Pharmaceutical Sciences & Analytical Research Journal
ISSN: 2640-6659

Academic Strive
Researcher's Perception is our Reality

Research Article *Volume 4 Issue 1*

Effects of Probiotics Isolated from Nigerian Indigenous Fermented Foods on Albino Wistar Rats

Akinnola OO[1]*, Oluwasola AM[1], Ajayi SA[1], Ogunleye OB[1] and Omeje M[2]

[1]Department of Biological Sciences, Covenant University, Nigeria

[2]Department of Physics, Covenant University, Nigeria

***Corresponding author:** Akinnola OO, Department of Biological Sciences, College of Science and Technology, Covenant University, Ogun State, Nigeria, Email: ola.akinnola@covenantuniversity.edu.ng

Received Date: May 24, 2021; **Published Date:** June 17, 2021

Abstract

Fermented foods have been the major carrier of probiotics. The fermentation process of foods is made possible by microorganisms. Probiotic lactic acid bacteria previously isolated from Ogi and Kunu-zaki was investigated for their effect on some biomarkers in healthy male albino Wistar rats. The two strains that showed good probiotic potentials were chosen based on tolerance to low pH and bile salt, and antibiotic susceptibility test. Thirty Albino Wistar rats were divided into six groups based on different treatments for each group. Four groups were administered orally with the two probiotic strains at different concentrations (1.0x109, 2.0x108). One group was administered with the combination of the two strains (2.0x108 each), while the last group, which was the control received normal saline for two weeks. The blood samples and livers were collected at the end of the treatment. The analysis of certain biomarkers (fasting blood glucose (FBG), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, bilirubin, total protein and antioxidant profile) was carried out. The ALT, AST and superoxide dismutase (SOD) activity in the test animals were found to improve significantly while no significant difference was observed in the activity of other biomarkers in the treated groups compared to the control. The results showed that the two strains had a positive impact on the health status of the rats with the combined group having the higher impact. However, more studies need to be done to explore other health benefits associated with these organisms.

Keywords: Fermented foods; Probiotics; Albino Wistar rats; Biomarkers; Health benefits

Abbreviations: LAB: Lactic Acid Bacteria, CHREC: Covenant Health Research Ethics Committee, SOD: Superoxide Dismutase.

Introduction

The global interest in harnessing and utilizing the beneficial properties of microorganism and their metabolites to improve human health and wellbeing, coupled with numerous benefits of fermented foods as probiotics make it significant to explore the probiotic properties of Lactobacillus species. Probiotics are live microorganisms which confer various benefits to human health when ingested in adequate amounts [1]. To confer beneficial properties on the host, the microorganism is expected to be able to colonize the gut and have tolerance to the harsh condition in the intestinal tract of the host [2]. Numerous Lactic acid bacteria (LAB) isolated from varieties of food sources in different countries around the world have been reported to confer health benefits and possess good probiotic characteristics [3,4]. Fermented foods have been

the major carriers of probiotics. Some of the health benefits related to probiotics are enhancement of lactose metabolism [5], reduction of serum cholesterol and enhancement of the normal functioning of the liver [6]. The fermentation process of foods is made possible by microorganisms and provides an easy, inexpensive and natural process to enhance the organoleptic and nutritional contents of cereals and other foods. Lactic acid bacteria, Bifidobacterium and few yeast species are the essential microorganisms involved in this process [7].

Lactobacillus species are part of the most essential bacteria in the food industry. They are mostly used as starter cultures for the production of fermented foods. They possess the ability to confer health benefits on their host, such as immune modulatory, antimicrobial, anti-allergic, anti-tumorigenic and antioxidant activities [8]. Some of Nigeria indigenous fermented cereals include; Ogi (fermented corn gruel), which is made using yellow maize (Zea mays) Kunu-zaki (fermented millet, Pennisetum typhoides), Ogi-baba made from guinea corn (Sorghum bicolor), fura made from fermented cow milk, and burukutu [9]. Various studies have revealed that a selection of autochthonous microbial strains used in traditional fermentation of cereals under controlled fermentation can enhance the quality, safety and stability of fermented products [10]. Other studies have also sought new probiotic strains which can confer health benefits. This study was therefore conducted to determine the probiotic potentials of Lactobacillus species isolated from Ogi and kunu-zaki through in vitro and in vivo techniques.

Materials and Methods

All chemicals and reagents were used as received without further purification.

Ethical Approval and Experimental Animals

Ethical approval was obtained from Covenant Health Research Ethics Committee (CHREC). Thirty male Albino Wistar rats were purchased, housed in plastic cages and allowed to acclimatize for one week. After which the rats were assigned to six groups.

Sample Collection: Samples for the isolation of probiotic organisms (freshly prepared Ogi and Kunu) were obtained from sales points in Ota, Ogun State.

Isolation of Lactobacillus SPP: For isolation of Lactobacillus spp, 1g and 1 ml of each sample (Ogi and Kunu) were mixed with 9 ml of sterile distilled water and a serial dilution of the homogenate was made up to six dilutions. A 0.1 ml aliquot of the third and sixth dilution were plated on MRS (de Mann, Rogosa, and Sharp) agar (Himedia) plates and these were incubated anaerobically with the application of (Gas Pak Anaerobic Systems, BBL) for 48 hours at 37°C. Single colonies were selected at random from MRS plates and streaked on MRS agar to obtain pure colonies.

Identification of Lactobacillus SPP: Identification of isolated organisms was carried out in line with stated morphological, biochemical, cultural and physiological characteristics listed in the Bergey's Manual of Systematic Bacteriology [11]. The tests carried out were Gram's reaction, catalase test, oxidase test, urease test and sugar fermentation test.

In-Vitro Characterization of Probiotic Properties

Tolerance to acidity: Lactic acid bacteria isolates were screened for growth in acidic condition as described with little modifications in the procedure [12]. LAB isolates were inoculated into MRS broth acidified with HCl to pH 2.0, pH 4.0, and non-acidified MRS broth at pH 6.8 and then incubated at 37°C for 48 h under anaerobic condition. Cell density was determined using a UV-VIS spectrophotometer at 600 nm.

Bile Tolerance Test: The resistance of the isolates to different bile salt concentrations was carried out following the procedure as explained [13] with some modifications. A 0.3 and 0.1% (w/v) of bovine bile was added to MRS broth, after that the strains were inoculated and incubated anaerobically at 37°C. The tested media were filtered through a 0.22 μm filter (Critical Syringe Filters; Critical Process Filtration Inc). The cultivated strains were collected in the absence of Ox-gall powder as a control. The following equation expressed the survival percentage of bile salts after 3 hours.

$$\text{Bile survival\%} = (\text{Log N1})/(\text{Log N0}) \times 100$$

N1 is the absorbance of cultures in MRS broth containing 0.1 and 0.3 % bile salts.

logN0 is the absorbance of cultures in MRS broth without bile salts.

Antibiotic Susceptibility Test: Each of the isolates that have tolerance to bile and acid was selected for antibiotic susceptibility test. This test was carried out by Kirby-Bauer disc diffusion technique [14]. Antibiotics used include; ciprofloxacin, erythromycin, cotrimoxazole, chloramphenicol, penicillin, tetracycline, neomycin, and gentamicin. A 100µl of freshly prepared isolates of acid and bile-tolerant LAB was plated on Mueller Hilton agar. Antibiotic discs were carefully placed on the culture medium, and the antibiotic discs were allowed to diffuse through the agar for 30 min at 4°C, and was incubated for 24h at 37°C in a CO2 environment. Resistance, intermediate and susceptibility to the test antibiotics were determined by the zone of inhibition which was measured in diameter [15].

Growth at Different Temperatures

A 50 µl of the freshly prepared LAB isolates was measured and inoculated into four different tubes containing 5ml of modified MRS broth medium. A drop of 0.12 g/l concentration

of bromocresol blue was added to the medium which serves as an indicator. Two separate tubes containing inoculated LAB was incubated at 15 and 45°C respectively. Growth at different temperature was observed after the incubation period, and color change of the broth from purple to yellow indicated that the organism was able to survive and grow at different temperatures.

Tolerance to Different NaCl Concentrations

Lactic acid bacteria isolates were examined for growth and tolerance at varied NaCl concentrations; 4 % and 6.5 % NaCl concentrations were used. Likewise, 5 ml of modified MRS broth was used to culture the isolates. The appropriate concentration of bromocresol purple indicator was prepared, and few drops were added to the broth. Four distinct test tubes containing 4 % NaCl and 6.5 % NaCl were prepared. A 50 µl of freshly prepared culture of LAB isolates was inoculated and incubated anaerobically at 37 °C for 7 days. The change in the colour from purple to yellow indicated cell growth.

Haemolysis Test

The determination of the haemolytic activity of the LAB isolates was carried out according to the methods explained [16]. Each of the LAB isolates was streaked on the surface of blood agar plates containing 5% (w/v) sheep blood and incubated anaerobically at 37°C for 48 h. After incubation, the plates were examined for beta-haemolysis, alpha-haemolysis, and non-haemolytic activities.

In-vivo Characteristics of Probiotics Properties of Lactobacillus Species

Probiotic administration and animal sacrifice: The experimental animals were placed into six groups. Group A - Control; Group B- Dose with (1x109) of Probiotic A; Group C - Dose with (2x108) of Probiotic A; Group D - Dose with (1x109) of Probiotic B; Group E - Dose with (2x108) of Probiotic B; Group F - Dose with (1x109) of Probiotic A and dose with (2x108) of Probiotic B. Two Lactobacillus species that exhibited the best in vitro probiotic properties were chosen for the in vivo studies. Oral solution of Lactobacillus spp K2 and K5 was prepared to contain a final concentration of 1x 109 CFU/ml and 2x108 CFU/ml and administered to the treatment groups. The control received normal saline. Probiotic treatment was for 2 weeks after which rats were fasted for 12 h and anaesthetized by intraperitoneal injection of 1 mL of ketamine hydrochloride (75 mg) and 1 mL of xylazine hydrochloride (5 mg) per kg body weight. At each evaluation point, faeces was collected. Blood samples were collected by cardiac puncture in the left ventricle, organs (kidney and liver) were harvested, weighed, and labelled for further analysis [17].

Determination of faecal bacteria count: Faecal samples from each rat were collected on the day of sacrifice into an aseptic tube. Faecal samples (1g from each group) was homogenized in distilled water, and serial dilution was carried out, plated on MRS agar and incubated between 24-48 h for isolation of Lactobacilli species that were present in the faecal samples.

Collection of blood and tissues: From each rat, 5ml of blood was collected into dried centrifuge tubes, and allowed to clot at room temperature. The serum was separated by centrifugation at 3500xg for 15mins, collected in clean tubes and stored at -20°C. Liver and kidney were collected, weighed and stored for further analysis.

Measurement of Oxidative Biomarkers in Liver and Kidney

Thiobarbituric acid reactive substance: Malondialdehyde (MDA) an index of lipid peroxidation was determined using the protocol described by Ohkawa H, et al. [18].

Superoxide dismutase (SOD): The SOD activity in kidney and liver homogenate was assayed according to an earlier procedure [19].

Glutathione peroxidase activities (GSH) : An earlier method was used to estimate the activity of GSH [20].

Liver Function Test: The following enzyme concentrations were measured from liver homogenates using standard kits purchased from Randox Laboratories Ltd following manufacturer's instructions.

Aspartate aminotransferase activity (AST): This test was done to investigate the influence of the administered Lactobacilli on liver function. The Reitman-Frankel method was used like that of ALT activity [21-23].

Alanine aminotransferase activity (ALT): This test was carried out to determine the effect of the Lactobacillus species on the rat's liver function. The method described by Randox diagnosis test kit was used for this test [21-23].

Statistical Analysis

All the measurements were performed in triplicates, and the results were expressed as mean standard deviation (SD). Data were analyzed by the one-way ANOVA plus post hoc Duncan's test by Graph Pad 8.0.

Results

Isolation of Lactic Acid Bacteria from Ogi and Kunu-Zaki

A total of 15 lactic acid bacteria were obtained from Ogi and Kunu-zaki samples (8 from Ogi and 7 from kunu-zaki). Only 7 isolates were considered to be Lactobacillus species. Alphanumeric letters were assigned to each isolate to differentiate them.

Physiological, Morphological and Biochemical Characteristics of the Isolates

The seven selected isolates were identified through their physiological, morphological and biochemical characteristics as shown in Table 1. All the isolates were Gram-positive, rod shaped, catalase-negative with creamy and large colonies. In the sugar fermentation test, all isolates were able to ferment glucose and lactose, while in the sucrose fermentation test, all the isolates were able to ferment sucrose, except isolate K5. In the Urease fermentation test, only 2 isolates (K2, and K4) were able to ferment urease. Out of the 7 isolates, 3 isolates produced gas from glucose (K1, K4, and K7), isolate (K2) produced gas from sucrose (Table 1).

				Morphology		Sugar Fermentation Test			
Isolate	Gram's reaction	Colony Morphology	Colony Color	Shape	Catalase	Sucrose	Glucose	Lactose	Urease
K1	+	Large	Creamy	Rod	-	+	+G	+	-
K2	+	Large	Creamy	Slender rod	-	+G	+	+	+
K3	+	Large	Creamy	Slender rod	-	+	+	+	-
K4	+	Large	Creamy	Slender rod	-	+	+G	+	+
K5	+	Large	Creamy	Slender rod	-	-	+	+	-
K6	+	Large	Creamy	Slender rod	-	+	+	+	-
K7	+	Large	Creamy	Slender rod	-	+	+G	+	-

Table 1: Physiological, morphological and biochemical characteristics of the isolates.

In Vitro Probiotic Properties of Isolates

Tolerance to low pH: Out of the 7 isolates tested for pH tolerance, 2 isolates (28.6%) were able to tolerate pH 2.0 for 3 h. At pH 4.0, all the 7 isolates (100%) survived (Table 2). Among the two isolates that survived at pH 2.0, one each (14.3%) was isolated from Ogi and kunu-zaki samples. Generally, the survival rates of all the isolates ranged from 15 to 47 % at pH 2.0 and 59 to 98% at pH 4.0 (Table 2). Isolates K2 and K5 showed the highest tolerance at pH 2.0, while at pH 4.0, isolates K2, K5 and K7 showed the highest tolerance. However, the survival rate of all the isolates reduced from pH 4.0 to pH 2.0 in a 3 h exposure period.

Isolates	pH 2.0	pH 4.0	0.1% Bile	0.3% Bile	4% NaCl	6.5% NaCl	15°C	45°C	Haemolysis activity
K1	15.00±1.155	66.60±2.887	91.01±2.884	87.71±1.798	+ve	+ve	+ve	+ve	-ve
K2	45.00±1.155	98.00±2.309	95.67±1.328	90.43±1.443	+ve	+ve	+ve	+ve	-ve
K3	27.53±2.728	59.00±1.270	95.57±2.656	88.51±1.732	+ve	+ve	+ve	+ve	-ve
K4	18.50±1.155	68.90±2.309	90.20±1.155	87.58±3.002	+ve	+ve	+ve	+ve	-ve
K5	55.00±1.155	95.00±1.732	97.50±1.443	89.22±2.598	+ve	+ve	+ve	+ve	-ve
K6	16.00±1.155	89.00±4.041	93.22±2.425	86.73±1.328	+ve	+ve	+ve	+ve	-ve
K7	23.00±1.155	98.00±2.309	98.57±1.386	84.57±2.425	+ve	+ve	+ve	+ve	-ve

Table 2: *In vitro* probiotic properties of *Lactobacillus sp.*

Tolerance to bovine bile salt: The entire seven isolates (K1, K2, K3, K4, K5, K6 and K7) survived 0.1% concentration of bovine bile salt (Table 2). However, at 0.3% of bovine bile salt, the survival rate of all the isolates reduced, with isolates K2 (90.4%) and K5(89.2%) surviving the most. In general, the growth of all the isolates reduced from 0.1% of bile salt to 0.3% bile salt.

Tolerance to NaCl: All the seven isolates were able to survive at 4% NaCl and 6.5% NaCl (Table 2). However, at 6.5% NaCl, the growth of the isolates reduced, with isolate K2 surviving.

Growth at Different Temperature: All isolates were able to grow at 15oC, while isolate K2 had the highest growth (Table 2). However, at 45°C, the growth of all the isolates reduced while isolate K5 had the highest growth rate.

Haemolysis Test: Table 2 shows the result obtained from haemolysis test. All the isolates had no haemolytic activity.

Antibiotic susceptibility pattern: Table 3 shows the result obtained from the antibiotic susceptibility test. All isolates were resistant to Ciprofloxacin and Cotrimoxazole with isolates K2, K3, and K5 being resistant to most antibiotics used. Isolate K2 was susceptible to 50% of the antibiotics used, while isolate K5 was sensitive to 10% of the antibiotics

(Table 3).

The diameter of inhibition zone (mm)								
ISOLATES	Gentamicin	Ciprofloxacin	Chloramphenicol	Penicillin	Neomycin	Co-trimoxazole	Tetracycline	Erythromycin
K1	S	R	S	S	I	R	I	S
K2	S	R	S	S	R	R	R	S
K3	R	R	R	R	I	R	R	R
K4	S	R	S	R	I	R	R	R
K5	I	R	R	S	I	R	R	R
K6	S	R	I	R	I	R	R	S
K7	I	R	S	S	I	R	R	S

Table 3: Antimicrobial Susceptibility Testing.

Faecal microbial count: Table 4 shows the result obtained from the faecal microbial count of the faecal samples collected at the end of the treatment period. Lactobacillus species were isolated from all the groups administered with isolate K2 and K5, which shows that they were able to survive in the gastrointestinal tract of Albino Wistar rats. This result indicates that they were able to colonize the gut which is evident in the faecal microbial count of the treated groups when compared with the untreated group (Control). The treated groups had a higher microbial count compared with the control (Table 4).

GROUPS	10^{-4} (CFU/ml)	10^{-6} (CFU/ml)
GRP. A (Normal Diet)	1.0×10^{6}	4.5×10^{7}
GRP.B (1.0×10^{9} Lac A)	6.0×10^{6}	2.0×10^{8}
GRP C: (2.0×10^{8} Lac A)	4.0×10^{6}	3.0×10^{8}
GRP D: (1.0×10^{9} Lac B)	2.6×10^{6}	2.0×10^{8}
GRP E: (2.0×10^{8} Lac B)	2.6×10^{6}	2.4×10^{8}
GRP F: (2.0×10^{8} Lac A + 2.0×10^{8} LacB)	6.0×10^{6}	2.0×10^{8}

Table 4: Faecal microbial count.

Effect of Probiotic Treatment on the Body Weight of Albino Wistar Rats

The average weight of the rats per group at different periods of treatment are shown in Table 5 The weight of the animals in the first week of treatment ranged from 98.20g -103.80g. However, at the end of the treatment period, the weight of all the groups fed with probiotics and the control group increased. Group C had the highest mean weight (158.40g), while group F (fed with combined treatment) had the lowest mean weight (142.20g). Generally, in the control group and the test groups, there was no significant difference ($p<0.05$) in the mean weight of the animals (Table 5).

	Weights of The Rats		Fasting Blood Glucose of The Rats	
GROUPS	Week 1 of treatment	End of treatment	Week 1 of treatment	End of treatment
GRP. A (Normal Diet)	95.80±8.991	143.80±6.719	102.20±2.557	105.40±4.589
GRP.B (1.0×10^{9} Lac A)	103.8±5.444	153.60±6.562	87.40±6.961	102.40±1.435
GRP C: (2.0×10^{8} Lac A)	118.2±7.716	158.40±7.440	102.80±1.428	105.00±2.966
GRP D: (1.0×10^{9} Lac B)	95.20±6.644	120.80±11.350	90.80±4.247	96.20±2.200
GRP E: (2.0×10^{8} Lac B)	101.20±4.576	155.40±6.918	98.00±5.070	97.40±1.364
GRP F: (2.0×10^{8} Lac A + 2.0×10^{8} LacB)	92.60±4.238	142.2±6.461	102.6±2.694	95.40±1.806

Table 5: Weight and Fasting blood glucose of the rats.

Effect of Probiotic Treatment on the Glucose Level of Albino Wistar Rats

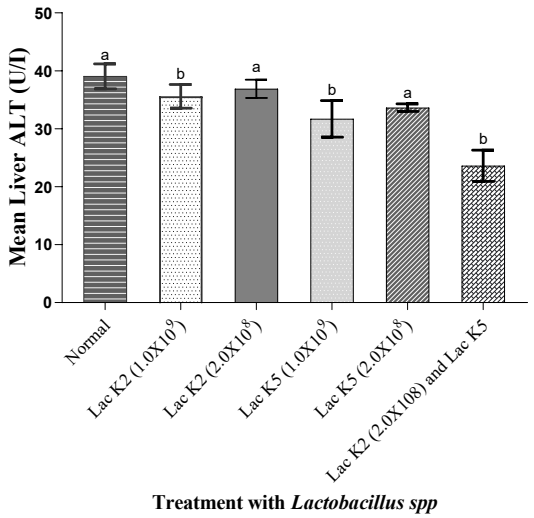
The result obtained from glucose level during the first week of treatment among the groups fed with probiotic ranged from (90.80-102.80 mg/dl), while the control group had a mean glucose level of (102.20mg/dl) (Table 5). However, at the end of the treatment period, the fasting glucose level of all the groups increased, except for the combined group (Group F) and Group E. The control group had a mean glucose level of 105.40mg/dl. In contrast, the tested groups (Group B, C, D, E, and F) fasting glucose level ranged from (95.40 - 105.00 mg/dl). However there was no significant difference in the fasting glucose level of the treated groups and control. Meanwhile, most of the values were within the normal range of fasting glucose level (<100mg/dl).

Effect of Probiotics on Liver Function

The results obtained from the liver function tests are shown in Table 6. The activity of alanine transaminases (ALT) in the control group (Group A) was the highest (39.05±2.16 U/I) in comparison with the test groups, while the lowest ALT activity was the combined treatment (23.62±2.701 U/I). Generally, there was a significant decrease in the mean ALT values of group B, C and F when compared to the control. However, the combined treatment gave the highest significant decrease in the ALT activity (Figure 1).

GROUP+TRT. (cfu/ml)	AST (U/I)	ALT (U/I)	ALP (U/I)	TP (G/DL)	CREAT. (MG/DL)	ALB. (G/DL)	DBIL. (MG/DL)
GRP. A (Normal Diet)	39.11±2.14[a]	39.05±2.16[a]	63.76±3.067[a]	5.484±0.12[a]	5.075±0.32[a]	0.524±0.05[a]	0.3600±0.12[a]
GRP.B (1.0 x10^9 Lac A)	40.26±3.49a	35.59±2.049[a]	68.32±4.07[a]	5.560±0.10[a]	6.699±0.83[a]	0.488±0.07[a]	0.1700±0.06[a]
GRP C: (2.0 x10^8 Lac A)	23.95±3.97[b]	36.89±1.58[a]	75.92±6.30[a]	5.497±0.160[a]	11.17±2.55[a]	0.444±0.02[a]	0.1814±0.08[a]
GRP D: (1.0 x10^9 Lac B)	33.42±3.38[a]	31.72±3.153[a]	68.35±2.78[a]	5.596±0.03[a]	6.293±1.18[a]	0.476±0.09[a]	0.2592±0.11[a]
GRP E: (2.0 x10^8 Lac B)	19.32±2.31[b]	33.64±0.663[a]	76.59±6.49[a]	5.437±0.21[a]	11.51±1.62[a]	0.622±0.06[a]	0.1613±0.04[a]
GRP F: (2.0 x10^8 Lac A + 2.0 x10^8 LacB)	16.53±1.92[b]	23.62±2.701[b]	75.30±6.19[a]	5.488±0.08[a]	12.18±5.40[a]	0.376±0.04[a]	0.2678±0.03[a]

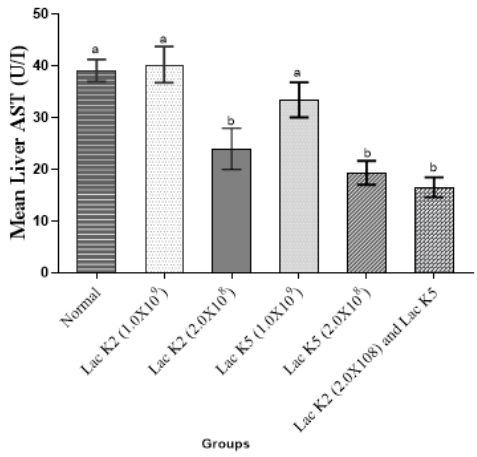
Table 6: Liver Function Tests.



a indicates no significant difference (p<0.05); b indicates significant difference (p<0.05)

Figure 1: Effect of probiotics on ALT activity in the liver.

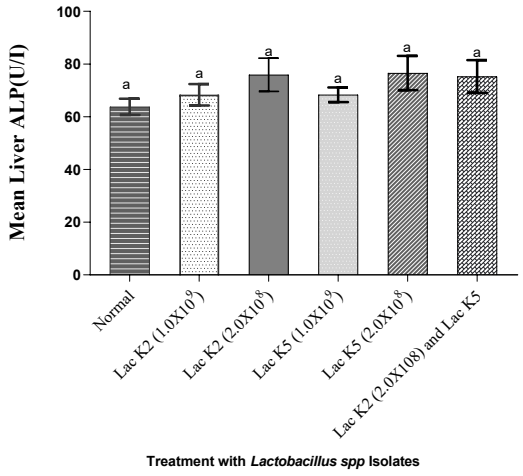
Similarly, in the activity of aspartate transaminase (AST), group B had the highest level of activity of AST (40.26±3.49 U/I), closely followed by the control group (39.11±2.14 U/I). While group F, the combined treatment group had the lowest AST activity (16.53±1.92 U/I). There was a significant decrease in the mean level of AST activity in group C, E and F, when compared with the control group. However, the combined treatment showed the highest significant decrease in the level of AST (Figure 2).



a indicates no significant difference (p<0.05)
b indicates significant difference (p<0.05)

The result obtained from the activity of alkaline phosphatase (ALP) ranged from (63.76±3.067 - 76.59±6.49 U/I). Group E

had the highest ALP activity, while the control group (Group A) had the lowest ALP activity. Generally, there was no significant decrease in the mean level of ALP activity in the test groups in comparison with the control group (Figure 3).



a indicates no significant difference ($p<0.05$); b indicates significant difference ($p<0.05$)

Figure 3: Effect of probiotics on ALP activity in the liver.

The result obtained from the Albumin level ranged from (6.699±0.83g/dl) in group B to (0.376±0.04g/dl) in the combined treatment group. The lowest level of albumin was obtained from the combined treatment group. However, between the control group and the test groups, there was no significant difference in the mean level of albumin (Figure 4).

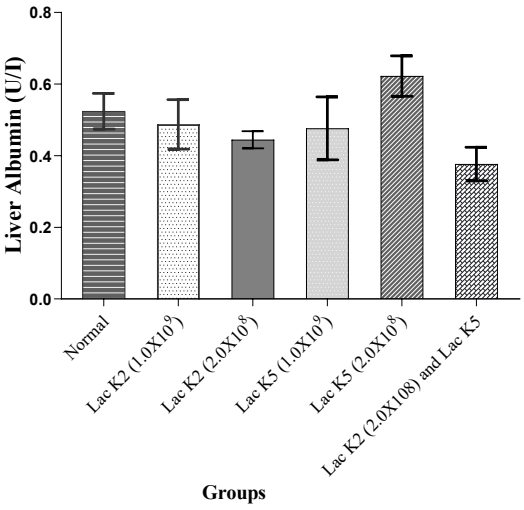


Figure 4: Effect of probiotics on albumin.

The result obtained from the direct bilirubin level ranged from (0.5155±0.13mg/dl) in the control group to (0.2678±0.03mg/dl) in the combined treatment group. The lowest level of direct bilirubin was obtained from the combined treatment group. However, in the control group and the test groups, there was no significant difference in the mean level of direct bilirubin (Figure 5).

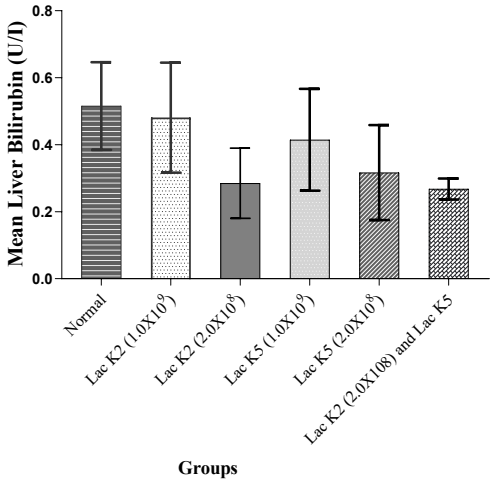


Figure 5: Effect of probiotics on direct bilirubin.

Antioxidant Profile

The result obtained from Malondialdehyde (MDA) shows that there was no significant difference in MDA activity in the liver, and kidney in groups administered with (1.0 x 109) of Lactobacillus K5 and (1.0 x 109) of Lactobacillus K5 when compared to the control group, which indicate that there was no lipid peroxidation (Figures 7 & 8).

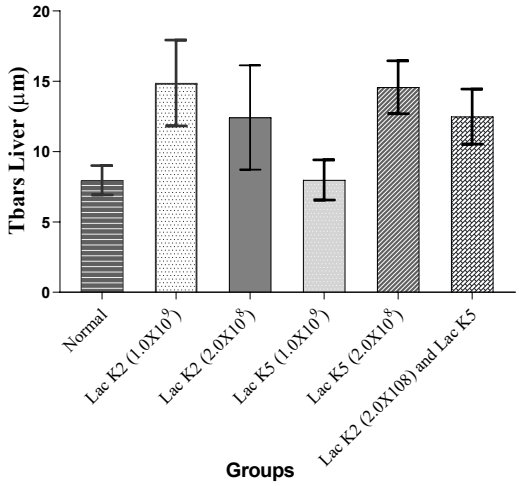


Figure 7: Antioxidant Profile (TBARS liver).

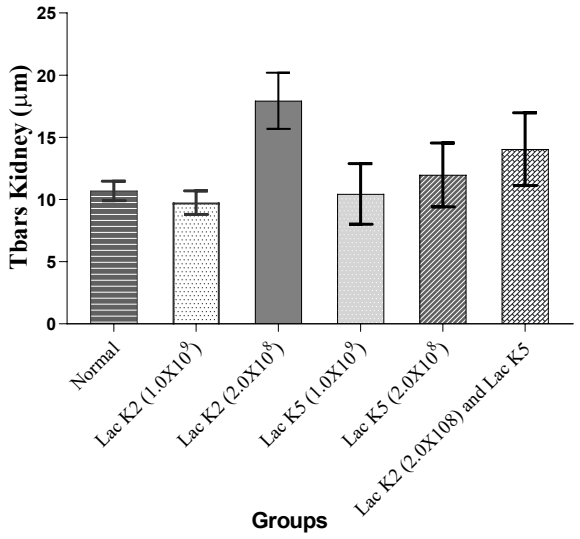


Figure 8: Antioxidant Profile (TBARS kidney).

The result obtained from the activity of superoxide dismutase (SOD) showed that there was a significant increase in SOD activity in the liver and kidney of the group administered with (1.0 x 10^9) of Lactobacillus K2 when compared to control. Other concentrations of Lactobacillus K2 and K5 showed no significant difference (Figures 9 & 10).

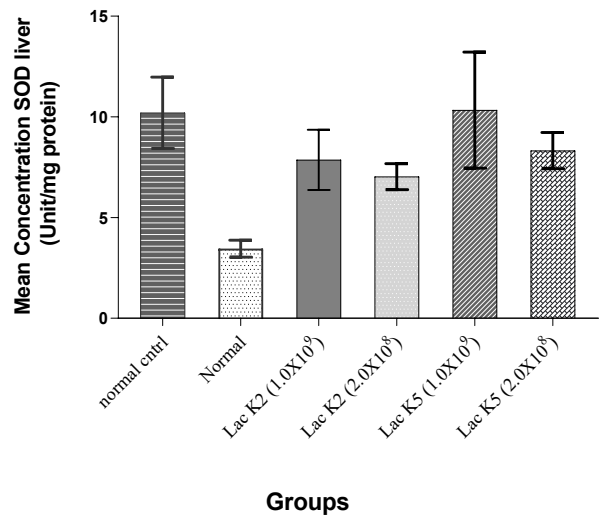
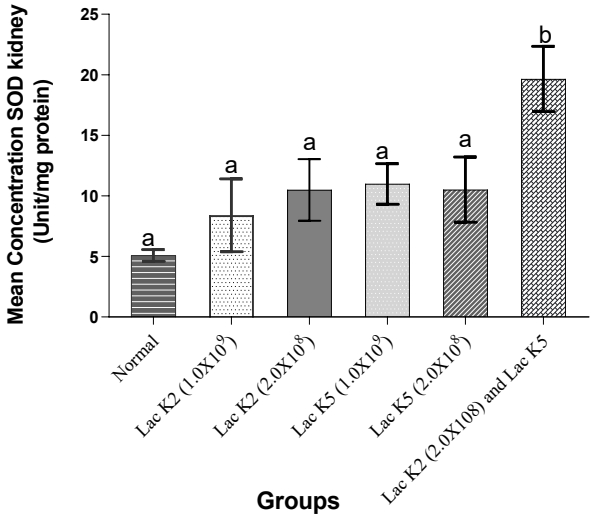


Figure 9: Antioxidant Profile Superoxide dismutase (Liver).



a indicates no significant difference ($p<0.05$); b indicates significant difference ($p<0.05$)

Figure 10: Antioxidant Profile Superoxide dismutase (Kidney).

The result obtained from Glutathione (GSH) activity showed that there was no significant difference in GSH activity in the liver and kidney of all the treatment group when compared with the control (Figures 11 & 12).

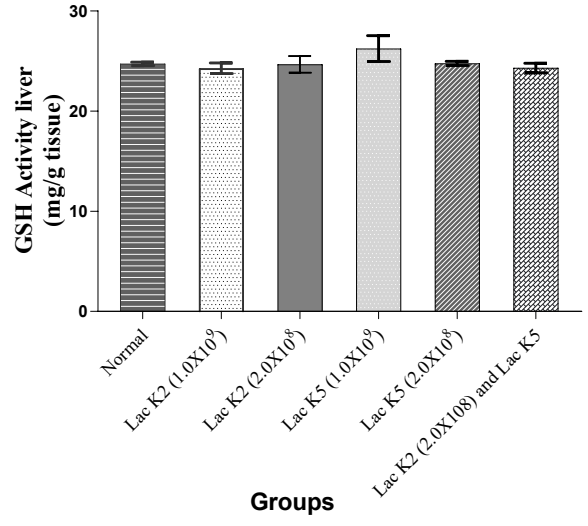


Figure 11: Antioxidant Profile Glutathione (Liver).

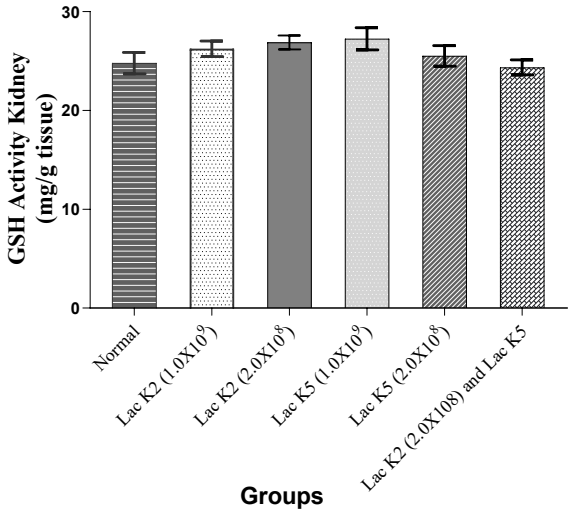


Figure 12: Antioxidant Profile Glutathione (Kidney).

Discussion

The intake of probiotic bacteria has shown various health benefits including maintenance of gut microbiota, immunomodulation, prevention and treatment of certain diseases such as diabetes, colon cancer and inflammatory bowel diseases. Fifteen lactic acid bacteria were isolated from the two fermented kinds of cereal (Ogi and Kunu) out of which seven were selected for in vitro probiotic characterization and two were eventually chosen for in vivo characterization. Several studies have reported the isolation of Lactobacillus species from fermented cereals [24,25].

For any microorganism to serve as probiotics, they should be able to survive the host GIT [1]. The in vitro selection criteria include the ability to grow in an acidic condition, tolerance to NaCl, growth at a different temperature, tolerance to bile salt, haemolysis test and antibiotic susceptibility test. In this study, only Lactobacillus K2 and K5 retained their survival rate after 3 hours of exposure to acidic environment. This is in accordance with earlier studies [26,27]. Similar to this present study, it has been demonstrated that Lactobacillus species isolated from fermented olives showed tolerance to pH 2.0 for 2h [28]. Other studies have also revealed that Lactobacillus species isolated from fermented cereals were found to be tolerant to low acid condition after incubation for 6h. However, there was a significant decrease in the survival rate of Lactobacillus species at low pH in another study [29]. The incubation period of 3 hours was used in tolerance to low pH because it replicates the residence time in the human stomach [30]. It is important to note that the survival rate of probiotics is also influenced by the buffering activity of food components [31]. In this present study, the survival rate of all the isolates in acidic condition was good. Isolate K2 and K5 show the highest viability in acidic condition.

Tolerance to bile salt is regarded as one of the essential criteria for colonization of the gut by probiotic strains. For a strain to serve as a probiotic, it must survive the bile salt concentration in the gut. Approximately about 1.0 litre of bile is released from the liver into the small intestine every day [32]. Bile salt is more poisonous to probiotics than low acidic condition [33]. In this study, Lactobacillus K7 showed high viability to 0.3% bile salt, while other strains had a decline in growth. Generally, at 0.1% bovine bile salt, all isolates showed high survival rates, while Lactobacillus K2 had the highest survival rate. The ability to resist bile salts is because they can de-conjugate bile salts which have been associated with the capacity of probiotics to extract cholesterol from the intestinal tract.

Antibiotic susceptibility testing has been classified as an essential safety characteristics and probiotic features to be examined when selecting probiotic bacteria. This is to prevent the emergence of multidrug-resistant pathogens, which is caused by the transmission of antibiotic resistance genes to pathogenic organisms in the gastrointestinal tract [1]. Isolate K2 and K5 were resistant to most antibiotics used in this study but were susceptible to gentamicin, chloramphenicol, and penicillin (Table 3). One of the essential probiotics properties is the safety for human consumption without possession of transferrable antibiotic resistance genes [5]. Probiotic bacteria are naturally resistant to some antibiotics which are usually intrinsic and non-transmissible to other pathogens. The use of antibiotics can cause a microbial imbalance in the gastrointestinal tract if they are able to destroy the microbial flora.

The faecal microbial count showed that the administered Lactobacillus isolates (K2 and K5) were able to survive the gastrointestinal tract of Albino Wistar rats. This confirmed the previous results on tolerance to low pH, tolerance to bile salts and tolerance to NaCl. The faecal microbial count of the combined group (Group F) and group A had the highest microbial count of 6.0 x106 CFU/ml while the normal control group which was fed with normal saline and basal diet had 1.0x106 CFU/ml. The result obtained from this study revealed that Lactobacillus species isolated from Nigerian fermented cereals (Ogi and Kunu-zaki) survived the gastrointestinal tract of Albino Wistar rats which is in accordance to other studies [2,5].

From this study, there was no significant difference in the mean weights of the test groups when compared with the control. This result is in accordance with other findings that observed an increase in weight [5]. The increase in the weight of the animals could be as a result of the

improvement in the digestibility and absorption of nutrients in the gastrointestinal tract of the rats due to the probiotic treatment.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are liver function biomarkers. Their increase in the general circulation of the body indicates liver problems. ALT is more specific for the liver while AST can be found in other organs such as the heart and kidney [34]. In this present study, these two enzymes activities were used to examine the normal functioning of the liver and kidney of Albino Wistar rats after the treatment period. The result obtained from this study showed a decrease in ALT and AST in the treated animals. This results is in accordance with the study carried out on male birds which demonstrated that as a result of probiotic treatment there was reduction in AST activity when compared with the control [35].

Alanine phosphatase (ALP) activity is also one of the vital liver function biomarker and an increase in ALP activity implies liver dysfunction, liver inflammation and blockage of bile ducts. This study does not report a difference in the mean level of ALP activity when compared with the control.

Bilirubin is a waste product from the breakdown of red blood cells in the liver. The increase in bilirubin level indicates that the inability of the liver to excrete bilirubin through the bile ducts [36]. There was no difference in the mean level of direct bilirubin in the treated groups when compared with the control group.

Albumin is a protein synthesized in the liver which carries various vitamins, hormones and enzymes throughout the body. The decrease in the level of albumin indicates the inability of the liver to produce the albumin protein [37]. The result obtained in this study showed no difference in the mean albumin level of the treated group when compared with the control group.

Creatinine is a waste product derived from the breakdown of creatine compound in the muscles and kidney. The result obtained from the level of creatinine in this present study showed no significant difference in the treated group when compared with the control group.

Oxidative stress is the imbalance of free radicals and antioxidants in the body, which usually leads to cell, tissue or DNA damage. Oxidative stress occurs when the release of free radicals in the body is more than the antioxidant [38]. Free radicals are unstable molecules in the body such as superoxide, reactive oxygen species and some nitrogen compounds [38]. At the same time, antioxidants are substances that help to prevent cell and tissue damage by interacting with free radicals. In this present study, the oxidative stress biomarkers such as Superoxide dismutase (SOD), Glutathione (GSH) and lipid peroxidation (malondialdehyde) were examined.

SOD is the enzyme that catalyzes the breakdown of superoxide radicals into an oxygen molecule and hydrogen peroxide and the result obtained from this study indicated that there was an increase in the mean activity of SOD in the combined treatment group when compared with the normal group. However, there was no difference in the level of GSH and MDA in the treated groups in comparison to the control.

Conclusion

In this study, the combined treatment of the two Lactobacillus species had the highest beneficial effect on the Albino Wistar rats. The result obtained from this study shows that Nigerian fermented cereals (Ogi and kunu-zaki) possess probiotic Lactobacillus species which are beneficial to human health and can be used in the management of metabolic disorders. However, further research still needs to be conducted to enhance the bioavailability of these organisms in ready-to-eat foods.

Conflict of interest

There is no conflict of interest.

Acknowledgement

We acknowledge all the laboratory staff for their contributions to the success of this study.

References

1. WHO (2004) Surveillance programme for control of food-borne infections and toxications in Europe 8th report 1999-2000, Country reports: Turkey.

2. Iyer R, Tomar SK, Kapila S, Mani J, Singh R (2010) Probiotic properties of folate producing Streptococcus thermophilus strains. Food Res 43(1): 103-110.

3. Bautista Gallego J, Arroyo Lopez FN, Rantsious K, Jimenez Diaz R, Garrido Fernandez A (2013) Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. Food Research International 50(1): 135-142.

4. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN (2012) Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 307(18): 1959-1969.

5. Okafor CA, Umeh, CN (2013) Studies on the Probiotic Properties of Some Lactobacillus Species Isolated from

Local Raw Cow Milk. Asian Journal of Biological Sciences 6(6): 277-291.

6. Kirpich IA, Solovieva NV, Leikhter SN, Natalia AS, Pavel IS, et al. (2008) Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: A Pilot Study. Alcohol 42(8): 675-682.

7. Ferri M, Serrazanetti DI, Tassoni A, Baldissarri M, Gianotti A (2016) Improving the functional and sensorial profile of cereal-based fermented foods by selecting Lactobacillus plantarum strains via a metabolomics approach. Food Research International 89(3): 1095-1105.

8. Ishida FK, Sato R, Goto S, Xiao PY, Hiroshi K, et al. (2007) Prevention of pathogenic Escherichia coli infection in mice and stimulation of macrophage activation in rats by an oral administration of probiotic Lactobacillus casei I-5. Biosci Biotechnol Biochem 71(4): 866-873.

9. Adisa AM, Enujiugha VN (2020) Microbiology and Safety of Ogi Fermentation: A Review. European Journal of Nutrition & Food Safety 12(5): 90-100.

10. Ogunremi OR, Banwo K, Sanni AI (2017) Starter-culture to improve the quality of cereal-based fermented foods: trends in selection and application. Current Opinion in Food Science 13: 38-43.

11. Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, et al. Bergey's Manual of Systematic Bacteriology. pp: 4.

12. Chou LS, Weimer B (1999) Isolation and characterization of acid-and bile-tolerant isolates from strains of Lactobacillus acidophilus. J Dairy Sci 82(1): 23-31.

13. Chen LS, Ma Y, Maubois JL, He SH, Chen LJ, et al. (2010) Screening for the potential probiotic yeast strains from raw milk to assimilate cholesterol. Dairy science & technology 90(5): 537-548.

14. Zhang S, Ren L, Li Y, Wang J, Yu W, et al. (2014) Bacteriology and drug susceptibility analysis of pus from patients with severe intra-abdominal infection induced by abdominal trauma. Experimental and therapeutic medicine 7(5): 1427-1431.

15. Vlková E, Rada V, Popelářová P, Trojanová I, Killer J (2006) Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Livestock Science 105(1-3): 253-259.

16. Dubey D, Rath S, Sahu MC, Rout S, Debata NK, et al. (2013) A report on infection dynamics of inducible clindamycin resistance of Staphylococcus aureus isolated from a teaching hospital in India. Asian Pac J Trop Biomed 3(2): 148-153.

17. Batista Souza CR, Reis SP, Castelo BJ, Carvalho L (2015) An overview of protein identification studies in cassava. Curr Protein Pept Sci 16(3): 219-227.

18. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2): 351-358.

19. Nishikimi, Rao N, Yapi K (1972) The occurrence of superoxide anion in the reaction of reduced phenozine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46(2): 849-853.

20. Prins GK, Loose JA (1969) GLUTATHIONE. Chapter 4 Biochemical methods in red blood cell genetics. Academic Press, N.Y.D. London, pp: 126-129.

21. Reitman S, Frankel SA (1957) Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28(1): 56-63.

22. Schmidt E, Schmidt FW (1963) Determination of serum GOT and GPT. Enzyme Biol Clin 3(1).

23. Englehardt A (1970) Measurement of alkaline phosphatase. Aerztl Labor 16: 42.

24. Adesulu Dahunsi AT, Jeyaram K, Sanni AI, Banwo K (2018) Production of exopolysaccharide by strains of Lactobacillus plantarum YO175 and OF101 isolated from traditional fermented cereal beverage. Peer J 6: e5326.

25. Mulaw G, Sisay Tessema T, Muleta D, Tesfaye A (2019) In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. International Journal of Microbiology.

26. Lee H, Yoon H, Ji Y, Kim H, Park H, et al. (2011) Functional properties of Lactobacillus strains isolated from kimchi. Int J Food Microbiol 145(1): 155-161.

27. Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, et al. (2010) Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. Food Control 21(5): 695-701.

28. Mourad K, Nour EK (2006) Physicochemical and microbiological study of "shmen", a traditional butter made from camel milk in the Sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. Grasas y Aceites 57(2): 198-204.

29. Guo W, Jia W, Li Y, Chen S (2010) Performances of Lactobacillus brevis for producing lactic acid from hydrolysate of lignocellulosics. Appl Biochem Biotechnol

161(1-8): 124-136.

30. Khalil R (2009) Evidence for probiotic potential of a capsular-producing Streptococcus thermophilus CHCC 3534 strain. Pol J Microbiol 58(1): 49-55.

31. Vinderola CG, Mocchiutti P, Reinheimer JA (2002) Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. J Dairy Sci 85(4): 721-729.

32. Begley M, Gahan CG, Hill C (2005) The interaction between bacteria and bile. FEMS Microbiology Reviews 29(4): 625-651.

33. Palencia PF, Werning ML, Sierra FE, Dueñas MT, Irastorza A, et al. (2009) Probiotic properties of the 2-substituted (1, 3)-β-D-glucan-producing bacterium Pediococcus parvulus 2.6. Appl Environ Microbiol 75(14): 4887-4891.

34. Ribeiro AJ, Yang X, Patel V, Madabushi R, Strauss DG (2019) Liver Microphysiological Systems for Predicting and Evaluating Drug Effects. Clin Pharmacol Ther 106(1): 139-147.

35. Babazadeh D, Vahdatpour T, Nikpiran H, Jafargholipour MA, Vahdatpour S (2011) Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (Coturnix japonica). Indian Journal of Animal Sciences 81(8):870.

36. Friedman LS, Martin P (2017) Handbook of Liver Disease E-Book. Elsevier Health Sciences.

37. Levitt DG, Levitt MD (2016) Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. Int J Gen Med 9: 229-255.

38. Pham Huy LA, He H, Pham Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 4(2): 89-96.