Iru, a Fermented Product of Parkia Biglobosa, Possesses Significant Hepatoprotective Effect in Wistar Rats During Treatment of Hypercholesterolaemia

***Ayowole Victor Atere[1, 2], Victor Olusegun Oyetayo[1], Felix Akinsola Akinyosoye[1]**

[1]*Depatrment of Microbiology, Federal University of Technology, Akure, P.M.B 704 Akure, Nigeria.*
[2]*Department of Biomedical Sciences, Nottingham Trent University, NG11 8NS Nottingham United Kingdom.*

***Corresponding author:** victor_efosa001@yahoo.com*

ABSTRACT

Background: *The liver is central to the body's metabolic health and serves as a key indicator of body toxicology. Therefore, this research was designed to investigate the effect of iru, used to treat hypercholesterolemia, on liver functionality.*

Methods: *Iru was produced from the seeds of Parkia biglobosa using starter culture of Bacillus subtilis, Lactobacillus plantarum and Leuconostoc mesenteroides. The rats were experimentally induced into hypercholesterolaemia using high lipid compounded feed. The hypercholesterolaemic rats were fed iru supplemented diet for 28 days and aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes were assessed in the serum. Histopathological study of the liver of experimental rats was also conducted.*

Results: *The induced untreated rats had 89.21 U/L of AST which is significantly higher ($p>0.05$) than the value of the control group (55.87 U/L), the groups fed with iru supplemented feed (47.63-58.54 U/L), and the group where standard drug was administered (46.79 U/L). The ALT values of the group fed iru supplemented diet (34.17 - 40.44 U/L) was significantly lower ($p<0.05$) than the control (49.10U/L). The untreated group had the highest ALP (62.60 U/L) and significantly higher than the control and all the treated groups. The histology of the liver tissue showed that the untreated groups displayed structural defect of cell deadness. In all the treated groups, cells structures were normal.*

Conclusion: *Fermented Parkia biglobosa seeds (iru) had hepato-protective effect on the liver tissues of rats with hypercholesterolaemia.*

Keywords: Iru, *Parkia biglobosa,* liver biomarker, Fermentation

Doi: https://dx.doi.org/10.4314/njns.v45i2.8

INTRODUCTION

Fermented seeds of *Parkia biglobosa* are used as condiment in several parts of sub-Sahara Africa. *Parkia biglobosa* is a leguminous plant belonging to the family Leguminoseae and sub-family Mimosadeae (1). Legumes have emerged as a vital source of daily protein requirements for rural populations due to their cost-effectiveness, ease of processing, and high energy content (2). Particularly in African countries, where many rural dwellers face challenges affording animal products like meat or dairy, which traditionally supply necessary protein, fermented legumes serve as a dependable dietary alternative (3).

Fermented seeds of *Parkia biglobosa* is known with different names in different part of Africa, such as Iru (South-Western Nigeria), Ogiri-igbo (South-Eastern Nigeria), Soumbala (Mali, Cote d'Ivoire, Guinea, and Burkina Faso), Dawadawa (Ghana, Niger, and Northern Nigeria) (4). Generally, Iru is consumed locally by rural dwellers especially in Southwestern

Nigeria. It is a good source of nutrients such as protein, fats, oil, carbohydrates, vitamin B2, and minerals such as calcium. The use of iru as a condiment in the sub-Sahara Africa dates back to the 14th century (1).

The production process of iru involves chance inoculation, where seeds of *Parkia biglobosa* are sorted, cooked, dehulled and fermented under uncontrolled conditions (5). However, the use of starter culture has been reported to give a stable product with a longer shelf-life (6).

Regular consumption of iru as a condiment may represent an alternative dietary control of hypercholesterolaemia and associated conditions (7). Iru has also being reportedly used in the treatment of eyes infections (8). Souleyman (9) reported that the fermented seeds of *Parkia biglobosa* is believed to be good for the treatment of arterial hypertension.

The liver is considered the largest internal organ and the largest gland in the body (1). Its role is crucial for sustaining life, as it eliminates wastes by using enzymes to break down bodily waste products, drugs, alcohol, and medications (10). Food samples are transformed by the liver into forms that could be assimilated by the body (1). The liver's health is determined by the level of indicator enzymes produced. These enzymes are referred to as liver function enzymes: they are aspartate transaminase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). The production of these enzymes by the liver is elicited by stress to the hepatic tissue or structural damage to the liver because of toxic substances (11). The histology of the hepatic cells also gives information on the structural wellbeing of the liver (12).

Iru has been reported to have a varying degree of hypocholesterolaemic effect on Wistar rats when the seeds were fermented with different starter culture (7). Based on the medicinal properties of iru prepared from the fermentation of *Parkia biglobosa* seeds, it is important to investigate the safety of this product using indicators for liver function. This research was therefore designed to investigate the effect of iru produced from the fermentation of *Parkia biglobosa* seeds on the liver functionality of Wistar rats, during the treatment of hypercholesterolaemia with the fermented samples.

METHODS

Source of *Parkia biglobosa* seeds

The raw seeds of *Parkia biglobosa* were obtained from a local market (Oja Oba) in Omuo-Ekiti, Southwestern Nigeria (7^0 46′ 0′′ North 5^0 43′ 0′′ East)

Seed processing

The seeds of *Parkia biglobosa* were processed according to the method (Fig 1) documented by Atere et al. (3). The temperature of cooking was 121°C during this process

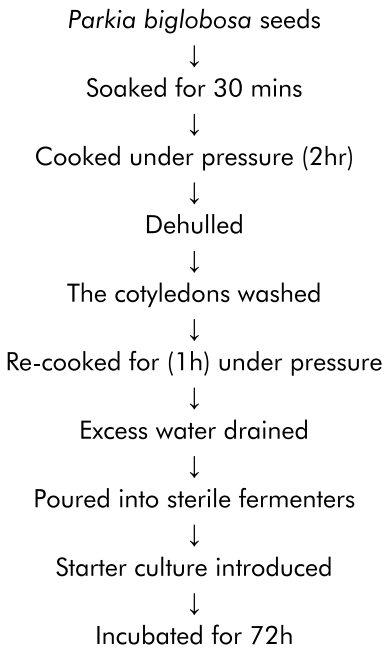


Fig 1: Flow chart for the production of Iru.

Experimental animals

Animal experimental study was carried out according to the guidelines for animal research approved by the University Institutional Animal Care and Use Committee (CERAD-2018 Approved MCB-1240). The laboratory Wistar rats used were of both sexes and numbered Forty-two. The body weight of the rats ranged between 94.3 and 112.4 g.

Effect of feeding on rats

The rats used in this research were 4 weeks old, acclimated for 14 days and randomly divided into two groups. The first group (control group) was fed commercial feed, while the second group (induced group) was given commercial feed supplemented with an average of 80 mg of pork per gram of pork and 1 ml of aqueous pork extract stock per gram of body weight daily for two weeks. Six groups were formed from rats induced into hypercholesterolaemia, each group contains six rats, the seventh group consisted of six rats that were not induced into hypercholesterolaemia and this group served as the control. Table 1 contains the details of the treatment and trials. At the end of 28

days of the feeding trial, six rats were scarified in each group. The blood was collected and analyzed for liver biomarkers and the liver was collected for histological studies.

Determination of Liver enzymes

Blood samples were collected in the heparinized bottle, centrifuged at 3000 r.p.m for 30 minutes. The toxicological assay was carried out on the plasma with the aid of Randox kits (13).

Determination of Aspartate Transaminase (AST)

To determine AST levels, a reagent blank was prepared by combining 0.5 ml of R1 (comprising phosphate buffer 100 mmol/l, l-aspartate 100 mmol/l, α-oxoglutarate 2 mmol/l) and 0.1 ml of distilled water in a test tube. Subsequently, 0.1 ml of the sample was added to sample test tubes followed by the addition of buffer R1. Incubation was carried out at 37°C for 30 minutes. Following incubation, 0.5 ml of R2 (2, 4-dinitrophenylhydrazine 2 mmol/l) was added to both the blank and sample tubes, and incubation continued for 20 minutes at 25°C. After this incubation period, 5 ml of 0.4 mol/l sodium hydroxide was added to both the blank and sample tubes, and left for 5 minutes at room temperature. The content in the blank tube was utilized for blanking the spectrophotometer at 546 nm before readings were taken from the samples. All absorbance readings were obtained within 60 minutes and matched with the standard curve.

Determination of Alanine Aminotransferase (ALT)

For determining ALT levels, a reagent blank was prepared in a test tube containing 0.5 ml of R1 (consisting of phosphate buffer 100 mmol/l, l-alanine 200 mmol/l, α-oxoglutarate 2 mmol/l) and 0.1 ml of distilled water. Subsequently, 0.1 ml of the sample was introduced into sample test tubes, followed by the addition of buffer R1. Incubation was carried out at 37°C for 30 minutes. Following incubation, 0.5 ml of R2 (2, 4-dinitrophenylhydrazine 2 mmol/l) was added to both the blank and sample tubes, and incubation continued for 20 minutes at 25°C. After this incubation period, 5 ml of 0.4 mol/l sodium hydroxide was introduced to both the blank and sample tubes, and left for 5 minutes at room temperature. The content in the blank tube was utilized for blanking the spectrophotometer at 546 nm before readings were taken from the samples. All absorbance readings were obtained within 60 minutes and matched with the standard curve.

Determination of Alkaline phosphatase (ALP)

To determine ALP levels, a mixture of diethanolamine buffer (1 mol/l) and MgCl2 (0.5 mmol/l) was combined with 10 ml of p-nitrophenyl phosphate (10 mmol/l). Subsequently, 0.5 ml of the mixture was added to a test tube containing 0.01 ml of the sample. Absorbance was measured immediately after adding the reagent and at intervals of 1, 2, and 3 minutes, using a wavelength of 405 nm. The change in absorbance was calculated and multiplied by 2760 to determine the ALP value in the plasma.

Histological Processing and Staining

Liver tissue was excised from each experimental animal and immediately transferred into 10% formalin and then taken to the laboratory for routine Haematoxylin and Eosin histological slide preparation for microscopic studies (12). Tissue preparation for histological studies was carried out using the following; 10 % formal saline, Absolute alcohol, 95 % Alcohol, 70 % alcohol, xylene, paraffin wax, egg albumen, oven, microtome, glass slides and microscope.

The excised liver tissues were trimmed and washed with normal saline solution and immediately fixed in 10 % formal saline. This was done separately for each group and labeled appropriately. After the fixation, the liver tissues were then washed in running water and were dehydrated with varying percentage of alcohol from 70 % to Absolute Alcohol. Sections were then cleared in xylene, infiltrated with molten paraffin wax for 2 hours in oven at 57 °C and embedded in molten paraffin wax.

Tissues were embedded in paraffin wax and sections were cut using the sliding microtome. The sizes of tissue sections were between 3-5 microns. The cut sections were floated on hot water bath and were picked on clean albuminized glass slides for staining. The staining was done using routine Heamatoxylin and Eosin staining technique. Stained sections were examined with the oil immersion lens where the photomicrography of the stained section was obtained.

Statistical Analysis

Statistical analysis was performed on the obtained data using Analysis of Variance (ANOVA), and Duncan's Multiple Range Test (DMRT) was employed to compare means at ($P < 0.05$), utilizing the Statistical Package for Social Sciences (SPSS, version 28.0.000(190) 2021).

RESULTS

Effect of treatment on the Toxicological enzyme biomarkers

The aspartate transaminase (AST) of the rats in the different treatment groups is presented in Figure 2. The rats in the control group had 55.87 U/L while the rats in the untreated groups had 89.20 U/L. The rats in the groups fed with iru had values ranging from 47.63 U/L to 58.53 U/L.

The least alanine aminotransferase (ALT) was recorded in the rats fed with iru sample fermented with *Bacillus subtilis* with a value of 34.17 U/L. The highest value (62.09 U/L) was recorded in the rats that were not treated (untreated group). The groups fed with iru had values ranging from 34.17 U/L to 40.44 U/L (Figure 3).

Alkaline phosphatase (ALP) distribution in the treatment groups is presented in Figure 4. The rats in the control groups had 23.00 U/L while the rats in the untreated group had a value of 62.60 U/L. The ALP of the rats where standard drug was administered had 31.21 U/L while the rats fed with iru supplemented diet had values between 39.38 U/L and 48.51 U/L.

Effect of treatments on Hepatocytes of rats

Histopathological studies of the rats' liver are presented in Figure 5. The section of the liver showed normal hepatic lobules composed of hepatocytes arranged in one-cell thick plate with sinusoids on the sides as shown in the liver of rats in the control group, the liver cell of the rats in groups fed with iru supplemented diets showed no structural defect, the hepatic lobules were normal. The rats in the untreated groups showed the presence of fibrosis and necrosis of the liver tissue as indicated on the slide as F and N respectively. The rats in the group where standard drug was used also showed normal cells.

Table 1: Groups of different feeding trials, showing the control condiment and standard medication.

Groups	Feeding composition
A control (uninduced)	Commercial feed
B induced (treated)	Commercial feed + 20% iru fermented naturally
C induced (treated)	Commercial feed + 20% iru fermented with *Bacillus subtilis*
D induced (treated)	Commercial feed + 20% iru fermented with *Leuconostoc mesenteroides*
E induced (treated)	Commercial feed + 20% iru fermented with *Lactobacillus plantarum*
F induced (treated)	Commercial feed + Flavostatin (standard)
G induced (untreated)	Commercial feed

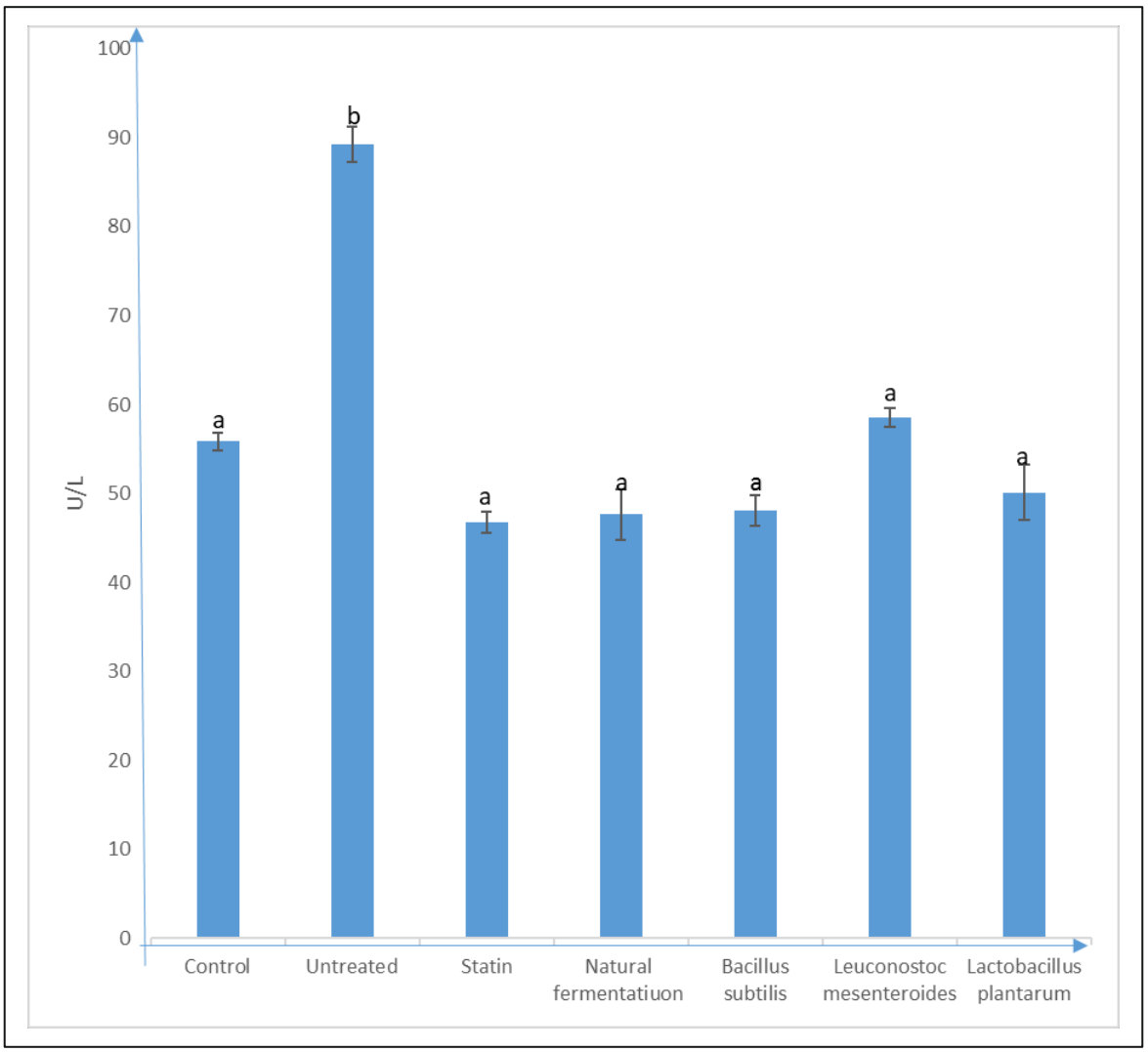


Figure 2: Distribution of aspartate aminotransferase (AST) in rats serum after feeding with diet supplemented with iru. Bars with different letters (a-b) are significantly different ($P < 0.05$).

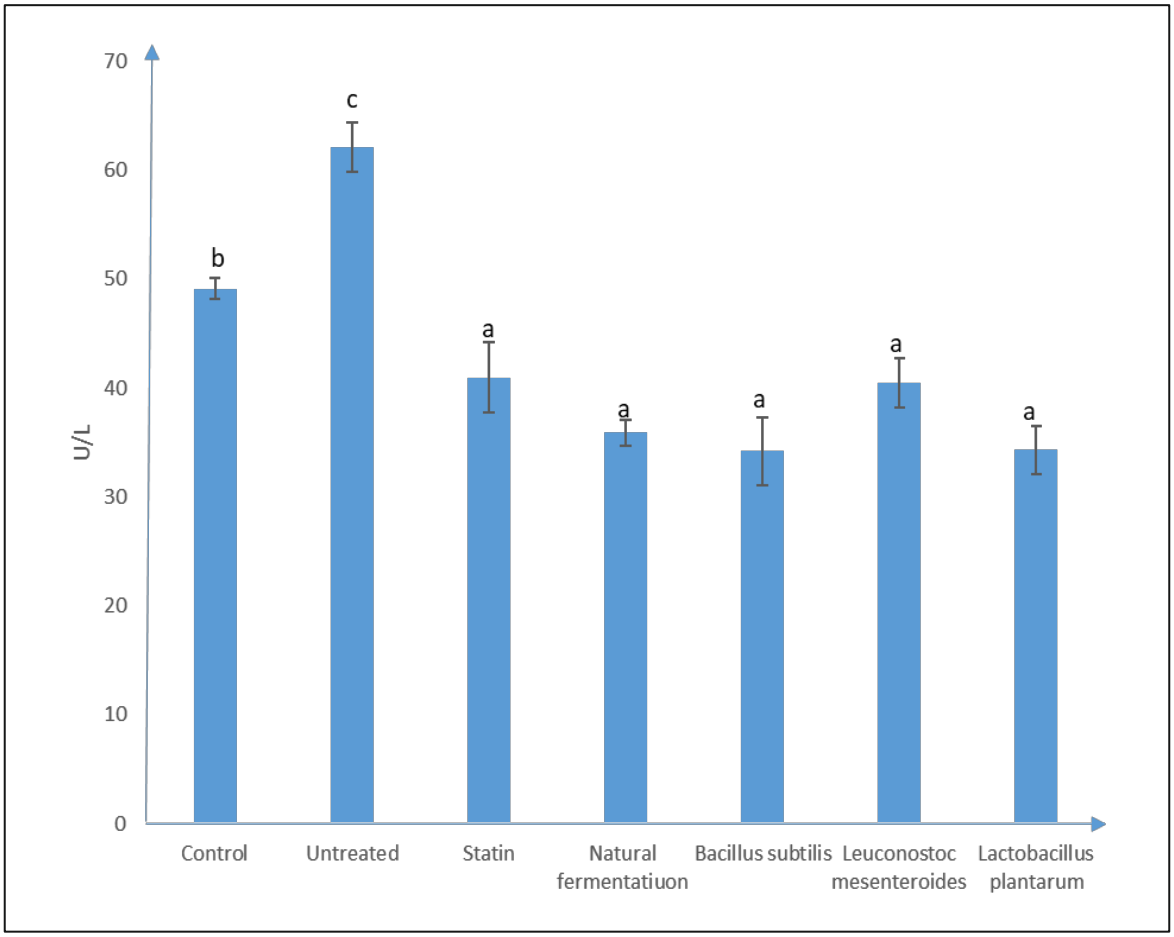


Figure 3: Distribution of Alanine aminotransferase (ALT) in rats serum after feeding with diet supplemented with iru. Bars with different letters (a-c) are significantly different ($P < 0.05$).

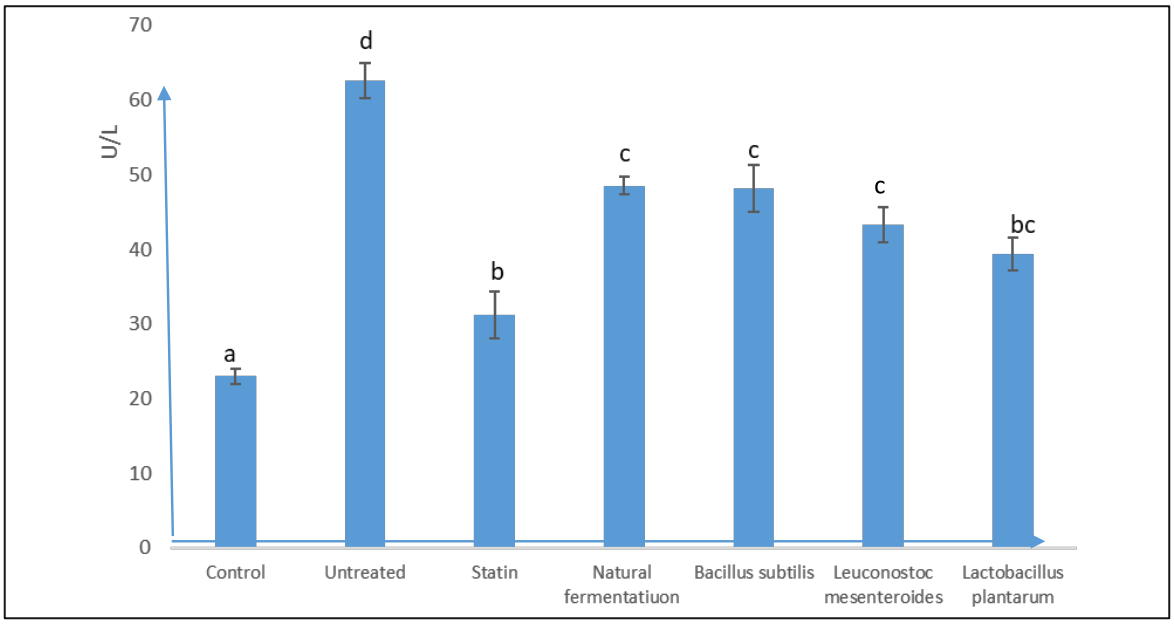
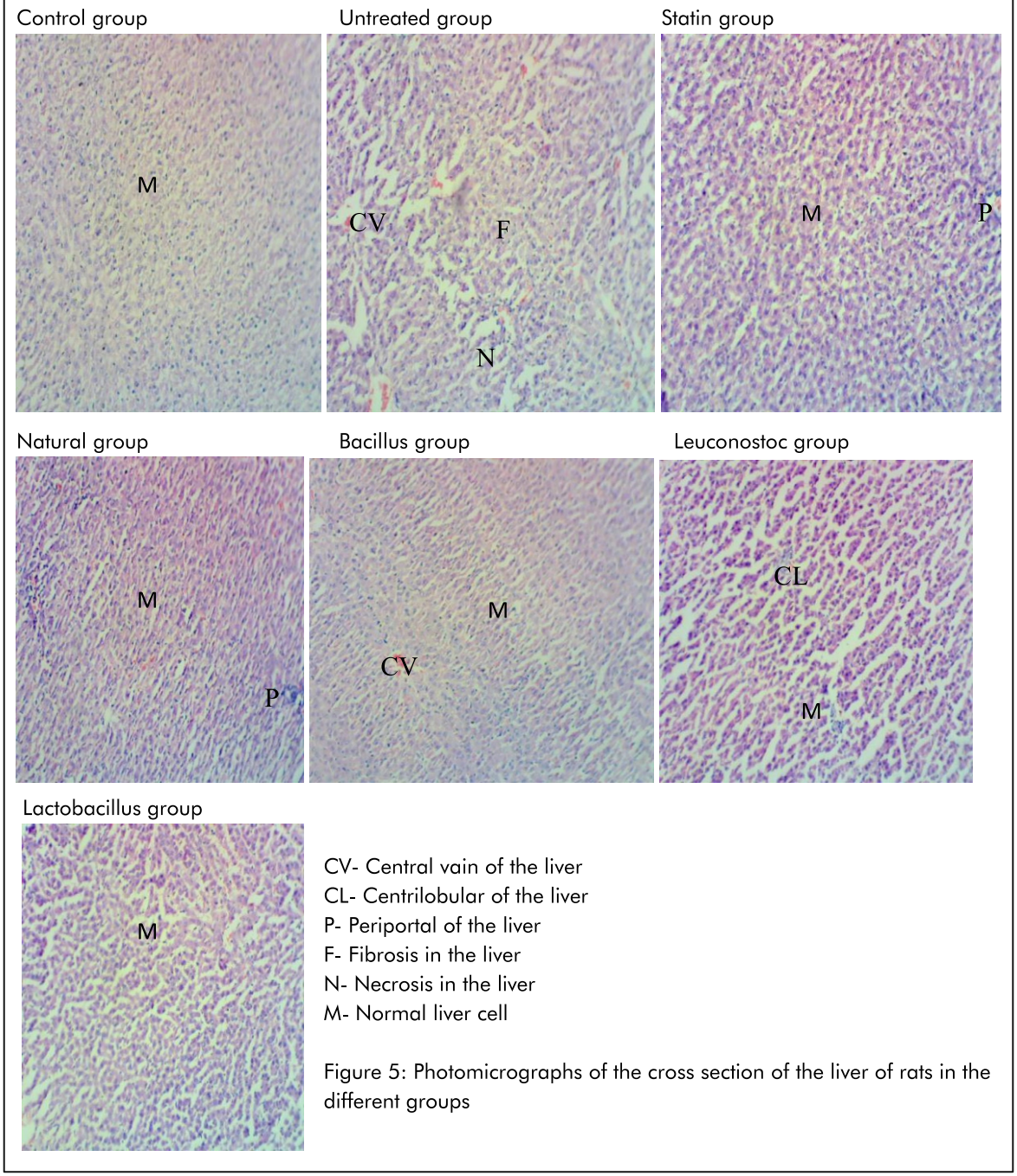


Figure 4. Distribution of Alkaline phosphatase (ALP) in rats serum after feeding with diet supplement with iru. Bars with different letters (a-d) are significantly different ($P < 0.05$).



CV- Central vain of the liver
CL- Centrilobular of the liver
P- Periportal of the liver
F- Fibrosis in the liver
N- Necrosis in the liver
M- Normal liver cell

Figure 5: Photomicrographs of the cross section of the liver of rats in the different groups

DISCUSSION

Hepatic injury is characterized by distortion in hepatic cells; this is often responsible for the high level of AST and ALT. An elevated level of aspartate aminotransferase (AST) (89.20U/L) in the untreated hypercholesterolaemic rat as recorded in this research is in line with the reports of Otunola et al. (14). These researchers reported a high AST (47.25 U/L) in hypercholesterolaemic rats when compared with rats treated for hypercholesterolaemia, though lower than what was observed in this research. Aderiye et al. (15) also reported AST value of 82.4 U/L in the induced hypercholesterolaemic rats which is similar to what was observed in this study. The observed increase in the AST in this study can be ascribed to the effect of elevated level of cholesterol on the liver. Exposure of the liver to stressors usually elicits the production of

the aspartate aminotransferase enzyme (14,16).

The elevated levels of serum ALT and AST are attributed to the leakage of these enzymes into the serum due to damage to liver cells. According to Pincus and Schaffner (17), AST and ALT are typically released into the serum in response to hepatic cell injury. Additionally, as reported by Ioannou et al. (18) elevated ALT levels in the absence of hepatitis viral infection or alcoholism may serve as a risk factor for cardiovascular disease. Aderiye et al. (15) reported that increase in the ALT/AST was an indication of damage to the liver. Damage to hepatic cell is often indicated by levels of AST and ALT as they serve as sensitive indicators of liver cell injury and are help recognize acute hepatocellular damages (19). However, the damage may likely not be only from the liver since other cells and tissue like heart, brain and kidney cells may also release the enzyme when there is an injury.

In untreated hypercholesterolemic-induced rats, serum levels of alkaline phosphatase (ALP) are higher. Elevated ALP is linked with liver diseases stemming from intra or extrahepatic cholestasis and damage to hepatic cell membranes (19). This increase in ALP is frequently observed in patients with both extrahepatic and intrahepatic bile duct obstruction. The heightened cholestasis triggers the synthesis of ALP by the bile duct cells, leading to increased levels of ALP in the bloodstream. In an earlier study on the neutriceutical effect of iru on recovery of malnourished rats, Biobaku et al. (20) reported that fermented *Parkia biglobosa* seeds have no adverse effect on the liver enzymes of the rats, but tend to reduce the biomarkers and protect the liver. The lower levels of AST, ALT and ALP observed in the iru treated groups which close to the control group, indicate that the fermented seeds of *Parkia biglobosa* is not toxic to the hepatic cells as well as the cardiac cells, but have a level of hepato-protective property which is evident in the low level of the liver biomarkers.

The histology of the liver section of rats fed iru supplemented feed showed that the tissue of rats treated with iru appeared normal like the cells of the rats in control group. However, the deadness of cells in the untreated group may have resulted from the effect of the hypercholesterolaemic diet (21). A similar report by Ayo-Lawal et al. (22) showed the recovery of liver cells when tyloxapol was used to induce hypercholesterolaemia in rats. The substituted meal preserved the liver of the rats; this is an indication of the protective capacity of the iru supplemented diets on the liver of the Wistar rats.

The level of protection of the liver cells by the standard drug (fluvostatin) is slower compared to the groups treated with iru samples fermented with *Lactobacillus plantarum*. This is evident in the reduced level of biomarkers as well as the structural integrity of the liver in the iru treated groups. This observation is similar to the report of Ayo-Lawal et al. (22) who reported a better effect of fermented condiment on the liver compared to the standard statin drug used in reducing hypercholesterolaemia in Wistar rats.

CONCLUSION

Iru, whether fermented naturally or with a bacterial starter culture, has demonstrated a promising hepato-protective effect characterized by low levels of toxicological biomarkers and the histopathological examination of rat cells. Using iru derived from the fermentation of *Parkia biglobosa* seeds for medicinal purposes appears to be safe, given its hepatoprotective properties. Furthermore, this condiment is cost-effective, natural, and readily available, making it an attractive option for consumption.

REFERENCES

1. Alfa, A. W., Abolude, D. S., Abdullahi S. A., Yaro, C. A. *et al*. (2018). Effects of Fermented *Parkia biglobosa* Seeds at Varied Dietary Inclusion on Liver Function of Albino Wistar Rat. Asian Journal of Biological Sciences, 11:186-191 DOI: 10.3923/ajbs.2018.186.191
2. Atere, A. V., Oyetayo, V. O. and Akinyosoye, F. A. (2020). Effect of Different Fermentation Time on the Microbial, Mineral and Proximate Composition of *Parkia biglobosa* Seeds Fermented for Iru Production. Sustainable Food Production 7:17-23 doi:10.18052/www.scipress.com/SFP.7.17
3. Atere, A. V. and Aderibigbe, E. Y. (2015). Studies on the causative factors of odour development during fermentation of *Parkia biglobosa*. 2nd International Conference and Exhibition (OWSD-FUTA), 98-103.
4. Sanya. E. A., Ahouansou R. H., Bagan, G., Vianou, A. *et al*. (2013). Effects of some pretreatments of African locust bean seeds (*Parkia biglobosa*) on delivered efficiency of a devised dehuller. Research Journal of Recent Science, 2:1-8.
5. Atere, A. V., Oyetayo, V. O. and Akinyosoye, F. A. (2019). Effects of starter culture on the proximate, antioxidant, antinutritional and mineral composition of fermented *Parkia biglobosa* seeds to produce iru. International Journal of Food

Science and Nutrition, 4:61-65.
6. Aderibigbe, E. Y., Visessanguan, W., Sumpavapol, P. and Kongtong, K. (2011). Sourcing starter cultures for *Parkia biglobosa* fermentation I: Phylogenic grouping of Bacillus species from commercial 'iru' samples. International Journal for Biotechnology and Molecular Biology Research, 2(7): 121-127.
7. Atere, A. V., Adedeji, A., Akinmoladun, A.C., Oyetayo, V. O. *et al*. (2020). Local Condiment, Iru, Obtained from the Fermentation of *Parkia biglobosa* Seed Substantially Reduced the Serum Cholesterol Level of Wistar Rats. Preventive Nutrition and Food Science, 25(2):153-157. https://doi.org/10.3746/pnf.2020.25.2.153
8. Aderiye, B. I. and Laleye, S. A. (2003). Relevance of fermented food products in southwest Nigeria. Plant Food Human Nutrition,56: 1-16.
9. Souleyman, T. (2009). Soumbala (the seed of African Locust Bean): A treatment of Hypertension. Nutrition World, 1: 1-4.
10. Akiyoshi, H. and Inoue, A. (2004). Comparative histological study of teleost livers in relation to phylogeny. Zoological Science 21:841-850.
11. Kim, W. R. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47:1363-1370.
12. Avwioro, O. G. (2014). Histochemistry and tissue pathology principle and techniques. Claverianum Press Nig. Ltd third edition pp 81-97.
13. Atere, A. V., Oyetayo, V. O. and Akinyosoye, F. A. (2022). Iru' Fermented with *Latobacillus plantarum* Significantly Reduced Cardiovascular Risks in Hypercholesterolaemic Rats. Korean Journal of Food and Health Convergence 8(6):1-10.
14. Otunola, G. A., Oloyede, O. B., Oladiji, A. T. and Afolayan, A. A. (2010). Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. African Journal of Biochemistry Research, 4 (6): 149-154
15. Aderiye, B. I., Laleye, S. A. and Odeyemi T. A. (2007). Hypolipidemic potential of *Lactobacillus* and *Streptococcus* sp. from some Nigeria Fermented food. Research Journal of Microbiology, 2(6):538-544.
16. Hongbao, M. A. (2006). Cholesterol and Human Health. The Journal of American Science, 2(1):46-50
17. Pincus, M. R. and Schaffner, J. R. (1996). Assessment of liver function in clinical diagnosis and management by laboratory methods. Philadelphia: W.B. Saunders Company
18. Ioannou, G. N., Weiss, N. S., Boyko E. J., Mozaffarian D. *et al*. (2006). Elevated serum Alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. Hepathology, 43(5):1145-1151.
19. Mohd, A. H., Marghoob, H. and Abdelmarouf, H. M. (2013). Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. European Journal of Experimental Biology, 3 (2):280-284
20. Biobaku, K. T. Thomas, F. C., Aremu, A., Asogwa, N. T., *et al.* (2017). Nutriceutical effects of fermented *Parkia biglobosa* seeds on recovery of malnourished rats. Ceylon Journal of Science, 46(3): 47-53 DOI:http://doi.org/10.4038/cjs.v46i3.7442
21. Laleye, S. A., Aderiye, B. I and David, O. M. (2016). Hypolipidemic Activity of Fermented Sorghum (*Sorghum bicolor* L. Moench) Gruel (Ogi) in Hypercholesterolaemic Rats (Rattusnervegicus). International Journal of Biochemistry Research and Review, 9(1): 1-15.
22. Ayo-Lawal, R. A., Osoniyi, O., Famurewa, A. J. and Lawal, O. A. (2014). Evaluation of antioxidant and hypolipidaemic effects of fermented *Parkia biglobosa* (Jacq) seeds in tyloxapol-induced hyperlipidaemic rats. African Journal of Food Science, 8:225-232. DOI;10.5897/AJFS2013.1124.