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Effects of *Azanza garckeana* Fruit Pulp on Metabolic Syndrome in Wistar Rats Fed on High Fructose Diet

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Abstract

The excessive consumption of high-energy dietary sweeteners is largely to blame for the widespread metabolic syndrome around the world. This study is aimed at in vivo evaluations of the ameliorative effects of A. garckeana fruit pulp on metabolic syndrome in Wistar rats. Twenty-four (24) adult male Wistar rats were divided into six (6) groups (n=4). Groups A, B, and C received standard, high-fructose, and 2% A. garckeana fruit pulp-supplemented standard diets, respectively. Groups D, E, and F were fed 5% A. garckeana fruit pulp-supplemented standard, 2% A. garckeana fruit pulp-supplemented high-fructose, and 5% A. garckeana fruit pulp-supplemented high fructose diets. In addition to weekly monitoring of weight changes, activities of serum antioxidant enzymes, lipid profile, and blood glucose level were determined. There were no significant changes in weight gain among the groups throughout the experimental period. Compared with the initial value of blood glucose level, only the group fed high fructose diet had significantly (P<0.05) higher blood glucose levels at the end of the experiment. The group fed 5% A. garckeana fruit pulp-supplemented high-fructose diet had significantly (P<0.05) higher serum concentration of total cholesterol and HDL-cholesterol in comparison with the control. The groups fed A. garckeana fruit pulp-supplemented diets had significantly (P<0.05) higher albumin concentrations than the group fed high fructose diet. The serum urea concentration was significantly (P<0.05) lower in the group fed 2% A. garckeana fruit pulp-supplemented high fructose diet when compared with the control. The group fed 5% A. garckeana fruit pulp-supplemented high fructose diet had significantly (P<0.05) higher activities of SOD and GSH activities compared with the group fed high fructose diet. Also, the group fed 2% A. garckeana fruit pulp-supplemented high fructose diet had significantly (P<0.05) higher activities of CAT when compared with the group fed high fructose diet. It can be concluded that A. garckeana fruit pulp has anti-hyperglycemic, anti-dyslipidemic, and antioxidant effects, which could be responsible for its ameliorative effects on metabolic syndrome.

Key Words: Metabolic Syndrome, Azanza garckeana, High Fructose Diet, Anti-hyperglycemic, Dyslipidemia, Antioxidant

INTRODUCTION

The consumption of high-calorie foods combined with inadequate metabolic activity results in an energy imbalance that produces the circumstances for metabolic dysregulation and raises body mass index (BMI). Insulin resistance, dyslipidemia, and type 2 diabetes can eventually result from this disruption in the metabolism of fats and carbohydrates (Srivastava, 2018). Previous studies also indicate that people increase their intake of high-energy snack foods when stressed, thereby leading to obesity and metabolic syndrome (Nderitu *et al.*, 2017). Metabolic Syndrome is characterized by a collection of interconnected factors that directly heighten the likelihood of developing coronary heart disease (CHD), various forms of cardiovascular atherosclerotic diseases (CVD), and diabetes mellitus type 2 (DMT2) (Kassi *et al.*, 2011). The underlying risk factors that promote the development of metabolic syndrome are overweight and obesity, physical inactivity, and an atherogenic diet (Grundy *et al.*, 2004). Plants have always been a source of drugs, and many of the currently available drugs have been derived from them (Patel *et al.*, 2012). Synthetically made therapeutics have, over the years, developed problems such as toxicity, resistance to micro-organisms allergy, superinfection, or even addiction, as well as being increasingly expensive; hence, this leads to the use of natural products as alternatives (Adamu *et al.*, 2013).

A. garckeana, commonly referred to as Goron Tula in the Hausa language, is a member of the *Malvaceae* family, which is cultivated mostly in Tula village, Gombe State, Nigeria (Bioltif *et al.*, 2020). This tropical African fruit serves various purposes and is considered both a medicinal and food plant, particularly in Northern Nigeria, where it is utilized in herbal medicine practices (Ahmed *et al.*, 2016) in remedies such as cough, chest pains, infertility, menstruation abnormalities, sexually transmitted infections, and hepatic impairments (Maroyi, 2017; Glew *et al.*, 2005). Numerous classes of bioactive metabolites, including amino acids, alkaloids, ascorbic acid, carotenoids, flavonoids, glucosides, phenols, lipids, tannins, and saponins, have been reported from *A. garckeana* (Yusuf *et al.*, 2020). An unhealthy lifestyle that predisposes to metabolic derangements and syndromes, such as excessive feeding, alcoholism, and consumption of sweetened drinks, causes visceral adiposity(obesity), and this activates inflammation and oxidative stress (Hwang *et al.*, 2015). This needs to be modified by eating and consuming food rich in antioxidants with lower calories (Idoko *et al.*, 2018). This current study aimed at *in vivo* evaluations of the effects of *A. garckeana* fruit pulp on metabolic syndrome in *Wistar* rats fed on a high fructose diet.

MATERIALS AND METHODS

Chemicals and feed ingredients

Methionine, pre-mixed minerals, and vitamins were purchased from Sabon Gari Market, Kano State. Lipid profiles, and antioxidant enzymes kits were bought from Randox Laboratories Limited. in the UK. Rice bran, maize starch, bone meal, soybean meal, and palm oil were locally purchased from Dutsin-Ma Central Market in Katsina State for use in the feed, as shown in Table 1.

Table 1: Formulated feed composition

Feed Composition	Control diet g/Kg	High-Fructose diet g/Kg
Corn Starch	55.45	-
Fructose	-	55.45
Cellulose	45	45
Palm oil	60	60
Bone meal	12.5	12.5
Salt mix	3.0	3.0
Vitamin/mineral mix	2.5	2.5
Methionine	2.5	2.5
Total	100	100

Fruit Pulp Supplement

The fruit of *A. garckeana* was obtained from Tula village, in Kaltungo Local Government Area, Gombe State, Nigeria, and identified in the Department of Plant Sciences and Biotechnology, Federal University, Dutsin-Ma, Katsina State with Voucher number FUDMA/PSB/00093. The fruit of *Azanza garckeana* containing the seed and pulp was washed and separated, and the pulp was air dried. The dried pulp was pulverized into powdered form using an electric mill.

The Standard rat diet's formulation

Control and high fructose diets were prepared according to the method of Siddiq and Abdullahi (2023). Corn starch, cellulose, soybean meal (SBM), vitamin, and mineral mix were mixed as summarized in Table 1.

Supplementation of formulated diet

The formulated standard diet was mixed with *Azanza garckeana* powder within the ratio 98:2 (w/w) and 95:5 (w/w) to obtain a 2% and 5% spice-supplemented diet, respectively.

Experimental design

Twenty-four (24) male Wistar rats weighing 120g - 150g were purchased from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were housed in metal cages (4 rats per cage). Animal handling and experimentation concord with the guidelines for laboratory animal use and care in the National Research Council (NRC, 2011). The rats were split into six (6) groups of four (4) rats each, as previously mentioned, and the prepared diets were distributed as follows:

Group A (Control): Rats maintained on standard diet
Group B: Rats maintained on a high fructose diet
Group C: Rats maintained on 2% inclusion of *Azanza garckeana* fruit pulp in a standard diet
Group D: Rats maintained on 5% inclusion of *Azanza garckeana* fruit pulp in a standard diet
Group E: Rats maintained on 2% inclusion of *Azanza garckeana* fruit pulp in a high fructose diet

Group F: Rats maintained on 5% inclusion of *Azanza garckeana* fruit pulp in a high fructose diet

Ad libitum feeding was provided for seven (7) weeks. (Idoko *et al.*, 2018).

Body weight changes

According to Idoko *et al.* (2018), the rats were weighed using a digital scale at the beginning of the experiment and then weekly for seven (7) weeks.

Determination of Blood glucose

The initial fasting blood glucose level was taken after fasting the rats overnight according to the method of Idoko *et al.*, 2018. The determination of fasting blood sugar was continued on a weekly basis and at the end of the feeding trial using Accu Chek® Active glucometer (Roche Diabetes Care, 25

South Africa Pty Limited, Hertford Office Park, 90 Bekker Road Vorna Valley, 1686, South Africa). ***Animal sacrifice and blood sample collection***

The animals were weighed and sacrificed after the 7 weeks by anesthetizing them in a jar filled with cotton wool drenched in chloroform. Blood was drawn into simple containers and centrifuged at 1500 rpm for 15 minutes in order to obtain serum for analysis. The serum was transferred to the laboratory for the required analysis while frozen.

Determining the Parameters of the Lipid Profile

Calculating Total Cholesterol (TC) in Serum

Spectrophotometric estimation of serum total cholesterol was performed using Randox kits in accordance with the manufacturer's instructions.1000 µl reagent was pipetted into three test tubes named reagent blank, standard, and sample. 10 µl distilled water, 10 µl standard, and 10 µl sample were added to the test tubes. The contents of the test tubes were mixed and incubated for 10 minutes at 20-25 ^{O}C. The absorbance of the test tubes was measured. This was determined according to the method described by Abel *et al.* (1951)

$Total\ Cholesterol\ (mg/dl)$ = $(Absorbance\ of\ sample/Absorbance\ of\ standard)\ x\ Concentration\ of\ standard$

Serum triglyceride (TG) measurement

The manufacturer-recommended Randox kits were used to perform an enzymatic hydrolysis technique to measure the serum triglyceride levels. 1000µl of the reagent (4-amonophenazone, ATP, lipases, glycerol-kinase, glycerol-3-phosphate oxidase, and peroxidase, PIPES buffer, 4-chloro-phenol, and magnesium ions), were added to 10 µl of a serum sample. The standard solution (1000µl of the reagent + 10µl of the standard reagent) and blank (1000µl of the reagent + 10µl distilled water) were prepared similarly. Following adequate mixing, the sample's absorbance was measured at 546 nm and compared to a blank. This was determined according to the method described by Rifai *et al.* (1999). Triglyceride concentration was calculated as follows:

$Triglyceride\ \ concentration\ (mg/dl)$=$(Absorbance\ of\ sample/Absorbance\ of\ standard)\ x\ Concentration\ of\ standard$

Determination of HDL-cholesterol (HDL-C)

HDL determination was based on quantitative precipitation of Low-density lipoproteins (LDL and VLDL) and chylomicron fractions by adding phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high-density lipoprotein) fraction, which remains in the supernatant, is determined. 1000µL precipitant reagent was pipetted into two test tubes labelled standard and sample. 500µL standard and 500µL samples were added to the test tube, respectively. The contents were mixed and allowed for 10 minutes at room temperature and later centrifuged for 10 minutes at 4000rpm. The supernatant was separated, and the cholesterol content was determined by the CHOD-PAP Method. Three (3) test tubes were arranged and labelled reagent blank, standard, and sample. 1000µL CHOD-PAP reagent was pipetted into the test tubes, which was then followed by the addition of 100µL of distilled water, 100µL standard, and 100µL sample supernatant respectively. The contents were mixed and incubated for 10 minutes at room temperature (Young, 1990). The absorbance of the sample and standard were measured against the reagent blank.

$HDL-C\ \ (mg/dl)\ =\ (Absorbance\ of\ sample/Absorbance\ of\ standard)\ x\ Concentration\ of\ standard$

Calculating Low-Density Lipoprotein (LDL-C)

Estimated results from TC, TG, and HDL testing were used to determine low-density lipoprotein cholesterol (LDL-cholesterol) (Friedewald *et al.*, 1972):

LDL= Total Cholesterol – (Triglyceride/5) – HDL

Measurement of the antioxidant enzyme levels in the serum

Superoxide dismutase (SOD) assay

This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. 1000 µl of the mixed substrate (R1) was pipetted into three test tubes

labelled standard, sample, and control. Ransod sample diluent 30 µl, 30 µl sample, and 30 µl control were pipetted into test tubes respectively. The contents of the text tubes were mixed, and 150 µl of xanthine oxidase was added into each test tube. The absorbance of A1 and A2 was measured at 505nm(Arthur and Boyne, 1985)..

Determination of serum catalase activity

Catalase determination was based on detoxifying hydrogen peroxide by catalase to oxygen and water molecules. 20ml catalase reagent was pipetted into two test tubes labelled with reagent blank and sample. The distilled water of 10ml and 10ml samples was pipetted into the test tubes, respectively. The contents were mixed, and catalase was selected on the screen of the RX Monza Machine.

Glutathione peroxidase activity measurement

Glutathione peroxidase was identified based on its ability to catalyze the oxidation of reduced glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to a reduced form with a concomitant oxidation of NADPH to NAD^+. Two test tubes were labelled sample and reagent blank. Reagent and cumene, 1.00ml and 0.04ml, respectively, were pipetted into the test tubes, whereas 0.02ml sample was added into the sample test tube and 0.02ml distilled water was added into the reagent blank test tube. The contents of the test tubes were mixed, and the initial absorbance of the sample and sample blank were measured. The absorbances were read again after 2 mins (Baumber and Ball, 2005).

Determination of glutathione reductase activity

Glutathione reductase catalyzes the reduction of Glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^{+.}$ The decrease in absorbance at 340nm was measured. Reagent 1 of 1000µl was pipetted into the test tube, and 40µl was added. The content of the tube was mixed. Reagent 2 of 200µl was then added. The initial absorbance and the final absorbance were read at 340nm (Goldberg. and Spooner, 1983)

Determination of serum protein level

Following the manufacturer's protocol, total protein, Albumin, and Globulin were determined using their respective assay kits from Randox Laboratory Limited (UK).

Determination of serum urea and creatinine level

According to the manufacturer's protocol, creatinine and urea were determined enzymatically using assay kits from Randox Laboratory Limited (United Kingdom).

Statistical analysis

Determinations were made in triplicate; Data was expressed as means ± standard error (SEM). One-way analysis of variance (ANOVA) was performed using IBM Corp.'s Statistical Package for Social Sciences software program for Windows version 16, while means were evaluated for significance using post hoc Ducan's new multiple range test at 95% confidence interval.

RESULTS

Weight Changes in Wistar rats fed *Azanza garckeana* fruit pulp-supplemented high-fructose diet.

Figure 1 shows the result for the average weight changes per group on a weekly basis. There were no significant changes ($P>0.05$) in weight gain among the groups throughout the experimental period.

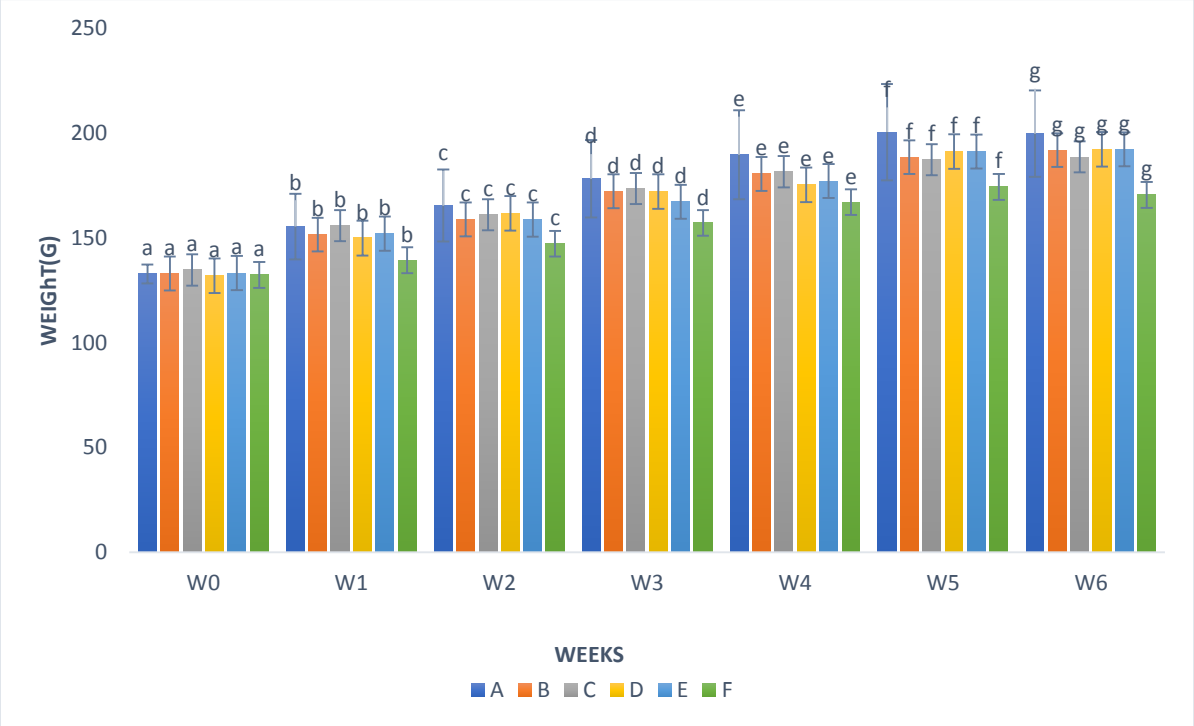

Figure 1: Average weekly weight of rats fed on high fructose diet supplemented with *Azanza garckeana* fruit pulp
Values are expressed as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly.

Blood Glucose Level in rats fed Azanza garckeana fruit pulp-supplemented high-fructose diet. Compared with the initial value, there was no significant variation ($P>0.05$) in the average blood glucose level in the group fed the standard diet. Although there were fluctuations in the average weekly blood glucose level in other groups, only the group that was given a diet high in fructose had significantly ($P<0.05$) higher blood glucose levels at the end of the experiment when compared with the initial value (Figure 2).

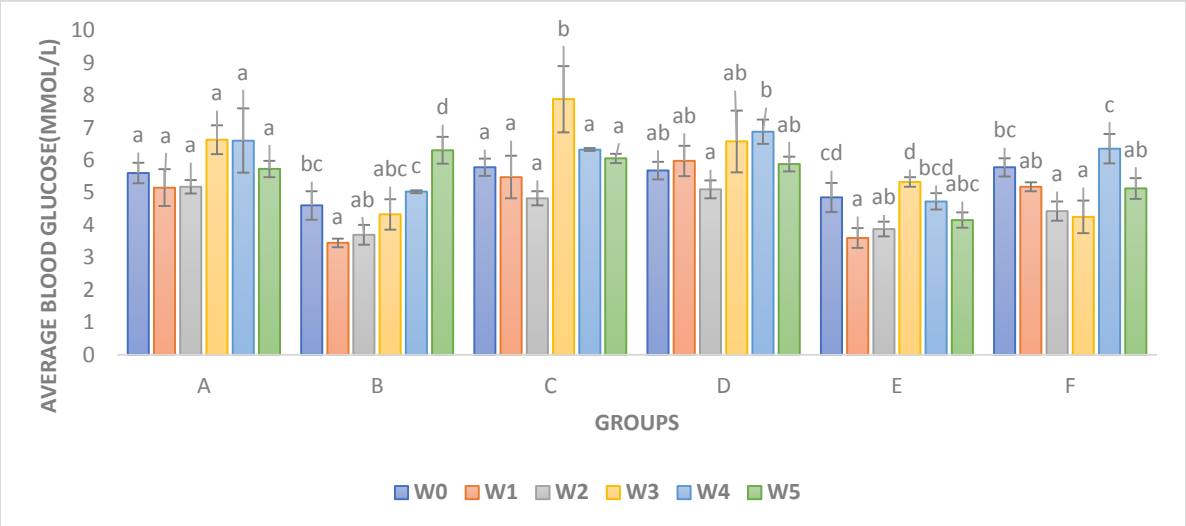

Figure 2: Average weekly blood glucose levels of Wistar rat fed on a high fructose diet supplemented with *Azanza garckeana* fruit pulp

Values are expressed as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences were observed at $P<0.05$ for values with distinct superscripts in the same column. W0=Week 1, W1=Week 2, W2=Week 3, W3=Week 4, W4=Week 5, W5=Week 6.

Lipid Profile concentration in rats fed Azanza garckeana fruit pulp-supplemented high-fructose diet.

With the exception of the group fed 5% *Azanza garckeana* fruit pulp-supplemented high-fructose diet, all other groups had significantly higher (P<0.05) concentrations of serum LDL when compared with the control. However, the group fed 5% *Azanza garckeana* fruit pulp-supplemented high-fructose diet had significantly (P<0.05) higher total cholesterol in comparison with the control. Furthermore, the group fed 5% *Azanza garckeana* fruit pulp-supplemented high-fructose diet had significantly (P<0.05) higher concentration of HDL when compared with the control (Table 2).

Table 2: Lipid Profile concentration in rats fed *Azanza garckeana* fruit pulp-supplemented high-fructose diet.

Group	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	97.00±2.00^{a}	91.33±4.63^{bc}	22.67±4.10^{ab}	56.07±4.95^{a}
B	107.00±4.04^{ab}	80.00±5.20^{bc}	13.50±0.87^{a}	77.50±4.21^{b}
C	106.67±3.84^{ab}	62.67±4.06^{ab}	21.33±2.33^{ab}	72.80±2.12^{b}
D	105.53±3.48^{ab}	42.00±8.62^{a}	18.67±2.33^{ab}	74.93±3.07^{b}
E	107.33±4.18^{ab}	66.33±7.17^{ab}	14.00±2.00^{a}	80.07±2.29^{b}
F	114.33±4.84^{b}	114.67±23.31^{c}	24.33±4.18^{b}	67.07±5.54^{a}

Values are expressed as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences were observed at P<0.05 for values with distinct superscripts in the same column.

Liver Function Enzymes activities in rats fed Azanza garckeana fruit pulp-supplemented high-fructose diet.

Table 3 shows that AST and ALT serum activities were significantly (P<0.05) higher in the group fed 5% A. garckeana fruit-pulp supplemented diet than in the control.

Table 3: Liver Function Enzymes Activities in rats fed *Azanza garckeana* fruit pulp-supplemented high fructose diet.

Group	AST (u/L)	ALT (u/L)
A	7.00±1.00^{a}	5.00±1.15^{ab}
B	11.00±2.31^{ab}	5.00±0.58^{ab}
C	9.67±0.33^{ab}	5.67±0.33^{ab}
D	6.67±0.88^{a}	4.33±0.33^{a}
E	11.67±1.45^{ab}	5.67±0.33^{ab}
F	12.67±2.60^{b}	7.67±1.86^{b}

Values are expressed as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences were observed at P<0.05 for values with distinct superscripts in the same column.

Serum Protein level in rats fed Azanza garckeana fruit pulp-supplemented high-fructose diet.

The groups fed the *Azanza garckeana* fruit pulp-supplemented diet had significantly (P<0.05) higher albumin concentration than the group fed high fructose diet. The albumin/globulin ratio in all the groups did not differ significantly (P<0.05) from the control (Table 4).

Table 4: Serum Protein, Albumin, and Globulin level in rats fed *Azanza garckeana* fruit pulp-supplemented high-fructose diet.

Group	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/Globulin (g/L)
A	72.33±6.39^{ab}	46.00±0.58^{d}	26.33±6.17^{ab}	1.94±0.42^{ab}
B	60.00±5.20^{a}	37.50±0.29^{a}	22.50±5.48^{a}	2.04±0.51^{ab}
C	77.00±7.51^{ab}	43.33±0.88^{c}	33.67±6.67^{ab}	1.37±0.21^{a}
D	60.67±1.20^{a}	44.67±0.88^{cd}	16.00±0.58^{a}	2.80±0.10^{b}
E	60.00±2.00^{a}	39.00±0.58^{ab}	21.00±2.52^{a}	1.91±0.23^{ab}
F	86.00±11.53^{b}	41.00±1.00^{b}	45.00±10.59^{b}	1.04±0.27^{a}

Values are given as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences were observed at P<0.05 for values with distinct superscripts in the same column

Serum Urea and Creatinine concentrations in Wistar rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The serum urea concentration was significantly (P<0.05) lower in the group fed a 2% *Azanza garckeana* fruit pulp-supplemented high fructose diet when compared with the control. However, there were no significant variations in the serum creatinine concentration among the groups (Table 5).

Table 5: Serum Urea and Creatinine concentrations in rats fed *Azanza garckeana* fruit pulp-supplemented high fructose diet.

Group	Urea (mmol/L)	Creatinine (umol/L)
A	12.40±0.82^{bc}	97.67±12.24^{a}
B	9.20±0.29^{a}	112.00±6.35^{a}
C	10.40±0.69^{abc}	101.33±10.67^{a}
D	9.93±1.33^{ab}	90.67±7.97^{a}
E	8.70±0.55^{a}	99.33±10.71^{a}
F	12.80±0.80^{c}	89.00±9.45^{a}

Values are given as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences are observed at P<0.05 for values with distinct superscripts in the same column

Serum antioxidant enzymes activities in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The group fed 5% *Azanza garckeana* fruit pulp-supplemented high fructose diet had significantly (P<0.05) higher concentration of SOD and GR activities compared with the group administered a diet rich in high fructose. Also, the group fed 2% *Azanza garckeana* fruit pulp-supplemented high fructose diet had significantly (P<0.05) higher concentration of CAT activities compared with the group administered a diet rich in high fructose (Table 6).

Table 6: Serum Antioxidant Enzymes Activities in rats fed *Azanza garckeana* fruit pulp-supplemented high fructose diet.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GR (U/mg protein)	GPX (U/mg protein)
A	20.20±2.29^{ab}	13.50±1.27^{ab}	54.17±10.73^{ab}	36.03±8.49^{a}
B	13.50±1.44^{a}	9.70±0.00^{a}	28.40±1.44^{a}	33.59±5.48^{a}
C	19.33±1.15^{ab}	12.40±1.32^{ab}	59.93±12.59^{b}	38.39±8.95^{a}
D	14.10±0.81^{a}	9.73±0.72^{a}	64.39±8.58^{b}	42.61±5.43^{a}
E	13.63±2.94^{a}	14.53±1.77^{b}	54.73±6.17^{ab}	60.32±22.37^{a}
F	27.20±4.63^{b}	11.77±1.82^{ab}	73.57±9.67^{b}	44.10±19.05^{a}

Values are given as the Mean ± SEM for three (3) determinations. Values along the same column with the same superscript do not differ significantly. Significant differences are observed at P<0.05 for values with distinct superscripts in the same column.

DISCUSSION

Weight change in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The observed non-significant difference in weight gain among the groups implies that the supplementation of the diet with *A. garckeana* fruit pulp at 2% and 5% inclusion levels may not affect the weights of the rats. The reason for the non-significant difference is unclear, as high fructose diet intake provides more calories than required and causes increased weight gain (MacDonald, 2016). Similarly, Elliott *et al.* (2002) reported that the combined effects of lowered circulating leptin and insulin in individuals who consumed diets high in dietary fructose could increase the likelihood of overfeeding and increased weight gain. It could, therefore, be inferred from the result that 7 weeks of feeding on a high fructose diet may be too short to produce a noticeable difference in weight gain.

The blood glucose level in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet

The result showed that supplementation with the *Azanza garckeana* fruit pulp could stabilize blood glucose. However, the stabilizing effect of this supplementation may take more than four (4) weeks before the manifestation of the stabilizing effect can be observed. This work agrees with the works of Alozieuwa *et al.* (2022), whose study shows that the experimental evidence offered substantiates the therapeutic effectiveness of the diethyl-ether fraction derived from *Thespesia garckeana* for treating diabetes.

In addition to augmenting the functions of antioxidant enzymes, the extract also impeded inflammatory reactions, thus showcasing antidiabetic effects in experimental models. Studies indicated that extracts from *A.garckeana* demonstrated a hypoglycemic effect, which can be ascribed to its substantial phenolic constituents (Lawal *et al.*, 2022). These constituents are a significant determinant of the numerous pharmacological effects of *A.garckeana* and form the foundation for its utilization in traditional medicine (Yusuf *et al.*, 2020). Previous research has reported the inhibitory activities of *A.garckeana* fruit pulp on alpha-amylase activities. Alpha amylase plays a crucial role as an enzyme in charge of the degradation of starch and sugar within the human body. Inhibitors targeting this enzyme have proven beneficial in treating diabetes in animals by acting as oral hypoglycemic agents, thereby aiding in regulating glucose levels and metabolic syndrome.

Lipid Profile in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The higher cholesterol serum level in the group fed a 5% *Azanza garckeana* fruit pulp-supplemented diet may be due to the high-fat content of the fruit pulp. A previous study by Sirajo *et al.* (2022) reported a high-fat content of 10% in the fruit pulp. Similarly, the elevation of total cholesterol and LDL may also be due to the activation of hydroxy-methyl-glutaryl-coenzyme A reductase (HMGCoA), an enzyme controlling the rate of cholesterol formation. Fortunately, the group fed a 5% *Azanza garckeana* fruit pulp-supplemented diet had a high level of HDL which could counter the potential danger of total cholesterol. This contrasts with the work of Jiang and Youling (2016), who suggested that *Azanza garckeana* and Melatonin supplementation could decrease TC and LDL-c through diminishing cholesterol synthesis and inhibition of the hydroxy-methyl-glutaryl-coenzyme A reductase (HMGCoA), an enzyme controlling the rate of cholesterol formation.

Liver Function Enzymes activities in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The 2% supplemented *Azanza garckeana* fruit pulp with animal diet may not distort the liver structure as there was no significant difference in the ALT and AST levels. ALT and AST normally leak into the liver when there is a liver problem. Increased ALT and AST levels were reported to be associated with the potential for developing metabolic syndrome and its associated elements (Kim and Han, 2018). Although high fructose is known to injure the liver (Muriel et al., 2021), the period taken for the experiment might have been responsible for the observed low effect. The increase in the enzyme activities in the group fed a 5% fruit pulp-supplemented diet is not yet clear on the activities of ALT and AST. However, increased activities of the enzymes could also be due to the increased synthesis and not necessarily due to enzyme damage.

Serum Protein, Albumin, and Globulin levels in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

The result obtained from this study showed that supplementing an animal diet with *Azanza garckeana* fruit pulp could help normalize the synthetic function of the liver. The liver synthesizes different proteins for specific hepatocyte function or peripheral tissue. Serum proteins include albumin and globulin. Albumin functions in the transportation of other molecules and also contributes to maintaining the blood's osmotic pressure (Spinella *et al.*, 2016; Busher, 1990). Globulin is a name given to globular serum protein, such as immunoglobulin, which functions in body immunity (Busher, 1990). This work agrees with the work of Yusuf *et al.* (2020) on the pharmacological activities of the extracts of *Azanza garckeana*, where methanol extracts of *Azanza garckeana* exhibited increased inhibition of protein denaturation and increased membrane stabilization effect with increased extract concentration in both Bovine Serum Albumin and Egg Albumin models which indicates the capability of the extract to ameliorate the denaturation of protein (albumin).

Serum Urea and Creatinine levels in rats fed Azanza garckeana fruit pulp-supplemented high-fructose diet.

The result of this study showed that there was an increase in the urea concentration and there was no significant difference in the creatinine level. These compounds are excreted by glomerular filtration at a constant rate. The increase in the urea concentration of the treated rats indicates that the supplementation of *Azanza garckeana* fruit pulp did not affect the normalization of renal function. This work agrees with Yusuf et al. (2020), who reported that serum urea concentration in both the air-dry and sun-dry methanol extract of A. garckeana pulp increased significantly compared to the control.

Serum antioxidant Enzymes activities in rats fed Azanza garckeana fruit pulp-supplemented high fructose diet.

e finding of this study showed that the supplementation of animal diet with *A. garckeana* fruit pulp exhibited antioxidant activity. This could result from the presence of flavonoids and phenolic compounds in the fruit pulp which could directly exert its effects in

scavenging reactive oxygen species. Phenolic compounds could also inhibit reactive oxygen species-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine oxidase (Compaoré *et al.*, 2022). Earlier reports demonstrated that the *Azanza garckeana* plant aids in reducing the generation of free radicals and promoting the production of antioxidant enzymes facilitated (Yusuf *et al.*, 2021; Christopher, 2016; Manna and Jain, 2015). Flavonoids are also known for their antioxidative and hepatoprotective potentials (Lawal *et al.*, 2022).

CONCLUSION

It can be concluded from this study that *Azanza garckeana* fruit pulp has anti-hyperglycemic, anti-dyslipidemic, and antioxidant effects, which could be responsible for its hepatoprotective activity, particularly at 2% supplementation.

RECOMMENDATIONS

Based on the findings in this result, it is recommended that longer duration studies on the effects of the diet supplementation with *Azanza garckeana* fruit pulp in experimental rats fed a high fructose diet is worthwhile.

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REFERENCES

Abel, L. L., Levy, B. B., Brodie, B. B., & Kendall, F. E. (1952). A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. The Journal of Biological Chemistry, 195(1), 357–366.

Adamu, H. M., Ushie, O. A., Lawal, D. S., & Oga, I. A. (2013). Phytochemical Screening of Fruit of Azanza garckeana and Root of Acacia macrothyrsa. International Journal of Traditional and Natural Medicines, 3(1), 19–25. www.ModernScientificPress.com/Journals/IJTNM.aspx

Ahmed, R.H., El Hassan, M.S., & El Hadi, H.M. (2016). International Journal of Advanced Research in Biological Sciences Potential capability of Azanza garckeana fruits aqueous extract on enhancement of iron absorption in Wistar albino rats. Int. J. Adv. Res. Biol. Sci, 3(3), 245–250.

Alozieuwa, U. B., Lawal, B., Sani, S., Onikanni, A. S., Osuji, O., Ibrahim, Y. O., & Babalola, S. B. (2022). Research Article Luteolin-Rich Extract of Thespesia garckeana F . Hoffm . (Snot Apple) Contains Potential Drug-Like Candidates and Modulates Glycemic and Oxidoinflammatory Aberrations in Experimental Animals. 2022(June).

Arthur, J. R., & Boyne, R. (1985). Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life sciences, 36(16), 1569–1575.

Baumber, J., & Ball, B. A. (2005). Determination of glutathione peroxidase and superoxide dismutase-like activities in equine spermatozoa, seminal plasma, and reproductive tissues. American Journal of Veterinary Research, 66(8), 1415–1419.

Bioltif, Y. E., Edward, N. B., & Tyeng, T. D. (2020). A Chemical Overview of Azanza garckeana. Biology, Medicine, & Natural Product Chemistry, 9(2), 91–95.

Busher, J. T. (1990). Serum Albumin and Globulin. Clinical Methods: The History, Physical, and Laboratory Examinations.

Christopher, Y. (2016). Cytotoxicity And Antioxidant Activity of Stem Bark Extracts of Azanza garckeana (kola of Tula). European Journal of Pure and Applied Chemistry, 3(2).

Compaoré, M., Lamien, C. E., Lamien-Meda, A., Vlase, L., Kiendrebeogo, M., Ionescu, C., & Nacoulma, O. G. (2012). Antioxidant, xanthine oxidase and lipoxygenase inhibitory activities and phenolics of Bauhinia rufescens Lam. (Caesalpiniaceae). Natural Product Research, 26(11), 1069–1074.

Elliott, S. S., Keim, N. L., Stern, J. S., Teff, K., & Havel, P. J. (2002). Fructose, weight gain, and the insulin resistance syndrome. American Journal of Clinical Nutrition, 76(5), 911–922.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6), 499–502.

Glew, R. S., Vanderjagt, D. J., Chuang, L. T., Huang, Y. S., Millson, M., & Glew, R. H. (2005). Nutrient content of four edible wild plants from West Africa. Plant Foods for Human Nutrition, 60(4), 187–193.

Goldberg, D.M. and Spooner, R.J. (1983) Assay of Glutathione Reductase. In: Bergmeyen, H.V., Ed., Methods of Enzymatic Analysis, 3rd Edition, Vol. 3, Verlog Chemie, Deerfiled Beach, 258-265.

Grundy, S. M., Hansen, B., Smith, S. C., Cleeman, J. I., Kahn, R. A., & Participants, C. (2004). Issues Related to Management. 551–556.

Huang, C., Mcallister, M. J., Slusher, A. L., Webb, H. E., Mock, J. T., & Acevedo, E. O. (2015). Obesity-Related Oxidative Stress : the Impact of Physical Activity and Diet Manipulation. Sports Medicine - Open, 1-12.

Idoko, A., Ayuba, A., & Ilouno, E. (2018). Physical and Biochemical Studies on Excess Weight-Induced Rats Maintained on Mixed Spices-Supplemented Diet. Nigerian Journal of Basic and Applied Sciences, 26(2), 9.

Jiang and Youling (2016). AG effect on TC and HDL. (n.d.). Kassi, E., Pervanidou, P., Kaltsas, G., & Chrousos, G. (2011). Metabolic syndrome : definitions and controversies. Appendix 1, 1–13.

Kassi, E., Pervanidou, P., Kaltsas, G., & Chrousos, G. (2011). Metabolic syndrome: definitions and controversies. BMC medicine, 9, 1-13. https://doi.org/10.1186/1741-7015-9-48

Kim, H. R., & Han, M. A. (2018). Association between serum liver enzymes and metabolic syndrome in Korean adults. International Journal of Environmental Research and Public Health, 15(8).

Lawal, B., Sani, S., Onikanni, A. S., Ibrahim, Y. O., Agboola, A. R., Lukman, H. Y., Olawale, F., Jigam, A. A., Batiha, G. E. S., Babalola, S. B., Mostafa-Hedeab, G., Lima, C. M. G., Wu, A. T. H., Huang, H. S., & Conte-Junior, C. A. (2022). Preclinical anti-inflammatory and antioxidant effects of Azanza garckeana in STZ-induced glycemic-impaired rats, and pharmacoinformatics of it major phytoconstituents. Biomedicine and Pharmacotherapy, 152, 113196.

Macdonald, I.A. (2016). A review of recent evidence relating to sugars, insulin resistance and diabetes. Eur J Nutr 55 (Suppl 2), 17–23.

Manna, P., & Jain, S. K. (2015). Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. In Metabolic Syndrome and RelatedDisorders.13(10):423-444.

Maroyi, A. (2017). Azanza garckeana Fruit Tree: Phytochemistry, Pharmacology, Nutritional and Primary Healthcare Applications as Herbal Medicine: A Review. Research Journal of Medicinal Plants, 11(4), 115–123.

Muriel, P., López-Sánchez, P., & Ramos-Tovar, E. (2021). Fructose and the Liver. International journal of molecular sciences, 22(13), 6969.

National Research Council (2011). Guide for the care and use of laboratory animals, National Academies Press. Washington, DC.

Nderitu, K. W., Mwenda, N. S., Macharia, N. J., Barasa, S. S., & Ngugi, M. P. (2017). Antiobesity Activities of Methanolic Extracts of Amaranthus dubius, Cucurbita pepo, and Vigna unguiculata in Progesterone-Induced Obese Mice. Evidence-Based Complementary and Alternative Medicine. 1, 1-10.

Patel, D. K., Prasad, S. K., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of Tropical Biomedicine, 2(4), 320–330.

Rifai, N., Bachorik, P.S., & Albers, J.J. (1999). Lipids, lipoprotein and apolipoprotein. In: Burtis CA, Ashwood E.R., editors. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W.B. Saunders company; 806-61

Siddiq, A., & Abdullahi, A. (2023). The inclusion of garlic and turmeric powder in high-fructose diets protects against the development of metabolic syndrome in Wistar rats. 3, 54–61.

Sirajo, K., Shehu, Z., & Usman, M. (2022). Study of Proximate Composition of Seed and Peel of (Azanza garckeana) Goron Tula. 1(2), 76–81.

Spinella, R., Sawhney, R., & Jalan, R. (2016). Albumin in chronic liver disease: structure, functions and therapeutic implications. Hepatology International, 10(1), 124–132.

Srivastava, R. A. K. (2018). Life-style-induced metabolic derangement and epigenetic changes promote diabetes and oxidative stress leading to NASH and atherosclerosis severity. 381–391.

Young, D.S. (1990). Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, AACC Press, Washington DC, 6-12.

Yusuf, A. A., Mohammed, B. A., Abafi, M. J., Garba, R., Usman, O. M., Ibrahim, J., Ariyeloye, D. S., & Berinyuy, E. B. (2020). Alterations in Serum Urea, Creatinine and Electrolytes Concentrations in Wister Rats Following Repeated Administration of Methanol Extracts Of azanza garckeana Pulp. Scholars International Journal of Biochemistry, 3(6), 127–131.

Yusuf, A. A., Yissa, T. D., Agboola, A. R., Balogun, S. M., Adeboye, P. O., Hassan, O. N., & Raji, I. S. (2021). Comparative free radical scavenging and hypoglycemic activities of n-hexane, ethyl-acetate, and aqueous fractions of Azanza garckeana pulp. AROC in Natural Products Research, 1(2), 1-8.