduced that effect of *Aspilia Africana* is dose dependent

The experimental groups 3, 4 and 5 showed an increase in calcium concentration compared to the normal control group (1.41 ± 0.13 mEq/L). However, it was only group 5 treated with the dosage of 150mg/kg that showed a significant increase. This increase in plasma calcium level in the experimental group may be attributed the presence of calcium element at a concentration of 11,550.65ppm in the plant [20]. Etim and Oguike [17], discovered similar increase in plasma calcium of lactating rabbit fed with *A. africana*. It has been reported by Ojo and colleagues Ojo *et al.*, [11] that unregulated alteration of plasma electrolytes may lead cardiovascular dysfunction.

CONCLUSION

Observation from this study showed that *A. africana* has dose dependent positive haemopoietic effect. The effect on plasma electrolytes shows that the plant extract may have impact on electrolyte transport, irrespective of individual concentration of each electrolyte in the extract.

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