

Evaluation of the Antioxidant, Antidiabetic and Hypolipidemic Potential of *Garcinia kola* Seed Tincture in Streptozotocin-Induced Diabetic Albino Wistar Rats.

Comment [N1]: Please clarify the type of the article, eg Research?

ABSTRACT

Aim: The aim of this study was to evaluate the antioxidant, antidiabetic and hypolipidemic potential of *Garcinia kola* seed tincture in streptozotocin-induced diabetic albino Wistar rats.

Study design: This study is a non-randomized experimental study

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Place and Duration of Study: This study was conducted at the Animal House of the Department of Applied and Environmental Biology, Rivers State University, Port Harcourt, Nigeria, between August and December, 2019.

Comment [N3]: See previous remark...

Methodology: A total of 46 male Albino rats were used. *Garcinia kola* seed was bought and 10g of the seed was macerated with 50mL of 43% alcoholic Gordon and stored at 4°C. Six rats were used for the pilot study to determine the concentration of streptozotocin that could induce diabetes, the remaining 40 rats were divided into 8 groups of 5 rats each, with different experimental treatments. "Streptozotocin diabetes" was achieved by intraperitoneally injecting the rats with 45mg/kg streptozotocin, glibenclamide and metformin doses were extrapolated from human doses and rats treated with *Garcinia kola* seed tincture were separately given low (1ml/kg), high (3ml/kg) of the tincture or in combination with the either glibenclamide or metformin once per day for 30 days. Rat ELISA kits were used to determine the levels of total antioxidant capacity (TAC), total oxidant status (TOS) and malondaldehyde (MDA). An autoanalyzer was used to assay for fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), HDL-cholesterol, while oxidative stress indices (OSI) and LDL-cholesterol were calculated. SPSS version 22.0 for Windows was used for statistical analysis and $P < .05$ was considered statistically significant.

Comment [N4]: Replace by 'Materials and Methods'

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Results: The results showed that there were significantly higher ($P < .001$) MDA, TOS, OSI and FBG levels after streptozotocin induction. On treatment with *G. kola* seed tincture, values were observed to have significantly reduced. Only TG level was significantly higher after induction and a significant lower TG level was also observed only in the group of rats treated with the higher dose of the tincture. Liver enzyme activities did not show any significantly higher values after induction, but values were significantly raised after treatment with tincture and alcohol separately.

Comment [N6]: Statistical model?

Conclusion: From the results, we conclude that *Garcinia kola* seeds tincture has both hypoglycemic and antioxidant attenuating effects in "streptozotocin diabetes" albino wistar rats.

Keywords: Antioxidant, Antidiabetic, *Garcinia kola*, Tincture, Streptozotocin-Induced Diabetic Albino, Rats.

1. INTRODUCTION

Diabetes mellitus is one of the most important diseases worldwide, reaching epidemic proportions. Global estimates predict that the proportion of adult population with diabetes will increase by 69% for the year 2030 [1]. The modalities that can be acquired to sustain the healthcare of the diabetics due to the aforementioned complications and side effects from the ingestion of orthodox drugs may be embraced with optimism if scientists look inward towards the traditional methods of alleviating disease conditions as it had long been reported that subsequent use of drugs as therapeutic measures for the pharmacological treatment of

disease began long ago with the use of herbs and these are readily available and less cost effective [2].

One of the clinical consequences of insulin resistance is chronic hyperglycemia due to the generation of nitrogen and reactive oxygen species. The overproduction [3], or insufficient removal of reactive oxygen as well as nitrogen species is known to be responsible for the development of hyperglycemia and its complications such as microvascular and macrovascular dysfunctions, causing severe damages to membrane lipids, cellular proteins and nucleic acids that can literally result to further chronic complications [4]. Chronic hyperglycaemia, as previously mentioned can lead to a variety of complications such as neuropathy, nephropathy and retinopathy and increased risk of cardiovascular disease, [5].

Comment [N7]: Reference(s)?

Growing proof supports the fact that oxidative stress plays a huge role in the pathogenesis of DM and its complications, [6]. Hyperglycaemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, that is, insulin action and insulin secretion. Also, anti-oxidative mechanisms become depleted in diabetes, which could further increase oxidative stress [7]. Although obesity and physical inactivity are the leading factors for the development of Type 2 DM, data from research suggests that oxidative stress may increase insulin resistance and impaire insulin secretion, thus contributing to its pathogenesis [8].

Bitter Kola scientifically known as *Garcinia kola* is a tropical flowering plant found in Western and Central Africa which produces brown nut-like seeds. It has been used in African culture for centuries for both traditional and medicinal purposes. It contains dimeric flavonoid, lipase inhibitor which is believed to have many healing benefits. Bitter Kola is a masticatory used in traditional hospitality, cultural and social ceremonies such as naming ceremonies and weddings. It is used in many tropical countries to fight infectious diseases such as AIDS and the Ebola virus. It is known to possess anti-inflammatory, antimicrobial and antiviral properties. It is often used to treat the symptoms of colds. It is particularly very effective for coughs, nasal congestions and help coagulate phlegm. Bitter kola is also effective in alleviating sore throat and sometimes believed to cure impotence. It increases blood flow to the Core area in men who have hardening of the arteries, [9]. *Garcinia kola* has been successfully used to treat patients suffering from knee osteoarthritis. It reduced pain and swelling and improved movement. *Garcinia kola* is known for its anti-inflammatory and antioxidant properties. It is used to prevent infections, particularly those caused by viruses. Bitter Kola has been known to be a natural hunger suppressant and also increases the urge to drink more water. It is used as a substitute for hops in brewing lager beer. It is especially useful in preventing beer spoilage, [9-10]

Several pharmacological effects of *Garcinia kola* have been reported and include anti-hypercholesterolemic activity, [11], antioxidant activity, [12] and hypoglycemic effects in diabetic animals, [13]. Accordingly, this study was designed to investigate the possible hypoglycaemic, hypolipidemic and antioxidant effects of *Garcinia kola* seed tincture in streptozotocin-induced diabetic rats.

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2. MATERIALS AND METHODS

2.1 Experimental Animals

Seventy (70) male albino rats of (150g to 200g) in weight were purchased from Department of Pharmacology, University of Port Harcourt, Port Harcourt. They were used throughout the course of this research work and were made to acclimatize for 14 days under standard laboratory conditions, fed with pelleted rat chow (Top Feed Finisher Mash, Nigeria) and tap water *ad libitum*.

Comment [N9]: Reference?

The rats were fed with high fatty feeds which was commercially prepared with margarine and sucrose (see appendix for preparation) in combination with the pelleted chow to initiate obesity, recent studies have reported that high fatty diets give out free radicals that contribute to the impairment of beta cells hence hyperglycemia and its subsequent complications [14].

2.2 Procurement of Herb

Garcinia kola seed was bought at Fruit Garden market, Port Harcourt and was authenticated by plant botanist, Dr. Samuel Adewole of Plant Science Department, University of Port Harcourt.

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2.2.1 Preparation of Hydroethanolic *Garcinia kola* Tincture

Tincture (ratio between drug and solvent 1:5) was prepared as follows:

10g of *Garcinia kola* seed was macerated with 50mL of 43% alcoholic Gordon Gin and allowed to stand for three weeks with 2 days intermittent shaking. After filtration, the tincture was stored at 4°C, and allowed to attain room temperature before use. The amount of the seed in 1ml of the alcohol was determined by evaporating the alcohol and measuring the amount of the seed in 1ml of the tincture.

Comment [N11]: Reference(s)?

2.3 Preparation of Reagents and Chemicals

2.3.1 Citrate Buffer Solution Preparation

The citrate buffer solution is a combination of citric acid salt and sodium citrate salt. About 1.47grams of the sodium citrate salt was measured and dissolved in 50ml of distilled water, this was followed by weighing 1.05gram of citric acid salt which was dissolved in 50ml of distilled water. The mixtures were thoroughly stirred to enable it evenly mixed together and a pH meter was used to check and adjust the pH buffer to 4.5

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2.3.2 Preparation of Glibenclamide

The administered dosage for Glibenclamide was extrapolated from human dose as shown below:

Human daily dose is 1 caplet (5mg) twice daily, that is, 10mg/day.

A 70kg man requires 10mg/day, a rat of 1kg would require $1/70 \times 10\text{mg} = 0.143\text{mg/kg}$ per day [15]. This was administered mg per kg body weight of the rats.

2.3.3 Preparation of Metformin

Human daily dose is 1 tablet (500mg) twice daily, that is, 1000mg/day.

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A 70kg man requires 1000mg/day, a rat of 1kg would require $1/70 \times 1000\text{mg} = 14.286\text{mg/kg}$ per day [15]. This dose was administered mg per kg body weight of the rats.

2.4 Pilot Study to Determine the Concentration of Streptozotocin for Induction of Diabetes

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After the two weeks of acclimatization, 6 rats (3 in each group) were selected and used as pilot study for the determination of the dose of streptozotocin (STZ) that would induce diabetes. The first group was injected 40mg/kg dose of streptozotocin, and the second group 45mg/kg streptozotocin, after a 6 hour fast, for the induction of diabetes. The injections were given intraperitoneally, with citrate buffer (pH 4.5) as vehicle. After 72 hours, diabetes was confirmed in the group of rats injected 45mg/kg STZ with mean fasting blood sugar levels of 22.1mmol/l. The group injected 40mg/kg STZ had a mean fasting blood sugar of 8.9mmol/l,

which is below 13.9mmol/l (250mg/dl), the diagnostic value for diabetes in rats [16], 45mg/kg STZ was chosen as the preferred dose in this study.

2.4.1 Induction of Diabetes using Streptozotocin

After two weeks of acclimatization, diabetes was induced in the male albino rats with streptozotocin (STZ, Sigma Chemical Company, St. Louis, Milestone). STZ was intraperitoneal (i.p.) administered in a dose of 45mg/kg dissolved in citrate buffer (0.1M, pH 4.5). Blood glucose concentrations were measured by Fine Test glucometer (Johnson & Johnson) after 48 hours and subsequently throughout the experiment after diabetes induction and glucose concentrations exceeded 250mg/dl or 13mmol/L confirmed the diabetic state [17].

2.5 Study Design

Forty rats were randomly divided into eight (8) groups of five rats per group:

2.5.1 Group 1 (Negative Control – NC)

Five (5) male rats were given pellet feeds and water *ad libitum* for 30 days.

2.5.2 Group 2 (Streptozotocin-Diabetes Model Control – SDMC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* for 30 days.

2.5.3 Group 3 (Alcohol Control – ALC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 1ml/kg b.wt/day of 43% alcoholic Gordon Gin for 30 days.

2.5.4 Group 4 (Glibenclamide Control-GC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 0.143mg/kg per day of Glibenclamide for 30 days.

2.5.5 Group 5 (Metformin Control-MC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 14.286mg/kg per day per day of Metformin for 30 days.

2.5.6 Group 6 (Garcinia kola High– Gkola High)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 3ml/kg b.wt. of *Garcinia kola* tincture for 30 days.

2.5.7 Group 7 (Garcinia kola Low – Gkola Low)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 1ml/kg b.wt. of *Garcinia kola* tincture for 30 days.

2.5.8 Group 8 (Garcinia kola + Metformin - Gkola+MC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 1ml/kg b.wt. of *Garcinia kola* tincture and 14.286mg/kg per day metformin tablet per day for 30

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days. The interval between the administration of *Garcinia kola* tincture and metformin equalled 40 min to minimize the interaction at the level of absorption.

2.5.9 Group 9 (*Garcinia kola* + Glibenclamide – Gkola+GC)

Five (5) male rats were induced intraperitoneally with a single dose of 45mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum* and given by gavage 1ml/kg b.wt. of *Garcinia kola* tincture and 0.143mg/kg Glibenclamide per day for 30 days. The interval between the administration of *Garcinia kola* tincture and Glibenclamide equalled 40 min to minimize the interaction at the level of absorption.

2.6 Collection of Sample for Laboratory Analysis

The rats were kept on fasting for 6 hours prior to the process of euthanasia, they were also weighed before the process started. The procedure followed the acceptable guidelines of euthanizing the rats, they were placed inside a glass euthanizing chamber that was pre-charged with ether and anesthetized which further confirmed the process of euthanasia. After the rat was observed to be loopy and finally unconscious, it was brought out of the chamber and placed on a board in a crucifix position with drove pins fixed unto its paws, Blood was collected by cardiac puncture after locating the heart through the rib cage through a careful insertion of the 5ml needle pierced into the still beating heart. Blood samples were collected for analysis into Lithium heparin anticoagulant bottles for biochemical test which was allowed to sit for some minutes and spun at 3,000r.p.m for 5minutes to get the serum for enzymes tests while blood was also collected in plain bottles and spun for other analysis such as the lipid tests.

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2.7 Biochemical Determination

The biochemical investigations that were carried out included: Fasting Blood Glucose (FBG), Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Malonaldehyde (MDA), Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein cholesterol (HDL-cho) and Low Density Lipoprotein cholesterol (LDL-cho).

Comment [N18]: Why did the Authors use such indices and not others? Reference(s)?

3.8.1 Determination of Oxidant and Antioxidant Enzymes

2.8.1.1 Determination of Malonaldehyde (MDA)

Malonaldehyde level was measured quantitatively by the sandwich-enzyme linked immunosorbent assay (ELISA) method [18] as described by Shanghai Korain Biotech Co., Ltd, China.

2.8.1.2 Determination of Rat Total Antioxidant Status (TAS) and Total Oxidant Status (TOS)

Total antioxidant status Total Oxidant Status (TOS) were measured quantitatively by the sandwich-enzyme linked immunosorbent assay (ELISA) method as described by Shanghai Korain Biotech Co., Ltd, China.

Comment [N19]: Reference(s)?

2.8.1.3 Calculation of Oxidative Stress Index (OSI)

Oxidative stress index was determined by dividing Total Oxidant Status (TOS) by Total Antioxidant Status (TAS) (TOS/TAS).

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3.8.2 Determination of Blood Glucose concentration [19]

The determination of blood glucose concentration in this study adopted the principle of 'Electro-Chemistry' a reaction described by [19]. The principle of the reaction is thus: Glucose is assayed for after an auto-encoding chemical reaction between the embedded electrodes in the test stripes as a result of the electric current present in the poles of the

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electrodes. The principle works solely on the reaction of glucose oxidase with the blood glucose to form gluconic acid that further reacts with ferrocyanide that enhances the generation of current directly proportional to the amount of glucose.

3.8.3 Determination of Serum Total Cholesterol [20]

Free cholesterols are determined through the hydrolysis and esterification of the enzyme 'cholesterol esterase'. The free cholesterol obtained gets oxidized to form hydrogen peroxide which reacts further with phenol and 4-aminoantiprine by the catalytic action of peroxides to form a red colored quinoneimine dye complex. The intensity of the color developed is directly proportional to the amount of cholesterol present in the sample which is read spectrophotometrically at 500nm.

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3.8.4 Determination of Triglycerides [21]

The determination of triglycerides in the samples was done by the Glycerokinase peroxidase method, a colorimetric enzymatic method described by [21]. Triglycerides are determined after the complete enzymatic hydrolysis with lipases. Under the catalytic initiation of peroxidases, an indicator of a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol will be obtained.

Comment [N23]: See previous remark...

3.8.5 Determination of High-Density Lipoprotein-Cholesterol [22]

The presence of the chylomicrons, low density lipoproteins as well as the very low-density lipoprotein fractions initiate the precipitation of the high-density lipoprotein as supernatant on the addition of phosphotungstic acid and magnesium chloride. The precipitated fraction of the high-density lipoprotein is subjected to the test for cholesterol following an enzymatic end point reaction. Two procedures are responsible for this reaction and all instructions according to the manufacturer's instruction was adhered to.

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3.8.6 Estimation of Low-Density Lipoprotein-Cholesterol [23]

The estimation of Low-density lipoprotein (LDL) was established using the formulae proposed by [23]:

$$\text{LDL -Cholesterol (mg/dl)} = \frac{\text{Total Cholesterol} - \text{High density lipoprotein} - \text{Triglycerides}}{5}$$

Comment [N25]: See previous remark...

3.9 Statistical Analysis

Statistical analysis was done with Statistical Package for Social Sciences (SPSS) of Windows Stat Pack (version 22). Data generated were recorded as mean and standard deviations (Mean \pm S. D), ANOVA (including Tukey's Multiple Comparative Test). p values less than 0.05 were considered to be statistically significant.

Comment [N26]: Weak and unreliable statistical model....

3. RESULTS AND DISCUSSION

In this study, there were significantly higher ($P < .001$) malonaldehyde (MDA), total oxidant status ($P < .001$), oxidative stress index ($P < .001$) and significantly lower ($P < .001$) total antioxidant capacity ($P < .001$) and levels when rats were induced with diabetes using streptozotocin, as compared with the negative control group (NC), (Table 1). The finding is a strong indication of involvement of reactive oxygen species in causing diabetes complications. It is also well known that lipid peroxidation provides a continuous supply of free radicals that play an important role in etiopathogenesis of diabetes and its complications, [24]. This agrees with previous studies [25-26], which stated that levels of MDA, SOD and OSI were significantly elevated in type 2 diabetes patients, [25]. Increasing evidence in experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of type 2 diabetes. Free radicals are formed disproportionately in diabetes by glucose degradation, nonenzymatic glycation of proteins, and the subsequent

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oxidative degradation which may play an important role in the development of complications in type 2 diabetes patients.

Some of these radicals are extremely reactive and therefore interact with some vital macromolecules including lipids, nucleic acids and protein [27]. ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT-enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [28].

Table 1: Malonaldehyde, Total Oxidant Status, Total Antioxidant Capacity and Oxidative Stress Indices of Albino Rats in Controlled Groups and Treatment Groups

Groups	MDA (ng/ml)	TOS (ng/ml)	TAC (ng/ml)	OSI
Grp 1 (NC)	80.62 ± 5.36	1.35 ± 0.10	2.75 ± 0.12	0.49 ± 0.053
Grp 2(SDMC)	319.15 ± 58.94 ¹	3.19 ± 0.25 ¹	1.33 ± 0.26 ¹	2.49 ± 0.627 ¹
Grp 3 (ALC)	239.24 ± 35.63	3.31 ± 0.24	1.63 ± 0.35	2.12 ± 0.595
Grp 4 (GC)	114.95 ± 8.18	2.11 ± 0.18	2.03 ± 0.06	1.03 ± 0.057
Grp 5 (MC)	91.79 ± 3.02	2.13 ± 0.14	2.23 ± 0.05	0.95 ± 0.088
Grp 6 (Gkola High)	116.65 ± 8.90 ^{2,3}	1.98 ± 0.10 ^{2,3}	2.39 ± 0.1 ^{2,3}	0.83 ± 0.061 ^{2,3}
Grp 7 (Gkola Low)	96.08 ± 6.97 ^{2,3}	1.82 ± 0.06 ^{2,3}	2.80 ± 0.05 ^{2,3}	0.65 ± 0.022 ^{2,3}
Grp 8 (Gkola + MC)	91.27 ± 3.24 ²	1.59 ± 0.16 ²	2.77 ± 0.02 ²	0.57 ± 0.066 ²
Grp 9 (Gkola + GC)	100.30 ± 1.35 ²	1.88 ± 0.06 ²	2.26 ± 0.07 ²	0.83 ± 0.001 ²
<i>p-value</i>	<0.001	<0.001	<0.001	<0.001
<i>F-value</i>	61.820	85.651	45.639	29.239
Remark	S	S	S	S

S-Significant, and numbers 1,2,3...signify significant *p* values when compared with the corresponding groups: Grp 1, Grp 2, Grp 3.... (Turkey's Multiple Comparison Test) @ *p*<.05.

In diabetic hyperlipemia, a series of bizarre lipoproteins and other lipids appear and interaction of this with oxidative stress and free radicals leads to enhanced lipid peroxidation in plasma, tissues and membranes, causing extensive tissue damage. As reported by some epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia [29].

However, when rats were treated with lower and higher concentrations of *G. kola* (groups Gkola Low and Gkola High), a significantly lower (*P*<.001) MDA, total oxidant status (*P*<.001), oxidative stress index (*P*<.001) levels were recorded (Table 1). This shows a strong antioxidant capacity of *G. kola* seed tincture. Most of *G. kola* biological activities have been attributed to the presence of the phytochemical constituent alkaloids, which is in line with the phytochemical analysis result of this study.

Over time, the antioxidant and scavenging activity of *Garcinia kola* has been investigated in a range of established *in vitro* assays involving reactive oxygen species. In a study carried out by Drury and colleagues, it was reported that *G. kola* elicited significant reducing power and a dose-dependent inhibition of oxidation of linoleic acid. *G. kola* inhibited H₂O₂, and was more effective than the standard antioxidants BHA and β-carotene and equivalent in power to α-tocopherol. [30]. Kolaviron also significantly scavenged superoxide generated by phenazine methosulfate NADH. Furthermore, *G. kola* scavenged hydroxyl radicals as revealed by significant inhibition of the oxidation of deoxyribose.

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Comment [N30]: Reference(s)?

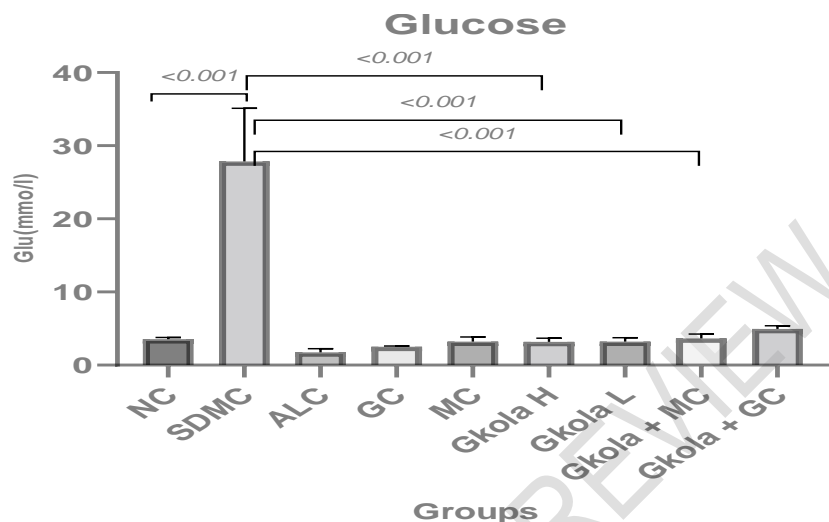


Fig. 1: Graphical Representation of Glucose Values for all Treatment Groups

It has also been reported that, *G. kola* seed scavenged hydroxyl radicals as revealed in the study, by significant inhibition of the oxidation of deoxyribose. The inhibitory activity of *G. kola* in deoxyribose assay may relate directly to prevention of the propagation of the process of lipid peroxidation and modulation of other biomarkers of oxidative stress in animal model, [12][31].

Generally, *G. kola* seed has been known to have antioxidant effect as stated above. However, some studies have reported otherwise. In study by [32] where rats were treated with *G. kola* extract, it was reported that there were increased lipid peroxidation and nitrite production. Diminished MDA production and decreased antioxidant enzymes activities of Superoxide dismutase as well as catalase in rats treated with *G. kola* seed were also reported.

As expected, rats that were treated with the antidiabetic drugs Metformin and Glibenclamide both produced significantly lower TOS, MDA and OSI with raised TAC levels, when compared with values for rats that were induced but not treated. This of course could be due to the ameliorative effect of the drugs on diabetic complications. Although, various therapeutic agents used in diabetes treatment available today achieve transiently regulated euglycemia but fail to prevent lipid and lipoprotein alterations, ultimately, exposing the diabetic humans and animals to cardiovascular complications, [32]. Feeding the rats with a combination of *G. kola* seed tincture and the diabetic drugs, both also produced significantly lower levels of all analysed oxidative stress markers and significantly higher antioxidant marker, TAC, with Metformin producing a better effect, both when administered individually and in combination with *G. kola* seed tincture. However, Glibenclamide when administered in combination with *G. kola* seed tincture had a better MDA reducing effect than when administered alone, (Table 1). This could mean that there was a synergistic herb-drug interaction between Glibenclamide and *G. kola* seed tincture to produce a stronger antioxidant effect.

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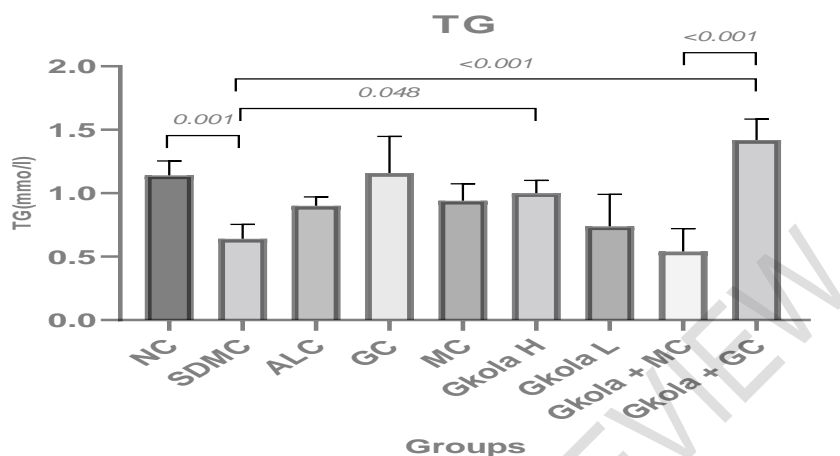


Fig. 2: Graphical Representation of Triglyceride Values for all Treatment Groups

Generally, there were no significant differences in the mean MDA, TOS, TAC and OSI values of rat group that was treated with alcohol only when compared with the SDMC group and a significantly lower ($P<.001$) levels of TOS, MDA and OSI when compared with values of groups treated with both *G. kola* seed tincture alone and with the antidiabetic drugs. This shows that the antioxidant effects observed in the latter groups were due to *G. kola* seed and not the alcohol with which it was tintured.

In this study, there was a significantly higher ($P<.001$) fasting blood glucose levels when the group of rats that were diabetes induced using streptozotocin was compared with the negative control group (NC), (Fig. 1). This confirms the effectiveness of the inducing agent. The significantly higher fasting blood glucose levels after induction of rats could be due to the diabetogenic potentials of streptozotocin. This finding is in agreement with other methods of streptozotocin induction of diabetes [34]. The finding also agrees with the reports of [35], in which intraperitoneal administration of streptozotocin effectively induced diabetes with significant hyperglycaemia. It is also in agreement with the works of [36], in which high fat diet in combination with a sub-diabetic dose of streptozotocin (35mg/kg), produced consistent hyperglycaemia in rats.

It has previously reported that "Streptozotocin diabetes" is basically caused by the specific necrosis of the pancreatic β -cells, and this agent is the first choice for diabetes induction in animals, [37-38]. Depending on the animal strain, dose, route of drug administration, and the life period in which STZ is administered in rats, severe diabetes (blood glucose greater than 25 -30mmol/l) [38] or mild diabetes (glycemia between 16 and 30 mmol/l) are generated [39]. For severe diabetes induction, STZ is administered at 40-50 mg/kg body weight intravenously or intraperitoneally during adulthood. After approximately three days, these animals present blood glucose levels greater than 30mmol/l, [40].

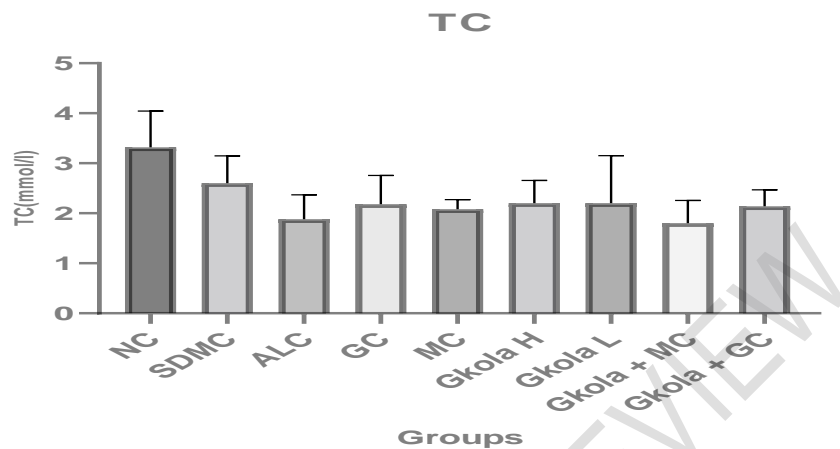


Fig. 3: Graphical Representation of Total Cholesterol Values for all Treatment Groups

Treatment of rats with lower and higher doses of *G. kola* seed tincture produced significantly lower ($P < .001$) fasting blood glucose values. There were also significantly lower fasting blood glucose levels when mean glucose levels for rats treated with metformin and glibenclamide when compared with values for SDMC group. The results also showed that the hypoglycemic effect of *G. kola* seed was not affected by the increase in the dose of the seed administered, as there was no significant difference between the mean fasting blood glucose values of rats in both groups (Gkola High and Gkola Low), (Fig. 1).

Comment [N33]: What about findings from previous similar studies?

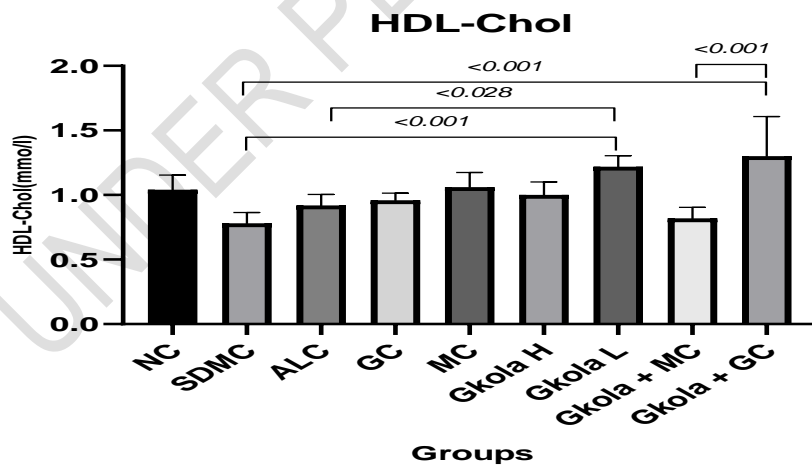


Fig. 4: Graphical Representation of HDL-Cholesterol Values for all Treatment Groups

From the findings of this study, *Garcinia kola* seed tincture could exert a beneficial effect in the diabetics by enhancing insulin secretion or improving the mimicking of insulin secretion

action [41], the plant phytochemical constituents include dimeric flavoid, biflavoid, xanthone and benzophenones [42]. Hydroxycitric acid is the principal acid of the fruit, this acid has shown to be a potent inhibitor of ATP-dependent citrate lyase which catalyses the cleavage of citrate to oxaloacetate and Acetyl-CoA [43].

The findings of this study showed that only mean triglyceride level was significantly lower ($P < .001$) when the mean value for SDMC group was compared with the NC group (Fig. 2). Other lipid profile parameters showed no significant difference (Figs 3-5), implying that the induction with streptozotocin possibly did not have much effect on the TC, HDL and LDL-Cholesterol levels of the rats. However, mean TC and LDL-cholesterol levels for SDMC were slightly lower than those of NC group, and there was also an increase in HDL-cholesterol level, but not significant ($P > .05$) (Fig. 4). Although, hyperlipidemia is a known complication of diabetes mellitus, [44] and coexists with hyperglycemia and is characterized by increased levels of cholesterol, triglycerides and phospholipids, and also changes in lipoproteins, [45]. Interest in the study of plasma lipids in diabetes arises from the widely acknowledged higher incidence of atherosclerotic disease which is a major cause of premature death in diabetic patients, [46]. The non-significant differences in some lipid profile parameters in this study in the diabetic rats, may possibly be due to the short period of time the experimental animals were exposed to streptozotocin, which probably wasn't enough to introduce diabetic dyslipidemia.

Comment [N34]: Reference(s)?

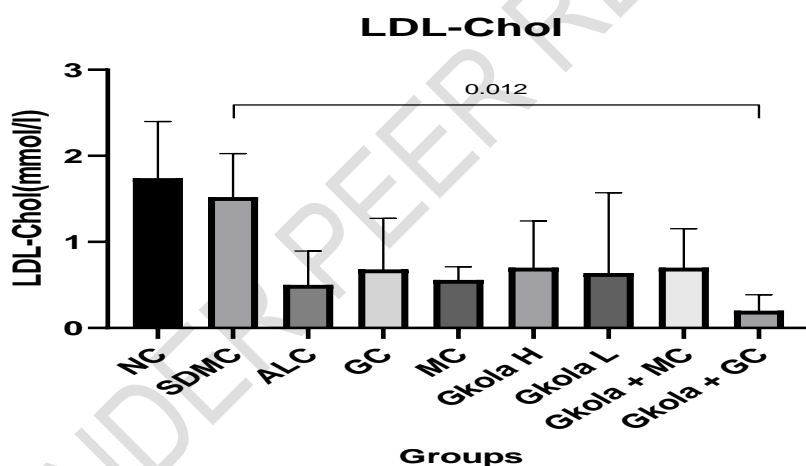


Fig. 5: Graphical Representation of LDL-Cholesterol Values for all Treatment Groups

Treatment of rats with the higher dose of *G. kola* after induction showed no significant differences in all lipid profile parameters except TG level, which was significantly lower ($P = .048$) (Fig. 2), when compared with the TG value for SDMC. Similarly, treatment with the lower dose (Gkola Low) also did not show any significant difference in lipid profile parameters except in HDL-cholesterol where there was a significantly higher ($P < .001$) HDL-cholesterol level when mean value for this group was compared with that SDMC. In other words, this implies that the higher dose of *G. kola* seed tincture was capable of reducing the total cholesterol levels of diabetic rats, while the lower dose of same plant seed caused an increase in the amount of "good cholesterol", the HDL-cholesterol to a significant level.

The finding of this study is to an extent in agreement with that of [40] where they reported that all prophylactic and therapeutic group treated sub-groups had significantly ($P < .05$) lower serum concentrations of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c) when compared to hyperlipidemic control. The *G. kola* seed tincture was also reported to have increased the levels of high-density lipoprotein cholesterol (HDL-c) (which is in line with the findings of this study), and HDL-c/TC ratio. The LDL-c/HDL-c and log (TG/HDL-c) level were lowered by the tincture.

4. CONCLUSION

From the findings of this study, it is therefore, concluded that *Garcinia kola* seeds tincture has both hypoglycemic and antioxidant attenuating effects in streptozotocin-induced diabetic albino rats. The induction of diabetes in this study did not significantly increase or decrease lipid profile levels, but the administration of the tincture significantly improved the blood HDL-cholesterol levels.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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