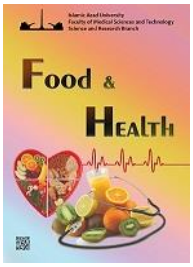

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In vivo assessment of probioticated African Yam Bean (*Sphenostylis stenocarpa*)-based milk analogue

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ABSTRACT

This study investigated the in vivo effect of administration of probioticated African Yam Bean (AYB) based milk analogues on albino rats. Vegetable milk extracts were obtained from processed African Yam Bean, Soybean Seeds, and Coconut. The samples were mixed at different ratios of 1:1:1, 3:1:1, and 5:1:1 (African Yam Bean: Soybean: Coconut) as A, B, and C, respectively. The blended milk analogues were fermented using *Lactobacillus delbrueckii* isolated from Kununzaki drink. The effect of the fermented milk analogues on the intestinal tract and the serum of the albino rats was also investigated. This strain inhibited the growth of *Escherichia coli*, a selected food-borne pathogen in vivo. Animals fed with only *E. coli* had the highest AST and ALT values of 79.31 and 24.59 IU/L respectively. Animals fed with sample B1 had the lowest ALT value of 16.24 IU/L. The weight gain was highest in animals fed with only probiotic drink sample. The histopathological examination showed the protective effect of the group dosed with the probiotic drink alone and the ones fed with a higher proportion of AYB. The study concluded that probioticated African yam bean drink exhibited health-promoting effect in vivo on the experimental animals and hence could be used as probiotic drink.

1. Introduction

Probiotic microorganisms are known to have health-promoting effects on their host after parenteral or oral administration (1). These probiotic microorganisms add to the intestinal microbial balance and contribute to sustaining health. Most commonly used are strains of the genera *Lactobacillus* and *Bifidobacterium*. Also, strains of *Bacillus*, *Pediococcus*, and some yeast have been added to these beneficial strains (2). Some of their valuable effects include prevention of intestinal infections, anticarcinogenic activities, control of serum cholesterol, enhancement of immunity, growth enhancement of animals (1, 3-5). Infectious agents like toxigenic *E. coli*, *Salmonella enteriditis*, *Entamoeba histolytica*, viruses, and antibiotics can result in gastrointestinal disorders (6). Both dairy and non-dairy products are the vehicles for probiotics administration. Usually, fermented dairy foods are the ideal food substrate for probiotics that are able to promote growth and enhance the viability of these organisms. Of these fermented foods, yogurt is the most popular, which gives greater amounts of protein, carbohydrate, calcium, and some B vitamins than milk (7). Some underutilized and under-exploited legumes may also be used as substrates for the production of non-dairy fermented foods. Adeniran *et al*. (8) produced and determined the viability of the probiotic organisms *in vitro* in a probiotic drink from milk blends of African Yam Bean (AYB), soybean, and coconut. Osundahunsi *et al*. (9) also determined the quality and consumer acceptability of soy-yoghurt with various fruit flavours. Ebhodaghe *et al*. (10) determined the physicochemical characteristics, viability, and inhibitory effect of Bifidobacterium in soymilk. Oyetayo *et al*., (1) assessed the safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* as probiotic agents and also the health-supporting potentials *Lactobacillus fermentum* and edible mushroom. Ikujenlola *et al*. (11) studied the chemical and sensory properties of probioticated drinks from milk analogues of African yam bean, soybean and coconut.

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These studies only evaluated the nutritional, physicochemical studies, and inhibitory effects of these probiotic drinks *in vitro*. Certain enzymes found in the liver, heart, pancreas, kidneys, red blood cells, and biliary ducts of the liver are used as biomarkers for liver injury. These include serum enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST). The levels of ALT and AST in serum are used to diagnose body tissues, especially the heart and the liver if it is injured or not (12). According to Hasan *et al*. (13), additional AST and ALT are released into the bloodstream and raise the serum enzyme level when body tissues are damaged. As a result, the levels of AST and ALT in the blood have a direct link with the amount of tissue damage. High AST and ALT ratio (>1.5) in acute viral hepatitis may give rise to severe condition (14). The *in vivo* assessment of the probiotic effect of these types of drinks have not been exhaustively dealt with. The ability of drinks to prevent damage or injury of the selected tissues/organs needs to be investigated. This study was aimed at eliciting some scientific information on the effect of probioticated drinks from the non-dairy product on the selected tissues/organs. The objective of the study, therefore, was to determine using *in vivo* method the effect of the probioticated African yam bean, soybean, and coconut milk analogues on the status of the gastrointestinal of the experimental animals.

2. Materials and methods

2.1. Material collection

The matured dried seeds of African yam bean (*Sphenostylis stenocarpa*), soybean (*Glycine max*), and coconut (*Cocos mucifera*) drupes were collected from the Institute of Agricultural Research & Training (IAR&T), Ibadan, Nigeria. *Lactobacillus delbrueckii* characterized and identified from *Kununzaki* drink produced in the Department of Food Science and Technology, OAU, Ile-Ife, Nigeria was used as the probiotic organism. The chemicals, AST, and ALT assay kits used were purchased from Sigma Aldrich, St. Louis, and Randox laboratory Ltd., UK respectively.

2.2. Methods

2.2.1. Preparation of the vegetable extracts

Milk extracts from the plant sources were obtained using the following procedures:

2.2.1.1. Preparation of African Yam Bean milk analogue

The seeds were carefully selected to remove extraneous materials. These were washed, soaked in warm water for 7 hours, drained and blanched for 5 min at 100 ^{o}C, dehulled, and milled with water (1:4) in a blender (Marlex, Excella model, India). The resulting slurry was filtered, kept for 5 min, and then boiled for 15 min with constant stirring and rapid cooling (15).

2.2.1.2. Preparation of soy milk

Wholesome and cleaned soybean seeds were processed by soaking in warm water to give a bean: water ratio of 1:3. After 6 h, the seeds were drained, rinsed with clean water, and blanched for 5 min at 100 ^{o}C, dehulled and milled with clean water. The resulting slurry was filtered through a muslin cloth and the extract/filtrate was pasteurized for 15 min with continuous stirring and rapid cooling (16).

2.2.1.3. Preparation of coconut milk

The back of the coconut flesh was scraped off and comminuted to enhance milling, blended with water in the ratio 2:1. The resulting solution was pasteurized at 90 ^{o}C for 10 min and refrigerated for 2 h at 0 ^{o}C. After freezing, coconut fat was scooped from the surface of the mixture. The remaining solution (coconut milk) was homogenized with coconut water for 2 min before use (17).

2.2.1.4. Formulation of milk blends

Milk analogues from African yam bean, Coconut, and Soybean were blended and formulated using the ratios of 1:1:1 (33.33% AYBM: 33.33% CM: 33.33% SM), 3:1:1 (60% AYBM: 20% CM: 20% SM), and 5:1:1 (71.43% AYBM: 14.29% CM: 14.29% SM), respectively. The milk samples were homogenized in a blender (Marlex, Excella model, India) for 2 min before use.

2.2.1.5. Selected milk blends fermented with probiotic strains

The milk blends were sterilized at 85 °C for 15 min and cooled to 45 ^{o}C in a water bath. *Lactobacillus delbrueckii* isolate was reconstituted by scrapping off 24 h old culture with a sterile loop into sterile distilled water. After proper shaking of the cloudy culture suspension, the concentration was standardized to 0.3 nm with a spectrophotometer (Spectrum α1506 plus, China) using the modified method of Mani-Lopez *et al.* (18). The milk blends were fermented/inoculated with 10 % (v/v) of *Lactobacillus delbrueckii* and incubated at 45±1 °C for 24 h. These fermented milk samples were refrigerated at 4 ±1 °C for an hour to stop the fermentation process, stirred using a sterile glass rod to break the curd formed, then bottled and stored as required before use (19). The flow chart used for the production of probioticated fermented milk samples from selected vegetable milk analogues is shown in Fig. 1.

2.2.2. *Experimental animal (in vivo) feeding procedure*

Forty (40) male Wistar rats (*Rattus norvegicus*) with an average weight of about 113.1 g were supplied by the Animal House, Faculty of Pharmacy, Obafemi Awolowo University,

Ile-Ife, Nigeria. The experimental animals were first acclimatized to the new environment in stainless steel metabolic cages with free access to drinking water and diet (UAC® Foods- growers' mash feed).

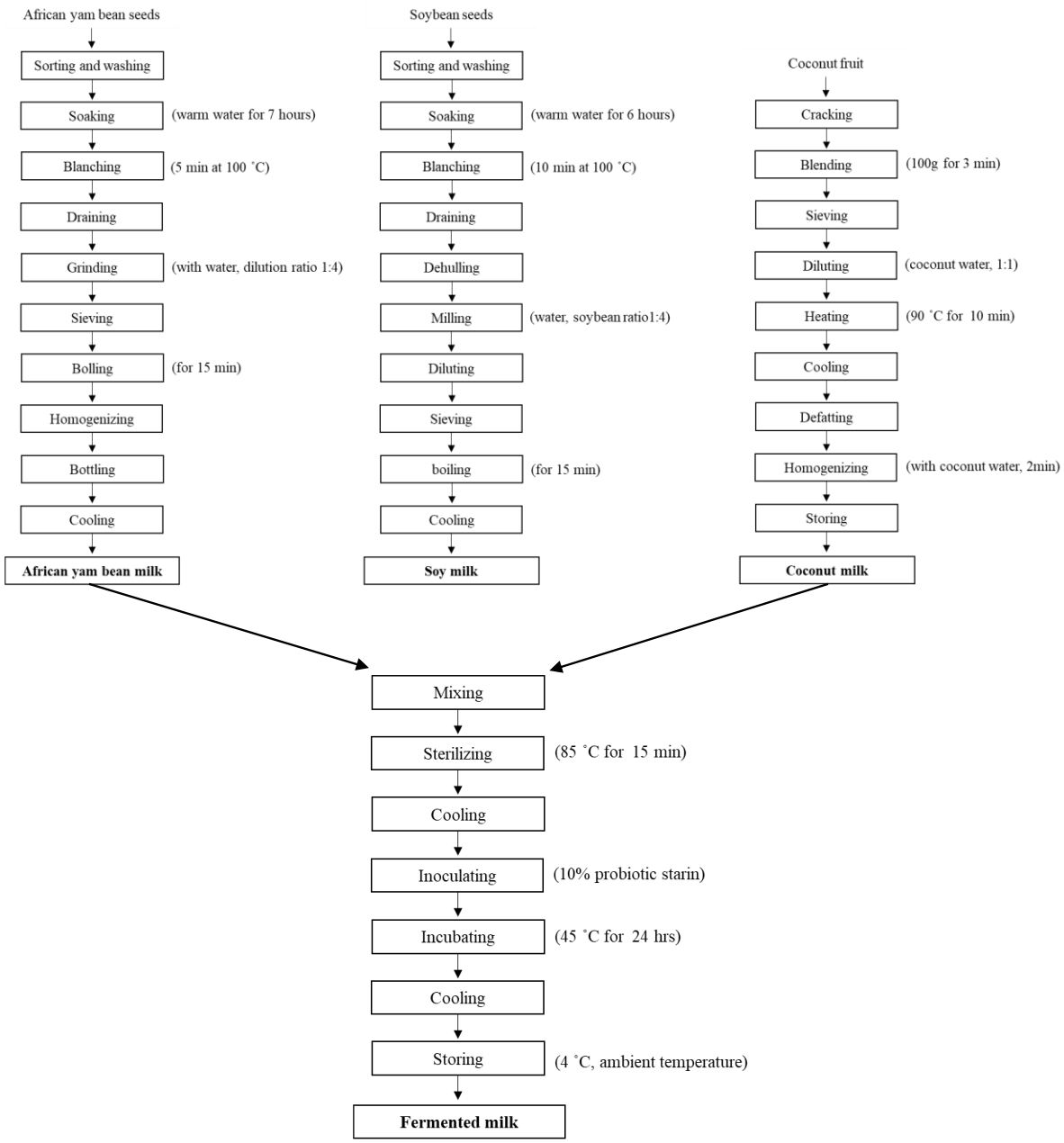

Fig. 1. The flow chart for the production of the probioticated (fermented) milk samples from vegetable milk analogues.

The room temperature was at 30±2 ◦C in a 12 h light/dark cycle. All the procedures/protocols were conducted under the guidelines and regulations approved by the Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria. The rats were distributed into seven groups of five animals per group. The groups were labelled A, B, C, D, E, F, and G each group received different treatments. Animals in Group A were inoculated with 0.3 ml of 10^5 CFU/ml of pathogenic *E. coli* and immediately dosed with 0.3 ml of 10^6 CFU/ml probiotic drink A1 (fermented 1:1:1 milk analogue). Group B animals were inoculated with 0.3 ml of 10^6 CFU/ml probiotic drink B1 (fermented 1:1:1 milk analogue). Group B animals were inoculated with 0.3 ml of 10^5 CFU/ml of pathogenic *E. coli* and immediately dosed with 0.3 ml of 10^6 CFU/ml probiotic drink B2 (fermented 3:1:1 milk analogue). Group C animals were inoculated with 0.3 ml of 10^5 CFU/ml of pathogenic *E. coli* and immediately dosed with 0.3 ml of 10^6 CFU/ml probiotic drink B3 (fermented 5:1:1 milk analogue). The animals in group D were inoculated with 0.3 ml of 10^5 CFU/ml of pathogenic *E. coli* only (no probiotic

drink added). This served as the negative control. Group E animals were fed only with the grower's feed. Group F animals were inoculated with 0.3 ml of 10^5 CFU/ml of pathogenic *E. coli* and immediately dosed with 0.3 ml of 10^6 CFU/ml of probiotic drink A1 (unfermented 1:1:1 milk analogue) while the animals in group G were dosed with 0.3ml of 10^6 CFU/ml of probiotic drink B1(fermented 1:1:1 milk analogue). Each group was supplied with 15g of the grower's feed with clean water every day throughout the experimental period. The feeding experiment lasted for 28 days during which growth, removal of fur, and some behavioral changes were monitored. The feed intake of individual animal and the individual body weight was recorded/monitored throughout the experiment. After the monitoring period, the animals were sacrificed by cervical dislocation and the blood samples of the rats were collected into EDTA bottles for analyses for serum biomarkers.

2.2.3. Biochemical Assay

"Assay kits (AST and ALT kits, Randox laboratory Ltd., UK) were used for the assessment of some major serum biochemical markers that can show the effects of the administered culture on the rat. The biomarkers assayed/assessed were aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the serum. A specified amount of each sample was automatically pipetted and applied on the test strip. The strip was inserted into the test chamber and the result was displayed after some seconds on the computer monitor. The activity of all serum enzymes was carried out using commercially available kits at 25 ºC, according to the manufacturer's instructions" (1, 20, 21).

2.2.4. Histopathological Analysis

"The histopathological test was carried out on the small intestines of the rats. The organ was fixed in 10% formaldehyde, dehydrated in increasing percentages of alcohol, cleared in xylene for 2 h for embedding. The embedded organ was sectioned using a microtome. The histopathological sections (3–5 µm) were stained with haematoxylin-eosin (H & E). The slides were coded and inspected by a single pathologist, who was unaware of the experimental conditions for each group. Sections were photographed directly using a stereo-microscope in 100 and 400 high power fields with Microsoft system" (6, 13). All the animal experiments were carried out according to the safety and ethics of Obafemi Awolowo University, Ile-Ife, Nigeria Animal Ethics Committee.

2.2.5. Statistical Analysis

The values that were obtained from each of the analyses are means of duplicate readings. The data obtained from biochemical analyses were subjected to analysis of variance (ANOVA) and the mean was separated by Duncan multiple range test (SPSS, version 20). Significance was determined at the 5 % level.

3. Results and discussion

3.1. In vivo feeding trial

The results on chemical composition and sensory characteristics of the probiotic drink had been reported in Ikujenlola *et al.* (11). Meanwhile, the results of the effect of the milk analogues (fermented and unfermented) and control samples on the bodyweight of the Wistar rats are presented in Fig. 2. The weights of animals in groups A, B, and C were higher than the weight gained by animals in group D but lower than those in groups E and G. This might be due to the effect of the probiotic drinks and the pathogenic *E. coli* administered to the rats. The weight gained by rats in group A was higher than that in group F but lower than that of group G. This was due to the probiotic effects of these drinks. In group G, the animals were fed with sample B1 (fermented 1:1:1 milk blends), the weight gain was higher than the control group E. In group C, the weight gained was less. This may be because there was a decrease in the viability of LAB in the drink B3 (fermented 5:1:1 milk blends) than in groups A and B. From this study, it can be inferred that as the level of African yam bean increased, the antimicrobial properties decreased.

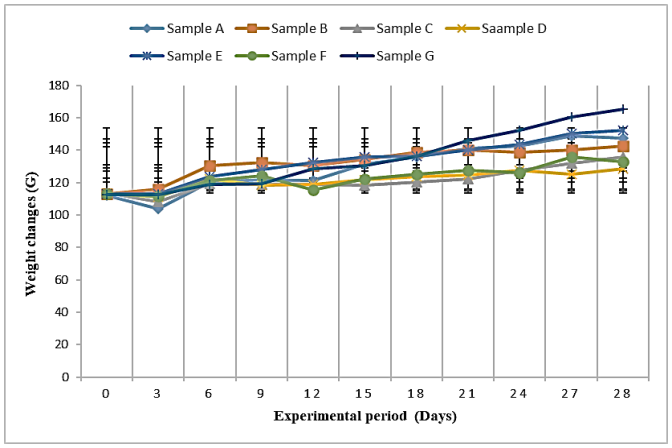

Fig. 2. Weight changes in rats during biological assessment of the probiotic drinks.
Keys: A=Group infected with *E. coli* and dosed with sample B1(fermented 1:1:1 AYB:SM:CM); B=Group infected with *E. coli* and dosed with sample B2 (fermented 3:1:1 AYB:SM:CM); C=Group infected with *E. coli* and dosed with sample B3 (fermented 5:1:1 AYB:SM:CM); D=Group dosed with *E. coli* only; E=Group fed with growers feed only; F=Group infected with *E. coli* and dosed with sample A1 (unfermented 1:1:1 AYB: SM: CM); G=Group dosed with sample B1 (fermented 1:1:1 AYB:SM:CM).

This agrees with the report of Oyetayo *et al.* (1) who observed an increase in weight when albino rats were fed with *L. acidophilus* and *L. casei* isolated from fresh cow milk in sterile water. The ability of the *Lactobacilli* to produce

antimicrobial metabolites such as hydrogen peroxide (H_2O_2), lactic acid, and bacteriocin has been suggested to be responsible for their ability to inhibit other bacteria (1, 22). Throughout the experiment, the albino rats appeared to be healthy, agile, and active. It was also observed in their food intake, weight gain, and general appearance. However, the rats in groups D and F were not as agile as other groups this suggests illness in the animals. There was no death recorded in any of the groups. The feces of rats in groups D and F were unformed, which might be an indication of diarrhea. They also exhibited falling furs and redness of the skin. These may all be symptoms of illness in the animals.

3.2. Biochemical Assay

Serum AST and ALT are the enzyme biomarkers to monitor the liver structural integrity and damage and aids in the clinical diagnosis of liver toxicity conditions (13). The effects of African yam bean-based milk analogues on the level of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are presented in Table 2.

Table 2. Biochemical assay of the serum.

Groups	AST (IU/L)	ALT (IU/L)
A	$67.72\pm2.15^{c*}$	22.41 ± 2.15^{ab}
B	61.83 ± 3.82^{c}	19.25 ± 2.90^{b}
C	53.74 ± 5.12^{d}	18.88 ± 2.58^{bc}
D	79.31 ± 1.75^{a}	21.78 ± 2.21^{ab}
E	52.36 ± 6.30^{d}	24.59 ± 3.51^{a}
F	73.40 ± 7.10^{b}	24.14 ± 2.15^{a}
G	49.38 ± 3.00^{e}	16.26 ± 4.85^{c}

*Means with different superscript within rows are significantly different at $p<0.05$

Keys: A=Group infected with *E. coli* and dosed with sample B1(fermented 1:1:1 AYB:SM:CM); B=Group infected with *E. coli* and dosed with sample B2 (fermented 3:1:1 AYB:SM:CM); C=Group infected with *E. coli* and dosed with sample B3 (fermented 5:1:1 AYB:SM:CM); D=Group dosed with *E .coli* only; E=Group fed with growers feed only; F=Group infected with *E. coli* and dosed with sample A1 (unfermented 1:1:1 AYB:SM:CM); G=Group dosed with sample B1 (fermented 1:1:1 AYB:SM:CM).

When there is an injury to the organs due to any reason, then these enzymes spill into the bloodstream and could be detected from blood samples. Therefore, AST and ALT levels in rat serum were examined. It was observed that the serum aspartate amino transferase (AST) activity of all rats treated with the fermented milk analogue and challenged with *E. coli* were higher in value and were significantly different ($p<0.05$) from the value for the control group (E). Rats in group A had an AST value of 67.72 IU/L while the value for group D (infected with only *E. coli*) was 79.31 IU/L. The lowest value of AST (49.38 IU/L) was observed in animals of group G, (animals fed with only the probiotic drinks; fermented 1:1:1 milk blends). As the proportion of AYB increased in the drink, the value of AST reduced. There was no significant difference ($p>0.05$) between the values of group C (animals fed with fermented 5:1:1 milk analogue) and the control group E (animals fed with growers' mash alone). Animals in group F (infected with *E. coli* and fed with unfermented 1:1:1 milk analogue) had an AST value of 73.40 IU/L. According to Hasan *et al*., (13), the reference ranges of AST and ALT are 50-150 and 10-40 IU/L (The AST values were within the range). The amount of AST and ALT in the blood is directly related to the amount of tissue damage (23, 24). The levels of AST and ALT in serum are used to detect body tissue injuries especially heart and liver tissues. *Lactobacillus* can translocate (25) and survive in the liver, spleen and lungs (1). In the case of their movement, they may cause the cellular injury that may cause an increase in the quantity of AST as observed in groups A, D and F. However, ALT (Alanine aminotransferase) was lowest in group G (animals fed with sample B1 fermented 1:1:1 milk analogue) when compared with other treated groups. ALT was highest in group F (animals infected with *E. coli* and fed with unfermented 1:1:1 milk analogue). Low values of ALT show liver function improvement likely brought about by *Lactobacillus delbrueckii* used to ferment the milk analogues. Serum alanine aminotransferase of the rats also showed that groups dosed with the fermented milk analogues and challenged with *E. coli* compared favorably with the control. ALT is mainly found in the liver and known to be more liver-specific than AST for detecting liver cell damage (1, 26). This study observed a reduction in the levels of serum AST and ALT in rats treated with probioticated drinks. This observation agrees with the findings of Tilg and Hotamisligi (27). The report indicates a significant decrease in serum ALT and AST is a result of protective effect on the liver by treatment with probiotics.

3.3. Histopathological findings

The results of the histology analysis are shown in Fig. 3 (a, b). It shows and confirmed the protective effect of the group dosed with the fermented milk analogue only and higher proportion of AYB (B3). In group A, the animals were dosed fermented milk analogue 1:1:1 (B1 and challenged with pathogenic *E. coli*, the villi are markedly eroded as a result of pathogenic infection and the intestinal wall is partially intact. In group B, the animals were infected with pathogenic *E. coli* and dosed with the fermented milk analogue 3:1:1 (B2), the villi and the wall are also eroded, but the degree of erosion was lower than the ones in group A. In group C, the villi were intact and directed towards the lumen. The probiotic drink B3 (fermented 5:1:1 milk analogue) conferred protection on the walls against pathogenic infection. In group D, the animals were infected only with pathogenic *E. coli*. The villi and the intestinal wall were markedly eroded. The goblet cells and the crypt of Lieberkühn were also observed to be shrunken. This signified great damage to the small intestine. In group E, the animals were fed only with growers' feed, the control, the crypt of Lieberkühn, the goblet cells and the walls were intact. In group F, the animals were fed with unfermented milk blends of ratio 1:1:1 and infected with *E. coli*. The slides showed that villi and goblet cells were markedly eroded. However, the intestinal wall was intact. In group G, the animals were fed with the probiotic drink (B1 fermented 1:1:1 milk analogue).

It was shown that the goblet cells, the crypt of Lieberkühn and the muscularis proporia were all intact. The probiotic drink conferred special protection on the lumen. The probiotic drink B3 (fermented 5:1:1 milk analogue) conferred protection on the intestinal walls against pathogenic infection. There are two stages in gastrointestinal pathogenic infection.

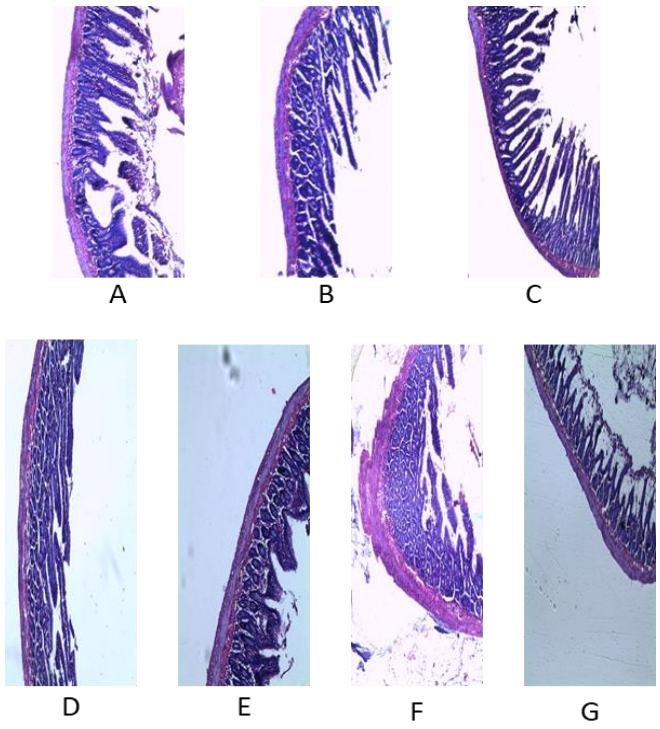

Fig. 3 (a). Histopathological section of the Small Intestine at Mag 100 using H&E stain.

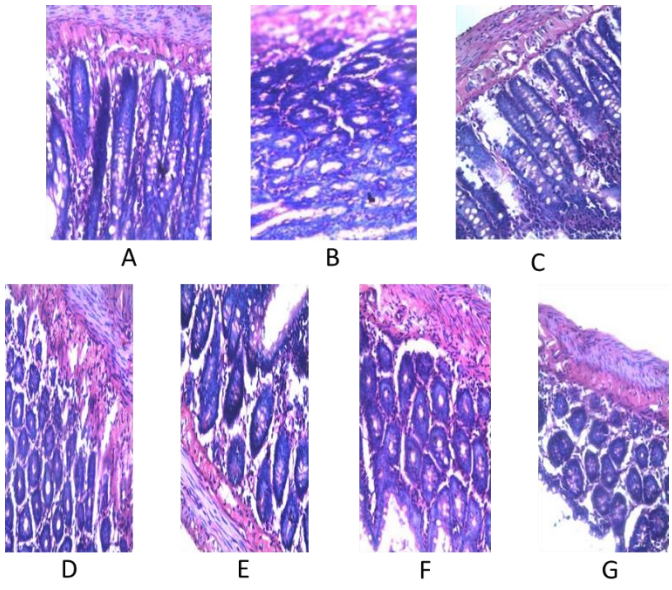

Fig. 3 (b). Histopathological section of the Small Intestine at Mag 400 using H&E stain.

At the first stage of the infection process, the pathogens adhere to the surfaces of intestinal epithelial cell structures which consist of glycoproteins and glycolipids. These serve as receptors for bacterial adhesion (28-30). The intestinal pathogenic effects of these pathogens disrupt epithelial barrier function and a loosening of the intestinal epithelium cells (31). These processes elevate the pathogenic or enterotoxic permeability of the mucosa wall. Probiotics are promoted for their ability to improve the intestinal barrier function by impeding the movement and adhesion of pathogenic bacteria to the intestinal epithelium (32). Nwachukwu *et al*. (33) showed in a rat model that the administration of *Bifidobacterium bifidum* may confer protection through the regulation of the main components of the mucous layer and improvement of intestinal integrity. Oyetayo *et al*. (1) established a protective effect on the small intestine of rat dosed with *L. acidophilus* isolated from fresh cow milk.

4. Conclusion

The study concluded that probioticated African yam bean-based drink exhibited probiotic effect *in vivo* on the experimental animals as evident in the prevention of the integrity of gastrointestinal tract (GIT) wall and the serum AST and ALT of the group of animals challenged with pathogenic *E. coli*. Lower values of both serum AST and ALT can be guaranteed with the use of probioticated drink from African yam bean blended with soybean and coconut milk analogues. Further work can be designed to look at the effect of the probioticated drink on the other organs.

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References

1. Oyetayo V, Adetuyi F, Akinyosoye F. Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent in vivo. *African Journal of Biotechnology*. 2003;2(11):448-52.
2. Soccol CR, Vandenberghe LPdS, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JDD, et al. The potential of probiotics: a review. *Food Technology and Biotechnology*. 2010;48(4):413-34.
3. Casas IA, Dobrogosz WJ. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial Ecology in Health and Disease*. 2000;12(4):247-85.
4. Bertazzoni M, Benini A, Marzotto M, Hendriks H, Sbarbati A, Dellaglio F, editors. Preliminary screening of health-promoting properties of new lactobacillus strain: in vitro and in vivo. HEALFO abstracts, Italy; 2001.
5. Chang CY, Stone MB. Effect of total soymilk solids on acid production by selected *Lactobacilli*. *Journal of Food Science*. 1990;55(6):1643-6.
6. Silva A, Bambirra E, Oliveira A, Souza P, Gomes D, Vieira E, et al. Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* subsp. *typhimurium* in conventional and gnotobiotic mice. *Journal of Applied Microbiology*. 1999;86(2):331-6.
7. Yilmaz-Ersan L, Kurdal E. The production of set-type-bio-yoghurt with commercial probiotic culture. *International Journal of Chemical Engineering and Applications*. 2014;5(5):402.

8. Adeniran H, Olaniyi O, Abiose S. Viability and effect of' probiotic' lactic acid bacteria isolated from locally fermented foods in a non-dairy beverage from African Yam Bean. *Ife Journal of Technology*. 2016;24(1):11-7.
9. Osundahunsi O, Amosu D, Ifesan B. Quality evaluation and acceptability of soy-yoghurt with different. *American Journal of Food Technology*. 2007;2(4):273-80.
10. Ebhodaghe S, Abiose S, Adeniran H. Assessment of physico-chemical characteristics, viability and inhibitory effect of *Bifidobacteria* in soymilk. *Journal of Food Research*. 2012;1(2):159.
11. Ikujenlola AV, Adurotoye EA, Adeniran HA. Chemical and sensory properties of probioticated drinks from blends of African yam bean, soybean and coconut milk analogues. *Acta Universitatis Cibiniensis Series E: Food Technology*. 2019;23(2):147-56.
12. Kasarala G, Tillmann HL. Standard liver tests. *Clinical Liver Disease*. 2016;8(1):13.
13. Hasan KMM, Tamanna N, Haque MA. Biochemical and histopathological profiling of Wistar rat treated with *Brassica napus* as a supplementary feed. *Food Science and Human Wellness*. 2018;7(1):77-82.
14. Botros M, Sikaris KA. The de ritis ratio: the test of time. *The Clinical Biochemist Reviews*. 2013;34(3):117.
15. Aminigo E, Lehtola P, Metzger L. Nutritive composition and physical characteristics of supplemented limitation milk from African yam bean (*Sphenostylis stenocarpa*). *Global Journal of Pure and Applied Sciences*. 2007;13(4):457-62.
16. Udeozor LO. Tigernut-soy milk drink: preparation, proximate composition and sensory qualities. *International Journal of Food and Nutrition Science*. 2012;1(4):18-26.
17. Sanful RE. The use of tiger-nut (*Cyperus esculentus*), cow milk and their composite as substrates for yoghurt production. *Pakistan Journal of Nutrition*. 2009;8(6):755-8.
18. Mani-López E, Palou E, López-Malo A. Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. *Journal of Dairy Science*. 2014;97(5):2578-90.
19. Amakoromo E, Innocent-Adiele H, Njoku H. Physicochemical quality of a yoghurt-like product from African yam bean. *Report Opinion*. 2012;4(4):58-61.
20. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28(1):56-63.
21. Schmidt E, Schmidt F, Möhr J. An improved simple chromatographic method for separating the isoenzymes of malic dehydrogenase and glutamic oxaloacetic transaminase. *Clinica Chimica Acta*. 1967;15(2):337-42.
22. Juven B, Schved F, Lindner P. Antagonistic compounds produced by a chicken intestinal strain of Lactobacillus acidophilus. *Journal of Food Protection*. 1992;55(3):157-61.
23. Rochling FA. Evaluation of abnormal liver tests. Clinical cornerstone. 2001;3(6):1-12.
24. Ode O, Asuzu O, Oladele G. The biochemical changes in rats following chronic toxicity with *Cassia singueana* leaf extract. *Journal of Pharmaceutical and Biomedical Sciences*. 2011;8:1-4.
25. Berg RD. Translocation of indigenous bacteria from the intestinal tract. *Human Intestinal Microflora in Health and Disease*. 1983;15:333-52.
26. Johnston DE. Special considerations in interpreting liver function tests. *American Family Physician*. 1999;59(8):2223.
27. Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: cytokine-adipokine interplay and regulation of insulin resistance. *Gastroenterology*. 2006;131(3):934-45.
28. Weinstein DL, O'Neill BL, Hone DM, Metcalf ES. Differential early interactions between *Salmonella enterica* serovar *typhi* and two other pathogenic *Salmonella* serovars with intestinal epithelial cells. *Infection and Immunity*. 1998;66(5):2310-8.
29. Schauer DB. Indigenous microflora: Paving the way for pathogens? *Current Biology*. 1997;7(2):R75-R7.
30. Thirabunyanon M. Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria. *Maejo International Journal of Science and Technology*. 2011;5(1):108.
31. Guttman JA, Li Y, Wickham ME, Deng W, Vogl AW, Finlay BB. Attaching and effacing pathogen-induced tight junction disruption in vivo. *Cellular Microbiology*. 2006;8(4):634-45.
32. Reiff C, Kelly D. Inflammatory bowel disease, gut bacteria and probiotic therapy. *International Journal of Medical Microbiology*. 2010;300(1):25-33.
33. Nwachukwu E, Umechuruba C. Changes in nutritional values of African yam bean, *Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms seeds due to seed-borne fungi. *Global Journal of Pure and Applied Sciences*. 1997;3(2):141-7.