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Variations in Blood Parameters of High Salt Loaded Rats Following Administration of *Moringa oleifera* Leaf Extract

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ABSTRACT

High salt loading is associated with myriad deleterious effects including alterations in blood parameters, cardiovascular risk and sudden death. This study explored the impact of *Moringa oleifera* on some blood parameters in high salt loaded rats. Twenty four male albino Wistar rats were assigned into 4 groups of 6 rats each. They were fed on either control diet, high salt diet (8% NaCl diet+1% NaCl drinking water) and/or *Moringa* extract (600 mg kg⁻¹ b.wt. orally, once daily) *ad libitum* for 6 weeks. Blood samples were obtained via cardiac puncture for full blood count measurement using automated blood counter. Results revealed that the high salt fed untreated rats had significant increases in their total WBC, RBC, platelet counts, PCV, lymphocytes, P-LCR, MPV and PDW. These increases were reversed to near control levels by *Moringa*. Also, *Moringa* reversed lowered, MCHC platelet and neutrophil counts in high salt loaded rats. Values obtained for control group include: RBC (7.02±0.16×10⁶ cell μL⁻¹), total WBC (8.08±0.80×10³ cell μL⁻¹), PCV (40.30±1.14%), platelet count (673.17±18.89×10³ cell μL⁻¹), RDW-SD (35.70±0.32 fL). In conclusion, *Moringa oleifera* extract prevents changes in total WBC, RBC count, packed cell volume (hematocrit), platelet count, lymphocytes, neutrophils, MPV, P-LCR and PDW in high salt fed rats, these parameters are determinants of the blood volume, hemostasis and the tendencies to excessive bleeding, cardiovascular diseases, hypertension and sudden death.

Key words: *Moringa oleifera* leaf, high salt load, blood cells, rats

INTRODUCTION

Salt (sodium chloride) is a vital component of our diets and it is important for proper functioning of different parts of the body. It has been estimated that 75% of the salt intake in the United State is derived from salt added during food processing or manufacturing, rather than from salt added at the table or during cooking. The lowest salt intakes are associated with diets that emphasize unprocessed foods, especially fruits, vegetables and legumes (Westphal *et al.*, 2012; FNBIM/SC., 2005; WHO., 2013).

High sodium intake decreases renal calcium reabsorption which in turn leads to greater urinary calcium excretions, osteoporosis and kidney stones (Heller, 1999; Audran and Legrand, 2000). Gastric cancer is another condition linked with high salt intake (Tsugane, 2005; Liu and Russell, 2008). Sodium intake is also associated with high blood pressure (hypertension) (Denton *et al.*, 1995). Death may also result from attempted use of salt solutions as emetics, forced salt intake and accidental mix-up of salt with sugar in child food. High salt loading in humans and experimental animals, increases the stiffness of conduit arteries and the activity

of resistance arteries (Simon and Illyes, 2001). Stroke is another effect of high salt intake (Xie *et al.*, 1992). High salt intake leads to platelet aggregation (Gow *et al.*, 1992).

Medicinal plants have been used for centuries to combat many ailments and are also useful component in pharmaceutical industries, among these plants is *Moringa oleifera*. *Moringa oleifera* plant is being studied extensively throughout the world, because of its numerous health benefits has accumulated by traditional and orthodox practitioners. *Moringa* leaves have been cited in several scientific literatures as having antioxidant, anti-inflammatory, antibiotic, hypertensive antispasmodic, antiulcer, hypoglycemic and hypocholesterolemic activities. Antitumour, hepatoprotective, hypoglycemic actions are other benefits of *Moringa oleifera* leaves cited in scientific literatures (Ghasi *et al.*, 2000; Bais *et al.*, 2014).

It is obvious that high salt loading will impact negatively on the blood homeostasis which may lead to deleterious effect on the body, with the existing scarcity of scientific information on the effect of *Moringa* leaf in high salt loading.

It is therefore, the main aim of this study to investigate on the impact of chronic administration of *Moringa* leaf extract on some hematological parameters in high salt loaded rats.

MATERIALS AND METHODS

Experimental animals: Twenty four male albino Wistar rats of initial body weights between 145-190 g were obtained from the animal house of the Department of Medical Physiology, University of Calabar, Nigeria were used for the experiment. The animals received their feed and drinking water *ad libitum*. The rats were housed in wooden cages under control environmental condition in line with standard laboratory practice. The experimental regimens lasted 6 weeks. The animals were maintained following international standard according to CCAC (2009).

Experimental plant: Fresh leaves of *Moringa oleifera* were purchased from the Botanical Garden of Calabar Municipality, Cross River State, Nigeria during the rainy season and were identified as authenticated by a botanist (Mr. Frank Adepoju) in the Department of Biological Sciences, University of Calabar, Calabar.

Preparation of plant extract: Fresh leaves of *M. oleifera* first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the ground sample (50 g) was weighed and Soxhlet extracted with 150 mL distilled water at 100°C for 9 h. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of distilled water. The extract was slowly evaporated to dryness in vacuo at 40°C using a rotary evaporator. A total yield of 31% was obtained. Weighed samples of the extract were then used to prepare the stock solution (Eno *et al.*, 2001).

Preparation of high salt diet: High salt diet containing 8% of sodium chloride was prepared using a standard diet containing 0.3% sodium chloride after the method of Obiefuna and Obiefuna (2001).

Experimental protocol: The twenty-four male albino Wistar rats were divided into 4 groups of 6 rats each. They were fed as follows: The group 1 (control) was fed on normal rat pellet+drinking water. The group 2 (NT) was fed on normal rat pellet+drinking water+600 mg kg⁻¹ b.wt. of *M. oleifera* orally once daily. The group 3 (SF) was placed on high salt diet (8% sodium chloride)+1%

sodium chloride drinking water. The group 4 (ST) received same as the third group+*M. oleifera* extract (600 mg kg⁻¹ b.wt.) orally once daily. The feeding regimens lasted for six weeks. At the end of the feeding period, the animals were sacrificed and blood sample collected for daily analysis. The animals were weighed daily.

Collection of blood samples: The animals were made unconscious inhaling chloroform anesthesia (3.5% soaked in cotton wool) and blood collected via cardiac puncture (blood was drawn from the heart) a modification of the method by Ohwada (1986). The samples were collected by the help of 5 mL syringe attached to needle (21 SWG) into plain capped bottles containing ethylene diamine tetra-acetate (EDTA). The samples were immediately used for the estimation of the different variables.

Measurement of blood parameters: Blood samples were analyzed using automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration according to the manufacturer's instruction (Coulter Electronics, 1979) using normal human blood and with complete profile for Red Blood Cell (RBC) count, total White Blood Cell (WBC) count, differential WBC count, hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), red blood cell distribution width (RDW), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Large Cell Ratio (P-LCR).

Statistical analysis: Data is presented as Mean±SEM. Data was analyzed using a one-way analysis of variance (ANOVA), significant data was then followed with a *post hoc* test (Least Square Deviation). p value of less than 0.05 (p<0.05) was accepted as statistically significant.

RESULTS

Comparison of the total white blood cell count in different experimental groups: As shown in Fig. 1, the total WBC count of the control, Normal Treated (NT), Salt Fed (SF) and Salt

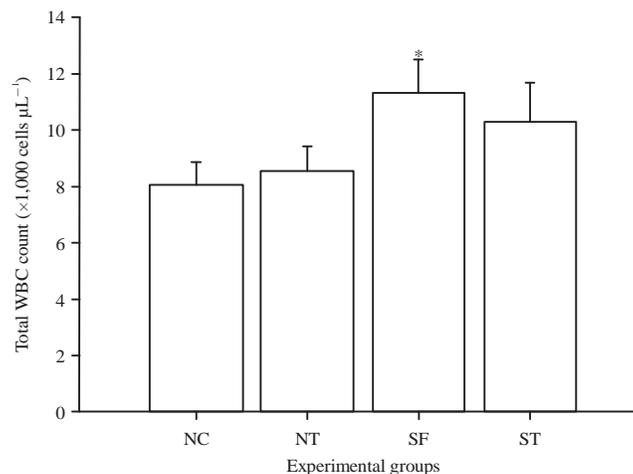


Fig. 1: Comparison of total white blood cell count in the different experimental groups. Values are expressed as Mean±SEM, n = 6, *p<0.05 vs normal control

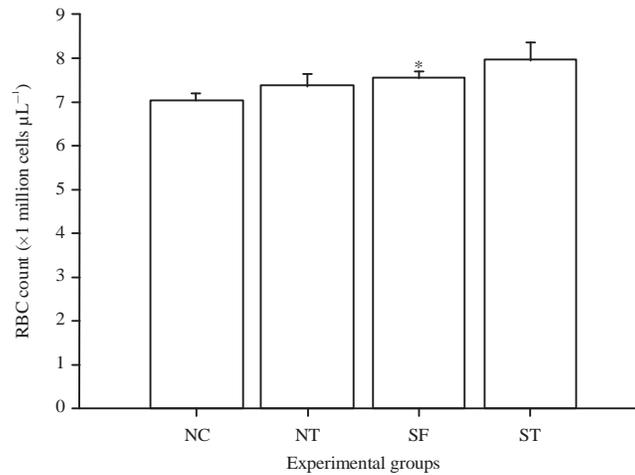


Fig. 2: Comparison of red blood cell count in the different experimental groups. Values are expressed as Mean \pm SEM, n = 6, *p<0.05 vs normal control

Treated (ST) groups were 8.08 ± 0.80 , 8.75 ± 0.87 , 11.33 ± 1.20 and $10.30\pm 1.32\times 10^3$ cell μL^{-1} , respectively. Total WBC count in NT and ST groups were not significant ($p>0.05$) compared with controls and ST groups but the SF group had significantly ($p<0.05$) higher total WBC count compared with control.

Comparison of the red blood cell count in the different experimental groups: The red blood cell count for the different experimental groups is illustrated in Fig. 2. The mean RBC count for the control was $7.02\pm 0.16\times 10^6$ cell μL^{-1} , it was not significant in NT ($7.36\pm 0.26\times 10^6$ cell μL^{-1}) and ST ($7.95\pm 0.40\times 10^6$ cell μL^{-1}) compared with control and SF. But it was significantly ($p<0.05$) higher in the SF group compared with control.

Comparison of hemoglobin concentrations in the different experimental groups: The mean concentrations of hemoglobin for the different experimental groups is illustrated in Fig. 3. The mean Hb concentrations of the different experimental groups were not significant ($p>0.05$).

Comparison of packed cell volume in the different experimental groups: As shown in Fig. 4, the PCV of the control, NT, SF and ST groups were 40.30 ± 1.14 , 45.18 ± 1.94 , 46.23 ± 2.13 and $44.25\pm 1.49\%$, respectively. Packed cell volume in NT, ST and control were not significant ($p>0.05$). SF had significantly ($p<0.05$) higher PCV compared with control.

Comparison of platelet count in the different experimental groups: Figure 5 shows the platelet count for the different experimental groups. The mean platelet count for the NT group ($805.00\pm 57.18\times 10^3$ cell μL^{-1}) was slightly higher compared with the control value ($673.17\pm 18.89\times 10^3$ cell μL^{-1}). The value obtained for in SF ($595.50\pm 33.66\times 10^3$ cell μL^{-1}) was significantly ($p<0.05$) lower compared with the control. Platelet count in ST ($696.67\pm 85.08\times 10^3$ cell μL^{-1}) groups was not significant ($p>0.05$) compared with control, NT and SF groups.

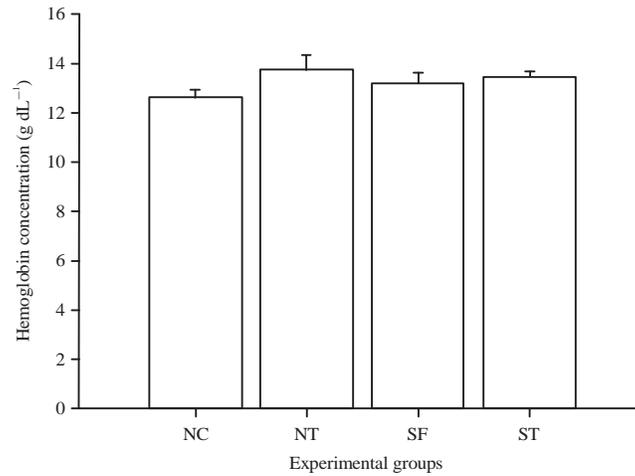


Fig. 3: Comparison of hemoglobin concentration in the different experimental groups. Values are expressed as Mean±SEM, n = 6

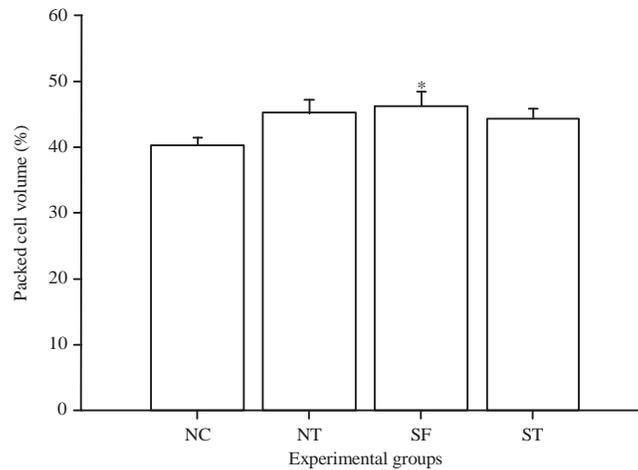


Fig. 4: Comparison of packed cell volume in the different experimental groups. Values are expressed as Mean±SEM, n = 6, *p<0.05 vs normal control

Comparison of red blood cell absolute values and indices in the different experimental groups: Results obtained for the red blood cell absolute values (MCV, MCH and MCHC) and indices (RDW-SD and RDW-CV) are summarized in Table 1.

The RDW-SD for the control, NT, SF and ST groups were 35.70±0.32, 38.67±1.37, 33.82±0.45 and 35.32±2.13 fL, respectively. It was significantly lower in SF group compared with control and NT groups. Value obtained for ST was not significant (p>0.05) compared with control, NT and SF groups.

The RDW-CV did not vary significantly (p>0.05) among the different experimental groups. The mean values of MCV and MCH obtained for the different experimental groups were also not significant among the different groups (p>0.05).

The MCHC was also significantly (p<0.05) lower in SF group compared with the control group, while values obtained for ST group did not vary significantly (p>0.05) compared with other groups.

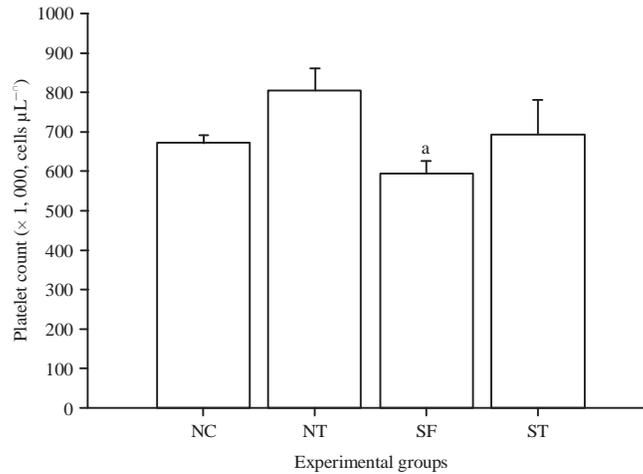


Fig. 5: Comparison of platelet count in the different experimental groups. Values are expressed as Mean±SEM, n = 6 *p<0.05 vs normal treated

Table 1: Comparison of red blood cell absolute values and indices in the different experimental groups

Groups	RDW-SD (fL)	RDW-CV (%)	MCV (fL)	MCH (pg)	MCHC (g dL ⁻¹)
Normal control	35.70±0.32	16.73±0.66	57.38±0.55	17.93±0.17	31.22±0.16
Normal treated	38.67±1.37	17.68±0.69	61.48±2.14	18.65±0.71	30.40±1.11
Salt fed	33.82±0.45** ^b	15.93±0.55	61.18±2.66	17.37±0.60	28.50±0.86*
Salt treated	35.32±2.13	15.17±1.28	56.34±3.20	17.15±1.22	30.43±1.09

Values are expressed as Mean±SEM, n = 6. *p<0.05, **p<0.01 vs normal control, b: p<0.01 vs normal treated, RDW-SD: Red blood cell distribution width-standard deviation, RDW-CV: Red blood cell distribution-coefficient of variance, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

Table 2: Comparison of differential white blood cells in the different experimental groups

Groups	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Mixed differential cells (%)
Normal control	60.68±0.72	34.18±0.62	4.30±0.19	0.83±0.06
Normal treated	62.73±1.77	32.10±1.72	4.20±0.39	0.97±0.08
Salt fed	69.12±1.52*** ^a	25.88±1.24*** ^a	4.13±0.34	0.87±0.08
Salt treated	63.15±1.11 ^x	31.92±1.03 ^x	3.95±0.28	0.98±0.14

Values are expressed as mean±SEM, n = 6. ***p<0.001 vs normal control, a: p<0.05 vs normal treated, x: p<0.05 vs salt fed

Comparison of differential white blood cell count in the different experimental groups:

Results obtained for the differential white blood cell count are summarized in Table 2. Lymphocyte counts were significantly raised in SF groups compared with control and NT groups (p<0.05). No significant variations in lymphocytes were observed between SF and control or NT groups.

Neutrophils were significantly (p<0.05) lower in SF group compared with control, NT and ST groups. It was not significant (p>0.05) in ST compared with control and NT groups.

Eosinophils and the mixed differential cells (monocytes and basophils) for control, NT, SF and ST groups were not significant (p>0.05).

Comparison of platelet indices in the different experimental groups:

Table 3 shows the summary of results obtained for platelet indices among the different experimental groups. The Platelet Distribution Wide (PDW) was significantly (p<0.05) higher in ST compared with SF groups. It was not significant (p>0.05) in NT, SF, ST compared with control.

The Mean Platelet Volume (MPV) was significantly higher in SF compared with control (p<0.05). No significant differences were observed between NT, SF, ST and control group.

The Platelet Large Cell Ratio (PLCR) was also significantly higher in SF group compared with control. It was not significant in NT, SF and ST groups compared with control.

Table 3: Comparison of platelet indices in the different experimental groups

Groups	PDW (fL)	MPV (fL)	P-LCR (%)	PCT (%)
Normal control	9.68±0.10	7.88±0.06	10.77±0.40	0.53±0.01
Normal treated	10.12±0.24	8.05±0.20	11.55±1.07	0.69±0.11
Salted	9.37±0.40	8.52±0.18**	12.73±0.37**	0.56±0.02
Salt treated	10.43±0.40	8.10±0.19	13.30±1.44	0.56±0.06

Values are expressed as Mean±SEM, n = 6. **p<0.01 vs normal control, PDW: Platelet distribution width, MPV: Mean platelet volume, P-LCR: Platelet large cell ratio, PCT: Plateletcrit

DISCUSSION

In this study, the effects of crude *Moringa oleifera* leaf extract on RBC, PCV, Hb, absolute values (MCV, MCH, MCHC and RDW), platelet count and platelet indices were measured in high salt fed rats. Blood is a tissue fluid which consists of fluid portion or plasma that is suspended by some formed elements (erythrocytes, leucocytes and thrombocytes). Blood provides the basic connections between the various organs and cells of the body and to maintain a constant cellular environment by circulating through every tissue delivering nutrients to them and removing waste products (Ganong, 2003; Sembulingam and Sembulingam, 2006).

The blood cells (erythrocytes, leucocytes and thrombocytes) are regulated so that excessive variation in their quality and quantity is prevented. This regulation is via some specialized feedback mechanism for the cells (Guyton and Hall, 2004; Sembulingam and Sembulingam, 2006). Our findings reveal that the crude *Moringa* extract had tremendous effect on the levels of the blood cells in high salt fed rats.

The high salt fed rats had significant increase in their total white blood cells, red blood cells and packed cell volume, the extract was observed to bring the elevated RBC, PCV and total WBC in the high salt-loaded rats to near control values. Therefore, with this stable level of red blood cells, it is very likely that the blood viscosity was kept unchanged by the crude extract. In other words, if the extract had caused an increase or decrease in RBC count, this could have led to a corresponding increase or decrease in viscosity.

Moringa extract also reversed low platelet count induced by high salt loading. Platelets are very useful in blood clotting, their reduction is associated with bleeding tendencies, hence the ability of the extract to ameliorate bleeding tendencies associated with thrombocytopenia, especially when triggered by high salt load. Mean Platelet Volume (MPV) is the indicator for platelet function (Jakubowski *et al.*, 1996), including aggregation, release of thromboxane A₂, platelet factor 4, beta-thromboglobulin (Sharp *et al.*, 1995) and expression of glycogen 1b and glycogen IIb/IIIa receptors (Tschoepe *et al.*, 1990; Giles *et al.*, 1994).

The MPV was significantly altered in this study following high salt loading. Mean platelet volume a determinant of platelet function; is a newly emerging risk factor for athero-thrombosis. Increase in MPV has been documented in patients with metabolic syndrome, stroke and Diabetes Mellitus (DM) (O'Malley *et al.*, 1995; Tavit *et al.*, 2007). Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischemia and transient ischemic attacks (Khandekar *et al.*, 2006; Kilicli-Camur *et al.*, 2005; Nadar *et al.*, 2004; McCabe *et al.*, 2004) and chronic vascular disease (Endler *et al.*, 2002). Extract of *Moringa* reversed the increase in MPV in high salt loaded rats.

Other platelet indices (platelet large cell ratio and platelet distribution width) also were reduced to near control level in high salt fed rats by *Moringa* extract.

The elevated lymphocyte and low neutrophil count in the high salt loaded rats were reversed to near control values by *Moringa* extract. The increase in lymphocyte count in high salt loaded rats is a reflection of perturbation of the immune system.

CONCLUSION

We conclude that *Moringa oleifera* extract prevents deleterious changes in total WBC, RBC count, packed cell volume (hematocrit), platelet count, lymphocytes, neutrophils, MPV, P-LCR and PDW in high salt fed rats, these parameters are determinants of the blood volume, hemostasis and the tendencies to excessive bleeding, cardiovascular diseases, hypertension and sudden death.

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