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'Iru' Fermented with Latobacillus plantarum Significantly Reduced Cardiovascular Risks in Hypercholesterolaemic Rats

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Abstract

Hypercholesterolaemia is one of the risk factors of coronary health in humans; hence this research was to investigate the effect of *Parkia biglobosa* seeds fermented with *Lactobacillus plantarum* on the cardiac risk factors of diet-induced hypercholesterolaemic Wistar rats. Hypercholesterolaemia in rats were experimentally induced and the hypercholesterolaemic Wistar rats were treated with iru samples. The total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), liver biomarkers and cardiac risks factors were determined after inducement and treatment with iru. Fourteen (14)-days after inducement, the rats in the group induced had the highest weight of 112.40 g while the control group had 94.30 g. The total cholesterol (TC) in the induced group was 100.80 mg/dl while the control had 51.40 mg/dl, triglyceride (TG) in the induced group was 111.75 mg/dl while the control group had 68.45 mg/dl. After 28 days of treatment, the group treated with fermented samples showed a reduction in the TC (100.80 to 56.99 mg/dl), Triglyceride (111.75 to 32.53 mg/dl), LDL (49.48 to 6.65 mg/dl), cardiac risk ratio (3.36 to 1.28), atherogenic coefficient (3.13-0.29) and atherogenic index (0.57 to 0.11). The result from this study reveals that fermented *Parkia biglobosa* sample reduced the cardiac risk of rats significantly.

Keywords: Parkia biglobosa, Lactobacillus plantarum, Hypercholesterolaemia, Cholesterol

Major or classificatios: Food Science (Food Nutrition, Healthy Food)

1. Introduction

Hypercholesterolemia is the presence of high level of cholesterol in the blood and has been reported as one of the major problems facing human societies. Hypercholesterolemia is a source of concern for public health sector because it constitutes one of the major risk factors of cardiovascular diseases (CVD) (Gerhardt & Gallo, 1998; Gomes, Tomaso, Nazarian, Bjarnason,

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Dietz & Hunter, 1998). Although cholesterols are essential in the body to insulate nerves, make cell membranes and in the production of some steroid hormones (Hongbao, 2006), yet excess cholesterol can stick to the wall of artery, causes total blockage of artery which may eventually lead to damage of the heart muscles.

The effect of fermented seeds of *Parkia biglobosa* on the cholesterol level has been documented (Atere, Adedeji, Akinmoladun, Oyetayo & Akinyosoye, 2020b); however, the relative effect of the fermented condiment in relation to the cardiovascular biomarkers will give a clearer picture of the effect of the fermented sample on the cardiovascular system. Therefore, this study was designed to assess the effect of *Parkia biglobosa* seeds fermented with *Lactobacillus Plantarum* on the cardiovascular biomarkers of diet induced hypercholesterolaemic Wistar rats.

2. Literature review

2.1 Relationship of hypercholesterolaemia and coronary disorder

Studies have shown that a high serum total cholesterol or low-density lipoprotein (LDL) cholesterol is a risk factor of new or recurrent coronary disorder (Aronow, 2013). To determine the risk level associated with high cholesterol, certain lipid ratio comparison is extensively used in predicting the effect of the cholesterol-based constituent on the cardiovascular system. Cardiac risk ratio presents precise result better than the ordinary LDL and HDL fractions. It is a biomarker of the potential of developing artery blockage. The atherogenic index has been described as a useful diagnostic tool for predicting cardiovascular diseases risk. As earlier reported, combining the markers of cardiac risk ratio, atherogenic index and atherogenic coefficient could be used for identifying people with higher risk of cardiovascular diseases (CVD) (Bhardwaj, Bhattacharjee, Bhatnagar, & Tyagi 2013; Sasikala & Kalyan, 2020).

2.2 Effect of Fermented products on body cholesterol

Food fermentation enhances the safety, taste, texture, and shelf life of raw materials. During food fermentation, microorganisms play vital and essential roles, contributing to improvement of the physiochemical and sometimes serve as probiotics (Atere, Oyetayo, & Akinyosoye, 2019). Fermentation usually improves the chemical composition of the fermented raw material, thereby making it easily digestible and less toxic. Researchers like Aremu, Ibrahim, Awala, Olonisakin, & Oko, (2015) reported a change in the fatty acid composition of *Parkia biglobosa* seed during fermentation, the observed changes were attributed to the effect of the fermenting bacteria on the short chain fatty acids which leads to accumulation of longer chains as well as the essential fatty acids.

Improvement in the amino acid concentration of fermented samples was reported by Atere, Oyetayo & Akinyosoye, (2020a) where the different increase in the essential amino acid content of the fermented *Parkia biglobosa* seeds samples were documented. The observed differences in the increment of essential amino acid after fermentation was attributed to the fermentation mechanisms of the starter culture used during the fermentation process. Samples of *Parkia biglobosa* seeds fermented with *Bacillus subtilis* was reported to have the highest essential amino acid followed by *Lactobacillus plantarum* and *Leuconostoc mesenteroidis.*

Iru is a fermented condiment, prepared from the seeds of *Parkia biglobosa*. This condiment is often used in cooking traditional dishes, such as melon soups, okra soups, and other vegetable soups. It is very popular among the Yoruba people of Nigeria as it is called ‘iru’ (Aderibigbe, Visessanguan, Sumpavapol & Kongtong, 2011). Iru has been seen as cheap source of protein and reportedly used in treating eye infection. It has also been reported in reducing cholesterol level (Aderiye & Laleye, 2003; Otunola, Oloyede, Oladiji & Afolayan, 2010; Atere et al., 2020b). Hence, the need to investigating the effect of this fermented condiment on the cardiovascular risk factor.

3. Materials and Methods

3.1 Source of P. biglobosa seeds

Raw *P. biglobosa* seeds were bought from a local market (Oja Oba) in Omuo-Ekiti, Southwestern Nigeria. The seeds were processed (Fig. 1) based on the method earlier described by Atere et al. (2019).

Parkia biglobosa seeds
↓
Soaked for 30 minutes
↓
Boiled under pressure for 2 hours (121°C)
↓
Excess water drained
↓
Dehulled
↓
Cooked for 1 hour
↓
Excess water drained
↓
Poured into sterile container
↓
Incubated at room temperature (30±2 °C) for 72 hours.
↓
'iru'

Figure 1: Flow chart for the production of Iru (Atere et al., 2019)

3.2 Experimental animals

The animal study was conducted in accordance with the guidelines for animal experiments approved by the University Institutional Animal Care and Use Committee. Fifty Wistar rats (both sexes) weighing between 45 and 57 g were used.

3.3 Composition of rat's meals

The proximate composition of the commercial feed (growers' marsh) was; moisture content 12 %, crude protein 15 %, crude fat 5 %, fiber 7 %, ash 7 %, carbohydrate 54 %. The body weight of the rats were taken at regular interval to monitor the changes in the rat weight.

3.4 Hypercholesterolemia induction in rats

The rats used in this research were acclimatized for 14 days and subsequently divided to two groups. The first group (control group) was fed with commercial feed while the second group (induced group) was fed with commercial feed compounded with an average amount of 80 mgpork/g pork and 1 ml of aqueous pork extract stock/g body weight of rats daily for two weeks. At the end of the 14 days hypercholesterolaemic inducement, the rats from each group were sacrificed and their serums were analyzed for hypercholesterolaemia and liver enzymes.

3.5 Effect of feeding on rats

In the second phase of the experiment, the induced rats were divided into two groups (Treated and Untreated groups). For the next 28 days, the treated group was fed with feed supplemented with 20 % of 'iru' fermented with *Lactobacillus plantarum*, while the untreated and the control groups were fed with the commercial feed. After the feeding trial, the rats were scarified. The serum was collected and analyzed for serum cholesterol, lipoproteins and liver enzymes.

3.6 Determination of cholesterolaemia level

The blood samples collected in the heparinized bottle was centrifuged at 3000 r.p.m for 30 minutes. The plasma was collected and used in the determination of the cholesterol assay using the Randox kits.

To determinate the total cholesterol level, a reagent blank and standard was prepared, the reagent blank test tube contains

the Reagent (pipes buffer 80 mmol/l; 4-aminoantipyrine 0.25 mmol/l; phenol 6 mmol/l; peroxidase 0.5 u/ml; cholesterol esterase 0.15 U/ml; cholesterol oxidase 0.10 U/ml) 1000 µl and 10 µl of distilled water. The standard test tubes contained the standard cholesterol solution (10 µl) and 1000 µl of the reagent. The samples test tubes contained 10 µl of the samples and 1000 µl of the reagent. The tubes containing the blank, standard and samples were incubated for 5 mins at 37 ºC. The reagent blank was used to zero (blank) the spectrophotometer at wavelength 546 nm. The absorbance of the standard and samples were taken within 60 minutes. The cholesterol level was calculated as follows:
Concentration of cholesterol in sample

$$\text{Cholesterol level} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} X\ concentration\ of\ standard\ (210\ mg/dl)$$

3.7 Determination of triglyceride

A working reagent was prepared from adding 10 ml of buffer solution R1 (pipes buffer 40 mmol/l; 4-chlorophenol 5.5 mmol/l; magnesium-ions 17.5 mmol/l) and enzyme reagent R2 (4-aminophenazone 0.5 mmol/l; ATP 1.0 mmol/l; lipases 150 U/ml; glycerol-kinase 0.4 U/ml; glycerol-3-phosphate oxidase 1.5 U/ml; Peroxidase 0.5 U/ml). From the working reagent a reagent blank was prepared by taking 1000 µl of the working reagent into a test tube. A standard was prepared by introducing 10 µl of standard triglyceride into a test tube and 1000 µl of the working reagent. A volume of 10 µl of the sample was added to 1000 µl of working reagent for the samples test tubes. The mixtures were incubated for 10 minutes at 25 ºC. The absorbance was determined using a spectrophotometer using the blank content to blank the spectrophotometer at wavelength of 546nm. The absorbance was taken within 60 minutes.

The triglycerides concentration was calculated as follows:

$$Triglycerides\ concentration = \frac{Asample}{Astandard} X\ standard\ concentration\ (199\ mg/dl)$$

3.8 Determination of high-density lipoproteins

A working reagent was prepared by mixing R1 and distilled water at ratio 1 to 4 (R= phosphotungestic acid) to give the working reagent 1. A 1000 µl of the reagent 1 was introduced into 200 µl of the serum. The mixture was centrifuged at 4000 rpm for 10 mins. A 100 µl of the supernatant was taken and 500 µl of the standard product was added. A reagent blank was prepared by adding 100 µl to the standard cholesterol reagent. The working standard was prepared by adding 100 µl of the standard supernatant to 1000 µl of the standard cholesterol reagent. For the samples, 100 µl of the sample supernatant was added to 1000 µl of the standard cholesterol reagent. It was incubated at 25 ºC for ten minutes. The absorbance was measured in the spectrophotometer. The HDL was calculated as

$$\text{HDL} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} X \frac{conc.standard}{}$$

(Cholesterol standard 210 mg/dl)

3.9 Determination of low density and very low-density lipoproteins

The low-density lipoprotein was calculated as follows.

$$LDL\ cholesterol = Total\ cholesterol - \frac{Triglyceride}{5} - HDL$$

The very low-density lipoprotein was calculated as

$$VLDL = \frac{Triglyceride}{5}$$

3.10 Determination of the Cardiac risk ratio, Atherosclerosis index and Atherogenic co-efficient.

The cardiac risk ratio was calculated as follows:

$$Cardiac\ Ratio = \frac{Total\ cholesterol}{HDL}$$

$$Atherosclerosis\ index = log10\ (\frac{TG}{HDL})$$

$$Atherogenic\ coefficient = \frac{non\ HDL}{HDL}$$

3.11 Determination of toxicological effect

The blood samples collected in the heparinized bottle was centrifuged at 3000 r.p.m for 30 minutes. The plasma was collected and used in the determination of the toxicological assay using the Randox kits.

3.12 Determination of AST

A reagent blank was prepared in a test tube containing 0.5 ml of R1 (phosphate buffer 100 mmol/l, l-aspertate 100 mmol/l, α-oxoglutarate 2 mmol/l) and distilled water 0.1 ml. A 0.1 ml volume of the sample was introduced into a sample test tubes and the buffer R1 was added. It was incubated at 37 ºC for 30 minutes. After incubation 0.5 ml of R2 (2, 4, dinitrophenylhydrazine 2 mmol/l) was added to both the blank and the samples tubes and incubated for 20 minutes at 25ºC. After the incubation, 5 ml of 0.4 mol/l of sodium hydroxide was introduced to both the blank and the samples. It was left for 5 minutes at room temperature. The content in the blank tube was used in blanking the spectrophotometer at 546 nm before the sample's readings were taken. All the absorbance were read within 60 minutes. The absorbance was matched with the standard curve.

3.13 Determination of ALT

A reagent blank was prepared in a test tube containing 0.5 ml of R1 (phosphate buffer 100 mmol/l, l-alanine 200 mmol/l, α-oxoglutarate 2 mmol/l) and distilled water 0.1 ml. A 0.1 ml volume of the sample was introduced into a sample test tubes and the buffer R1 was added. It was incubated at 37 ºC for 30 minutes. After incubation 0.5 ml of R2 (2, 4, dinitrophenylhydrazine 2 mmol/l) was added to both the blank and the samples tubes and incubated for 20 minutes at 25ºC. After the incubation, 5 ml of 0.4 mol/l of sodium hydroxide was introduced to both the blank and the samples. It was left for 5 minutes at room temperature. The content in the blank tube was used in blanking the spectrophotometer at 546 nm before the sample's readings were taken. All the absorbance were read within 60 minutes. The absorbance was matched with the standard curve.

3.14 Determination of ALP

Diethanolamine buffer (1 mol/l) and $MgCl_2$ (0.5 mmol/l) were mixed with 10 ml of p-nitrophyenyl phosphate (10 mmol/l). A 0.5 ml volume of the mixture was introduced into a test tube containing 0.01 ml of the sample. The absorbance was measured at wavelength 405nm immediately after adding the reagent and after 1, 2 and 3 minutes. The change in the absorbance was calculated and multiplied by 2760 to give value of ALP in the plasma.

3.15 Statistical analysis

Statistical analysis was carried out on data obtained, using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used to compare means at $p<0.05$, with Statistical Package for Social Sciences (SPSS version 16.8).

4. Results

The initial average weight of the rats was 49.30 g and increased to 69.40 g after two weeks of acclimatization. The average weight of the rats after 14 days of induction was 112.40 g in the induced group and 94.30 g in the control group as shown in Figure 2. The physiological changes observed after induction was heavy removal of the rat's fur (hair) in the induced group which was not observed in the control.

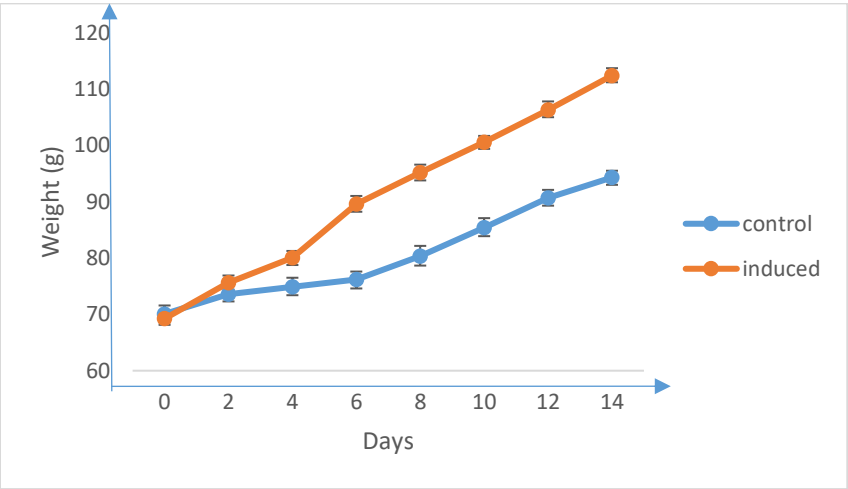

Figure 2: Weight of rats during inducement.

Figure 3 shows the changes in the cholesterol factors of the rats induced into hypercholesterolaemia and the uninduced group. In the group induced into hypercholesterolaemia, the serum cholesterol level was 100.80 mg/dl after 14 days of diet inducement of the rats. The control group had cholesterol level of 51.40 mg/dl. The triglyceride of the induced group was 111.75 mg/dl while the control group had 68.45 mg/dl. The induced group had 29.97 mg/dl of high-density lipoprotein (HDL) while the control group had 28.26 mg/dl. Low-density lipoprotein (LDL) was lower in the control group with a value of 9.80 mg/dl, while the induced group had 49.45 mg/dl.

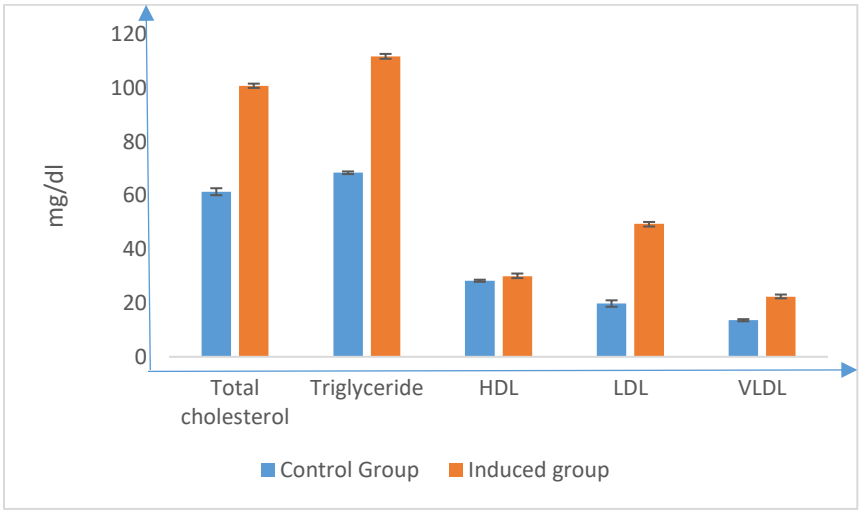

Figure 3: Cholesterol factors of induced and control rats
Key: HDL- High Density Lipoprotein
LDL- Low-Density Lipoprotein
VLDL- Very Low-Density Lipoprotein

Figure 4 shows the toxicological biomarkers in the rats group. Aspartate transaminase (AST) of the rats in the control group was 54.43 U/L while the rats in the induced group had 86.43 U/L. The value of ALP in the control group was 25.02 U/L while in the induced group had 38.66 U/L. The cardiac risk ratio, atherosclerosis index and atherogenic coefficient increased in the hypercholesterolaemic induced rats as shown in table 1.

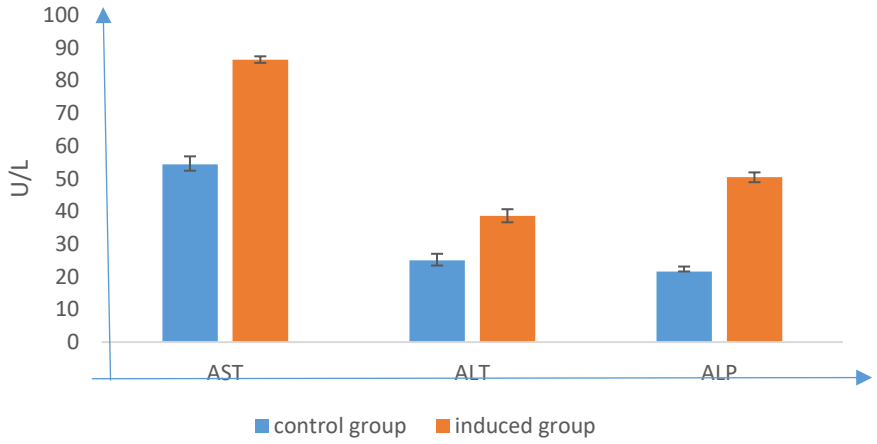

Figure 4: Toxicological Biomarkers of induced and control rats
Key: AST- Aspartate transaminase
ALT- Alanine aminotransferase
ALP- Alkaline Phosphatase

Table 1: Effect of inducement on the Cardiac risk ratio, Atherosclerosis index and Atherogenic coefficient of the control and the hypercholesterolaemic induced rats.

Cholesterol Factors	Control	Induced
Cardiac risk ratio	1.82±0.12	3.36±0.11
Atherosclerosis index	0.38±0.01	0.57±0.02
Atherogenic co-efficient	1.17±0.02	3.13±0.21

Table 2 shows that the total cholesterol was 56.99 mg/dl in the rats induced into hypercholesterolaemia and treated with iru diet, this result is significantly lower than the control and the untreated groups with 68.56 mg/dl and 119.45 mg/dl respectively. The triglyceride is significantly lower in the treated group (32.53 mg/dl) when compared with the rats in the control and the untreated group. Similarly, the HDL was significantly higher in the iru treated rats with a value of 44.03 mg/dl. Both LDL and VLDL were significantly lower in the treated group when compared with the control and untreated group. Both AST and ALT were significantly lower in the treated group while ALP was least in the control group. The cardiac risk ratio was found highest in the untreated group with a value of 4.13 and least in the treated group (1.28). The atherogenic index was least in the treated group with a value of 0.11 and highest in the untreated group with a value of 0.63. The atherogenic coefficient was lowest in treated group and highest in the untreated.

Table 2: Effect of treatment on the cholesterol factor, toxicological factor cardiac risk ratio, atherosclerosis-index and atherogenic coefficient of Wistar rats

	Treated group	Control group	Untreated group
TC (mg/dl)	56.99 ± 0.90^{a}	68.57 ± 0.20^{b}	119.45 ± 1.10^{c}
TG (mg/dl)	32.53 ± 0.81^{a}	79.19 ± 0.56^{b}	124.60 ± 1.31^{c}
HDL (mg/dl)	44.03 ± 0.62^{c}	39.73 ± 0.33^{b}	28.93 ± 0.42^{a}

LDL (mg/dl)	6.65±0.30[a]	16.83±0.32[b]	62.36±0.34[c]
VLDL (mg/dl)	6.47±0.61[a]	15.84±0.50[b]	24.91±0.61[c]
AST (U/L)	50.11±0.71[a]	55.86±0.60[b]	89.26±1.09[c]
ALT (U/L)	34.30±0.22[a]	49.10±0.11[b]	62.09±0.50[c]
ALP (U/L)	39.38±0.50[b]	23.00±0.50[a]	62.60±0.50[c]
Cardiac risk ratio	1.28±0.08[a]	1.73±0.10[b]	4.13±0.10[c]
Atherogenic index	0.11±0.01[a]	0.30±0.01[b]	0.63±0.01[c]
Atherogenic coefficient	0.29±0.05[a]	0.73±0.01[b]	3.13±0.11[c]

key: TC- Total cholesterol, TG- Triglycerides, HDL- High density lipoprotein, LDL- Low-density lipoprotein, VLDL- Very low-density lipoprotein, AST- Aspartate transaminase, ALT- Alanine aminotransferase, ALP-Alkaline phosphatase.

5. Discussion

The body weight of the rats significantly increased in both groups as the feeding days progressed. This observed increase in rats' weight was reported to be the resultant effect of assimilation of nutrient (Otunola et al., 2010). However, the hypercholesterolaemic rats had higher body weight than the control, the observed difference in the body weight may be attributed to increase in fat and protein contents in the diets fed to the induced rats. Edem et al. (2003) earlier reported that increased fat and protein content of meals supplemented in rat often results in increase in the body weight.

Fatty meals have been reported to increase the cholesterol content of the body. The increased cholesterol observed in the induced hypercholesterolaemic rats is like the report of Aderiye et al. (2007) who reported serum total cholesterol of 109.0 mg/dl when rats were fed with pork and pork extract. The reason for the increased cholesterol might be linked to the fact that Acetyl- CoA, a precursor for cholesterol biosynthesis is often elicited by diets rich in saturated fatty acids, which in turn increases the activity of HMG-CoA reductase, which is an enzyme that determines the rate at which cholesterol are produced in the body, thereby increasing cholesterol in the body system (Hillgartner et al., 1995).

The observed increase in the triglyceride is similar to the report of Otunola et al. (2010) where an increase in the triglyceride was reported during dietary inducement of hypercholesterolaemia in rats. The increase in triglyceride in the induced group might be attributed to the effect of cholesteryl ester transfer protein (CETP) which is an enzyme that increased the transfer of cholesteryl esters from HDL to triglyceride rich particles which eventually lead to increased serum concentration of triglycerides (Swati et al., 2014). The increase in the serum triglyceride and total cholesterol in the rats might also have resulted from the enlargement of the intestinal absorption of these lipids (Mihoko et al., 2003). The increase in LDL has been reported to be one of the risk factors of atherosclerosis and related cardiovascular diseases (Getz and Reardon, 2006).

The observed increase in aspartate aminotransferase (AST) after inducement of hypercholesterolaemia in rats is similar to the report of Otunola et al. (2010) where an increase in the AST (47.25 U/L) was observed during induced hypercholesterolaemia in rats using soybeans oil. The increase in the AST can be attributed to the effect of the high cholesterol level on the liver. The liver was stressed and therefore elicit the production of the enzymes.

The report of increase in the ALT was also in line with the report of Sheyla et al. (2005). The increase in the serum ALT and AST was attributed to leakage of the enzyme into the serum because of damage of the heart and liver cells. According to Pincus and Schaffner (1996), AST and ALT are often released into serum when there is an injury on the hepatic cell. In a report of Ioannou (2006), an elevated ALT in the absence of hepatitis viral infection or alcoholism might be a risk factor of cardiovascular disease. Hongbao (2006) also reported that cholesterol is a risk factor of cardiovascular disease.

Alkaline phosphatase (ALP) increased in the serum of the hypercholesterolaemia induced rats. Elicited ALP is associated with liver disease caused by intra or extra hepatic cholestatis and some destruction of hepatic cell membrane (Mohd et al., 2013). Increase in the ALP is often common in patient with extra-hepatic and intra hepatic bile duct obstruction. Increased cholestatis stimulates the synthesis of ALP by the bile ductless cell providing more ALP which ultimately enters the blood.

The observed increase in the cardiac risk ratio, atherosclerosis-index and atherogenic coefficient in the induced group has been found to be an indicator of disorders in lipid metabolism which is a potential risk of the entire spectrum of lipoprotein fractions (Mertz, 1980; Bhardwaj et al., 2013).

With the supplementation of fermented *Parkia biglobosa* seeds into the rats' feeds, a clear reduction in the TC, TG, LDL

and VLDL was observed. The observed reduction is in line with the earlier report of Atere et al. (2020b) and was linked to the ability of fermenting bacteria to produce secondary metabolites which helps in the bioconversion of low-density lipoprotein to high density lipoprotein, this mechanism may have accounted for the observed increase in HDL of the rats in the treated group. Previous researchers (Rasic et al., 1992; Noh et al., 1997; Aderiye et al., 2007) had earlier reported that *Lactobacillus* spp can break cholesterol in the serum, this may have accounted for the observed decrease in cholesterol level (Maki et al., 2012).

The AST, ALT and ALP are enzymes indicators of the health state of the liver, As observed in this study, the treatment of hypercholesterolaemia using fermented seeds of *Parkia biglobosa* is safe since the rats in the group treated had low level of AST ALT and ALP which is an indication that the iru sample is not toxic to the hepatic cells.

Cardiac risk ratio which is an indicator of the potential of developing arterial blockage in the heart was observed to have reduced after treatment. The observed reduction is an indication of the effectiveness of the supplemented fermented feed sample in reducing atherosclerosis.

Atherogenic index and atherogenic coefficient are biomarker of plasma atherosclerosis and has been reported to be directly related to the development of atherosclerosis. As observed in this study, both were reduced significantly in the treated group compared with the untreated, which is an indication of the potential of the fermented seeds of *Parkia biglobosa* in preventing the development of atherosclerosis.

6. Conclusion

This research revealed that pork and pork extracts are effective in inducing hypercholesterolaemia in rats within a short period of 14 days without necessarily using cholic acid. This submission is based on the raised level of total cholesterol and the LDL-C in the induced rats. The diet led to hepatic and cardiovascular stress in the induced rats which is evident in the increased cardiac risk ratio, atherosclerosis-index and atherogenic coefficient. Hence, the result obtained in this study can be used in atherogenesis studies. Data gathered from this treatment revealed that iru diet reduced both the cholesterol as well as cardiovascular stress in Wistar rats, hence *Lactobacillus plantarum* fermented *Parkia biglobosa* seeds has cardiovascular protecting capacity and can be seen as a cheap remedy to cardiovascular diseases elicited by high level of cholesterol.

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