ORIGINAL RESEARCH ARTICLE

EFFECT OF HIGH SALT DIET AND HABISCUS SABDARIFFA (ZOBO) ON THE HISTOLOGY OF THE AORTA IN WISTAR RATS

ABSTRACT

Effect of salt diet and *hibiscus sabdoriffa* (sobo) on the histology of the aorta of wistar rats was investigated. A total of 20 adult wistar rats weighing 162-220 g were randomly assigned into four groups (l-IV) of five animals each. Group I served as the control , group II received 8g of salt, group III received 8gm of salt with 600ml of *sobo* while group IV received 600ml of *zobo* . The salt was administered in lOOg of feed for eight weeks, at the end of which blood was obtained for haematological analysis and sacrificed to obtain the aorta for histological analysis. The results obtained in this study showed a significant decrease in the body weight of rats taking high salt and *sobo* and an increase in body weight in rats taking normal diet and *sobo* compared to the control. The histological architecture of the aorta in the group taking salt and water shows a large thick wall and a moderate thickening in group taking salt and *sobo.* There is an increase level of AST in rats that received salt compared with Group I, III, IV and shows no significant changes in PCV and HB between the groups (l-IV). This study showed that high salt diet increased the blood pressure and volume in form of increase amount of water intake. It also caused thickening of the wall of the aorta.

Keywords: salt, diet, zobo, hibiscus sabdariffa

INTRODUCTION

Hypertension has been recognized as a multifactorial trait resulting from the effects of a combination of both environmental and generic factors. Vast majority of people suffering from hypertension have been attributed to genetic factors, high dietary salt intake and possible psychological factor (Sofawara, 1982; Khosh & Khosh, 2001; Obatomi, *et al,* 1994). An excess dietary salt intake is the most common environmental factor that contributes to the pathogenesis of hypertension (Gu, *et al,* 1998; Sacks *et al,* 2001; Aviv, 2001; Florich *et al,* 2005; & Meneton *et al,* 2005). It is a well-known fact that blood pressure is controlled primarily by salt and water balance, and the balance is regulated by the kidney. Therefore, whenever the kidney failed to maintain this balance due to excess salt intake, elevation of plasma volume is the result, leading to increased blood pressure.

The mechanism of dietary salt - induced hypertension and renal injury has been attributed to mutations affecting synthesis and circulating level of mineralocorticoids (Lifton *et al,* 2009). Other reports showed that, blood vessels are more sensitive to salt retention. They proposed that, whenever there is increase in salt retention, vascular reactivity to Angiotensin II increased and therefore, increased blood pressure

(Sandler et *al*, 2004; Zangari *et al*, 2004). Yu et al recently showed that administration of 80% NaCl diet to normotensive rats and spontaneous hypertensive rats' increases transforming growth factor. B_1 (TGF-B_1 and produced fibrosis in the kidney and left ventricles.

In another study, administration of 8.0% NaCl diet to fisher/Lewis rats after orthotropic renal transplantation nephropathy cause an increased tubulointerstitial fibrosis and glomeruli sclerosis. There was also associated increased urinary excretion and renal cortical levels of TGF-B1 (Sanders *et al*, 2001). Increasing salt intake also promotes an increase in production of TGF- B_1 from aortic ring preparation, and this effect was lost after removal of endothelium (Ying & Sanders, 1998). This study provided direct evidence that the endothelium responded to salt intake and TGF- B_1 production that result into sclerosis and organ dysfunction. (Chen and Sanders, 1991). Vascular endothelin (ET-I) is up regulated in several forms of salt- induced hypertension. It is also observed that expression of ET-I MRNA by aortic wall is increased in response to chronic high salt diet in Wistar rats (Redgrave *et al*, 1985). This study seeks to throw light on the impact of high salt diet on the histology of the aorta and some biochemical parameters in Wistar rats.

Hibiscus Sabdarifa (Hs) belongs to the family *Malvacae.* The leaves, calyx and corolla of the plant are used traditionally in many West African countries to control high blood pressure (Adegunloye et *al*, 1996). Earlier report showed petal crude extract of the plant has a direct relaxant effect on the aortic smooth muscle of rats (Obiefuna et al, 1994). The aortic relaxant effect was found to be mediated through chronic nitric oxide synthesis inhibition (Dikko, 2003). Apart from attenuating hypertension, chronic administration of aqueous extract of *Hibiscus Sabdariffa* has been reported to reverse cardiac hypertrophy in renovascular hypertensive rats (Odigie *et al*, 2003). Despite several studies on salt induced hypertension and the use of *Hibiscus sabdariffa* to ameliorate this effect, there is pulsity of information on the effect of high salt diet on the histology of blood vessels and biomarkers. Therefore, this study aimed in investigating the effect of high salt diet on the histology of blood vessels (Aorta); effect of *Hibiscus Sabdariffa* on anticipating changes of the blood vessels and the effect of the plant on any biomarker changes.

Hypertension is the commonest cardiovascular disease (Lawai and False, 1988) and a major cause of morbidity and mortality among adult Nigerians (Balogun and Ladipo, 1998). It is one of the leading causes of death and disability due to complications such as coronary heart diseases, stroke, congestive heart failure, end-stage renal disease and peripheral vascular disease (Khosh and Khosh, 2001). In vast majority of people suffering from hypertension, the major cause or predisposing factor is not clearly defined but has been attributed to genetic factors, high dietary salt intake and possibly psychological factors (Sofowara, 1982), (Khosh and Khosh, 2001, Obatomi *etal*, 1994). There is also considerable epidemiological evidence in humans linking the ingestion of large quantities of salt to the development of hypertension (Intersalt, 1988, Stamber *et al*, 1991). A similar association has been reported from studies of experimental animals fed high levels of salt and this particularly marked in the genetically selected Dahl salt sensitive rats (Dahl *et al*, 1962).

The mechanisms linking high salt intake to hypertension appears to be complex and to involve alteration in both reflex function (Ferrari and Mark, 1987) and in the contractile properties of the vascular smooth muscle (Mulvany *et al,* 1978; Adegunloye & Sofola, 1997; Nishida *et al,* 1998). The effect of salt loading has been inconsistent (Obiefuna *et al,* 1991, Lenda *et al,* 2000). However, part of the inconsistency may have been due to the different vessels studied; aortic rings by Obiefuna *et al* (1991), spino-trapezius vessels by Lenda *et al* (2000). In mesenteric vessels which are known to be of major importance in blood pressure control. There is some evidence that in addition to endothelial derived picric oxide, endothelium-derived hyperpolarising factor (EDHF) may also be of importance (Ebeighe et *al,* 1990; Adeagbo & Triggle, 1993). It is not known, however, whether the actions mediated by EDNO or by EDHF are affected by the level of salt intake. The mechanism responsible for elevation of blood pressure function released from endothelium of the resistant vessels (Sofola et *al,* 2002). Dilatation may be affected by endothelia released of Nitric oxide and this may be stimulated invitro by acetylcholine and normal response to acetylcholine has been used as an index of normal endothelia function (Vanhoutte *et al,* 1995). It is becoming apparent that vasodilation may be mediated through different mechanisms depending both on the vessels studied as well as on the conditions. For example, Lenda *et al,* (2000) reported that a high salt diet resulted in reduced relaxation in vessels of spino-trapezius muscle and suggested that this was due to production of reactive oxygen radicals.

The aorta is the main trunk of a series of vessels which convey the oxygenated blood to the tissues of the body for their nutrition. The wall of the artery is composed of 3 layers tunica intima (the inner layer), tunica media (the middle layer) and tunica adventitia (the outer layer). Hypertension induces pressure overload resulting in cardiac hypertrophy. Hypertension is a major independent risk factor for the development of heart failure (Subramanian & Lip, 2009 and Arguedas *et al,* 2009). Different plants extracts and photochemical have been recently screened for antihypertensive and antihypertrophic properties (Seymore *et al,* 2008 and Ardiansyah *et al,* 2008). Cardio protection of resveratrol (Trans -3, 4'5 - trihydroxystilbene) a polyphenol found predominantly in grapes, and berries, or its analogue has been overload. (Liu *et al* and Juric *et al* 2007). The report also showed that neither Sino aortic denervation (Masson & Aoki, 1996) nor renal nerve denervation (Dzielak & Norman, 1985) prevent the generation of salt dependent forms of hypertension. One of the effects of high salt intake is increased blood pressure, which was clearly demonstrated in chimpanzees fed with a salt diet (Elliot *et al,* 2007). Increased in salt in modern diet is strongly associated with health risk, particularly for those individuals with salt sensitivity in response to increased salt diet.

MATERIALS AND METHOD

MATERIALS

Some of the materials used for this study include digital weighing balance, syringes, dissecting set, rubber bowl, hand gloves, sample bottles, microscope, gavage, RBC pipette, counting chamber, Hayem's diluting fluid or RBC diluting fluid, sterile disposable lancet, cover slips, WBC pipette, WBC diluting fluid, dry clean glass slides, Leishmans stain, distilled water, ceder wood oil, heparinized capillary tubes, plastin, haematocrit, centrifuge, razor blade, Haemocytometer, platelet diluting fluid, haematoxylin and eosin stain, other solvents, rat cages, rotary microtome, Mounting medium, and fluoride oxylate container (anticoagulant).

EXPERIMENTAL DESIGN

Twenty (20) male Wistar rats weighing between 160-180g were used for the experiment. They were obtained at Human Anatomy animal house. They were maintained under standard nutritional and environmental conditions throughout the experiment and had access to food and water *ad libitium.* The animals were divided into four groups of five animals per group labelled Group I, II, III and IV. Group I served as Control and received water and feed only, group II (salt and water group) were given feed contain 8gm of salt, Group III (salt and sobo group) receive 8gm salt diet and zobo instead of water, While, Group IV (Normal diet and zobo) receive standard food with sobo also. The animals were treated for 8weeks. The animals were housed in laboratory animal cages inside the cross ventilated room in the department of Human Anatomy animal house under 12 hours light/ 12 hours dark cycle. Saw dust was used for the bedding of the cages. The bedding was changed for every after 2days throughout the period of the research. The initial weight of the animals was obtained at the beginning of the experiment and the final weight was taken before the termination of experiment.

SALT ADMINISTRATION

Dangote iodized salt was used for the experiment obtained in the regular market. 8gram of the salt was measured on digital weighing balance and mixed in 100grams of feed per meal given to experimental groups of animals taking.

COLLECTION OF *HIBISCUS SABDARIFFA* (ZOBO)

Dried leaves (calyxes) of *Hibiscus sabdariffa* (Roselle plant) were obtained in bulk from nkwo market in Nnewi and it verified at the specimen herbarium of the department of pharmacology, Nnamdi Azikiwe University, Agulu campus.

EXTRACTION PROCEDURE

100gram of dried leaves of *Hibiscus sabdariffa* (HS) was measured on a digital weighing balance and boiled in 150cl of distilled water for 15 mins at 100°C on a hot plate. It's kept to cool at room temperature for 30mins and filtered with pitman paper. The filtrate is kept at 4 c to serve as a stock solution for further dilution, while the residue is $_{dri}$ed at room temperature and measured after drying.

ZOBO ADMINISTRATION

400mls from stock solution (concentrated crude zobo) was mixed with 800mls of distilled water, make a total of 1200mls, which is given to experimental groups taking sobo i.e. Group III (salt and zobo) and Group IV (normal feed and zobo) 600mls each, through bottle feeder. At end of every 2days, the remnant was measured and subtracted from the volume given to them.

TERMINATION OF EXPERIMENT AND HISTOLOGICAL PROCEDURE

The animals were weighed and sacrificed at the end of the 8^{th} week of the experiment. The animal chests were opened through sternal bone and the heart and aorta were identified. The aortas were free from connective tissue and cut and transfer into 10% formalin. The head was also open through the vertebrae and the skull, the basilar artery were also identified and cleanly removed and also transferred into 10% formalin for fixation. The tissues were then dehydrated through graded alcohol solutions, clean in Xylene, infiltrated and embedded in molten paraffin wax. Serial sections were obtained at 5um thickness from a rotary microtome and subjected to haematoxylin and Eosin (H&E). The sections were examined with the light microscope and photomicrographs of the sections were taken for further analysis.

PARAMETERS ANALYZED

Determination of packed cell volume (PCV)

The packed cell volume was determined in quadruplicate using Hawksley micropillary tubes and centrifuge at 1200xg for 5minutes before reading with haematocrit reader (Abudu and Sofola, 1994). Haemoglobin (HB) was determined using standard method described by Dacie (1984). Serum Aspertate amino transaminases (AST), Alanine aminotransferase (ALT), protein, albumin and alkaline phosphate were determined at the University of Maiduguri teaching hospital, Maiduguri.

STATISTICAL ANALYSIS

Data obtain were expressed as means ±SEM of 5 rats per group. The mean were compared using student t-test, $p< 0.05$ is considered statistically significant.

RESULTS

AVERAGE WATER INTAKE AND ZOBO CONSUMPTION

The observation of unequal water in both control and salt diet rats, attract the attention to determine the water intake towards the end of the experiment. A comparable water intake in both control and salt diet animal group shows that, daily intake of water in control group (147.5±3.96) was significantly lower ($p<0.01$) than salt diet group (243.8±1.16).

Table la: MEAN DIFFERENCES IN WATER INTAKE

GROUP	GROUP I (CONTROL)	GROUP II (SALT + WATER)
Mean ± SEM	147.5 ± 3.969	243.75 ±1.16

ZOBO INTAKE:

The mean zobo *(Hibiscus sabdariffa)* consumption in salt diet rats after 2days was also found to be significantly higher (516.36±11.62) compared with the rats taking normal diet and zobo (HS) as fluid in every 2days (364.71±15.43).

Table lb: MEAN DIFFERENCE IN *SODO* (HS) INTAKE.

GROUP	GROUP IV	GROUP III
Mean ± SEM	364.71 ± 15.4	516.36 11.615

RESULT OF BODY WEIGHT CHANGES

TABLE 2a: Percentage Mean body weight gain per group

Groups	TREATMENT		Mean Body Weight Gain (%)
	SALT	*ZOBO*	

1	0	0	21 + 5.357%
II	8gm	0	18.6 ± 3.919%
III	8gm	600 ml	9.86 ± 2.630%
IV	0	600 ml	24.2 ± 5.911%

Results are presented as MEAN ± SEM, N= 5

Table 2a: shows the percentage mean body weight gain of animals after 8weeks in control (21±5.36), group II (18.6±3.92), salt and zobo group (9.86±2.63) and normal diet with zobo (24.2±5.91). The mean body weight gain was significantly higher ($p<0.05$) in group that consume normal diet and zobo (Group IV) compared with the groups (GROUPS I, II, III), while a least mean body weight gain (9.86+2.63) was observed in group 2 (salt and zobo). The difference is significant ($p<0.05$). Group II (salt+water) have less body weight gain (18.6+3.92) than control (21±5.36). However, the difference is not significant.

Table 2b: The overall percentage body weight gain in each group was found to be higher (26.76%) in group IV (normal diet and zobo), while a less percentage body weight gain was observed in group taking salt with zobo (9.84%).

Table 2b: Percentage body weight gain per group

GROUP	Group 1	GROUP II	GROUP III	GROUP IV
Body Weight gain (%)	24.20%	19.11%	9.84%	26.76%

EFFECT OF SALT AND *HIBISCUS SABDARIFFA* ON BLOOD PARAMETERS

Mean value of packed cell volume concentration in control (42.00±1.23), salt diet and water (39.60±2.46), salt and zobo (37.80±3.99) and normal diet and zobo (39.00+0.81) group. While mean Haemoglobin concentration ranges between 10.50±0.30 (control group) and 9.45±0.20 (salt and zobo group) as shown in table 3. This result shows that salt diet and administration of zobo, with salt diet and normal diet do not have a significant effect ($p>0.05$) in either PCV or HB concentration in rats, though there is a slight decrease in concentration of PCV in experiment groups.

TABLE 3: Mean packed cell volume and Haemoglobin concentration

GROUPS	PCV (%)	HB (g/dl)
GROUP 1 (CONTROL)	42.00 ± 1.230	10.50 ±0.310
GROUP ll(SALT + WATER)	39.60 ±2.460	9.90 ±0.615
GROUP III (SALT + SOBO)	37.80 ±3.990	9.45 ± 0.203
GROUP IV (NORMAL + ZOBO)	39.00 ±0.810	9.85 ±0.200

Table 4: EFFECT OF SALT AND *ZOBO* (HS) ON THE BIOCHEMICAL PARAMETERS

GROUP	Mean ± SEM % Fragility				
	ALT (mmol/l)	AST (mmol/l)	ALT (mmol/l)	PROTEIN (g/l)	ALBUMIN (g/l)
GROUP 1	59.00±0.000	54.80±5.774	25.20±2.691	62.60±3.108	31.80±1.772
GROUP II	59.00±0.316	86.00±10.310	32.80±0.970	64.80±2.871	34.00±1.225
GROUP III	59.20±0.200	64.00±8.933	30.60±3.750	62.80±3.513	33.00±0.447
GROUP IV	59.20±0.200	57.40±8.022	25.40±3.487	62.00±1.342	32.00±0.775

Table 5 shows the result of Aspertate amino transaminases (AST), Alanine amino transferase (ALT), Alkaline Phosphate, Protein and Albumin in Group I (Control) group and Experimental Groups. It was observed that, the level of aspertate amino transferase (AST) was raised in both the group that received salt (86.00±10.30) and those that received salt with zobo (64.00±8.93), with highest serum level of AST observed in rats that received salt, when compared with group IV (normal and sobo) and control. The differences are significant. However, there was no significant difference between the control group (54.80±5.77) and the group that received *zobo* only (57.40±8.02). This result also demonstrated that no

difference in alkaline phosphate, ALT, protein, and albumin in control group when compared with experimental groups.

Calculated ASAT and ALT ratio is higher in experimental group II and group III i.e. salt group and salt + zobo group. When AST / ALT ratio >0.2, suggested non-alcoholic tissue damage. (Spech and Lieh, 1983).

Table 5: BIOCHEMICAL FEATURE OF AST AND ALT

PARAMETER (mmol/l)	GROUP 1	GROUP II	GROUP III	GROUP IV
AST	54.80	86.00	64.00	57.40
ALT	25.20	32.80	30.60	25.40
RATIO	2.175	2.622	2.092	2.260

HISTOLOGICAL OBSERVATION

Following the histological preparation with H&E, microscopic observation were carried out with binuclear light microscope and the auto micrograph of each slide were taken using photographic set. The following observations were made; the aortic wall of the control rats appeared normal with normal vasculature, containing RBCs. (Fig: 1); the section of aorta in rats the administered high salt diet and water shows a large thick wall containing RBCs. (Fig: 2); the section of aorta rats the administered high salt diet and *sobo* shows a large vessel with moderate thickening of its wall. (Fig: 3); the section of aorta in the rats administered normal diet and z*obo* a large vessel with mildly thicken wall that shows no or less difference in what observation in the aortic section of the rats taking in control. (Fig: 4).

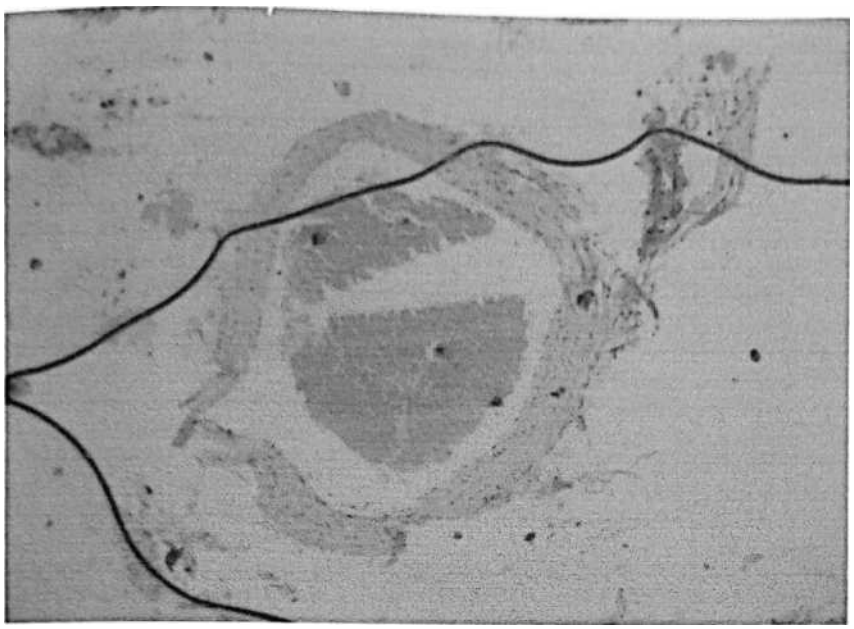

Fig. 1 Photomicrograph of rat aorta shown in control H&E xlOO. (Control)

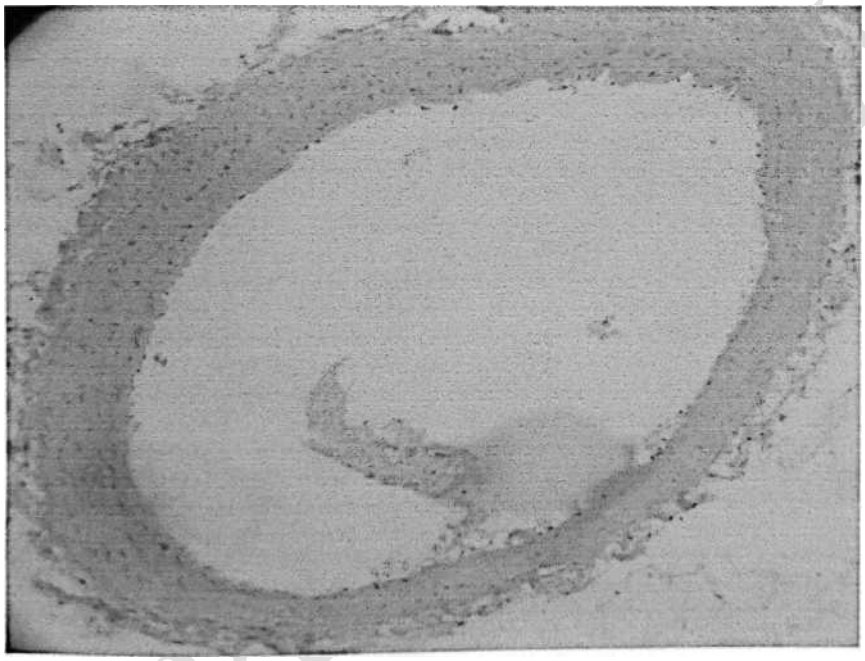

Fig. 2 Photomicrograph of aorta of rats administered with high salt diet, H&E x100. (GP II) shows a large thick wall

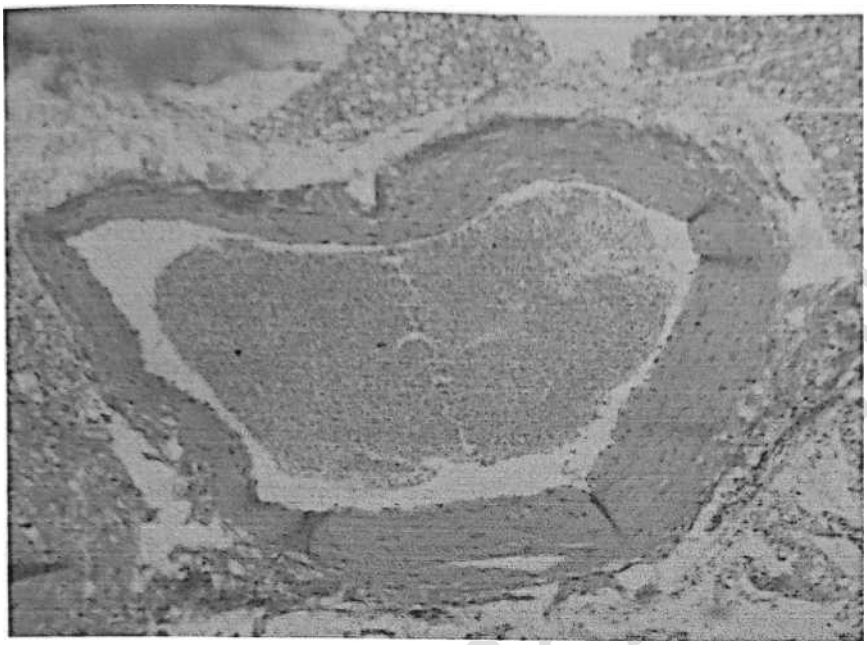

Fig. 3 Photomicrograph of aorta of rats taking high salt diet and *sobo* (GP III) H&E x100. Showing a large vessel with moderate thicken wall.

Fig. 4 Photomicrograph of rat aorta showing large with mildly thicken vascular channel H&E x100 in rats that received *sobo* (GP IV).

DISCUSSION AND CONCLUSION

DISCUSSION

Several reports show that increased salt intake expands blood volume and blood flow (Redgreeve et *al,* 1985; Greene *et al,* 1990; Bech *et al,* 1998;) in experimental animals. Therefore, the increase in water intake observed in the present experiment could be attributed to increase in the sodium chloride load in the blood which stimulated the animal to take in more water and sobo juice when compared to the control and the animals that were on normal diet (i.e. without salt). The volume overload resulting from the increase in fluid intake observed in the present study might be one of the mechanisms involved in the development of high salt diet. Such finding has not been reported in the literature on the high- salt induced hypertension. Previous studies in animals have shown that a high-salt diet intake significantly increased renal excretion of water due to increase in water intake (Cowley et *al,* 1983; luft et *al,* 1983). The mechanism by which high salt diet can increase water intake was put forward by Hladky and Rink (1986). They showed that high-salt diet increased the plasma sodium and plasma osmolarity, which is known to stimulate thirst and antidiuretic hormone secretion. This increase in fluid intake will reduce plasma osmolarity and increase urine volume. Humans experiment also demonstrated that the greater salt intake, the greater water consumption and the excretion of water (Feng *et al,* 2001).

The comparative effect of high-salt diet on the body weight of the experimental animals also showed a less body weight gain in the group that consumed high salt diet with *sobo,* probably indicating the importance of taking ordinary water than taking *sobo juice.* The increase in water intake observed in this experiment might be the reason the kidneys excrete more salt and water in rats taking high-salt diet as demonstrated by Guyton and colleagues (1991) on the pressure natriusis and diuresis relationship in an attempt to decrease extracellular and plasma volume. The increase water intake can be taken as evidence of hypertension because; Jian-wei Gu *et al* (2008) demonstrated that after 6, 7 and 8weeks of high-salt intake the systemic blood pressure would increase significantly. Clinical studies also suggested that a long- term high intake of salt can increase blood pressure in both normotensive and hypertensive humans (Weinberger, 1996; Lifton, 1996).

The results of the biochemical parameters showed that the level of alkaline phosphatase, Alanine amino transferase, Protein and Albumin were not significantly different between the controls and the experimental groups. The level of L-aspertate amino transferase was raised, especially in the group that consumed salt and water.

ALT and AST are jointly known as transaminases. They are associated with inflammation and/or injury to the liver cells, a condition known as hepatocellular liver injury (Melissa, 2004). Damage to the liver resulted to leakage of Ast and Alt into the blood stream. However, raised Ast does not specifically mean that there is liver damage. Damage to the muscles and heart can also raise the

Ast level in the blood. High ratio of AST and ALT obtained in this study confirmed the assumption that the actual damage might be in liver, muscle or the heart. This is supported by the claim that if the ratio of AST to ALT is >0.2 the damage must be non-alcoholic.

The histological findings showed normal architecture of the aorta in the group that took normal diet with *sobo.* However, there were changes in the microscopic characteristics of the aorta of the rats on high-salt diet and water. They had large, thick wall with the lumen greater than that of the aorta in rats that were on high-salt diet and sobo. But these features were less prominent in the aorta of rats fed with the high-salt diet and *sobo.* The enlarged lumen of the aortic section (dilatation) may be due to increased aortic flow. The same observation was made in tetralogy of Fallot (Capelli *et al,* 1982; Marelli *et al,* 1994). It was well documented that in rats with high-salt diet, increase plasma volume and blood flow was recorded, a phenomenon that could probably result into aortic dilatation and thickening as observed in the present study.

5.2. CONCLUSION

This study showed that high salt diet increased the blood pressure and volume in form of increase amount of water intake. It also caused thickening of the wall of the aorta. However, *sobo* was able to ameliorate some of these effects caused by the intake of high- salt diet. It was also observed that high-salt diet increased AST and the AST/ALT ratio suggesting non-alcoholic liver damage.

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