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Glycemic and Biochemical Effects of Phytosterol in Female Wistar Rats

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ABSTRACT:

Phytosterols are like [cholesterol](#) and maintain structural components of plant cell membrane. The present study aims to investigate the glycemic and biochemical effects of phytosterol using Wistar rats as experimental models. Fifteen (15) female Wistar rats were divided randomly into three (3) groups, each consisting of five (5) rats. Group I served as control and rats in this group were administered distilled water. Groups II and III served as the treatment groups and received 1000 and 2000mg/kg body weight of phytosterol respectively. Phytosterol was orally administered daily for twenty-eight (28) days in the morning hours (8-9am daily). During the study, serum blood glucose level was determined twice via tail vein puncture technique: firstly, before the administration of phytosterol (pre-treatment blood glucose: Day 0); secondly, at the end of the study (post-treatment blood glucose: Day 29). At the end of treatment, (Day 29), blood samples were collected via direct cardiac puncture and stored in lithium heparin tubes for analysis of biochemical indices. Findings from this study indicate that phytosterol treatment resulted in a significant and dose dependent decrease in blood glucose, total cholesterol, triglyceride, low-density lipoprotein, and malondialdehyde levels in the experimental groups relative to control ($p < 0.05$). Conversely, a significant and dose dependent increase was observed in the mean animal weight, high-density lipoprotein, glutathione, superoxide dismutase and catalase concentrations among the experimental rats compared to control ($p < 0.05$). Evidence from this study suggests that phytosterol treatment lowers blood glucose level, decrease lipid profile and basal oxidative stress markers by decreasing glycemic indices, cardiovascular risks and oxidative disruptions. This effect is probably achieved via beta cell stimulation and decreased reactive oxygen species radicals.

Keywords:

Phytosterol; glucostatic; biochemical; oxidative stress; lipid indices.

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Introduction

Phytosterols are like [cholesterol](#) and maintain structural components of plant cell membrane (Moreau *et al.*, 2018). Over 250 sterols and closely related compounds have been identified, characterized and isolated (Akhisa and Kokke, 1991). They essentially have a fused polycyclic structure which differ in carbon side chains and or presence/absence of a [double bond](#) (Patterson, 2006). They are further divided into 4-monomethyl phytosterols, 4,4-dimethyl phytosterols, and 4-desmethyl phytosterols on account of the position of the methyl group (which is usually at the carbon-4 position) (Zhang *et al.*, 2020). Stanols are [saturated](#) sterols, with no double bonds in the structure. The naturally occurring sources of phytosterols are mostly vegetable oils and products emanating from them. Sterols exist in the free form and [glycolipids](#) and as [fatty acid esters](#). The bound is hydrolysed in the gastrointestinal tract by [pancreatic enzymes](#) (Moreau and Hicks, 2004). The remaining sterols are removed during deodorization without changing their chemical composition (Patterson, 2006). Cereal products, berries, vegetables and some fruits are also important sources of phytosterols (Valsta *et al.*, 2007). Phytosterol is reportedly one of the major components of the leaves of *Cratererispermum schweinfurthi*, constituting 34.66% of its entire content (Saronee *et al.*, 2023). The European Foods Safety Authority (EFSA) and the FDA alluded that [blood cholesterol](#) can be lowered if a person consistently consumes plant sterols and stanols for a period of 2 to 3 weeks (EFSA, 2008). Based on this and other available data, the EFSA scientific panel provided the following advisory: "Plant sterols have demonstrated considerable reduction in blood cholesterol, blood cholesterol reduction may lower associated cardiovascular insults" (EFSA, 2008).

Despite documented [LDL cholesterol](#)-lowering effects due to long-term consumption of phytosterols, there is inadequate evidence for its effect on blood glucose and oxidative stress markers emphasizing the need for further research (Genser *et al.*, 2012; Salehi-sahlabadi, 2020). While studies on phytosterol have provided conflicting data on its biologic and pharmacologic effectiveness, the mechanisms underlying its therapeutic efficacy on body weight, blood glucose, lipid profile and oxidative stress need further investigation, especially with graded doses of phytosterol and a prolong period of administration. Literature reports that phytosterol has a wide range of therapeutic efficacies including: anticarcinogenic, anti-inflammatory, immunomodulatory, anti-cholesterol, aphrodisiac, and tonic effects (Bouic, 2001; Awad *et al.* 2008; Cabral and Klein, 2017; Lesma *et al.* 2018; Saronee *et al.*, 2024). However, a broad estimation of its effects on [body weight, blood sugar](#), lipid parameters and oxidative stress markers remain relatively scarce. Also, the inconsistent research findings underscore the need for the investigation of its application and usefulness on blood glucose, lipid profile and oxidative stress to address visible knowledge void. The present study therefore aims to evaluate the glycemic and biochemical effects of phytosterol using Wistar rat as experimental models.

Materials and Methods

Source of Phytosterol

Phytosterol was procured from Wakunaga of America Co., LTD. Mission Viejo, CA92691 U.S.A. Capsules were dissolved in tween 80 and constituted into 1000 and 2000mg/kg body weight.

Sourcing and handling of experimental rats

15 adult female Wistar rats weighing between 100-250g were procured from PAMO University of Medical Science animal house, Port Harcourt, Rivers State, Nigeria. Purchased rats were placed in cages, one for each study group and cared for under standard laboratory conditions (National Research Council of The National Academies, 2011; Albus, 2012). After two (2) weeks of acclimatization, experimental animals were subsequently used for the study.

Acute toxicity

Acute toxicity level of phytosterol was found to be >3000mg/kg body weight as previously reported by Carlos and Ma'rcia-regina, (2017) and Saronee *et al.*, (2024).

Experimental design

Fifteen (15) female Wistar rats were divided randomly into three (3) groups, each consisting of five (5) rats. Group I served as control and rats in this group were administered distilled water. Groups II and III served as the treatment groups and received 1000 and 2000mg/kg body weight of phytosterol respectively. Phytosterol was orally administered daily for twenty-eight (28) days in the morning hours (8-9am daily: West African Time). Animals were throughout the duration of the experiment granted unhindered access to standard rat chow and water for proper nutrition and hydration. During the study, serum blood glucose level was determined twice via tail vein puncture technique as earlier described by Saronee *et al.*, (2019): firstly, before the administration of phytosterol (pre-treatment blood glucose: Day 0); secondly, at the end of the study (post-treatment blood glucose: Day 29).

Blood collection and assay of biochemical parameters

At the end of treatment, (Day 29), experimental animals were anesthetized using diethyl ether and blood samples collected via direct cardiac puncture and stored in lithium heparin tubes for analysis of biochemical indices. Biochemical indices were assayed using standard laboratory test kits following established protocols. Lipid parameters (Total Cholesterol, Triglyceride, High Density Lipoprotein and Low-Density Lipoprotein) were determined to assess possible lipidemic and cardiogenic effects while glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) were assayed to determine the degree of pero-oxidation and serum concentration of antioxidant enzymes.

Statistical analysis

Statistical analysis was performed using SPSS version 25 to determine mean values and standard error of mean (SEM). An ANOVA test was performed to determine the mean

differences among all treatment groups, followed by a post hoc test using LSD. Data were expressed as mean $\pm$ standard error of mean and a p-value <0.05 was considered significant statistically.

Results

Changes in body weight and blood glucose level are shown in table 1. Significant increase in body weight was observed amongst Groups 2 (low dose) and 3 (High dose) rats administered 1000mg/kg and 2000mg/kg body weight respectively of phytosterol compared to Group 1 (Control) rats ($P < 0.05$): indicating a potential steroidogenic effect. Lower but insignificant blood glucose values (-1.0%) were observed among Group 2 rats treated with 1000mg/kg of phytosterol, however, administration of 2000mg/kg body weight of phytosterol among Group 3 rats caused a significant reduction in blood glucose level (-13.55%) compared to Group 1 (Control) rats ($P < 0.05$), suggesting a possible hypoglycemic effect of phytosterol at the administered dose.

Table 1. Values of body weight and blood glucose level after phytosterol administration

GROUPS	TREATMENT	Body Weight (kg)	Pre-treatment Blood Glucose level (Mmol/l)	Post-treatment Blood Glucose level (Mmol/l)	Percentage Difference in Blood Glucose level (%)
GROUP 1	Control	187.60 $\pm$ 0.92	4.95 $\pm$ 0.05	5.03 $\pm$ 0.02	1.6
GROUP 2	Low Dose (1000mg/kg)	199.00 $\pm$ 0.07*	4.98 $\pm$ 0.01	4.93 $\pm$ 0.03	-1.0
GROUP 3	High Dose (2000mg/kg)	215.40 $\pm$ 0.22*	4.87 $\pm$ 0.19	4.21 $\pm$ 0.04*	-13.55

Values expressed as Mean $\pm$ SEM. n=5. * = significant difference compared to Control ($p < 0.05$)

Table 2 shows values of lipid parameters after phytosterol administration. Interestingly, significantly lower values of total cholesterol, triglyceride and low-density lipoprotein but higher values of high-density lipoprotein level were observed amongst experimental rats treated with 1000mg/kg (Group 2) and 2000mg/kg body weight (Group 3) of phytosterol when compared to Group 1 (Control) rats ($P < 0.05$): suggesting a possible hypolipidemic effect of phytosterol at the administered doses in female Wistar rats.

Table 2. Values of lipid parameters after phytosterol administration

GROUPS	TREATMENT	Total Cholesterol (Mmol/l)	Triglyceride (Mmol/l)	High Density Lipoprotein (Mmol/l)	Low Density Lipoprotein (Mmol/l)
GROUP 1	Control	2.57±0.04	0.60±0.03	0.78±0.03	1.64±0.03
GROUP 2	Low Dose Phytosterol Group (1000mg/kg)	2.21±0.03*	0.47±0.01*	0.84±0.03*	1.51±0.03*
GROUP 3	High Dose Phytosterol Group (2000mg/kg)	1.99±0.01*	0.30±0.03*	0.91±0.05*	1.03±0.04*

Values expressed as Mean ± SEM. n=5. * = significant difference compared to Control (p<0.05)

Table 3 highlights changes in oxidative stress markers after phytosterol administration. Surprisingly, Compared to Group 1 (Control) rats, significantly higher values of glutathione, superoxide dismutase and catalase concentrations but lower values of malondialdehyde level was observed in phytosterol treated rats amongst Groups 2 and 3, indicating a potential reactive oxygen species scavenging effects of our intervention.

Table 3. Changes in oxidative stress markers after phytosterol administration

GROUPS	TREATMENT	Glutathione (u/ml)	Superoxide Dismutase (u/ml)	Malondialdehyde (Umol/ml)	Catalase (umol/H ₂ O ₂ /min)
GROUP 1	Control	0.92±0.05	0.46±0.03	0.49±0.02	1.61±0.03
GROUP 2	Low Dose Phytosterol Group (1000mg/kg)	1.11±0.04*	0.56±0.04*	0.30±0.02*	1.97±0.04*
GROUP 3	High Dose Phytosterol Group (2000mg/kg)	1.56±0.09*	0.81±0.03*	0.20±0.02*	2.30±0.03*

Values expressed as Mean ± SEM. n=5. * = significant difference compared to Control (p<0.05)

Discussion

Phytosterols are structural components of plant cell membrane commonly consumed due to its anecdotally reported antihypercholesterogenic effects (Moreau *et al.*, 2018). Literature reports a wide range of therapeutic efficacies of phytosterol (Bouic, 2001; Awad *et al.* 2008; Cabral and Klein, 2017; Lesma *et al.* 2018; Saronee *et al.*, 2024). In the present study, glycemic and biochemical effects of phytosterol were assessed using female Wistar rats as models. Glycemic potentials of phytosterol, a common derivative and product of plant origin is tested in this study for its anecdotally reported glycemic control, hypolipidemic and antioxidant properties. Diabetics, particularly those in developing countries of the world with palpable food insecurity, poor agricultural technologies, insufficient and poorly funded

healthcare facilities are faced with enormous challenges in making food choices in addition to other numerous afflictions associated with diabetes mellitus. In this study, we observed that phytosterol treatment significantly increased animal weight which may be due to regeneration of adipocytes and steroidogenic effects. This finding is consistent with previously reported studies involving plant products ([Niwa et al., 2011](#); [Ijaola et al., 2014](#)). Our study outcome further showed a significant reduction in systemic glucose level in phytosterol treated animals compared to control rats. This observation agrees with previous reports ([Ijaola et al. \(2014\)](#); [Saronee et al., \(2019\)](#)). The possible mechanism by which phytosterol elicits its glucostatic modulatory effects may be through the potentiation of insulin effects, either by increasing the pancreatic secretion of insulin from cells of islets or its release from bound insulin, hence, decreasing serum glucose concentration in animals.

Evaluation of cardiovascular risk has become the mainstay of cardiovascular diseases prevention and management. However, atherogenesis is multifaceted, lipoprotein metabolism abnormalities are key factors, amounting to about 50% of the global population-attributable risk of cardiovascular disease development (Yusuf *et al.*, 2004; Ijeoma *et al.*, 2020; Saronee *et al.*, 2020). In spite of palpable progress recorded in cardiovascular disease management in recent times, there is a seeming unanimous agreement or understanding among clinicians and epidemiologists that coronary risk assessment based majorly on basic lipid indices including total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein is a major diagnostic tool, particularly in individuals at intermediate risk (Yusuf *et al.*, 2004). Efforts have been made in seeking emergent or new interventions to improve cardiovascular disease management (Yusuf *et al.*, 2004; Saronee *et al.*, 2024). Lipid indices serve as elementary medical screening tool for cardiovascular dysfunction; obtained results may be useful, particularly in identifying hidden cardiovascular diseases and associated complications. There are reports suggesting that an increase in low-density lipoprotein cholesterol level is atherogenic and elevated high-density lipoprotein level is cardioprotective (Castelli *et al.*, 1986; Ijeoma *et al.*, 2020; Saronee *et al.*, 2020; Saronee *et al.*, 2024). Findings from the present study suggest a significant and dose dependent effect of phytosterol on assessed lipid indices for the study duration. This is indicative that phytosterol may have lipid lowering functions and can reduce cardiovascular risks.

Furthermore, our findings show that phytosterol administration significantly increased mean levels of Glutathione, Superoxide Dismutase and Catalase and reduced Malondialdehyde level among all treatment groups compared to group 1 (control) ($P < 0.05$). Malondialdehyde is the principal product of unsaturated fatty acid peroxidation and a biological indicator of cell/ tissue damage eliciting oxidative stress (Gbaranor *et al.*, 2024; Saronee *et al.*, 2024). The observed decrease in Malondialdehyde level shows that phytosterol may ameliorate tissue and cellular damage. Oxidative stress occurs in a situation whereby the concentration of deleterious reactive oxygen species (ROS) is greater than the body's antioxidant protective capacity. Phytosterol may have discouraged the generation of harmful ROS responsible for the imbalance between oxidation and antioxidant defense mechanisms. This may hinder possible cellular disruptions guiding against lipid peroxidation (LPO) and subsequent

oxidative damage to membranous proteins and DNA (Obiandu *et al.*, 2019; Saronee *et al.*, 2024). Glutathione, superoxide dismutase and catalase are important antioxidant agents responsible for neutralizing harmful reactive oxygen species thereby mitigating potential oxidative injury (Chinko and Umeh, 2023; Saronee *et al.*, 2024). The increased Glutathione, superoxide dismutase and catalase levels suggest that phytosterol administration may prevent possible detoxification impairment responsible for oxidative damage. With an increase in these antioxidants, the body's ability to detoxify ROS is significantly improved, preventing oxidative stress, cellular damage, and tissue peroxidation. Furthermore, oxidative stress leading to diminished antioxidant enzyme function has been correlated to various abnormalities, including nephrotoxicity, hepatotoxicity and neurotoxicity (Zhang *et al.*, 2023). Therefore, the observed improvement in antioxidant defense represents a basic and rudimentary mechanism underlying phytosterol activity, highlighting the need for antioxidant supplementation.

Conclusion

Evidence from this study suggest that phytosterol supplementation increased animal weight and reduced serum glucose level resulting in regeneration of adipocytes and muscle tissues and pancreatic beta cells stimulation. Phytosterol reduced lipid parameters by improving the free radical scavenging properties of high-density lipoprotein. It also indicates that phytosterol could mitigate harmful reactive oxygen species thereby preventing lipid peroxidation and associated cellular damage. These findings underscore the need for phytosterol supplementation.

Ethical Consideration

Experimental animals were treated in strict compliance with standard ethical protocols guiding the use of laboratory animals in scientific research (National Research Council of The National Academies, 2011; Albus, 2012). The protocol guiding the research, with experimental design and methods, were reviewed and given approval by the PAMO University of Medical Sciences Research, Grants and Ethics Committee vide a communication referenced PUMS/REC/2025030.

Conflict of Interest

Authors declare that there are no conflicts of interest related to this study

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