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Effect of *Aloe vera* gel on lipid profile and some serum biomarkers of liver function in high salt fed Wistar rats

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Abstract : *This study examined the effect of Aloe vera gel on lipid profile and some serum biomarkers of liver function in high salt-fed wistar rats. Twenty male wistar rats (200-250g) were randomly assigned into four groups (n=5): 1 (control) received rat feed and water; 2 (ALOE) received 600mg/kg of Aloe vera orally once daily; 3 (SALT) received high salt diet (8% NaCl feed + 1% NaCl drinking water); 4 (SALT+ALOE) received high salt diet + Aloe vera. The feeding regimen lasted for six weeks after which blood samples were collected for analysis. Total protein and globulin concentration was significantly (p<0.001) increased in ALOE and SALT groups compared with control. AST, ALT and ASP were significantly (p<0.001) increased in ALOE and SALT+ALOE groups compared with control. Total and conjugated bilirubin concentration was significantly (p<0.001) increased in SALT and SALT+ALOE compared with control and ALOE. Unconjugated bilirubin concentration was significantly decreased in SALT compared with control (p<0.01) and ALOE (p<0.001). MDA was significantly (p<0.05) decreased in SALT+ALOE compared with SALT. TC and LDL-c were significantly decreased in SALT+ALOE compared with SALT. Aloe vera gel therefore exhibited hepatoprotective effect and has the potential to treat hyperlipidaemia and hyperbilirubinaemia associated with high salt intake.*

Keywords - *Aloe vera gel, high salt diet, lipid, liver, rats*

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I. Introduction

Salt (sodium chloride) is an essential component of life but excessive intake of it is dangerous to health. High salt intake has been reported to cause several deleterious effects in the body [1-4]. Studies have associated these deleterious effects of high salt intake with oxidative stress [5, 6] and hepatocytes are not spared from the menace of reactive oxygen species [7].

The liver is one of the largest and vital organs of the human body. It is involved in biotransformation, detoxification and excretion of various endogenous and exogenous compounds. These activities are affected when the liver is diseased or damaged. Liver disease is still a global health problem [8]. The synthetic or conventional drugs used to treat liver disease are not adequate and sometimes have serious side effects [8]. This has led to the employment of herbal medicines in the treatment of liver diseases [9-11]. *Aloe vera* is a plant that has recorded hepatoprotective effect [8, 12-17]. It is a cactus-like plant of the family, Asphodelaceae and grows in hot dry climate [15]. *Aloe vera* can be separated into two basic products: gel and latex. *Aloe vera* gel has recorded other therapeutic effects aside hepatoprotective effect [8, 18-23].

However, to date, the effect of *Aloe vera* gel on liver function of rats exposed to high salt diet has not been studied. In view of the high demand for traditional herbal medicines in underdeveloped countries [16] and the paucity of information on the effect of *Aloe vera* gel on the liver following high salt diet, this study sought to examine the effect of *Aloe vera* gel on some serum biomarkers of liver function in Wistar rats exposed to high salt diet. Additionally, the study investigated the effect of *Aloe vera* gel on lipid profile of Wistar rats fed on high salt diet on the background that high salt intake is associated with cardiovascular complications [4].

II. MATERIALS AND METHODS

1.1 Experimental Animals

Twenty (20) male wistar rats weighing 200-250g were bought from the Department of Agriculture, University of Calabar. Ethical standards laid down in 1964 declaration of Helsinki [24] were employed in handling the animals. The animals were acclimatized for seven (7) days, given rat feed and water *ad libitum* and exposed to 12/12 hours light/dark cycle. They were kept in properly ventilated transparent plastic cages in the animal house of Physiology Department, University of Calabar.

1.2 Plant Material and Preparation of *Aloe vera* Gel Extract

Aloe vera plant was harvested from a garden in University of Calabar and the fresh leaves thoroughly washed using tap water to remove dirt. The base and apex were cut with surgical blades and the leaves sliced open along the margin to reveal the transparent mucilage. A spatula was then used to scoop the mucilage. An electric blender was used to blend the mucilage for twenty minutes to obtain a greenish gel-like liquid. The liquid obtained was allowed to settle for twenty minutes and thereafter a Whatman filter paper was used to sieve it to obtain a particulate-free gel [25]. After use each day, the extract was refrigerated at 4-6°C for three days.

2.3 Preparation of High Salt Diet

High salt diet was prepared as described by Obiefuna and Obiefuna [26].

2.4 Experimental Design and Extract Administration

The twenty male wistar rats were randomly assigned into four groups of five (5) rats each. Group 1 served as control and received rat feed and water. Group 2 was the Aloe vera (ALOE) group and received *Aloe vera* (600mg/kg) orally once daily. Group 3, the Salt-fed (SALT) group received high salt diet (8% NaCl feed + 1% NaCl drinking water). Group 4, the salt+Aloe (SALT+ALOE) group received in addition to high salt diet, *Aloe vera* at 600mg/kg orally once daily. All groups had access to rat feed and water throughout the duration (six weeks) of the experiment.

2.5 Collection of Blood Samples

The rats were anaesthetized using 3.5 % chloroform and blood samples collected via cardiac puncture using 5mL syringe attached to 21G needle into plain capped bottles. The samples were left for two hours to clot after which they were centrifuged at 10,000 rpm for 10 minutes and the serum collected for analysis of lipid profile and some biomarkers of liver function.

2.6 Determination of Serum Proteins Concentration

2.6.1 Total Protein

Total protein was determined by analysing the blood samples using Biuret method. It is based on the principle that cupric ions in alkaline solution react with peptide bonds present in proteins yielding violet colour that is proportional to the amount of protein present.

2.6.2 Albumin

Serum albumin concentration was determined as described by Reinhold [27]. This is based on the principle that albumin binds with bromocresol green at a PH of 4.2 causing a slight in absorbance of the yellow BCG dye. A blue green colour is formed in the process and is proportional to albumin concentration when measured photo metrically between 580-630nm with maximum absorbance at 625nm measured.

2.6.3 Globulin

Globulin concentration was calculated as the difference between the total plasma protein and serum albumin concentration.

Globulin = Total plasma protein – serum albumin concentration.

2.7 Determination of Serum Enzymes Concentrations

2.7.1 Aspartate aminotransferase and Alanine aminotransferase

AST and ALT were measured as described by Reitman and Frankel [28]. This is based on the principle that transamination by ALT produces pyruvate which reacts with 2,4-dinitrophenylhydrazine to produce a brown-coloured hydrazine which is measured in the colorimeter at 510nM.

The reaction with AST forms an oxaloacetate which decarboxylates spontaneously to pyruvate which is also measured by formation of hydrazine. The calculation is thus:

$\frac{T-TB}{S-SB}$ X 67/Umol/min/L for AST

$\frac{T-TB}{S-SB}$ X 133/mol/Umin/L for ALT

Where T = Test

TB =Test Blank

S = STD

SB = STD Blank

2.7.2 Alkaline Phosphatase

Alkaline phosphatase concentration was determined according to the modified method of King and Armstrong [29]. The principle is that phenol released by enzymatic hydrolysis from phenylphosphate under defined conditions of time, temperature and PH is estimated colorimetrically. A test tube containing a mixture of 1mL of buffer and 1mL of phenylphosphate substrate was placed in a water bath at 37°C for 3 minutes. 0.1ml of serum was added, mixed gently and incubated for 15 minutes and the reaction was halted by adding 0.8mL of 0.5N sodium hydroxide

Control: In a test tube, 1mL substrate was mixed with 0.8mL of 0.5N sodium hydroxide followed by 0.1mL of serum

Standard: 1.1mL of buffer was mixed with 0.1mL of phenol standard (1mg/100mL) and 0.8mL of 0.5N sodium hydroxide.

Blank: 1.1mL of buffer, 1.0mL of water and 0.8mL of 0.5N sodium hydroxide was mixed. To all tubes, 1.2mL of 0.5N $NaHCO_3$ was added with 1mL of $K_3(Fe(CN)_6)$. The content of each tube was well mixed after each addition and the successive additions adjusted the PH to develop the colour. The 0.0 of reddish-brown colours of 510 nM (nanometer) was read avoiding exposure to strong sunlight.

The calculation is thus:

$$\text{Serum ALT (King} - \text{Armstrong Units/100ml} = \frac{\text{Reading of unknown} - \text{Reading of control}}{\text{Reading of standard} - \text{Reading of blank}} X\ 100$$

2.8 Estimation of Serum Bilirubin Concentration

Serum bilirubin concentration was estimated using Sherlock and Lunec [30] method.

2.9 Determination of Hepatic Malondialdehyde (MDA) and Glutathione Peroxidase (GPx) Concentration

The liver of each rat was removed and homogenized using a Potter-Elvehjem homogenizer at 0°C in phosphate buffer containing 1nM ethylenediaminetetracetate (EDTA) and centrifuged at 10,000g for 15minutes at 4 °C. The supernatant was collected for MDA and GPx assays.

Lipid peroxidation was quantified as MDA as described by Ohkawa *et al.* [31]. Hepatic GPx activity was determined using hydrogen peroxide as substrate according to the method of Luchese *et al.* [32].

2.10 Determination of Serum Lipid Profile

Serum total cholesterol concentration was determined using enzymatic calorimetric method of Siedel *et al.* [33]. Serum triglyceride concentration was determined using the method described by Negele *et al.* [34]. Siedel *et al.* [33] method was used to measure serum high density lipoprotein cholesterol concentration. Very low density lipoprotein cholesterol concentration was determined by dividing the serum TG concentration by 2.2.

$$\text{VLDL (mg/dL)} = \frac{\text{Serum TG}}{5}$$

Low density lipoprotein cholesterol was determined using Friedewald's relationship [35]:

LDL = TC – (HDL + VLDL).

2.11 Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) along with post hoc multiple comparison test (least square difference). Computer software, Statistical Package for Social Sciences (SPSS) (version 21, Microsoft Company, USA) was used for the analysis. $p<0.05$ was considered statistically significant.

III. Results

3.1 Comparison of Serum Proteins Concentration in the Different Experimental Groups

Serum total protein concentration (g/L) for control, ALOE, SALT, AND SALT+ALOE groups was 63.50 ± 1.26, 79.50 ± 1.71, 89.50 ± 0.65 and 66.75 ± 0.48 respectively. Total protein concentration was significantly ($p<0.001$) increased in ALOE and SALT groups compared with control. It was significantly ($p<0.001$) increased in SALT group compared with ALOE group. Serum total protein concentration was significantly ($p<0.001$) decreased in SALT+ALOE group compared with ALOE and SALT groups (Figure 1).

Serum albumin concentration (g/L) for control, ALOE, SALT and SALT+ALOE groups was 38.25 ± 1.31, 36.50 ± 1.26, 33.00 ± 1.29 and 41.00 ± 1.29 respectively. Albumin concentration was significantly ($p<0.05$) decreased in SALT group compared with control. It was significantly increased in SALT+ALOE group compared with ALOE ($p<0.05$) and SALT ($p<0.001$) groups (Figure 2).

Serum globulin concentration (g/L) for control, ALOE, SALT AND SALT+ALOE groups was 25.25 ± 1.11, 43.00 ± 0.58, 56.50 ± 1.19 and 25.75 ± 1.55 respectively. Serum globulin concentration was significantly

($p<0.001$) increased in ALOE and SALT groups compared with control. It was significantly increased ($p<0.001$) in SALT group compared with ALOE group and decreased ($p<0.001$) in SALT+ALOE group compared with ALOE and SALT groups (Figure 3).

Albumin-globulin ratio for control, ALOE, SALT AND SALT+ALOE groups was 1.53 ± 0.10, 0.85 ± 0.02, 0.59 ± 0.03 and 1.62 ± 0.14 respectively. Albumin-globulin ratio was significantly ($p<0.001$) decreased in ALOE and SALT groups compared with control. It was significantly ($p<0.001$) increased in SALT+ALOE group compared with ALOE and SALT groups.

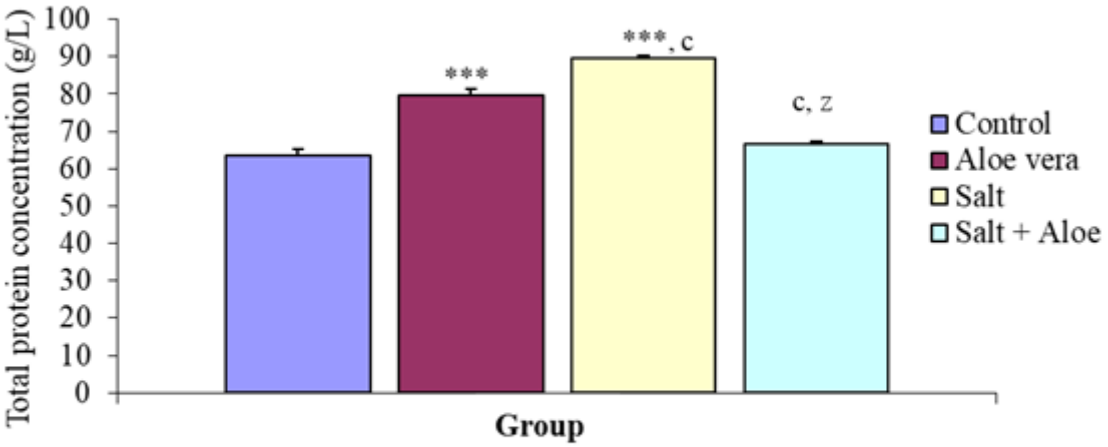

Figure 1: Comparison of total protein concentration in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera

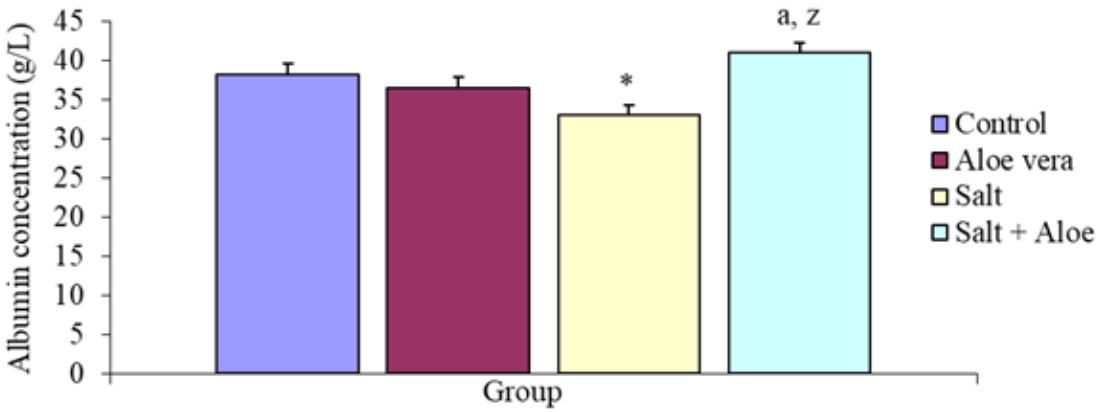

Figure 2: Comparison of serum albumin concentrations in control and test groups.

Values are expressed as mean ± SEM, n=5
*p<0.05 vs Control
a = p<0.05 vs Aloe vera
z = p<0.001 vs Salt

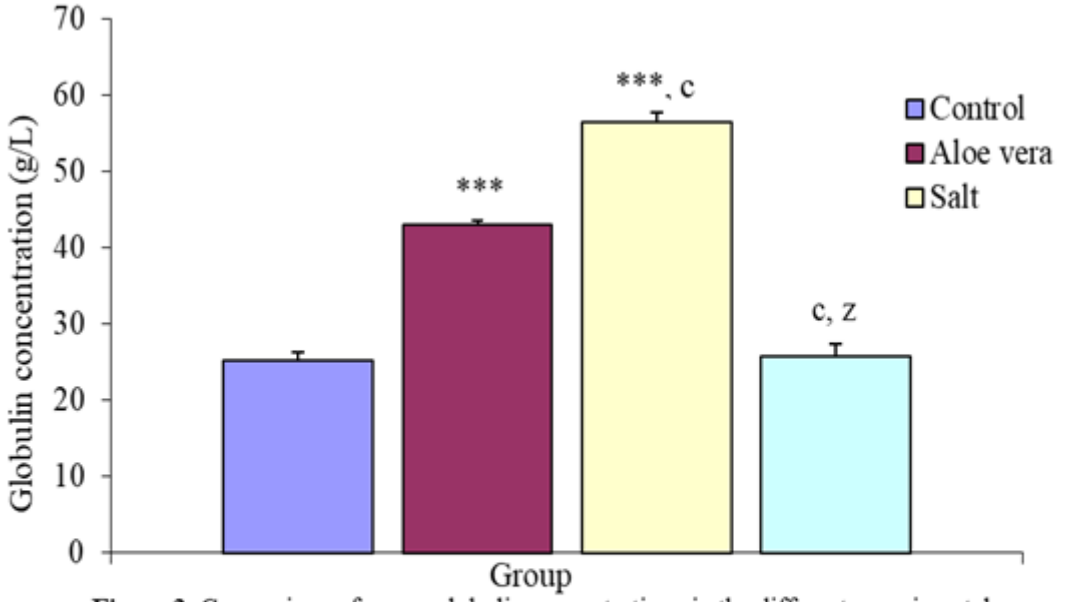

Figure 3: Comparison of serum globulin concentrations in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera
z = p<0.001 vs Salt

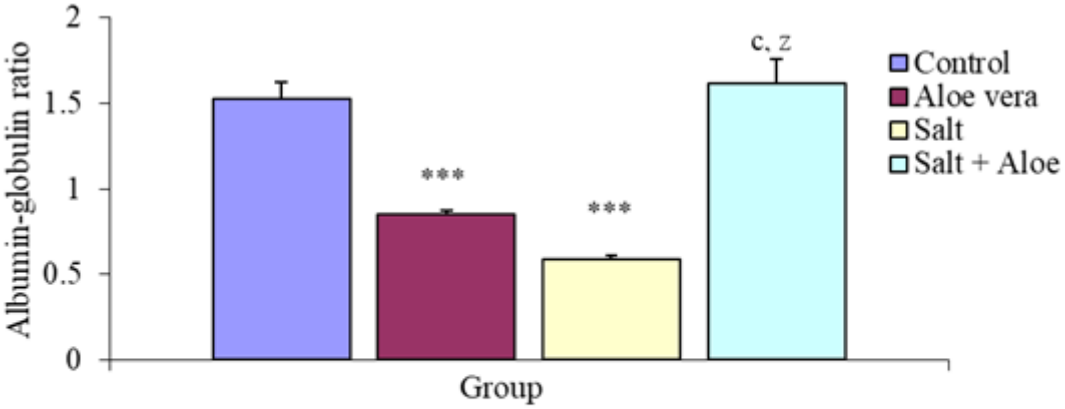

Figure 4: Comparison of albumin-globulin ratio in the different experimental groups.

Values are expressed a s Mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera
z = p<0.001 vs Salt

3.2 Comparison of Serum Enzymes Concentration in the Different Experimental Groups

Serum aspartate aminotransferase (AST) concentration (IU/L) for control, ALOE, SALT AND SALT+ALOE groups was 295.00± 2.35, 364.25± 5.98, 256.50± 1.76 and 499.00± 3.03 respectively. AST concentration was significantly ($p<0.001$) decreased in SALT group compared with control and ALOE groups. It was significantly ($p<0.001$) increased in ALOE and SALT+ALOE groups compared with control. AST was also significantly ($p<0.001$) increased in SALT+ALOE group compared with ALOE and SALT groups (Figure 4).

Serum alanine aminotransferase (ALT) concentration (IU/L) for control, ALOE, SALT AND SALT+ALOE groups was 214.50± 4.19, 255.25± 5.85, 218.00± 5.10, and 453.25± 2.69 respectively. ALT concentration was significantly ($p<0.001$) increased in ALOE and SALT+ALOE groups compared with control. It was significantly decreased ($p<0.001$) in SALT group compared with ALOE group and increased ($p<0.001$) in SALT+ALOE group compared with ALOE and SALT groups (Figure 5).

Serum alkaline phosphatase (ALP) concentration (IU/L) for control, ALOE, SALT AND SALT+ALOE groups was 258.25 ± 4.48, 365.75 ± 6.49, 449.50 ± 4.27 and 681.00 ± 11.37 respectively. ALP concentration was significantly ($p<0.001$) increased in all treatment groups compared with control. It was also significantly ($p<0.001$) increased in SALT and SALT+ALOE groups compared with ALOE group. ALP concentration was also significantly increased in SALT+ALOE group compared with SALT group (Figure 6).

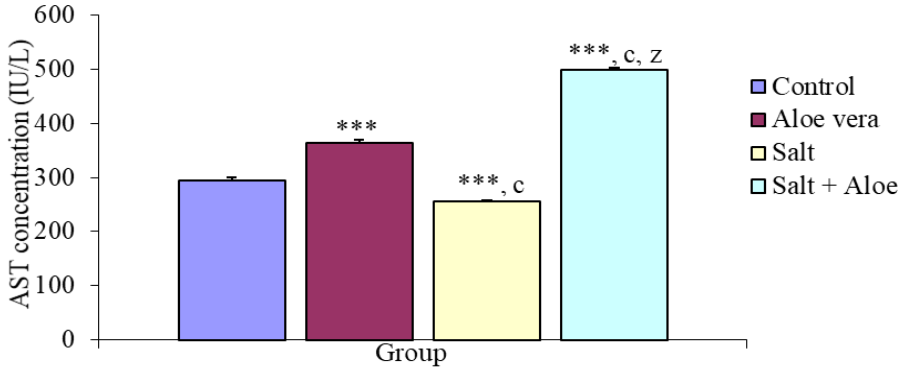

Figure 4: Comparison of aspartate aminotransferase concentration in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera
z = p<0.001 vs Salt

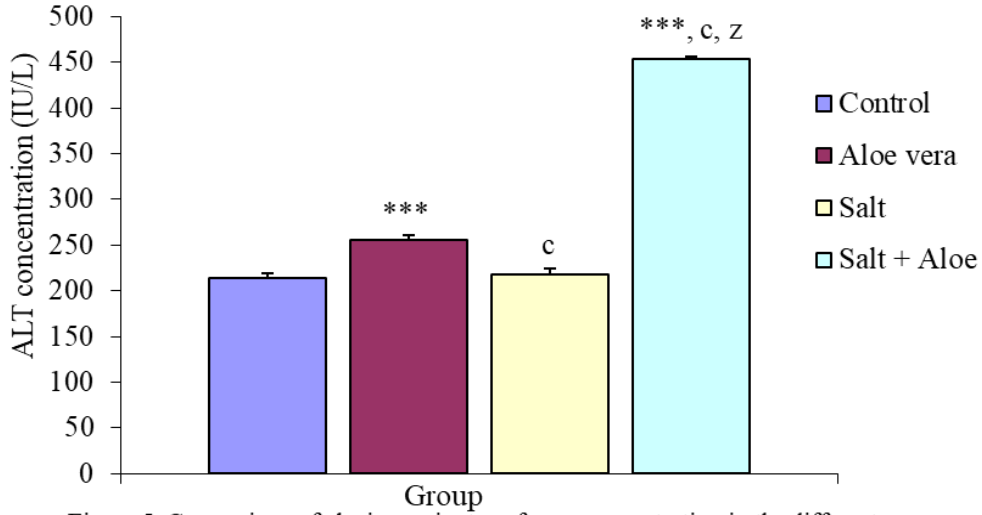

Figure 5: Comparison of alanine aminotransferase concentration in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera
z = p<0.001 vs Salt

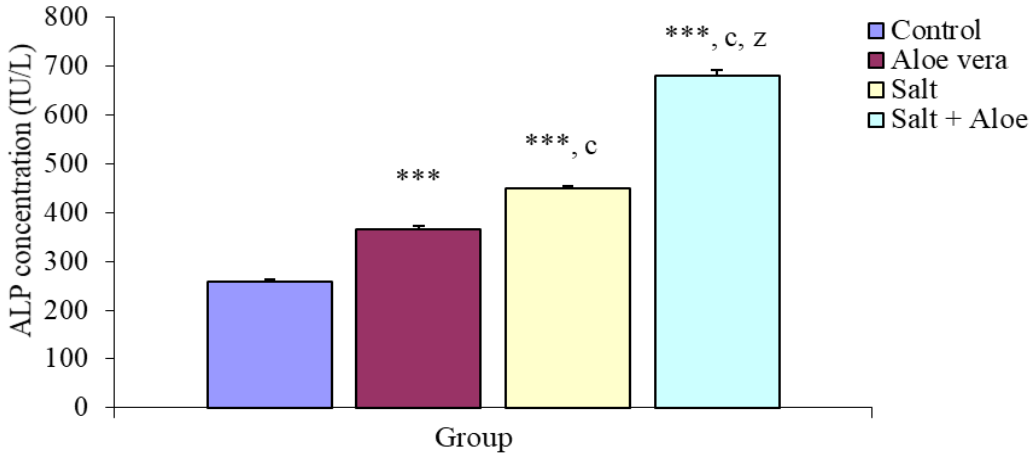

Figure 6: Comparison of alkaline phosphatase concentrations in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs Control
c = p<0.001 vs Aloe vera
z = p<0,001 vs Salt

3.3 Comparison of Bilirubin Concentrations in the Different Experimental Groups

3.3.1 Mean Total Bilirubin Concentration

Mean total bilirubin concentration (µmol/L) for control, ALOE, SALT, and SALT+ALOE groups was 3.50±0.17, 4.00±0.36, 5.60±0.14 and 6.13±0.14 respectively. Mean total bilirubin concentration was significantly ($p<0.001$) increased in SALT and SALT+ALOE groups compared with control and ALOE groups. It was not significantly different between control and ALOE groups (Figure 7).

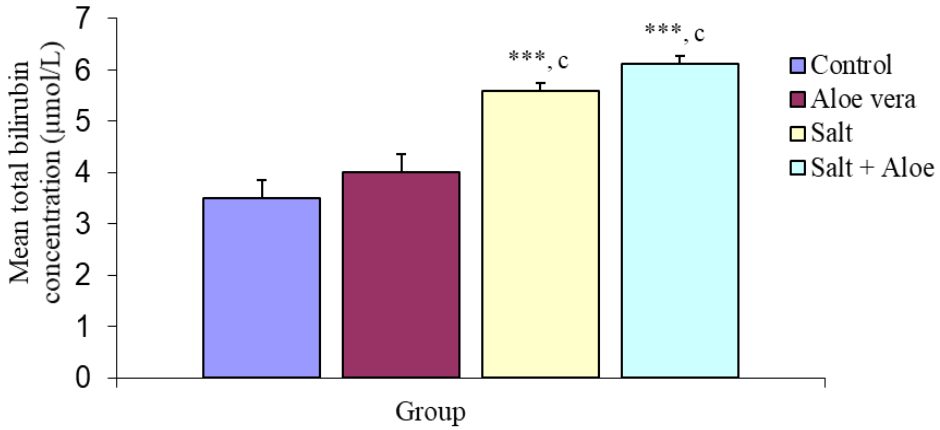

Figure 7: Comparison of mean total bilirubin concentration in the different experimental groups.

Values are expressed as mean ± SEM, n=5
***p<0.001 vs control
c= p<0.001 vs Aoe

3.3.2 Mean Conjugated Bilirubin Concentration

Mean conjugated bilirubin concentration (µmol/L) for control, ALOE, SALT, and SALT + ALOE groups was 2.08±0.11, 2.23±0.17, 4.93±0.26 and 4.20±0.15 respectively. Mean conjugated bilirubin concentration was significantly ($p<0.001$) increased in SALT and SALT+ALOE groups compared with control and ALOE groups. It was significantly ($p<0.05$) decreased in SALT+ALOE group compared with SALT group. Mean conjugated bilirubin concentration was not significantly different between control and ALOE groups (Figure 8).

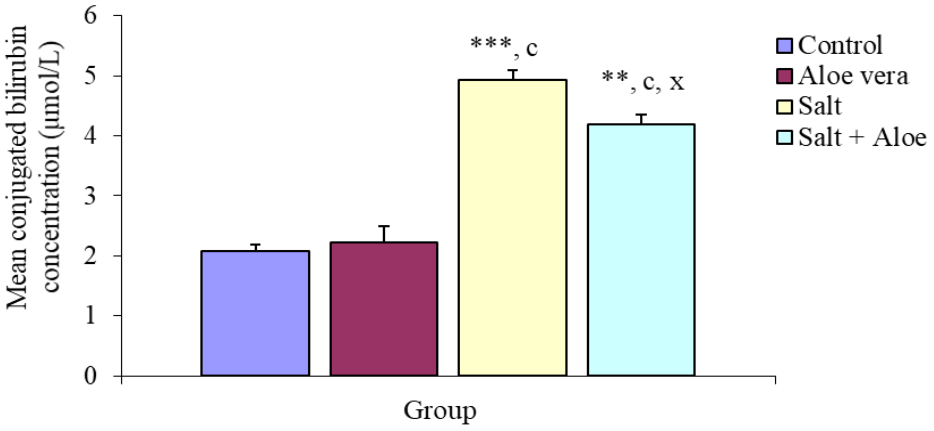

Figure 8: Comparison of mean conjugated bilirubin concentration in the different experimental groups

Values are expressed as mean ± SEM, n=5
***p<0.001 vs control
c= p<0.001 vs Aloe, x = p<0.05 vs Salt

3.3.3 Mean Unconjugated Bilirubin Concentration

Mean unconjugated bilirubin concentration (µmol/L) for control, ALOE, SALT, and SALT+ALOE groups was 1.43±0.14, 1.78±0.24, 0.68±0.15 and 1.93±0.03 respectively. Mean unconjugated bilirubin concentration was significantly decreased in SALT group compared with control ($p<0.01$) and ALOE ($p<0.001$) groups. It was significantly increased in SALT+ALOE group compared with control ($p<0.05$) and SALT ($p<0.001$) groups (Figure 9).

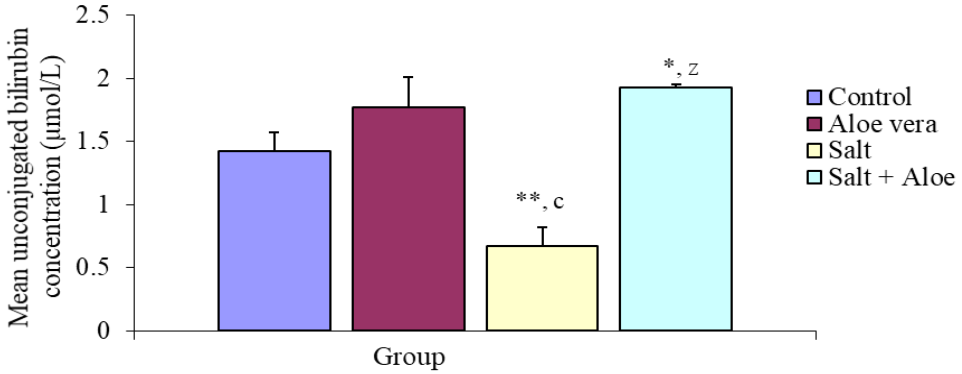

Figure 9: Comparison of unconjugated bilirubin concentration in the different experimental groups.

Values are expressed as mean ± SEM, n=5
*p< 0.05, **p<0.01 vs control
c = p<0.001 vs Aloe, z = p<0.001 vs Salt

3.4 Comparison of Hepatic Malondialdehyde and Glutathione Peroxidase concentration in the Different Experimental Groups

Table 1 shows malondialdehyde (nmol/mg protein) and glutathione peroxidase (nmol/mg protein) concentration for control, ALOE, SALT and SALT+ALOE groups. Malondialdehyde (MDA) concentration was significantly ($p<0.001$) increased in SALT group compared with control and ALOE group. It was significantly ($p<0.05$) decreased in SALT+ALOE group compared with SALT group.

Glutathione peroxidase (GPx) concentration was significantly ($p<0.01$) decreased in SALT group compared with ALOE group. It was not significantly different between control, ALOE and SALT + ALOE groups.

Table 1: Comparison of hepatic malondialdehyde and glutathione peroxidase concentration in the different experimental groups

Parameter	Control	ALOE	SALT	SALT+ALOE
MDA (µmol/mg protein)	10.00±0.95	9.60±0.6^{ns}	14.60±1.30^{***,c}	11.20±0.66^{x}
GPx (µmol/mg protein)	725.00±40.63	885.00±73.06^{ns}	613.60±50.11^{b}	745.80±67.02^{ns}

values are expressed as mean ± SEM, n = 5.
ns = not significant vs control
***p<0.001 vs control
b = p<0.01, c = p<0.001 vs ALOE
x = p<0.05 vs SALT

3.5 Comparison of Lipid Profile in the Different Experimental Groups

Table 2 shows lipid profile (mmol/L) for control, ALOE, SALT and SALT+ALOE groups.

Serum total cholesterol concentration was significantly ($p<0.001$) increased in SALT and SALT+ALOE groups compared with control and ALOE groups. It was significantly decreased in SALT+ALOE group compared with SALT group.

Serum triglyceride concentration was significantly increased ($p<0.001$) in ALOE group and decreased ($p<001$) in SALT and SALT+ALOE groups compared with control. It was also significantly ($p<0.001$) decreased in SALT+ALOE group compared with SALT group.

Serum high density lipoprotein was significantly ($p<0.001$) decreased in SALT group compared with control and ALOE groups. It was significantly ($p<0.001$) increased in SALT+ALOE group compared with control, ALOE and SALT groups.

Serum low density lipoprotein concentration was significantly decreased in ALOE ($p<0.001$), SALT ($p<0.05$) and SALT+ALOE ($p<0.01$) compared with control. It was significantly increased in SALT ($p<0.001$) and SALT+ALOE ($p<0.05$) groups compared with ALOE group.

Very low density lipoprotein was significantly ($p<0.001$) increased in all treatment groups compared with control. It was significantly ($p<0.001$) increased in SALT and SALT+ALOE group compared with ALOE group and decreased ($p<0.001$) in SALT+ALOE group compared with SALT group.

Table 2: Comparison of lipid profile in the different experimental groups

Parameter	CONTROL	ALOE	SALT	SALT+ALOE
TC	1.25± 0.01	1.23 ± 0.02	1.72± 0.02^{***,c}	1.36± 0.01^{***,c,z}
TG	0.73± 0.01	0.97± 0.01***	1.61± 0.02^{***,c}	1.07± 0.01^{***,c,z}
HDL-c	0.31±0.00	0.31± 0.00	0.28± 0.00^{***,c}	0.34± 0.00^{***,c,z}
LDL-c	0.60 ± 0.01	0.49± 0.01***	0.56± 0.001^{*,c}	0.53± 0.01^{**,a}
VLDL-c	0.34± 0.01	0.44± 0.00***	0.73± 0.01^{***,c}	0.49± 0.01^{***,c,z}

values are expressed as mean ± SEM, n = 5.
ns = not significant vs control
*p<0.05, **p<0.01, ***p<0.001 vs control
a= p<0.05, c= p<0.001 vs ALOE
z= p<0.001 vs SALT

IV. Discussion

High salt intake is associated with cardiovascular complications [4], liver toxicity and fibrosis [36, 37]. *Aloe vera* is a cactus-like plant of the family, Asphodelaceae. It has been reported to alleviate cardiovascular system disorder [38] and exhibit hepatoprotective effect [8, 12-14]. This study examined the effect of *Aloe vera* gel on lipid profile and some serum biomarkers of liver function in rats fed on high salt diet.

Total protein concentration was significantly increased in ALOE and SALT groups compared with control. This is contrary to Ofem *et al.* [37] who reported significant decrease in total protein concentration following high salt diet. Albumin concentration was not significantly different between control and ALOE groups whereas globulin concentration was significantly increased in ALOE and SALT groups compared with control and albumin concentration decreased in SALT group compared with control. From these results, it is

clear that high salt diet and *Aloe vera* administered separately improved globulin secretion by the liver which could be the cause of the increase in total protein observed in ALOE and SALT groups. Albumin forms a larger proportion of all serum and plasma proteins. The decrease in albumin concentration observed in the SALT group is an indication that high salt intake impacted negatively on the liver. *Aloe vera* however demonstrated a protective effect as albumin concentration and albumin-globulin ratio were significantly increased in SALT+ALOE group compared with SALT group. The results of this study indicate that separate administration of *Aloe vera* gel and high salt diet promote the synthesis of globulin whereas their combination decrease and increase globulin and albumin synthesis respectively.

Serum AST concentration was significantly increased in ALOE and SALT+ALOE groups compared with control. It was significantly decreased in SALT group compared with control and ALOE group. Serum ALT concentration was significantly increased in ALOE and SALT + ALOE groups compared with control and decreased in SALT group compared with ALOE group. ALP was significantly increased in all treatment groups compared with control. It was significantly increased in SALT and SALT+ALOE groups compared with Aloe group. AST, ALT and ALP are normally released into the blood when hepatocytes are damaged [39]. Our findings are consistent with Akpan *et al.* [40] who reported significant increase in serum AST and ALT following *Aloe vera* administration. It is normal to think that these elevation of serum AST and ALT in ALOE group indicates liver damage caused by *Aloe vera* administration. But this may not be the case. In a previous study, histological examination of the liver tissue following *Aloe vera* administration showed no serious defect [40]. Results from the present study also show that liver MDA and GPx concentration was not significantly different between ALOE and control groups (Table 1) indicating that *Aloe vera* did not cause damage to the liver. *Aloe vera* may be increasing serum AST and ALT concentration through a mechanism other than liver damage. Increased AST and ALT have been associated with increase in body weight. *Aloe vera* gel had been reported to increase body weight in normal animals without increasing food intake [41].

Hepatic MDA was significantly increased in SALT group compared with control and ALOE groups. Hepatic GPx was significantly decreased in SALT group compared with ALOE group. It was also decreased in SALT group although not significant compared with control. These results indicate that high salt diet caused oxidative damage to the liver and *Aloe vera* is able to protect the liver from damage associated with high salt intake. This is seen in the SALT+ALOE group that had significantly reduced MDA concentration compared with SALT group. The SALT+ALOE group also presented an increase although not significant in GPx concentration compared with control and SALT groups. Liver damage is associated with increase in AST and ALT concentration but AST is a more sensitive marker of hepatocellular damage than ALT [42]. Owing to the fact that high salt intake causes liver damage, it is not clear from the present study why AST was significantly decreased in SALT group compared with control and the concentration of ALT was almost the same in these groups.

Total, conjugated and unconjugated bilirubin concentrations were not significantly different between control and ALOE groups. This suggests the presence of negative feedback mechanism which enables *Aloe vera* to maintain bilirubin concentrations within normal limits.

Total and conjugated bilirubin concentrations were significantly increased in SALT and SALT+ALOE groups compared with control and ALOE groups. This is consistent with earlier reports [37,43] that high salt diet increased total and conjugated bilirubin concentrations in rats. These results indicate that chronic consumption of NaCl is a risk factor for hyperbilirubinaemia. Conjugation of unconjugated bilirubin with glucoronic acid in the liver is catalyzed by uridinediphosphateglucuronyltransferase (UDP-GT). The resulting conjugated bilirubin is excreted by the liver in bile into the gut where some is lost in faeces and the rest reabsorbed back into the blood through the mucosa of the intestine. Elevated level of conjugated bilirubin is indicative of biliary obstruction which can cause obstructive jaundice [44]. Our result for increased serum conjugated bilirubin concentration in the SALT group suggests that chronic consumption of NaCl may have enhanced conjugation but caused obstruction of the bile ducts due to damaged hepatocytes or precipitation of NaCl in the bile duct which probably led to the hyperbilirubinaemia. Elevated serum bilirubin concentration may arise from damage to liver, presence of immature red blood cells or Gilbert syndrome [45]. High salt intake has been reported to cause damage to hepatocytes [36, 37]. *Aloe vera* however demonstrated the potential to reduce the possible obstruction of bile ducts which may be associated with high salt intake as conjugated bilirubin concentration was significantly decreased in SALT+ALOE group compared with SALT group (Figure 2). This feature is probably attributed to the phytochemicals in *Aloe vera. Aloe vera* contains cellulase, alkaline phosphatase, lipase, amylase, carboxypeptidase, catalase and peroxidase [46]. *Aloe vera* has been previously reported to reduce serum bilirubin concentration in rabbits [16].

Unconjugated bilirubin on the other hand was significantly decreased in SALT group compared with control and ALOE group. This suggests that the increase in total bilirubin concentration observed in the SALT group was not due to red blood cell (RBC) haemolysis. Previous study shows that high salt intake increases RBC count and packed cell volume [47]. Our result suggests that high salt intake facilitated the rate of bilirubin

conjugation which is why the concentration of the unconjugated bilirubin was low and that of the conjugated bilirubin, high. Mean unconjugated bilirubin concentration was however significantly increased in SALT+ALOE group compared with control and SALT groups. It was also increased although not significant in ALOE group compared with control. These results indicate that *Aloe vera* also reduced serum conjugated bilirubin concentration by probably reducing the rate of bilirubin conjugation in the liver or causing a deficiency in the transport of bilirubin across the liver as presented in Gilbert's syndrome [48].

Lipid profile is an important index that shows whether or not a person is likely to develop cardiovascular disease. The result of this study shows the hypercholesterolaemic effect of high salt intake as total cholesterol was significantly increased in SALT group compared with control. Administration of *Aloe vera* gel demonstrated anti-hypercholesterolaemic effect as total cholesterol was significantly decreased in SALT+ALOE group compared with SALT group. This decrease in serum total cholesterol could be attributed to the phytosterols contained in *Aloe vera*. These compounds are similar in structure to cholesterol and help to reduce serum cholesterol concentration by reducing cholesterol absorption from the gut by competing for the limited space for cholesterol in mixed micelles [49,50].

Triglyceride concentration was also significantly decreased in SALT+ALOE group compared with SALT group. HDL-c was significantly decreased in SALT group compared with control and ALOE group and significantly increased in SALT+ALOE group compared with SALT group. Decrease in HDL-c and increase in LDL-c increase the risk of developing cardiovascular diseases [51]. Our results show that high salt intake increases the risk of developing cardiovascular complications whereas *Aloe vera* demonstrated anti-atherogenic effect as serum HDL-c and LDL-c were significantly increased and decreased respectively in SALT+ALOE group compared with SALT group. VLDL-c was also significantly increased in SALT group compared with control and ALOE groups and decreased in SALT+ALOE group compared with SALT group. We suggest that *Aloe vera* gel has a beneficial role in preventing atherosclerosis. *Aloe vera* gel had been previously reported to possess anti-atherosclerotic effect [20].

V. Conclusion

High salt intake causes hyperlipidemia, oxidative damage to hepatocytes and hyperbilirubinaemia. It facilitates the conjugation of bilirubin in the liver but reduces bilirubin excretion by the liver through the small intestine by probably obstructing the bile ducts. *Aloe vera* gel reduces the rate of bilirubin conjugation and has the potential to treat salt-induced hyperbilirubinaemia that may be associated with biliary obstruction. *Aloe vera* gel also has hepatoprotective effect and the potential to treat hyperlipidaemia associated with high salt intake.

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