The Effect of Hydro-Methanolic Seed Extract of *Azanza garckeana* (Goron Tula) on Lipid Profile and Oxidative Stress Markers of Male Wistar Rats

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Abstract

The prevalence of cardiometabolic conditions has risen globally over the years even in the low- and middle-income countries. The present study was aimed at investigating the effects of hydro-methanolic seed extract of Azanza garckeana on the lipid profile and oxidative stress markers of male wistar rats. A total of 20 wistar rats were separated into four groups of five rats each; including group 1 which served as control. Groups 2, 3, and 4 respectively received a daily dose of 250mg/kg, 500mg/kg, and 1000mg/kg of the extract. The body weight of the animals was taken on day 1 and day 28. The experiment lasted for 28 days, thereafter, the animals were sacrificed and blood samples collected for the determination of lipid profile and oxidative stress markers. The result showed that 1000mg/kg of the extract caused a significant increase in total cholesterol, low-density lipoprotein, and very low-density lipoprotein levels but no significant effect on the triglyceride, and high-density lipoprotein. The percentage of weight gain was significantly reduced compared to control. 250mg/kg and 500mg/kg of the extract did not cause significant changes in the lipid profile parameters. There were also no significant changes in the oxidative stress parameters. This therefore suggests that at higher doses hydro-methanolic seed extract of Azanza garckeana despite causing dyslipidaemia, may have a weight protective effect and would not potentially increase lipid peroxidation in male wistar rats.

Keywords: *Azanza garckeana, lipid profile, oxidative stress markers.*

Introduction

The use of natural products in the prevention and treatment of certain medical conditions is becoming widely accepted in many cultures globally. One of such natural products is *Azanza garckeana* which has proved to be a useful remedy for some medical conditions including respiratory and reproductive disorders [1]. In Nigeria, the plant is mainly cultivated within the North where it is commonly known as ''Goron Tula'' (kola of Tula) in Hausa language [2]. Tula being a community in Gombe state, Nigeria where the plant is believed to have numerous medicinal benefits especially its capability to enhance sexual performance. *Azanza garckeana* as a versatile edible fruit has been shown to possess alkaloids, phenols, saponin, tannin, carotenoids

and other beneficial phytochemicals [3,4,5,6,7]. These constituents confer the antioxidant properties and other effects associated with consumption of the plant. Oxidative stress and abnormal lipid metabolism have been primarily implicated in the pathogenesis of a number of human diseases. Oxidative stress is characterized by an imbalance between the production of free radicals (such as reactive oxygen species) and the body's antioxidant defences [8,9].

The global prevalence of cardiovascular diseases is increasing dramatically even in low-income countries [10] due to a shift from the traditional minimally refined meals to the highly refined westernized diets. The burden of cardiovascular diseases can be reduced by developing techniques and guidelines for the management of blood lipids, oxidative stress and inflammation [11]. The use of synthetic drugs has over the years helped to reduce the morbidity and mortality associated with these cardiovascular diseases. However, these drugs are expensive and not readily affordable by people residing in local communities of developing countries. Natural products such as *Azanza garckeana* are found within the communities, readily accessible and affordable with possible less side effects. The aim of the present study is to determine the effect of methanolic seed extract of (*Azanza garckeana* on the lipid profile and oxidative stress parameters of male wistar rats.

Materials and Methods

Plant Material and Preparation of Extract

Azanza garckeana fruit was procured from Tula village, in Gombe State, Nigeria via a known vendor at the Mile 3 Market in Port Harcourt. The plant was identified and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, Rivers State University, Nigeria with herbarium number (RSUPbH0157).

The pulp of *Azanza garckeana* fruit was first removed and the seeds sun dried. The dried seeds were crushed into a powdery form using an electric grinder. Subsequently, extraction was done according to the method used by Dadaya *et al*., 2023 [12]. The obtained seed extract was stored in a refrigerator at 2-8°C for experimental use.

Experimental Animals

Twenty male wistar rats were procured from the animal house of the faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria and acclimatized for one week under standard laboratory conditions. The animals had free access to clean water and were fed *ad libitum* with normal rat chow during the period of the study. Animal handling and experimentation were in accordance with the standards of the Rivers State University and in compliance with internationally accepted principles for the use of laboratory animals.

Drugs, and chemicals

The reagents as well as the organic solvent used for extraction were purchased from reputable chemical stores in Port Harcourt. All reagents were of analytical grade.

Experimental Design

The wistar rats were separated into four groups of five rats each including the control group which received distilled water, while the treatment groups received respective doses of the extract orally for 28 days as follows;

Group 1 (control).
Group 2 (250mg/kg of the extract)
Group 3 (500mg/kg of the extract)
Group 4 (1000mg/kg of the extract).
The animals were weighed on days 1 and 28 of the experiment and recorded accordingly.
Sample collections and processing.
At the end of the treatment period, the rats were fasted overnight, and anesthetized, and blood samples were collected via cardiac puncture for biochemical analysis.

Biochemical Analysis

Parameters measured in serum lipid Profile [including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C)] were determined by standard colorimetric methods using commercial kits. Oxidative stress markers [Malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)] were also measured in plasma using established standard protocols.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 25). Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were made using one-way ANOVA followed by Tukey's post hoc test. A *p-value* < 0.05 was considered significant.

RESULTS

Table 1: Effect of hydro-methanolic seed extract of *Azanza garckeana* on body weight.

GROUP	Initial weight (g)	Final weight (g)	% Change in weight
1 (Control)	63.40±1.57	142.40±16.15	125.58±27.88
2 (250mg/kg extract)	84.40±2.11	174.60±2.46	107.32±5.31
3 (500mg/kg of extract)	93.40±1.40	164.20±13.57	75.52±13.27*
4 (1000mg/kg of extract)	108.20±1.32	160.60±12.74	48.21±11.09*

Values are presented in mean ± SEM, n=5, *P < 0.05 statistically lower compared to the control.

Table 2: Effect of hydro-methanolic seed extract of *Azanza garckeana* on lipid profile

GROUP	TC(mmol/l)	TG(mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)
1 (Control)	2.46±0.15	1.19±0.08	1.30±0.06	1.70±0.15	0.54±0.04
2 (250mg/kg)	2.54±0.10	1.03±0.07	1.27±0.03	1.74±0.14	0.46±0.03
3 (500mg/kg)	2.50±0.10	1.03±0.07	1.21±0.07	1.77±0.08	0.48±0.03
4 (1000mg/kg)	2.96±0.20*	1.40±0.06	1.38±0.08	2.22±0.19*	0.64±0.03*

Values are presented in mean ± SEM, n=5, *P<0.05 statistically different compared to control.

Table 3: Effect of hydro-methanolic seed extract of *Azanza garckeana* on oxidative stress

GROUP	GPX (µmol/l)	CAT (U/mg protein)	SOD (U/mg protein)	MDA (µmol/l)
1 (Control)	0.04±0.00	1.73±0.08	0.39±0.02	0.39±0.03
2 (250mg/kg)	0.04±0.00	2.03±0.29	0.32±0.03	0.48±0.02
3 (500mg/kg)	0.04±0.01	1.51±0.31	0.38±0.03	0.43±0.05
4 (1000mg/kg)	0.05±0.01	2.04±0.34	0.37±0.01	0.42±0.02

Values are presented in mean ± SEM, n= 5.

Discussion

The administration of hydro-methanolic seed extract of *Azanza garckeana* in different doses revealed significant alterations in body weight. This effect of the extract on body weight was in a dose-dependent fashion such that increasing the concentration of the seed extract causes a decline in the in the percentage weight gain. The higher doses (500 mg/kg and 1000 mg/kg) respectively caused significant reduction in the percentage weight gain compared to the control group. Thus, suggesting that the *A. garckeana* seeds may have potential weight protective effect when consumed at higher doses. This effect is useful in the maintenance of healthy weight, preventing the extremes of weight and their consequences. The reduction in weight gain may be due to the effect of its bioactive compounds on appetite suppression, increased metabolic rate, or modulation of lipid metabolism and weight regulating hormones. Other studies suggest that weight regulation is essential in the dietary management of diabetes mellitus [13,14], that protects against diabetes-induced dyslipidaemia.

This study also showed that daily administration of the extract in lower doses of 250mg/kg and 500mg/kg respectively had no significant effects on lipid profile of wistar rats. However, at a very high concentration (1000 mg/kg), the extract caused significant increase in TC, LDL-C and VLDL-C levels compared to the control group. Elevated LDL is associated with high risk of cardiovascular diseases [15]. Therefore, despite the numerous beneficial effects of *A. garckeana*, it should be consumed in moderation to avoid its possible dyslipidaemic tendency at very high concentrations. Lipid-lowering effect of the plant extract was demonstrated in other studies involving lower doses of the commonly consumed part of the fruit, the pulp [7,14]. The method of extraction can also influence the effect of the fruit on lipid metabolism. Iyoja *et al*., 2022 [16] in their study showed elevated levels of LDL as well as HDL using 500mg/kg of ethyl acetate extract

of the fruit pulp. These results should serve as a basis for cautious consumption of very high concentrations of the fruit especially the seed. The observed changes in lipid profiles following extract administration, particularly at higher doses, suggest that the extract may either cause increased synthesis and/or reduced plasma clearance of LDL.

Previous studies using the fruit pulp proved the antioxidant potentials of *Azanza garckeana* extract [14,17,18]. The present study using the seed, did not suggest any significant effect on the oxidative stress parameters. The superoxide dismutase (SOD) levels were relatively stable across all doses. There were no significant changes in malondialdehyde (MDA) levels, suggesting that the seed extract does not have a significant effect on lipid peroxidation.

Conclusion

Our research findings provide valuable insights into the dose-dependent effects hydro-methanolic seed extract of *Azanza garckeana* on lipid profile and oxidative stress markers. While lower doses may offer beneficial effects, higher doses could potentially increase lipid levels, warranting careful consideration of dosage in therapeutic applications. Also, it suggests that at higher doses despite causing dyslipidaemia, it may have a weight-protective effect and would not potentially increase lipid peroxidation in male wistar rats.

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