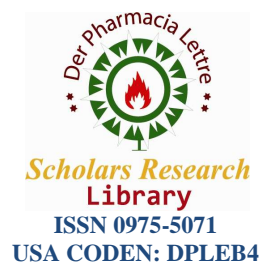




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## Effect of masfon aloe vera gel on some blood parameters in high salt loaded rats

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### ABSTRACT

High salt loading is associated with myriad cellular damage, hypertension and death. It is not known if Masfon aloe vera gel could ameliorate the menace of high salt loading. This study aimed to investigate the effect of Masfon aloe vera gel on some blood parameters in high salt loaded rats. Twenty four (24) albino Wistar rats were randomly assigned into 4 groups of 6 rats each. The rats took either on normal rat chow, high salt (8% NaCl feed + 1% NaCl drinking water) diet and/or Masfon aloe vera gel 6 weeks. Blood samples obtained into EDTA sample bottles for full blood counting using automated blood counter. RBC and total WBC counts increased significantly ( $p < 0.05$ ) in the tests groups compared to the control. The control values of RBC and total WBC were  $6.07 \pm 0.24 \times 10^3/\mu\text{L}$  and  $7.01 \pm 0.11 \times 10^6/\mu\text{L}$ . The Hb concentration in the control was  $12.80 \pm 0.20\text{g/dL}$ , it was significantly ( $p < 0.05$ ) raised in the salt treated (ST) group compared with other groups. PCV increased significantly ( $p < 0.05$ ) in the salt fed untreated (SF) and ST groups compared with control. The normal treated (NT) group had significant ( $p < 0.05$ ) increase in platelet count compared with other groups, but their red cell distribution width was significantly ( $p < 0.05$ ) reduced compared with other groups. Mean corpuscular volume (MCV) did not later significantly among the groups, but the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) decreased significantly ( $p < 0.05$ ) in SF group compared with other groups. Lymphocytes count increased, while neutrophils decreased significantly ( $p < 0.05$ ) in all the tests groups compared with the control. PDW, MPV and P-LCR decreased significantly in the extract treated groups (NT and ST) compared with other groups. In conclusion, high salt loading and treatment with Masfon aloe vera gel increased the total WBCs, lymphocytes, RBCs, Hb and PCV. However, Masfon aloe vera reversed elevated PDW, MPV and P-LCR and the low MCH and MCHC induced by high salt loading in rats.

**Key words:** Masfon aloe vera gel, blood parameters, high salt intake, rats.

### INTRODUCTION

Aloe vera is a plant of the family Asphodelaceae, although it was formerly thought as part of the lily family (Liliaceae). Cosmetic and some medicinal products are made from the mucilaginous tissue in the centre of the Aloe vera leaf and called Aloe vera gel.<sup>[1]</sup> Aloe vera has anti-inflammatory, anti-arthritic, anti-bacterial and hypoglycaemic effects.<sup>[2]</sup> Aloe gel is effective in treating radiation-induced dermatitis.<sup>[3]</sup> It reduces ulcer injuries in experimental animals.<sup>[4,5]</sup> Ingestion of Aloe gel also lowers serum cholesterol, serum triglyceride, and serum phospholipids, which when elevated causes atherosclerosis, and cardiovascular disease<sup>[6]</sup>, it also inhibitstumours growth in mice.<sup>[7,8]</sup> Aloe gel contains substances which stimulates phagocytosis, and immune boosters.<sup>[9]</sup>

Although scientists agree that a minimal amount of salt is required for survival, the health implications of excess salt intake represent an area of continued investigation among scientists, clinicians, and public health experts.<sup>[10]</sup> High salt loading in experimental animals has been associated with endothelial dysfunction<sup>[11,12]</sup>, increase in plasma brain natriuretic peptide concentration, perivascular inflammation<sup>[13,14]</sup>, down regulation of cytochrome P-450 in the brain

of stroke-prone hypertensive rats, deactivation of ATP – Sensitive potassium channels and Na<sup>+</sup>/K<sup>+</sup> ATPase pumps on the vascular smooth muscle membrane.<sup>[15]</sup> High salt intake increases arterial blood pressure and cardiovascular disease<sup>[10]</sup>, it increases insulin resistance /decrease insulin sensitivity.<sup>[16]</sup> The blood cells are not spared by the deleterious effects of high salt loading.

However, because of these several scientific studies undertaken in animal models on the beneficial actions of ingested Aloe vera gel coupled with paucity of studies on the direct effect of Aloe vera on blood parameters following high salt intake. The aim of this study is therefore to investigate the effect of Masfon Aloe vera on haematological parameters in high salt loaded rats.

## MATERIALS AND METHODS

### Experimental animals

Twenty four (24) male albino Wistar rats weighing initially between 140 to 180g obtained from the animal house of the Department of Medical Physiology, University of Calabar, Nigeria were used for the experiment. The animals provided feed and drinking water freely without restraint. They were kept under controlled environmental condition. The experimental feeding period lasted 42 days.

### Experimental plant extract

The Masfon Aloe vera gel used for this experiment was bought from the University of Calabar Teaching Hospital, Calabar-Nigeria.

### 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 *et al.*<sup>[15]</sup> and Adigun and Akinyanjuola<sup>[17]</sup>.

### 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 + 3mL/kg body weight of Masfon aloe vera gel 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 group 3 + Masfon aloe vera gel (3mL/kg body weight) 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.<sup>[18]</sup> The samples were collected by the help of 5mls syringe attached to needle (21 SWG) into plain capped bottles containing ethylene diaminetetraacetate (EDTA). The samples were immediately used for the estimation of the different variables.

### Analysis of blood samples

Blood samples were analyzed using automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration according to the manufacturer's instruction using normal human blood and with complete profile for red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR).

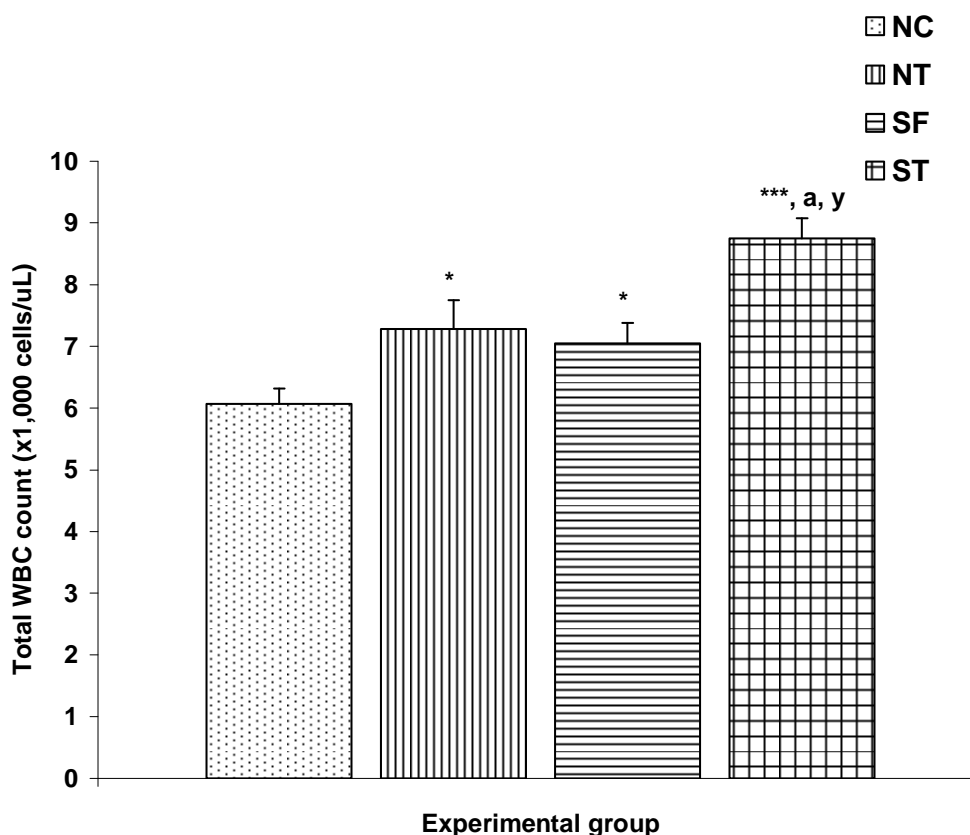
### Statistical analysis

Data were presented as mean ± SEM. Data were analysed using a one way analysis of variance (ANOVA) then followed with post hoc test (Least Square Deviation). P-value of less than 0.05 was declared as significant statistically.

## RESULTS

As shown in figures 1, the total WBC count of the control, normal treated (NT), salt fed (SF) and salt treated (ST) groups were 6.07 ±0.24, 7.28 ±0.47, 7.05 ±0.33 and 8.75 ±0.33 x10<sup>3</sup> cell/μL respectively, showing significant

( $p < 0.05$ ) increases in NT, SF and ST groups compared with controls. ST in turn had a significantly higher total WBC count compared with SF and NT groups.

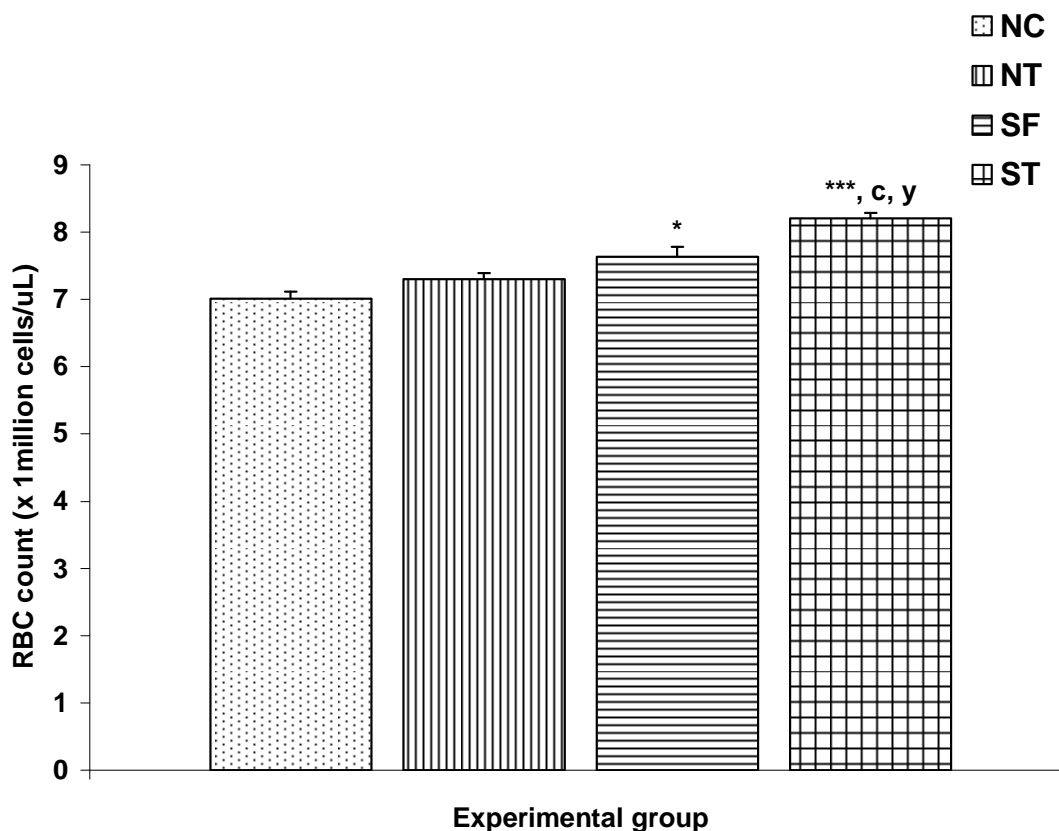


**FIG. 1: Comparison of total white blood cell count in the different experimental groups. Values are expressed as mean  $\pm$  SEM, n = 6.**  
 \* $p < 0.05$ , \*\*\* $p < 0.001$  vs normal control (NC);  
 a =  $p < 0.05$  vs normal treated (NT);  
 y =  $p < 0.01$  vs salt fed (SF).

The red blood cell count for the different experimental groups is illustrated in figure 2. The mean RBC count for the control was  $7.01 \pm 0.11 \times 10^6$  cell/ $\mu$ L, it was higher in NT ( $7.31 \pm 0.09 \times 10^6$  cell/ $\mu$ L), SF ( $7.63 \pm 0.15 \times 10^6$  cell/ $\mu$ L) and ST ( $8.20 \pm 0.08 \times 10^6$  cell/ $\mu$ L). The increase in NT group was not significant compared with control. But values obtained for SF and ST groups were significant ( $p < 0.01$ ) compared with control values. It was also significantly higher in ST compared with SF groups ( $p < 0.05$ )

The mean concentrations of haemoglobin for the different experimental groups is illustrated in figure 3. The control group had a mean Hb concentration of  $12.80 \pm 0.20$ g/dL, the values in NT ( $13.18 \pm 0.12$ g/dL) and ST ( $13.00 \pm 0.28$ g/dL) were comparable with the control value ( $p > 0.05$ ). But the increase in Hb concentration observed in the SF group was significant compared with the control, NT, and SF groups.

As shown in figure 4, the PCV of the control, NT, SF and ST groups were  $38.03 \pm 0.58\%$ ,  $38.82 \pm 0.48\%$ ,  $41.77 \pm 0.54$  and  $46.28 \pm 1.17\%$  respectively. Showing no significant statistical differences between the NT and control groups, but significant ( $p < 0.001$ ) increase in SF and ST groups compared with control and NT. Values in ST were in turn significantly higher compared with SF group.

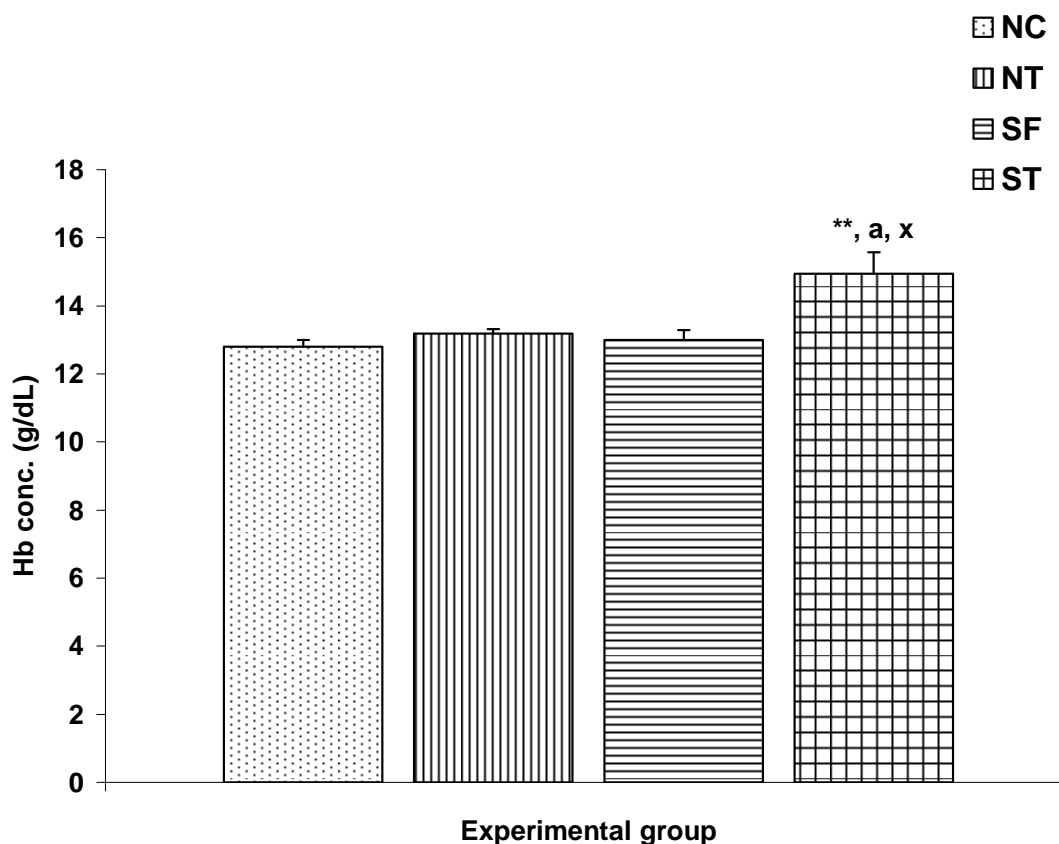


**FIG. 2: Comparison of red blood cell count in the different experimental groups. Values are expressed as mean  $\pm$  SEM, n = 6.**  
**\*p<0.05, \*\*\*p<0.001 vs normal control (NC);**  
**c = p<0.001 vs normal treated (NT);**  
**y = p<0.01 vs salt fed (SF).**

Figure 5 shows the platelet count for the different experimental groups. The mean platelet count for the NT group ( $1085.17 \pm 33.27 \times 10^3$  cell/ $\mu$ L) was significantly higher compared with the control value ( $905.33 \pm 53.27 \times 10^3$  cell/ $\mu$ L). The value obtained for in SF ( $866.83 \pm 76.83 \times 10^3$  cell/ $\mu$ L) and ST ( $904.67 \pm 31.64 \times 10^3$  cell/ $\mu$ L) groups were not significantly different compared with the control ( $p > 0.05$ ) but was significantly ( $p < 0.05$ ) lower compared with the NT group.

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  $33.73 \pm 0.69$ ,  $30.68 \pm 0.65$ ,  $35.50 \pm 0.89$  and  $38.13 \pm 2.34$ fL). It was significantly lower in NT group compared with control, SF and ST groups. Values obtained for SF and ST were slightly higher compared with control and not significant ( $p > 0.05$ ). RDW-CV in the NT group ( $15.37 \pm 0.70\%$ ) was significantly lower compared with values obtained for control ( $9.12 \pm 0.18\%$ ) and ST ( $19.02 \pm 0.73\%$ ) groups. While values obtained for SF ( $17.20 \pm 0.96\%$ ) and ST were comparable with control values.

The mean values of MCV obtained for the different experimental groups were not significant among the different groups ( $p > 0.05$ ). The MCH was significantly ( $p < 0.05$ ) lower in SF group compared with the control and NT groups. Values obtained for ST group was not significantly different compared with control, NT and SF groups. MCHC was also significantly ( $p < 0.01$ ) lower in SF group compared with the control and NT groups, while values obtained for ST group did not vary significantly compared with other groups.



**FIG. 3: Comparison of haemoglobin concentrations in the different experimental groups. Values are expressed as mean  $\pm$  SEM, n = 6.**

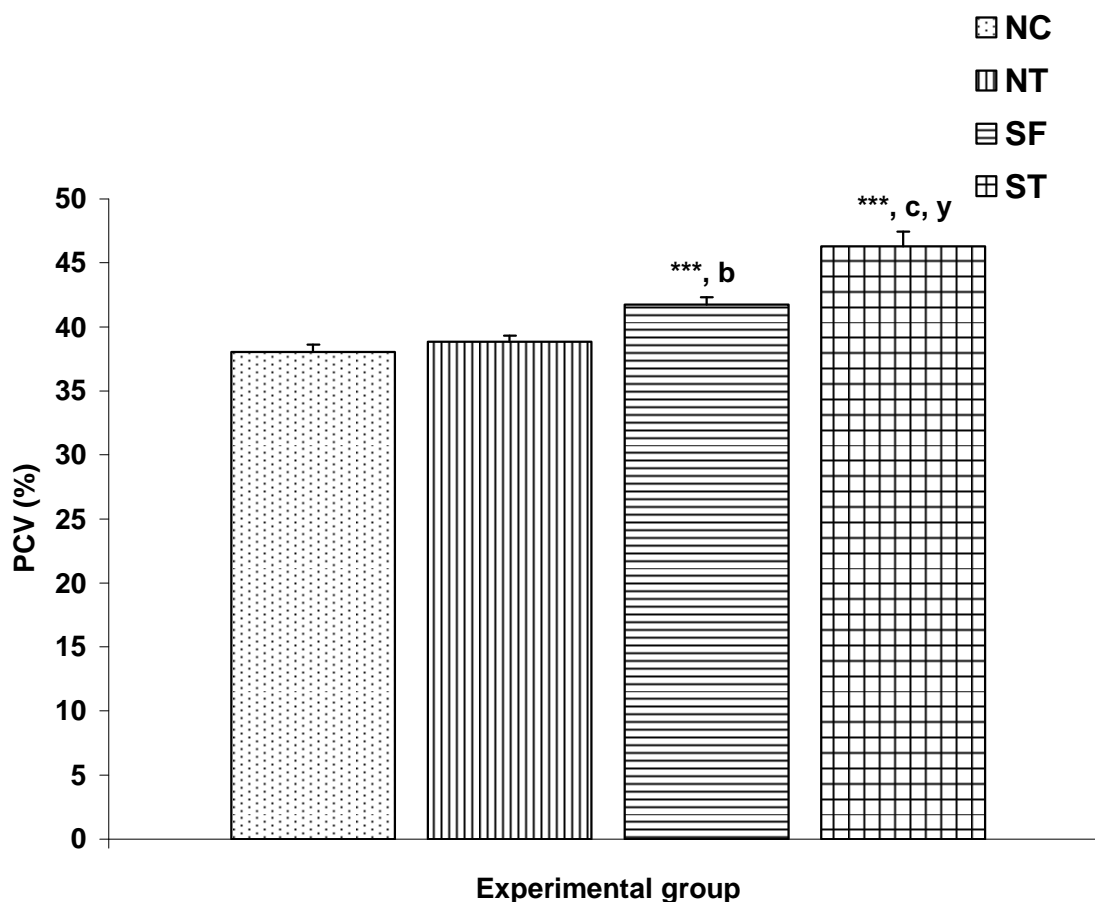
**\*\*p<0.01 vs normal control (NC);**

**a = p<0.05 vs normal treated (NT);**

**x = p<0.05 vs salt fed (SF).**

Results obtained for the differential white blood cell count are summarized in table 2. Lymphocyte counts were significantly raised in NT, SF and ST groups compared with control ( $p<0.05$ ). Neutrophils were significantly lower in NT, SF and ST groups compared with control. Eosinophils and the mixed differential cells (monocytes and basophils) for control, NT, SF and ST groups were comparable among the groups ( $p>0.05$ ).

Table 3 shows the summary of results obtained for platelet indices among the different experimental groups. The platelet distribution wide (PDW) was significantly ( $0.05$ ) lower in NT and ST groups compared with control and SF groups. The mean platelet volume (MPV) was also significantly lower in NT and ST groups compared with control and ST groups ( $p<0.05$ ). Same results were obtained for the platelet large cell ratio (PLCR).



**FIG. 4: Comparison of packed cell volume in the different experimental groups. Values are expressed as mean  $\pm$  SEM, n = 6. \*\*\*p<0.001 vs normal control (NC); b = p<0.01, c = p<0.001 vs normal treated (NT); y = p<0.01 vs salt fed (SF).**

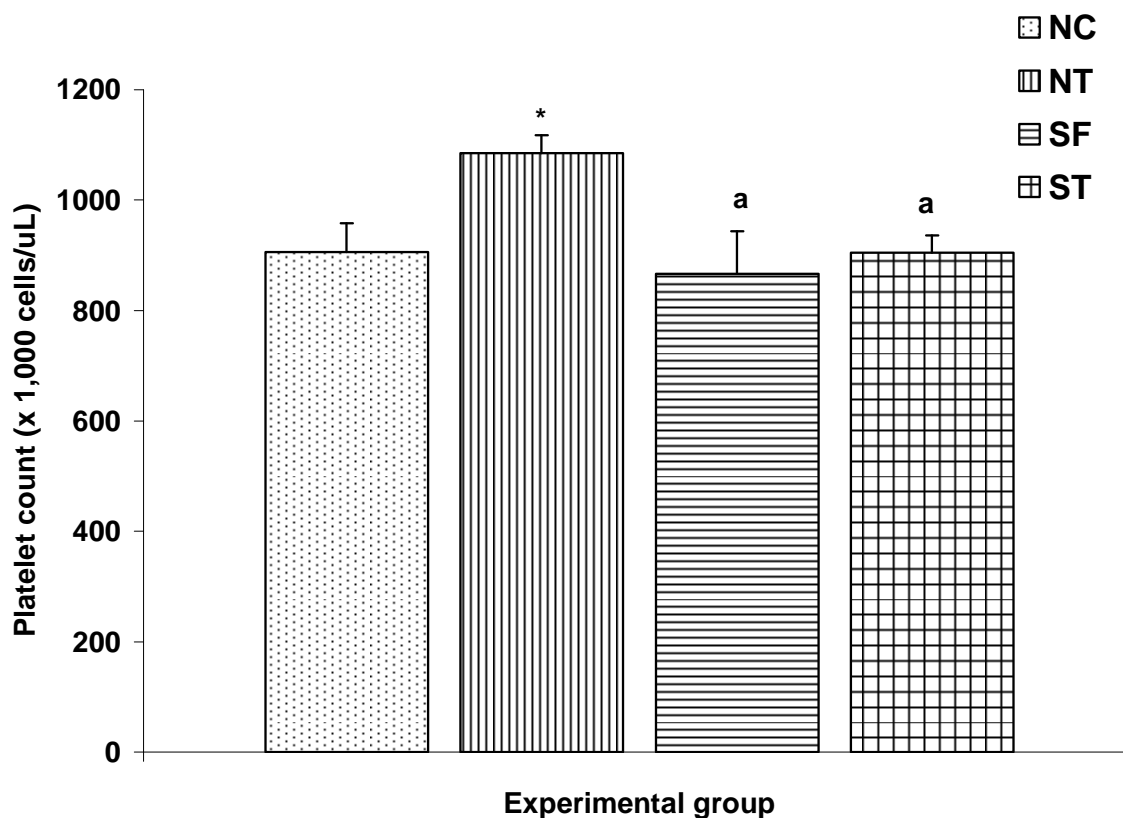
**TABLE 1: Comparison of red blood cell absolute values and indices in the different experimental groups**

	RDW-SD (fL)	RDW-CV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Normal control	33.73 $\pm 0.69$	17.20 $\pm 0.42$	54.23 $\pm 0.59$	18.23 $\pm 0.17$	33.67 $\pm 0.06$
Normal treated	30.68 $\pm 0.65^{**}$	15.37 $\pm 0.70^*$	53.15 $\pm 0.61$	18.05 $\pm 0.09$	33.97 $\pm 0.23$
Salt fed	35.50 $\pm 0.89^b$	17.20 $\pm 0.96$	54.85 $\pm 1.52$	17.05 $\pm 0.41^{*,a}$	31.13 $\pm 0.65^{**},b$
Salt treated	38.13 $\pm 2.34^a$	19.02 $\pm 0.73^b$	54.92 $\pm 1.17$	18.05 $\pm 0.25$	34.10 $\pm 1.17$

Values are expressed as mean  $\pm$ SEM, n = 6.

\*p<0.05, \*\*p<0.01 vs normal control;

a = p<0.05, b = p<0.01 vs normal treated



**FIG. 5: Comparison of platelet count in the different experimental groups. Values are expressed as mean  $\pm$  SEM, n = 6.**  
**\*p<0.05 vs normal control (NC);**  
**a = p<0.05 vs normal treated (NT).**

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

	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Mixed differential cells (%)
Normal control	63.05 $\pm 0.98$	32.67 $\pm 0.77$	3.30 $\pm 0.22$	0.98 $\pm 0.17$
Normal treated	68.58 $\pm 2.21^*$	26.73 $\pm 1.79^*$	3.73 $\pm 0.51$	0.95 $\pm 0.08$
Salt fed	69.88 $\pm 1.16^{**}$	25.45 $\pm 1.23^{***}$	3.80 $\pm 0.32$	0.87 $\pm 0.08$
Salt treated	69.72 $\pm 1.79^{**}$	25.77 $\pm 1.70^{**}$	3.62 $\pm 0.37$	0.90 $\pm 0.10$

Values are expressed as mean  $\pm$  SEM, n = 6.  
 \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs normal control

**TABLE 3: Comparison of platelet indices in the different experimental groups**

	PDW (fL)	MPV (fL)	P-LCR (%)
Normal control	9.12 ±0.18	7.37 ±0.06	8.57 ±0.46
Normal treated	8.22 ±0.26*	6.92 ±0.14*	6.77 ±0.21**
Salt fed	9.47 ±0.15 <sup>b</sup>	7.77 ±0.22 <sup>b</sup>	9.52 ±1.01 <sup>a</sup>
Salt treated	7.82 ±0.50* <sup>x</sup>	6.90 ±0.18* <sup>x</sup>	8.70 ±0.73 <sup>a</sup>

Values are expressed as mean ±SEM, n = 6.

\* $p < 0.05$ , \*\* $p < 0.01$  vs normal control;

$a = p < 0.05$ ,  $b = p < 0.01$  vs normal treated;

$x = p < 0.05$  vs salt fed.

## DISCUSSION

In this study, the effects of Masfon aloe vera gel on haematological parameters which are major determinants of blood viscosity and arterial blood pressure were measured in high salt fed rats.

Blood is a tissue which consists of fluid plasma in which is suspended a number of formed elements (erythrocytes, leucocytes and thrombocytes). Its primary functions is to provide a link 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.<sup>[19]</sup> The blood parameters exist at fairly constant levels, suggesting the existence of feedback mechanism for the cells.<sup>[19]</sup> In this study, the evidence is convincing that the Masfon aloe vera gel had tremendous effect on the levels of the blood parameters in high salt loaded rats.

In this present study, the red blood cell count and PCV were significantly increased following high salt loading. This finding is consistent with earlier report by Ofem *et al.*<sup>[20]</sup> that increase in RBC and PCV following high salt load could be due to dehydration. The increase in this parameters could be possibly lead to increase viscosity and the tendency to predispose to hypertension. However, salt fed rats that received Masfon aloe vera gel also had elevated RBC count and PCV. Showing that the extract was unable to reverse the increase in RBC and PCV induced by high salt loading. Hb was only significantly raised in the salt treated group.

The mean corpuscular volume was not adversely altered following the various treatments. But MCH and MCHC was significantly lowered in the high salt fed untreated group compared with control. This depicts iron deficiency anaemia in the high salt fed rats, but treatment with the extract ameliorated this condition.

Platelet count was only raised in the normal rats treated with the Masfon aloe vera gel. Salt loading in this study did not alter platelet count adversely. Previous study by Imoru *et al.*<sup>[21]</sup> and Ofem *et al.*<sup>[22]</sup> reported increase platelet count following high salt load. The increase was attributed to dehydrated induced by high salt intake and possible attenuation of nitric oxide synthase in the endothelium of high salt fed rats.<sup>[23]</sup>

Nevertheless, the platelet indices were tremendously altered in high salt fed rats. Therefore, it would seem likely that the gel contains some compounds that are capable of causing the release of a thrombopoietin. Platelets play an important role in the maintenance of normal homeostasis and MPV is an indicator of platelet function, including platelet aggregation; release of thromboxane A<sub>2</sub>, platelet factor 4, and beta- thromboglobulin; and expression of glycogen 1b and glycogen IIb/IIIa receptors.

In this study, MPV, PDW and P-LCR decreased in recipients of high-dose *O. gratissimum*. MPV, as a determinant of platelet function, is a newly emerging indicator of risk for athero-thrombosis. Increase in MPV and PDW has been documented in patients with metabolic syndrome, stroke, and diabetes mellitus.<sup>[24,25]</sup> Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischemia/transient ischemic attacks, and chronic vascular disease.<sup>[26,27,28,29,30]</sup>

PDW, MPV and P-LCR were significantly raised in high salt fed rats. Showing increased risk of cardiovascular disease.



The total WBC count and lymphocyte count of both Masfon aloe vera gel and salt fed rats increased significantly. The increase in WBC observed in high salt fed rats could be due to agitation of the immune system following high salt load.

In conclusion, both high salt loading and treatment with Masfon aloe vera gel increased the total WBCs, lymphocytes, RBCs, Hb and PCV. However, Masfon aloe vera reversed elevated PDW, MPV and P-LCR and the low MCH and MCHC induced by high salt loading in rats.

#### REFERENCES

- [1] Ni Y, Turner D, Yates KM, Tizard I. *Int J Immunopharm* **2004**;4:1745-1755.
- [2] Habeeb F, Shakir E, Bradbury F, Cameron P, Taravati MR, Drummond AJ, Gray AI, Ferro VA. *Methods* **2007**; 42: 315-320.
- [3] Jani GL, Shah DP, Jain VC, Patel MJ, Vithalan DA. *Pharmaceut Tech* **2007**;31:90-98.
- [4] Yusuf S, Agunu A, Diana M. *J Ethnopharmacol* **2004**;93:33-37.
- [5] Dagne, E., Bisrat, D., Viljoen, A., Van Wyk, B-E. (2000) *Curr Org Chem* **2000**;4:1055-1078.
- [6] He Q, Changhong L, Kojo E, Tian Z. *Food Control*. **2005**;16: 95- 104.
- [7] Reynolds T, Dweck AC. *J. Ethnopharmacol* **1999**;68:3-37.
- [8] Turner CE, Williamson DA, Stroud PA, Talley DJ. *Inter J Immunopharmacol* **2004**;4:1727- 1737.
- [9] Boudreau, MD, Beland FA. *J Envi Sc Health* **2006**;24:103-154.
- [10] Briganti EM, Shaw JE, Chadban SJ, Zimmet PZ, Welborn TA, McNeil JJ, Atkins RC. *MJA*. **2003**;179:135-139.
- [11] Barthan NP, Laurant D, Hayoz-Fellmann HR, Brunner A, Berthelot D. *Can J Physiol Pharmacol* **2002**; 80: 553-561
- [12] Robert P, Heaney M. *J Am Coll Nutr* **2006**;25:2715-2765
- [13] Cheng ZJ, Vaskonen T, Tikkanen I, Nurminen K, Ruskoaho H, Vapaatalo H, Muller D, Park JK, Luft FC, Mervaala EM. *Hypertension* **2001**;37: 433-439.
- [14] Braquhat EA, Dela S. *Journal of Clinical Hypertension* **2002**;4:41 - 46.
- [15] Obiefuna PCM, Obiefuna IP. *West Indian Med J* **2001**;50:17-21.
- [16] Sharma P, Varma MVS, Chawla HPS, Panchagnula R. *IIFarmaco*. **2005**;60:874-883.
- [17] Adigun SA, Akinyanjuola OB. *Nig J Physiol Sc* **1991**; 7: 88-99.
- [18] Ohwada K. *Jikken Dodutsu*, **1986**;35(3)353-355
- [19] Guyton AC, Hall JE. Text book of medical physiology (10<sup>th</sup> Ed.) Saunders, Pp. 345-356, **2004**.
- [20] Ofem OE, Ani EJ, Okongor EY, Okot-Asi A, Eno AE, Ibu JO. *Nig J Health Biomed Sc* **2008**; 7(1):1-5.
- [21] Imoru J, Unoh FB, Nkanu EE, Ofem OE, Ibu JO. (2005). *Nig J Health Biomed Sc* **2005**;4:139-145.
- [22] Ofem OE, Eno AE, Imoru J, Nkanu E, Unoh F, Ibu JO. *Ind J Pharmacol* **2007**;39(1):15-19.
- [23] Klinge JM, Topf H, Trusen B, Rauh M, Rascher W, Dotsch J. *Critical Care Medicine* **2003**;31:2010-2014
- [24] O'Malley T, Langhorne P, Elton R. *Stroke*. **1995**;23:345-352.
- [25] Tavit Y, Sen N, Yazici HU. *Thrombosis Research* **2007**;120:245-250.
- [26] Khandekar MM, Khurana AS, Deshmukh SD. *J Clin Path* **2006**;9:146-149.
- [27] Kiliçli-Camur N, Demirtunç R, Konuralp C, Eskiser A, Başaran Y. *Med Sc Monitor* **2005**;11: CR387-CR92.
- [28] Nadar SK, Lip GY, Blann AD. *Throm Haem* **2004**;92: 1342-1348.
- [29] McCabe DJ, Harrison P, Sidhu PS, Brown MM, Machin SJ. *Bri J Haematol* **2004**; 126:861-869
- [30] Endler G, Klimesch A, Sunder-Plassmann H. *Bri J Haematol* **2002**;117:399-404.