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EVALUATION OF THE EFFECT OF A LOCALLY FORMULATED HIGH-SALT AND HIGH-LIPID DIET ON THE LIVER FUNCTION STATUS, BLOOD PRESSURE AND LIPID PROFILE OF ALBINO *WISTAR* RATS

ANIONYE JC[1*], ONYENEKE EC[2], EDOSA RO[1], EGILI S[1], OGUNSANYA OO[1], ONOVUGHAKPO-SAKPA OE[3], ANEKWE AI[2] AND OFOHA PC[2]

[1]Department of Medical Biochemistry, College of Medical Sciences, University of Benin, Benin City, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
[3]Department of Chemical Pathology, College of Medical Sciences, University of Benin, Benin City, Nigeria

***Corresponding Author: Dr. Anionye John Chukudi**

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ABSTRACT

Cardiovascular diseases are on the increase in sub-Saharan Africa. Most animal studies trying to understand the role of a high salt and/or a high fat diet in the pathophysiology of cardiovascular diseases use a rather "expensive purified diet" or a modification of it, to induce hypertension and/or hypercholesterolaemia in rats. This study therefore focuses on determining if hypertension and hypercholesterolaemia can be induced in rats by a modified locally available and "cheap" rat chow.

The basal diet, a locally available standard pelleted grower's mash, was modified by a replacement of soy protein common in standard growers mash with other alternative protein sources. The combined high-salt/high-fat diet was made by adding to a proportionate quantity of the basal diet, 8% NaCl, 2% cholesterol, 0.5% bile salts and 20% butter. Twelve male *Wistar* rats divided into two groups of 6 rats each was used for this 6 weeks study. The control group was fed the modified basal diet, while the experimental group was fed the combined high-salt/high-fat diet. Using a "tail-cuff" blood pressure instrument, the blood pressure of the rats were determined at the beginning of the 1st week, at the end of the 4th week and at the end of the 6th week. The rats were then sacrificed, and some organs and blood collected for the evaluation of their relative organ weight, liver function indices and plasma lipid profile using standard assay kits. The results reveal that the experimental diet promoted a significant increase ($p<0.05$) in blood pressure, LDL-cholesterol, total cholesterol and a significant decrease ($p<0.05$) in HDL-cholesterol, without negatively affecting the hepatic

function of the rats. The experimental diet therefore was able to induce hypertension and hypercholesterolaemia in the *Wistar* rats meaning they could help in further studies of the risk factors related to cardiovascular diseases.

Key words: Cardiovascular diseases, hypertension, hypercholesterolaemia, high-fat diet, high-salt diet

INTRODUCTION

The World Health Organisation (WHO) in 2002 estimated that every year about 12 million people worldwide die from cardiovascular diseases, with most of them from the developing world (1). However, a study recently carried out by researchers led by the Institute of Health Metrics and Evaluation, IHME, at the University of Washington, USA, and published in the New England Journal of Medicine titled "Demographic and Epidemiologic Drivers of Global Cardiovascular Mortality", shows that the number of deaths from hypertension, stroke, heart attacks and other circulatory/cardiovascular diseases is on the rise, climbing from 12.3 million in 1990 to 17.3million in 2013 (2). A world cardiology study conducted in Oyo, Katsina, Kwara and Enugu States of Nigeria, shows that the population of people living with hypertension is about 27% in Katsina, 36.6% in Ilorin, 20.8% in Ibadan rural and 46.6% in Enugu rural (3). The death rates of cardiovascular diseases are higher in Nigeria due to late detection of cases, adoption of a western diet (high in salt and saturated fat) and other risk factors such as high blood cholesterol, high blood pressure, smoking, diabetes, obesity and sedentary living (3). This is not surprising, as the 2002 report from the World Health Organisation, shows for the first time that the main risk factors for cardiovascular disease—high blood pressure, high blood cholesterol level, and smoking—which are traditionally linked to an affluent lifestyle, are now being seen in middle income and poorer countries (1).

The pathogenesis of hypertension in humans is not fully understood. This disease of persistent elevation of blood pressure is a multifactorial combination of genetic and environmental factors. To better understand the specific mechanisms involved, as well as to research treatments for prevention of hypertension, various animal models have been developed to mimic the hypertensive responses seen in humans (4). Historically, the preferred small animal model for hypertension research has been the rat. This may be due to the amount of published physiological data, relative small size, and robust responses seen in some genetic strains. Because of the polygenic nature of hypertension, numerous rat models have been developed including selective bred homozygous hypertensive rat strains (e.g. spontaneously hypertensive rat [SHR] and Dahl salt sensitive [1] and outbred strains (e.g. Sprague Dawley and *Wistar* rats) to elucidate the desired hypertensive phenotype (4). As the form of hypertension can differ between strains, researchers need to not only be aware of the form of hypertension that the individual strain exhibits but also the impact that a particular type of diet may have on the phenotypic response (4).

High sodium diets are commonly used to study diet induced hypertension, since increasing levels of circulating sodium cause cells to release water (due to osmotic pressure) which elevates the pressure on blood vessel walls. A diet containing 8% NaCl has been shown to be adequate in inducing hypertension in rats (4). Lewis Dahl developed, from selectively inbred Sprague Dawley rats, the Dahl salt-sensitive (Dahl SS) and Dahl salt-resistant (Dahl SR) rats based on their response to an 8% NaCl diet (5). Some weaned Dahl SS rats can rapidly develop elevated blood pressure (>180 mm Hg) when fed an 8% NaCl diet in as little as 2 weeks, though the average for the strain is usually closer to 4-6 weeks (5-8). When fed lower levels of NaCl, hypertension along with vascular and renal lesions still develop, though the length of time is typically longer (9, 10). The development of salt induced hypertension seems to have something to do with the age of the animal. Dahl SS rats 3 or 6 months after weaning developed elevated blood pressure at a slower rate compared to those at weaning, when both were placed on a high salt diet (8%). Spontaneously hypertensive rats (SHR) develops hypertension spontaneously with increases in blood pressure beginning early in life (5-6 weeks) (11). This strain has been considered a good model of essential hypertension because the peripheral resistance and normal cardiac output exhibited by the SHR are similar to human hypertension (12). An additive effect has been seen to take place in terms of an increase in the blood pressure of the SHR when there is an increase in vascular resistance with the addition of NaCl (~7%) to the diet and/or saline solution of 1% NaCl (13, 14).

As a model of diet-induced hypertension, the mouse is not widely used. Inbred mice such as the C57BL/6 can develop elevated blood pressure on purified diets high in NaCl (8%), though the time frame for this appears to be on the order of several months (4, 15).

Sodium is not the only dietary factor that can play a role in the degree and onset of hypertension in animals. When 4% NaCl was added to both a chow diet and a purified ingredient diet, Dahl SS rats fed the purified diet had higher blood pressure and more renal damage compared to chow-fed rats (16). This seem to indicate that the background diet (chow vs. purified) affected the outcome. The reasons are not clear but may be related to fundamental differences between chows and purified diets in the levels of minerals such as sodium and potassium, protein sources, presence or absence of phytochemicals, carbohydrate type, and/or the level and type of fibre (4). Typical purified ingredient diets contain about 0.1% Na (0.25% NaCl), while chow diets contain about 0.3-0.4% Na (0.75-1.0% NaCl) (4). The chow based diets contains soy protein (a common protein source in chows) which has been shown to attenuate the development of hypertension in the spontaneous hypertensive rat (SHR) in comparison to diets containing casein (the primary protein source used in purified diets) (17). Soy protein does contain the phytoestrogen, genistein, which has been shown to blunt the increase in blood pressure due to NaCl induced hypertension (18). These phytoestrogens are not contained in casein, so the differences seen in blood pressure between the rats fed chows and purified diets may be partially due to the variable levels of genistein and other phytoestrogens within chow (19). Modifying rat chow by replacing the soy protein in it completely with another protein source may therefore allow the chow to be supportive of the development of hypertension.

Other dietary factors like sucrose, fructose and vitamins have also been implicated in hypertension. Dahl rats fed high sucrose or fructose diets have been seen to exhibit hypertension (4). When a high level of salt (6%) is combined with high sucrose/fructose there is an exacerbation of this hypertension and a pronounced increase in mortality (20, 21). Outbred rat strains such as the Sprague-Dawley and *Wistar* rats also used for obesity research have been seen to develop numerous components of metabolic syndrome (hypertension, insulin resistance, and hypertriglyceridemia) on diets high in fructose (60%) (22, 23) as well as hypertension concurrent with the development of obesity (24-26).

Just as one can elevate blood pressure through dietary measures, so also can hypertension be attenuated by way of dietary measures. It has been shown that the hypertension resulting from feeding an 8% NaCl diet to both humans and animals, can be prevented by supplementing the diet with extra potassium (6, 27). Dietary supplementation with antioxidants (such as vitamins E and C) has been shown to lower blood pressure in the stroke-prone, SHR or the Dahl SS rat (28, 29, 30). Therefore, this suggests that low intake of these micro-nutrients could promote hypertension (4).

High blood cholesterol (hypercholesterolaemia) has been recognized as one of the major risk factors for the development of cardiovascular diseases. In humans, the consumption of high amounts of saturated fat has been implicated in blood lipid abnormalities (hyperlipidaemia) especially hypercholesterolaemia and hypertriglyceridaemia often associated with cardiovascular diseases (1). This has led to attempts at reproducing such high lipid blood levels in laboratory animals, in order to understand better the relationship between disorders in cholesterol metabolism and atherogenesis and to test possible treatment or prevention strategies with the ultimate goal of reducing circulating cholesterol levels. A great number of animal models, such as pigeons, chickens, swine, cats, dogs, non-human primates, mice, rabbits and rats, have been tested (31,32). For inducing hypercholesterolemia in rats, triglyceride-rich diets containing cholesterol, with or without cholic acid have been used (33); the level of cholesterol used in these diets as well as the fat sources varied. The fat sources varied from lard to soybean, to canola and sunflower oils. Most of these diets were with a purified or semi purified diets as base. Commercial rations supplemented with cholesterol have also been used (34, 35). Over the years various models in terms of diets that can induce hypercholesterolaemia have been designed while focusing on designing the models that should be capable of promoting the highest hypercholesterolemia possible without affecting negatively the development of the rats. Those that achieve this while promoting an increase in the LDL-cholesterol, a decrease in the HDL-cholesterol fraction, with minimal effect on the hepatic function of the animals were frequently put to use (36).

Various model diets relating to the study of hypercholesterolemia in rats can be found in the literature. They vary from commercial rations supplemented with 1% cholesterol, to diets with variations in the lipid and carbohydrate portions, such as different fat sources and contents, containing or not containing cholic acid, and partial substitution of sucrose for starch (36). Blood Cholesterol levels result from a combination of the cholesterol absorbed from dietary cholesterol (exogenous absorption from food in the gastrointestinal tract) and the endogenous *de novo* biosynthesis of cholesterol. [2] considered rats as hypo-responsive to dietary cholesterol, hence they suggested that cholic acid should always be used in the formulation of hypercholesterolaemic diets for rats in order to induce hyperlipidaemia/hypercholesterolaemia and atherosclerosis in this species (37). As much as it is true that cholesterol is used to synthesize bile acids (cholic acid) and so including cholic acid in the diet will help preserve circulating cholesterol, hence increase plasma cholesterol concentration, evidence has recently been presented which do not recommend the utilization of cholic acid in hypercholesterolaemic models in rats (33).

In a study carried out by Matos *et al.,* (36) aimed at finding a dietetical model capable of promoting the highest hypercholesterolemia without affecting the development of the rats, four model hypercholesterolaemic diets were designed [with the basal AIN-93M diet having constituents similar to the semi-purified diet of Reeves et al., (38)] with cholesterol and different contents of soybean oil, starch, casein, micronutrients and fibre (36). The best result was obtained with the diet containing 25 % soybean oil, 1.0 % cholesterol, 13 % fibre, given to the rats for 8 weeks, since it promoted an increase in LDL-cholesterol, a decrease in the HDL fraction and affected less the hepatic function of the animals. In this same study, there was no increase in the systolic blood pressure in the animals receiving hypercholesterolaemic diets after eight weeks of observation. This gives the impression that dietetic cholesterol has no influence on blood pressure. The increase in LDL-cholesterol and reduction in HDL-cholesterol have been pointed out as risk factors for the development of atherosclerosis and related cardiovascular diseases; the higher the ratio of LDL/HDL promoted by a dietetical model, the more likely that it will be atherogenic and hence precipitate cardiovascular diseases (36).

In a recent research carried out by Soliman (39), where he tried to study the effects of nuts (Pistachio or Almonds) consumption on the lipid profile of hypercholesterolaemic rats, the control (basal) diet he used was prepared according to the method of [3], while the induction of hypercholesterolaemia was carried out by addition of 2% cholesterol and 0.25% bile salts to the basal diet for 4 weeks (39).

In an outstanding research carried out by [4]., (40), it was demonstrated again that cholesterol-fed normotensive *Wistar* rats (WKY) and spontaneously hypertensive rats (SHR), developed comparable levels of severe hypercholesterolaemia. The basal semi-purified diet used here again was the AIN-93M diet (38) and hypercholesterolaemia was induced by supplementing the basal diet for 12 weeks with 1% cholesterol, 0.5% cholic acid and 20% butter (40). The final composition of purified diets and hypercholesterolaemic diets derived from it is given in Table 1 (40)

Table 1: Composition of experimental diets based on the AIN93M (38) formulation, g/kg diet

Ingredient	Control	Hypercholesterolaemic
Maize starch	622.49	447.49
Casein (95% N x 6.25)	140.00	140.00
Sucrose	100.00	100.00
Soyabean oil	40.00	-
Fibre[1]	50.00	50.00
Mineral mix[2]	35.00	35.00
Vitamin mix[3]	10.00	10.00
Butter	-	200.00
Choline bitartrate[4]	2.50	2.50
Tert-butylhydroquinone[5]	0.008	0.008
Cholesterol[6]	-	10.00
Cholic acid[7]	-	5.00

[1]Alpha cellulose powder - Sigma (St. Louis, MO, USA); [2]AIN-93M-MX (38); [3]AIN- 93M-VX (38); [4,5]Sigma (St. Louis, MO, USA); [6]Aldrich (Steinheim, Gemany); [7]Fluka (Buchs, Switzerland).

This semi-purified diet used as the control and basal diet for inducing both hypertension and hypercholesterolaemia is expensive to formulate in Nigeria, especially because of the cost of imported Casein (95% N x 6.25), hence, this research is designed using a modification of the locally available rat chow in Benin City to evaluate if hypertension and hypercholesterolaemia could be simulated in male normotensive *Wistar* albino rats.

MATERIALS AND METHODS

Materials

Chemicals/Reagents: The reagent kits and other reagents used were of standard quality and were purchased from their manufacturers' representative in Nigeria (Randox Laboratories UK).

Methods

Animals of the study: Male albino rats of the *Wistar* strain were obtained from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The rats were housed in a well-ventilated room in the animal house of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria, with the room temperature ranging between 27-33^{o}C. They were allowed the diurnal 12-hr light and 12-hr dark cycle. The rats were fed ad-libitum with standard pelleted mash and clean tap water for an acclimatization period of two weeks. All the experimental animals of this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research, 1984 edition (41).

Formulation of the Experimental Diets: The control or basal diet is a modified standard pelleted grower's mash which was designed following the suggestions put forward by [4]and [5]. They suggested a replacement of soy protein common in standard growers mash with other alternative protein sources as it contains phytoestrogens, genistein in particular, which has been shown to attenuate the development of hypertension in spontaneous hypertensive rats and blunting of the increase in blood pressure due to NaCl induced hypertension in other species of rats like the albino *Wistar* rat (17, 18). The combined high-salt/high-fat diet (hypertension-inducing diet/hypercholesterolaemia-inducing diet) was made by adding to a proportionate quantity of the basal diet, 8% NaCl (to make the diet high in salt for the purpose of making the rats hypertensive) (4, 42) and 2% cholesterol + 0.25% bile salts + 20% butter (to make the diet high in lipids/cholesterol for the purpose of making the rats hypercholesterolaemic) (38, 39, 40, 43).

The final composition of both the basal and combined diets was as indicated in Tables 2 and 3

Table 2: Composition of the basal diet (g/1000g) based on a slight modification (17, 18) of the standard pelleted growers mash of Jerrison Agro Allied Services, Benin City, Nigeria.

Ingredients	Basal Diet (g)
Maize	260.0
Wheat offal	300.0
Palm Kernel cake	232.0
Groundnut cake	128.0
Fish meal (65%)	12.0
Lysine	1.6
Bone meal	10.0
Limestone	52.0
Grower premix	2.4
Salt	2.0

Table 3: Composition of the Combined diet (g/1000g) (hypertension-inducing diet/hypercholesterolaemia-inducing diet) based on specific adaptations (4, 38, 39, 40, 42, 43)

Ingredients	Combined Diet (High-salt/High-fat Diet) (g)
Basal Diet in Table 1	707.5
Butter	200.0
Pure analytical grade cholesterol	10.0
Sodium taurocholate (bile-salt)	2.5
Pure analytical grade NaCl	80.0

Experimental Design:

Twelve (12) male albino rats of the *Wistar* strain weighing between 125-175g, were used for this study. After 14 days acclimatization, the rats were weighed and divided into two (2) groups of six (6) rats each, making sure that the weights of those in a group was representative of the weight range of all the rats, such that the average weight of the groups at the onset of the experimental period was approximately 150g. The rats were fed ad-libitum during the entire course of the 6-weeks (42 days) study. Group 1 was fed the basal diet (modified standard pelleted mash) and clean tap water, while Group 2 was fed the combined high-salt/high fat diet, and clean tap-water. Care was taken to determine the quantity of feed consumed daily. The rats were housed in wooden cages with a tiny-wire mesh/iron gauze flooring to allow the rat-excreta to be collected into another steel tray receptacle below covered with a bedding material to prevent coprophagy. The cages, their surroundings, the receptacle tray below with its bedding, were cleaned and disinfected daily.

Body Weight:

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, then on day 1 before commencement of the study, once weekly during the 6-week period of the study, (day 7, 14, 21, 28, 35) and on the day of sacrifice at the end of the 6-week study (44). The results were used to determine the average daily weight gain per rat in each group and subsequently the food efficiency.

Food Consumption:

Using a sensitive balance, the quantity of feed given to each group of rats daily was determined by subtracting the quantity of feed left the next morning from that given the day earlier. From the results the average quantity consumed weekly by the rats was determined. The result was used to determine the weekly feed consumption by each group and the quantity of feed consumed by each rat daily (44) and this was subsequently used to determine the food efficiency.

Food Efficiency:

This was determined using the formula:

$$\text{Food efficiency} = \frac{\text{Weight gain/day/rat}}{\text{Feed consumed/day/rat}} \times 100 \quad \ldots(36)$$

Systolic Blood Pressure Measurement:

The systolic blood pressure of the rats was measured in conscious restrained rats with a photoelectric sensor and a tail-cuff sphygmomanometer, using the Ugo Basile Blood Pressure Recorder Model 58500, on day 1, day 28 and day 42 of the study, following the procedure described by [6]., (45) and [7] (36). Thirty minutes before the measurements, the rats will be placed into a preheated restrainer, with the tail exposed. The tail cuff will be pushed up to the base of the tail and fit closely but freely on the tail and the pulse sensor will be placed just behind the tail cuff. The cuff will then be inflated and it will deflate itself automatically until a pulse signal is obtained and onwards (45). The initiation of the pulse signals, after the inflation peaks, will be correlated with the pressures in the occlusion cuff to obtain the mean systolic blood pressure readings for each animal (36). The pressure in the occlusion cuff and the pulse signal will be displayed automatically on the screen of the instrument and printed out on a paper chart.

Blood Sample Collection and Preparation:

By 7pm on the 42nd day, all meals were stopped for both groups; the rats were then placed on an overnight fast. The next day morning, following chloroform anaesthesia, the animals were opened up via a laparotomic incision which was later extended to the lower thoracic cavity. Blood samples were collected from the animals via the inferior vena cava/portal vein and cardiac puncture, into already labeled lithium heparin bottles. The samples were then mixed by gentle inversion and were centrifuged at 4000r/min for 10mins to obtain plasma. The plasma supernatants were then separated into sterile plain bottles and were used for assay of the required parameters.

Tissue Sample Collection and Determination of the Relative Organ Weight:

At the end of the 42-day and overnight fast, just after the rats have been weighed, sacrificed (using chloroform anaesthesia) and blood samples collected from them, the liver, heart and kidneys, of the respective rats from both groups were carefully dissected out and weighed in grams (this weight was designated as the absolute organ weight). The relative organ weight was then calculated using the formula:

$$\text{Relative organ wt.} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100 \quad (44)$$

Determination of Plasma Lipid Profile

The parameters assayed were total cholesterol, triacylglycerol, HDL-cholesterol, using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers and the LDL-cholesterol and VLDL-cholesterol using the Frieldewald equation (46, 47).

Determination of the Liver Function Status:

The parameters assayed were total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma-glutamyl transferase, using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers (48, 49, 50, 51, 52).

Statistical Analysis

The results are expressed as mean±SEM. Data was subjected to appropriate statistical analysis using the students paired t-test from the computerized statistical package for the social sciences, edition 18 (SPSS 18). $p<0.05$ was considered significant.

RESULTS

From the results presented in Table 4, the feed consumed, weight gain and food efficiency of the rats fed the combined high-salt and high-fat diet did not differ significantly ($p>0.05$) from those fed the basal diet.

From the results presented in Table 5, the relative organ weights of the liver, heart and kidney of the rats fed the combined high-salt and high-fat diet did not differ significantly ($p>0.05$) from those fed the basal diet.

From the results shown in Table 6, the combined diet at 6 weeks is yet to impact any significant effect ($p>0.05$) on the liver function status of the rats when compared to those fed the basal diet.

From the results in Table 7, from the 4^{th} week up till the 6^{th} week, the combined diet due to its high salt content caused a significant increase ($p<0.05$) in the blood pressure of rats when compared to those fed the basal diet.

From the results presented in Table 8, the combined diet due to its high fat content caused a significant ($p<0.05$) increase in the plasma LDL- and total- cholesterol and a significant decrease ($p<0.05$) in the plasma HDL-cholesterol in the rats when compared to those fed the basal diet. Though it also caused an increase in the plasma VLDL-cholesterol and the plasma triglyceride levels in the same rats, these increases were not statistically significant ($p>0.05$) at the end of the study.

Table 4: Feed Consumption, Weight Gain and Food Efficiency Per Rat Per Day

Parameter determined	Group 1	Group 2
Feed consumed per rat per day (g)	27.19±1.00[a]	27.85±1.00[a]
Weight gain per rat per day (g)	1.57±0.10[a]	1.51±0.11[a]
Food efficiency per rat per day (g)	5.78±0.14[a]	5.69±0.21[a]

Values are represented as mean ± SEM of six (6) determinations. Means with different superscripts on the same row differ significantly ($p<0.05$) from one another.

Table 5: Relative Organ Weight of Some Organs of the Rats

Organ	Group 1	Group 2
Liver	3.02±0.07[a]	3.88±0.23[a]
Heart	0.34±0.04[a]	0.37±0.02[a]
Kidney	0.63±0.02[a]	0.63±0.03[a]

Values are represented as mean ± SEM of six (6) determinations. Means with different superscripts on the same row differ significantly ($p<0.05$) from one another

Table 6: Liver Function Indices of the Rats

Group	Group 1	Group 2
ALT (IU/L)	10.00±0.90[a]	10.17±1.00[a]
AST (IU/L)	10.50±0.90[a]	8.17±0.50[a]
ALP (IU/L)	29.83±2.00[a]	31.50±3.00[a]
Albumin (g/dl)	2.65±0.60[a]	2.63±0.20[a]
Total protein (g/dl)	4.57±0.40[a]	5.18±0.20[a]
Total bilirubin (mg/dl)	0.95±0.20[a]	1.03±0.20[a]
Direct bilirubin(mg/dl)	0.42±0.10[a]	0.47±0.10[a]
γ-GT (IU/L)	1.10±0.20[a]	0.95±0.02[a]

Values are represented as mean ± SEM of six (6) determinations. Means with different superscripts on the same row differ significantly ($p<0.05$) from one another

Table 7: Systolic Blood Pressure of the Rats

Groups	Day 1/1st Week (mmHg)	Day 28/4th Week (mmHg)	Day 42/6th Week (mmHg)
1	115.83±5.69[a]	119.17±4.55[a]	121.67±3.80[a]
2	120.00±5.32[a]	142.50±6.29[b]	174.17±9.35[b]

Values are represented as mean ± SEM of six (6) determinations. Means with different superscripts on the same column differ significantly ($p<0.05$) from one another.

Table 8: Lipid Profile Indices

Group	Group 1	Group 2
VLDL-cholesterol (mg/dl)	10.17±0.50[a]	11.80±0.90[a]
LDL-cholesterol (mg/dl)	49.00±3.00[a]	75.97±4.00[b]
HDL-cholesterol (mg/dl)	33.67±2.00[a]	23.67±2.00[b]
Total-cholesterol (mg/dl)	92.83±3.00[a]	111.50±4.00[b]
Triglycerides (mg/dl)	50.83±2.00[a]	59.00±4.00[a]

Values are represented as mean ± SEM of six (6) determinations. Means with different superscripts on the same row differ significantly ($p<0.05$) from one another

DISCUSSION

The correlation between a high salt diet and hypertension on one hand and high fat diet and hypercholesterolaemia on the other hand with cardiovascular diseases, has made the study of animal models of hypertension and hypercholesterolaemia using a high salt and a high fat diet, important (1,4,36). Additions to a known diet like increasing the salt and fat content of a diet can result in reduced palatability of the diet and reduced consumption by rats (44). The results from this study where the feed consumed, weight gain and food efficiency of the rats fed the combined high-salt and high-fat diet did not differ significantly (p>0.05) from those fed the basal diet indicates a balance and that the additions to the diet did not adversely affect consumption. This means the food formulation in this study is good for research for conditions related to a high salt and high fat diet. This outcome is similar to the findings of Matos et al., (36).

Although there was an increase in the weight of the liver, heart and kidney which is in keeping with fatty liver, left ventricular and renal hypertrophy associated with prolonged consumption of a high salt and a high fat diet (53, 54), the relative organ weight of the liver, heart and kidney of the rats fed the combined high-salt and high-fat diet in this study did not differ significantly (p>0.05) from those fed the basal diet. The results of this study also show that the combined diet at 6 weeks was yet to have any significant (p>0.05) effect on the liver function status of the rats when compared to those fed the basal diet. Though the plasma lipids of this study show a dyslipidaemia, this has not yet resulted in general liver dysfunction even as the liver is the major organ responsible for the maintenance of homeostasis related to lipid metabolism. This lack of effect of the combined diet on the relative organ weights and the liver function status of these rats, may be as a result of the relatively short duration the diet was consumed, compared to that of other studies (53, 54, 58). The aim of the formulation of the diet of this study was to induce high blood pressure (hypertension) and high blood cholesterol (hypercholesterolaemia) at minimal cost to the functionality of these organs in the rats. The focus was the formulation of a locally available diet that will be able to induce cardiovascular disease risk factors, hypertension and hypercholesterolaemia in particular, without immediately causing obvious macroscopic deleterious effects on the liver, heart and kidney or compromising their functions. It is however possible or likely that at the microscopic level, fatty changes in the liver and other deleterious changes to the liver, heart and kidneys may have commenced, which would have been more obvious if the diets were continued for a longer duration (53, 54, 55).

In a study carried out by Matos et al., (36), there was no increase in the systolic blood pressure in the animals receiving hypercholesterolaemic diets after eight weeks of observation. This gives the impression that dietetic cholesterol or a high fat diet on its own at the initial stages has no influence on blood pressure. It can therefore be said that as from the 4th week up till the 6th week of this study, the combined diet due to its high salt content, caused a significant (P<0.05) increase in the blood pressure of rats when compared to those fed the basal diet. This is in keeping with the fact from other researchers whose results also show that a diet containing 8% NaCl is adequate in inducing hypertension in rats (4). High sodium diets are commonly used to study diet induced hypertension, since increasing levels of circulating sodium cause cells to release water (due to osmotic pressure) thereby increasing intravascular volume which elevates the pressure on blood vessel walls. The result of this study is in keeping with the results derived by other researchers who used local standard diets in their area (rat chow) in place of the purified diets commonly recommended (53).

In humans, dietary saturated fat and cholesterol play a major role in the development of cardiovascular diseases (CVD) because they contribute to hyperlipidaemia which is one of the known risk factors for CVD (1, 36, 43). Hyperlipidaemia, with its increasing rates of incidence and prevalence, is a major risk factor of coronary heart disease, and one of the most important public health problems (56, 57). Cardiovascular diseases are by nature progressive chronic and metabolic diseases which begin commonly in adults and progresses to morbidity and mortality throughout the remainder of their lifespan. Hyperlipidaemia has an important effect on development and progression of various cardiovascular diseases and atherosclerosis. Both moderate hyperlipidaemia and severe hyperlipidaemia are associated with cardiovascular disease (58). This is why a diet that can induce hyperlipidaemia/ hypercholesterolaemia in animal models is of utmost importance to the study of the pathophysiology of this risk factor associated with CVD so as to design ways to effectively put it in check. The combined diet within the 6 weeks of this study due to its high fat content was able to cause a significant increase in the LDL- and total- cholesterol and a significant ($p<0.05$) decrease in the HDL-cholesterol in the rats when compared to those fed the basal diet. Though it also caused an increase in the VLDL-cholesterol and the triglyceride levels in the same rats, these increases at the end of the study were not statistically significant ($p>0.05$). This result is in keeping in principle, with the results of other related research involving formulation of diets that can induce hypercholesterolaemia in rats (36, 39, 43).

CONCLUSION

The feeding of albino *Wistar* rats with combined high-salt/high-fat diet made by adding 8% NaCl and 2% cholesterol + 0.25% bile salts + 20% butter to a proportionate quantity of the basal diet (already modified by replacing soy protein in locally available rat chow with other protein products), in this study, was found to have promoted a significant increase ($p<0.05$) in blood pressure, plasma LDL-cholesterol, plasma total cholesterol and a significant decrease ($p<0.05$) in plasma HDL-cholesterol in the albino *Wistar* rats without affecting negatively the hepatic function of these rats. By this result the experimental diet can be said to have been able to induce hypertension and hypercholesterolaemia/hyperlipidaemia in the rats. These are known risk factors for cardiovascular diseases, indicating that the diet could help in further studies of these risk factors related to cardiovascular diseases.

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