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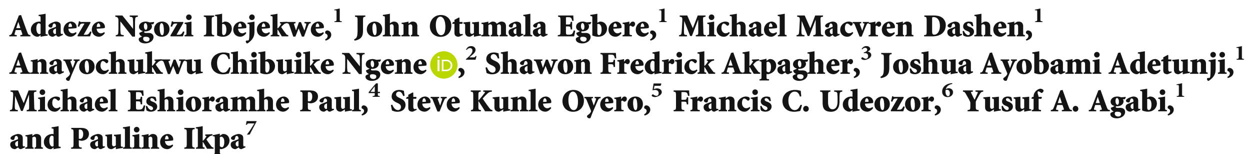
Research Article

Preliminary Investigations on the Therapeutic Efficacy and Safety of Mixed Probiotic Lactic Acid Bacteria on Albino Rats Challenged with *Shigella dysenteriae*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This study investigates the potential of lactic acid bacteria (LAB) from the Nigerian beverage “kunun zaki” as alternative therapeutic agents against *Shigella dysenteriae* infections. In light of rising antibiotic resistance, the decline in probiotic usage prompted interest in LAB’s role in countering bacterial dysentery. *Shigella dysenteriae*, a significant cause of dysentery in developing nations, prompted this research which aims to carry out a preliminary investigation on the therapeutic efficacy and safety of mixed probiotic lactic acid bacteria on albino rats challenged with *Shigella dysenteriae*. Lactic acid bacteria, known for treating infections, were isolated from the beverage and tested against *Shigella dysenteriae*. The study employed 15 albino rats for in vivo trials, inducing diarrhea and treating with *Lactococcus lactis* and *Lactobacillus brevis* separately (T1 and T2) and combined in a 1 : 1 ratio (T3). Clinical parameters were observed before and after treatment. This revealed that *L. lactis* and *L. brevis* administration lowered rectal temperature from an average of 42°C caused by infection to 36.5°C. Stool consistency improved from light brown loose to dark brown semisolid, signifying reduced diarrhea. Bacteriological analysis displayed significant reduction ($p < 0.05$) in *Shigella* counts in rat intestines across all treatments—$220 \pm 2.88\,CFU/g$ to $19.00 \pm 1.77\,CFU/g$, $8.33 \pm 0.88\,CFU/g$, and $65.00 \pm 2.88\,CFU/g$ for T1, T2, and T3, respectively. The mixed LAB treatment was notably effective. Lactic acid bacteria counts increased significantly in *Shigella*-treated rats versus the positive control. Hematology and liver function parameters showed no significant differences between treatments and untreated controls. Lactic acid bacteria from “kunun zaki” exhibited curative potential, individually or combined, against *Shigella dysenteriae*. These lactic acid bacteria also positively influenced gut microbiota in *Shigella*-infected albino rats.

1. Introduction

Diarrhea is one of the most common diseases that cause infant death in developing countries, it has been killing children for several decades, approximately 1.6 million death occurs each year, and it has attained an endemic status according to [1]. The burden of diarrhea illness sits firmly in the developing world, both for morbidity and mortality. Malnutrition, lack of portable water, sanitation, and hygiene highlight the stark inequalities that exist within our world

[2]. This illness is characterized by an increase in frequency and passage of loose (unformed) stools [3]. It is the second leading killer of children under five years. Diarrhea is caused by both infectious and noninfectious agents. Infectious diarrhea is caused by the consumption of pathogens. These pathogens include *Escherichia coli*, *Salmonella* species, and *Shigella species* [3]. Infection with *S. dysenteriae* usually progresses to the most severe form of dysentery with life-threatening complications especially with those whose immune system is weakened [1], and 30-60% of these patients develop diarrhea due to infection with enteric pathogens, *Shigella dysenteriae* and *E. coli* O157 inclusive [4]. The gut microbiota promotes effective digestion and fights infection. Antibiotics taken to address diarrhea-related problems may attack these healthy bacteria and further interfere with the intestine's ability to work properly [5]. There are an estimated 27,000 antibiotic-resistant *Shigella* infections in the United States each year. Anyone can get sick with antibiotic-resistant *Shigella*. Some people are more likely to get antibiotic-resistant infections, including international travelers, gay and bisexual men, and those with weakened immune systems like HIV and AID patients [6]. In an era of increasing antimicrobial resistance, an update on the appropriate empirical therapy for shigellosis in children and the immunocompromised persons is necessary, taking into account susceptibility patterns, cost, and risk of adverse events [7].

Probiotics are microorganism that are claimed to provide health benefits when consumed, which are considered generally safe but may cause bacteria host interactions mostly strains of *Lactobacillus* spp. [2]. Probiotic is defined as live microorganisms that, when administered in sufficient amounts, have a positive effect on the host's microbiota and benefit the health of the host [8]. Most probiotics are lactic acid bacteria generally referred to as LAB. Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, nonrespiring, non-spore-forming cocci or rods, which produce lactic acid as the major end product of fermentation [9]. *Lactobacillus lactis* and *Lactobacillus brevis* are some of the predominant microorganisms involved in the spontaneous fermentation of sorghum during "kunun zaki" production. These microorganisms are responsible for the production of lactic acid which account for the flavor and taste of "kunun zaki" [10]. In a study by [11], it was shown that LAB strains isolated from fermented cereal product—ogi and kunun zaki—have antimicrobial effect against diarrhea causing bacteria such as *Escherichia coli*, *Salmonella*, and *Shigella*. "Kunun zaki" is produced all year round, and its consumption is fast spreading from the northern to the southern part of Nigeria. It is also cheaper and more satisfying than the carbonated beverages to the low-income earners in the society [12]. [13] reported several beneficial activities of LAB which include immunomodulatory, antiallergic, antimicrobial, antihypertensive, and antitumorigenic effects. LAB also modifies the composition of intestinal microorganisms, thereby acting as deterrents for pathogenic enteric bacteria; thus, this necessitates the search for affordable, efficient, and effective LAB as a therapy against diarrheagenic organisms. On the part of economy, probiotics is one of the fast growing in the European economy; probiotics are an important concept for health care in the 21st century. The probiotic market is predicted to grow at a CAGR of 7.32% over the forecast period, from its current value of USD 63942.6 million in 2021–2022 to USD 97673.84 million by 2030. In Asia and Europe, probiotics are widely used as health foods and medicines. In the global probiotic market, the European market is the largest and the fastest growing with an average annual growth rate of around 20% (https://www.grandviewresearch.com/industry-analysis/probiotics-market, http://www.marketwatch.com/globalprobioticmarket).

The study is aimed at investigating the therapeutic efficacy of a mixture of probiotic lactic acid bacteria (LAB) on albino rats that are challenged with *Shigella dysenteriae*. *Shigella dysenteriae* is a bacterium that causes dysentery, a severe form of diarrhea. The researchers conducted experiments on albino rats, which were divided into different groups. One group served as the control, while the other groups were orally administered with the mixed probiotic LAB. The LAB mixture contained various strains of lactic acid bacteria known for their probiotic properties.

2. Materials and Methods

2.1. Morphological Identification of Lactic Acid Bacteria. The lactic acid bacteria used in this study was obtained from the Isolate Bank of the Molecular Biology Laboratory of the University of Jos. The test carried out will only confirm the LAB.

2.2. Gram Staining. This was a vital technique used to distinguish bacteria into Gram-positive, appearing purple due to retaining the primary stain (crystal violet), and Gram-negative, appearing pink due to retaining the counterstain [14]. The process involved the following steps, as described by [15].

First, a smear was prepared using a 24-hour-old bacterial culture. Next, a loopful of sterile distilled water was transferred onto a clean, grease-free glass slide, and a colony from a 24-hour-old culture was evenly spread to create a thin preparation. Subsequently, the smear was allowed to air dry, and the edge of the glass slide was passed over a Bunsen flame about three times to heat fix the specimen. Afterward, the primary stain, crystal violet, was applied to the smear and left for approximately 60 seconds. Following this, the stained smear was rapidly rinsed with distilled water. Gram's iodine solution was then added to the smear for another 60 seconds and washed with distilled water. The smear was further decolorized with 95% alcohol for 5 seconds and washed with water. Finally, the smear was counterstained with safranin dye for 60 seconds and washed with distilled water. The prepared smear was examined using a microscope equipped with a 100× oil immersion objective lens, allowing for detailed observation.

2.3. Biochemical Tests

2.3.1. Catalase Test. This test detects the presence of catalase enzymes, which convert hydrogen peroxide into water and oxygen. It helps distinguish lactic acid bacteria from other species based on their positive or negative reaction to hydrogen peroxide solution. A smear was created on a slide, and 3 drops of 3% hydrogen peroxide solution were added. The production of effervescent oxygen bubbles indicated a positive reaction [14].

Table 1: Identification of *Lactobacillus* species based on carbohydrate fermentation profiles using API 50 CHL database.

Lactobacillus sp.	API identification	ID (%)
Gb8 4b	*Lactobacillus brevis* 1	86
TM8	*Lactococcus lactis* ssp. *lactis 1*	71.9
GB3 4b	*Lactococcus lactis* ssp. *lactis 1*	93.8
GB2 5b	*Lactococcus lactis* ssp. *lactis 1*	79.8
GB3 4a	*Lactococcus lactis* ssp. *lactis 1*	71.3

ID: identity (%); the percentages following the scientific names of species represent the similarities from the computer-aided database of the apiweb™ API 50 CHL Vs. 1 software.

2.3.2. Sugar Fermentation Test. This test is aimed at identifying isolates to the species level. It involved observing the production of turbidity and gas from various carbohydrates (lactose, glucose, sucrose, fructose, maltose, and mannitol) [16]. To prepare, one gram of each sugar was dissolved in 50 ml of distilled water. The solutions were filtered using membrane filtration, and the filtered solutions were used for analysis. Nutrient broth was prepared by dissolving 3.3 grams of nutrient powder in 250 ml of distilled water, with the addition of 2.5 ml of phenol red. Subsequently, 8 ml of this broth was transferred into sterile test tubes, with Durham tubes inserted. They were autoclaved at 121°C for 15 minutes. After cooling, 2 ml of each filtered solution was added to the sterilized nutrient broth and inoculated with purified colonies under aseptic conditions. Incubation was carried out anaerobically at 37°C for 48 hours, and color changes were observed.

2.4. API Test: Identification of Lactic Acid Bacteria (LAB) by Using Analytical Profile Index (API) 50 CH Kit (bioMérieux). All isolates that were catalase negative and Gram-positive rods and cocci were further identified based on their carbohydrate fermentation profile using API 50 CH system. The procedure is as follows:

The isolates were harvested from MRS plate using sterile swab sticks and emulsified in two millimeters of sterile distilled water until a thick suspension is obtained. Two millimeters of the isolate suspension was drawn out with sterile pipettes and introduced in drop into test tubes containing five millimeters of sterile distilled water. The drops of the isolate suspension introduced into the five millimeters of sterile distilled water were counted, and turbidity of the suspensions was checked until it equals that of McFarland standard 2 (bioMérieux SA, Marcy-l'Étoile, France). Twice the number of drops of the isolate suspension that was equivalent to McFarland standard 2 was pipetted into ampoules of ten millimeters API CHL medium. The tiny holes on the incubation trays of the API 50 CH system were filled with sterile distilled water, and the API test strips were chronologically arranged in the trays. Sterile syringes were used to fill the 50 wells of the API test strips with the inoculum of the isolates contained in the API 50 CHL medium. All the wells were sealed with paraffin oil.

The trays were covered and incubated aerobically at 35°C for 48 h. The results were read after 24 h and 48 h and recorded in the result sheet provided. A change in color to yellow indicates a positive test while the color remains purple in a negative test. The results obtained were fed into the apiweb™ software which gave the identity of the isolate [17, 18].

All the identified isolates were preserved on MRS agar slants at 4°C and in MRS broth supplemented with 10% glycerol at -20°C.

Table 2: Antimicrobial efficacy of LAB on *Shigella dysenteriae*.

	Zones of inhibitions(mm)
LAB	*Shigella dysenteriae*
L. brevis	10.66 ± 0.67^{a}
L. lactis	12.33 ± 1.45^{b}

Means tagged with different alphabet letters under the same column are significant at $p < 0.05$.

Figure 1: Metabolic cage housing the albino rats.

2.5. Antimicrobial Assessment of LAB. Antimicrobial effects of the strains on diarrheagenic bacteria were determined by the agar diffusion method. The test bacteria, *Shigella dysenteriae*, were obtained from Jos University Teaching Hospital, Jos, Plateau State. Supernatants were collected from overnight grown probiotic cultures and were neutralized with 1N NaOH to pH 6.5. The neutralized supernatants of the strains of lactic acid bacteria species were checked for antibacterial activity against pathogenic bacteria inoculated into Mueller-Hinton agar (HiMedia, India); a bacteriocin of concentration of 10 mg/ml was used for comparison. A 50 ml of cell-free supernatants was filled into 5 mm diameter wells cut in the Mueller-Hinton agar. Once solidified, the plates were stored for 2 hours in a refrigerator. The inoculated plates were incubated for 24 hours at 37°C, and the diameter of the zone of inhibition was measured in millimeters [19].

2.6. Determination of In Vivo Effects of LAB against Test Microorganisms on Albino Rats. Pathogen-free albino rats within the age of 4-5 weeks were purchased from the animal house in the Faculty of Pharmacy, University of Jos, and the procedure as described by [20] will be followed. The acclimatization of the animal was for one week, with adequate food and water and suitable temperature and pressure. The rats were divided into treatment groups (T) and control groups. After 1 week of adaptive feeding, the albino rats in the treatment (T) groups were induced orally with 0.5 ml/10^6*E. coli* O157 for three days and the same process for

Table 3: Physical assessment on albino rats before Inducing with *Shigella dysenteriae*.

	Rectal temperature (°C)	Frequency of stool	Texture of stool	Feed consumed (g)	Inference
Positive control	36.0 ± 0.00	Normal	Brown and hard pellets	17.0 ± 0.00	No diarrhea
Negative control	36.6 ± 0.20	Normal	Brown and hard pellets	17.3 ± 1.20	No diarrhea
Treatment 1	36.5 ± 0.11	Normal	Brown and hard pellets	16.7 ± 1.75	No diarrhea
Treatment 2	36.5 ± 0.21	Normal	Brown and dark pellets	17.0 ± 1.70	No diarrhea
Treatment 3	36.8 ± 0.00	Normal	Brown and dark pellets	17.5 ± 1.52	No diarrhea

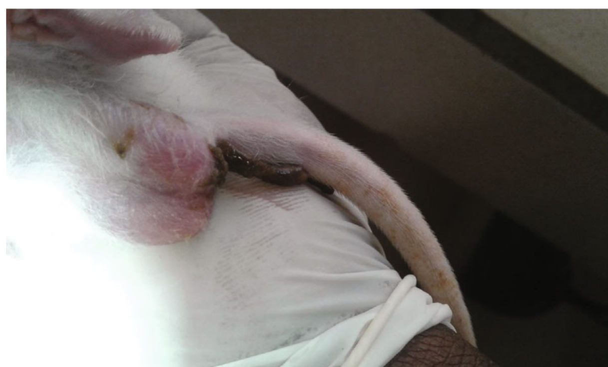

Figure 2: Loosed stool indicating diarrhea: increase in frequency in stooling.

Figure 3: Positive control (induced diarrhea).

Shigella dysenteriae. At the onset of diarrhea, lactic acid bacteria species tagged as LAB 1 (treatment 1), LAB 2 (treatment 2), and mixture of LAB 1 and 2 (treatment 3) were used as diarrheal intervention curatively at 0.5 ml/10^6. The control group was divided into two groups, the negative control group which was provided with adequate water and food and 0.9% sterile saline instead of bacteria and a positive control group, where the albino rats were infected with *Shigella dysenteriae* for three days without the LAB intervention until they were euthanized. There were three rats in each experimental group. All animal handling and experiment were approved by Animal Care Ethics, Department of Pharmacology, University of Jos, Jos, Nigeria. Rats were anesthetized by intraperitoneal injection of 20% arababital and sterile saline solution 1 : 3 *v*/*v* ratio [20]. Following anesthesia, cervical dislocation was used to kill the rats.

2.7. Collection of Stool Samples from Albino Rats. Fresh fecal samples were collected from the cage floor (immediately after defecation) of the rats, before treatment and after treatment with lactic acid bacteria [3].

2.8. Bacteria Analysis of Stool Samples. 1 g of mice fecal samples was added into 9 ml of MRS broth and incubated at 37°C under microaerophilic condition (CampyGen™ Oxoid, UK) for 24 hours, the culture was appropriately plated out on MRS agar (Oxoid, UK), and viable cells were counted. Distinct morphologically different colonies were picked from each plates and subcultured to obtain pure cultures. Gram-positive and catalase negative isolates were preserved in slant cultures. The same process was carried out in enumeration of Salmonella/Shigella and total plate count using MacConkey, Salmonella-Shigella agar (SSA).

2.9. Determination of Biochemical Parameters. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total bilirubin, and alkaline phosphate were determined calorimetrically according to the method described by [21]. The hematological analysis and full blood count analysis were carried out to determine the volume of blood cells present in the whole blood sample. Automated method of full blood count anticoagulated blood which is suckled through a narrow tube equipment. The equipment then counts the type of cells via two types of sensors: light detectors and electrical impedance.

2.10. Statistical Analysis. Results obtained were analyzed by simple percentage table and one-way analysis of variance (ANOVA). All data were expressed as mean ± SD ($n = 5$), and differences between groups were considered statistically significant at 95%.

Table 1 shows the API identification (analytical profile index (API) 50 CH kit, bioMérieux). The five lactic bacteria isolates were subjected to fifty sugars; GB2 5B had *Lactococcus lactis* and *Lactococcus lactis* scores of 79.8% and 19%, respectively; GB3 4B had *Lactococcus lactis* and *Lactobacillus brevis* scores of 93.8% and 6.0%, respectively; TM8 had *Lactococcus lactis* and *Lactobacillus brevis* scores of 71.9% and 27.8%, respectively; GB8 4B had *Lactococcus lactis* and *Lactobacillus brevis* scores of 86.6% and 13.1%, respectively.

Table 2 shows the antimicrobial efficacy of LAB on diarrheagenic bacteria. It was shown that there was a significant difference in the effect of LAB on diarrheagenic bacteria at $p < 0.05$. With respect to *Shigella dysenteriae*, *L. lactis* showed the highest inhibitory activity (12.33 ± 1.45 mm), followed by *L. brevis* (10.66 ± 0.67 mm) (Figure 1).

Table 4: Physical assessment of albino rats after inducing *Shigella dysenteriae* without lactic acid bacteria treatment.

	Rectal temperature (°C)	Frequency of stool	Texture of stool	Feed consumed (g)	Inference
Positive control	42.0 ± 0.60	More frequent	Light brown and semisolid	15.0 ± 0.00	Diarrhea
Negative control	36.6 ± 0.20	Normal	Brown and hard pellets	17.3 ± 1.20	No diarrhea
Treatment 1	45.5 ± 0.11	More frequent	Light brown and semisolid	16.7 ± 1.75	Diarrhea
Treatment 2	41.5 ± 0.20	More frequent	Light brown and semisolid	16.2 ± 1.70	Diarrhea
Treatment 3	39.8 ± 0.00	More frequent	Light brown and semisolid	15.5 ± 1.52	Diarrhea

Table 5: Physical assessment of albino rats after inducing with *Shigella dysenteriae* and treating with lactic acid bacteria.

Group	Rectal temp (°C)	Frequency of stooling	Texture of stool	Feed conversion ratio (g)	Inference
Negative control	36.8 ± 1.50	Normal	Brown and hard pellets	60.00	No diarrhea
Positive control	39.1 ± 0.77	More frequent	Light brown and loose	30.26	Diarrhea
Treatment 1	36.7 ± 0.73	Normal	Light brown and soft pellets	41.60	No diarrhea
Treatment 2	36.5 ± 0.90	Normal	Dark brown and hard pellets	44.90	No diarrhea
Treatment 3	36.9 ± 2.00	Normal	Dark brown and hard pellets	52.10	No diarrhea

Table 6: Bacteria analysis of stool samples before treatment and after treatment for *Shigella dysenteriae*.

Treatment	Coliform count	Total plate count	Salmonella/Shigella count	LAB count
Negative control (before)	239.67 ± 5.48	12.00 ± 1.15	230.00 ± 6.92	251.00 ± 6.35
Negative control (after)	323.00 ± 4.93*	136.00 ± 1.73*	220.00 ± 2.88	314.67 ± 2.40*
Positive control (before)	12.33 ± 2.03	10.00 ± 1.73	260.00 ± 5.77	47.67 ± 2.02
Positive control (after)	64.33 ± 3.17*	92.66 ± 1.45*	45.66 ± 2.60*	20.33 ± 2.33*
Treatment 1 (before)	14.33 ± 1.45	480.00 ± 5.77	380.00 ± 5.77	38.00 ± 2.30
Treatment 1 (after)	25.33 ± 4.66*	114.33 ± 2.33*	19.00 ± 1.73*	1290 ± 20.81*
Treatment 2 (before)	78.33 ± 3.51	260.00 ± 5.77	422.66 ± 37.77	67.00 ± 1.15
Treatment 2 (after)	184.00 ± 5.19*	268.00 ± 1.73*	8.33 ± 0.88*	103.33 ± 1.85*
Treatment 3 (before)	323.33 ± 6.01	241.33 ± 6.06	400.00 ± 6.92	342.00 ± 1.73
Treatment 3 (after)	162.33 ± 1.45*	1321.66 ± 4.41*	65.00 ± 2.88*	418.00 ± 1.15*

*Significantly compared to before treatment at 95% C.I.

Table 7: Percentage change in the bacterial load of stool samples of albino rats challenged with *Shigella dysenteriae*.

S/no.	Treatment	Coliform counts % increase	Total plate counts % increase	Salmonella/Shigella counts % decrease	LAB counts % increase	Inference
1	Positive control	26.00	91.18	40.35	20.10	Not improved
2	Negative control	81.25	89.21	82.44	57.35	Normal
3	Treatment 1 (T1)	43.43	decrease 76.25	95.00	97.00	Greatly improved gut
4	Treatment 2 (T2)	57.43	2.98	98.03	35.00	Normal
5	Treatment 3 (T3)	49.79	15.74	83.75	18.20	Not improved

Table 3 shows the physical assessment of albino rats before inducing with *Shigella dysenteriae*. The following physical parameters were evaluated on the five cages containing three albino rats each before inducing with *Shigella dysenteriae* (diarrheagenic organism): rectal temperature, frequency of stooling, texture of stool, and feed conversion ratio. It was observed across the groups that all examined parameters were normal (Figures 2 and 3).

Table 4 shows the physical assessment of albino rats after inducing with *Shigella dysenteriae* without lactic acid bacteria treatment. It was observed that all the groups had diarrhea after *Shigella dysenteriae* was administered. There was a sharp increase in rectal temperature across the group except for the negative control (36.5 ± 0.20) where others had 38.5 to 45 ± 0.11°C. Stooling became more frequent across the groups except for the negative control that had

Figure 4: Picture showing the blood sample collection for hematological and liver function test at the University of Jos animal house (Feb 2020).

normal frequency. Texture of stool across the groups changed from dark brown and hard pellets to light brown and semisolid except for the negative control group that retained its normal texture. There was a sharp decrease in the feed conversion ratio.

Table 5 shows the physical assessment of albino rats after inducing with *Shigella dysenteriae* and treated with lactic acid bacteria. It was observed that rectal temperature dropped to normal (36.8 to 36.5°C) across the group except for positive control group which remained at 39.1 ± 0.77. The frequency in stooling returned to normal except for the positive control which showed more frequency in stooling; the stool texture after treatment returned to normal from light brown and loose to brown and hard pellets/soft pellets except for the positive control which still remains light brown and very loose.

Table 6 shows the bacteria analysis of stool samples before treatment and after treatment for *Shigella dysenteriae*. It was shown that there was a significant difference observed across all parameters counted when compared to before and after treatment at $p < 0.05$. With respect to coliform counts, the negative control after treatment was significantly higher (323.00 ± 4.93) compared to the negative control before treatment (239.67 ± 5.48). The positive control after treatment was higher (64.33 ± 3.17) compared to that before treatment (12.33 ± 2.03). On T1, coliform count was higher after treatment (25.33 ± 4.66) compared to before treatment (14.33 ± 1.45). On T2, coliform count was also higher after treatment (184.00 ± 5.19) compared to before treatment (78.33 ± 3.51). There was switch on T3. Coliform count was higher before treatment (323.33 ± 6.01) compared to after treatment (162.33 ± 1.45). In respect to total plate count, negative control after treatment was significantly higher (136.00 ± 1.73) compared to negative control before treatment (12.00 ± 1.15). Positive control after treatment was higher (92.66 ± 1.45) compared to that before treatment (10.00 ± 1.73). On treatment 1, total plate count was higher before treatment (480.00 ± 5.77) compared to after treatment (114.33 ± 2.33). On T2, total plate count was higher after treatment (268.00 ± 1.73) compared to before treatment (260.00 ± 5.77). On T3, total plate count was higher after treatment (1321.66 ± 4.41) compared to before treatment (241.33 ± 6.06). For *Salmonella/Shigella* count, negative control before treatment had no significant difference (230.00 ± 6.92) compared to negative control after treatment (220.00 ± 2.88). The positive control before treatment was higher (260.00 ± 5.77) compared to that after treatment (45.66 ± 2.60). On T1, *Salmonella/Shigella* count was higher before treatment (380.00 ± 5.77) compared to after treatment (19.00 ± 1.73). On T2, *Salmonella/Shigella* count was higher before treatment (422.66 ± 37.77) compared to after treatment (8.33 ± 0.88). On T3, *Salmonella/Shigella* count was higher before treatment (400.00 ± 6.92) compared to after treatment (65.00 ± 2.88). With respect to LAB count, negative control after treatment was significantly higher (314.67 ± 2.40) compared to positive control before treatment (251.00 ± 6.35). The positive control before treatment was higher (47.67 ± 2.02) compared to that after treatment (20.33 ± 2.33). On T1, LAB count was higher after treatment (1290 ± 20.81) compared to before treatment (38.00 ± 2.30). On T2, LAB count was higher after treatment (103.33 ± 1.85) compared to before treatment (67.00 ± 1.15). On T3, LAB count was higher after treatment (418.00 ± 1.15) compared to before treatment (342.00 ± 1.73).

Table 7 shows the percentage change in the bacterial load of stool samples of albino rats challenged with *Shigella dysenteriae.* For treatment 1, there is a 95% decrease in Salmonella/Shigella counts, and treatment 2 shows 98.03% decrease in Salmonella/Shigella counts, while treatment 3 shows 83.75% decrease in Salmonella/Shigella counts (Figure 4).

Table 8 shows the hematological analysis before treatment for *Shigella dysenteriae* and after treatment for *Shigella dysenteriae*. It was shown that all hematological indices were compared similarly without any significant difference before and after treatment at $p > 0.05$, respectively.

Table 9 (liver function test) shows that the effect of treatment when compared to the negative control on aspartate aminotransferase, total plasma protein, and ALB was the same at $p > 0.05$. With respect to alkaline phosphatase, positive control (17.66 ± 3.7), T1 (48.33 ± 2.86), T2 (15.00 ± 4.04), and T3 (53.66 ± 1.85) all compared significantly were lower than the negative control (68.66 ± 2.83).

3. Discussion

Two *Lactic acid bacteria* species were identified as *L. lactis* and *L. brevis* from "kunun zaki." [22] isolated similar lactobacillus species from "kunun zaki." The API results identified *L. lactis* and *L. brevis* as the LAB with the highest identification percentage and thus were selected as the preferred probiotics for the study. [23] did a similar study on the isolation and identification of *Lactococcus lactis* and *Weissella cibaria* strains from fermented beetroot and an investigation of their properties as potential starter cultures and probiotics where the results obtained from the present study align with the above reports.

The antibacterial activity of *L. lactis* and *L. brevis* showed an optimal zone of inhibition against the pathogen *Shigella dysenteriae*. [24] demonstrated the antipathogenic properties of *Lactobacillus* strains against anaerobic pathogens of the digestive system. An increase in the rectal temperature was observed in all the rats after treatment with LAB for the period of five days. The increase in temperature could be as a result of the alteration caused by the

Table 8: Hematological analysis before treatment for *Shigella dysenteriae* and after treatment for *Shigella dysenteriae*.

Hematological parameters	Positive control	Negative control	Treatment 1	Treatment 2	Treatment 3
WBC (μl) before	7.50 ± 0.50	7.65 ± 0.55	10.05 ± 3.90	9.50 ± 2.50	9.65 ± 4.85
WBC (μl) after	7.55 ± 0.45	7.50 ± 0.50	8.57 ± 4.3	8.50 ± 1.40	9.75 ± 3.25
RBC (μl) before	7.35 ± 0.15	6.85 ± 0.25	7.10 ± 0.90	7.70 ± 0.30	7.50 ± 0.50
RBC (μl) after	5.10 ± 1.83	5.35 ± 0.85	5.93 ± 0.56	7.15 ± 1.35	6.34 ± 0.15
HCT before	38.05 ± 4.05	39.60 ± 0.60	32.4 ± 12.40	40.65 ± 1.65	42.75 ± 5.75
HCT after	34.45 ± 10.05	32.25 ± 7.80	38.27 ± 1.82	41.35 ± 2.45	41.2 ± 0.40
HGB before	11.80 ± 1.80	12.55 ± 1.55	12.25 ± 2.75	14.25 ± 0.75	12.15 ± 12.8
HGB after	9.20 ± 1.80	7.30 ± 1.19	10.97 ± 0.22	10.77 ± 0.77	11.23 ± 0.73
MCV before	58.30 ± 1.80	60.35 ± 2.35	61.40 ± 1.70	55.75 ± 5.75	62.10 ± 1.90
MCV after	67.10 ± 2.30	63.15 ± 1.15	66.97 ± 0.47	68.12 ± 0.87	66.85 ± 1.15
MCH before	18.90 ± 0.10	19.75 ± 2.25	17.90 ± 0.90	18.45 ± 6.55	19.50 ± 3.50
MCH after	19.85 ± 0.15	18.05 ± 1.65	19.90 ± 0.10	17.20 ± 2.80	19.75 ± 0.45
MCHC before	32.55 ± 0.55	31.55 ± 3.55	31.10 ± 2.10	29.40 ± 0.80	31.55 ± 1.45
MCHC after	29.60 ± 0.40	28.00 ± 2.20	29.65 ± 0.35	25.40 ± 4.90	29.40 ± 0.10
LYM before	59.45 ± 0.55	64.00 ± 1.00	63.25 ± 0.75	66.00 ± 1.00	59.25 ± 10.75
LYM after	79.20 ± 0.20	75.10 ± 0.20	75.95 ± 2.05	76.32 ± 1.62	68.35 ± 5.75
MXD before	5.75 ± 1.25	8.30 ± 0.50	6.30 ± 0.30	5.95 ± 0.05	6.35 ± 1.45
MXD after	6.50 ± 0.50	6.95 ± 0.05	4.85 ± 1.15	6.77 ± 2.92	7.90 ± 0.10
PLT before	271.00 ± 131.0	210.0 ± 30.0	200.0 ± 2.00	206.0 ± 74.0	218.0 ± 86.0
PLT after	205.50 ± 0.50	174.6 ± 29.3	205.2 ± 4.7	246.1 ± 20.1	221.8 ± 0.80
NEUT before	25.85 ± 4.15	22.50 ± 4.50	23.35 ± 0.35	21.50 ± 3.50	31.95 ± 5.95
NEUT after	28.65 ± 0.35	19.30 ± 1.10	22.00 ± 2.00	23.25 ± 0.15	29.25 ± 0.25

Significantly compared to before treatment at 95% C.I.

Table 9: Liver function test on experimental rats induced with *Shigella dysenteriae*.

Treatment	Aspartate amino transferase	Alanine amino transferase	Alkaline phosphatase	Total plasma protein	TBil	ALB
Negative control	13.33 ± 4.70	9.00 ± 2.30	27.66 ± 3.7	79.33 ± 3.13	4.33 ± 1.20	38.33 ± 3.8
Positive control	21.33 ± 1.74	12.33 ± 3.76	68.66 ± 2.83	107.00 ± 7.37	6.76 ± 4.12	44.66 ± 2.96
Treatment 1	12.66 ± 7.34	22.00 ± 5.03	48.33 ± 2.86*	103.33 ± 2.03	4.33 ± 3.95*	40.00 ± 5.50
Treatment 2	13.66 ± 0.66	8.66 ± 1.85	25.00 ± 4.04*	110.66 ± 5.67	5.00 ± 3.07*	41.66 ± 3.75
Treatment 3	12.00 ± 2.50	12.33 ± 4.09	53.66 ± 1.85*	98.66 ± 3.33	4.26 ± 0.81	40.00 ± 5.77

Means tagged with * under the same column is significantly compared to negative control at 95% C.I.

pathogenic *Shigella dysenteriae*. The frequency of stool and change in stool texture exhibited by the positive control group indicates *Shigella dysenteriae* infection on the groups. On feed consumption, the treated animals consumed more feeds than the positive control, showing that the animals recovered after treatment with lactic acid bacteria: *Lactobacillus lactis* and *Lactobacillus brevis*. This result affirms that several probiotic agents are able to inhibit the adherence of pathogenic bacteria to the intestinal epithelial cells through their ability to increase the production of intestinal mucins [11, 25, 26].

Studies have shown that the proportion of the intestinal microbiota differs between healthy albino rats and sick ones. The bacteria analysis of the albino rats stool samples shows microbial composition before and after treatment with LAB (see Table 5). The results showed that treatment with the mixed LAB had effects on the infected rats with *S. dysenteriae* ($4.0 \times 10^2 \pm 6.92$ CFU/g for *Shigella*/*Salmonella* count before treatment and 65.00 ± 2.88 CFU/g after treatment). Coliform count had $3.0 \times 10^2 \pm 1.0 \times 10^1$ CFU/g, thus a decrease of 83% and49%, respectively, and *L. lactis* (T1), *Lactobacillus brevis* (T2), and mixture of the two species

(T1 and T2) were all curative against *S. dysenteriae* and also restored the gut which shows $3.4 \times 10^2 \pm 1.0 \times 10^1$ CFU/g for before treatment and $4.2 \times 10^2 \pm 1.0 \times 10^1$ CFU/g for after treatment for T3. However, T1 had a better LAB increase in the gut with $3.0 \times 10^0 \pm 2.0 \times 10^0$ before treatment and $1.3 \times 10^3 \pm 2.0 \times 10^0$ after treatment for T1, thus increase of 18% for T3 and 97% for T1. To this effect, there was a better enrichment of the gut by the treatment as single than as mixed treatment. There are factors that could bring about the low performance of the treatments as mixed such as antagonism of the two organisms [22]. Meanwhile, the percentage of LAB count was significantly higher than the total plate count though the reason is not clear at this conventional stage of the study.

[27] in their study revealed the probiotic potential against induced *Shigellosis* in experimental rats which also agrees with the present study as LAB cures *shigellosis:* a life-threatening diarrhea.

It was also revealed that the intestinal flora of rats treated with mixed LAB was not enriched in LAB count, but better gut restoration was observed in *L. lactis* (T1) where *L. brevis* (T2) also showed restoration effect on the gut and cured the symptoms of *shigellosis* (causing diarrhea) infection improved after five days of administration This study agrees with [20] who carried out similar study in mice infected with *S. aureus*, treated with LAB. Increase in LAB count was observed possibly due to LAB colonization of the intestinal tract by *L. lactis* and *L. brevis.*

All parameters checked for hematology and liver function test had no significant difference with control. Hematological parameters are important indices of the physiological and pathological status for both animals and humans [28]. There was no significant difference between the pack cell volume and white and red blood cells before and after treatment when compared.

All parameters for liver function test had no significant difference with control, and all hematological indices were compared similarly without any significant difference before and after treatment at $p > 0.05$, respectively.

4. Conclusion

In the context of Nigeria, the traditional beverage "kunun zaki" has transcended its role as a mere thirst quencher. It is now recognized for its valuable probiotic attributes, holding potential not only for refreshment but also as a prophylactic and remedial agent within animal systems. This study has undertaken a comprehensive exploration of the therapeutic impacts of potent lactic acid bacteria, highlighting their probiotic potential and their capacity to foster improved gut health and mitigate dysbiosis in albino rats.

This revelation underscores the importance of fostering awareness among the public regarding the significance of producing "kunun zaki" within hygienic environments. This practice can ensure a higher intake of beneficial probiotics compared to potential pathogens. Encouraging the consumption of "kunun zaki" for its probiotic properties can contribute to enhanced well-being.

Notably, the research establishes the robust inhibitory influence of *L. lactis* and *L. brevis* strains isolated from "kunun zaki." In essence, this study advances our understanding of the therapeutic capabilities of "kunun zaki" through its probiotic components. Promoting the consumption of this traditional beverage underlines the potential to bolster animal health and address dysbiosis-related concerns. Further research endeavors, as outlined, will refine these insights, potentially paving the way for valuable contributions to both animal care and human health.

4.1. Recommendation. Based on the results of the current study, lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus brevis*, and mixed LAB) from kunun zaki had shown a notable difference in the composition and diversity of the intestinal microbiota of the albino rats before their treatment and after their treatment; thus, *L. lactis* cured *E. coli* O157:H7-induced diarrhea and also significantly enriched gut microbiota, suggesting that they could be potential probiotics for inclusion in the fermentation of beverages in their specific forms:

(i) *Lactococcus lactis* or/and *Lactobacillus brevis* should be one of the choicest probiotic

(ii) That mixed *L. lactis* and *L. brevis* should be preferred when gut enrichment in number and diversity is required

(iii) That further studies should be done on the modulatory effects of mixed *L. lactis* and *L. brevis* on gut microbiota using metagenomics sequencing so as to get better precision of its activity in the gut

(iv) To broaden the applicability and credibility of these findings, the authors recommend an extension of the study. This could involve employing antibiotics as a control, conducting experiments on a larger cohort of albino rats, and extending the treatment duration

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Adaeze Ngozi Ibejekwe was responsible for the conception, analysis, writing, and data acquisition. John Otumala Egbere was responsible for the supervision. Michael Macvren Dashen was responsible for the supervision. Shawon Fredrick Akpagher was responsible for the data analysis. Joshua Ayobami Adetunji was responsible for the data interpretation and acquisition. Michael Eshioramhe Paul was responsible for the analysis. Steve Kunle Oyero was responsible for the critical review. Francis C. Udeozor was responsible for the critical review. Yusuf Agabi was responsible for the

critical review. Pauline Ikpa was responsible for the design. Anayochukwu Chibuike Ngene was responsible for the data curation, writing, and critical review.

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