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HAEMATOLOGICAL PROFILE IN SALT LOADED EXPERIMENTAL RABBITS TREATED WITH ETHANOL EXTRACT OF ACALYPHA WILKESIANA LEAVES

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ABSTRACT

This study evaluates the haematological profile of salt loaded rabbits treated with *Acalypha wilkesiana* ethanolic leaf extract. Twenty-four rabbits were randomized into four groups (A - D) of six animals each. Group A-C were fed salt-loaded diet for 35 days, subsequently group B were treated with extract for 7 days. Group C received the salt-loaded diet until the 42nd day, while group A received normal feed in place of salt-loaded diet for 7 days. Group D were fed normal feed only. Blood was collected on days 35 and 42 for analysis. There was significant ($p < 0.05$) increases in total white cell count and neutrophil count, significant ($p < 0.05$) decreases in lymphocyte count, monocytes count (MC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration and platelets count. However, no significant ($p > 0.05$) effect in packed cell volume (PCV), haemoglobin concentration (HC), red cell count, and mean corpuscular haemoglobin. Administration of extract at 250 mg/kg body weight, caused significant ($p < 0.05$) increases in PCV, MC, HC, and a significant ($p < 0.05$) decrease in MCV. *Acalypha wilkesiana* may be safe, as its administration did not trigger immune response in the animals.

INTRODUCTION

Excessive intake of sodium chloride has long been suspected to increase blood pressure.[1] It also increases the number of strokes, the severity of cardiac failure and the tendency for platelets to aggregate.[2] Herbal medicine, also called botanical medicine or phytomedicine refers to the use of plants or plant parts for medicinal purposes. Herbal medicine is used in the management of many conditions such as cardiovascular diseases, hypertension and cancer, among others. In the treatment of hypertension, one of the plants that are commonly used is *Acalypha wilkesiana*.[3] It belongs to the family, Euphorbiaceae (spurge family). Its common names are; copper leaf, Joseph's coat, fire dragon and beef steak plant.[4] The preliminary phytochemical screening of the leaves of *A. wilkesiana* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins, all of which have potential health promoting effects, at

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least under some circumstances.[5][6] Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. In Nigeria, the cold extracts of the leaves is used to bath babies with skin infection.[7] Its leaf extract is active against Gram positive bacteria, the extracts of the seed have been reported by Bussing *et al*., [8] to have immune-modulating properties which work against some tumours. In this study, the haematological profile of salt loaded experimental rabbits, treated with ethanol extract of *A. wilkesiana* leaf was investigated.

MATERIALS AND METHODS

Experimental Animals

Twenty-four (24) rabbits of the New Zealand strain were used for the study. The animals were housed in clean disinfected cages in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-hour light and dark cycle. They were allowed free access to feed (standard pelletized growers feed from UAC- Vital Feed, Jos, Plateau State) and water throughout the duration of the experiment. The animals were allowed to acclimatize to the new environment for a period of three (3) weeks. They were then randomized into four (4) groups of six (6) animals each (i.e. groups A - D).

Experimental design

The experimental animals were randomized into four groups (A to D) and treated as follows;

Group A: salt-loaded and non-treated

Group B: salt-loaded and treated with ethanol extract of A. wilkesiana leaves.

Group C: Continuous Salt-loading

Group D: Non-loaded and Non-treated (Control)

The animals in Groups A, B, and C were fed with the salt-loaded diet for a period of five (5) weeks (35 days), after which group B animals were treated with ethanol extract of *A. wilkesiana* for a period of seven (7) days (i.e. till the forty-second day). Group C animals were given the salt-loaded diet continuously until the forty-second (42^{nd}) day, while group A animals were given normal feed in place of the salt-loaded diet for seven (7) days (i.e. till the forty-second day) . Group D was neither given salt-loaded diets nor treated with the extract (Control).

Preparation of salt-loaded diet

The salt-loaded diet (8%NaCl) was prepared by mixing eight grams (8g) of analytical NaCl (from BDH Chemicals, England) with ninety-two grams (92g) of the feed.

Plant Materials

Fresh *Acalypha wilkesiana* leaves were obtained from local gardens within Igbinedion University, Okada. The leaves were properly washed, air-dried and ground into fine powder.

Preparation of Ethanol extract

Two hundred (200g) of the powdered leaves was soaked in 800ml of ethanol for 48 hours (2 days), after which the mixture was filtered using a sintered funnel (which is equivalent to four folds of cheese cloth). The filtrate (extract) was dried using a rotary evaporator, weighed and a known amount suspended in distilled water for administration to the experimental animals.

Extract administration

The extracts were administered orally at a dose of 250mg/kg body weight.

Collection of blood sample
Three (3) ml of blood sample was collected from the ear lobe of the rabbits using a 5ml syringe on days 35 and 42 respectively. The blood samples were stored in EDTA bottles and used for haematological analysis.

Haematological Analysis and Principles
Haematological analysis was carried out using the Abacus Junior Hematology Analyzer S/N 111749 P/D 02/2009, Diatron, GmbH, Wein Austria. Blood cells were diluted in a buffered electrolyte solution. A measured volume of the sample was passed through an aperture tube between the two electrodes. Interruption of the current by the non-conducting blood cells altered the electrical charge and a pulse was produced. The amplitude of each pulse was proportional to the volume of the cell which caused it. A threshold circuit ensured only those pulses that exceeded the pre-set threshold level were counted. The cell count was determined from the total number of pulses obtained from a measured volume of blood.

Statistical Analysis of Data
Data are represented as Mean ± S.E.M (n = 6). Significance of Difference was tested by Student t-Test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical Significance was set at $P < 0.05$.

RESULTS
The effects of ethanol extract of Acalypha wilkesiana leaf on the haematological parameters of salt loaded rabbits are as indicated in the tables below;

TABLE 1: Blood Packed Cell Volume (%) and Haemoglobin Concentration (g/dl) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Packed Cell Volume (PCV) (%)		Haemoglobin Concentration (HC) (g/dl)	
	35 (Days)	42 (Days)	35 (Days)	42 (Days)
A (Salt + No Ext)	34.83 ± 0.75^{ax}	33.00 ± 1.13^{ax}	11.85 ± 0.31^{ax}	11.43 ± 0.14^{ax}
B (Salt + Et. Ext)	34.17 ± 0.95^{ax}	41.50 ± 1.46^{by}	11.67 ± 0.38^{ax}	13.77 ± 0.35^{by}
C (Cont. Salt)	33.17 ± 0.75^{ax}	33.50 ± 0.92^{ax}	11.32 ± 0.21^{ax}	11.52 ± 0.21^{ax}
D (Control)	36.33 ± 1.50^{ax}	39.00 ± 1.13^{bx}	12.30 ± 0.42^{ax}	13.02 ± 0.22^{bx}

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b}$ superscripts, along column, are significantly different ($P < 0.05$). Means with different letter $^{x, y}$ superscripts, along row are significantly different ($P < 0.05$).

Salt loading resulted in non-significant ($P > 0.05$) decreases in blood packed cell volume (%) and haemoglobin concentration (g/dl) of the experimental animals (groups A, B and C), as compared with the control group (group D), after day

35 of salt loading (Table 1). After a week of treatment with ethanol extract of A. wilkesiana, at a dose of 250mg/kg body weight, the ethanol extract resulted in significantly ($p < 0.05$) higher packed cell volume and haemoglobin concentration (group B) as compared with the other groups (A and C) given salt load.

TABLE 2: Blood Red cell Count (10^9 cells/l) and Blood Total White Cell Count (10^9 cells/l) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Red Cell Count (RCC) (10^9 cells/l)		Blood Total White Cell Count (TWCC) (10^9 cells/L)	
	35 (Days)	42 (Days)	35 (Days)	42 (Days)
A (Salt + No Ext)	4.09 ± 0.09^{ax}	4.01 ± 0.05^{ax}	6.15 ± 0.35^{ax}	5.88 ± 0.23^{ax}
B (Salt + Et. Ext)	4.02 ± 0.11^{ax}	4.33 ± 0.05^{ax}	6.17 ± 0.45^{ax}	6.00 ± 0.08^{ax}
C (Cont. Salt)	3.90 ± 0.83^{ax}	4.03 ± 0.03^{ax}	6.13 ± 0.62^{ax}	6.10 ± 0.47^{ax}
D (Control)	4.24 ± 0.15^{ax}	4.28 ± 0.05^{ax}	5.33 ± 0.35^{bx}	5.82 ± 0.17^{ax}

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with letter $^{x, y}$ superscripts, along row are significantly different ($p < 0.05$)

In table 2, salt loading or treatment with ethanol extract of *A. wilkesiana* leaves, at a dose of 250mg/kg body weight, resulted in no significant decrease or increase, respectively, on red blood red cell count (10^9 cells/l) of the experimental animals, as compared with the control.

Salt loading resulted in significant ($p < 0.05$) increases in blood total white cell count (10^9 cells/l) of the experimental animals (groups A, B and C), as compared to those not given salt load (group D), after 35 days (Table 2). However, treatment with *A. wilkesiana* leaf extracts resulted in no significant difference in red cell count (group B) as compared with the other salt loaded groups (groups A and C).

TABLE 3: Blood Neutrophil Count (%) and Blood Lymphocyte Count (%) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Neutrophil Count (NC) (%)		Blood Lymphocyte Count (LC) (%)	
	35 (Days)	42 (Days)	35 (Days)	42 (Days)
A (Salt + No Ext)	73.00 ± 2.94^{ax}	71.50 ± 1.63^{ax}	24.17 ± 2.34^{ax}	25.33 ± 1.94^{ax}
B (Salt + Et. Ext)	70.33 ± 4.58^{ax}	70.33 ± 3.40^{ax}	26.33 ± 3.82^{ax}	24.33 ± 2.54^{ax}
C (Cont. Salt)	69.80 ± 4.14^{ax}	68.30 ± 3.69^{ax}	28.00 ± 3.85^{ax}	27.83 ± 2.97^{ax}
D (Control)	54.30 ± 4.77^{bx}	58.70 ± 4.36^{bx}	40.50 ± 4.05^{bx}	37.83 ± 4.07^{bx}

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with letter $^{x, y}$ superscripts, along row are significantly different ($p < 0.05$).

In table 3, salt loading resulted in significant ($p < 0.05$) increases in blood neutrophil count (%) of the experimental animals (groups A, B and C), as compared with the control (group D). However, treatment with *A. wilkesiana* leaf extracts resulted in no significant difference ($p > 0.05$) in blood neutrophil count (%) (Group B) as compared with the other salt loaded groups (groups A and C). Also, at day 42, the experimental groups showed blood neutrophil count (%) that were significantly ($p < 0.05$) higher than that of the control. Salt loading also resulted in significant ($p < 0.05$) decreases in blood lymphocyte count (%) of the experimental animals given salt loaded diets (groups A, B and C), as compared to the control (group D), after 35 days. At day 42, after treatment with *A. wilkesiana* leaf extracts, there was a non-significant ($p > 0.05$) decrease in blood lymphocyte count (%) of the treated group (group B).

TABLE 4: Blood Monocyte, Eosinophil and Basophil Count (%) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Monocyte Count (MC) (%)		Blood Eosinophil Count (EC) (%)		Blood Basophil Count (BC) (%)	
	35 (Days)	42 (Days)	-	-	-	-
A (Salt + No Ext)	3.00 ± 0.77^{ax}	3.67 ± 0.76^{ax}	-	-	-	-
B (Salt + Et. Ext)	3.33 ± 0.84^{ax}	5.33 ± 0.95^{by}	-	-	-	-
C (Cont. Salt)	2.50 ± 0.56^{ax}	3.83 ± 0.75^{ax}	-	-	-	-
D (Control)	5.83 ± 1.35^{bx}	4.00 ± 0.77^{ax}	-	-	-	-

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with letter $^{x, y}$ superscripts, along row are significantly different ($p < 0.05$).

In table 4, salt loading resulted in significant ($p < 0.05$) decreases in blood monocyte count (MC) (%) of the experimental animals given salt loaded diets (groups A, B and C), as compared to the control (group D), after 35 days. However, at day 42, treatment with *A. wilkesiana* leaf extract resulted in a significant ($p < 0.05$) increase in blood monocyte count (MC) (%) of the treated group (group B). Eosinophils and basophils were absent in all the groups.

TABLE 5: Blood **Mean Corpuscular Volume** (fl) and Blood **Mean Corpuscular Haemoglobin** (pg/cell) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Mean Corpuscular Volume (MCV) (fl)		Blood Mean Corpuscular Haemoglobin (MCH) (pg/cell)	
	35 (Days)	42 (Days)	35 (Days)	42 (Days)
A (Salt + No Ext)	73.33 ± 2.93^{ax}	73.00 ± 1.69^{ax}	3.93 ± 0.15^{ax}	3.83 ± 0.17^{ax}
B (Salt + Et. Ext)	72.33 ± 4.08^{ax}	70.00 ± 3.72^{by}	3.73 ± 0.13^{ax}	3.88 ± 0.14^{ax}
C (Cont. Salt)	71.50 ± 2.73^{ax}	72.33 ± 3.07^{ax}	3.60 ± 0.26^{ax}	3.83 ± 0.26^{ax}
D (Control)	80.67 ± 4.39^{bx}	79.83 ± 2.48^{cx}	4.15 ± 0.25^{ax}	4.17 ± 0.10^{ax}

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b, c}$ superscripts, along column, are significantly different ($p < 0.05$). Means with letter $^{x, y}$ superscripts, along row are significantly different ($p < 0.05$).

In table 5, salt loading resulted in significant ($p < 0.05$) decreases in Blood Mean Corpuscular Volume (MCV) (fl) of the experimental animals given salt loaded diets (groups A, B and C), as compared to the control (group D), after 35 days. At day 42, treatment with *A. wilkesiana* leaf extract resulted in a significant ($p < 0.05$) decrease in blood mean corpuscular volume (MCV) (fl) of the treated group (group B). Salt loading and treatment with ethanol extract of *A. wilkesiana* leaf, also, resulted in no significant difference ($p > 0.05$) in blood mean corpuscular haemoglobin (MCH) (pg/cell) of the experimental animals (groups A, B and C) as compared with the control animals (group D).

TABLE 6: Blood **Mean Corpuscular Haemoglobin Concentration** (g/dl) and Blood **Platelet Count** ($\times 10^4$ cells/mm^3) of salt-loaded rabbits; salt-loaded and non-treated (A), salt-loaded and treated with ethanol extracts of *A. wilkesiana* leaves (B), continuous salt loading (C), non-loaded and non-treated (D).

GROUP	Blood Mean Corpuscular Haemoglobin Conc.(MCHC)g/(dl)		Blood PlateletsCount(PC) ($\times 10^4$ cells/mm^3)	
	35 (Days)	42 (Days)	35 (Days)	42 (Days)
A (Salt + No Ext)	32.50 ± 1.54^{ax}	32.00 ± 1.34^{ax}	179.67 ± 8.23^{ax}	180.67 ± 6.16^{ax}
B (Salt + Et. Ext)	31.67 ± 1.50^{ax}	31.00 ± 0.45^{ax}	178.00 ± 13.25^{ax}	177.67 ± 14.25^{ax}
C (Cont. Salt)	31.33 ± 1.50^{ax}	33.00 ± 2.18^{ax}	172.00 ± 16.66^{ax}	168.83 ± 15.48^{ax}
D (Control)	36.00 ± 2.30^{bx}	39.50 ± 2.25^{bx}	199.17 ± 11.69^{bx}	184.5 ± 7.86^{bx}

Data represents Mean ± S.E.M (n = 6). Means with different letters $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with letter $^{x, y}$ superscripts, along row are significantly different ($p < 0.05$).

After 35 days of salt loaded diets, the blood mean corpuscular haemoglobin concentration (g/dl) and blood platelet count ($\times 10^4$ cells/mm^3) of the experimental animals (groups A, B and C), were significantly ($p < 0.05$) lower than that of the control (group D). Treatment with ethanol extract of *A. wilkesiana* leaf, for one week, resulted in no significant difference in mean corpuscular haemoglobin concentration and blood platelet count ($\times 10^4$ cells/mm^3) of the treated group (group B) (Table 6).

DISCUSSION

Haematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health.[9] Assessment of haematological parameters can be used to determine the extent of poisonous effect of foreign compounds including plant extracts on the blood constituent of an animal.[10] It can also be used to explain blood related functions of chemical compounds and plant extract.[11] In this study, salt loading resulted in significant ($p < 0.05$) increases in blood total white cell count (TWCC) and neutrophil count (NC), and significant ($p < 0.05$) decreases in blood lymphocyte count (LC), monocytes count (MC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelets count (PC). Some diseases trigger a response by the immune system and cause an increase in the number of WBCs. Other conditions affect the production of WBCs by the bone marrow or the survival of WBCs in the circulation, resulting in either an increase or decrease in the number of circulating WBCs. [12] Damage or inflammation of tissues, sudden kidney failure and stress can cause a high neutrophil count. [13] This may also result from excess salt in the diet as shown from the effects of salt loading on these parameters.

However, salt loading resulted in no significant ($p > 0.05$) effect in blood pack cell volume (PCV), haemoglobin concentration (HC), red cell count (RCC), and mean corpuscular haemoglobin (MCH). Haemoglobin, red blood count (RBC) and packed cell volume (PCV) are associated with the total population of red blood cells.[14] They are considered as an integral part of a person's complete blood count results. These were however not affected by the excess salt in the diet of the experimental animals, as indicated.

There was a significant increase in the PCV and haemoglobin concentration of animals treated with ethanol extract of *A. wilkesiana* leaf (group B) when compared to that of the control group (D), the group given continuous salt (C) and the non-treated group (A). The significant increase in PCV and haemoglobin concentration of the treated group (B) in day forty-two (42) when compared to day thirty-five (35) could be due to the positive haematopoietic function of the plant. Administration of ethanol leaf extract of *A. wilkesiana* showed no significant effect on red blood count (RBC), white blood cell count (WBC), neutrophil, lymphocyte, monocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration, and platelet. Eosinophils and Basophils were absent in all groups.

MCH, MCHC and MCV relates to individual red blood cells.[14] The non-significant effect of the plants extract on these parameters including RBC is likely an indication that the rates of production and destruction of the blood corpuscles were not altered.[16] The non-significant effect of the treatment with the plant extract on all white blood cells of the treated group showed that the plant had no effect on the immune system of the animal, either by boosting immunity or causing infection.

CONCLUSION

Salt loading resulted in significant increases in blood total white cell count (TWCC) and neutrophil count (NC), significant decreases in blood lymphocyte count (LC), monocytes count (MC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelets count (PC), and no significant effect in blood pack cell

volume (PCV), haemoglobin concentration (HC), red cell count (RCC), and mean corpuscular haemoglobin (MCH). Administration of ethanol leaf extract of *A. wilkesiana* resulted in significant increases in the PCV and haemoglobin concentration, and no significant effect on red blood count (RBC), white blood cell count (WBC), neutrophil, lymphocyte, monocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration, and platelet count of the salt loaded experimental rabbits. Thus, salt loading resulted in significant effects on the haematological parameters of the experimental animals while treatment with the ethanol extract of *Acalypha wilkesiana* leaves resulted in no significant effect on the haematological parameters of the experimental rabbits. Hence, *A. wilkesiana* may not trigger immune response in experimental animals, which portends its relative tolerability by the animals.

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