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Effect of Fermented Nunu on Serum Lipid Profile of Female Wistar Rats

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ABSTRACT

Several studies have established the relationship between an individual's lipid profile and risk of cardiovascular diseases which accounts for a high mortality rate in women. This study delves into the effect of fermented nunu in female Wistar rats while exploring the possibility of reducing cardiovascular risks. Four different samples of fermented milk namely; yogurt, laboratory fermented milk (nunu), locally fermented milk, and the normal feed and water (as control) was used to feed the female Wistar rats for 14 days after which they were sacrificed. Blood samples were collected and analyzed for HDL, LDL, triglycerides, and total cholesterol. The result of the summation of the mean for the LDL (low-density lipoprotein) analysis are as follows; 56.416(group 1), 52.785(group 2), 48.479(group 3), and 53.810mg/dl (group 4). The result for the summation of the mean for the total cholesterol is as follows 80.892(group 1),78.411(group 2), 82.891(group 3), 76.764mg/dl (group 4). The result of the summation of the mean for the triglycerides analysis is as follows 57.42(group 1), 55.425(group 2),60.197(group 3), 56.654mg/dl (group 4). The result of the summation of the mean for the HDL (high-density lipoprotein) analysis is as follows 51.267(group 1), 53.385(group 2),46.073(group 3), 52.269 mg/dl (group 4).The effect of fermented nunu on the total cholesterol, triglycerides, HDL, and LDL was more remarkable in rat subjects fed Yoghurt and Laboratory Fermented Nunu.

KEYWORDS: Cardiovascular Diseases; Effect; Fermented Nunu; Female Wistar Rats; Lipid

1. Introduction

Milk fermentation is a very common practice in Africa. Among the Hausa community in Nigeria, fermented milk is known as Nunu. Fermented foods have been part of human diets and they provide health benefits. These health benefits provided by fermented foods are related to the bioactive compounds and other compounds formed during fermentation (Ivey *et al.*, 2015; Sanlier *et al.*, 2017). Nunu is one of the fermented yogurt-like milk consumed in west Africa; it is a product from raw milk obtained from cows, sheep, or goats that are hardly pasteurized. It is yogurt-like in taste (a sharp, acid taste) and it can be taken alone. They can be fermented from milk naturally or by the use of starter culture to produce the desirable milk products (Owusu-Kwarteng *et al.*, 2017).

The most common examples of fermented milk are yogurt, cultured cream and buttermilk, and kefir, although many variations of these products exist

based on historical practices, geography, and type of milk. The popular ones in Africa, Syria, India, America, and Nepal include Cheese, nunu, buttermilk, yogurt, irgo, kadam, laban, shenineh, dahi, shirkand, mahi, etc. (Ajayi, 2006). The local production of fermented milk in Nigeria and other African countries does not involve the use of starter cultures, suggesting that the fermentation arises spontaneously from microbes originating from the environment, processing equipment, or processors (Eyassu *et al.,* 2012). The Nunu is kept unrefrigerated and its acceptability depends on the texture, flavor, and taste which are in turn dependent upon the inherent microbial constituents (Obi *et al.,* 2007). During the production of nunu, the fermenting microorganisms interact with the milk components and transform the liquid milk into a yogurt-like product. These fermenting microorganisms may produce beneficial metabolites or may themselves positively interact with the host, referred to as the probiotic effect (Roupas *et al.,* 2009).

Beyond the refreshing benefit, they can also be explored for their health benefits. The role of fermented milk products on human health has been the subject of extensive research, includingepidemiological, observational, and clinical studies; some of which include boosting immunity; reducing the occurrence of some illnesses lowering cholesterol levels amongst others. (Adebolu *et al.,* 2007; Maltock, 2007; Savaiano *et al.,* 2021). These roles have been explored to determine the effects of fermented nunu on lipid profile. A lipid profile is often used to assess a person's possibility of developing cardiovascular diseases (Miller *et al.,* 2011). Hyperlipidemia is characterized by a rise in either one or all of the following factors; total cholesterol (TC), Low-density Lipoproteins (LDL), or Triglycerides (TG), and also associated with a decrease in High-density Lipoprotein (HDL). (Liu *et al.,* 2010). Various studies have associated increased risk of cardiovascular diseases with high levels of TC, LDL, TG, and low level of HDL; hence the parameters can be used as biomarkers (Upadhyay, 2015; Dayimu *et al.,* 2019). While cholesterol triglycerides, and high-density lipoproteins are important constituents of the lipid fraction of the human body, any abnormality could be dangerous to the health, and early detection is an effective way of tackling, managing, and preventing untimely deaths (WHO 2017).

Cardiovascular diseases (CVD) continue to be the leading cause of death among women in the United States, accounting for approximately one of every three deaths in females and becoming more prominent in younger women (Garcia *et al.,* 2016; Young and Cho, 2019). With the high rate of consumption of nunu in Nigeria and Africa at large, there is a need to ascertain if the product has some effect on the lipid profile of female individual particularly the possibility of reducing the risk of cardiovascular diseases considering the lipid profiles as will be seen in this study using female rat subjects. If found so, it could easily be incorporated into diets since it's readily available.

Although the incidence of CVD in women is usually lower than in men, women have shown to have higher mortality and possible worse verdict after acute cardiovascular events. (Gao *et al.,* 2019). The mortality rate is said to be due to some factors which include hormonaldifferences. Also, because a percentage of women do not easily adapt to drugs or medical procedures needed to manage the condition (Bots *et al.,* 2017; Rodgers *et al.,* 2019). This presents the need to explore the possibility of management through diet. This study explores the effect of fermented nunu on the serum lipid profile of female Wistar rats.

2. Materials and methods

2.1 Sample preparation

The batch of laboratory fermented nunu used was processed in the laboratory by the addition of *lactobacteria* to sterilized fresh milk The locally fermented nunu was purchased from local sellers at Ikare junction, Owo, South-western Nigeria. It was kept in a clean keg. The Yoghurt was purchased from a grocery store in Akure, Southwestern Nigeria.

2.2 Experimental design

A total of 40 wistar strained female rats were obtained from the animal house of the Department of Medicine, Ekiti State University, Ekiti State, South-western Nigeria. The animals wereacclimatized for two weeks. The animals were housed and maintained under standard conditions (12h light/dark cycles).

2.3 Experimental Setup

The study spanned 14 days. The rats were randomly selected and divided into 4 groups containing ten (10) animals each as follows:

Group 1 received normal feed and distilled water only.

Group 2 received 400ml of laboratory fermented nunu.

Group 3 received 400ml of locally fermented nunu. Group 4 received 400ml of yogurt.

2.4 Collection of blood sample

At the end of the experimental period which was fourteen (14) days. The animals were sacrificed and blood samples were collected through heart puncture. The blood samples were collected into EDTA bottles. Blood was spun for 10 minutes at 4000rpm to obtain serum. Approximately 1 mL of blood was taken from each rat, transferred to sterile tubes, and kept on ice for 30 min.

2.5 Biochemical analysis

2.5.1 Total cholesterol

Total cholesterol was measured using the Total Cholesterol Assay Kit by Randox Laboratories Ltd., Crumlin, UK. The kit utilizes a modified CHOD-PAP method for the measurement of total cholesterol as described by Trinder (1969). The results were obtained and the absorbance was read on a spectrophotometer at 500nm.

2.5.2 Triglycerides

Triglyceride concentration was measured using Triglyceride Assay Kit by Randox Laboratories Ltd., Crumlin, UK. The kit utilizes a GPO-PAP modified method described by Trinder (1969). The results were obtained and the absorbance was read on a spectrophotometer at 500nm.

2.5.3 High-density Lipoprotein

High-density lipoprotein was determined using Randox direct HDL-cholesterol kit by Randox Laboratories Ltd., Crumlin, UK. The results were obtained and the absorbance was read on a spectrophotometer at 500nm.

2.5.4 Low-density Lipoprotein

Low-density lipoprotein was determined using Randox direct LDL-cholesterol kit by Randox Laboratories Ltd., Crumlin, UK. The results were obtained and the absorbance was read on a spectrophotometer at 500nm. The reason for choosing this kit over the Friedewald equation as described by Friedewald *et al.*, (1972) is because it eliminates errors that could occur from the presence of triglycerides.

2.5.5 Statistical analysis

Data obtained from the study were analyzed by descriptive statistics and presented as mean ± standard deviation. Using the statistical software package (SPSS) for the Windows version, the difference was separated by using the analysis of variance (ANOVA) and multiple comparison tests

3. Results and discussion

Total cholesterol

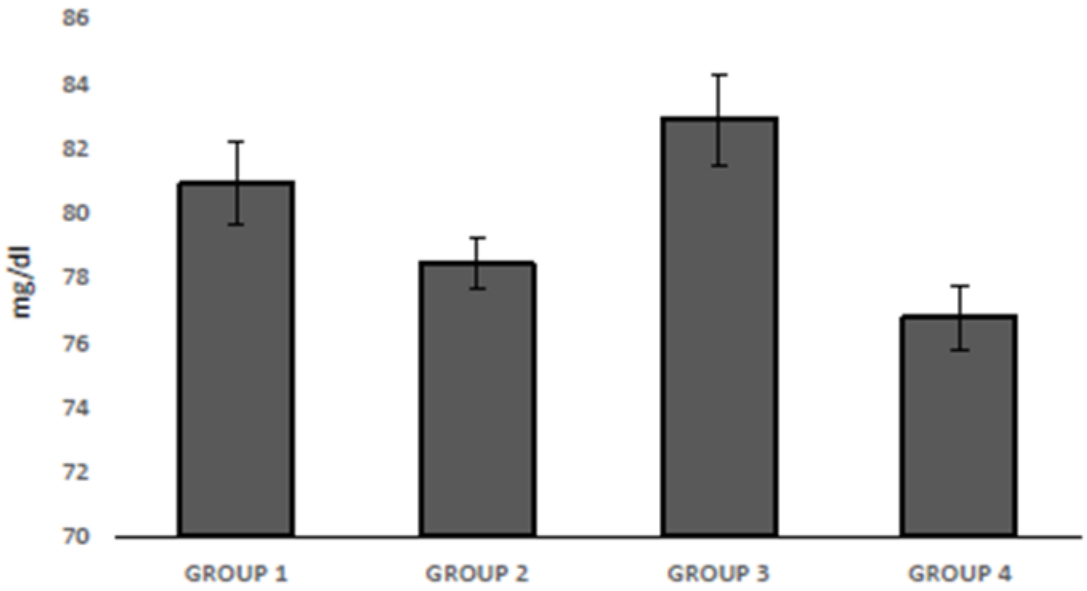

Figure 1: Total cholesterol levels of female Wistar rats

Triglycerides

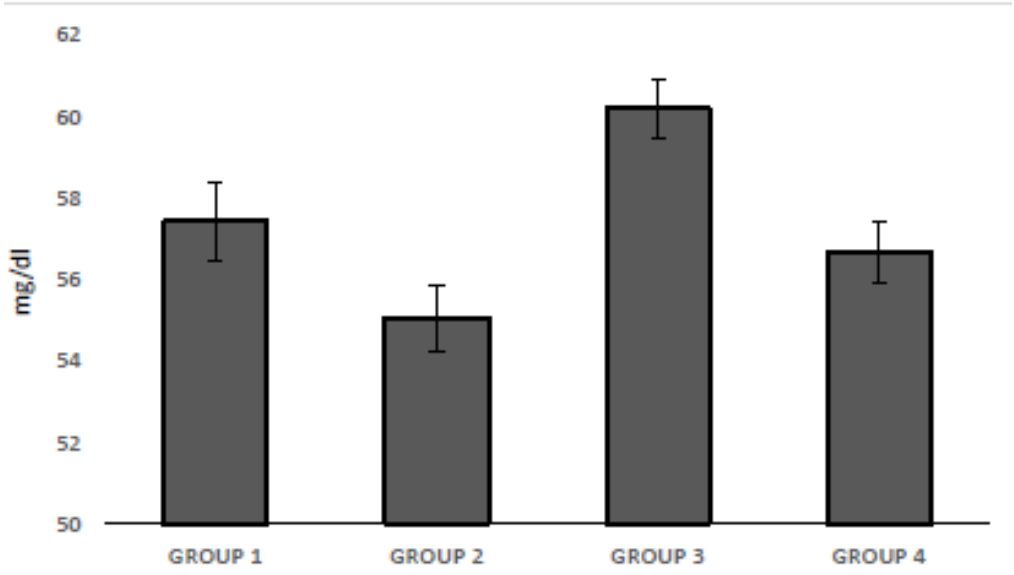

Figure 2: Triglyceride levels of female Wistar rats

High-Density Lipoprotein

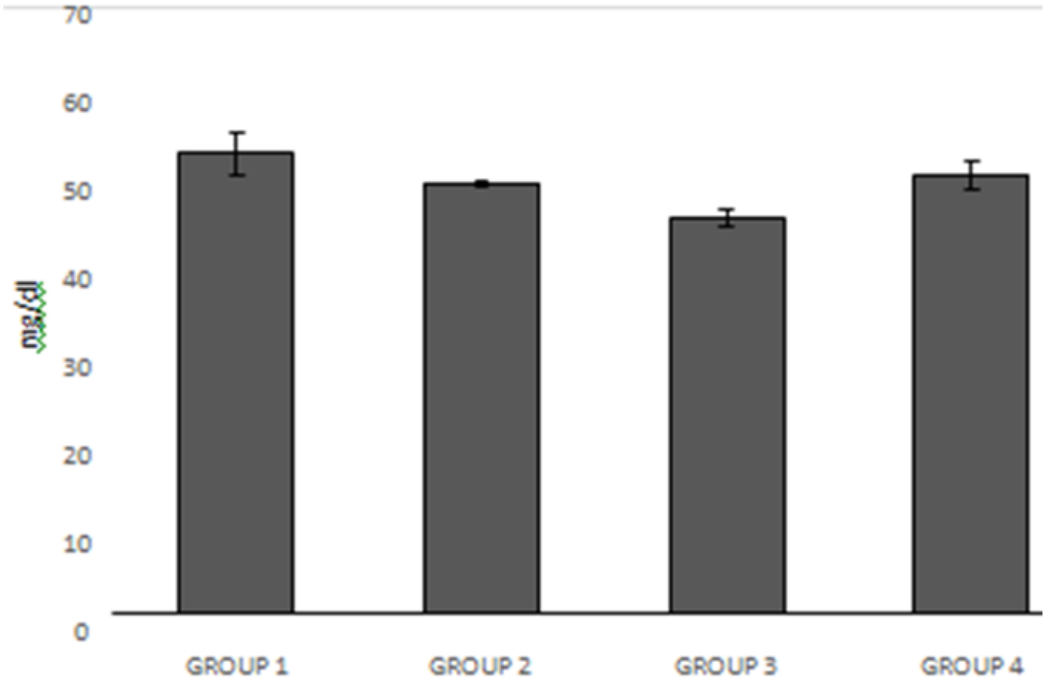

Figure 3: High-density lipoprotein levels of female Wistar rats

Low-density Lipoproteins

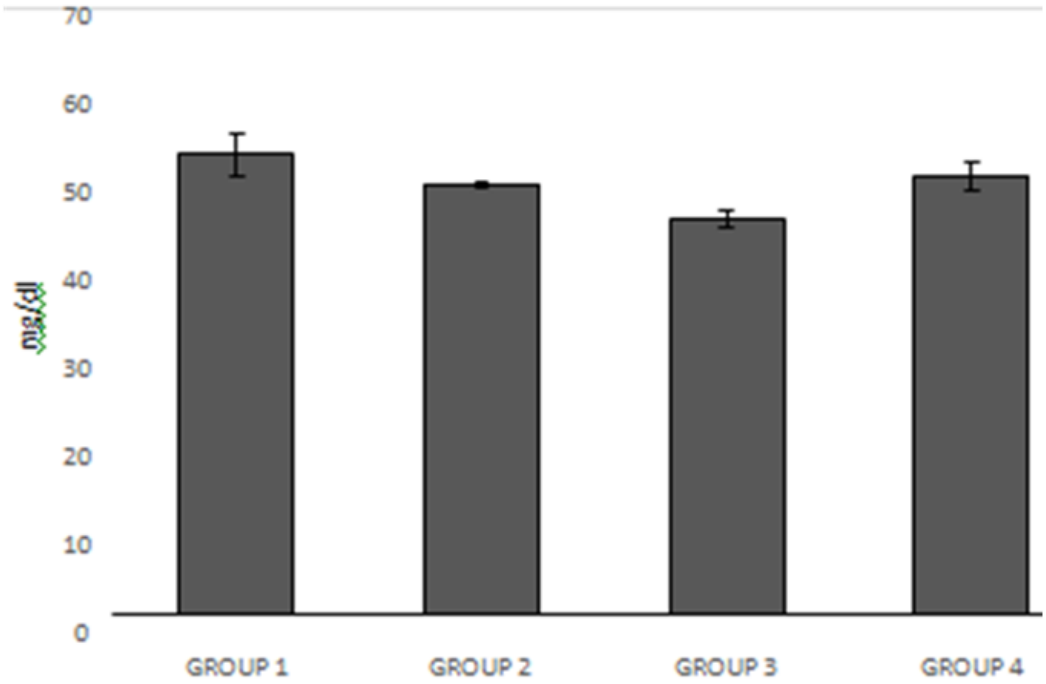

Figure 4: Low-density lipoprotein levels of female Wistar rats

4. Discussion

4.1 Total cholesterol

A decrease in Total Cholesterol was observed in groups 2 and 4 which are the laboratory fermented nunu and Yoghurt fed respectively as shown in Figure 1. The group fed with locally fermented nunu showed a higher level than the control group. A study by Jeoung *et al., (*2018) indicated that increased cholesterol levels were associated with a high risk of cardiovascular diseases while postulating that lower levels would depict reduced risk. The results obtained for Total Cholesterol agrees with Shimizu *et al.,* (2015) and Pourrajab *et al.,* (2019) that fermented milk products are effective in decreasing TC levels.

4.2 Triglycerides

The result is shown in Figure 2. An increase was observed in group 3 which was fed with locally fermented yogurt. Groups 2 and 4 which were fed laboratory fermented nunu and yogurt showed levels slightly lower than the control group indicating an average reduced level of triglycerides. This is considered positive because a continuously high triglycerides level increases the risk of heart diseases even with an optimal LDL level (Miller *et al.,* 2011; Scherer and Nicholls, 2015; Ye *et al.,* 2019).

4.3 High-Density Lipoprotein

As seen in Figure 3, the groups fed laboratory fermented nunu and yogurt gave results higher than the control. Groups 2 and 3 which were fed laboratory fermented nunu and yogurt showed a higher level of HDL compared to the control group, with the locally fermented nunu presenting lower levels compared to the control. The HDL transports cholesterol from the arteries to the blood while the LDL does the opposite. The HDL inhibits cellular uptake of LDLand also serves as a carrier that removes cholesterol from the peripheral tissues and transports it back to the liver for catabolism and excretion (Agbedana, 1997; Maisto *et al.,* 1999; Georgi, 2002).

The Journal of the American college of cardiology established that lower HDL cholesterol levels were associated with a higher risk of death from cardiovascular causes. High serum HDL levels have been proven to protect LDL from oxidation. It is also indicative of a healthy metabolic system if other complications do not exist. Hence, an increase in HDL allows more cholesterol to be transported to the blood and can also be used in energy generation in the cells when there is a drop in the level of triglycerides. This will consequently lead to a drop in the level of cholesterol in the body which is beneficial to the body.

4.4Low-density Lipoproteins

The result is shown in Figure 4. A reduced level of LDL was observed across all groups with the laboratory-fermented nunu and yogurt-fed groups showing the closest LDL levels to the control group. Elevated levels of LDL characterize hypercholesterolemia. It is postulated that nunu can lower LDL cholesterol by stimulating the hepatic LDL receptor (Agbedana, 1997; Prestamo *et al.,* 2002). Some other studies postulate that the decrease is achieved by the conversion of cholesterol to bile acids (Banjoko *et al.,* 2012).

The reduction of LDL has been associated with a decrease in cardiovascular morbidity and mortality. Martin *et al.,* (2012) and Pedro-Botet, and Pintó, (2019) established that the lower the LDL, the better.

The results obtained imply that fermented nunu lowers LDL cholesterol and it agrees with the earlier findings by Prestamo *et al.,* (2002); Shimizu *et al.,* (2015), and Pourrajab *et al.,* (2019) that there was a significant decrease in the serum low-density lipoproteins in rats fed with nunu. This further agrees with the finding of Sonestedt *et al.,* (2011); Magdy *et al.,* (2017), and Savaiano and Hutkins, (2021) that probiotic fermented milk has hepatoprotective effects. The study showed a decrease in the Cholesterol, Low-density lipoproteins (LDL), and Triglycerides values as well as an increase in High-density lipoproteins for the laboratory fermented nunu and yogurt fed female Wistar rats as compared with the control group. The reasons for the deviation and differences observed from the locally fermented nunu fed group in some of the assays could be due to the absence of

regulation during the fermentation process as compared with the laboratory and industrial fermentation process for yogurt.

5. Conclusion

Our study supports that the laboratory fermented nunu provides health benefits beyond its good taste. It could be deduced that fermented nunu has the potential to protect against cardiovascular diseases, maintain healthy lipid levels and improve cardiovascular health generally. Thus, fermented nunu can be incorporated into the diet to manage cardiovascular issues in females. However, further studies are advised.

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