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HEAMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL RESPONSES OF INFECTED *Rattus albus* (albino Wister rats) FED WITH PROBIOTICS (*Lactobacillus* sp.)

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ABSTRACT

For years, *Lactobacilli* have been used as probiotics. They are believed to play important role in maintaining a state of immunological homeostasis within host. This research was aimed at evaluating immunity-boosting potential of lactic acid bacteria isolated from kunu and palm wine; using standard experimental/scientific methods. Results revealed the bacterial isolates, *Lactobacillus fermentum*, *Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus casei* to influence the immune system of pathogen-infected albino Wister rats. The pathogen treated rats without probiotic did not survive shortly after the post-acclimatization treatment, while the pathogen treated rats (fed with probiotic) survived; with a mean weight ranging from 128.33 g – 149.19 g for pathogen treated rats, 140.18 g – 149.65 g for *Lactobacillus* species + pathogen treated rats and 150.90g – 188.31g for control rats (non-infected). The haematological parameters (PCV and Hb) of the *Lactobacillus* species + pathogen treated rats (ranging 45.6 – 53.4 %; 15.5 -17.8 mg/dl) were higher compared to the control rats (ranging 20.7 – 32.9 %; 6.9 – 11.0 mg/dl). Enzymes (AST and ALT) activities of the *Lactobacillus* species + pathogen treated rats ranged from 0.091 - 0.108 U/L and 0.092 – 0.094 U/L respectively; and the control rats ranged from 0.083 – 0.098 U/L and 0.072 – 0.095 U/L respectively, with no significant difference. The histological examination of the rats' liver showed hepatic changes in the pathogen treated rats, unlike those of the *Lactobacillus* species + pathogen treated rats and the control rats, suggestive of the beneficial role of *Lactobacillus* species in the pathogen treated rats. However, further in-vivo research to study a more encompassing health benefits and the mechanism of actions of these *Lactobacillus* species is recommended.

Keywords: *Kunu, Lactobacillus, Palm* wine, *Probiotics, Rattus albus*

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INTRODUCTION

Probiotics are bacteria that, when taken, provide positive health benefits (Hill *et al*., 2014). Currently, the word is used to describe meals containing microorganisms that provide advantages for both humans and animals (Magdalena *et al*., 2006). On the other hand, probiotics are defined by the World Health Organization (WHO) as "live microorganisms that, when supplied in sufficient concentrations, confer health benefits on the host" (Schlundt, 2012). A number of microorganisms are now being tested for their probiotic potential and used as supplementary cultures in different kinds of food items or medicinal preparations. These microorganisms are mainly food grade lactic acid bacteria (LAB) and *bifidobacteria*. Probiotics need to fulfil a number of requirements in order to be helpful to human health, including being able to survive transit through the upper gastrointestinal tract (GIT) and functioning in the gut environment. Additionally, probiotics must have the capacity to endure and remain viable in food during the processing and storage of food. As a result, a thorough assessment of each potential probiotic strain becomes a crucial step before further application as a culture supplement.

The LAB are the organisms that are most frequently used in probiotic products. Probiotics must be alive when provided (Fuller, 1989; Fuller, 1991; Fuller, 1992). One of the issues raised in the scientific literature is the viability and repeatability of the observed findings on a large scale, as well as their viability and stability during usage and storage. Another problem is the capacity to survive in stomach acids and subsequently in the intestinal ecology (Rijkers *et al*., 2011). To prove that probiotics have a positive impact on health in the target host, controlled trials must have been conducted. Probiotic products may only be defined as those that include living organisms that have been proven to improve health in human trials and that can be reproduced (O'Hara *et al*., 2006; Reid *et al*., 2010; Rijkers *et al*., 2011). In order to have the desired impact on the host, there must be a sufficient supply of probiotics, which is measured by their ability to do so. This relies on the intended outcome, the method, the matrix, and the strain specificity. The majority of the claimed advantages associated with conventional probiotics have been seen following consumption of a concentration of around 107 to 108 probiotic cells per gramme, with a serving size of approximately 100 to 200 mg per day (Rijkers *et al*., 2010). The intestinal lactic acid bacteria *Lactobacillus rhamnosus*, *Lactobacillus casei* and *Lactobacillus johnsonii* were among those marketed as probiotics in the next decades with claims of health benefits (Tannock, 2003).

The purpose of this study was to use conventional physical, chemical, and microbiological techniques to identify and assess the probiotic potential of lactic acid bacteria isolated from locally fermented foods and products in *Rattus albus* (albino Wister rats).

MATERIALS AND METHOD

Sample collection

Fresh quantities of kunu (kunuzaki) and palm wine were purchased in sterile plastic containers from the Oluku and Oba Markets in Benin City, Edo State, respectively, and were then immediately taken to the laboratory for microbiological investigation.

Microbiological Analysis of the Lactic Acid Bacteria (LAB)

To culture the samples, ten millilitres (10 ml) of defrosted samples were dispensed in 90 ml of sterile distilled water to obtain a 10-1 dilution. In order to achieve 10-3 dilutions, more dilutions were created by adding 1 mL to 9 mL of distilled water. The pour plate technique was used to inoculate an aliquot portion (1 ml) of each dilution onto De

Mann, Rogosa, and Sharpe (MRS) agar, and plates were then incubated for the required amount of time and temperature (Cheesbrough, 2000). Upon incubation, the bacterial counts were estimated in colony forming units per millilitre (cfu/ml), the total count was given. The bacterial isolates were subjected to a number of biochemical assays, including oxidase, coagulase, urease, indole, citrate utilisation, catalase, and sugar fermentation tests. Bacterial isolates were identified according to methods of Cheesbrough (2000).

Isolation and Collection of Bacterial Pathogens

To obtain bacteria pathogen, one millilitre (1 ml) of 24-hours stock cultures of the following pathogens were revived and used for the experimentation: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Animals Used in Experiments

Nine (9) *Rattus albus* (Wister rats) in good health, both male and female, were purchased from the animal house, University of Benin, Benin City, Edo State.

Preliminary Assessment for the Presence of Lactic Acid Bacteria in *Rattus albus*

From their faeces samples, a preliminary evaluation for the presence of lactic acid bacteria in each *Rattus albus* was conducted. One gram (1g) of faeces from each *Rattus albus* was homogenised and serially diluted in nine millilitres of ordinary saline. The pour plate method was used to plate each of the serially diluted samples onto De Mann, Rogosa, and Sharpe (MRS) agar. For the purpose of lactic acid bacteria enumeration and characterization, plates were incubated at 37 °C for 24 hours.

Preparation and Treatment of *Rattus albus*

Each set of three *Rattus albus* was housed in three distinct wooden cages (cages 1, 2, and 3). Prior to receiving treatments with pathogens and lactic acid bacteria, each rat's weight was measured, and they were provided a regular meal and water for two weeks (fourteen days). Only after the two weeks of acclimation were all of the *Rattus albus* in cage 2 were given an oral dosage of 1 ml of the lactic acid bacteria isolated from the fermented meal samples. Following the acclimatisation period, sterile pasture pipettes were used to administer a 1 ml (44×10^5 cfu/ml) sample of a 24-hour culture of each of these pathogens (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*) to all of the *Rattus albus* in cages 1 and 2 only. All of the *Rattus albus* in cage 3, which served as the experimental control, received no antimicrobial treatment.

Determination of Weight of Rattus albus

Before the rats were sacrificed, the *Rattus albus* in cages 1, 2, and 3 were all weighed before acclimatization, 14 days after acclimatisation, and 14 days after the end of the treatment.

Sacrificing of *Rattus albus*

After two weeks of microbial treatment, the rats were chloroform-suffocated in cotton wool swabs in a desiccator. Heart blood was drawn using a 5 ml sterile syringe and needle, and then the samples were put into appropriately labelled EDTA and plain sample containers, respectively, before being cut apart with the use of a dissecting kit to harvest the liver.

Determination of Packed Cell Volume (PCV)

The micro-haematocrit technique was used to calculate the packed cell volume. Blood from the EDTA sample vial was placed in a capillary tube, filled to the middle, sealed with sealant, and spun for five minutes at 15000 RPM in a

haematocrit centrifuge. A haematocrit reader was used to read the red cell column of the spined capillary tube, and the value was represented as a percentage (%) (Cheesbrough, 2000).

Determination of Haemoglobin (Hb)

The Cheesbrough (2000) cyanomethamoglobin technique was used to determine the haemoglobin content. The iron (II) contained in haemoglobin, oxyhaemoglobin, and carboxyhaemoglobin is oxidised by ferricyanide in Drabkins solution to iron (III), which results in methaemoglobin. Cyanomethaemoglobin is a stable red molecule that can be measured photometrically at 540 nm. A test tube containing 5 ml of Drabkins solution and 0.20 ml (20 ul) of blood was filled, mixed, and left to stand at room temperature for 10 minutes to allow for the full conversion of haemoglobin to cyanomethamoglobin. At 540 nm, the solution's absorbance was measured using Drabkins solution as a blank. The value of the unknown was extrapolated from the previously created calibration curve (Cheesbrough, 2000).

Liver Function Test (LFT)

Assay of Aspartate Aminotranferase (AST) activity

The AST was calculated using the Reitman and Frankel (1957) technique. The reagent blank (B) and sample test (T) tubes received 0.5 ml of the AST substrate buffer, respectively. Only the sample test tube (T) was used, and the serum sample (0.15 mL) was introduced and carefully mixed. To the reagent blank, 0.01 ml of distilled water was then added (B). After thoroughly mixing the entire reaction medium, it was heated to 37 °C in a water bath for 30 minutes. 2, 4 - of dinitrophenyllydrazine (0.5 ml) was added to the reagent blank (B) and sample test (T) tubes after incubation, completely mixed, and then left to stand for precisely 20 minutes at 250C. Finally, each test tube containing reagent and a blank was filled with sodium hydroxide solution (5.0 ml) and carefully mixed. After 5 minutes, the absorbance of the sample (A sample) was measured against the reagent blank.

Assay of Alanine Aminotransferase (ALT) activity

The ALT was calculated using the Reitman and Frankel (1957) technique. In two sets of test tubes marked B (Sample Blank) and T (Sample Test), the ALT substrate phosphate buffer (0.5 ml) was pipetted. Only the sample test (T) was introduced to and correctly mixed with the serum sample (0.1 ml). The tubes were then incubated at 37 0C in a water bath for exactly 30 minutes. After the incubation, 0.5 ml of 2,4-dinitrophenylhydrozine was immediately added to both test tubes. Additionally, the serum sample (0.1 ml) was solely put in the sample blank (B). The entire medium was well mixed before being allowed to stand at 25 0C for exactly 20 minutes. Then, 5.0 ml of 0.4 N sodium hydroxide (NaOH) was added to each test tube, and the mixture was well mixed. After 5 minutes, the reagent blank was used to compare the sample's (A sample) absorbance to the sample blank.

Hepatological Examination

The livers were taken from all the groups, mounted in molten paraplast at 58 to 62°C, dehydrated in escalating grades of ethyl alcohol, cleaned in xylol, and preserved in 10% formalin in saline. A light microscope was used to examine thin sions (4 to 5 mm) that had been cut using a microtome and stained with hematoxylin and eosin. The presence of edoema, an inflammatory cell infiltration, bleeding, and ulceration were graded as histological alterations in the liver, as were the organ's architecture and these other conditions. A field with a portal vein with a diameter of about 100 m in the centre was selected for examination under the light microscope at a magnification of 200×. A digital camera was used to digitise the field and five random fields of the same size for computer analysis. To determine the level of fibrosis in each case, the average of the three fields was determined.

RESULTS AND DISCUSSION

The genus *Lactobacillus* is home to the majority of probiotic strains. Future generations of probiotics appear to have a bright future based on the encouraging results of a first generation of probiotic bacteria that were tested in animal models as well as in naturally occurring illnesses in both animals and people. The most researched types of diarrhoea include those brought on by antibiotics, traveler's, paediatric, and more recently, inflammatory bowel disease and irritable bowel syndrome (Odunfa and Adeyele, 1985). Future probiotics are likely to include blends of strains with complementing traits, designed specifically for certain gastrointestinal illnesses; vaginoses for vaccine delivery systems; immunoglobulins; and other protein-based therapeutics. Four (4) lactic acid bacteria (LABs) were isolated and characterised from the palm wine and kunu samples used in the study. These LABs mostly belonged to the *Lactobacillus* genus. As shown in Table 1, the isolates of the LABs were *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus casei*. The *Lactobacillus* species recovered from the samples is consistent with observations made earlier using comparable samples by Odunfa and Adeyele (1985).

The colony counts of the separated *Lactobacillus* species in Table 2, shows the highest counts recorded with L. fermentum (1.46×107 cfu/ml) and L. casei recording the lowest (7.0×105 cfu/ml).

Table 3 shows the mean weight of the experimental rats along with the effects of the pathogen and *Lactobacillus* species treatments administered to the rats

Table 1: Lactic acid bacteria isolates from palm wine and kunu samples

Samples	Isolates
Palm wine	*Lactobacillus fermentum*
	Lactobacillus plantarum
Kunu	*Lactobacillus acidophilus*
	Lactobacillus casei

Table 2: Colony count of lactic acid bacteria (cfu/ml)

Isolates	Colony count
Lactobacillus fermentum	1.46×10^{7}
Lactobacillus plantarum	4.5×10^{6}
Lactobacillus acidophilus	5.3×10^{6}
Lactobacillus casei	7.0×10^{5}

While all rats were usually gaining weight steadily, the mean weights of the control rats without receiving microbial treatments were substantially greater than the mean weights of the rats receiving microbial treatments. This highlights the impact of the microbial treatment on the experimental animals even more. The constant provision of dietary nutrients to the rats as well as the enhancement of nutrient digestion and absorption in the rats' intestines by the *Lactobacillus* species may be responsible for the overall increase and weight gain across all rats throughout the trial period. This weight gain is comparable to the published guinea pig research by Okwu *et al*. (2016). This increased

pattern of weight gain is also consistent with the reports of Singh *et al*. (2016). This increased pattern of weight gain is also consistent with Singh *et al*. (1999).

According to results presented in Table 4, the packed cell volume (PCV) values of the experimental rat blood treated with pathogens and *Lactobacillus* species ranged from 46.6% to 53.4%, while those of the control rats ranged between 20.7% and 32.9%. The packed cell volume of the treated rats significantly increased when compared to the control rats.

Similar to Table 5, the blood of the microbially treated rats had considerably higher levels of haemoglobin (ranging from 15.5 mg/dL to 17.8 mg/dL) than the control rats (whose levels ranged from 6.9 mg/dL to 11 mg/dL). The rats' ability to absorb nutrients may be responsible for the rise in the proportion of packed cells and the blood's level of haemoglobin. Additionally, these high levels of packed cell volume and haemoglobin concentration, especially in the microbially treated rats, strongly suggested a decreased risk or case of anaemia in the experimental rats because of an increase in nutritional values and immunological benefits that the rats received from the administration of probiotics (*Lactobacillus* species). This finding is consistent with the research conducted and published by Iyer *et al*. (2010) utilising strains of *Streptococcus thermophilus.*

Table 3: Weights of *rattus albus* before, during and post-acclimitization treatment (g)

Replicates	Before (day 0)	During (day 14)	After (day 28)	Mean
Pathogen Treatment				
1	127.96	170.42	Nil	149.19
2	110.83	145.83	Nil	128.33
3	113.90	158.61	Nil	136.26
Pathogen + Lactic acid bacteria treatment				
1	103.19	146.78	179.14	143.04
2	115.73	150.09	183.12	149.65
3	107.98	131.43	181.13	140.18
Control				
1	101.28	152.60	198.83	150.90
2	117.96	173.37	222.42	171.25
3	124.83	182.54	257.57	188.31

Table 4: Determination of packed cell volume (%)

Replicates (Pathogens)	Cage 1	Cage 2 (LAB + Pathogens)	Cage 3 (Control)
1	Nil	46.6	20.7
2	Nil	53.4	32.9
3	Nil	50.0	27.0

Table 5: Determination of haemoglobin (Hb) concentration (mg/dl)

Replicates (Pathogens)	Cage 1	Cage 2 (LAB + Pathogens)	Cage 3 (Control)
1	Nil	15.5	6.9
2	Nil	17.8	11.0
3	Nil	16.7	9.0

Liver function measurements determined were ALT and AST (alanine aminotransferase and aspartate aminotransferase, respectively). Their increased circulation throughout the body increases the risk of liver disease. Indicating liver issues with greater specificity is alanine aminotransferase (Denniston *et al*., 2004; Cheesbrough, 2005). Aspartate aminotransferase and alanine aminotransferase enzyme activities are shown in Tables 6 and 7, respectively. When compared to the activities of the control rats that received no microbial treatments, neither enzyme neither activity nor bacterial composition significantly differed between the rats treated with pathogens and *Lactobacillus* species combined. The results for the treated rats ranged from 0.091 U/L to 0.108 U/L for the AST and 0.092 U/L to 0.094 U/L for the ALT. These findings suggest that the probiotic *Lactobacillus* species the rats received caused normal liver function in the pathogen-infected rats as compared to the control group of rats that received no pathogen treatment. This could be closely related to findings that were made using broiler chicks in a research study by Islam *et al.* (2004). The degree of the host system's infection directly affects the liver's ability to function or malfunction. According to the liver examination of all the rats, those exposed to pathogens showed some degree of irregular hepatologic changes, whereas those exposed to *Lactobacillus* species and pathogens and the rats in the control group that received no microbial treatment did not exhibit any notable changes in their liver micrograph. Rats in cages 2 and 3 (which received pathogen and *Lactobacillus* species treatment) survived the entire study period, but rats in cage 1 (which received pathogen treatment alone) did not. These findings revealed dependable connections between the rats' immunological health state and consumption of the isolated *Lactobacillus* species (*Rattus albus*).

As lactic acid bacteria, the *Lactobacillus* genus has been one of the most widely used bacteria for its probiotic effect, even though the health benefits of consuming probiotics continue to be an essential method of strengthening and boosting the immune system of the host against pathogenic infections. More research should be done in order to possibly determine the probiotic potential of other *Lactobacillus* species from locally fermented products and their mechanisms of action in order to improve human health at a relatively lower cost. This is because the *Lactobacillus*

species isolated from palm wine and kunu samples have demonstrated probiotic potential. However, further research is necessary before any commercial applications.

Table 6: Determination of aspartate aminotransferase (AST) activity (u/l)

Replicates	**Cage 1**	**Cage 2**	**Cage 3 (Control)**
(Pathogens)		**(LAB + Pathogens)**	
1	Nil	0.091	0.083
2	Nil	0.108	0.097
3	Nil	0.100	0.098

Table 7: Determination of alanine aminotransferase (ALT) activity (u/l)

Replicates	**Cage 1**	**Cage 2**	**Cage 3 (Control)**
(Pathogens)		**(LAB + Pathogens)**	
1	Nil	0.092	0.072
2	Nil	0.094	0.094
3	Nil	0.093	0.095

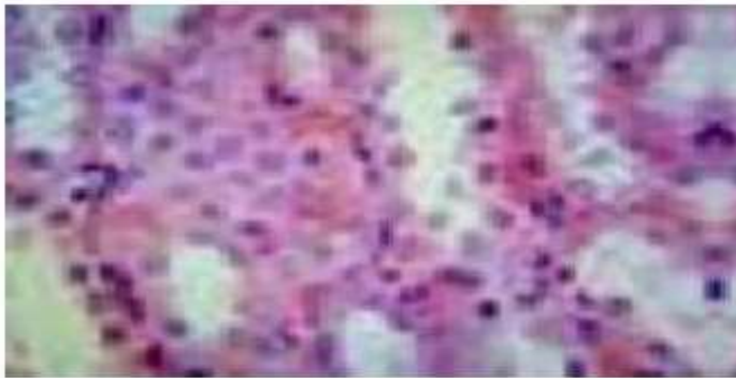

Figure 1: Micrograph of the pathogen- infected rat's liver

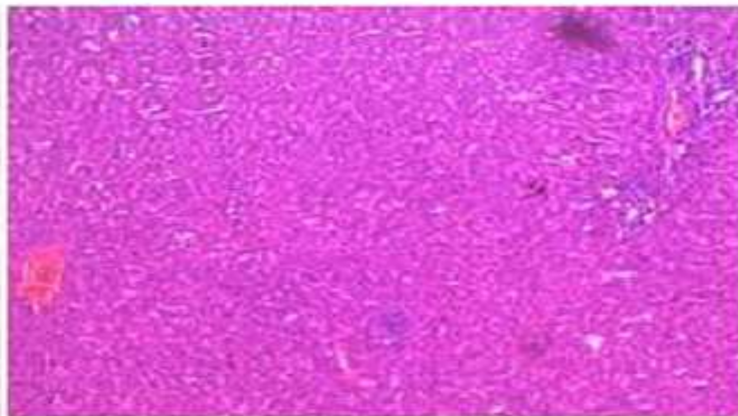

Figure 2a: Micrograph of probiotic + pathogen-infected rat's liver

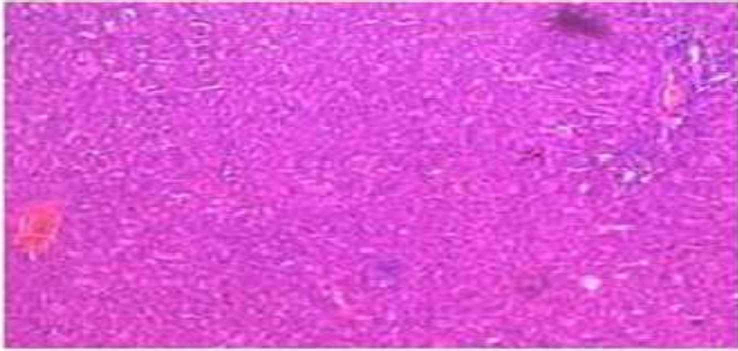

Figure 2b: Micrograph of probiotic + pathogen-infected rat's liver

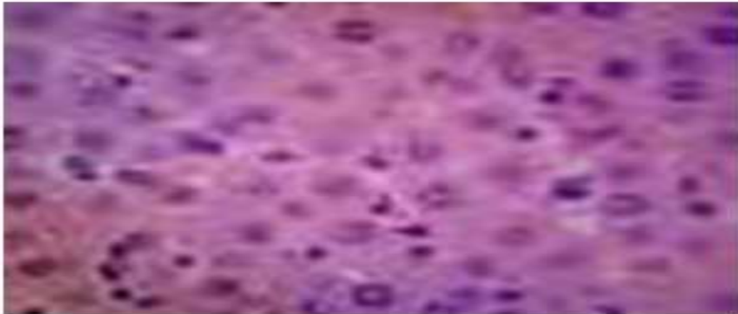

Figure 3a: Micrograph of experimental control rat's liver

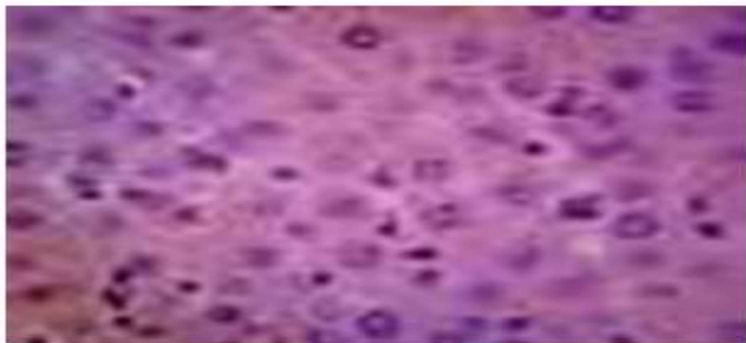

Figure 3b: Micrograph of experimental control rat's liver

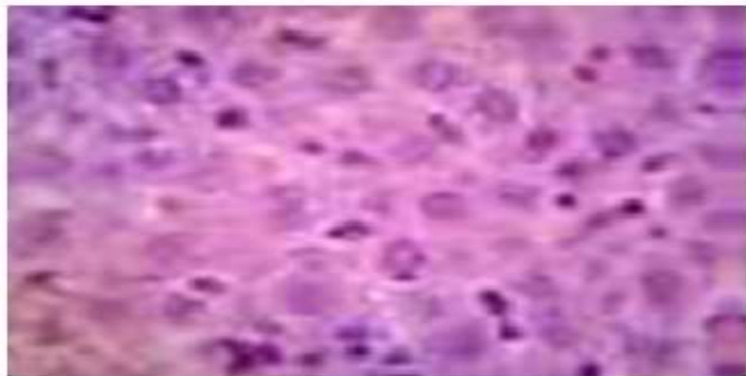

Figure 3c: Micrograph of experimental control rat's liver

CONCLUSION

In conclusion, it is important to fully investigate the potential health benefits (i.e., immune system augmentation) that the naturally occurring *Lactobacillus* species found in locally produced fermented foods may have on their host. This may be done by regularly consuming these fermented foods on their own in sufficient and suitable proportions or by combining them with antibiotics to treat microbial infections.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests

REFERENCES

Cheesbrough, M. (2000). Measurement of Hemoglobin: in *District Laboratory Practice in Tropical Countries*. Cambridge University Press Ltd, England Part II: 299-302.

Cheesbrough, M. (2005). *"Discrete Laboratory Practice in Tropical Countries Part 1, Cambridge Second Editions.* Published by Press Syndicate of the University of Cambridge, Chapter 5, England. p 247-258.

Denniston, K. J., Topping J. J. and Caret, R. L. (2004). *"General, Organic and Biochemistry"*. 4th Edition, McGraw Hill Co., New York, p 432-433.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology.* **66**: 365–378.

Fuller, R. (1991). Probiotics in human medicine. *Gut Journal* **32**(4): 439–442.

Fuller, R. (1992). *"Probiotics: The Scientific Basis".* Chapman & Hall, London. http://dx.doi.org/10.1007/978-94-011-2364-8

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C. and Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology and Hepatology* **11**(8): 506–514.

Islam, M. S., Kabir, M. S., Khan, S. I., Ekramullah, M., Nair, G. B., Sack, R. B. and Sack, D. A. (2004). Wastewater-grown duckweed may be safely used as fish feed. *Cancer Journal of Microbiology* **50**(1): 51-56.

Iyer, R., Tomar, S. K., Kapila, S., Mani, J. and Singh, R. (2010). Probiotic properties of folate producing *Streptococcus thermophiles* strains. *Food Research International* **43**: 103-110.

Magdalena, A., Catherine, S., Lorenzo, M., Gregor, R. and Maya, P. (2006). *"Probiotics in food: health and nutritional properties and guidelines for evaluation".* Combined Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.

Odunfa, S. A. and Adeyele, S. (1985). Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *Journal of Cereals Science* **3**: 173-180.

O'Hara, A. M., O'Regan, P., Fanning, A., O'Mahony, C., Macsharry, J., Lyons, A., Bienenstock, J., O'Mahony, L. and Shanahan, F. (2006). Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. *Immunology Journal* **118**(2): 202–215.

Okwu, G. I., Obazi, H. A. K., Amaobi, C. B., Amengialue, O. O. and Akpe, A. R. (2016). Physiological changes in probiotics (*Latobacillus* spp) fed infected guinea pigs. *American Journal of Research Communication* **4**(4): 85-96.

Reid, G., Gaudier, E., Guarner, F., Huffnagle, G. B., Macklaim, J. M., Munoz, A. M., Martini, M., Ringel-Kulka, T., Sartor, B., Unal, R., Verbeke, K. and Walter, J. (2010). Responders and non-responders to probiotic interventions: how can we improve the odds? *Gut Microbes Journal* **1**(3): 200–204.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamate-oxaloacetic acid and glutamate-pyruvic acid transaminases. *The American Journal of Chinese Pathology* **28**: 56-63.

Rijkers, G. T., Bengmark, S., Enck, P., Haller, D., Herz, U., Kalliomaki, M., Kudo, S., Lenoir-Wijnkoop, I., Mercenier, A., Myllyluoma, E., Rabot, S., Rafter, J., Szajewska, H., Watzl, B., Wells, J., Wolvers, D. and Antoine, J. M. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *Journal of Nutrition* **140**(3): 671–676.

Rijkers, G. T., De Vos, W. M., Brummer, R. J., Morelli, L., Corthier, G. and Matheau, P. (2011). Health benefits and health claims of probiotics: bridging the gap between science and marketing. *British Journal of Nutrition* **106**(9): 1291-1296.

Schlundt, J. (2012). *Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. FAO/WHO.

Singhi, S. C. and Kumar, S. (2016). Probiotics in critically ill children. *Research Review* **5**: doi:10.12688/f1000research.7630.1

Tannock, G. W. (2003). Probiotics: time for a dose of realism. *Current Issues in Intestinal Microbiology* **4**(2): 33–42.