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Hypolipidemic Activity of Fermented Sorghum (*Sorghum bicolor* L. Moench) Gruel (*Ogi*) in Hypercholesterolemic Rats (*Rattus nervegicus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAL and BIA designed the study, wrote the protocol and supervised the work. All Authors managed the analyses of the study. Author SAL wrote the first draft of the manuscript. Authors BIA and OMD managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Fermented gruel (*Ogi*) has been reported to have high lactic acid bacteria (LAB) with proven probiotic properties. This fermented food has also has different therapeutic usages.
Objective: The activities of fermented sorghum (*Ogi*) on induced hypercholesterolemia in albino rats were investigated.
Materials and Methods: The LAB strains were isolated from traditionally fermented sorghum gruel *Ogi.* Also the ability of the isolates to tolerate bile and produced bile salt hydrolase was also determined. The hypolipidemic activity of fermented gruel (*Ogi*) with and without LAB strains in hypercholesterolemic rats was determined using standard methods.
Results: The lactic acid bacteria (LAB) count in sorghum *Ogi* ranged between 5. 0792 $\log_{10}$ CFU/g

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and 5.6990 log_{10} while the average values of pH and titratable acidity (TTA) were 3.8 and 0.38 respectively. There was no significant difference between the weights of the animals in the control group (Basal Diet) and the experimental groups (at $p<0.05$). The mean values of the low density lipoproteins cholesterol (LDLC) in the rats were 188.60±7.27 mg/dl in the animals fed with HLF while the least value was recorded in the Basal diet supplanted with *Ogi* (BD+*Ogi*) (110.80±26.93 mg/dl). The mean high density lipoproteins cholesterol in the serum of the rats in cholesterol free feed supplemented with BD+*Ogi* diet group was the highest (52.600 mg/dl). The amount of the total lipid in the animal fed with high lipid diet (HLF) was significantly higher than the amount in the organs of the animals feed with diets containing BD+*Ogi*, HLF+ *L. lactis* and HLF+ *L. brevis* (at $\alpha = 0.05$). In decreasing order, the amount of the glycerides in high lipid feed diets supplemented with *Ogi*, *Lactobacillus lactis* and *L. brevis*. The mean value of the Aspartate transaminase in the animals in Basal Diet group was higher at the commencement of the experiment (275.45±34.43 IU/L).while the level of the alanine transaminase was not significantly different (at $p< 0.05$). The histo-pathological micrograph of the various organs revealed the hypolipidemic potential activity of fermented sorghum.

Conclusion: The diets supplemented with the fermented food showed better activity against hypercholesterolemia in the rat groups than their microbial isolates. It would therefore be safe to recommend these foods to individuals with this disease situation and those with incidence of coronary heart disease in Nigeria.

Keywords: Hypolipidemic activity; Ogi; hypercholesterol; lactic acid bacteria; probiotes; triglyceride.

1. INTRODUCTION

Some African fermented food products have been reported to have therapeutic values [1].These food products have been used in the treatment of diarrhoea or dysentery. *Ogi* is a major indigenous traditional weaning food in West Africa. Also one of the most frequently consumed fermented foods in southwestern Nigeria [1]. The processing of *Ogi* involves either soaking the whole grain in water contained in a fermenting vat for a length of time or an initial reduction of the grain size by milling in the wet form before being allowed to ferment at room temperature for 2-3 days [2,3]. *Lactobacillus* species are the predominant bacteria isolated from *Ogi* [4,5]. The major lactic acid bacteria (LAB) responsible for the production of lactic acid are *L. plantarum.* Teniola and Odunfa [6] isolated the following species of lactic acid bacteria from fermented *Ogi; L. plantarum, L. brevis, Pediococcus* and *Pentococcus* spp.

Lactic acid bacteria (LAB) are a broad group of Gram positive, non-motile catalase negative rods and cocci that ferment carbohydrates and form lactic acid as the major end product [7]. Strains of *Lactobacillus* have been reported to be involved in the fermentation of Ogi and most other foods consumed in Africa [3,6,8]. Sufficient numbers of viable LAB alter the intestine microbiota by implantation or colonization in a compartment of the host. They are generally regarded as safe due to their long history of safe use in foods [3,5,6].

High serum cholesterol level has been linked to increased consumption of dairy and animal fats. Hypercholesterolemia is considered to be one of the major factors predisposing atherosclerotic heart disease, and has been widely accepted that diet can play a significant role [9]. The hypercholesterolemic effect of probiotes has been reported by many authors. Lin and Chen [10] reported the cholesterol lowering activity of *L. acidophilus* while Tabuchi et al. [11] showed the hypercholesterolemic effect of *Lactobacillus* in hypercholesterolemic rats.

Cholesterol found in plasma membranes can be extracted from the body by the High Density Lipoproteins (HDL). By esterification HDL carries cholesterol away from the arteries back to the liver, where it is passed out of the body. Hypercholesterolemia refers to high levels of cholesterol in the blood. A normal or desirable cholesterol level in man is described as less than 200 mg of cholesterol per deciliter of blood (mg/dl). It is considered to be at borderline when it is in the range of 200 to 239 mg/dl and elevated when it is 240 mg/dl or above in humans. De Rodas et al. [12] showed that dietary supplementation with *Lactobacillus acidophilus* reduced serum cholesterol and low density lipoprotein (LDL) cholesterol in pigs previously fed high cholesterol diet more than in pigs not receiving *L. acidophilus*. The

colonization of the intestine by *Lactobacillus* was concluded to be responsible for this the cholesterol lowering effects of LAB [11,13]. Gilliland et al. [14] referred to this phenomenon as cholesterol assimilation.

Fermented sorghum gruel (*Ogi*) contains high level of LAB and has been used for the treatment of different ailments in the south western Nigeria [1]. Despite this acclaimed therapeutic usage for the treatment of microbial infections, there is dearth of scientific information on the empirical proofs on its hypolipidemic activity. This study therefore aimed at investigating the ability of fermented sorghum gruel and LAB isolated from it to treat (reverse/reduce) the level of cholesterol in hypercholesterolemic rat and to detect the enzymatic changes in the rat.

2. MATERIALS AND METHODS

2.1 Source of *Ogi*

Fermented sorghum gruel *Ogi* samples were purchased from a single producer to ensure consistency in the quality of the product. The samples were carried in the ice pack and transferred within two hours to the Microbiology Laboratory of the Adekunle Ajasin University Akungba-Akoko for analyses. The pH and the titratable acidity of the sample were determined by the method of AOAC [15].

2.2 Isolation and Identification of Lactic Acid Bacteria (LAB)

The *Ogi* samples were serially diluted and one ml of appropriate dilution was aseptically plated, using pour plate technique, on Plate Count Agar (PCA) for total viable bacterial count. The method of Harrigan and McCance [16] was used to isolate LAB. Discrete colonies on de Man Rogosa Sharpe (MRS) agar (Oxoid) plates were selected and the method of Savadogo et al. [17] was used to identify the isolates. The results were interpreted according to Holt et al. [18].

The LAB strains were harvested by centrifuging at 4000 rpm for 30 min at 15ºC after washing the cells three times in physiological saline for 10 min. The washing of the bacterial cells was done to remove slime layer and nutrient from the growth medium. The cells were re-suspended in sterile normal saline solution (0.85% NaCl). The absorbance of the bacteria suspensions at 600 nm was adjusted to a reading of 0.500 with an average cell count of 1.5 x 10^9 CFU/mL. Standardized identified LAB strains were sub-cultured on MRS broth supplemented with 0.5 % taurodeoxycholic acid (TCDA) (Sigma) and incubated for 24h at 37ºC. Representative tubes with growth was inoculated onto the MRS agar supplemented with 0.5 % (TCDA) and incubated for 24 h at 37ºC.

2.3 Acid and Bile Tolerance Test

The ability of the isolates to tolerate bile was determined according to Liong and Shah [19]. The pH of MRS broth was adjusted by 1 M HCl (BDH) to achieve pH values of 3.0, 4.0 and 5.0 and the standardized LAB strains were inoculated. The broth was incubated for 90 min at 37ºC. The optical density of the broth was read at 650nm before and after the incubation by a spectrophotometer (Jenway 6505, England). All the experiments were conducted in triplicates and repeated twice. The effects of bile on the growth of probiotic LAB strains were examined using method of Meei-Yn and Tseng-Wei [20]. Each of the standardized isolates was inoculated into MRS broth and supplemented with 0.5 ml (% v/w) sterile fresh bovine bile. The culture was incubated at 37ºC for 18 h. The optical density of the broth was read at 650 nm before and after the incubation.

2.4 Bile Salt Hydrolase (BSH) Assay

According to Moser and Savage [21] was used to detect the isolates that produced bile salt hydrolase (BSH). Briefly, MRS agar was supplemented with 0.5% taurodeoxycholic acid (TDCA) and the isolates were inoculated onto the agar and incubated anaerobically at 37ºC for 5 days and the plates observed for precipitation of deoxycholic acid around the colony. Isolates with pronounced activity were selected for further studies (Table 1).

2.5 Formulation of Diets for the Animal Feed Experiment

Albino rats of Wister strains were induced by feeding on a high lipid cholesterol diet (HLF) for two weeks. Following hypercholesterolemia, the feeds were supplemented with the fermented foods and later fed to the rats for another eight weeks. As shown in Table 2, the diets were composed of four test groups and one control, the basal diet (BD). The four diet groups comprised high lipid feed (HLF), high lipid feed

with *Ogi* (HLF+*Ogi*), high lipid feed with different LAB strains (containing 5.6 x 10^{10} CFU/ml) (HLF+LAB) and the basal diet with Ogi (BD+Ogi). Diets HLF, HLF+*Ogi* and HLF+LAB were supplemented with 20 g/100 g lard (sourced from fatback) and 1.0 g/100 g cholesterol.

The pre-mixed vitamin and pre-mixed mineral were prepared as follows: Vitamin mixture (g/kg per diet) contained thiamine (0.02), riboflavin (0.03), pyridoxine (0.10), vitamin B12 (0.00003), niacin (0.001), calcium pantothenate (0.10), p-aminobenzoic acid (0.01) Vitamin A acetate (0.04), ergocalciferol (0.4) and choline-HCl (2.0). The mineral mixture (g/kg per diet) contained $CaCO_3$ (15.56) $CaCl_2.6H_2O$ (0.001), $CuSO_4.5H_2O$ (0.019), $FeSO_4.7H_2O$ (1.078), $MgSO_4$ (2.292), and $MnSO_4.2H_2O$ (0.025). The weight of each of the rats in the groups was taken twice a week and recorded. The difference between the initial and the final weights was taken to be the weight gained by the rat. The mean weight gained by the rats in each diet group was determined.

2.6 Source and Feeding of the Experimental Animals

Seventy post weaning healthy albino rats of Wister strains (*Rattus nervegicus*) with initial average body weight of 83.83±3.34 g were obtained from the Preclinical Animal House, University of Ibadan, Nigeria. The experimental animals were managed according to the National Research Council's Guide for the Care and Use of Laboratory Animals [22]. The animals were acclimatized for five days, fed with grower's mash (TopFeed®) and supplemented diets (Table 1) and adequately supplied with distilled water *ad libitum*. The animals were randomly assigned to each of the diet groups and they (n=5/group) were maintained at 27ºC with about 13:11 h of light and dark respectively. The animals were randomly assigned (in cages) into five groups. They were fed for 8 wk and the weight taken weekly throughout the experiment. Food and water were withdrawn from the animals 18 h prior to the termination of the experiment.

Table 1. Diet composition (g/Kg) of animal feed to evaluate the hypolipidemic activity of *'ogi'*

Group	Diets					
	Basal diet	**BD+'OGI'**	**HLF**	**HLF +'OGI'**	**HLF+ *L. lactis***	**HLF+ *L. brevis***
Corn starch	64.50	--	48.5	--	48.5	48.5
'Ogi'	--	65.5	--	48.5	--	--
Soybean	21.0	21.0	21.0	21.0	21.0	21.0
Fish meal	6.50	6.50	6.50	6.50	6.50	6.50
Vegetable oil	5.0	5.0	--	--	--	--
Lard	--	--	20.0	20.0	20.0	20.0
Mineral mix*	1.5	1.5	1.5	1.5	1.5	1.5
Cholesterol	--	--	1.0	1.0	1.0	1.0
Vitamin mix*	1.0	1.0	1.0	1.0	1.0	1.0
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Choline Chl.	0.2	0.2	0.2	0.2	0.2	0.2
≈10 $\log_{10}$ CFU/ml	--	--	--	--	*L. lactis*	*L. brevis*

**Premix, Chl: chloride*

Table 2. Growth characteristics of lactic acid bacteria isolates with probiotic attributes

Organisms	Cell morphology	Growth at acidic pH (3.0)	Growth at fresh bovine bile	Ability to deconjugate TCDA
Lactobacillus bulgaricus	Rod	++	+++	++
Lactobacillus plantarium	Rod	+++	+++	+++
Lactobacillus sp. (JBI 1)	Rod	+++	+++	NG
Lactobacillus lactis	Rod	++	+++	+++
Lactobacillus sp. (JBI 4)	Rod	+	+++	++
Lactobacillus brevis	Rod	+++	+++	+++
Lactococcus sp. (JBI 9)	Coccus	+++	+++	+
Lactococcus sp. (JBI 12)	Coccus	+++	+++	NG

+++: Dense growth; ++: Moderate growth; +: Poor growth; NG: No growth

2.7 Blood Collection and Determination of Serum Cholesterol Types

Blood was collected from the rats by cardiac puncture after a deep anesthesia. One-ml syringe using 22 gauge needle was inserted 5mm from the center of the thorax towards the animal's chin, 5 to 10mm deep; the syringe was held at 25 to 30º away from the chest. Serum total cholesterol and high-density lipoprotein (HDLC) cholesterol were determined according to Grove [23] while low-density lipoprotein (LDLC) cholesterol, and triacylglycerol were measured enzymatically by the methods of Meiattini [24] and Buccolo and David [25] respectively.

2.8 Determination of Lipid and Marker Enzymes in the Organs of the Rats

The different organs (heart, kidney and liver) were removed and separately homogenized before lipid and enzyme analyses. Total lipids were determined using the method of Folch [26]. The fat contents were extracted with chloroform: methanol mixture (2:1 v/v). The aliquots of the organic phase were analyzed for total cholesterol calorimetrically using glacial acetic acid-$FeSO_4$-H_2SO_4 while the remainder of the organic phase was evaporated to dryness and weighed to give the amount of liver total lipids. Aspartate transaminase (AST) and alanine transaminase (ALT) were determined using the Randox® enzyme kits.

2.9 Histopathological Processes

The animals were sacrificed eight weeks after the commencement of the experiment and the organs (kidney, heart and liver) were extracted and processed for histopathological assessment according to the method of Gentry-Weeks *et al*. [27]. The slides were examined under the microscope and the microphotographs taken at a magnification of X 400 as described by Baker and Silverton [28].

2.10 Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software. Data are expressed as mean ± SD. Data were analyzed using one-way ANOVA, followed by the Turkey Test. The result was considered significantly different at level of $p < 0.05$.

3. RESULTS

Eight LAB strains which grew well on fresh bovine bile were isolated from sorghum *Ogi*; six *Lactobacillus* species and two *Lactococcus* spp. Only two of these LAB strains ((*Lactobacillus* sp. (JBI 1) and *Lactococcus* sp. (JBI 2)) could not deconjugate TCDA. However, all the isolates are acid tolerant, with at least moderate growth at pH 3 (Table 2) but the strains of *Lactobacillus* sp. (JBI 1, JBI 4: 60%, 68.75%) and *Lactococcus* sp. (JBI 9) grew very poorly even at pH 4 (Table 3).

Table 3. The percentage survival of LAB isolates from '*ogi*' at different pH*

LAB isolates	pH 3	pH 4	pH 5
L. bulgaricus	92.59	94.74	96.08
L. plantarum	95.38	95.00	96.00
Lactobacillus sp. (JBI 1)	50.43	60.00	73.33
L. lacti	94.00	96.51	96.88
Lactobacillus sp. (JBI 4)	37.75	68.75	92.42
Lactobacillus brevis	93.33	93.75	93.82
Lactococcus sp. (JBI 9)	42.86	16.67	28.57
Lactococcus sp. (JBI 12)	44.44	80.00	89.36

** Microbial growth at 37ºC for 24 h*

The total bacterial count in ogi ranged between 7.7762 $\log_{10}$ CFU/g and 7.9631 $\log_{10}$ CFU/g while the LAB count in the samples were between 5.0792 $\log_{10}$ CFU/g and 5.6990 $\log_{10}$. LAB was dominant in the fermented gruel. The number of non-lactic acid bacteria that could survive in the sample ranged from 28.43% to 34.48% of the total bacterial load in the *ogi* sample (Table 4). The average values of pH and total titratable acidity (TTA) in all the *ogi* samples were 3.8 and 0.38 respectively (data not shown).

The weight of the animals increased with the duration of feeding. At the end of the feeding experiment animals in HLF group had the highest average weight followed by those in the Basal Diet group (Fig. 1). The animals in the HLF+*L. lactis* diet group had the least mean weight at the end of the experiment. There was no variation between the weights of the animals in the control group (Basal Diet) and the experimental groups (at 0.05 level of significance).

The amount of the total lipid in the animals fed with high lipid diet (HLF) was significantly lower than those obtained in the organs of the animals fed with diets containing BD+*Ogi*, HLF+ *L. lactis* and HLF+ *L. brevis* (at α = 0.05) (Fig. 2). At the probability of 0.001 the cholesterol concentration in the organs of the rats fed on HLF diet differs significantly from those fed with Basal diet, BD+*Ogi* and HLF+ *L. lactis*. While the levels in HLF +*Ogi* and HLF+ *L. brevis* diet groups were statistically lower than those of animals in the HLF group. The mean values of the LDLC in the rats were 188.60±7.27 mg/dl in the animals fed with HLF while the least value was recorded in the 110.80±26.93mg/dl. The values of LDLC in the animals fed Basal Diets were significantly different from those fed on HLF diet (P<0.01) while the difference in the serum LDLC of the animals fed HLF was significantly different from those on HLF+*Ogi* and BD+*Ogi* diets (at P<0.05 and P<0.001) respectively (Fig. 3).

Table 4. Lactic acid bacterial growth on taurodeoxycholic acid supplemented media

Organisms	Growth in TCDAsupplemented MRS broth	Viable count on TCDA supplemented MRS agar (CFU/ml)
Lactobacillus bulgaricus	+++	2.5 X 10^5
Lactobacillus plantarium	+++	5.0 X 10^5
Lactobacillus sp (JBI 1)	+*	1.5 X 10^4
Lactobacillus lactis	+++	2.5 X 10^5
Lactobacillus sp. (JBI 4)	++*	2.5 X 10^5
Lactobacillus brevis	+++	2.0 X 10^5
Lactococcus sp. (JBI 9)	NG	NG
Lactococcus sp. (JBI 12)	+	1.0 X 10^3

**Showed growth indicated by turbidity but there was no sign of precipitation; +: slightly turbid, ++: turbid, +++: highly turbid, NG: no growth. Data are the mean of three determinations*

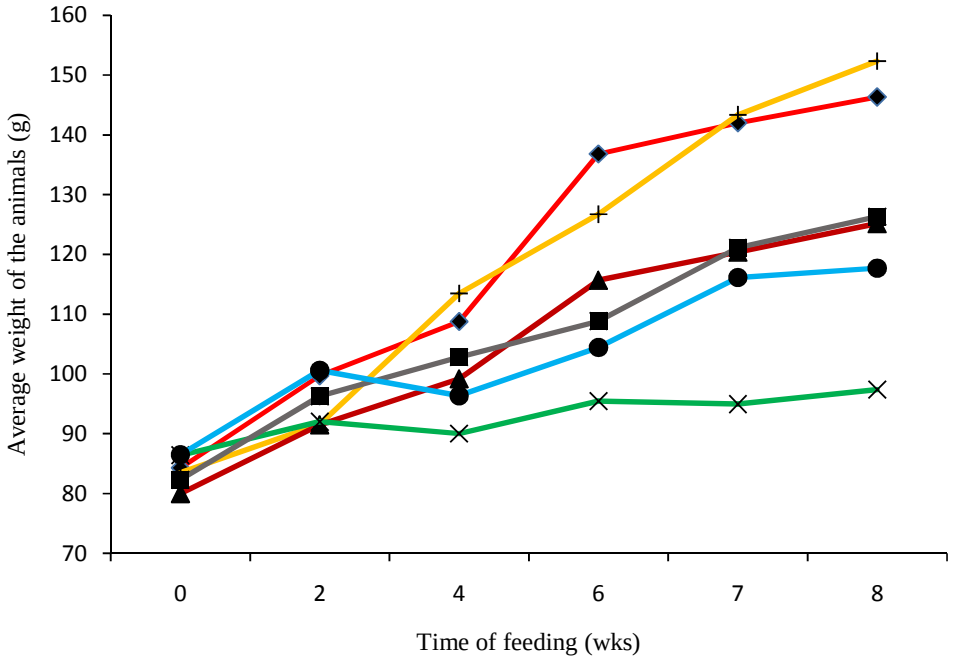

Fig. 1. Change in the body weight of rats fed high lipid cholesterol feed supplemented with *'ogi'* and *Lactobacillus* spp.

The diets include (■)HLF+L. brevis, (▲) HLF + 'OGI', (♦) Basal diet (●) BD+'OGI', (+) HLF and (×) HLF + L. lactis

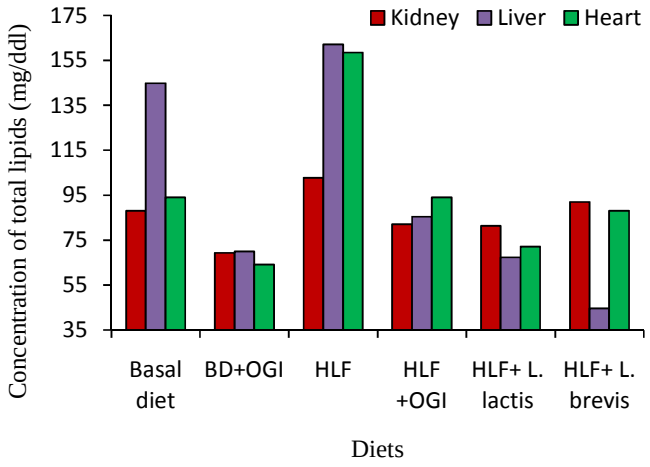

Fig. 2. The total lipid concentration in the organs of experimental rats

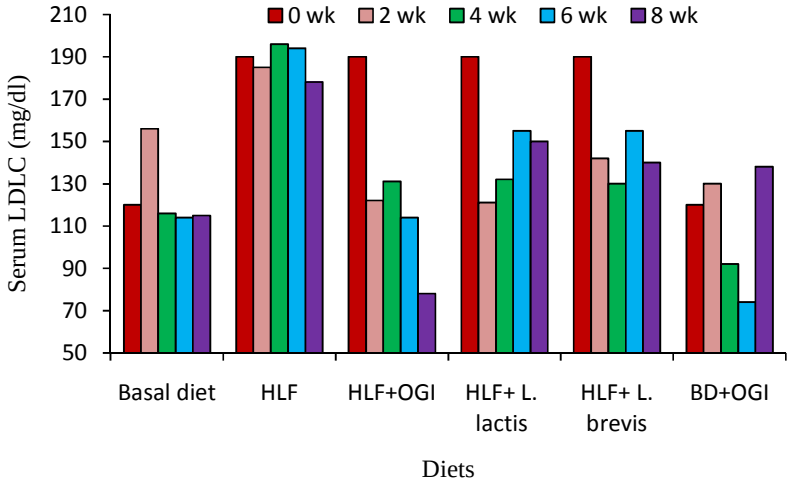

Fig. 3. The concentration of serum low density lipoproteins cholesterol in the serum of the experimental rats

The mean high density lipoprotein cholesterol (HDLC) in the serum of the rats in BD+*Ogi* diet was the highest (52.600 mg/dl) compared to the Basal Diet (49.400 mg/dl) while the HLF group had the least (24.800 mg/ml). HDLC concentration in HLF differed significantly from the basal diet, HLF+*L. brevis* and BD+Ogi at P<0.001 and was also significantly different from HLF +*Ogi* and HLF+*L. lactis* at P<0.01 (Fig. 4). As shown in Fig. 5 the highest amount of glycerides was recorded in the HLF group (100.40 ± 21.29 mg/dl) while the least amount of triglyceride was observed in the animals in the Basal Diet group (31.200±7.190 mg/dl). The levels of glycerides in the Basal and HLF diets were significantly different (P<0.001). Glyceride level in the HLF group was higher than the HLF+*L. lactis* and BD+*Ogi* groups (at P<0.05).

The mean value of alanine transaminase (ALT) in the animals in the Basal Diet group was higher at the commencement of the experiment (275.45±34.43 IU/L) compared to the value obtained after 8wks (144.27±38.63 IU/L). Meanwhile ALT activity (340.99±45.83 IU/L) was highest in the rats fed with the Basal diet supplemented with *Ogi* after 8wks of feeding. Compared to the ALT activity in rats fed on high lipid diet (HLF), the AST activity in rats fed on the Basal Diet +Ogi, HLF + Ogi, HLF+ *L. lactis* and HLF+ *L. brevis* were lower (at p<0.05). The level of AST activity in the animals in all the groups

was not significantly different at 0.05 probability level (Table 5).

The histo-pathological micrographs of the various organs (heart, kidney and liver) of hypercholesterolemic and the non-induced rat groups are presented in Plates 1A to J. The heart of rats fed on the normal feed and those on HLF diets with or without *Ogi* showed no visible lesion (Plate 1). In rats fed on HLCF and *Ogi* multiple necrotic tissues of the heart muscles were observed while those on HLCF supplemented with *Ogi* showed some multiple foci of fibre necrosis and haemorrhage with mild cellular infiltration by macrophages. Plates 1E and F showed the tissues of normal and afflicted kidney. All the rats fed on the different diet regimes showed no visible lesion in the kidneys except those fed on HLF and *L. brevis* where the lumen of the tubules was distended (Plate 1F). The liver tissues of most of the rats showed no visible lesions but in some rat groups there were observable cellular changes in the central vein and portal triad of the organ while those fed on HLF were appreciably affected.

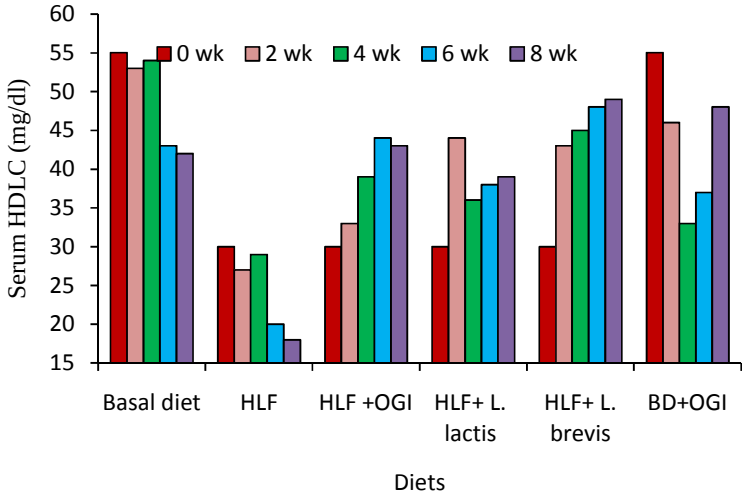

Fig. 4. The concentration of serum high density lipoproteins cholesterol in the serum of the experimental rats

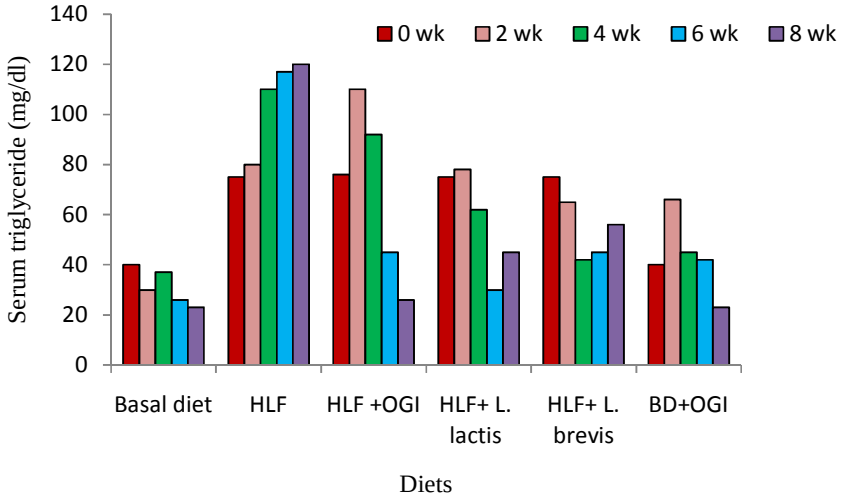

Fig. 5. The level of triglyceride in the serum of the experimental rats

Table 5. The concentration of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) in the experimental rats

Weeks	Enzymes	Diets					
		Basal diet	**Basal Diet + 'OGI'**	**HLF**	**HLF + 'OGI'**	**HLF+ *L. lactis***	**HLF+ *L. brevis***
0	ALT	275.45±34.43	270.42±72.49	90.39±17.45	90.45±28.34	94.44.±19.35	90.34±23.41
	AST	80.78±12.32	90.27±18.42	95.78±14.59	90.34±16.36	93.51±28.34	105.33±41.32
2	ALT	195.27±28.39	190.44±55.27	73.52±26.49	95.22±15.43	73.23±24.31	72.34 ±22.17
	AST	60.30±11.11	250.39±59.23	150.35±48.29	152.24±33.28	103.32±32.33	242.32±34.59
4	ALT	164.49±19.15	100.35±19.46	80.69±29.43	92.34±43.14	140.12±34.22	140.42±34.11
	AST	140.49±27.38	410.34±83.42	230.00±45.81	135.46±25.34	125.23±41.12	250.39±41.39
6	ALT	100.38±28.55	176.84±17.49	121.32±39.10	120.34±25.33	120.33±31.51	110.33±51.93
	AST	150.39±51.53	180.11±45.28	250 .34±34.25	190.34±16.99	220.33±13.42	175.27±31.52
8	ALT	144.27±38.63	340.99±45.83	125.56±42.14	102.12±52.21	110.54±31.65	90.46±25.77
	AST	163.19±48.24	178.33±19.46	264.34±24.62	165.45±23.34	245.23±43.34	150.36±18.36

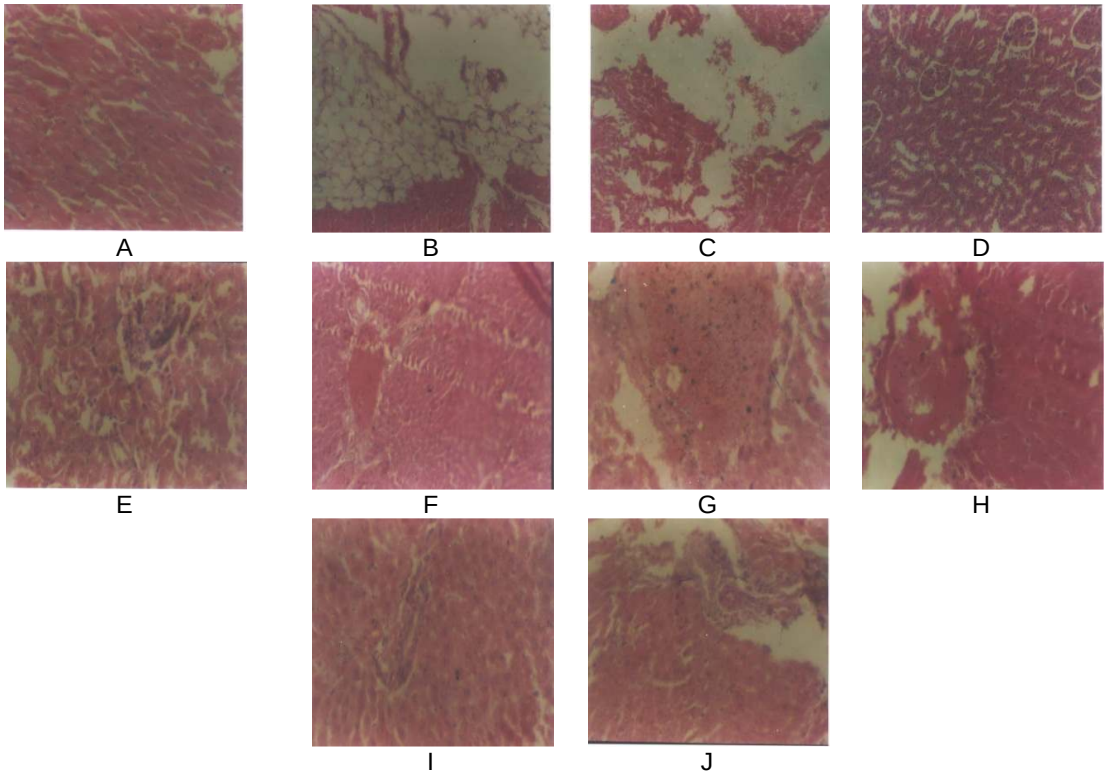

Plate 1. Histopathologic examinations of different organs of animals showing lesions due to hypercholesterolemic situation created by the HLF diet

A: Heart tissues of animals fed with Basal Diets with no visible lesion (Mag. X250), B: Heart of animal in diet group HLF+'ogi' (Mag. X250), C: Multiple necroses into the heart muscles for rats on HLF+'ogi' (Mag. X125), D: Kidney of rat on Basal Diet showing no visible lesion (Mag. X250), E: Kidney of rat in HLF+L. brevis with lumen of the tubules distended (Mag. X125), F: Liver-mild congestion of central vein of rats fed HLF+L. brevis (Mag. X250), G: Distended central vein of the liver of rats on HLF diet group (Mag. X250), H: Mild fibroplasia of portal triad in liver of rats on high lipid cholesterol (Mag. X125), I: Liver of rat on HLF+OGI with normal morphology (Mag. X125) J: Liver of rat on HLF+L. lactics having moderate cellular infiltrations of portal triad by macrophages (Mag. X125)

4. DISCUSSION

Lactic acid fermented foods have made up a significant portion of food intake by humans for a long time and are still available in the developing countries [1,2,6]. *Ogi* which is an example of lactic acid bacteria fermented food product in Nigeria was investigated for its ability to reduce the serum cholesterol level in induced hypercholesterolemic rats. The total lactic acid bacteria counts ranged between 7.7762 $\log_{10}$ CFU/g and 7.9631 $\log_{10}$ CFU/g. This high lactic acid bacteria count observed in *Ogi* may be as a result of the growth of the associated microbiota on the grains and their low pH range of between 3.5 and 4.5. This confirms the reports of Hounhouigan et al. [29], Teniola and Odunfa [6], and Adebayo and Aderiye [5], which showed the dominance of lactic acid bacteria in fermented maize for *Ogi* production. Also the viable count of LAB in *Ogi* may be due to their known complex nutritional requirements: Substrate rich in carbohydrate [30,31].

The low pH and the TTA of 3.8 and 0.38 respectively observed in *Ogi* may be attributed to the secondary metabolites (lactic acid) produced by the lactic acid bacteria involved in the fermentation of the foods. Teniola and Odunfa [6] reported that metabolites produced during the fermentation of maize (*Ogi)* are usually responsible for the changes in the physical and chemical parameters. They concluded that the changes may impact both desirable and undesirable characteristics on the products.

In all, six different lactic acid bacteria isolates were selected and used in the study. The

bacteria are all catalase negative and fermented glucose with or without the production of gas. The rod-shaped isolates were identified as *Lactobacillus* spp. while the cocci were the *Lactococcus* spp. The identification of these isolates as *Lactobacillus* species is in line with Tannock [32] definition of lactobacilli as members of the Lactic acid bacteria group that are characterized by the formation of Lactic acid as sole end product and are Gram positive, catalase negative, non-spore-forming rods or coccobacilli. He described them as been strictly fermentative with complex nutritional requirement.

Bile tolerance is an important criterion for the selection of probiotes [33]. A bile concentration of 0.2% (w/v) in MRS medium has been reported to prevent growth of lactobacilli for at least 4 hrs, but the cells remained viable after 4 h incubation [34]. In this study, *L. plantarum, L. bulgaricus, L. brevis* and *L. lactis* showed considerable growth when the medium was supplemented with bovine bile. This medium and the survival of these four organisms at different pHs had been employed to simulate conditions in the upper intestinal tract of man [35]. These conditions (i.e. the acid and bile tolerance) which are required for an efficacious probiote and variability in the survival rates may help to explain some beneficial propensities of these organisms [36].

The growth of these organisms at pH 3, 4, and 5 is an indication that they are likely to survive the acidity of the stomach when ingested with foods. The proliferation of these organisms at these pH values and the ability to tolerate bile are important indices to categorize them as probiotes. Their tolerance to high acidity may be attributed to the presence of a constant gradient between extracellular and cytoplasmic pH [37].

For these organisms to be used as probiotes in humans, Moser and Savage [21] postulated that they must be able to overcome the conjugated bile acids of the duodenum. Bile acids are synthesized from cholesterol and conjugated to either glycine or taurine in the liver [38]. Isolates from the fermented foods used in this study displayed varying growth abilities in taurodeoxycholic acid (TCDA) supplemented medium. The inability of some of the isolates to precipitate TCDA may be that they cannot express bile salt hydrolase activity as suggested by Savage et al. [38] and Tanaka et al. [39].

Hypercholesterolemia refers to levels of cholesterol in the blood that are higher than the normal levels in animals. Cholesterol circulates in the blood streams and it is a major building block of cell membranes. It is essential in the formation of bile, vitamin D, other steroids and hormones [40]. All the groups of rats fed on high lipid cholesterol diet (HLCF) showed high level of serum cholesterol after 2 weeks of feeding. This may be as a result of the addition of pork fat which increased the lipid content of the feed to 20% and also the incorporation of cholesterol in the diet. Diets rich in saturated fats and contain high level of cholesterol raises serum cholesterol [41].

When compared to the control group which are fed on the normal feed and took water *ad libitum,* all the hypercholesterolemic rats were active throughout the period of the experiment. This indicates that hypercholesterolemia does not have any noticeable visible symptom(s), unless those diagnosed through blood analysis or when heart attack or stroke eventually occurred [42].

There were noticeable changes in the body of the rats in the groups in which hypercholesterolemia was induced (HLF and *Ogi*) when compared to those fed on the normal feed and *Ogi*. Rats fed on HLF supplemented with *Ogi* and lactobacilli had lower body weights compared to those fed with the Basal and HLF diets. Feeding the rats with the fermented foods did not show much difference in their body weights. It could be deduced that *Ogi* could be an antidote for the reduction of body weight. This may therefore mean that the consumption of *Ogi* had a way of enhancing the conversion/removal of the excess lipid in the body of the rats. This may also probably be attributed to the activity of the organisms contained in the fermented food which reflected on their body weight values (Fig. 1).

The cholesterol profile in the various groups showed a decline as a result of the supplementation of the diet. The animals whose diets were supplemented with the bacterial isolates also had significant reduction in their serum cholesterol concentration at the end of the feeding period. The decrease in the serum cholesterol concentration may be attributed to the ability of these organisms to take up cholesterol into their cellular membrane [13,14,36].

Rats fed on normal feed or HLF supplemented with *Ogi* showed decrease in the serum cholesterol level after the 4^{th} week, and this

continued until the 8th week. This observation can be attributed to the co-precipitation of cholesterol with deconjugated bile acids. Klaver and van der Meer [43] showed that the solubility of cholesterol is dependent on the bile salts present.

The low density lipoprotein cholesterol (LDLC) values were low in all the rat groups fed on diets supplemented with *Ogi* and *Lactobacillus* isolates. Low density lipoprotein cholesterol is a product of the liver through the packaging of triglycerides with cholesterol, phospholipids and apoprotein [44]. Very high LDLC tend to stick to the lining of the blood vessels, leading to the hardening of the arteries. The lowering effect was more pronounced in the groups fed with HLF and *Ogi.* This finding confirmed an earlier report of Fletcher et al. [45] that the principal strategy for lowering LDLC levels was to replace cholesterol raising fatty acids (FA) with dietary carbohydrates. Aderiye and David [46] reported that probiotics from African fermented food *iru* increased the concentration of the HDL and reduced the LDL.

The serum triglyceride content of the rats was also lower in all the animal groups fed with diets supplemented with the fermented foods and the organisms. This was expected since triglyceride determines LDLC composition, physical properties and cell specific binding in culture cells of organism [47]. The reduction may also be as a result of the fact that the supplements (*Ogi* and the lactobacilli) did not in any way increase the saturated fat content of the diet.

There was an increase in the high density lipoprotein cholesterol (HDLC) values in all the rat groups. High HDLC levels in the animal and human blood protect against heart attack [19]. The high values reported are in agreement with the findings of Minelli et al. [48] who observed that the administration of *Lactobacillus* sp. in animal models induced a cholagogic effect, favouring an easier bile flux and improvement in some metabolic indices of hepatic and lipidic functionality. HDLC have also been reported to increase triglyceride catabolism [49].

Alanine and Aspartate aminotransferase are normally found in diversity of tissues including the liver, heart, kidney, and brain [50]. They are found in the cells of these organs and may leak out to the blood when the cells are injured. The results from this work showed an initial increase and then a decrease in the values of the two enzymes. The reduction in activity of these enzymes over the supplementation period may indicate an improvement in the recovery of the organ injury. This may be as a result of the ability of the lactic acid bacteria present in the fermented foods to produce polyamines from amino acids [51]. Our findings agree with the report of Adawi et al. [52] that supplementation of the diet with *L. plantarum* and arginine reduced the hepatocellular necrosis and the inflammatory cell infiltration in the liver, which was reflected in the low concentrations of the enzymes released.

The lesions observed in the histopathologic plates in the different organs of the animals may probably be as a result of the hypercholesterolemic situation created by the HLF diet. The degree of the severity of the lesions could explain the impact the supplements had on arrest of the situation of the disease condition. This is further confirmed by the fact that the group fed on the normal diet only had no noticeable lesion(s) in all the organs.

5. CONCLUSION

In this study, one can attribute the reduction of serum cholesterol to the assimilation and binding of the cholesterol by the different organisms that survived the passage through the stomach and finally colonized the intestine. The organisms prevented the reabsorption of the cholesterol in the diet and thereby resulting in low values in the serum. Other ailments such as Diabetes mellitus may be challenged with these fermented foods to ascertain if there will be any effect.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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