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The Hypocholesterolemic and Glucose Modulatory Effect of Fibre Enriched Snack in Hyperlipidemic/Hyperglycemic Rat Model Induced by High Cholesterol and High Fructose Diet

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Abstract

Dietary interventions have been used in the management of hyperlipidemia. Incorporation of dietary fibre into food products enhances their nutritional value. This study was done to investigate the effect of a fibre enriched snack on the blood glucose level and lipid profile in hyperlipidemia-hyperglycemia - induced wistar albino rats. The rats (n=25) were divided into 5 groups, 1 - fed standard laboratory diet (Control), 2 - fed hyperlipidemic diet and 10% fructose solution, 3 - fed hyperlipidemic diet, 10% fructose solution alongside fibre enriched snack, 4- fed hyperlipidemic diet which was withdrawn before feeding fibre enriched snack, 5 - fed standard laboratory diet and fibre enriched snack, 6 - fed hyperlipidemic diet, 10% fructose solution and a conventional antilipemic drug (CAD). The duration of the experiment was for 6 weeks. Statistical analysis was done using graph pad prism 6.01 version. At the end of the experiment, it was found that the fibre enriched snack stabilized blood glucose levels, reduced serum total cholesterol (TC) ($p<0.01$) and low density lipoproteins (LDL) ($p< 0.01$) in the hyperlipidemic diet fed group. There was, however, no significant change in the triglycerides (TG) as well as the high-density lipoproteins (HDL) in the hyperlipidemic group fed with the fibre enriched snacks. The results indicate that the fibre enriched snack has hypoglycemic and hypolipidemic potential and could, therefore, serve as an effective dietary intervention in the management of diabetic dyslipidemia

Keywords: Antilipemic; Dietary; Cholesterol; Diabetic; Fibre; Glucose

Introduction

Hyperlipidemia has significantly contributed to the increased risk of cardiovascular disease burden, the leading cause of mortality. Globally, hyperlipidemia is associated with more than 50% of ischemic heart disease and over 4 million deaths are recorded annually due to the diseases it degenerates into [1]. More so, defects in insulin action and hyperglycemic conditions caused by hyperlipidemia leads to the pathogenesis of diabetes [2]. Globally, the diabetic population has been projected to increase by 156% in 2045 [3]. Hyperlipidemia and hyperglycemia affect lipid and carbohydrate metabolism causing elevations in total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG) and glucose with reduction in high density lipoprotein (HDL). The elevations in glucose, TC and LDL causes an

increased inflammatory response and triggers oxidative stress which further constricts the vascular endothelium causing complications [4]. The sustained inflammatory state in hypercholesterolemia deactivates the insulin signaling receptors in pancreatic beta cells causing insulin resistance, majorly responsible for pathogenesis and progression of complications in patients with type 2 diabetes mellitus [5].

The incorporation of dietary fibre into food products has been found to be effective, safe and readily available in comparison to the use of most conventional medications in the management of hypercholesterolemia [6]. Dietary fibre has been reported to reduce blood cholesterol and stabilize blood glucose levels by delaying glucose/ fatty acid absorption, increasing cholesterol and bile acid excretion [7]. Dietary fibre also reduces systemic inflammation by lowering pro-inflammatory cytokines and C-reactive protein (CRP) [8]. Snacks enriched with fruit fibres have been shown to improve insulin sensitivity, atherogenic index and demonstrate protective effect against oxidative stress related damage in brain, kidney and liver of diabetic rats [8-10]. Therefore, this research work was directed towards evaluating the effects of a fibre enriched snack on serum lipid profile, blood glucose and serum insulin levels in an experimental model using Wistar rats fed on a hyperlipidemic-hyperglycemic diet.

Materials and Method

Fibre Enriched Snacks

Doughs produced from a composite flour of wheat and fruit fibres were baked as snacks, using Federal Institute of Industrial Research Oshodi (FIIRO) baking protocol [11].

Chemicals

Cholesterol, cholic acid and fructose powder (manufactured by Sigma Aldrich, Co, U.S.A) and a CAD were purchased from a Chemical store in Lagos, Nigeria.

Experimental Animals

Healthy male albino rats were obtained from the Animal house of the Physiology Department, College of Medicine and University of Lagos, Nigeria. The animals were kept and maintained under standard conditions (12 h light and dark cycles, with room temperature at 25°C), fed on standard laboratory diet and water adlibitum and used for the study, after an acclimatization period of 2 weeks.

Experimental Design for Study: Male albino rats (n= 30), weighing between 80 -100g, were divided into 6 experimental groups consisting of 5 rats each.

Group 1 / Normal Control Diet (NCD) Group - This group received the standard laboratory diet (normal rat chow) and distilled water for 6 weeks.

Group 2 / Hyperlipidemic Diet (HCD) / Hypercholesterolemic Diet Control - This group received the hypercholesterol diet (standard laboratory diet + 1% cholesterol + cholic acid) administered alongside 10% fructose solution (replaced the water for drinking) for 6 weeks.

Group 3 (HCD + FES): This group received the hyperlipidemic diet + 10% fructose solution for the first 3 weeks and subsequently the fibre enriched snack (FES) was administered alongside HCD for another 3 weeks.

Group 4 (HCDW + FES): This group received the hyperlipidemic diet + 10% fructose solution for the first 3 weeks and subsequently the fibre enriched snack (FES) was administered with the withdrawal of HCD for another 3 weeks.

Group 5 (NCD + FES): This group received the standard laboratory diet for the first 3 weeks and subsequently the fibre enriched snack (FES) was administered alongside NCD for another 3 weeks.

Group 6 (HCD + CAD): This group received the HCD + 10% fructose solution for the first 3 weeks and subsequently a conventional antilipemic drug was administered alongside HCD for another 3 weeks.

Determination of Fasting Blood Glucose

The blood glucose level of the animals was determined after 12 hours overnight fast. ''The tails of the animals was punctured with the set Accu-check Softclix (Roche Diabetes Care, Germany) and the blood was put on the glucose strips inserted in the Accu-check active blood glucose meter'' [12]. The blood glucose level was measured before the start of the experiment, 3 weeks after induction of hypercholesterolemia and at the end of treatment.

Determination of Blood Lipid Level

The rat blood samples were taken through plexus Retroorbitalis in the eye. The blood was allowed to clot and then centrifuged at 3000rpm for 15 minutes. The lipid analysis was then carried out on the plasma samples obtained. The Biolab assay kit and automated chemistry analyser (SFRI, BSA, France) was used to analyze the triglycerides (TG), total cholesterol (TC) and High Density Lipoproteins (HDL) with slight modifications in the type of analyzer used [13]. "The

Low Density Lipoprotein was estimated using the Friedwald equation'' [14]. The Friedwald equation is as follows:

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

Determination of Insulin

The serum insulin was determined using Enzyme linked immunosorbent assay (ELISA) method [15].

Statistical Analysis

Statistical analysis of the differences between the mean values of different experimental groups was calculated using Graph Pad Prism (6.01). Data was subjected to one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. In all cases, p values < 0.05 was regarded as statistically significant.

Results

The results obtained from this study are presented in Figures 1-5 and Table 1

EXPERIMENTAL GROUPS	INSULIN (µg/ml)
Group 1 (NCD)	0.59 ± 0.04
Group 2 (HCD)	0.47 ± 0.04#
Group 3 (HCD + FES)	0.56 ± 0.06
Group 4 (HCDW + FES)	0.62 ± 0.04**
Group 5 (NCD + FES)	0.61 ± 0.05**
Group 6 (HCD + CAD)	0.38 ± 0.06

Table 1: Serum Insulin Levels of Different Experimental Groups.

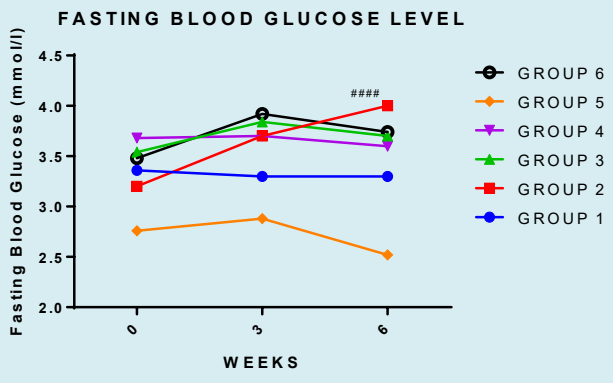

Figure 1: Fasting blood glucose level (mg/dl) of animals in different experimental groups. Values are represented as mean + SD, N=5 ####P < 0.0001 when week 6 was compared to week 0.

Figure 1 shows the fasting blood glucose levels (mg/dl) of animals in the different treatment groups. There was a significant increase in fasting blood glucose in Group 2 (HCD group) at week 6 (the final week of the experiment) compared with week 0 (the first week). There were, however, no significant differences across all the other experimental groups when week 6 was compared with week 1.

Blood cholesterol levels (mg/dl) of animals in the different treatment groups are presented in Figure 2.

The cholesterol level was significantly increased (P < 0.001) in group 2 (HCD) compared with group 1 (NCD). There was, however, a significant reduction in cholesterol level in group 3 (HCD + FES) and group 4 (HCDW + FES) at P < 0.001 as well as in group 5 (NCD +FES) P < 0.01 and Group 6 (HCD + CAD) P< 0.0001 compared with group 2.

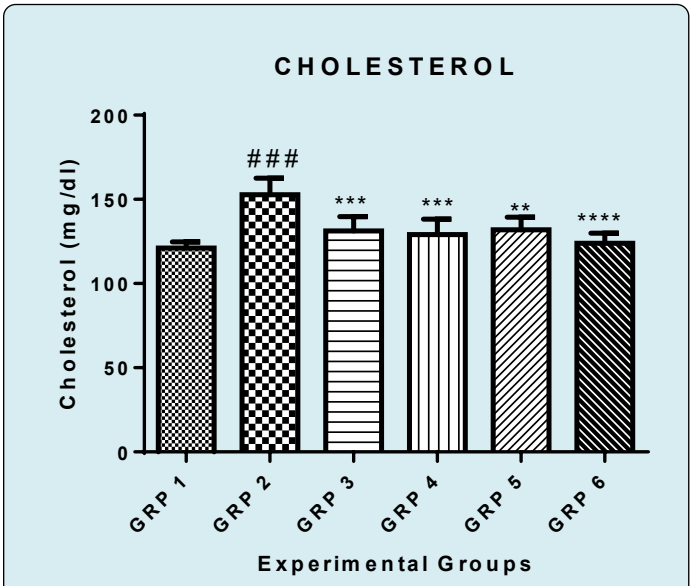

Figure 2: Blood cholesterol level (mg/dl) of animals in different experimental group. Values are represented as mean + SD, N=5 ###P < 0.001 when compared to Group 1, ****P < 0.0001 when compared to Group 2, ***P < 0.001 when compared to Group 2, **P < 0.01 when compared to group 2.

The LDL level (mg/dl) of animals in the different treatment groups are presented in Figure 3. It can be observed that the LDL level was significantly increased (P < 0.0001) in group 2 in comparison to group 1. It was, however, significantly decreased in groups 3, 4 and 5 (P < 0.01), as well as in group 6 (P < 0.0001), compared with group 2.

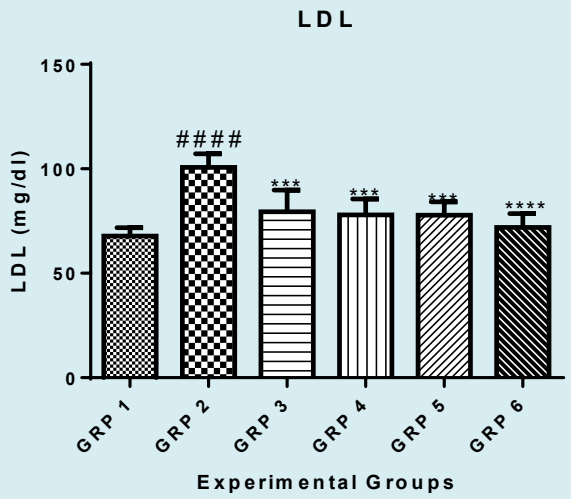

Figure 3: LDL level (mg/dl) of animals in different experimental group. Values are represented as mean + SD, N=5 ####P < 0.0001 when compared to Group 1, ***P < 0.001 when compared to Group 2, **P < 0.01 when compared to Group 2.

Figure 4 shows HDL levels (mg/dl) of animals in the different treatment groups. The HDL level was significantly decreased (P < 0.01) in group 2 compared with group 1. There was no significant difference in HDL in groups 3, 4 and 6 compared with group 2, however, there was a significant increase (p< 0.05) in HDL level in group 5 in comparison with group 2.

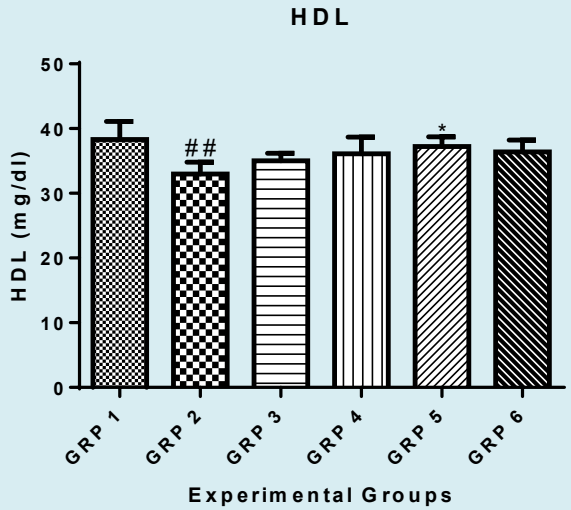

Figure 4: HDL level of animals in different experimental group. Values are represented as mean + SD, N=5 ##P < 0.01 when compared to Group 1, *P < 0.05 when compared to Group 2.

The LDL level (mg/dl) of animals in the different treatment groups are presented in Figure 3. It can be observed that the LDL level was significantly increased (P < 0.0001) in group 2 in comparison to group 1. It was, however, significantly decreased in groups 3, 4 and 5 (P<0.01), as well as in group 6 (P < 0.0001), compared with group 2.

Triglyceride level (mg/dl) of animals in the different treatment groups are presented in Figure 5. A significant increase (P < 0.001) in triglyceride levels was observed in group 2 in comparison to group 1. There was a significant decrease in triglyceride level in group 3 (p< 0.05) and group 4 (p < 0.001) compared with group 2. There was also a significant decrease in triglyceride level in Group 5 and 6 (P < 0.01) compared with group 2.

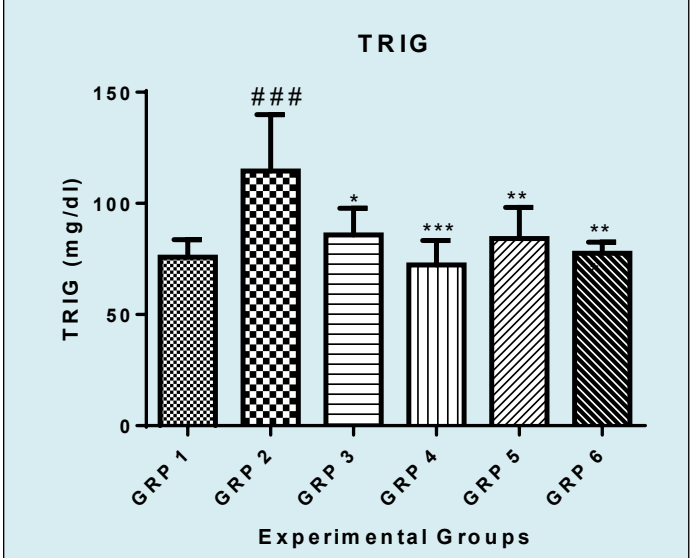

Figure 5: Triglyceride level (mg/dl) of animals in different experimental group. Values are represented as mean + SD, N=5 ###P < 0.001 when compared to Group 1, *P < 0.05 when compared to Group 2, **P < 0.01 when compared to Group 2, ***P < 0.001 when compared to Group 2.

Serum Insulin level (ug/ml) of animals in the different treatment groups are presented in Table 1. There was a significant decrease (p< 0.05) in serum insulin levels in Group 2 (HCD) in comparison with Group 1 (NCD). The levels were significantly increased (p< 0.01) in Group 4 (HCDW + FES) and group 5 (NCD + FES) in comparison with Group 2 (HCD).

Discussion

The economic cost of managing diseases associated with hyperlipidemia and hyperglycemia are quite enormous.

Notwithstanding the continuous rise in the number of populations associated with these conditions especially the diabetic and obese population. The increase in diabetic population globally an especially in developing country is of great concern among the myriad of other non-communicable diseases affecting developing countries with their poor health infrastructures. Moreso, most conventional medications used in the management of such hyperlipidemic/ hyperglycemic conditions are not readily available and present a wide range of adverse effects such as diabetes, liver/ renal damage, muscle myopathy, insomnia, drowsiness and digestive disturbances. This has led to the quest for effective, safe, affordable and readily available treatment options. Incorporation of dietary fibre in snacks reduces their caloric content and enhances their nutritional value. In this study, we investigated the ability of fibre enriched snacks to reduce blood lipid and glucose level, while boosting blood insulin levels in hyperlipidemia-hyperglycemia induced rats.

Chronic hyperglycemia is a well-known hallmark for patients with type 2 diabetes causing further metabolic complications such as hyperlipidemia in such pathological state. Hyperlipidemia increases the levels of free fatty acids which impairs the function of pancreatic beta cells and induces insulin resistance facilitating increased blood glucose levels [16]. The significant increase of fasting blood glucose in the group fed on a high cholesterol diet and fructose solution (Figure 1) justifies this, such as has been confirmed by Afzal, et al. [17]. This corroborates the trend in the increased consumption of cholesterol rich fatty foods and sugary drinks causing an increase in diabetic population. The stability of blood glucose levels in the groups fed the fibre enriched snacks suggests hypoglycemic activity. This finding is similar to that of Yesmin, et al. [18], where the ingestion of dietary fibre along with a glucose drink reduced the sharp and sustained rise of postprandial glucose. Dietary fibre especially the soluble fibre slows the absorption of carbohydrates, improving blood sugar levels [19].

The significant reductions in cholesterol level and LDL by 13.74% and 21.21% respectively, (Figure 2 and Figure 3), suggests that the fibre enriched snacks was able to achieve a marked reduction in blood cholesterol and LDL levels. Administration of the fibre enriched snacks alongside normal diet was also able to significantly increase HDL levels (Figure 4). A similar study involving another variety of fibre enriched snacks showed that they significantly reduced cholesterol and LDL levels in diabetic rats [10], thus, suggesting the significant role of fiber enriched snacks in reducing blood lipid levels. Soluble dietary fibre has been reported to facilitate cholesterol reduction through various mechanisms involving an interplay of delayed intestinal absorption of glucose and lipids, binding of cholesterol to bile acids and increased excretion of bile acids [20], [21]. The CAD was observed to show a more significant reduction in lipid levels especially triglycerides (Figure 5), being a more targeted drug. However, various adverse effects, such as muscle aches, myalgia, headaches, nausea, abdominal cramping, increased risk of diabetes mellitus and renal damage, however, have been reported with the administration of the drug [22-24]. This justifies the use of alternative means, such as fibre enriched diet, as safer and more reliable especially in long term treatment of hyperlipidemia.

Studies have reported that hypercholesterolemia is capable of reducing insulin levels by inducing pancreatic β cell apoptosis through the oxidative stress pathway, enabling type 2 diabetes [25-26]. The significant reduction of blood insulin concentration in group 2 (Table 1) was caused by the hypercholesterolemic state of the animals. The non-significant difference in insulin levels for the group administered the conventional antilipemic drug (CAD) indicates that the CAD used had no effect in stabilizing insulin levels in hypercholesterolemic animals. In fact, the class of CAD used has been suggested to impair β-cell insulin secretion and promote insulin resistance, thus increasing the risk of developing type 2 diabetes especially in high risk patients such as hyperglycemic patients and postmenopausal women [27]. Fibre enriched snacks boosts blood insulin concentrations with the withdrawal of the hyperlipidemic-hyperglycemic diet. This was observed with the significant increase in insulin concentration observed in the group that had the fibre enriched snack with the antilipemic diet withdrawn and the group administered the fibre enriched snack alongside normal diet. Lummela, et al. [28] have also reported that dietary fibre favorably affects insulin in healthy individuals. Dietary fibre exerts an anti-inflammatory effect and reduces oxidative stress at cellular and molecular levels boosting insulin concentrations in normal subjects.

Conclusion

This study has shown that incorporation of fibre enriched snacks in the diet caused significant reduction in cholesterol, LDL concentration and stabilized blood glucose after six weeks in hyperlipidemic test animals. Additional benefits of increasing HDL concentration and boosting insulin levels were also recorded, when the fibre enriched snacks was administered with the withdrawal of the hypercholesterol- hyperglycemic diet. The fibre enriched snacks can thus serve as an effective dietary intervention in managing hyperlipidemic conditions, especially in patients with type 2 diabetic dyslipidemia.

Further studies involving longer duration of exposure to high fibre snacks is hereby recommended.

Ethical Approval

All authors hear by declare that the ARRIVE guidelines were followed and the animal experiment was carried out in accordance with the U.S Public Health Service Policy on humane care and use of laboratory animals. "All experiments have been examined and approved by the animal care and use research ethics committee in the institution in which it was performed".

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Author Contributions

Adetoboye Olubunmi Olajumoke: Methodology, formal analysis, investigation, writing-original draft. Osibanjo Adetokunbo: Methodology, Writing-Review and editing, Supervision Erukainure Ochuko: Conceptualization, methodology, validation. Obode Okukwe: methodology, investigation, Eboagwu Ijeoma: Investigation, Familola Oluwatosin: Investigation. Odega Joy: Investigation. Efuribe Nnenna: Investigation. Oke Oluwatoyin: Product development. Olonode Titilayo: Product development. Oluwole Oluwatoyin: Supervision, Project administration. Elemo Gloria: Funding acquisition.

All authors have revised and approved the final submitted version of this work

Author Disclosures

"Competing interests: None"

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