EFFECT OF PROLONGED ADMINISTRATION OF HIGH FAT DIET ON SERUM BIOCHEMICAL, HAEMATOLOGICAL AND ORGAN HISTOLOGY IN RATS

ABSTRACT

Background: Numerous health challenges have been associated with lipid peroxidation andoxidative damage due to increased consumption of fatty diets.

Aim: This study evaluated serum biochemical and haematological parameters, tissue antioxidant activities and histopathological changes inwistar rats administered high fat diet.

Methods: Forty rats of both sexes weighing 120-150g and assigned to 4 groups of 10 rats each were used. Groups 1 and 2 served as female and male controls respectively, while groups 3 was the female test group which received high fat diet. Group 4 was the male test group which also received high fat diet. Treatment lasted 16 weeks before animals were sacrificed to collect blood and tissue samples forserumbiochemical analyses including liver function, renal function and antioxidants tests. Slides of liver and kidney samples were also prepared for histopathological examination.

Results: When compared with control results obtained in the high fat treated rats showed significant increase inbody weight gain ($p<0.05$), increased lipid peroxidation activity (MDA), significant decrease in activities of tissue antioxidant enzymeslikeglutathione transferase (GST), glutathione peroxidase (GPx), catalase (CAT) andsuperoxide dismutase (SOD), PON 1 and reduced glutathione (GSH) concentration with higher PCO values ($p<0.05$). Liver function parameters including aspartate aminotransferase(AST), alanine aminotransferase(ALT), alkaline phosphatase (ALP), total protein and bilirubin had higher values in the high fat treated rats than control. Total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein concentrations were also higher in the high fat treated groups but values of high density lipoprotein cholesterol did was not significantly altered following treatment ($p>0.05$). Haematological parameters like number of red blood cells, packed cell volume and haemoglobin concentration, all significantly reduced in the high fat groups ($p<0.05$), but number of white blood cells increased significantly. Histopathological examination of liver tissues showed areas with fibrosis, steanosis and lobular inflammations in the high fat groups.

Conclusion: Increased lipid peroxidation and decline in antioxidant defense may account for higher cardiovascular disease risks and its associated complications commonly seen among high fat consumers.

Key words: Lipid peroxidation, high fat diets, antioxidants, liver function, histopathology

1.0 INTRODUCTION

Lipid Peroxidation is oxidative damage that affects cellular membrane lipoproteins and other lipid containing molecules. This lipid peroxidation is a free-radical mediated chain of reactions that, once initiated, results in an oxidative deterioration of polyunsaturated lipid (1). Consequently, free radicals are produced in the body as by-products of normal metabolism or as a result of exposure to radiation and some environmental pollutants. The imbalance between the prooxidant load and the adequacy of antioxidant defense is susceptible to free radical mediated damage in the body (2)

This study is determined to explore the effect of high lipid peroxide diet on antioxidant defense in rats using the following parameters; thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST), in the liver, kidney and heart of rats fed with the high peroxidizable fat diet and compared to the control rats. Oxidative stress is highly correlated with a wide variety of inflammatory and metabolic disease states (3, 4,5). It is highly correlated with cumulative damage in the body done by free radicals inadequately neutralized by antioxidants (6). Oxidative damage is aggravated by the decrease in antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT), glutathione s-transferase (GST), and glutathione peroxidase (GPX), which act as free radical scavengers in conditions associated with oxidative stress (7). Paraoxonase (PON1) is another antioxidant enzyme closely associated with high-density lipoproteins. It is a calcium-dependent esterase, which detoxifies lipid peroxides, and is widely distributed in many tissues, including the liver, brain, lung, heart, kidneys, small intestine and aorta (3). Evidence suggests that a clustering of sources of oxidative stress exits in obesity; hyperglycemia, increase tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, and chronic inflammation (8).

It was therefore against this background that this study was designed to evaluate the effects of prolonged administration of high fat diet on serum biochemical parameters, haematological parameters and organ histopathological changes in rats, with a view to provide further information on the systemic impact of high fat diet on life.

2.0 MATERIALS AND METHODS

2.1 Experimental design

This study was carried out on 40 white male and female albino rats, with weight range of 120-150g which were obtained from the breeding unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were kept in wire mesh cages with adequate access to water. The room temperature was about 22-24^{0}C and the animals were exposed to 12:12 hours light dark cycles. After one week of acclimatization, the animals were randomly divided into four equal groups. Each of the rats were painted at the head with indelible dyes of different colours and then put into different cages.

-Group 1(control Female): Received only normal diet for 16 weeks.

-Group 2 (control male): Received only normal diet for 16 weeks.

-Group 3 (high fat female): Received the peroxidizable fat diet for 16 weeks.

-Group 4 (high fat male): Received the peroxidizable fat diet for 16 weeks.

By the end of the experimental period, all rats were sacrificed and the abdomen and the thorax were opened and both liver, kidney were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue MDA, PCO, GSH levels and the activity of GST, GPX, CAT and PON1 enzymes.

2.2 Experimental diet formulation

Experimental diets (g/kg) were formulated and they include:

(i) **The normal diet for control rats:** fat 5% (corn oil 5%), carbohydrates 65% (corn starch 15% and sucrose 50%), proteins 20.3% (casein 20% and DL-methionine 0.3%) fiber 5%, salt mixture 3.7%, and vitamins mixture 1%).

(ii)**The high fat diet contains:** fat 46% (corn oil 25.5% and beef tallow 20.5%) carbohydrates 24% (corn starch 6% and sucrose 18%), proteins 20.3% (casein 20% and DL-methionine 0.3%, fiber 5%, salt mixture 3.7% and vitamin mixture 1%) (Kim *et al*, 2005).

2.3 Preparations of tissue homogenates.

Preparation of tissue homogenates specimens from organ were separated into three parts. Each piece was weighed and homogenized separately with a potter-Elvehjem tissue homogenizer.One part of tissue (liver, kidney) was homogenized in phosphate buffer saline

(PBS) 50m M PH (7.4) for estimation of protein content of GST, CAT enzymes activities and GSH levels.The second part of the tissue was homogenized in potassium phosphate buffer 10mM PH (7.4) for estimation of MDA, PCO levels and GPX activity. The third part of the tissue was homogenized in tris-HCL 100mM, PH (8) for estimation of PON1 activity. The crude tissue homogenate was centrifuged at 10,000 rpm, for 15 minutes in cold centrifuge and the resultant supernatant was used for the different estimations.

2.4 Determination of antioxidant parameters on tissue homogenate and other biochemical parameters on serum samples

Catalase (CAT) activity on tissue homogenate was determined using the method of Shina (9). Superoxide dismutase (SOD) activity, reduced glutathione and malondialdehyde concentrations were assayed in accordance with the method of McCord and Fridouich (10). Other biochemical parameters including lipid profile parameters (total cholesterol, high density lipoprotein cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol), liver function parameters (alanine aminotransferase activity, alkaline phosphatase activity, aspartate aminotransferase activity, total protein, total bilirubin and albumin concentrations), kidney function parameters (urea, creatinine, and electrolytes) were assayed using their respective commercial test kits (Randox laboratories, UK).

2.5 Histopathological examination.

The tissues of the kidney, liver and heart of Wistar rats were carefully removed and they were fixed with 10% neutral formalin. The kidney and liver were disserted, embedded in paraffin and 5um sections were cut by using a rotary microtome and the samples were then stained with haematoxylin and eosin (H&E) for microscopic examination

2.6 Statistical analysis.

Collected data were subjected to statistical Analysis of Variance (ANOVA) with the Statistical Package for Social Sciences (SPSS) for window version 22.0. The Duncan post hoc test was used to identify the means that differ significantly at $p < 0.05$. Results were expressed as Mean ± standard error of the mean (SEM).

3.0 RESULTS

The result of body weight changes as shown in Table 1 reveals that rats subjected to a high-fat diet experienced significantly higher weight gain and percentage weight gain compared to the control groups. High-fat female rats gained 150.46±13.81g (114.04±14.58%), and high-fat male rats gained 134.88±14.32g (108.95±13.65%), both of which were significantly different from their respective control groups ($P \leq 0.05$).

The result of the liver homogenate test, as shown in Table 2, reveals that high-fat diet groups exhibited significantly higher TBARS, MDA, and PCO levelscompared to control groups. Specifically, high-fat females and males had elevated TBARS (5.07±0.10 and 4.86±0.04,

respectively), MDA (5.91±0.05 and 6.02±0.18, respectively), and PCO (1.66±0.05 and 1.40±0.03, respectively). Conversely, antioxidant enzymes such as GST, GPx, CAT, SOD, and GSH were significantly reduced in the high-fat groups.

The result of the kidney homogenate test, as shown in Table 3, reveals that high-fat groups displayed significantly increased MDA and PCO levels, indicating elevated oxidative stress, with MDA and PCO being highest in high-fat males (5.08±0.07 and 2.06±0.06, respectively). Antioxidant enzymes GPx, CAT, GSH, and SOD were significantly reduced in the high-fat groups compared to controls($P \leq 0.05$).

The result of liver function, as shown in Table 4, reveals that high-fat diet groups exhibited significantly increased TP, AST, ALT, ALP, and bilirubin levels compared to controls. High-fat females (8.51±0.09, 64.40±1.60, 54.80±1.62, 73.20±3.12, and 0.90±0.02, respectively) and males (8.72±0.06, 66.40±2.29, 56.80±2.82, 80.60±2.80, and 1.16±0.19, respectively) showed elevated values.

The result of kidney function, as shown in Table 5, reveals that high-fat diet groups exhibited significantly increased Na^+ and Cl^- levels compared to controls, with high-fat females (140.14±0.47 and 102.95±1.73, respectively) and males (140.50±1.39 and 113.98±1.32, respectively) showing elevated values. K+ levels were slightly increased in high-fat females (5.36±0.12), while urea and creatinine levels showed slight elevations in high-fat groups, particularly creatinine in high-fat males (0.67±0.04).

The result of lipid profile, as shown in Table 6, reveals that high-fat diet groups had significantly elevated total cholesterol (T.Chol), triacylglycerol (TAG), LDL, and VLDL levels compared to controls. High-fat females showed T.Chol (178.04±2.64), TAG (178.98±2.50), LDL (145.09±4.26), and VLDL (35.80±0.50), while high-fat males showed T.Chol (173.52±4.23), TAG (177.46±2.41), LDL (139.29±3.10), and VLDL (35.49±0.48). HDL levels were similar across all groups.

The result of the hematological examination, as shown in Table 7, reveals that high-fat diet groups exhibited reduced RBC, PCV, Hb, and MCHC levelscompared to controls. High-fat females showed RBC (6.58±0.14), PCV (41.80±0.73), Hb (12.96±0.17), and MCHC (31.02±0.21), while high-fat males showed RBC (6.30±0.16), PCV (40.20±1.07), Hb (12.16±0.37), and MCHC (30.24±0.22). Conversely, WBC and PLT levels were significantly elevated in the high-fat diet groups. MCV and MCH levels remained relatively unchanged across groups ($P \leq 0.05$).

Table 1: Body weight

Groups	Treatment	Initial Body Weight (g)	Final Body Weight(g)	Weight gain(g)	% Weight gain
1	Control Female	139.56±4.93	240.82±14.50	101.26±10.03	72.05±5.28

2	Control Male	135.48±4.53	205.14±8.20	69.66±8.43	51.94±7.02
3	High Fat Female	131.94±6.76	282.40±9.62	150.46±13.81	114.04±14.58
4	High Fat Male	125.08±4.86	259.96±13.92	134.88±14.32	108.95±13.65

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05).

Table 2: Liver homogenate test.

Groups	Treatment	TBARS	GST (U/mg)	GPx (U/mg protein)	CAT (U/mg)	MDA (nM/mg protein)	GSH (U/mg)	SOD (nM/mg)	PCO (nM/mg)	PON (nM/mg)
1	Control Female	0.74±0.03	21.69±0.38**	26.22±0.30	48.58±0.56	2.39±0.06	28.40±0.26	28.71±0.29	0.86±0.02	3.81±0.04
2	Control Male	0.82±0.03	23.13±0.34*	26.05±0.60	43.66±0.45	2.68±0.04	27.75±0.34	27.86±0.31	0.84±0.01	3.81±0.04
3	High Fat Female	5.07±0.10**	17.29±0.42**	21.17±0.28**	42.29±0.66*	5.91±0.05**	23.87±0.40**	12.64±0.08**	1.66±0.05**	3.27±0.06**
4	High Fat Male	4.86±0.04**	18.68±0.27**	23.59±0.51**	29.12±3.28**	6.02±0.18**	23.50±0.48**	12.23±0.41**	1.40±0.03**	3.64±0.12

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05)

Table 3: Kidney homogenate test

Groups	Treatment	GPx (µg/ml)	CAT (nm/ml)	MDA (Mmol/L)	GSH (µg/ml)	SOD (u/mg)	PCO (nMole/mg protein)	PON(mIu/mg protein)
1	Control Female	24.16±0.22**	48.37±0.14	2.59±0.05	26.84±0.14**	14.51±0.21	0.96±0.02	3.74±0.04**
2	Control Male	25.35±0.11*	47.96±0.50	2.69±0.21	25.65±0.20*	14.21±0.22	0.95±0.01	3.59±0.05*
3	High Fat Female	21.44±0.13***	41.39±0.18***	4.34±0.03***	22.95±0.27***	10.80±0.16***	1.86±0.04***	3.39±0.02***
4	High Fat Male	20.76±0.23***	43.39±0.51***	5.08±0.07***	22.89±0.24***	10.45±0.05***	2.06±0.06***	3.23±0.02***

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05).

Table 4:Liver function

Groups	Treatment	TP (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)
1	Control Female	7.96±0.14	33.40±1.08	16.80±0.97	57.20±2.08	0.44±0.04
2	Control Male	7.93±0.20	34.20±2.01	18.20±1.28	62.00±3.08	0.57±0.02
3	High Fat Female	8.51±0.09* **	64.40±1.60* **	54.80±1.62* **	73.20±3.12* **	0.90±0.02* **
4	High Fat Male	8.72±0.06* **	66.40±2.29* **	56.80±2.82* **	80.60±2.80* **	1.16±0.19* **

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05)

Table 5: Kidney function

Groups	Treatment	Na^+ (mEq/L)	K^+ (mEq/L)	Urea (mg/dl)	Creatinine (mg/dl)	Cl^- (mEq/L)
1	Control Female	130.70±0.81	5.04±0.10	15.48±0.41**	0.55±0.04	84.81±2.78
2	Control Male	132.36±1.29	5.11±0.04	17.05±0.52*	0.55±0.01	92.45±1.56
3	High Fat Female	140.14±0.47* **	5.36±0.12*	15.77±0.31	0.63±0.02	102.95±1.73
4	High Fat Male	140.50±1.39* **	5.26±0.08	15.79±0.52	0.67£0.04* **	113.98±1.32

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05)

Table 6: Lipid profile

Groups	Treatment	T.Chol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	Control Female	112.86±2.21	129.74±0.71	65.65±1.81	73.15±3.11	25.95±0.14
2	Control Male	107.62±1.79	128.18±0.74	67.37±0.69	65.88±2.38	25.64±0.15
3	High Fat Female	178.04±2.64***	178.98±2.50**	68.74±2.11	145.09±4.26**	35.80±0.50**
4	High Fat Male	173.52±4.23***	177.46±2.41**	69.72±2.80	139.29±3.10**	35.49±0.48**

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05)

Table7: Haematological Examination

Groups	Treatments	RBC ($x10^6/mm^3$)	PCV (%)	Hb (g/dl)	WBC ($x10^3/mm^3$)	PLT($x10^3/mm^3$)	MCV (*fl*)	MCH (*pg*)	MCHC (g/dl)
1	Control Female	6.63±0.11**	42.20±0.73	13.22±0.37**	9.26±0.28	513.20±12.30	63.61±0.31	19.91±0.28	31.32±0.53
2	Control Male	7.02±0.04*	44.20±0.37	14.22±0.09*	9.94±0.45	513.20±6.58	62.96±0.41	20.26±0.13	32.18±0.13
3	High Fat Female	6.58±0.14**	41.80±0.73**	12.96±0.17***	13.54±0.80***	556.00±19.29	63.57±0.43	19.72±0.24	31.02±0.21**
4	High Fat Male	6.30±0.16**	40.20±1.07**	12.16±0.37****	14.02±0.65***	546.80±17.19	63.85±0.31	19.31±0.13**	30.24±0.22* **

Results are represented as Means±SEM. Means with one astericks (*) are significantly different from Control Female while means with two astericks(**)are significantly different from Control Male (P≤0.05)

Histopathological examination

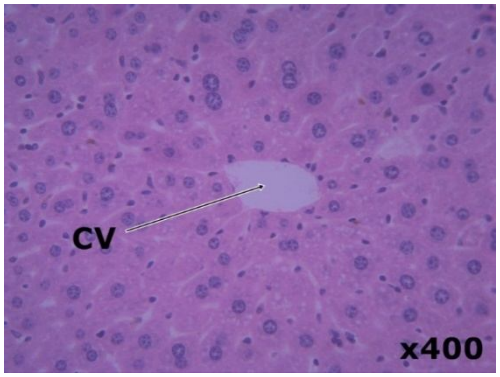

Plate 1: Photomicrograph of liver: an architecture from rats in normal control female (Group 1). This shows well preserved liver architecture. The portal triads are evenly spaced around a central vein (CV) and there is neither portal inflammation nor fibrosis. There is no steatosis.

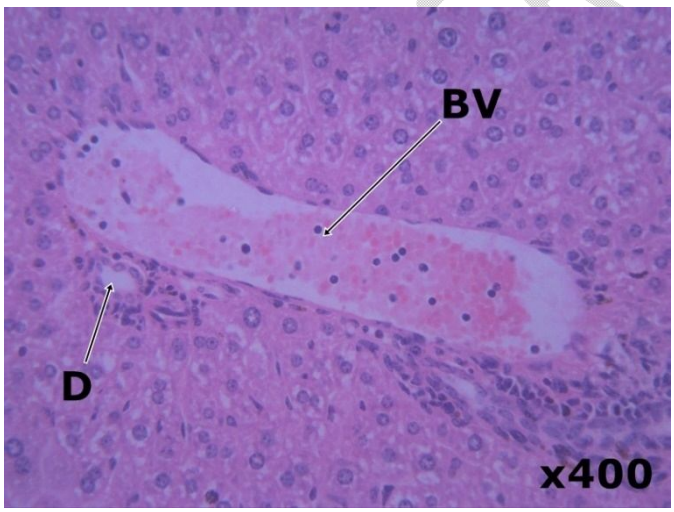

Plate 2: Photomicrograph of liver: an architecture from rats in normal control male (Group 2). This shows well preserved liver architecture. The portal triads are evenly spaced around a central vein (CV) and there is neither portal inflammation nor fibrosis. There is no steatosis. The blood vessels and hepatic ductile are free from arteriosclerosis. There is no pathology

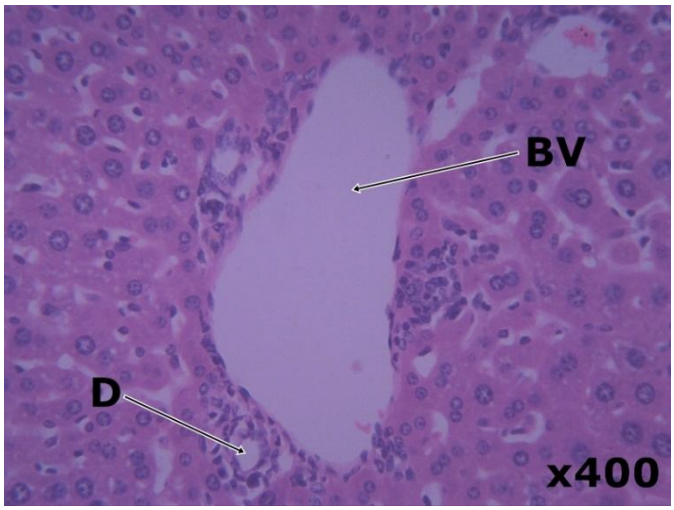

Plate 3: Photomicrograph of liver: an architecture from rats in high fat female (Group 3). This shows well preserved liver architecture. When the sections examined, fibrosis in portal area, steatosis in hepatocytes and lobular inflammation in the portal area were observed in the group.

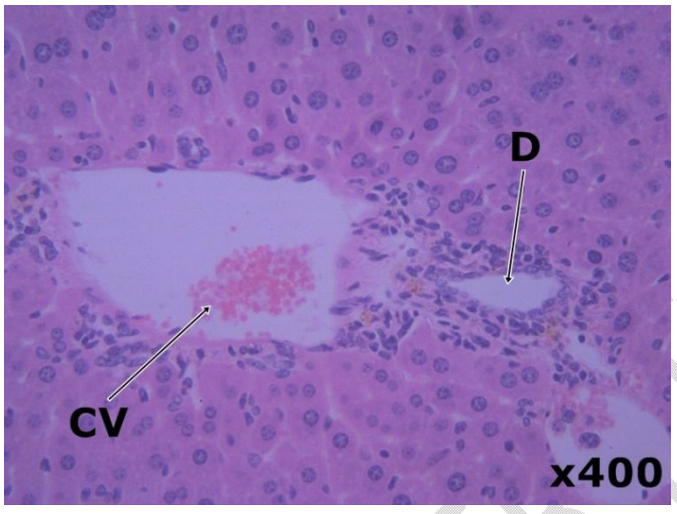

Plate 4: Photomicrograph of liver: an architecture from rats in high fat male (Group 4). This shows well preserved liver architecture. When the sections examined, fibrosis in portal area, steatosis in hepatocytes and lobular inflammation in the portal area were observed in the group.

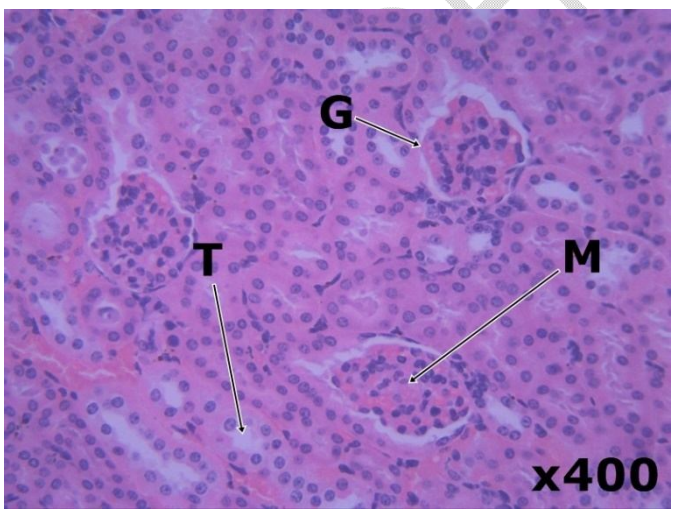

Plate 5: Photomicrograph of kidney: an architecture from rats in normal control female (Group 1). Photomicrograph shows evenly distributed glomeruli (G), of similar size, with normal mesangial (M) cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules (T) are of normal density and tubular epithelium is viable.

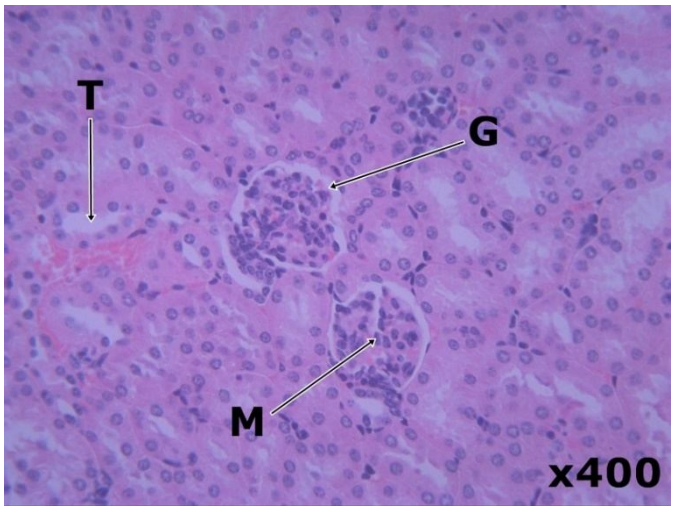

Plate 6: Photomicrograph of kidney: an architecture from rats in normal control male (Group 2). Photomicrograph shows evenly distributed glomeruli (G), of similar size, with normal mesangial (M) cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules (T) are of normal density and tubular epithelium is viable.

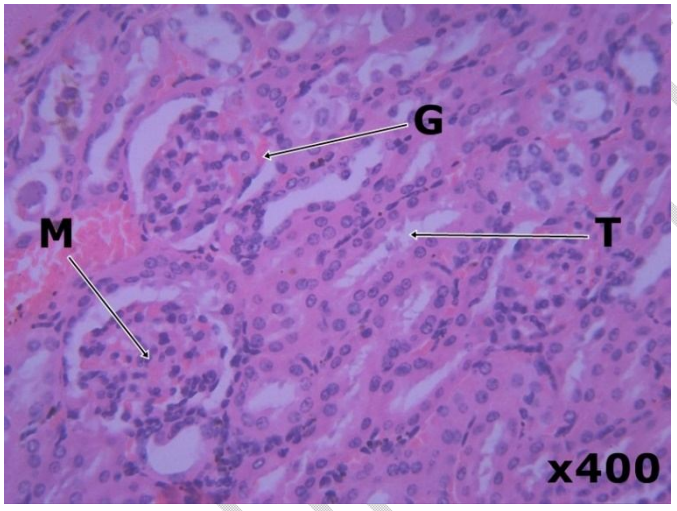

Plate 7: Photomicrograph of kidney: an architecture from rats in high fat female (Group 3). Photomicrograph shows that the kidney tissue of rats fed with high fat diet presented several alterations including variable size of glomeruli, bleeding and congested blood vessels and deformations of several tubular structures. light microscopic evaluation of kidney sections detected bleeding and congestion in the blood vessels and dilation in glomerular capillaries. Also, the Bowman's space was enlarged and several tubular structures were defected.

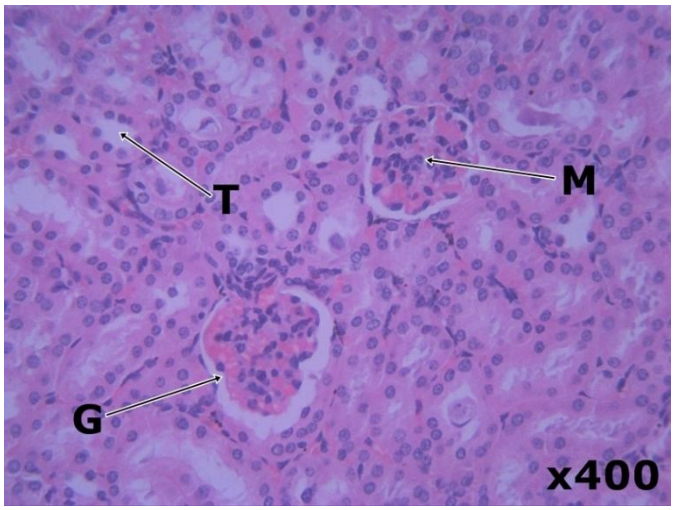

Plate 8: Photomicrograph of kidney: an architecture from rats in high fat male (Group 4). Photomicrograph shows that the kidney tissue of rats fed with high fat diet presented several alterations including variable size of glomeruli, bleeding and congested blood vessels and deformations of several tubular structures. light microscopic evaluation of kidney sections detected bleeding and congestion in the blood vessels and dilation in glomerular capillaries. Also, the Bowman's space was enlarged and several tubular structures were defected.

4.0 DISCUSSION

The findings from the study on body weight changes in rats subjected to a high-fat dietalign with existing literature indicating that such diets often lead to significant weight gain compared to control groups. While some studies suggest that high-fat diets can induce obesity through increased energy intake and prolonged exposure, others, like the one by Chen *et al.* (11) and Chen *et al*. (12) indicate that weight gain may not be immediate and can depend on factors such as the age of the rats and duration of the diet. For instance, it has been noted that metabolic homeostasis can be maintained for several weeks before noticeable weight gain occurs, suggesting a complex relationship between dietary fat content and body weight regulation (13). Additionally, the energy density of high-fat diets is often cited as a critical factor influencing weight gain; these diets are typically more palatable and energy-dense, which can lead to higher caloric intake over time, thereby exacerbating weight gain (14).

The results from the liver homogenate test indicate that rats on a high-fat diet exhibited significantly elevated levels of TBARS, MDA, and PCO, alongside reduced antioxidant enzyme activities. This finding is consistent with previous studies that have demonstrated high-fat diets lead to increased oxidative stress in the liver, primarily through enhanced lipid peroxidation and protein oxidation, which are critical markers of cellular damage (15). Other research supports these observations, showing that chronic high-fat consumption induces oxidative stress by upregulating reactive oxygen species (ROS) production, resulting in mitochondrial dysfunction and hepatocyte apoptosis (16). Moreover, the observed decrease in antioxidant enzymes such as GST and SOD in high-fat diet groups aligns with findings that suggest a depletion of the liver's antioxidant defenses under oxidative stress conditions (17). Collectively, these results highlight the detrimental effects of high-fat diets on liver health, underscoring the importance of antioxidant mechanisms in mitigating oxidative damage.

The kidney homogenate test results indicate that rats on a high-fat diet exhibited significantly elevated levels of malondialdehyde (MDA) and protein carbonyls (PCO), particularly in high-fat males, suggesting increased oxidative stress. This finding is consistent with previous research that demonstrates a strong correlation between high-fat diets and oxidative stress, where increased lipid peroxidation and reduced antioxidant enzyme activity were observed in similar experimental setups (18,19). Specifically, studies have shown that high-fat diet-induced oxidative stress leads to a marked decline in antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), mirroring the significant reductions noted in your findings ($P \leq 0.05$) (20). Furthermore, the elevated oxidative stress markers in this study align with the notion that prolonged exposure to high-fat diets exacerbates renal oxidative injury and metabolic dysfunction, reinforcing the critical role of dietary composition in modulating oxidative stress responses (18,20).

The liver function results indicate that rats subjected to a high-fat diet exhibited significantly elevated levels of TP, AST, ALT, ALP, and bilirubin, suggesting hepatic dysfunction. This

observation is consistent with previous studies that have documented similar increases in liver enzymes as indicators of liver injury and inflammation due to high-fat diets (21). Specifically, the elevation in AST and ALT levels is often associated with hepatocellular damage and steatosis, conditions frequently observed in models of diet-induced obesity (22). Furthermore, the rise in ALP and bilirubin levels may reflect cholestatic liver injury or impaired bile flow, which has been reported in other research examining the effects of high-fat diets on liver health (15).

The results of the kidney function analysis in rats subjected to a prolonged high-fat diet (HFD) indicate significant alterations in electrolyte levels and markers of renal function when compared to control groups. Specifically, the elevated sodium (Na^+) and chloride (Cl^-)levels in high-fat diet groups suggest a potential disruption in renal homeostasis, which aligns with findings from other studies indicating that HFD can lead to renal lipotoxicity and metabolic dysregulation, ultimately compromising kidney function (23,24). The slight increase in potassium (K^+) levels, particularly in high-fat females, may reflect adaptations or imbalances in renal handling of electrolytes under dietary stress (25). Furthermore, the observed elevations in urea and creatinine levels, especially notable in high-fat males, are consistent with literature that associates HFD with increased markers of renal injury and impaired glomerular filtration rate (eGFR) (23). These findings corroborate previous research indicating that long-term consumption of high-fat diets can induce structural and functional changes in the kidneys, contributing to conditions such as glomerulopathy and proximal tubular injury (24).

The findings from the lipid profile analysis in rats subjected to a prolonged high-fat di*et* align with existing literature that indicates such diets lead to significant alterations in serum lipid levels. Specifically, the elevated levels of total cholesterol, triacylglycerol, LDL, and VLDL observed in both male and female rats are consistent with previous studies that have documented similar increases in hyperlipidemic conditions induced by high-fat diets (26,27). For instance, research has shown that high-fat diets can lead to dyslipidemia characterized by increased triglycerides and cholesterol, which is a well-established risk factor for cardiovascular diseases (14). Interestingly, the lack of variation in HDL levels across groups suggests a potential protective mechanism or a metabolic adaptation that warrants further investigation, as other studies have reported contrasting effects on HDL in similar dietary contexts (28).

The findings from the hematological examination indicate significant alterations in blood parameters due to prolonged high-fat diet exposure, aligning with existing literature that highlights similar trends. Specifically, reductions in red blood cell (RBC), packed cell volume (PCV), hemoglobin (Hb), and mean corpuscular hemoglobin concentration (MCHC) have been consistently reported in studies involving high-fat diets, suggesting a potential link to obesity-related anemia characterized by chronic inflammation and oxidative stress (29,30). Conversely, the observed elevation in white blood cell (WBC) and platelet (PLT) counts may reflect a compensatory inflammatory response to the dietary-induced metabolic derangements, corroborating findings from other research that documented increased leukocyte counts in high-

fat diet models (14). Interestingly, the stability of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) across groups suggests that while the high-fat diet impacts overall hematological health, it may not uniformly affect all erythrocyte indices, a phenomenon also noted in related studies (28).

The histopathological examination of liver and kidney tissues from rats subjected to a prolonged high-fat diet reveals significant alterations when compared to control groups. In the liver, both female and male rats on a high-fat diet exhibited fibrosis, steatosis, and lobular inflammation, which aligns with findings from prior studies indicating that high-fat diets lead to nonalcoholic fatty liver disease (NAFLD) characterized by increased lipid accumulation and inflammatory responses in hepatocytes (21,31). The well-preserved architecture observed in control groups, with evenly spaced portal triads and absence of steatosis, contrasts sharply with the pathological changes noted in the high-fat diet groups, highlighting the detrimental effects of dietary fat on liver health (32, 31). Similarly, kidney tissues from high-fat diet rats demonstrated glomerular alterations, including variable glomeruli size, vascular congestion, and tubular deformation, consistent with previous research linking high-fat intake to renal damage and dysfunction (33, 23).

4.1 Conclusion

The findings of this study reveal that prolonged consumption of a high-fat diet induces significant alterations in serum biochemical and hematological parameters, characterized by elevated markers of lipid peroxidation, decreased antioxidant enzyme activities, and compromised liver and kidney function. Tissue antioxidant activities were markedly reduced, while histopathological analyses showed evidence of liver fibrosis, steatosis, lobular inflammation, and extensive renal damage, including glomerular deformation and tubular injury. These results highlight the detrimental effects of a high-fat diet on systemic and organ-specific health, emphasizing the need for dietary moderation to prevent associated metabolic and oxidative stress-related disorders.

ETHICAL APPROVAL:
This study was carried out in compliance with international guidelines for care and use of laboratory animals as approved by the ethical committee of the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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