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Original Article

Ameliorative effect of Masfon Aloe Vera gel on altered proteins, bilirubin levels and body weight in high salt loaded Rats

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ABSTRACT

Salt loading is association with numerous cellular damages occasioned by generation of free radicals, the liver which is the site for the production of vital substances including the serum proteins and bilirubin is not spared from this menace. Aloe vera is a medicinal plant with huge healing potentials. With paucity in scientific literature on the impact of Masfon aloe vera gel on serum proteins, bilirubin levels (which are indicators of liver integrity) and body weight following high salt intake. It was therefore, the aim of this study to elucidate the effect of Masfon aloe vera on serum proteins, bilirubin levels and body weight in high salt loaded rats. Twenty four (24) male albino Wistar rats were divided into 4 groups of 6 rats each. The animals took normal rat chow, high salt diet (8% NaCl feed + 1% NaCl drinking water) and/or 3mL/kg b.w. of Masfon aloe vera gel for 42 days (6 weeks). The initial body weights of the control, normal treated (NT), salt fed (SF) and salt treated (ST) groups were 185.83±4.12g; 180.50 ± 1.71g; 185.33±2.35g and 176.50±5.38g respectively, showing no significant differences among the groups. The final body weights of SF (135.83±2.01g) reduced significantly ($p<0.01$) compared to other groups. The total protein level in the SF (55.00±1.03g/L) was significantly ($p<0.05$) higher compare to the control (50.33±1.76g/L) and NT (48.50 ±0.85g/L). No significant statistical differences were observed in serum albumin levels among the different experimental group ($p>0.05$). But the mean serum globulin concentration in the SF group (15.00±0.82g/L) increased significantly ($p<0.01$) compared to other experimental groups. The albumin to globulin ratio for the control group was 4.33 ±1.20, it was significantly ($p<0.05$) lower in ST compared to NT. The mean concentrations of total, conjugated, unconjugated bilirubin as well as the conjugated to unconjugated bilirubin ratio in control group were 10.95 ±0.62mmol/L, 7.53 ±0.58mmol/L, 3.50 ±0.42mmol/L and 2.49 ±0.41 respectively. SF group had significantly ($p<0.05$) higher levels of total and unconjugated bilirubin, but presented lower conjugated bilirubin levels compared to control. However, teatment with Masfon aloe vera reversed these changes in salt fed rats to near control values. Masfon aloe vera gel ameliorates the adverse effect of high salt diet on the total serum total protein, globulin, and bilirubin levels in rats. The gel also improved the body weight of high salt loaded rats.

Keywords: Masfon Aloe Vera, bilirubin levels, high salt loaded Rats

INTRODUCTION

Aloe vera belongs to the Liliacea family, is a succulent perennial that grows in a clump and has spiky, green leaves. The genius of *Aloe vera* contains at least 324 species of herbs, trees and shrubs. It originates from south and East African with some Arabian and Madagascans antecedents, this plant tarts in dry warm climate such as the Mediterranean [1,2]. However, a Greek physicians and a Roman naturalist, were the first to state the broad spectrum uses of the *aloe vera*, the leaves are mostly used for medicine, but several parts of the leaf can be used for different purpose [3].

Aloe gel is composed of 99% water, the remaining amount being solid materials. It contains over 75 different ingredients which includes materials, vitamins, enzymes, amino acid, salicylic acid, phenolic compounds, sterol, sugars, Kalilon B-sitosterol and lupeol. These ingredients are responsible for the healing properties of the gel of *Aloe vera*. Aloe vera is an immune-modulator capable of retarding the immunity [4,5], treatment of burns [6], wound healing [7-9], anti-diabetic [10-13], hypo-glycaemic and hypo-lipidaemic [14,15], Anti-cancer [16-17]. The extract of *Aloe vera* used in this study is called Masfon-*Aloe vera* gel. It is produced by Ahishua Ventures and is

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enclosed in a light blue plastic bottle that is 1L. Salt is an essential component of our diet. It is essential for contraction of muscle, nerve conduction, maintenance of osmotic pressure of extracellular fluid etc. However, excess of sodium chloride in the body is associated with incidence of stomach cancer in human population and gastric tumor in experimental animals. Also, salt loading generates free radicals giving rise to oxidative stress and associated oxidative damages such as reno-vascular, cardiovascular pathologies, and homeostatic imbalance [18-20]. Previous works have shown that more than 70% of the free radical trapping activity of serum was due to human serum albumin [21]. Bilirubin also acts as a cellular anti-oxidant to mop up free radicals [22,23]. It is true that high salt could alter the concentrations of serum proteins and bilirubin which are mainly produced by the liver, these endogenous substances are also used as indices to assess liver performance.

Although, so much has been reported in scientific literature on the impact of aloe vera gel on the body, very few information is available on the physiological role of Masfon aloe vera gel especially on serum proteins and bilirubin concentrations during salt loading. It was therefore the aim of this study to elucidate the effect of Masfon *Aloe vera* gel on serum proteins (total protein, albumin and globulin) and bilirubin (total, conjugated and unconjugated bilirubin) concentrations in high salt loaded rats.

MATERIALS AND METHODS

Experimental animals: Twenty four (24) male albino Wistar rats weighing initially between 160 to 200g were obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria were employed for this study for 6 weeks. The animals were allowed free access to their feed and drinking water. The rats were weighed before commencement of the feeding experiment and thereafter were weighed daily. They were nursed under control environmental conditions.

Experimental plant extract: The experimental plant extract used for this study was Masfon Aloe vera gel. It was purchased 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 standard methods [24,25].

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 the third group + 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 using chloroform anesthesia and blood collected via cardiac puncture (blood was drawn from the heart) a modification of the method described by an erudite scholar [26]. The samples were collected by the help of 5mls syringe attached to needle (21 SWG) into plain capped bottles. The samples were immediately used for the estimation of the different variables.

Extraction of sera: Blood samples from each rat was collected separately into clean capped plain tubes and allowed to stand for 2 hours for effective clotting to occur. These were then centrifuged at 2500 g for 15 minutes. The serum was extracted into clean test tube for the analysis of sodium, potassium, chloride, and bicarbonate ions.

Measurement of serum protein concentrations

Measurement of total protein: The blood samples were analyzed using Biuret method.

Principle: Cupric ions in alkaline solution react with peptide bonds in proteins producing violet colour that is proportional to the amount of protein present.

Measurement of serum albumin:

Principle: Albumin binds with bromocresol green (Bch) at pH 4.2 causing a slight in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin present when measure photometrically between 580 – 630nm with maximum absorbance at 625nm measured [27].

Calculation of serum globulin: Globulin concentration will be calculated as the difference between the total plasma protein and serum albumin concentration.

Globulin = Total plasma protein – Serum Albumin.

Estimation of serum bilirubin

Principle: Serum bilirubin is present in two forms, conjugated (mostly with glucoronic acid), and unconjugated (free bilirubin). Both give purple azo-

bilirubin with diazotized sulphanilic acid. Conjugated bilirubin reacts in aqueous solution (direct reaction), whereas the unconjugated bilirubin requires an accelerator or solubilizer, such as benzoate urea as in this method or alcohol used in some other methods (indirect reaction) [28].
Calculation: T/S x 8mg/100ml
8mg/100ml = 136.8µmol/L

Statistical analysis: The data were presented as mean ± standard error of mean. The data were analysed using one way analysis of variance (ANOVA) followed with a post hoc test (least square deviation test). These were done with assistance of computer software, SPSS version 17.0 and Excel for windows, p-values of less than 0.05 were accepted as statistically significant.

RESULTS

Mean weekly body weights of the different experimental groups: As shown in figures 1 and 2, the initial body weights of the control, normal treated (NT), salt fed (SF) and salt treated (ST) groups were 185.83 ±4.12, 180.50 ±1.71, 185.33 ±2.35 and 176.50 ± 5.38g respectively, showing no significant statistical differences among the different experimental groups. However, on the last day of feeding (i.e. on the 42nd day), the mean body weights (final body weights) of the control, NT, SF and ST groups were 246.67 ±2.47, 211.67 ± 4.01, 135.83 ±2.01 and 156.67 ±2.47g respectively. The final body weight of the SF group was significantly ($p<0.001$) lower compared with the other experimental groups, also the final body weight of the ST group reduced significantly ($p<0.001$) compared with the control and NT groups.

Comparison of the mean growth rate of the different experimental groups: The mean growth rate of the different experimental groups is illustrated in figure 3. The mean growth rate for the control was 1.45 ±0.15g/day, the NT growth had a growth rate of 0.74 ±0.10g/day, showing a significant reduction compared with the control group. However, the SF group had a negative growth rate (weight loss) of -1.18 ±0.06g/day. ST group also had a negative growth rate of -0.47 ±0.14g/day, although it was significantly higher compared with SF group.

Comparison of total protein concentrations in the different experimental groups: The mean concentrations of total protein for the different experimental groups is illustrated in figure 4. The control group had a mean total protein concentration of 50.33 ±1.76g/L, the value in NT group, 48.50 ±0.85g/dL was comparable with the control value ($p>0.05$). The SF group had a significantly higher mean protein concentration (55.00 ±1.03g/dL) compared with the control and NT groups, while the total protein concentration of the ST group was 50.33 ±0.76g/dL, significantly lower compared with SF group, but was comparative with control and NT values.

Comparison of serum albumin concentrations in the different experimental groups: As shown in figure 5, the mean serum albumin concentrations of the control, NT, SF and ST groups were 40.17 ±0.95, 40.50 ±1.23, 40.00 ±0.93 and 40.50 ±0.76g/dL respectively. Showing no significant statistical differences among the different experimental groups, ($p>0.05$).

Comparison of serum globulin concentrations in the different experimental groups: The mean serum globulin concentration of the NT group (8.00 ±1.75g/dL) was not significantly different from the control value (10.17 ±1.74g/dL), it was significantly higher in the SF group (15.00 ±0.82g/dL) compared with the control ($p<0.05$) and NT ($p<0.01$) groups. ST group had a significant reduction in serum globulin concentration (9.83 ±1.80g/dL) compared with SF group, but was comparable to control and NT values ($p>0.05$), figure 6.

Comparison of albumin:globulin ratio of the different experimental groups: The albumin to globulin ratio for the control group was 4.88 ±1.20, values obtained for the NT, SF and ST groups were not significantly different compared with the control group ($p>0.05$), however, SF had a significantly ($p<0.05$) lower albumin:globulin ratio compared with NT group, figure 7.

Comparison of total bilirubin concentration in the different experiment groups: The mean total bilirubin concentration was 19.95 ± 0.62, 11.05 ± 0.42, 13.22 ± 0.49 and 11.88 ± 0.69 mmol/L for control, NT, SF and ST group respectively. The mean total bilirubin concentration was significantly ($p<0.001$) increased in the SF compared to NC and NT group. The reduction in mean total bilirubin concentration following treatment of the salt fed rats was not significantly, Table 1.

Comparison of conjugated bilirubin concentration in the different experiment groups: The mean conjugated bilirubin concentration was 7.53 ± 0.58, 6.87 ± 0.31, 5.80 ± 0.38 and 6.83 ± 0.34 mmol/L for control, NT, SF and ST group respectively. Mean conjugated bilirubin concentration was significantly ($p<0.05$) lower in SF group when compared to NC and NT

groups; the increases observed following treatment of salt fed rats was not significant, table 1.

Comparison of unconjugated bilirubin concentration in the different experiment groups: Values obtained for mean unconjugated bilirubin concentration were 3.50 ± 0.42, 4.18 ± 0.57, 7.42 ± 0.81 and 5.05 ± 0.74 mmol/L for control, NT, SF and ST group respectively. The mean unconjugated bilirubin concentration was significantly ($p<0.01$) higher in the SF group compared to control and NT groups. It was also significantly ($p<0.05$) lower in the ST group compared to SF group, table 1.

Comparison conjugated to unconjugated bilirubin ratio in the different experiment groups: The ratio of conjugated to unconjugated bilirubin concentration was significantly lower ($p<0.05$) in the ST group compared to other experimental groups.

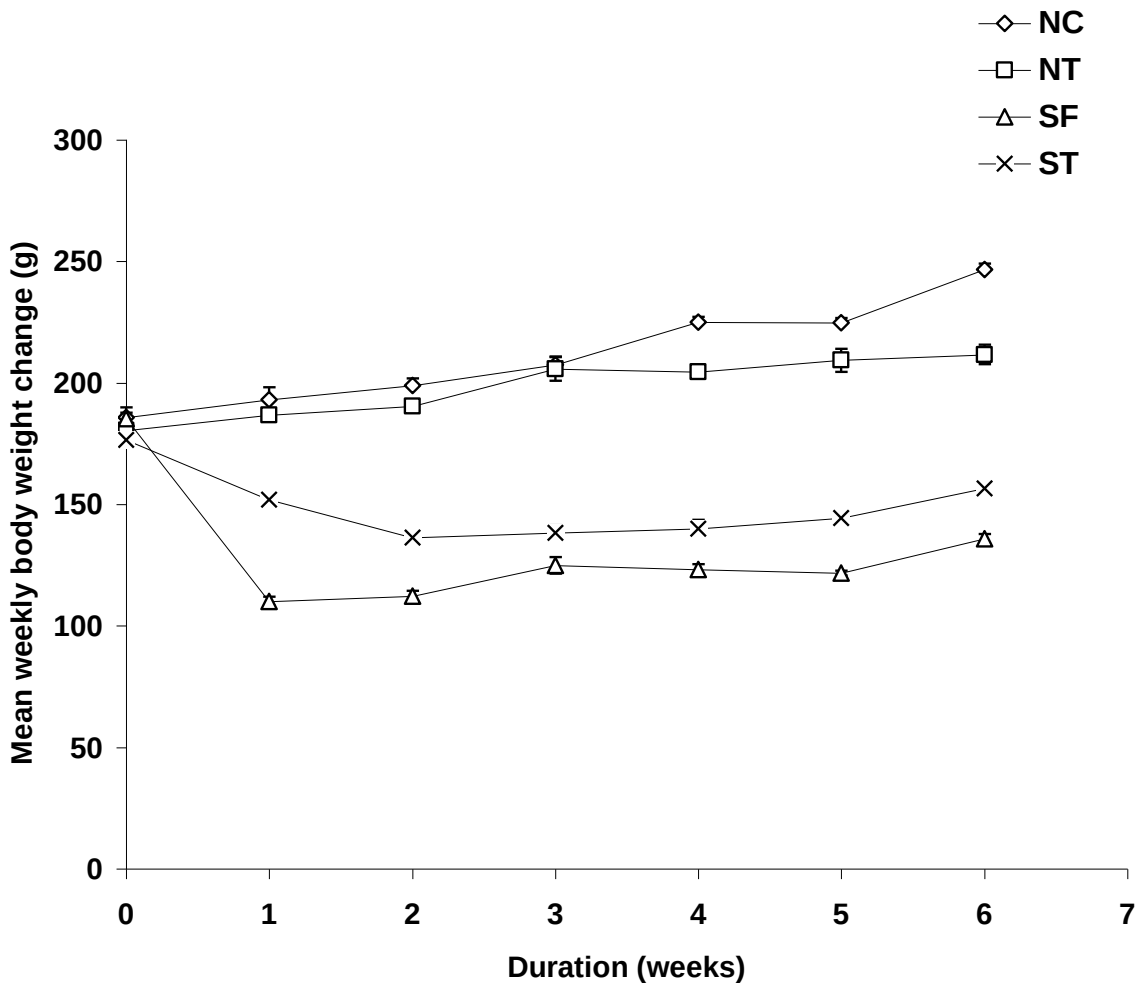

FIG. 1 Mean weekly body weight change of the different experimental groups. Values are mean ± SEM, n = 6.

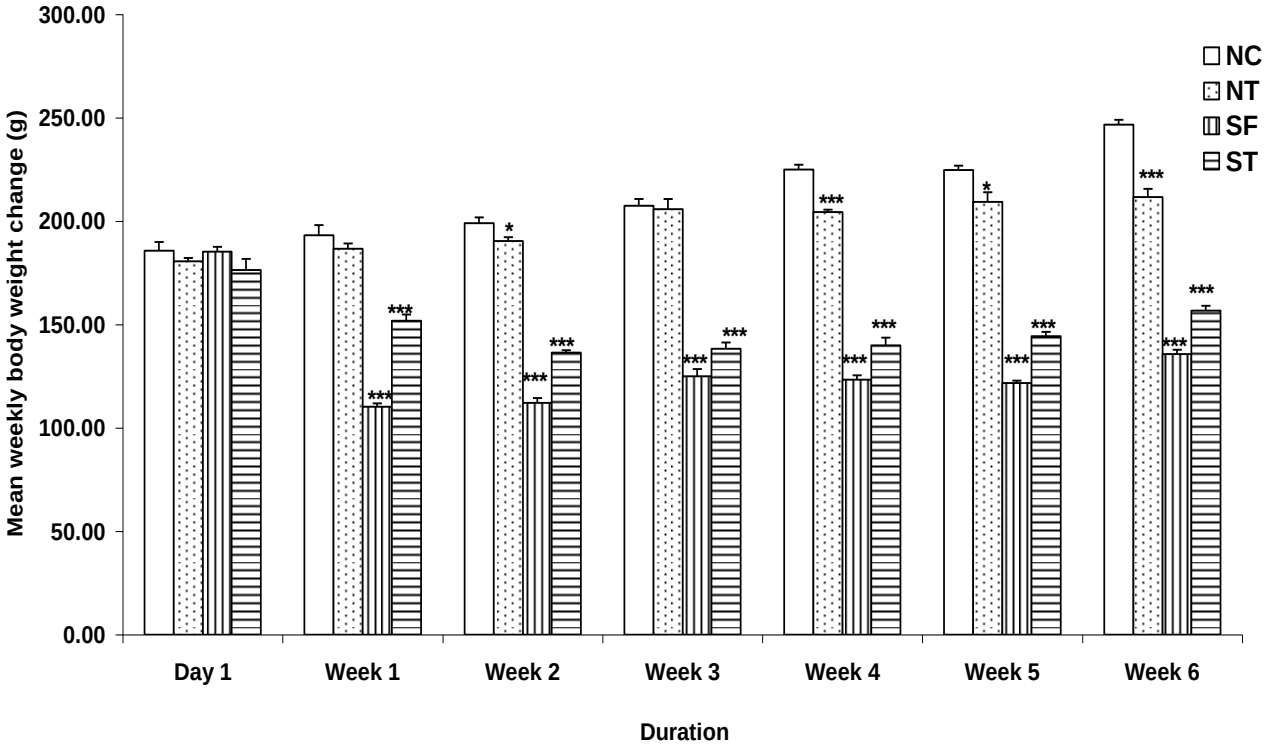

FIG. 2: Mean weekly body weight change of the different experimental groups. Values are mean ± SEM, n = 6. *p<0.05, ***p<.001 vs normal control (NC).

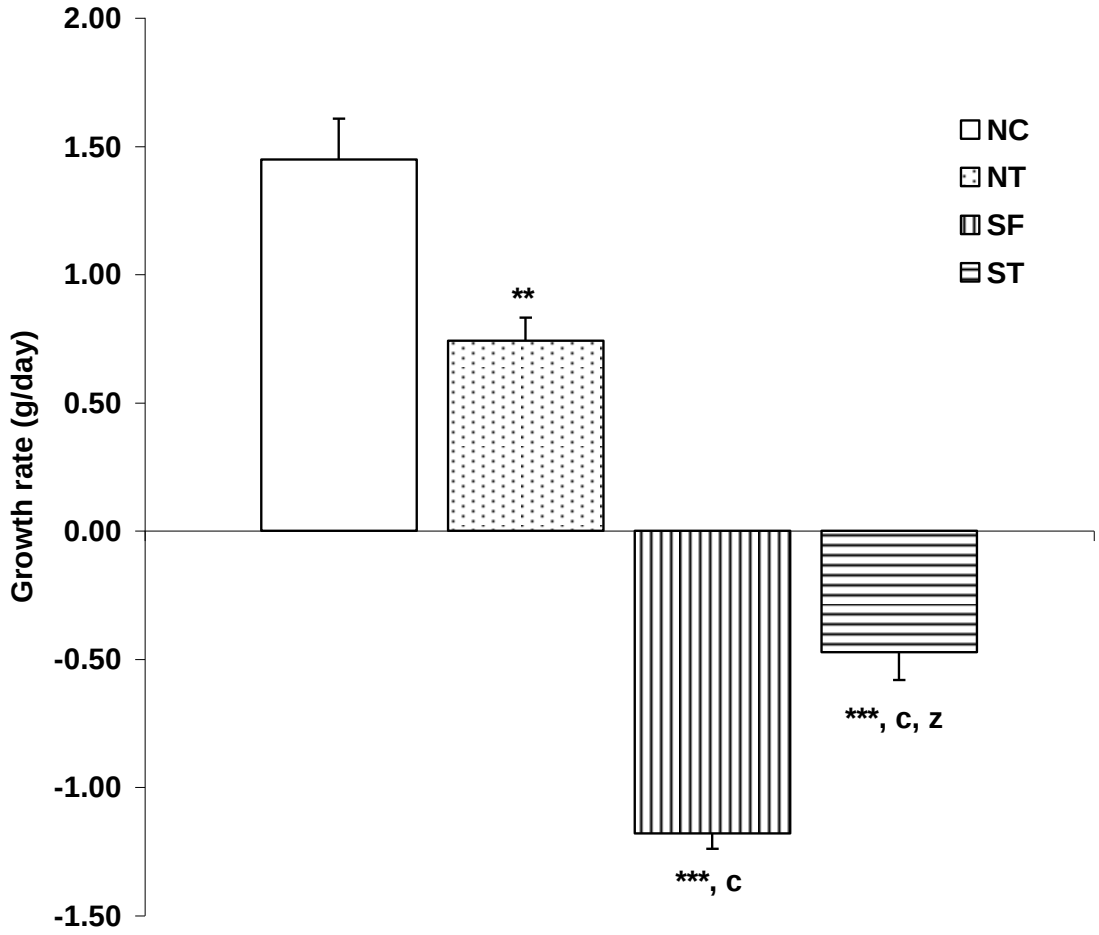

FIG. 3: Growth rate of the different experimental groups. Values are mean ± SEM, n = 6.
****p<0.01, ***p<0.001 vs normal control (NC);**
c = p<0.001 vs normal treated (NT);
y = p<0.001 vs salt fed (SF).

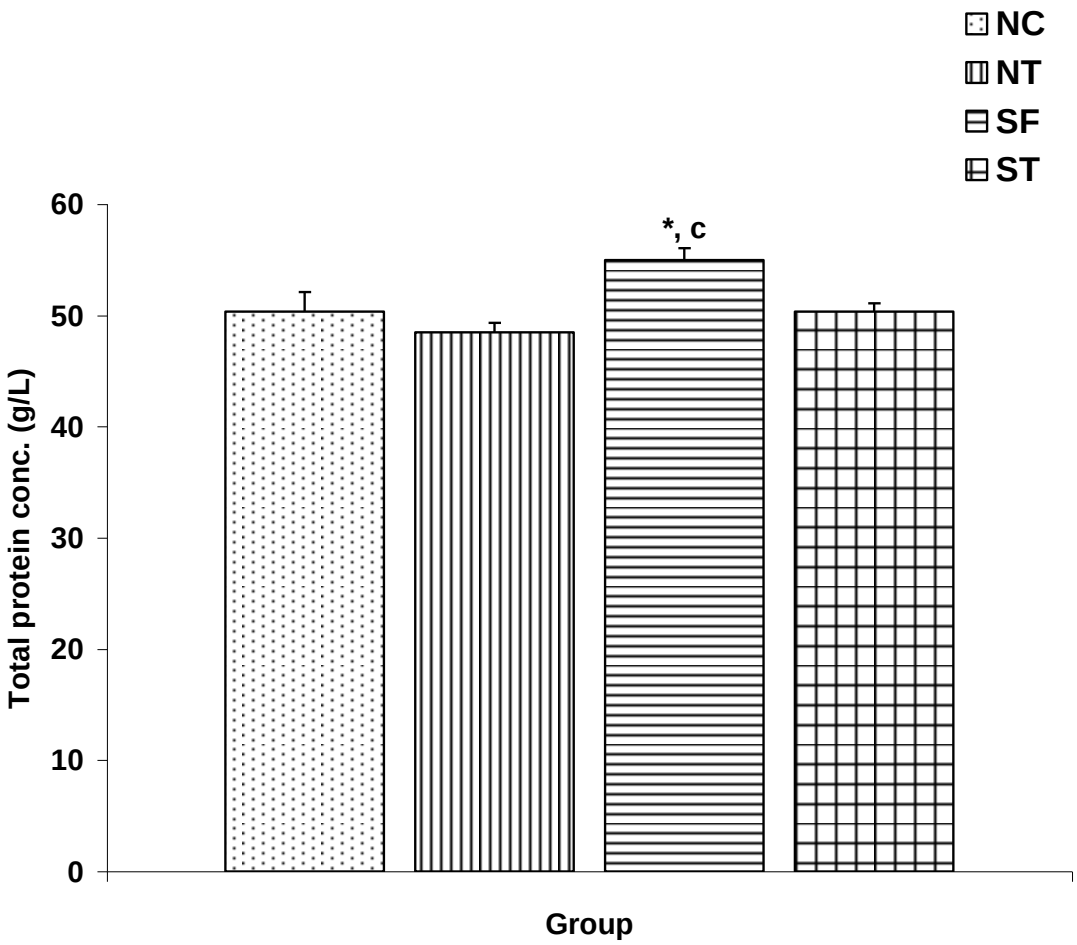

FIG. 4: Comparison of total protein concentrations in the different experimental groups. Values are expressed as mean ± SEM, n = 6.
***p<0.05 vs normal control (NC);**
c = p<0.001 vs normal treated (NT).

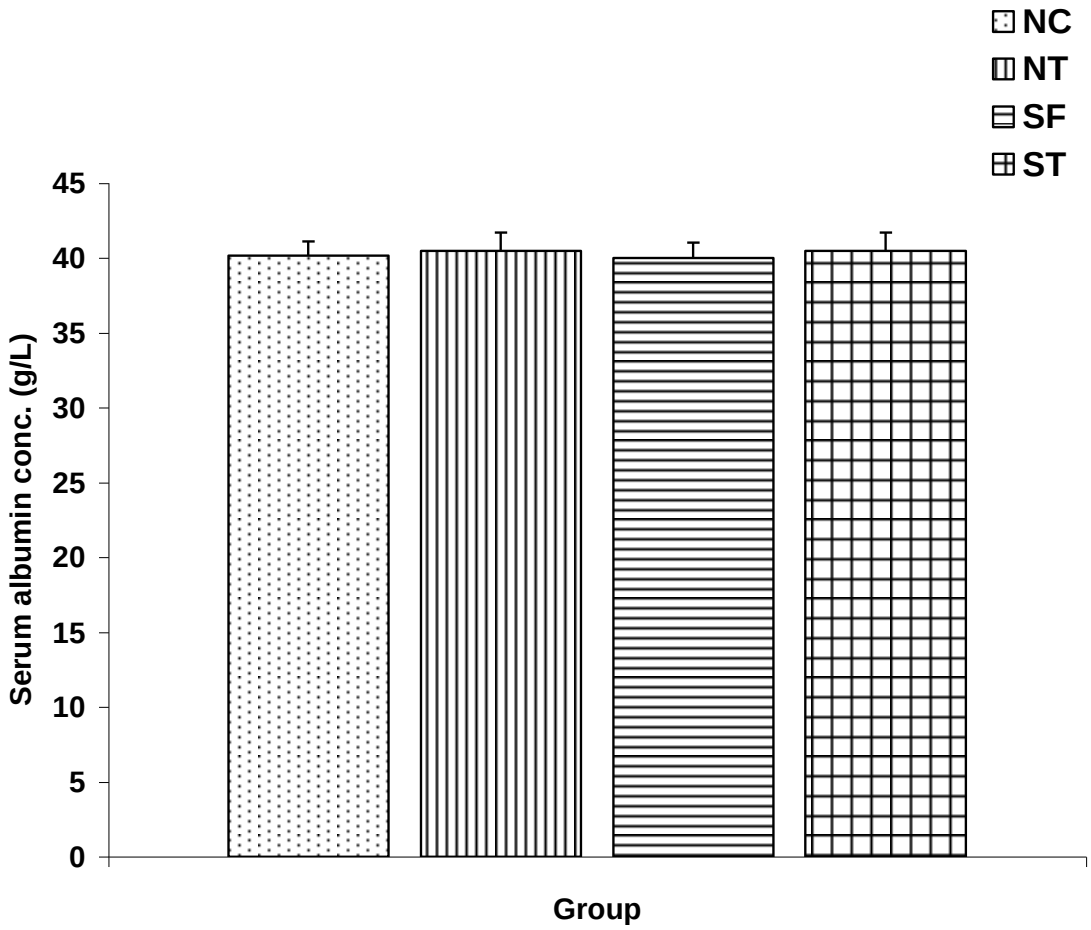

FIG. 5: Comparison of serum albumin concentrations in the different experimental groups. Values are expressed as mean ± SEM, n = 6.

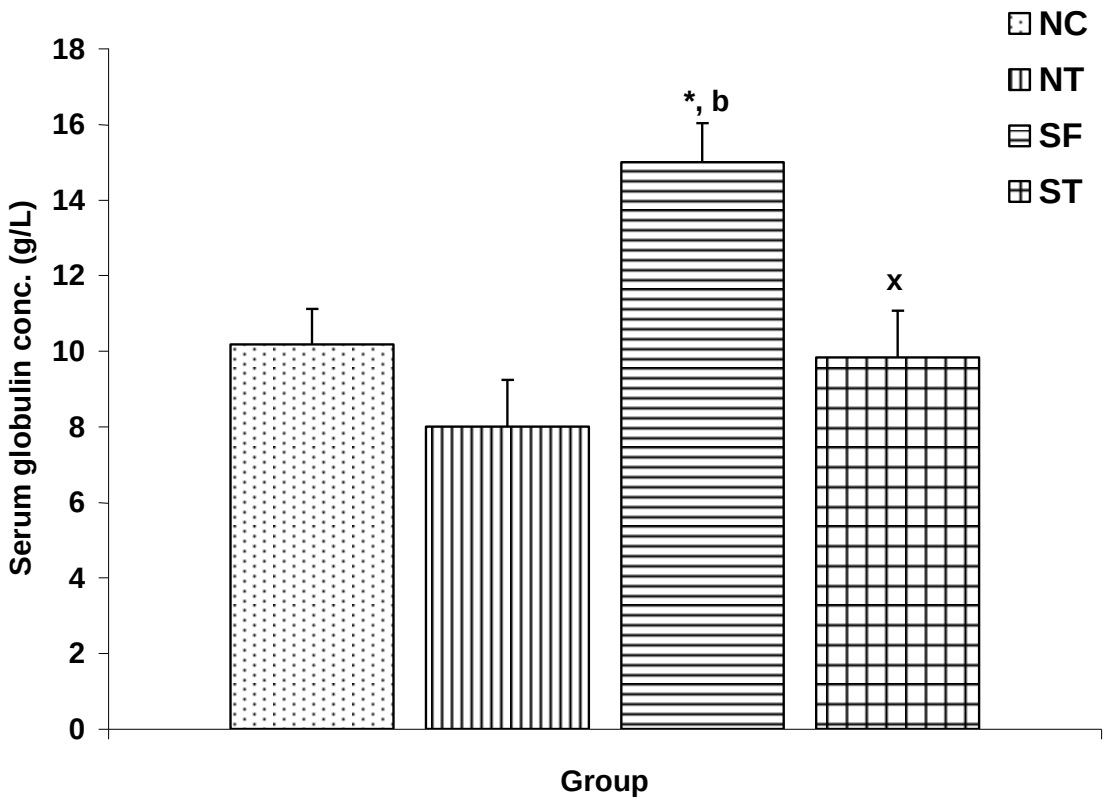

FIG. 6: Comparison of serum globulin concentrations in the different experimental groups. Values are expressed as mean ± SEM, n = 6.
***p<0.05 vs normal control (NC);**
b = p<0.01 vs normal treated (NT);
x = p<0.05 vs salt fed (SF).

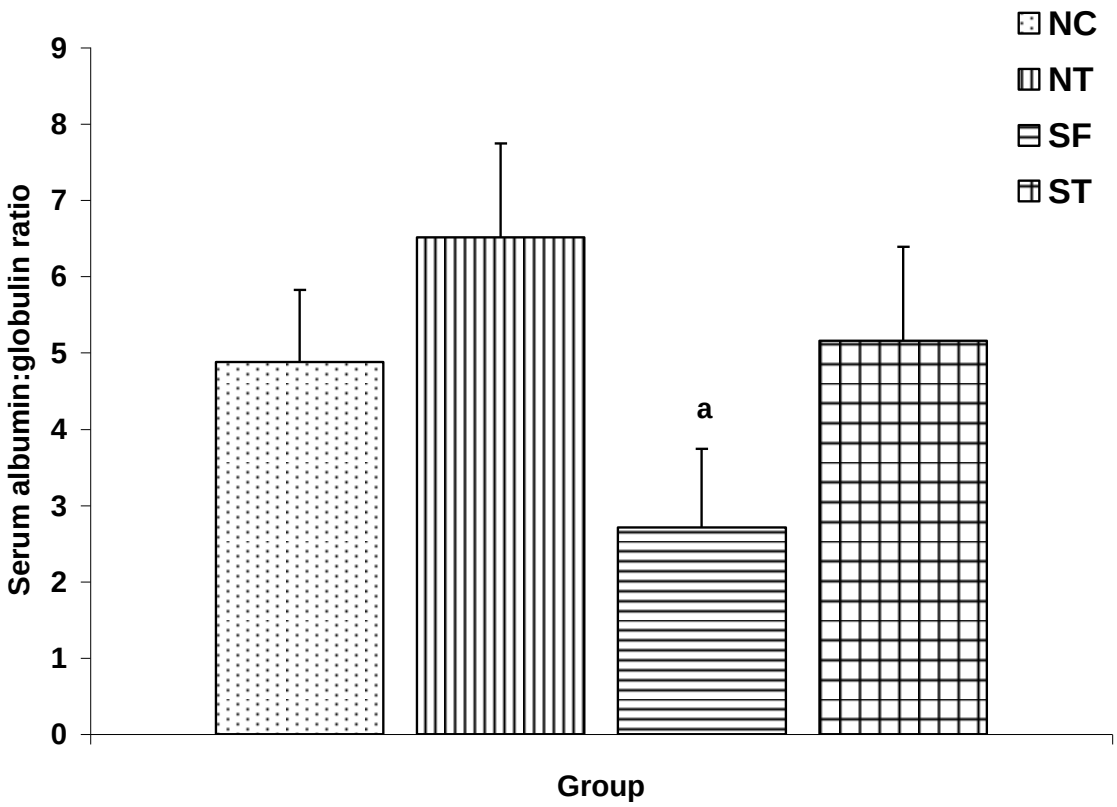

FIG. 7: Comparison of serum albumin:globulin ratio in the different experimental groups. Values are expressed as mean ± SEM, n = 6.
a = p<0.05 vs normal treated (NT)

TABLE 1: Comparison of total, conjugated and unconjugated bilirubin concentrations in the different experimental groups.

	Total bilirubin concentration (mmol/L)	Conjugated bilirubin concentration (mmol/L)	Unconjugated bilirubin concentration (mmol/L)	Ratio of conjugated to unconjugated bilirubin
Control	10.95 ±0.62	7.53 ±0.58	3.50 ±0.42	2.49 ±0.41
Masfon Aloe	11.05 ±0.42	6.87 ±0.31	4.18 ±0.57	1.87 ±0.34
Salt fed	13.22 ±0.49*, b	5.80 ±0.38*, a	7.42 ±0.81**, b	0.88 ±0.18**, a
Salt + Masfon Aloe	11.88 ±0.69	6.83 ±0.34	5.05 ±0.74x	1.48 ±0.19x

Values are expressed as mean ±SEM, n = 6.
*p<0.05, **p<0.01 compared with control;
a = p<0.05, b = p<0.01 compared with Masfon aloe;
x = p<0.05 compared salt fed.

DISCUSSION

The effect of Masfun *Aloe vera* on serum proteins and billirubin concentrations in high salt loaded fed rats was carried out in this study. The changes in growth rate, body weight, total protein, albumin and globulin concentration were used to assess the effect of high salt in rats. This study shows that high salt loading leads to loss of body weight in rats. This has previously been reported [29,30], as a consequence of insulin resistance following high salt load. Insulin resistance reduces the sensitivity of the tissues to insulin, leading to inability of the body to utilize glucose, this results in the use of proteins for energy production and tissue wasting. Although, it was observed that the salt fed rats did not each much, probably due to the non-palatability of their feed, rather they drank too much of the salt water. Their furs were wet and constantly erect, with perforated skin. It has been shown that high salt diet caused decreased food intake accompanied with slow growth rate and decrease body weight [31].

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 [32,33]. The plasma protein in blood exists at fairly constant levels, suggesting the existence of feedback mechanism for the cells [33]. In this study, the evidence is convincing that the Vitamins C and E supplementation had tremendous effect on the levels of the plasma protein concentration in high salt loaded rats.

In this study, high salt loading was observed to increase total plasma protein, and globulin concentrations. High salt intake has been reported as one of the causes of liver cirrhosis, [34,35]. The increased level of globulin in the salt fed rats would possibly be due to liver production of globulin sequel to cirrhosis from excessive salt loading. Cirrhosis of the liver has been associated with increased liver gamma-globulin producing cells and hence elevated plasma levels of globulin [36]. It is also possible that the increase in total protein observed in the salt loaded group could be due to both increase in globulin concentration and the attractive forces of sodium (cations) on the plasma protein to help achieve ionic equilibrium of plasma. The salt fed treated group had a lower globulin concentration compared to the salt fed group, this point to the tendency of the vitamins to tilt the balance of plasma protein level towards normal values. Although, it has been reported that high salt loading increases the load on the kidneys, leading to increase in the GFR and the tendency to excrete proteins, especially albumin in urine [37] and a fall in albumin levels in the high salt fed rats, this contradict our finding. We did not observe any significant changes in the albumin levels following high salt intake.

Chronic consumption of salt-rich (sodium chloride) diet significantly increased serum total bilirubin and unconjugated bilirubin concentrations. This is in line with previous reports [36] that high salt diet increased serum bilirubin concentration. Apart from the fact that chronic consumption of sodium chloride is a risk factor for hypertension, it is evident from our study that chronic consumption of sodium chloride is also a risk factor for hyperbilirubinemia. Elevated serum unconjugated bilirubin concentration most often indicates hepatic damage. In this condition, the hepatocytes can no longer conjugate bilirubin with glucuronide. Consequently, the unconjugated bilirubin re-enters circulation. High level of unconjugated bilirubin is also seen in severe haemolytic anaemia, when excessive unconjugated bilirubin overwhelms the liver's conjugating mechanisms. Increased serum conjugated bilirubin concentration on the other hand is indicative of possible biliary obstruction. Administration of Masfon aloe vera to the salt-fed treated group significantly reduced serum bilirubin levels when compared to the salt-fed untreated group (SF). Administration of Masfon aloe vera to the normal treated (NT) group did not significantly influence the serum bilirubin concentrations when compared to control (NC). This is suggestive of the presence of a negative feedback mechanism that ensures bilirubin concentration does not fall below normal, despite treatment with Masfon aloe vera.

Summarily, high salt loading led to increased levels of total protein, serum globulin, total and unconjugated bilirubin concentration which are implicated in the etiology of hepatic disorder. However, treatment with extract from Masfon *Aloe vera* reversed the adverse effects caused by high salt and consequently leads to improved body weight.

Conclusion

Masfon *Aloe vera* extract ameliorated the adverse effect of high salt diet on total serum protein, globulin, and bilirubin concentrations in rats

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