Palm Wine and Orijin Bitters Severed Hyperlipidemia and Immunomodulatory Responses in Atherogenic-diet Fed Male Wistar Rats

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Abstract

Purpose: This research investigated some health implications of the daily consumption of palm wine and orijin bitters in male Wistar rats fed with atherogenic diet. *Materials and Methods:* One hundred and fifteen rats were allotted into two groups comprised of 65 and 50 rats, and fed with atherogenic diet (AD) or non-atherogenic diet (N) for six weeks respectively. The rats were administered palm wine and orijin bitters daily, such that: A (AD), B (AD + palm wine), C (AD + orijin bitters), D (AD + simvastatin), E (N), F (N + palm wine), G (N + orijin bitters) for twelve weeks. *Results:* AD reduced feed intake, which increased after alcoholic beverages were administered ($p<0.05$). Serum HDL-C and esterified cholesterol were increased ($p<0.05$) in alcoholic beverages only administered rats, while other lipid parameters were increased ($p<0.05$) in the AD fed rats. AD and alcoholic beverages increased ($p<0.05$) total cholesterol and triglycerides in the liver, small intestine and heart. Increases ($p<0.05$) were obtained in oxLDL-C in the heart and plasma in the AD fed rats, while paraoxonase, glutathione peroxidase and reduced glutathione were reduced ($p<0.05$). Malondialdehyde was increased ($p<0.05$) in the liver, heart, small intestine and blood. Increases ($p<0.05$) were recorded in prealbumin, interleukin-6, TNF-α and C-reactive protein in all the rats, and AD reduced ($p<0.05$) total immunoglublin and phagocytic capabilities. *Conclusions*: From the foregoing, it was evident that the administration of the alcoholic beverages severed the hyperlipidemic responses precipitated by the AD and down modulated the immune response. Therefore, the indulgence in alcoholic beverages is highly discouraged with AD.

Keywords

1. Introduction

In the developed and developing countries, there has been observed escalations in the overwhelming consumption of fast foods, such as pizzas, hot dogs, grilled meat and chicken, barbeque, sausages etc., that were attributed to non availability of the alleged long time to be expended on cooking and the stressful cooking processes. The occurrence has been alleged to be the resultant effects of the tight work schedule, hasty lifestyles, stressful routine and the 'typical classy' life styles in urbanized areas. However, almost all typical fast-foods that are consumed by humans are atherogenic in nature. An atherogenic diet is a food that

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contained high fat content, especially saturated fats, trans-fats and cholesterol, and salt (Allison, 2011). This diet is renowned to promote inflammatory disorders, loss of organ functions, atheromas, which are inflamed plaques that are formed on the insides of arteries ('hardening' or 'furring' of the arteries), the hallmark of atherosclerosis, heart attack and stroke. Although non-dietary factors, such as non exercise, sedentary lifestyle, over feeding etc had been implicated in these health conditions (Johnatham, 2005).

In the same vein, the indulgence in the consumption of alcoholic beverages to complement diets, for various reasons, such as medical, recreational, artistic inspiration, as aphrodisiacs, euphoric effects, a replacement of water and relaxant etc.; and the rising popularity of phyto-medicines due to the alleged advantages of being efficacious, highly prophylactic and also a cheap source of medical care have resulted in impressive patronage and usage of alcohol (Oyewo *et al.*, 2013). Although, most of the herbal mixtures are often found as alcoholic suspensions with characterized bitter taste, however, a few are also suspended in non alcoholic media.

Orijin bitters is an example of an alcoholic polyherbal mixture prepared from a mixture of many plants parts and plant species. It is made by Guinness Nigeria Plc, which is owned by the multinational drinks company, San Diageo. Orijin Bitters aims to differentiate itself from the competitive market by having a bittersweet taste with various alleged health benefits, making it is an absolute must drink for many people in Nigeria.

Palm wine is a milky alcoholic beverage, also referred to as palm toddy or simply toddy that is obtained from the sap of the tropical plants of the *Palmae* family. It is a fermented liquid known as emu, nkwu, oguro in Nigeria, nsamba in the Democratic Replubic of Congo, nsafufo in Ghana, montago in Cameroun, tuak in North Samatra, Indonesia and tuba in Phillipines and Bornoe. It is a beverage commonly consumed in the Southern and Eastern parts in Nigeria (Elijah *et al.*, 2010). Palm wine had been alleged to help in enhancing sight because it contains the bacteria, yeast and other useful chemical properties that are very good for treating some eye problem, measles, etc. (Ogbulie *et al.*, 2007; Adeleke *et al.*, 2015).

From the foregoing and the vast numbers of scientific reports on the health benefits of the moderate-regular consumption of alcoholic beverages with food, especially grape wines (Renaud *et al.*, 1999; Volcik *et al.* 2008), there has been massive indulgence the consumption of alcoholic beverages. However, recently, there have been increased calls for concerns in the societies as well as by health professionals, due the close correlation between heavy use of alcohol beverages and lipid metabolic anomalies (WHO, 2014). The occurrences informed a school of thought on likely pathological relationship(s) that could exist between the frequent consumption of these alcoholic beverages and atherogenic diets, which are routinely, consumed food by humans in the ever busy modernized world. Therefore, this research work evaluated the relationship(s) inherent in the daily consumption of palm wine and Orijin bitters in atherogenic diet fed male Wistar rats.

2. Materials and Methods

2.1. Materials

2.1.1. Experimental Animals

One hundred and fifteen male Wistar rats weighing between 155 and 172g were purchased from the animal house, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

2.1.2. Cholesterol Salt

Na-cholate was a product of products of Bulk supplements.com, 7511 Eastgate Road, Henderson, NV89011

2.1.3. Bone Marrow and Groundnut Oil

These were obtained locally in the Ogbomoso, southwest region in Nigeria.

2.1.4. Palm Wine

Fresh Palm wine was obtained locally in Owode area, Ogbomoso, the southwest region in Nigeria.

2.1.5. Orijin Bitters

Orijin bitters was a product of Guiness Nigeria Plc, 24, Oba Akran Avenue, Ikeja, Lagos State, Nigeria.

2.1.6. Reagent Kits

Quantitative assay kits for the determination of total cholesterol, free cholesterol, glutathione peroxidase, triglycerides and high density lipoprotein-cholesterol and pre-albumin were products Fortress Diagnostic Laboratory, unit 2c, Antrim Technology Park, AntrimBT41, United Kingdom. The assay kits for interleukin 6, tumour necrosis factor-α and C-reactive protein determinations were products of RayBio Technology, inc. USA. Oxidized low density lipoprotein-cholesterol was a product of CUSABIO BIOTECH CO. LTD and paraoxonase was produced by Cloud-Clone Corp -Uscn Life Sciences Inc. 11271 Richmond Avenue Suite H104, Houston, TX77082, USA.

2.1.7. Carbon Ink Suspension

The carbon ink suspension was a product of Pelica AG, Germany.

2.1.8. Drugs and Chemicals

All drugs, chemicals and reagents were of analytical grade, either a product of the British Drug House (BDH) Poole

England, or Sigma Aldrich, Wisconsin U.S.A.

2.2. Methods

2.2.1. Preparation of Atherogenic Diet

The atherogenic diet was prepared following the previous methods of Adekunle *et al*. (2013) with slight modifications as illustrated in Table 1.

Table 1. Composition of standard and atherogenic diets.

Constituents	Weights(kg) Standard diet (Kg)	Atherogenic diet (Kg)
Maize	4.485	4.485
Soya Beans	1.875	1.875
Brewery Dry Grain –BDG	8.750	8.750
Wheat Bran	2.500	2.500
Rice bran	6.250	6.250
Oyster Shell	0.500	0.500
Bone shell	0.250	0.250
Common Salt	0.0625	0.0625
Methione	0.0250	0.0250
Fish Meal	0.250	0.250
Groundnut Oil	-	0.300
Bone Marrow*	-	1.000
Na-cholate*	-	0.250

(Adekunle *et al*., 2013) with slight modification *

2.2.2. Animal Handling and the Administration of Palm Wine and Orijin Bitters

The rats were kept in wooden cages and allotted into two groups (65 and 50 rats, respectively), allowed access to standard feed and tap water *ad libitum*. The cages were cleaned daily and the rats were kept under condition of uniform humidity and temperature on a 12 hours light-dark cycle and were allowed to acclimatize for 14 days, during which, they were monitored closely for any changes in their behaviour and activities. They were subsequently fed with either atherogenic diet (65 rats) or non atherogenic diet (50 rats) for a period of six (6) weeks, after which five rats were picked from each group and sacrificed by mild anaesthesia with diethyl ether after an overnight fast. The chest region was quickly opened and plasma and serum were prepared from the blood drawn by puncturing the heart. Organs of interest, such as the livers, small intestine, heart, kidney and brain were harvested, cleansed of blood and rinsed in normal saline solution and the weight recorded. The organs were minced, and stored in physiological buffer and kept frozen or in fixing solution as appropriate.

The remaining 105 rats were allotted accordingly into seven compartments comprised of fifteen rats each, and were administered with palm wine and orijin bitters as indicated in Table 2. The doses of the palm wine and orijin bitters administered were calculated using Reagan (2008) conversion of the human equivalent dosage extrapolation to animal equivalent dosage:

HED (mg/kg) = Animal dose (mg/kg) * (Animal Km: Human Km). Where Km is the ratio of body weight in kg to surface area in m^2.

Table 2. The various groups of the male Wistar rats.

Groups	Diet	Substance administered with dose	
A	Atherogenic	Distilled water	(1.1 ml)
B	Atherogenic	Palm wine	(1.7 ml)
C	Atherogenic	Orijin Bitter	(1.1 ml)
D	Atherogenic	Simvastain	(2 mg/ml)
E	Non atherogenic	Distilled water	(1.1 ml)
F	Non atherogenic	Palm wine	(1.7 ml)
G	Non atherogenic	Orijin Bitter	(1.1 ml)

The alcoholic beverages were administered orally with the use of metal cannula and the doses spread into three, such that, palm wine; 0.6, 0.6 and 0.5 ml and orijin bitters; 0.4, 0.4 and 0.3 ml between 17:30 hours ± 1 hour GMT. The administrations of the palm wine and orijin bitters lasted for twelve (12) weeks, during which the feed intake, water intake and body weights of the rats were monitored and recorded. After six (6) weeks of administration of the palm wine, orijin bitters and simvastatin, a set of five rats were sacrificed by a repeat of the procedure stated earlier. After twelve (12) weeks of the administrations, a set of five rats in each group were administered the carbon ink intraperitoneally at the tail region, anaesthetized and blood was collected by retro orbital bleeding using glass capillaries and were sacrificed with the last set of five rats, using the same procedure stated earlier.

* This study was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH, 1985).

2.2.3. Bioassays

The concentrations of various biological parameters were determined by standard methods, such as total cholesterol, free cholesterol, triglycerides, (Tietz *et al*, 1995), high density lipoprotein cholesterol (Natio, 1984), very low density lipoprotein cholesterol, low density lipoprotein cholesterol (Friedewald *et al*., 1972), reduced glutathione (Sedlak and Lindsay, 1968), glutathione peroxidase (Thomas *et al*., 1990), malondialdehyde (Varshnney and Kale, 1990), prealbumin (Guder *et al*., 1996), carbon clearance test (Gokhale *et al*., 2003), zinc sulphate turbidity test (Pfeiiffer *et al*., 1977), interleukin-6, C-reactive protein, tumor necrosis factor-alpha, paraoxonase and oxidized low density lipoprotein-cholesterol (Sandwich ELISA as contained on the instruction manuals in RAYbiotech, Cloud-Clone Corp-Uscn Life Sciences Inc. and CUSABIO BIOTECH reagent kits respectively).

2.3. Statistical Analysis

This work was a Completely Randomised Design (CRD), in which the results were expressed as mean ± standard error of mean (S.E.M) of at least four determinations. The results

were subjected to ANOVA at p<0.05, after which the least significance difference test was utilized to identify the variation(s) within treatment groups, also at p<0.05.

3. Results

3.1. Appearance, Behaviour and Mortality

The rats fed with atherogenic diet had fuller furs, were robust (looked bigger) and were more active than the rat fed with the non-atherogenic diet. The rats administered the alcoholic beverages attended to the feed and water than their counterparts, were very active, aggressive and alert. However, the stools of the atherogenic diet fed rats were semi-solid and pale (yellowish) in colour, while those of the alcoholic beverages were solid and compared well to that of the non-atherogenic diet only fed rat. No mortalities were recorded through the span of the research work.

Table 3. Feed and water intake of the palm wine, orijin bitters and atherogenic diet fed male Wistar rats.

	Feed intake (g)						
Week	A	B	C	C	E	F	G
0	162.32 ± 5.67^a	-	-	-	154.58 ± 5.05^a	-	-
1	107.33 ± 2.66^b	-	-	-	162.67 ± 4.06^a	-	-
2	110.51 ± 4.71^b	-	-	-	169.25 ± 6.04^a	-	-
3	101.56 ± 5.60^b	-	-	-	181.55 ± 6.78^a	-	-
4	122.73 ± 9.22^b	-	-	-	172.46 ± 4.95^a	-	-
5	117.68 ± 8.45^b	-	-	-	180.50 ± 8.09^a	-	-
6	123.67 ± 6.78^b	-	-	-	182.30 ± 8.34^a	-	-
7	138.50 ± 5.81^b	126.44 ± 4.41^c	121.50 ± 6.11^c	121.89 ± 7.61^c	181.56 ± 11.01^a	170.89 ± 7.31^a	167.55 ± 5.48^a
8	133.61 ± 8.17^c	139.63 ± 4.28^c	142.96 ± 7.94^c	118.54 ± 3.89^d	177.22 ± 7.91^a	156.30 ± 6.76^b	161.66 ± 5.94^b
9	140.52 ± 7.78^c	154.78 ± 7.22^b	151.32 ± 5.06^b	112.32 ± 4.44^d	189.59 ± 8.34^a	$175..82 \pm 5.98^a$	180.09 ± 6.51^a
10	141.78 ± 8.65^c	157.01 ± 6.07^c	162.11 ± 4.36^b	128.32 ± 7.21^d	171.71 ± 9.04^a	187.42 ± 8.34^a	180.05 ± 7.55^a
11	130.06 ± 6.96^c	159.56 ± 7.04^b	157.88 ± 7.99^b	128.03 ± 10.25^c	178.65 ± 7.36^a	185.68 ± 9.12^a	188.77 ± 6.95^a
12	132.56 ± 6.76^d	149.29 ± 10.11^c	152.77 ± 8.10^c	120.55 ± 7.76^e	179.22 ± 8.38^a	192.67 ± 7.82^b	199.08 ± 7.05^b
13	121.58 ± 7.88^d	154.88 ± 9.45^c	149.66 ± 7.71^c	117.81 ± 9.31^d	188.89 ± 7.74^a	203.01 ± 6.98^b	208.45 ± 8.80^b
14	117.44 ± 7.19^e	156.73 ± 8.09^c	138.04 ± 8.11^d	129.78 ± 6.44^d	200.87 ± 6.89^a	217.85 ± 9.11^b	225.54 ± 8.91^b
15	108.37 ± 5.31^e	151.22 ± 5.53^c	137.89 ± 9.06^d	118.46 ± 7.01^e	202.67 ± 8.94^a	215.74 ± 8.42^b	225.76 ± 7.11^b
16	105.83 ± 7.08^f	155.89 ± 8.72^d	124.65 ± 7.08^e	120.67 ± 6.43^e	206.71 ± 7.05^a	217.66 ± 7.70^b	231.01 ± 7.98^c
17	112.32 ± 5.67^f	160.44 ± 7.43^d	115.23 ± 8.89^f	127.86 ± 8.02^e	195.74 ± 6.23^a	212.56 ± 6.98^b	222.71 ± 8.61^c
18	110.33 ± 4.66^f	159.56 ± 8.88^d	113.77 ± 6.61^f	131.91 ± 4.78^e	199.90 ± 4.28^a	212.87 ± 8.91^b	239.64 ± 6.05^c
Water intake (ml)							
Week	A	B	C	D	E	F	G
0	91.45 ± 7.21^a	-	-	-	94.55 ± 7.41^a	-	-
1	110.85 ± 4.51^b	-	-	-	90.97 ± 5.60^a	-	-
2	115.69 ± 8.12^b	-	-	-	87.89 ± 7.63^a	-	-
3	107.02 ± 6.39^b	-	-	-	88.60 ± 6.19^a	-	-
4	98.45 ± 4.08^b	-	-	-	89.45 ± 5.18^a	-	-
5	103.54 ± 6.06^b	-	-	-	91.87 ± 4.64^a	-	-
6	112.43 ± 4.38^b	-	-	-	82.58 ± 3.89^a	-	-
7	105.31 ± 6.76^b	110.65 ± 6.03^{bc}	121.41 ± 6.71^d	114.83 ± 5.95^c	86.75 ± 5.01^a	101.93 ± 6.28^b	104.99 ± 6.73^b
8	105.11 ± 5.06^b	118.08 ± 5.77^{bc}	122.09 ± 7.45^c	119.76 ± 7.31^{bc}	86.26 ± 6.28^a	107.22 ± 4.71^b	113.52 ± 8.60^b
9	110.57 ± 4.86^{bc}	116.95 ± 4.35^c	120.67 ± 6.82^c	118.23 ± 7.05^c	88.81 ± 3.90^a	105.54 ± 6.06^b	118.58 ± 4.39^c
10	102.66 ± 4.62^b	114.07 ± 5.89^b	127.86 ± 6.12^c	121.68 ± 4.89^c	91.45 ± 5.07^a	104.63 ± 5.38^b	118.17 ± 7.08^b
11	105.28 ± 5.63^{bc}	117.21 ± 7.13^c	125.95 ± 4.89^d	115.59 ± 5.04^c	84.81 ± 4.28^a	98.58 ± 5.18^b	110.61 ± 6.22^c
12	103.22 ± 3.64^b	121.67 ± 5.27^c	128.62 ± 6.08^c	125.22 ± 5.92^c	85.99 ± 4.33^a	100.71 ± 5.19^b	109.97 ± 6.07^b
13	99.66 ± 4.79^b	120.13 ± 5.83^c	125.56 ± 4.79^c	119.73 ± 5.82^c	90.45 ± 6.09^a	103.42 ± 4.90^b	118.25 ± 5.74^c
14	100.45 ± 6.04^b	111.30 ± 6.29^c	118.05 ± 3.62^c	114.17 ± 4.89^c	78.85 ± 8.27^a	100.21 ± 6.76^b	111.56 ± 4.25^c
15	96.07 ± 5.85^b	112.23 ± 6.97^c	124.44 ± 5.71^d	121.36 ± 6.14^d	81.73 ± 6.32^a	97.85 ± 6.27^b	113.77 ± 6.09^c
16	93.38 ± 5.51^b	110.87 ± 3.71^c	120.80 ± 6.77^d	120.65 ± 5.21^d	76.22 ± 6.06^a	98.92 ± 4.36^b	110.48 ± 4.59^c
17	90.40 ± 4.74^b	111.37 ± 6.38^c	112.78 ± 7.40^c	117.29 ± 6.82^c	72.66 ± 3.54^a	91.59 ± 6.76^b	96.23 ± 3.97^b
18	96.85 ± 2.78^b	106.30 ± 4.69^c	118.58 ± 5.06^d	106.31 ± 5.89^c	74.52 ± 6.04^a	93.01 ± 6.00^b	99.58 ± 5.55^b

Values are means ± SEM; n=6. (Mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet only, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

3.2. Feed, Water Intake and Body Weight

In Table 3, the introduction of the atherogenic diet to the rats resulted in a decrease (p<0.05) in the feed consumption from week 1 till the end of the administration (p<0.05), compared to the non atherogenic diet fed rats. Administrations of the palm wine and orijin bitters presented an initial decreases (p<0.05) in the feed intake in both the atherogenic and non-atherogenic diets fed rats at week 7, but increased (p<0.05) later from week 8 and were sustained till the end of administration, except in

the group C that presented no changes ($p<0.05$) at week 17 and 18 in the atherogenic diet fed rats. In the simvastatin administered rats, a rather non-consistent increase ($p<0.05$) trend was obtained in the feed intake (Table 3).

The water intake was increased ($p<0.05$) on the introduction of the atherogenic diet that was sustained further ($p<0.05$) with the administrations of palm wine and origin bitters. In the same vein, a similar trend was obtained in the non atherogenic diet fed male rats that were administered with palm wine and orijin bitters (groups F and G) (Table 3).

The atherogenic diet did not alter significantly ($p>0.05$) the body weight of the rats until the 3rd week ($p<0.05$) of the feed administration (Figure 1). However, immediate increases ($p<0.05$) were recorded in the body weights of the male rats on the introduction of the of palm wine and orijin bitters in both the atherogenic and non atherogenic diets fed rats from the 7th week and were maintained till the end of the experiment, except in the orijin bitters administered rats (group G) that were reduced ($p<0.05$) after week 14th to other rats fed the atherogenic diet only (group A) (Figure 1). Surprisingly, the non atherogenic diets fed rats, administered with palm wine and orijin bitters recorded weight gains ($p<0.05$) than the rats fed the atherogenic diet only. The rats administered simvastatin (group D) presented reductions ($p<0.05$) in the weight of the atherogenic diet fed rats, which were inconsistently non significant ($p>0.05$) with the non atherogenic diet only fed rats (Figure 1).
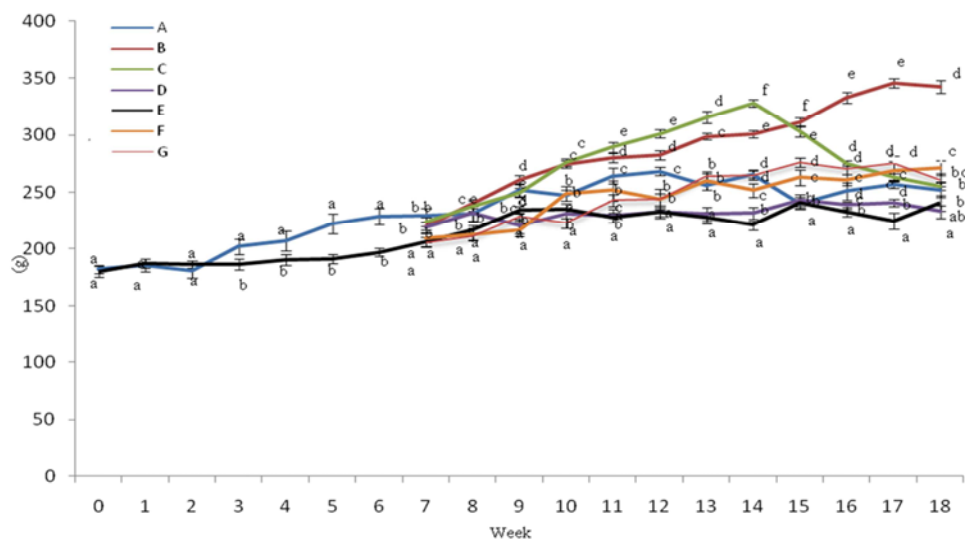
Figure 1. Body weight of atherogenic diet fed male rats administered palm wine and orijin bitters.

Values are means ± SEM; n=6. (Mean values bearing different alphabets are significantly different ($p<0.05$). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
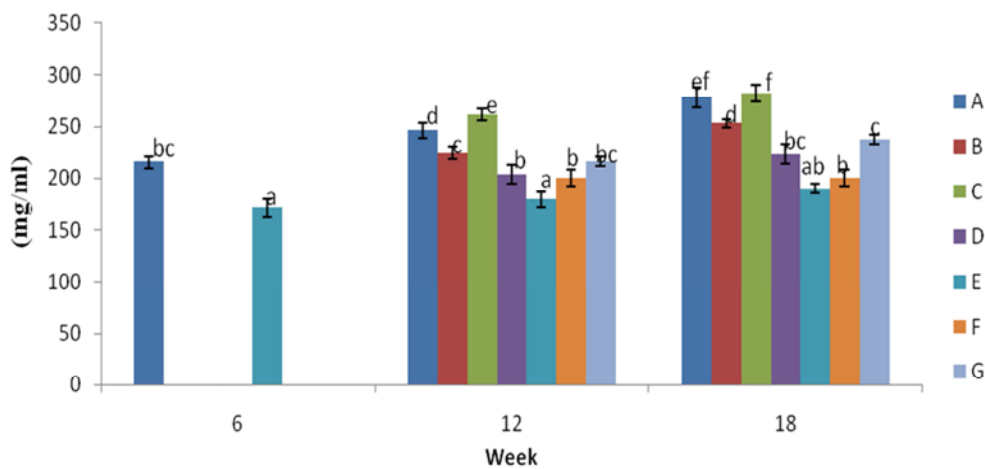

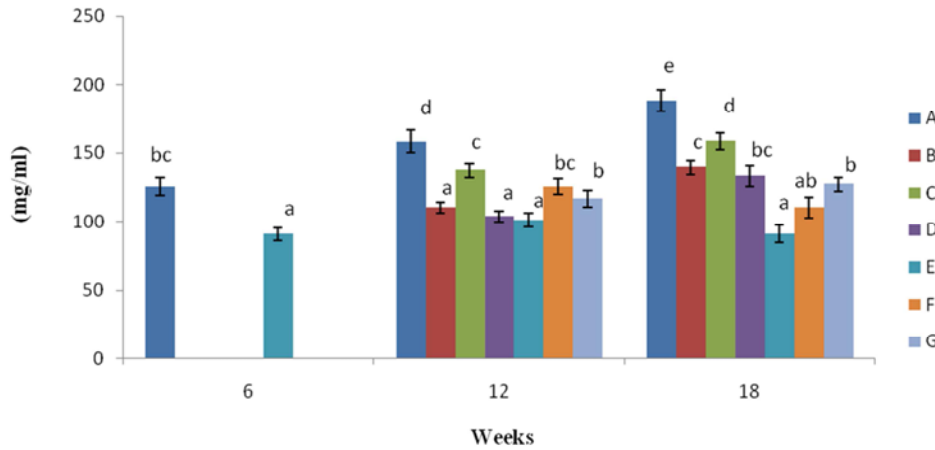**Figure 2.** Total cholesterol concentration in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different ($p<0.05$). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet +simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

3.3. Lipid Profile

A general increase (p<0.05) was recorded in the results of the lipid profile in the serum, such as, in the concentrations of total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol in atherogenic diet fed rats and were increased further (p<0.05) with the administration of palm wine and orijin bitters

(Figures 2, 5, 7 and 8). In a similar manner, the administration of palm wine and orijin bitters only (groups F and G) presented increases (p<0.05) in the aforementioned parameters. However, a significant reduction (p<0.05) was obtained in the simvastatin administered rats that were not different (p>0.05) from the atherogenic diet only fed rats (Figures 2, 5, 7 and 8).

Figure 3. Concentration of free cholesterol in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

High density lipoprotein cholesterol concentrations in the serum were not different (p>0.05) in the atherogenic diet only, atherogenic diet + simvastatin and non-atherogenic diet rats (groups A, D and E). On the other hand, palm wine and orijin bitters increased the concentration of high density lipoprotein cholesterol in the rats fed both diets (Figure 6).

The concentrations of free cholesterol in the serum were increased (p<0.05) in all the groups, except in the palm wine and simvastatin (groups B and D) at 12 weeks (Figure 3), while the esterified cholesterol didn't increase (p>0.05) in the atherogenic diet fed rats only (Figure 4).
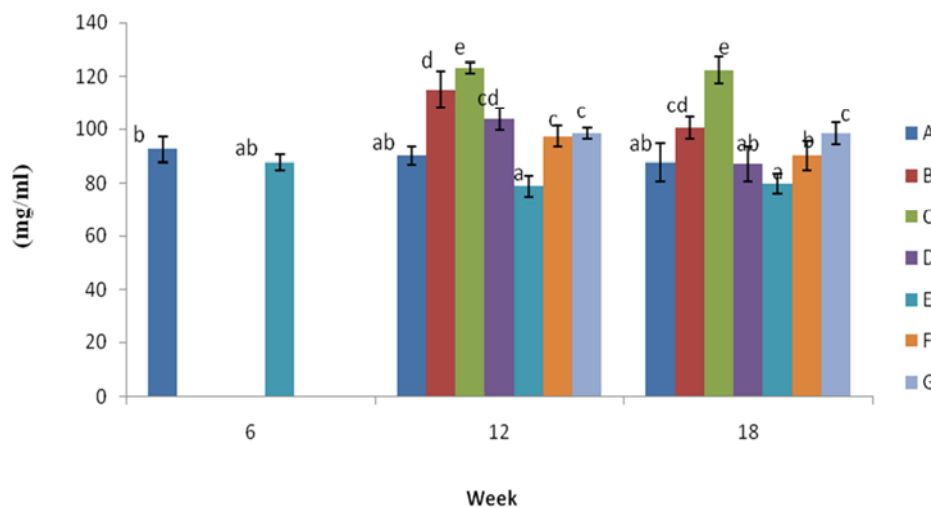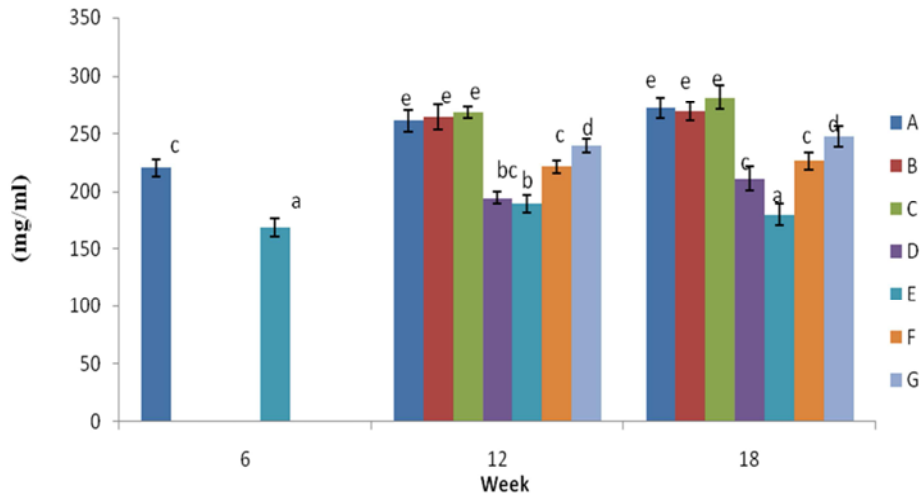

Figure 4. Serum concentration of esterified cholesterol in rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
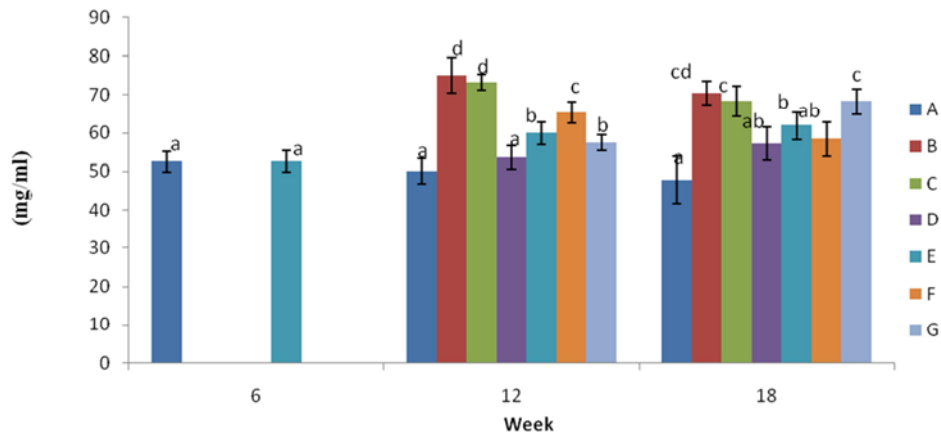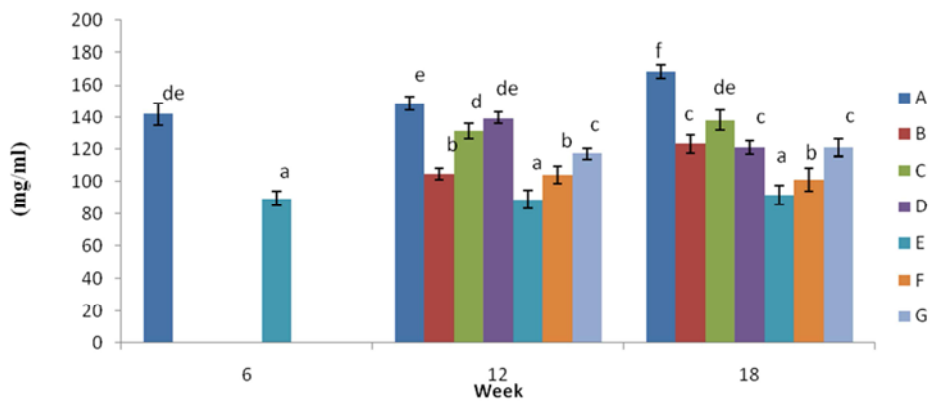
Figure 5. Concentration of triglycerides in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

Figure 6. High density lipoprotein cholesterol concentration in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
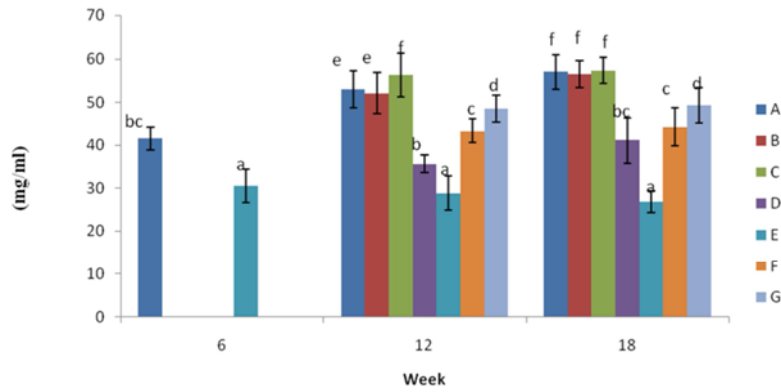

Figure 7. Concentration of low density lipoprotein cholesterol in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
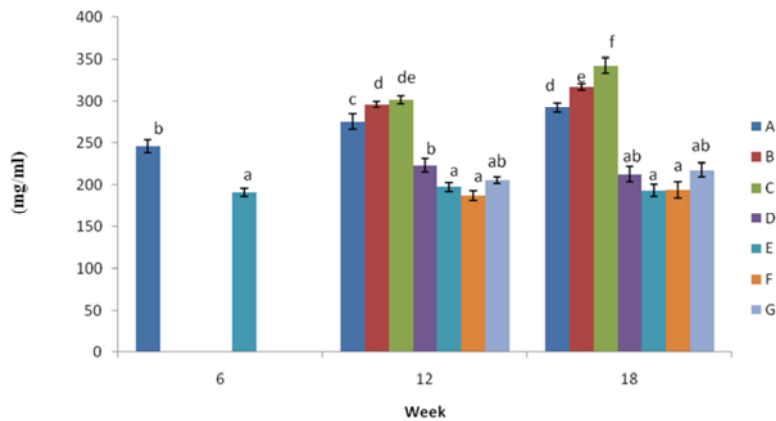
Figure 8. Serum concentration of very low density lipoprotein cholesterol in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

Figure 9. Total cholesterol concentration in the liver of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

The administrations of atherogenic diet, palm wine and orijin bitters increased (p<0.05) the total cholesterol concentrations in the liver, small intestine and heart in the rats, except (p>0.05) in the liver of non-atherogenic diet fed with palm wine (Figure 9) and in the small intestine (Figure 11), but the triglycerides increased (p<0.05) in all these organs (Figures 10, 12 and 14).
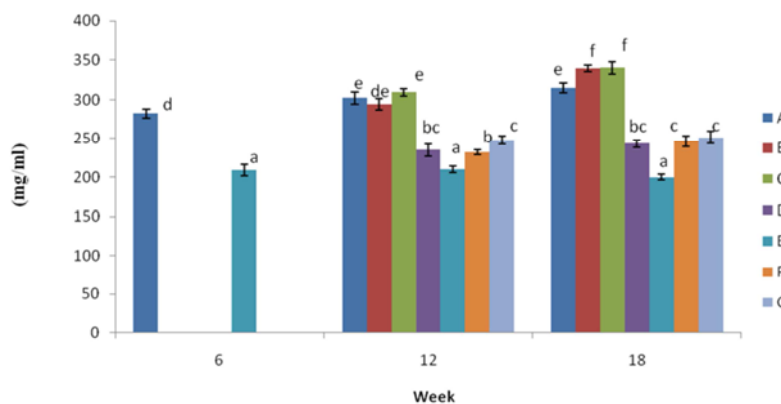

Figure 10. Concentration of triglycerides in the liver of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
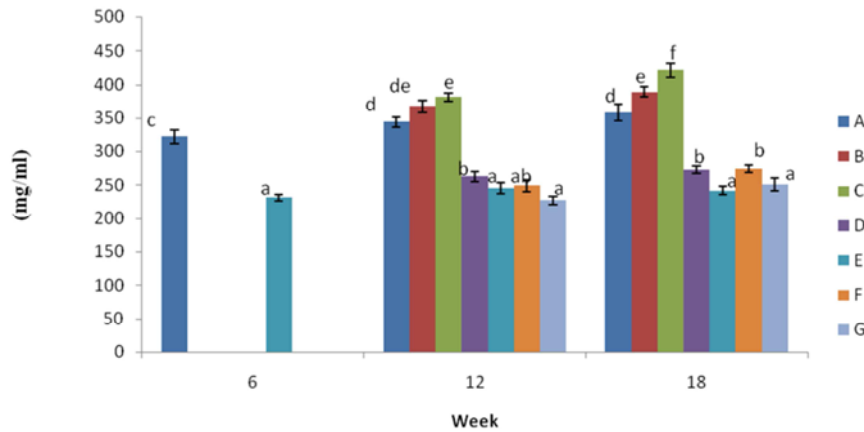
Figure 11. Total cholesterol concentration in the small intestine of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
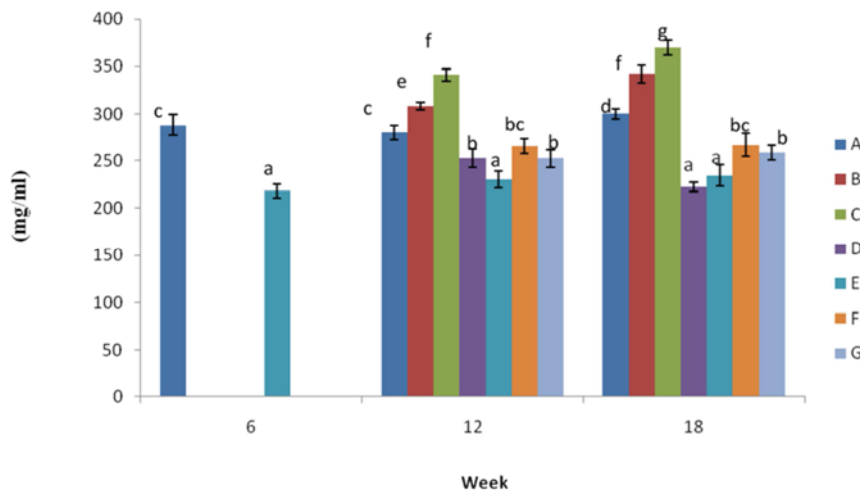

Figure 12. Concentration of triglycerides in the small intestine atherogenic diet, palm wine and orijin bitters fed male rats.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
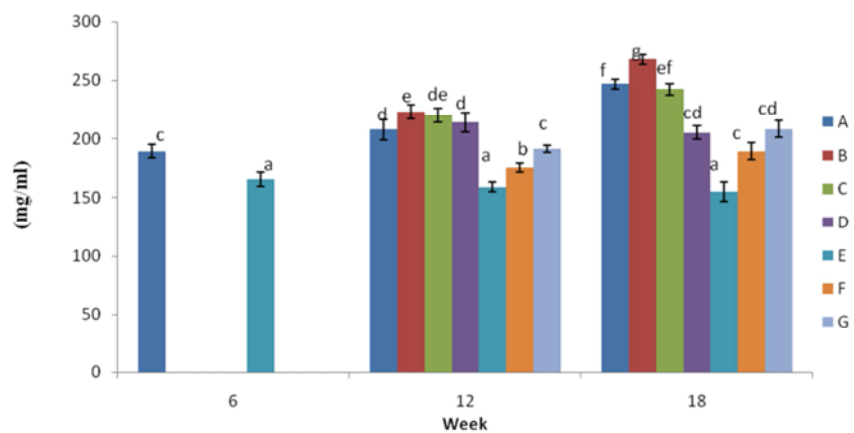

Figure 13. Total cholesterol concentration in the heart of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
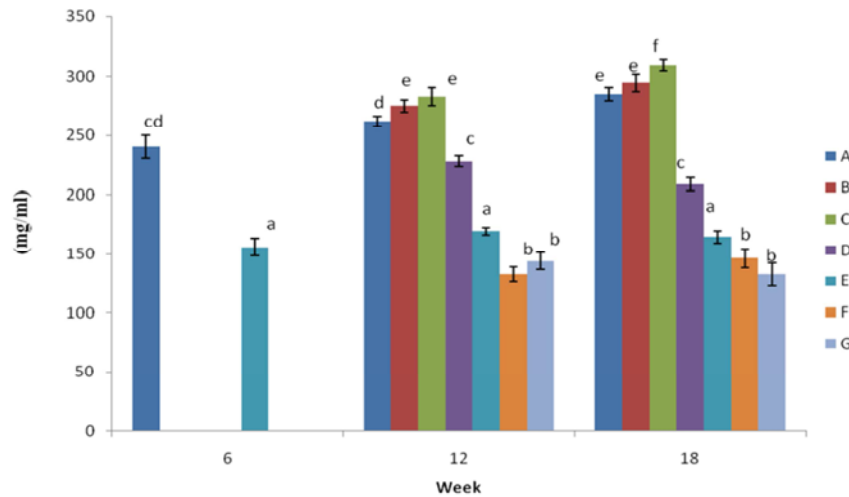
Figure 14. Concentration of triglycerides in the heart of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=5, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

Table 4. The trends of the oxidative stress indices in male Wistar rats administered with palm wine and origin bitters

			Groups						
			A	B	C	D	E	F	G
Malondialdehyde (nmole/mg of protein)	Liver	(x)	5.39± 0.31[c]	6.05± 0.65[cd]	6.44± 0.48[d]	6.11± 0.28[d]	2.78±0.60[a]	4.13± 0.44[b]	3.90± 0.51[b]
		(y)	6.27± 0.28[cd]	8.51± 0.18[e]	9.20±0.43[e]	6.03±0.33[cd]	2.85± 0.81[a]	6.81± 0.37[d]	6.17± 0.19[cd]
	Heart	(x)	3.77± 0.51[d]	3.46± 0.20[d]	2.89±0.41[c]	3.18±0.56[cd]	1.97±0.22[a]	2.65±0.18[c]	1.68± 0.17[a]
		(y)	4.04± 0.23[e]	6.21± 0.52[f]	4.07± 0.15[e]	3.97±0.90[d]	1.86± 0.14[a]	2.59± 0.15[c]	1.90±0.23[a]
	Small intestine	(x)	5.05± 0.35[c]	5.45± 0.51[cd]	4.88± 0.16[c]	3.61±0.62[b]	2.13±0.32[a]	3.01±0.09[b]	2.87±0.44[ab]
		(y)	5.92±0.24[d]	5.77±0.63[cd]	4.79±0.53[c]	5.01±0.57[c]	2.44±0.71[a]	3.78±0.55[b]	3.00±0.61[ab]
	Blood	(x)	3.56±0.18[b]	3.44±0.34[b]	3.98± 0.71[c]	4.37±0.45[c]	1.97±0.08[a]	3.09±0.29[b]	2.55± 0.11[ab]
		(y)	4.17±0.22[c]	4.28±0.41[c]	4.89± 0.37[c]	4.71±0.55[c]	1.85± 0.60[a]	3.76±0.36[b]	2.78±0.21[ab]
Liver	Glutathione peroxidase (µmol/ml) (x)		8.78 ±1.21[c]	8.22 ± 1.67[c]	11.05± 0.70[a]	7.38±1.40[c]	12.83±1.06[a]	13.04±1.12[a]	16.01±1.21[b]
		(y)	8.17±0.99[c]	7.05±1.01[c]	12.76±1.28[a]	8.56±1.32[d]	12.91±0.87[a]	12.16±1.10[a]	15.11±2.37[ab]
	Reduced Glutathione (mg/ml) (x)		32.11± 2.58[cd]	35.87±1.29[c]	40.08± 1..95[ac]	33.02±2.01[c]	43.35±1.89[a]	46.78±2.02[ab]	50.73±1.79[b]
		(y)	30.50±1.45[d]	35.19±2.11[c]	38.77± 1.06[ac]	34.50± 1.76[c]	42.50±2.01[a]	45.00±2.12[a]	48.57± 2.15[ab]
Oxidized LDL-C (µmol/ml) Plasma		(x)	163.70 ± 5.04[cd]	148.55± 8.35[c]	146.08± 6.94[c]	157.83± 7.29[c]	118.10± 9.34[a]	102.48± 5.17[b]	107.32± 8.35[ab]
		(y)	171.65± 7.89[d]	166.21± 11.21[cd]	151.44± 8.85[c]	162.44± 7.91[cd]	125.44± 6.72[a]	121.07± 7.54[a]	110.88± 7.12[a]
Oxidized LDL-C (µmol/ml) Heart		(x)	151.09± 9.42[c]	164.72± 6.85[cd]	182.82± 10.90[d]	161.43± 7.15[c]	88.55±8.61[a]	106.91± 9.52[b]	100.41± 9.98[ab]
		(y)	180.56± 7.33[d]	178.74± 9.21[d]	160.43± 10.66[c]	148.10± 9.34[c]	98.08± 11.69[a]	118.44± 6.34[b]	100.52± 9.70[ab]
Paraoxonase (ng/ml) Plasma		(x)	122.10± 4.88[c]	101.28± 6.09[de]	121.43± 8.47[c]	108.98± 9.66[d]	171.90± 7.92[a]	146.05± 6.68[b]	159.77± 10.15[ab]
		(y)	114.67± 6.37[cd]	113.53± 8.59[cd]	127.31± 10.40[c]	96.16± 7.38[e]	162.42± 9.96[a]	147.87± 7.03[b]	156.16± 10.02[ab]

Values are means ± SEM; n=3-5. (Mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet only,
B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters. X= 12 weeks and y= 18 weeks.

3.4. Immonomodulatory Indices

3.4.1. Oxidative Stress Indices

The concentrations of reduced glutathione in the liver indicated consistent decreases (p<0.05) in the rats administered atherogenic diet, palm wine and simvastatin (groups A, B and D), while it was increased (p<0.05) in the orijin bitters rats (group G) (Table 4). Activities of glutathione peroxidase in the liver presented significant increases (p<0.05) in the orijin bitters rats (group G) and decreases (p<0.05) in rats in groups A and B. Likewise, in Table 4, a product of lipid per-oxidation, malondialdehyde concentration presented non significant increases (p>0.05) in the heart, small intestine and blood in rats fed the non atherogenic diet and orijin bitters (group G). In the liver, the concentration of malondialdehyde increased (p<0.05) in all the groups in a time dependent manner. The administrations of palm wine and orijin bitters were shown to reduce

(p<0.05) the level of oxidised low density lipoprotein-cholesterol in the heart and plasma in the groups of atherogenic diet fed male Wistar rats (Table 4), but not in the non-atherogenic diet fed group (p>0.05). However, the plasma activities of paraoxonase reduced (p<0.05) in all the rats, except in the non-atherogenic diet fed rats administered with orijin bitters (Table 4).

3.4.2. Acute Phase Proteins, Immunoglobulin Levels and Phagocytic Index

The serum concentration of a stressor protein of the liver, prealbumin, indicated increases (p<0.05) in the serum of atherogenic diet fed rats, while it was reduced (p<0.05) in the

rats fed orijin bitters only (group G) (Figure 15). The concentrations of interleukin-6, tumour necrosis factor-alpha and C-reactive protein increased in all the atherogenic diet fed male Wistar rats; while the administrations of the palm wine and orijin bitters reduced (p<0.05) tumor necrosis factor-alpha concentration in the non-atherogenic diet rats with concomitant increases in the concentrations of interleukin-6 (Figures 16, 17 and 18). The abilities of the rats to phagocytose pathogens and total immunoglobulin levels in the plasma were reduced (p<0.05) in rats fed the atherogenic diet with significant increases (p<0.05) in the non-atherogenic diet fed rats administered with orijin bitters (Figure 19 and 20 respectively).
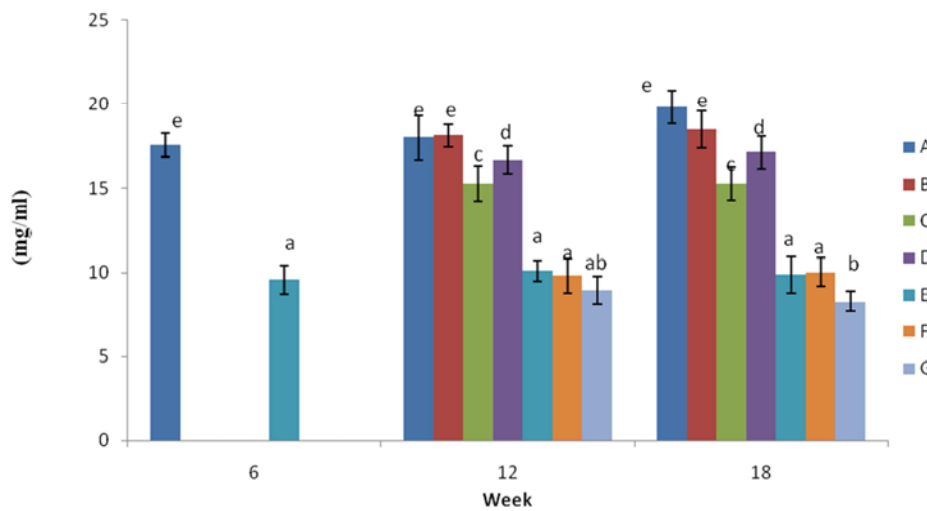

Figure 15. Prealbumin concentration in the serum of rats fed with atherogenic diet, palm wine and orijin Bitters.

Values are means ± SEM; n=4, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
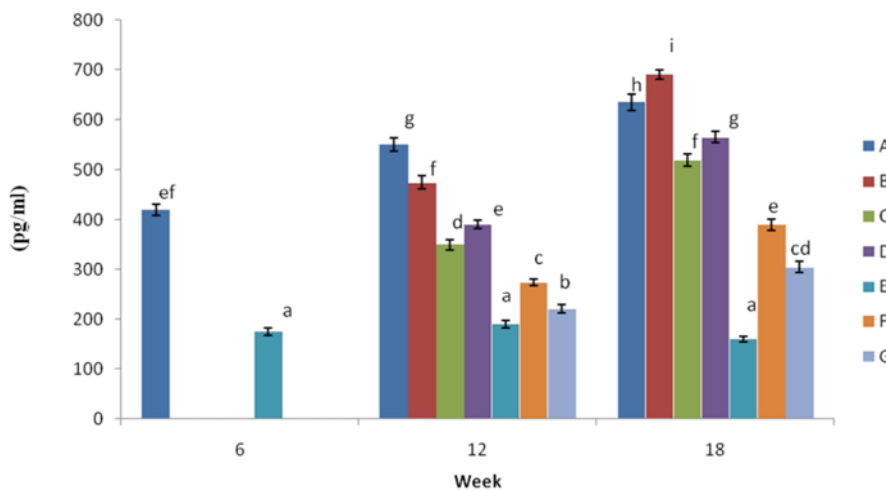

Figure 16. Concentration of interleukin-6 in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=3, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
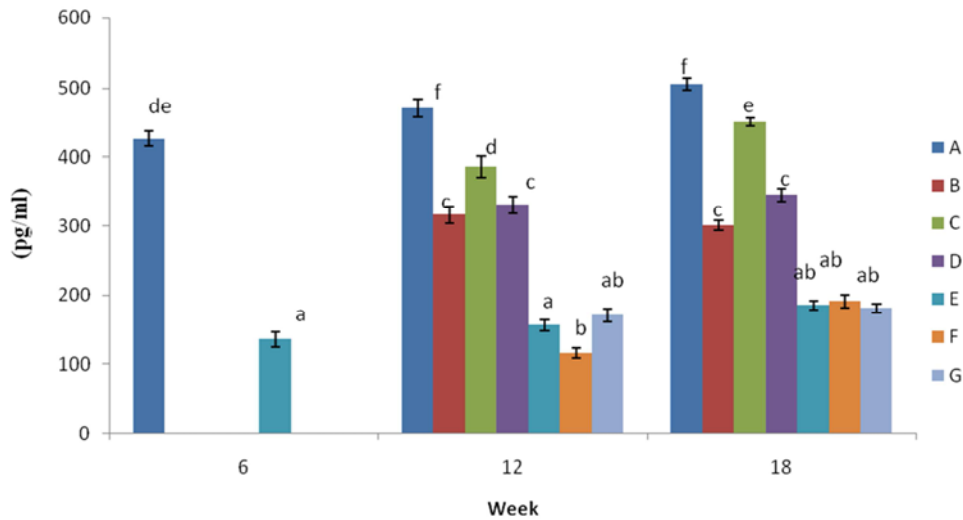
Figure 17. Concentration of tumor necrosis factor-α in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=3, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
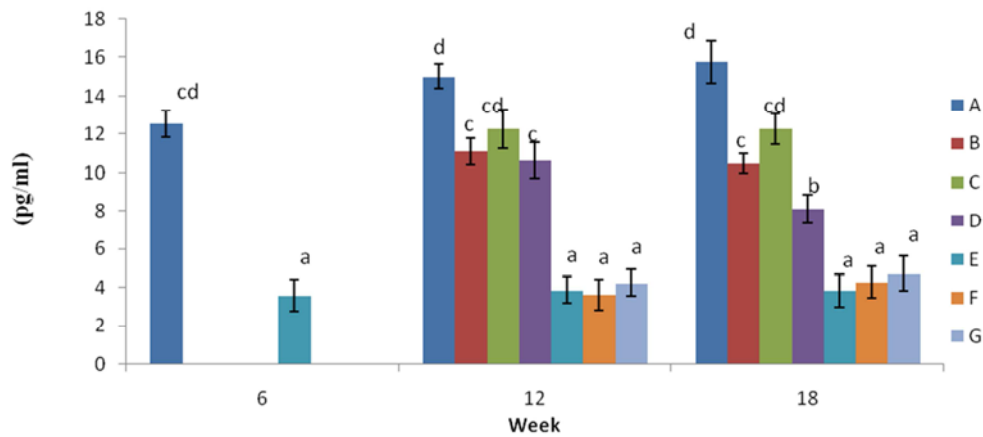

Figure 18. C-reactive protein concentration in the serum of rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=3, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.

Figure 19. Carbon clearance test in rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=4, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
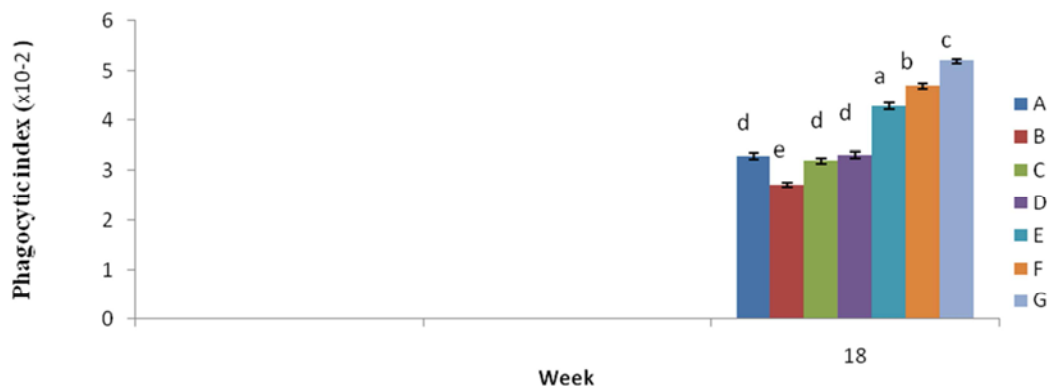
Figure 20. Zinc turbidity test in rats fed with atherogenic diet, palm wine and orijin bitters.

Values are means ± SEM; n=4, and mean values bearing different alphabets are significantly different (p<0.05). A= atherogenic diet, B = atherogenic diet + palm wine, C = atherogenic diet + orijin bitters, D = atherogenic diet + simvastatin, E = non atherogenic diet, F = non atherogenic diet + palm wine and G = non atherogenic diet + orijin bitters.
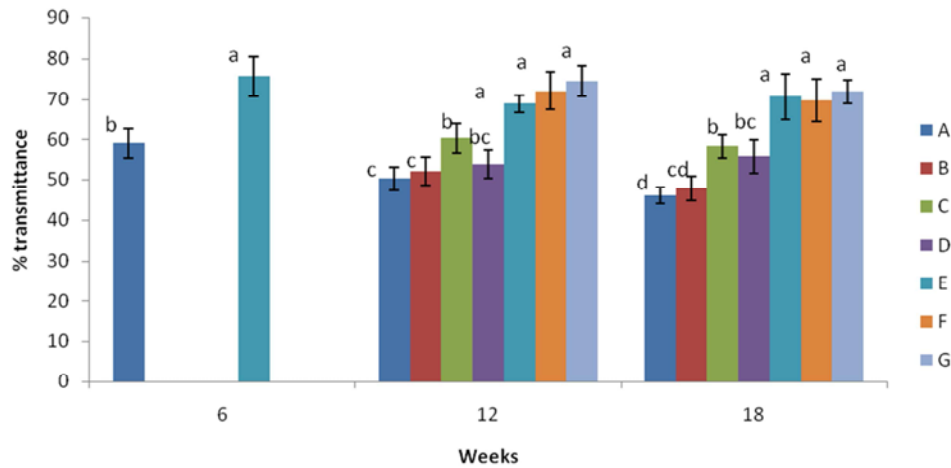
4. Discussion

Hyperlipidaemia is one of the major health problems in developed and developing countries. It has been shown to lay the foundation of very many inflammatory disorders, degenerative process and diseases, organ failure and death (Horejsi, 2000; Adekunle *et al*., 2013). Independent studies have reported separately the clinical roles of over indulgence in alcoholic beverages and atherogenic diets in the progression of hyperlipidemic states and the consequent pathological conditions (Assadi *et al*., 1989; Renaud *et al*., 1999; Assman and Gotto, 2004; Anja *et al*., 2004).

The trends obtained in the fed intake of the rats before the 6th week (Table 3) indicated that the introduction of the atherogenic diet might be non palatable to the rats, could have delayed absorption and or, activated up-regulation of satiety. Lipid dense food substances are renowned for the feeling of fullness due to the non-palatability by masking the taste bud and the inhibition of the leptin satiety pathway. The decreases recorded in the feed intake immediately the alcohol beverages were introduced to the atherogenic and non-atherogenic diets fed rats could be adduced to up-regulation of fullness or a result of the physiological reactions to the alcoholic beverage. Alcoholic beverages were reported by Cordain *et al*. (1997) and Renaud *et al*. (1999) to trigger the physiologic mechanisms that regulate the feeling of fullness in the short term and or, long-term, depending on the doses. The increases presented later in feed intake (8th week) could indicate that the alcoholic beverages stimulated the down-regulation in the satiety of the rats. It was plausible that the doses of the alcoholic beverages administered to the rats were not in the high consumption ranges, which served as an activator of leptin secretion and the inhibition of satiety in the hypothalamus. Although, the decreases recorded in the feed intake in the orijin bitters administered rats (group C) was not in agreement with the latter, but rather the up-regulation of satiety or possible digestion and or, absorption disorders. However, alcohol consumption on short-term, is considered an appetite stimulant, influencing neurochemical and peripheral systems utilized to control appetite, such as, leptin inhibition, glucagon-like-peptide-1 and serotonin, and enhancing the effect of gamma-aminobutyric acid, endogenous opioids and neuropeptide (Yeomans *et al*., 2010).

The increases recorded in the water intake in the atherogenic diet fed rats (Table 3) might be as a result of the increased demand for hydration due to the hydrophilic nature of the diet, which could prolong the digestion and absorption in the gastrointestinal tracts by masking the endothelial surfaces. This is in support of the previous report that lipid/energy dense food substances increase the need for hydration due to the role of aqueous surroundings during the digestion of dietary lipid in the duodenum (Champe *et al*., 2005). The trends in the water intakes of the rats administered the alcoholic beverage might be as a result of dehydration. This can't be farfetched because alcoholic beverages have been reported to inhibit the release of the anti-diuretic hormone, thereby increasing fluid loss in the tubule during urine formation (Sanofi, 2013). The orijin bitters administered rats presented a highest demand for water than the palm wine administered rats, which might be due to the bitter taste. This is logical because the rats might need to ingest more water so as to compensate or wear off the bitter taste of the orijin bitters.

The patterns obtained in the body weight of the rats fed the atherogenic diet and alcoholic beverages (Figure 1) might be

due to increased deposition of lipids in the tissues of the rats. This observation was consistent with the previous findings of Harnafi *et al.* (2009) and Otunola *et al.* (2010), but not with Ramachandran *et al.* (2003) that reported a loss in body weight in the atherogenic diet fed rats. However, the decreases later recorded in the body weights of the rats in group A and C might indicate possible adverse metabolic reactions, either with ketogenicity and or, tissue intoxication. The observation was supported by the works of Ramachandran *et al.* (2003) that reported a similar trend in the body weight of rats fed atherogenic diet only. The long term consumptions of atherogenic diet and alcohol could be implicated in the 'slow down' or 'lock up' of the ingestion, digestion, absorption and assimilation of food substances, and also the utilization of the fuel from ketone bodies accumulation from the mobilization of fats in the adipose tissue and acetoacetate by acetate condensation in alcohol metabolism. Interestingly, the groups of rats administered with palm wine did not support this explanation, but rather maintained a steady deposition of fats in the tissues that presented the consistent gain in body weights (Figure 1). The trend obtained in the feed intake supported the patterns present in the body weight of the rats.

The increases recorded in the serum lipid profile (Figures 2-8) in the rats fed the atherogenic diet and alcoholic beverages (both single and combined) might be adduced to increase in the lipid availability and consequent metabolism in the rats. Atherogenic diets and chronic alcohol consumption are implicated in hyperlipidaemic clinical conditions, which are key players in the progressions of "atheroma". Various reports have it that the consumption of lipid rich diets correlates with increases in serum lipid levels (Hu *et al.*, 2001; Adekunle *et al.*, 2013). Therefore, the increased serum levels of lipid rich lipoproteins (LDL-C and VLDL-C) and protein rich lipoprotein (HDL-C) indicated that more cholesterol and triglyceride are being transported from the liver to the extra-hepatic tissues to be taken up by those tissues and vice versa. This might be a response by the body to maintain lipid homeostasis *in situ*. This is because an increased intake in dietary lipids, under normal physiological conditions proportionately increases the needs for lipoproteins for effective distribution and metabolism of the exogenous lipids. Although, the alcoholic beverage, especially orijin bitters presented the most beneficial trends in the good cholesterol levels in the serum (Figures 4 and 6), the concomitant levels of the bad cholesterol were also alarming (Figures 7 and 8).

The trends obtained in the total cholesterol and triglyceride concentrations in the liver, small intestine and heart are all indicators of hyperlipidaemia attributable to the increased availability of lipid, which must have been due to the increased lipids in the atherogenic diet, coupled with the addition of calories from the alcoholic beverages (Figures 9-14). These do not relay any good news in the pathogenesis of lipid metabolic disorders, as 'tissue steatosis' is the hallmark of fatty infiltration that could end in fibrosis and necrosis of the tissue, which are the least wanted processes in clinical studies and health. However, the trends are expected when correlated with the patterns obtained in the serum lipids. Hyperlipidaemia is a proven major problem in the clinical diagnosis of many lipid metabolism abnormalities, inflammatory disorders and diseases (Matos *et al.* 2005; Afolabi *et al.*, 2013). Interestingly, the patterns obtained in the rats administered with the alcoholic beverage only could be beneficial to the rats (Figures 9-14). This was supported by the report of Volcik *et al.* (2008) that alcohol consumption induced alterations in the lipid profile, thereby, reducing the probability of cardiovascular problems, the incidence of strokes, such as brain haemorrhages and subarachnoid and necrosis in tissues.

Perturbations in the endogenous free radicals scavenging units from the uncontrolled formation of free radicals during metabolic processes may result in oxidative stress. The trends obtained in the oxidative stress indices in this study indicated that the atherogenic diet must have induced the production of free radicals, which depleted the levels of reduced glutathione in the liver (Table 4). The liver serves as the reservoir of glutathione in the reduced form that are the required usable form of glutathione. A reduction in the level of reduced glutathione in the liver might be an indicator of impeding oxidative imbalances (Oyewo *et al.*, 2013). The trends in the reduced glutathione level in the liver must definitely be the precipitant of the patterns obtained in the glutathione peroxidase activities in the liver. Interestingly, the rats administered with alcoholic beverages only, especially the orijin bitters preserved these important endogenous anti-oxidants in the liver (Table 4). This might be due to the herbal constituents (chamomile and thyme) in Orijin bitters, renowned for the anti-oxidative properties.

Hyperlipidaemia has been shown to help the progression of oxidative stress that could be seen as increases in the products of lipid peroxidation, such as malondialdehyde, carbonyl proteins, oxidized low density lipoprotein cholesterol, etc. (Kallol and Biswadev, 2009). The results obtained in the malondialdehyde concentrations in the various tissues in the rats indicated an overall disposition to oxidative stress by the atherogenic diet and the alcoholic beverages both singly and combined, except in the heart, small intestine and blood in the rats administered with orijin bitters only. These results supported the trends obtained in the endogenous anti-oxidants in the liver. In addition, chronic hyperlipidemia has been shown to activate innate immune cells, such as macrophages

and dendritic cells, which generate reactive oxygen species (H_2O_2, O_2^-, NO etc.) and consume NADPH (H^+) via NADPH oxidases, 'respiratory burst'. The continuous stimulation of these immune cells could result into insulin resistance depositing inflammatory disorders, tissue damage, ageing and degenerative diseases, such as *Diabetes mellitus*, cardiovascular diseases, arthritis, various cancers etc (Doulia *et al.*, 2013; Kelly and O'Neill, 2015).

Paraoxonase is another anti-oxidative enzyme that is associated with high density lipoprotein cholesterol, which prevents the auto-oxidation of low density lipoprotein cholesterol in the serum. The administrations of the atherogenic diet and alcoholic beverages contributed to the mop up of paraoxonase in the serum, which indicated the onslaught of oxidative stress in the rats. A decrease in the activity of paraoxonase could predict an increase in the rate of the auto-oxidation of low density lipoprotein cholesterol and foam cell formations, the hallmark of atheroma. Therefore, the patterns presented in the oxidized low density lipoprotein cholesterol indicated that the atherogenic diet was undoubtedly a predisposal to oxidative stress, which scenario couldn't be ameliorated by the alcoholic beverage, despite some anti-oxidative properties displayed by the rats administered the alcoholic beverage only (Table 4). However, the result obtained in the levels of oxidized LDL-C in the hearts of rats administered the alcoholic beverages still sounded a 'note of warning', because the oxidation of low density lipoprotein cholesterol is the activator of 'atheroma' formation, which is the "building blocks" of the very many vascular clinical problems. Institutively, the results obtained in the oxidized lipoprotein were supported by the results of the serum LDL-C and HDL-C concentrations reported (Figures 6 and 7), as well as the trends of the endogenous anti-oxidation processes.

Immunosurveillance is a routine process carried out by the immune cells to extrude the body system of damaged, senescent and pre-malignant cells perfectly through the actions of immune modulators. It is very evident that the alterations in the molecular and cellular circuitries that underpin immunosurveillance aggravate or ameliorate the course of chronic and degenerative diseases, cancer, loss of organ functions, etc. (Oyewo *et al.*, 2013; Goubran *et al.*, 2014). The trends presented in the serum concentrations of prealbumin, interleukin-6, tumour necrosis factor-alpha and C-reactive protein in the rats indicated that the atherogenic diet induced tissue(s) injury that must have increased the release of these acute phase proteins (APP). Prealbumin and C-reactive protein are stressor proteins (APP) released by the liver during injury, mainly related to hepatic challenges, and are meant to cause exponential inflammatory reactions that are short lived to contain the challenge, due to their short half

lives. However, the continuous release of these proteins in the blood is implicated in oxidative stress, inflammatory disorders and diseases, ageing, etc. (Oyewo *et al.*, 2013; Kelly and O'Neill, 2015). A critical examination of the results presented on these proteins in the serum (Figures 15 and 18) showed that the administration of the alcoholic beverages only might not be deleterious to the health of the liver or induced any form of inflammatory responses.

In conjunction with the foregoing, the administration of the atherogenic diet must have led to some injuries in the tissues that are of muscular origins and, or liver and cell death (Figures 16 and 17). However, the sole administrations of the alcoholic beverages did not support the death of cells in the rats (Figure 16), but rather tissue(s) injury (Figure 17) that kept the serum concentrations of the pro-inflammatory cytokines high. Although, immune responses are preceded by inflammatory responses, which help in chemotaxis of the immune cells during immunosurveillance and, or phagocytosis of the antigens and sensitization in the productions of myeloid cells (immunoblast) to proliferate and differentiate into mature immune cells and contain the situation (Oyewo *et al.*, 2013; Kelly and O'Neill, 2015).

The persistent marked increases in the level of these cytokines would have over stimulated the immune systems in the rats, which could precipitate into inflammatory disorders or diseases, degenerative diseases, loss of organ functions, etc due to the autoimmune response characteristics of the persistent high levels of these cytokines in the blood. This hypothesis is further supported by the patterns obtained in the phagocytic index of the reticulo-endothelia systems (Figure 19) and the humoral immunity (total immunoglobulins level) in the rats (Figure 20). The decreases in the serum immunoglobulin levels in the rats fed the atherogenic diets indicated an overwhelming call up of the antibodies with a concomitant drop in the abilities of the rats to phagocytose antigens, injured and, or dead cells. These reductions could be the causes of the reported trends of the oxidized low density lipoprotein cholesterol in the atherogenic fed rats (Table 4), which must have enhanced the formation of atheromas and subsequently foams cells, the hallmark of vascular diseases and various inflammatory disorders. However, the alcoholic beverages when administered to non atherogenic diet fed rats were potent modulators of the immune systems.

5. Conclusion

In this line, our study has implicated the roles of the atherogenic diet in combinations with alcoholic beverages in the severities of hyperlipidemic, oxidative stress, inflammatory and immunomodulatory responses in the

models. Therefore, the frequent indulgence in the consumptions of fat dense food substances alone and in combinations with alcoholic beverages is hazardous to health and is discouraged.

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Author's Contribution and Competing Interests

OEB designed the research work, was involved in the collection of data and data analysis, as well as the interpretation of data and the preparation of the manuscript.

AAS was involved in the interpretation of the result and guided the preparation of the final manuscript.

AOK provided some chemicals and was involved in the data analysis and interpretation of the result

OPT sourced for materials, both online and manually for the interpretation of data and the collection of data and data analysis

AMA guided the preparation of the final manuscript.

The authors declare that no conflict of interest exist in the organization, results, presentation and the finance of the research article.

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