European Journal of Biotechnology and Bioscience

Volume: 3, Issue: 4, 40-48
April 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

Osheke Shekins Okere
Department of Biochemistry,
Bingham University,
Nasarawa State, Nigeria

Moses Dele Adams
Department of Biochemistry,
Bingham University,
Nasarawa State, Nigeria

Danjuma Kassim Abdullahi
Department of Chemistry,
Bingham University,
Nasarawa State, Nigeria

Correspondence:
Moses Dele Adams
Department of Biochemistry,
Bingham University,
Nasarawa State, Nigeria

Chemopreventive and hepatoprotective properties of *Garcinia kola* seeds in alloxan-induced diabetic rats

Osheke Shekins Okere, Moses Dele Adams, Danjuma Kassim Abdullahi

Abstract
The chemopreventive and hepatoprotective properties of different doses of aqueous extract of *Garcinia kola* seeds on alloxan-induced diabetic rats were studied. Thirty six male Wistar rats weighing 140.25±3.56 g were randomly divided into six groups (A-F) comprising six animals each. Group A, served as the normal control (no treatment). Animals in groups B, which served as the diabetic control, were induced with diabetes by diabetogenic dose of alloxan (200 mg/kg body weight) intraperitoneally. Animals in groups C-F which were induced with diabetes were also administered 1 ml of Glibenclamide (reference antidiabetic drug), 40, 80 and 120 mg/kg body weight of aqueous extract of *Garcinia kola* seeds respectively. Treatment with the extract lasted for 14 days. Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, proteins, glycosides, carbohydrates, reducing sugars, starch, steroids and triterpenoids. Administration of the extract at 120 mg/kg body weight cleared the glucose in urine by 75% on day 14. The extract at 120 mg/kg body weight significantly ($p<0.05$) reduced the levels of blood glucose and serum cholesterol from 458.40±13.94 mg/dL to 117.12±10.55 mg/dL (90%) and 208.64±1.35 mmol/L to 162.29±1.00 mmol/L (30%) respectively on day 14. Administration of the extract at 120 mg/kg body weight significantly ($p<0.05$) decreased serum bilirubin concentration from 30.73±0.38 µg/100ml to 20.52±0.21 µg/100ml (50%) on day 14. Furthermore, the extract at 120 mg/kg body weight significantly ($p<0.05$) reduced the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver of the animals from 41.66±2.10 IU to 23.24±1.16 IU (45%) and 77.53±0.55 IU to 25.73±1.16 IU (35%) on day 14. Of all doses investigated, only the 120 mg/kg body weight of *Garcinia kola* seeds produced the most significant effect in all biochemical parameters analyzed. This dosage is found to possess hypoglycaemic potentials and thus can be recommended for use in the treatment of diabetes as well as in control of some of the metabolic disorders of liver normally associated with diabetes.

Keywords: *Garcinia kola*, Diabetes Mellitus, Blood Glucose, Alloxan, Glibenclamide

1. Introduction
Diabetes mellitus is a chronic disorder of carbohydrate, lipid, and protein metabolism characterized by persistent elevation of fasting blood glucose above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action [1]. This disorder is a worldwide public health problem and one of the main chronic syndromes currently affecting mankind, regardless of socioeconomic status and geographic location [2]. Presently, projections for world global prevalence of diabetes mellitus has it that 366 million people will be diabetic in 2030 as against the 191 million estimated in 2000 [3]. It is also predicted that it will affect approximately 4% population worldwide, which is expected to increase by 5.4% in 2025 [4]. In Nigeria, World Health Organization [5] as well as [6] put the incidence at 1995 as 576,000 while it is projected to rise to 685,000 by 2010. Diabetes is associated with complications such as blindness, stroke, heart and blood vessel disease, kidney failure, retinopathy, amputation, neuropathy, ulceration and gangrene of extremities [7, 8]. Several management options for diabetes are available but are associated with serious shortcomings such as gastrointestinal irritation, nausea, diarrhea, cramps, and increased flatulence [9, 10]. This is because, none of these antidiabetic drugs could give a long term glycaemic control without causing any adverse side effects [11, 12]. Therefore, it is imperative to explore options in herbal medicine for the management of the disease. One plant claimed to be used in the management of diabetes mellitus in folk medicine of Nigeria is *Garcinia kola*.
Garcinia kola, a dicotyledonous plant belonging to the Clusiaceae or Guttiferae family is commonly found in the subtropical and tropical forests of some countries of West and Central Africa such as Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Ghana, Liberia, Nigeria, Senegal and Sierra Leone, Asia and Europe [13, 14]. It is a medium-sized tree growing up to 12 m tall and 1.5 m wide and usually found in the rain forest of Nigeria [15].

The seed is popularly known as bitter kola or male kola in English. In Nigeria, the seed is commonly called "*Namiji goro*" in Hausa, "*Aku'ilu*" in Igbo and "*Orogbo*" in Yoruba [16]. It is a brown nut-like seed, used in traditional medicine. The seeds when chewed have a bitter astringent taste [17].

Previous studies by Adaramoye and Adeyemi [18] have reported the hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin-induced diabetes mellitus rats while Azu and Osinubi [19] worked on the hypoglycaemic activities of extract of *Garcinia kola* seeds in normal, hypoglycaemic and alloxan-induced diabetic rats. Adaramoye [20] saw the antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds in Wistar rats while Iwu *et al* [21] assayed for anti-diabetic and aldose reductase activities of biflavanones of *Garcinia kola*. Furthermore, Udenze *et al* [22] reported the pharmacological effects of *Garcinia kola* seeds powder on blood sugar, lipid profile and atherogenic index of alloxan-induced diabetic rats while Chinedu *et al* [23] worked on acute administration of aqueous extract of *Garcinia kola* on daily blood glucose level and selected biochemical indices in longevity Wistar albino rats. Despite these studies, there is still dearth of information on the ethnobotanically acclaimed doses (40, 80 and 120 mg/kg body weight) investigated in this study. Therefore, the present study intends to provide information on the chemopreventive and hepatoprotective properties of aqueous extract of *Garcinia kola* seeds on alloxan-induced diabetic male rats.

2. Materials and Methods

2.1 Materials

2.1.1 Plant Materials and Authentication

Garcinia kola seeds which were purchased from Mararaba Main Market in Nasarawa State were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher specimen number (F.H.I. 10847).

2.1.2 Experimental Animals

Male albino rats (*Rattus norvegicus*) weighing 140.25±3.56 g were obtained from the Veterinary and Livestock Studies Division of Nigerian Institute for Trypanosomiasis Research, Vom, Jos, Plateau State, Nigeria. This was used for both the antidiabetic and toxicity study.

2.1.3 Assay Kits and Drugs

The assay kits for the determination of AST and ALT were products of Randox Laboratory Ltd, Co-Atrim, UK. Alloxan monohydrate (1, 3-Diazinane-2, 4, 5, 6- tetrone) and glibenclamide were products of Sigma-Aldrich, St. Louis, USA and Kemei Laboratories Ltd., Mumbai, India respectively. Other reagents used were of analytical grade (BDH, UK) and were prepared with distilled water unless otherwise stated.

2.1.4 Glucose Meter and Test Strips

One Touch Blood Glucose Monitoring System (glucometer) and On-Call-Redi Blood Glucose Test Strips and were products of Schiffgraben Diagnostic, Hannover, Germany and Acon Laboratories Inc., San Diego, USA respectively. Medi-Test Combi 10 SGL urine test strips was obtained from BHR Pharma, LLC, Herdon, VA, Washington DC.

2.2 Methods

2.2.1 Preparation of Aqueous Extract of *Garcinia kola* Seeds

Dried seeds of *Garcinia kola* were peeled to remove the testa. This was cut into smaller sizes and thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil) to obtain smooth powder. A known weight (20 g) of the powder was extracted in 100 ml of distilled water for 48 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a steam bath to give the required brownish-black residue (4.5 g). This was then reconstituted in distilled water to give the required doses (40, 80 and 120 mg/kg body weight) used throughout the experimental period.

2.2.2 Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, proteins, glycosides (cyanogenetic glycosides, cardiac glycosides, anthracene glycosides), carbohydrates, reducing sugar, starch, steroids and triterpenoids were carried out by adopting the procedures described by Trease and Evans [24].

2.2.3 Determination of Blood Glucose and Induction of Diabetes

After 14-16 hours of over-night fasting, the fasting blood glucose was determined by drawing blood samples (0.6 μL) from the sharply cut tail vein, placing it on the test strip that had been inserted into the glucometer. Thereafter, diabetes mellitus was induced intraperitoneally in the experimental rats by the administration of diabetogenic dose of alloxan monohydrate (200 mg/kg body weight). Alloxan crystals weighing 1.2 g were dissolved in 10 ml normal saline to give 12 mg/ml solution. Then 0.3 ml of the alloxan solution was administered as the diabetogenic dose following the methods described by Bowman and Rand [25] and Guyton and John [26]. Only animals with fasting blood glucose level higher than 200.00 mg/dL [27] were used for the study.

2.2.4 Experimental Design

Thirty six male albino rats weighing 140.25±3.56 g were completely randomized into six groups (A-F) comprising 6 animals each. Animals in group A which served as the normal control were administered 1 ml of distilled water. Animals in groups B, C, D, E and F which were all induced with diabetes mellitus by intraperitoneal administration of alloxan monohydrate (200 mg/kg body weight) were in addition respectively administered distilled water, glibenclamide (a reference antidiabetic drug), 40, 80 and 120 mg/kg body weight of the aqueous extract of *G. kola* seeds once daily. Hyperglycemia was confirmed 48 hours after induction using one touch glucometer before treatment with the aqueous extract of *G. kola* seeds commenced. Treatment with the aqueous extract lasted for 14 days during which blood glucose level and selected biochemical parameters were determined.

2.2.5 Treatment of Experimental Animals

The animals were allowed to acclimatize for two weeks prior to commencement of the experiment. The animals were allowed free access to rat pellets (Vital Feed®, Grand Cereals Ltd, Jos, Plateau State, Nigeria) and tap water *ad libitum*, and were also administered aqueous extract of *G. kola* seeds,

with respect to the experimental design. The amount of extract administered to the rats was based on the work of Heinz [28] where the treatment was for 14 days using a metal oropharyngeal cannula. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123 [29].

2.2.6 Determination of Urine Glucose

Fresh urine, collected three times on the designated days (Days 1, 7 and 14), by slightly pressing the tail and back of the rats was centrifuged at 224 *g* x 10 min. The urine glucose was determined according to the procedures outlined in the assay kit using Medi-Test Combi 10 SGL urine test strips as described by Trinder [30].

2.2.7 Collection of Blood, Preparation of Serum and Tissue Homogenate

Under ether anaesthesia, the veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were cut with a sterile scapel blade and 5 ml of the blood was collected into clean and dry centrifuge tubes. The blood was then left undisturbed for 10 minutes at room temperature to clot. The tubes were thereafter centrifuged at 224 g x 10 minutes using Uniscope Laboratory Centrifuge (Model 800D, New Life Medical Instrument, England). The sera were later aspirated with Pasteur pipettes into dry sample bottles and used within 12 hours of preparation for the biochemical assays. The animals were thereafter quickly dissected and the liver was removed. The organ was later blotted with a clean tissue paper, weighed and homogenized in 0.25 M sucrose solution (1:5 w/v). The homogenate was then centrifuged at 1340 x g for 15 minutes. The supernatant was frozen overnight [31] before being used for the determination of selected biochemical parameters.

2.2.8 Determination of Serum Total Bilirubin (Vander Bergh's Reaction)

The method of Malloy and Evelyn [32] was selected for modification, as it was the simplest available and most suited for this purpose.

Procedure: Serum (0.4 ml) was pipette into each of the test tubes A and B. 1.0 ml of diazo reagent was added to A and 1.0 ml of blank solution added to B. After 5 minutes, absorbance was read at 540 nm of A using B as blank (E_1). Methanol (5 ml) was added to A and B. It was mixed thoroughly and left for 30 minutes. The absorbance was read using B as blank at 540 nm (E_2). The absorbance of standard was also read at 540 nm using water as blank (E_s). Concentration of the test sample was calculated using the colorimetric equation below:

$$\text{Bilirubin Concentration } (\mu g/100ml) = \frac{A_{test} \times \text{Conc.}_{std}}{A_{std}}$$

2.2.9 Determination of Serum Total Cholesterol (Libermann-Burchardt's Test)

Total cholesterol was determined in the serum of the animals by adopting the procedure described by Allan and Peter [33].

Principle: Cholesterol reacts with concentrated acid as a typical alcohol to produce coloured substance. In this method, acetic acid and acetic anhydride were used as solvents and dehydrating reagents. Concentrated sulphuric acid was employed as a dehydrating and oxidising agent. The absorbance of the final bluish- green colour obtained was read at 570 nm.

Procedure: Blood samples (3 ml) were collected into a plain container with the aid of a syringe (2 ml) from the heart having anaesthetized pinned and dissected the experimental rat under study on a dissecting-board. The blood sample was allowed to clot before it was centrifuged to obtain blood serum. Test tubes were labelled; sample (six test samples), standard and blank. To each of these test tubes was added 0.2 ml of serum, cholesterol standard and distilled water respectively. Thereafter, 5.0 ml acetic acid-acetic anhydride reagent was added to each of the test tubes and the solution were thoroughly mixed, and then allowed to stand for a period of 5 minutes. Then, 0.1 ml of concentrated sulphuric acid was added alongside to each of the tubes, mixed thoroughly and kept in cold water for a period of 10 minutes. The absorbance was then read against the blank. Concentration of the test sample was calculated, using the colorimetric equation:

$$\text{Cholesterol Concentration (mmol/L)} = \frac{A_{test} \times \text{Conc.}_{std}}{A_{std}}$$

2.2.10 Determination of Liver Aspartate Aminotransferase (AST) and Alanine Amino Transferase (ALT)

Principle: Enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) act on the appropriate substance to yield the products oxaloacetate and pyruvate respectively. The ketoacids (oxaloacetate and pyruvate) were assayed colorimetrically coupling with 2,4-dinitrophenyl hydrazine in an alkaline medium to yield the corresponding hydrazones. Liver enzyme activity was done using the assay kits stated above.

2.2.11 Statistical Analysis

Results were expressed as the mean ± SEM of six determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student's t test. The differences were considered statistically significant at $p<0.05$. All the analyses were done using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, proteins, glycosides, carbohydrates, reducing sugars, starch, steroids and triterpenoids. Phytochemical screening also revealed that the most abundant phytoconstituents in *Garcinia kola* seeds are flavonoids (Table 1).

Table: 1 Phytochemical Constituents of *Garcinia kola* Seeds

Phytoconstituents	*Garcinia kola* Seeds
Alkaloids	-
Saponins	+
Tannins	+
Flavonoids	++
Proteins	+
Glycosides:	
a. Cyanogenetic glycosides	-
b. Cardiac glycosides	+
c. Anthracene glycosides	+
Carbohydrates	+
Reducing sugar	+

Starch	+
Steroids and triterpenoids	+

Key: + = Present; - = Absent

Administration of the extract at 40 and 80 mg/kg body weight reduced the urine glucose level from 150 mg to 100 mg and 150 mg to 50 mg respectively (Table 2). However, the extract at 120 mg/kg body weight significantly ($p<0.05$) reduced the urine glucose level from 150 mg to 0 mg (normal) when compared with the normal control animals (Table 2).

Table: 2 Urine Glucose Concentration (Normal Range- Nil)

Treatment Groups	Day 1 (mg)	Day 7 (mg)	Day 14 (mg)
Normal Control (No Treatment)	Nil	Nil	Nil
Diabetic Control 1 (No Treatment)	+++	+++	+++
Diabetic Control 2 (Glibenclamide)	+++	+++	++
Diabetic Rats + 40 mg/kg Body Weight	+++	+++	++
Diabetic Rats + 80 mg/kg Body Weight	+++	+++	+
Diabetic Rats + 120 mg/kg Body Weight	+++	++	Nil

Key: Nil = Normal Range; + = 50mg; ++ = 100mg; +++ = 150mg

After 14 days of treatment, the extract of *Garcinia kola* seeds at 40 and 80 mg/kg body weight significantly ($p<0.05$) reduced blood glucose level from 510.75±17.85 mg/dL to 200.42±3.75 mg/dL and 507.29±9.80 mg/dL to 147.63±10.30 mg/dL (Table 3). The extract at 120 mg/kg body weight significantly ($p<0.05$) reduced blood glucose level from 458.40±13.94 mg/dL to 117.12±10.55 mg/dL when compared with the normal control animals (Table 3).

Table: 3 Blood Glucose Concentration (Normal Range: 75-115 mg/dL)

Treatment Groups	Day 1 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)
Normal Control (No Treatment)	84.43±1.20 ^{a}	106.25±1.38^{b}	101.45± 2.30^{c}
Diabetic Control 1 (No Treatment)	503.71±9.60^{a}	497.23±10.50^{b}	408.45±14.35^{c}
Diabetic Control 2 (Glibenclamide)	522.39±15.30^{a}	433.46±7.05^{b}	211.15±2.64^{c}
Diabetic Rats + 40 mg/kg Body Weight	510.75±17.85^{a}	438.65±10.10^{b}	200.42±3.75^{c}
Diabetic Rats + 80 mg/kg Body Weight	507.29±9.80^{a}	343.96±14.30^{b}	147.63±10.30^{c}
Diabetic Rats + 120 mg/kg Body Weight	458.40±13.94^{a}	234.41±4.45^{b}	117.12±10.55^{c}

n =6 ± SEM;

$^{a-c}$Values carrying superscript different from their respective control for each day are significantly different ($p<0.05$).

After 14 days, administration of the extract at 40 and 80 mg/kg body weight significantly ($p<0.05$) reduced cholesterol concentration in the serum of the animals (Table 4). After 7 days, the extract at 120 mg/kg body weight which did not significantly ($p>0.05$) alter the concentration of cholesterol in the serum of the animals when compared with the value obtained at day 1, significantly ($p<0.05$) reduced cholesterol concentration in the serum of the animals when compared with the normal control animals. Administration of the Glibenclamide (reference antidiabetic drug) also significantly ($p<0.05$) reduced cholesterol concentration in the serum of the animals (Table 4).

Table: 4 Serum Cholesterol Concentration (Normal Range - >200 mmol/L)

Treatment Groups	Day 1 (mmol/L)	Day 7 (mmol/L)	Day 14 (mmol/L)
Normal Control (No Treatment)	135.43±0.25^{a}	147.55±0.75^{b}	150.83± 1.00^{c}
Diabetic Control 1 (No Treatment)	236.46±2.25 ^{a}	221.39±1.70^{b}	259.20±1.20^{c}
Diabetic Control 2 (Glibenclamide)	208.58±0.90 ^{a}	193.51±3.20^{b}	171.49±2.25^{c}
Diabetic Rats + 40 mg/kg Body Weight	210.37±1.40^{a}	206.24±2.90^{b}	192.66±0.40^{c}
Diabetic Rats + 80 mg/kg Body Weight	221.19±1.15^{a}	203.87±1.40^{b}	187.42±1.75^{c}
Diabetic Rats + 120 mg/kg Body Weight	208.64±1.35^{a}	208.62±2.10^{a}	162.29±1.00^{b}

n =6 ± SEM;

$^{a-c}$Values carrying superscript different from their respective control for each day are significantly different ($p<0.05$).

After 14 days, administration of the extract at all doses significantly ($p<0.05$) reduced serum bilirubin concentration when compared with the normal control animals (Table 5).

Table: 5 Serum Bilirubin Concentration (Normal Range: 0 – 17µg/100ml)

Treatment Groups	Day 1 (µg/100ml)	Day 7 (µg/100ml)	Day 14 (µg/100ml)
Normal Control (No Treatment)	5.73±1.01[a]	9.45±0.38[b]	6.79± 0.07[a]
Diabetic Control 1 (No Treatment)	38.65±0.75[a]	35.93±0.05[b]	42.53±0.01[c]
Diabetic Control 2 (Glibenclamide)	36.87±0.22[a]	29.11±0.02[b]	25.38±0.02[c]
Diabetic Rats + 40 mg/kg Body Weight	33.51±0.04[a]	28.54±0.08[b]	27.33±0.01[c]
Diabetic Rats + 80 mg/kg Body Weight	37.28±0.05[a]	29.62±0.08[b]	24.21±0.16[c]
Diabetic Rats + 120 mg/kg Body Weight	30.73±0.38[a]	24.20±0.04[b]	20.52±0.21[c]

n =6 ± SEM;

[a-c]Values carrying superscript different from their respective control for each day are significantly different (p<0.05).

After 14 days, administration of the extract at all doses significantly (p<0.05) reduced the activity of liver aspartate aminotransferase (AST) when compared with the normal control animals (Table 6).

Table: 6 Liver Aspartate Aminotransferase (AST) Activity (Normal Range: 6 – 25 IU)

Treatment Groups	Day 1 (IU)	Day 7 (IU)	Day 14 (IU)
Normal Control (No Treatment)	13.60±2.10[a]	16.50±1.55[b]	10.53± 1.6[c]
Diabetic Control 1 (No Treatment)	67.19±1.16[a]	52.36±2.10[b]	59.23±2.50[c]
Diabetic Control 2 (Glibenclamide)	59.36±2.58[a]	41.34±2.50[b]	31.29±2.10[c]
Diabetic Rats + 40 mg/kg Body Weight	52.16±2.45[a]	47.50±1.16[b]	36.57±1.58[c]
Diabetic Rats + 80 mg/kg Body Weight	52.76±1.16[a]	41.92±2.10[b]	27.10±2.41[c]
Diabetic Rats + 120 mg/kg Body Weight	41.66±2.10[a]	31.73±1.58[b]	23.24±1.16[c]

n =6 ± SEM;

[a-c]Values carrying superscript different from their respective control for each day are significantly different (p<0.05).

After 14 days, administration of the extract at all doses significantly (p<0.05) reduced the activity of liver alanine aminotransferase (ALT) when compared with the normal control animals (Table 7).

Table: 7 Liver Alanine Aminotransferase (ALT) Activity (Normal Range: 6 – 25 IU)

Treatment Groups	Day 1 (IU)	Day 7 (IU)	Day 14 (IU)
Normal Control (No Treatment)	17.50±1.58[a]	12.33±1.16[b]	21.43± 1.58[c]
Diabetic Control 1 (No Treatment)	83.29±1.16[a]	72.62±2.50[b]	77.11±1.61[c]
Diabetic Control 2 (Glibenclamide)	67.51±2.58[a]	52.49±2.10[b]	43.15±2.50[c]
Diabetic Rats + 40 mg/kg Body Weight	77.36±0.55[a]	57.50±0.33[b]	39.31±2.00[c]
Diabetic Rats + 80 mg/kg Body Weight	72.33±0.30[a]	48.67±0.55[b]	34.12±2.10[c]
Diabetic Rats + 120 mg/kg Body Weight	77.53±0.55[a]	39.12±0.30[b]	25.73±1.16[c]

n =6 ± SEM;

[a-c]Values carrying superscript different from their respective control for each day are significantly different (p<0.05).

4. Discussion

Diabetes mellitus is often referred to a condition in which the body either does not produce enough, or does not properly respond to insulin, a hormone produced in the pancreas [34]. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides. Alloxan, a beta cytotoxin, destroys β-cells of islets of Langerhans of pancreas resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues [35, 36, 37, 38]. Therefore, the determination of concentration of glucose content in the blood of diabetic laboratory animals induced with alloxan is a useful quantitative index of diabetes. On the other hand, the reference antidiabetic drug, glibenclamide, is a commonly used anti-hyperglycaemic agent which acts via several mechanisms such as binding to the ATP-sensitive potassium channel and in the process, lower glucose levels, suppressing hepatic glucose production, increasing insulin sensitivity of extrapancreatic tissues and fatty acid oxidation, enhancing peripheral glucose uptake, decreasing hepatic glycogenolysis and gluconeogenesis as well as the absorption of glucose from the gastrointestinal tract [39].

Phytochemical screening revealed that the most abundant phytoconstituents in *Garcinia kola* seeds are flavonoids. Flavonoids (specifically, biflavonoids) have been found to be the most abundant phytoconstituents of *G. kola* seeds [40]. Examples of typical biflavonoids isolated from *G. kola* seeds include kolaviron, amentoflavonone, kolaflavanone, GB-1, GB-2 and GB-1a. These biflavonoids have been implicated for most of the properties of *G. kola* seeds [21, 40, 41, 42, 43]. Alloxan (2,4,5,6-tetraoxypyrimidinetetrone) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution [38] and has been shown to produce prompt and highly selective coagulative necrosis of the beta-bells of the islet of Langerhans, resulting in a syndrome resembling clinical diabetes [25, 44, 45]. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to Type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study

suggests that alloxan does not cause diabetes in humans [38].
According to Barbato and Landan [46], Bowman and Rand [25] and Guyton and Hills [47], diabetes was induced in the experimental rats by the administration of diabetogenic dose of alloxan (200 mg/kg body weight). Hyperglycemia was confirmed forty-eight hours after induction by the quantitative determination of urine and blood glucose levels. Urine glucose levels increased from normal (normal rats) to 150 mg in the (diabetic rats); while blood glucose level increased from 97 mg/dL (normal rats) to 368 mg/dL (diabetic rats).
The crude aqueous extract of *Garcinia kola* seeds was able to reduce alloxan-induced hyperglycaemia. The potency was measured in terms of level of reduction in urine glucose levels. There was significant decrease in urine and blood glucose levels after 14 days of oral administration of the extract to the alloxan-induced diabetic rats. This can be compared with reports of Dhar *et al* [48] and Vohora *et al* [49] on the hypoglycaemic effects of the crude extracts of *Anacardium occidentale and Brassica olerancea*. The extracts of these two plants were able to reduce and normalize glycaemia in alloxan-induced diabetes by 42% in rabbits.
The crude extract of *Garcinia kola* seeds reduced alloxan-induced hyperglycaemia in urine by 75% following oral administration of 120 mg/kg body weight of the extract (Table 2). In the blood, hyperglycaemia was reduced by 90% with respect to oral administration of 120 mg/kg body weight of the aqueous extract of *Garcinia kola* seeds (Table 3).
According to Mooradian [50], the major goal of treating diabetes mellitus is controlling elevated blood sugars without causing abnormally low levels of blood sugar. The results of the present study is in agreement with the observation which showed a significant reduction in urine and blood sugar levels, following the oral administration of the aqueous extract of *Garcinia kola* seeds without necessarily causing an abnormally low level of blood sugar. The aqueous extract of *Garcinia kola* seeds at 120 mg/kg body weight has therefore been proven to be antidiabetic in alloxan-induced diabetic rats, which is similar to Type 2 non-insulin dependent diabetes.
Alloxan-induced diabetes in rats caused a significant increase in serum cholesterol levels. Hypercholesterolemic effects in rats can be partly accounted for, by increased cholesterogenesis in the diabetic rats as a result of the increased activity of the HMG-CoA reductase enzyme in the intestine of alloxan-induced diabetic rats. Reports by Nakayama and Nagakawa [51] and Mordes *et al* [52] agreed with result of the present study carried out on alloxan-induced animals. According to lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia); that is, higher concentrations of LDL and lower concentrations of functional HDL are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the LDL particle [53]. LDL particles are sometimes referred to "bad cholesterol". These balances are mostly genetically determined but can be changed by body build, medications, food choice and other factors [54].
Elevated levels of the lipoprotein fractions: LDL, IDL and VLDL are regarded as atherogenic (prone to cause atherosclerosis). Levels of these fractions, rather than the total cholesterol level, correlate with the extent and progress of atherosclerosis. On the converse, the total cholesterol can be within normal limits, yet be made primarily of small LDL particles, under which conditions atheroma growth rates would still be high. In contrast, however, if LDL particle number is low (mostly large particles) and a large percentage of the HDL particles are large, then atheroma growth rates are usually low, even negative, for any given total cholesterol concentration. Recently, a post-hoc analysis of the IDEAL and the EPIC prospective studies found an association between high levels of HDL cholesterol (adjusted for apolipoprotein A-1 and apolipoprotein B) and increased risk of cardiovascular disease, casting doubt on the cardioprotective role of "good cholesterol".
It was observed that the aqueous extract of *Garcinia kola* seeds significantly lowered the level of cholesterol by 30% after 14 days of treatment with 120 mg/kg body weight of the extract. According to Adoga [55], the hypocholesterolaemic action of *Garcinia kola* seeds extract corresponds to the result of the hypocholesterolemic action of aqueous extract of *Garcinia kola* seeds observed in the present study (Table 4).
Bilirubin (formerly referred to as hematoidin) is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principal component of red blood cells. Bilirubin is excreted in bile, and its levels are elevated in certain colour of bruises and the yellow discoloration in jaundice [56].
In the liver, it is conjugated to glucuronic acid, making it soluble in water. Much of it goes into the bile and thus into the small intestine. Some of the conjugated bilirubin remains in the large intestine and is metabolized by colonic bacteria to urobilinogen, which is further metabolized to stercobilinogen, and finally oxidized to stercobilin. This stercobilin gives faeces its brown colour. Some of the urobilinogen is reabsorbed and excreted in the urine along with an oxidized form, urobilin [57].
Normally, a tiny amount of bilirubin is excreted in the urine, accounting for the yellow colour [58]. If the liver's function is impaired or when biliary drainage is blocked, some of the conjugated bilirubin leaks out of the hepatocytes and appears in the urine, turning it dark amber. The presence of this conjugated bilirubin in the urine can be clinically analyzed, and is reported as an increase in urine bilirubin [59]. However, in disorders involving hemolytic anemia, an increased number of red blood cells are broken down, causing an increase in the amount of unconjugated bilirubin in the blood.
There was a significant increase in total bilirubin concentration in alloxan-induced rats, following induction after 48 hours. However, administration of 120 mg/kg body weight of *Garcinia kola* seeds extract produced a statistically significant decrease (50%) in bilirubin concentration in the extract treated rats after 14 days of treatment (Table 5). The fact that the extract caused a reduction in bilirubin levels, suggests that the extract has a potential to protect the integrity of liver cells from damage.
The liver helps to maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissue

such as the liver are an early manifestation of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycaemia. The precise genetic, environmental and metabolic factors and sequence of events that lead to the underlying insulin resistance, however, is not fully understood [60].

In animal models, chronic hyperinsulinemia is found to predispose the liver to relative resistance to insulin. This is characterized by a failure of insulin to signal, an increase in insulin receptor substrate-2. Upregulation of sterol regulatory element-binding protein 1c (SREBP-1c) also occur, leading to increased lipogenesis [61]. Despite down-regulation of the insulin receptor substrate-2-mdiated insulin signaling pathway in insulin-resistant states, the up-regulation of SREBP-1c and subsequent simulation of *de novo* Lipogenesis in the liver leads to increased intracellular availability of triglycerides, promoting fatty liver. This also increases VLDL assembly and secretion [60]. Thus, hyperinsulinemia might directly lead to hepatic insulin resistance with associated fatty changes.

The aminotransferases, AST and ALT, are called marker enzymes because they can be used in clinical diagnosis to indicate liver or sometimes heart damage/injury [62, 63]. The aminotransferases are involved in the transfer of amino group from α-amino to α-keto acids and in the biochemical regulation of intracellular amino acid pool. There was a significant increase in the activity of these enzymes (AST and ALT) in alloxan-induced diabetic rats (Tables 6 and 7). However, administration of the *Garcinia kola* seeds aqueous extract at 120 mg/kg body weight produced a statistically significant decrease in the levels of these enzymes in the treated rats. The activity of these enzymes increased significantly over normal conditions of tissue damage (liver tissue). Administration of extract would seem to have protected the cell membrane, by stopping or reducing the release of enzymes as it was observed when 120 mg/kg body weight of extract was given after 14 days. Liver AST activity was significantly reduced by 45% (Table 6) while liver ALT activity was also significantly reduced (Table 7) after 14 days of administration of 120 mg/kg body weight of extract.

5. Conclusion

Garcinia kola seeds extract significantly reduced hyperglycemia, hypercholesterolemia, hyperbilirubinemia as well as elevated liver AST and ALT activities after 14 days of administration to alloxan-induced diabetic rats. The 120 mg/kg body weight of *Garcinia kola* seeds extract produced the most significant reductive effect in all parameters analyzed. This dose may therefore be recommended to patients suffering from diabetes. The extract may also be explored in the control of some of the liver metabolic dysfunctions normally associated with diabetes.

6. Suggestion for Further Studies

Further studies should be directed towards bioassay (fractionation) and characterization of the principal active ingredient that confers this hypoglycaemic effect on the aqueous extract of *Garcinia kola* seeds.

Competing Interests

Authors have declared that no competing interests exist.

References

1. Murray M, Pizzorno J. Encyclopedia of Natural Medicine, 2nd edition; Prima Health Publishing, Rockling, U.S.A. 1997. Pp 401.
2. Barcelo A, Rajpathak S. Incidence and prevalence of diabetes mellitus in the Americas. Rev Panam Salud Publica 2001, Nov; 10(5): 300-8.
3. Wild SG, Roglic A, Green R, King H. Global prevalence of diabetes estimated for the year 2000 and projection for 2030. *Diabetes Care*. 2004; 27, 1047-1054.
4. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998; 21: 1414-1431.
5. WHO. Geneva World Health Organization Conference: Definitions, diagnosis and classification of diabetes mellitus and its complications. 1999; No. 146-150.
6. Ebonyi E, Fakeye T. Prevalence of Diabetes Mellitus in Rural Nigerian Population. Nigerian Medical Practitioners. 1988; 15: 22-26.
7. McCarthy M, Frognel P. Genetic approaches to the molecular understanding of type 2 diabetes. Am. J. Physiol. Endocrinol. Metab. 2002; 283: E217-E225.
8. Rotshteyn Y, Zito SW. Application of modified *in vitro* screening for identifying herbals possessing sulfonylureas-like activity. Journal of Ethnopharmacology. 2004; 93: 337–344.
9. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol*, 2002; 81. 81-100.
10. Dey L, Xie JT, Wang A, Wu J, Maleckar SA, Yuan CS. Anti-hyperglycemic effects of ginseng: comparison root and berry. *Phytomed*. 2003; 10, 600-605.
11. Kim YW, Kim KH, Choi HJ, Lee DS. “Anti-diabetic activity of beta-glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*”. Biotechnology letters. 2005; 27(7):483-7.
12. Nayak BS, Marshall JR, Isitor G, Adogwa A. Hypoglycemic and hepatoprotective activity of fermented fruit juice of *Morinda citrifolia* (Noni) in diabetic rats. *Evid-Based Compl. Altern. Med.*, (in press). 2011.
13. Plowden CC. A manual of plants names. 3rd Edn. George Ltd. London. 1972; 239.
14. 14. Adesuyi AO, Elumm IK, Adaramola FB, Nwokochs AG. Nutritional and phytochemical screening of *Garcinia kola*. Advanced Journal of Food Science and Technology. 2012; 4(1):9-14.
15. Iwu MW. Handbook of African Medicinal Plant. CRC Press. Boca Raton. Ann Arbor London. Tokyo. 1993; 183–184.
16. Esomonu UG, El-aalu AB, Anuka ND, Salim MA, Atiku MK. Effect of ingestion of ethanolic extract of *Garcinia kola* seed on erythrocytes in Wistar rats. *J. Phy. Sciences*. 2005; 20(1 and 2):30-32.
17. 17. Braide VB, Vitrotio G. Histological alterations by a diet containing seeds of *Garcinia kola*: Effects on liver, kidney and intestine in the rat. In. Gedenhaurs Morphjol.1989; 5:95-101.
18. 18. Adaramoye OA, Adeyemi O. Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin-induced diabetes mellitus rats. Journal of Pharmacy and Pharmacology, 2006; 58:121-128.

19. 19. Azu OO, Osinubi AA. Hypoglycaemic activities of extract of *Garcinia kola* seeds in normal, hypoglycaemic and alloxan-induced diabetic rats. West African Journal of Medicine 01/2005; 8: 141-149.
20. Adaramoye OA. Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats. African Health Sciences. 2012; Vol. 12 Issue 4: 498-506.
21. 21. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Anti-diabetic and aldose reductase activities of biflavanones of *Garcinia kola*. *J Pharm Pharmacol*. 1990; 42:290-292.
22. Udenze ECC, Braide VB, Okwesilieze CN, Akuodor GC. Pharmacological effects of *Garcinia kola* seeds powder on blood sugar, lipid profile and atherogenic index of alloxan-induced diabetic rats. *Pharmacologia*. 2012; 3(12): 693-699.
23. Chinedu I, Uhegbu FO, Imo CK, Ifeanacho NG, Osuocha KU, Chibuike I. Acute administration of aqueous extract of *Garcinia kola* on daily blood glucose level and selected biochemical indices in longevity Wistar albino rats. International Journal of Microbiology and Mycology. 2013; Vol 1, No 2, p. 7-12.
24. Trease GE, Evans MC. Text book of Pharmacognosy, 13th edition, Bailler Tindal, London. 1983; 247-762.
25. Bowman WC, Rand MI. Textbook of Pharmacology. (3rd ed). London: Blackwell Scientific Pub. 1985. Pp. 1943-1964.
26. Guyton Arthur, John Hall John. Textbook of Medical Physiology, Saunders, September, 1996; ISBN 978-0-7.
27. 27. Osheke Shekins Okere, Moses Dele Adams, Uju Dorathy Ejike, Eunice Ogunwole, Ejike Daniel Eze. Hypoglycaemic Potentials of Frog oil in Alloxan-Induced Diabetic Rats. American Journal of BioScience. Vol. 2, No. 4, 2014, Pp. 115-121. doi: 10.11648/j.ajbio.20140204.12.
28. Heinz E. Plant glycolipids: structure, isolation and analysis. In *Advances in Lipid Methodology* - 3, 1996; Pp. 211–332 (ed. W.W. Christie, Faty Press, Dundee).
29. European Treaty Series. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series, Strasbourg, 2005; ETS-123.
30. 30. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 1969; 6:24-27.
31. Ngaha EO, Akanji MA, Madusolumuo MA. Studies on correlation between chloroquine-induced tissue damage and serum changes in rats. *Experientia* 1989; 45:143.
32. 32. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem. 1937; 119: 481-490.
33. 33. Allan CD, Peter JGD. Enzymic Assay of Total Cholesterol Involving Chemical or Enzymic Hydrolysis. A Comparison of Methods. Clin. Chem. 1979; 25/6, 976-984.
34. Rother KI. ”Diabetes treatment-bridging the divide”. The New England Journal of Medicine. 2007; 365(15): 1499-501.
35. Yamamoto H, Uchigata Y, Okamoto H. Streptozotocin and alloxan induce DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets. Nature. 1981; 294. 284-6. 14.
36. Wilson GL, Patton NJ, McCord JM, Mullins DM, Mossman BT. Mechanism of streptozotocin and alloxan-induced damage in rat β-cells, *Diabetologia*. 1984; 27(6): 587-591.
37. Dhanabal SP, Raja MK, Ramanathan M, Suresh B. Hypoglycemic activity of *Nymphaca stellata* leaves ethanolic extract in alloxan- induced diabetic rats. Fitoterapia. 2007; 78. 288-91.
38. Lenzen S. The mechanism of alloxan and streptozotocin-induced diabetes. *Diabetologia*. 2008. Volume 51, Pp. 216-226.
39. 39. Wadkar KA, Magdun CS, Patil SS, Naikwade NS. Antidiabetic potentials of Indian medicinal plants. *J. Herbal Med. Toxicol*. 2008; 2(1), 45-50.
40. 40. Iwu MM, Igboko AO. The flavonoids of *Garcinia kola*. J. Nat. Prod., 1982; 45: 650-651.
41. Iwu MM, Igboko OA, Onwuchekwa UA, Olaniyi CO. Evaluation of activities of biflavonoids of *Garcinia kola* seed. J Ethnopharmacology. 1987; 21(2): 127-138.
42. Braide VB. Antihepatotoxic and biochemical effects of kolaviron, a biflavonoid of *Garcinia kola* seeds. *Phytother. Res*. 1991; 4: 39-41.
43. Adegoke GO, Kumar MV, Sambaiah K, Lokesh BR. Inhibitory effect of *Garcinia kola* on lipid peroxidation on rat liver homogenate. Indian J Exp Biol. 1998; 36(9): 907-910.
44. Barnes CD, Eltherington LG. Drug dosages in Laboratory animals: a handbook. Berkeley, CA: University of California Press, p.53. 1973.
45. Haffner SM, Miettinen H, Stern MP. Insulin secretion and resistance in non-diabetic Mexican American and Non-Hispanic whites with a parental history of diabetes. J Clin Endo Metab. 1996; 81(5): 1846-1851.
46. 46. Barbato C, Landan M. Drug induced endocrine disorder. London: Churchill Livingstone, P 120. 1997.
47. Guyton AC, Hills EJ. Textbook of Medical Physiology. (9th ed). London: Saunders Co. 1996. Pp 926-972.
48. Dhar ML, Bhawan BA, Ray C. Screening Indian plants for biological activity. Part 1. Indian J Pharmacy. 1968; 23:128-129.
49. Vohora SB, Rizwan M, Khan AJ. Medicinal uses of common Indian vegetables. Planta Medicus. 1973; 23: 382-393.
50. Mooradian AD. Drug therapy of non-insulin dependent diabetes mellitus in the elderly. Drugs. 1996; 51: 931-941.
51. Nakayama H, Nagakawa S. Influence of STZ-induced on intestinal 3-HMG-CoA reductase activity in rats. Diabetes. 1977; 26: 439-444.
52. 52. Mordes JP, Desemone J, Rossini AA. The BB rat. Diabetes Metab. Rev. 1987; 3(3): 725-750.
53. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL. “Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation”. Diabetes Care. 2008; 31 (4): 811- 22.
54. Durrington P. ”Dyslipidaemia”. Lancet 2003; 362 (9385): 717-31.
55. Adoga GI. Effects of garlic oil on some enzymes in STZ-induced diabetic rats. Med. Sci. Res. 1990; 18: 523.
56. Libor NL. Impact of Serum Bilirubin on Human Diseases. Pediatrics. 2005; 115:1411-1412.

57. 57. Moudgil KD, Narang BS. The liver and biliary system. In:Textbook of Biochemistry and Human Biology (2nd edition), GP Talwar, LM Srivastava and KD Moudgil (eds). Prentice-Hall of India. Private Ltd. 1989. Pp.271-273.
58. 58. Berk PD, Korenblat KM. Approach to the patient with jaundice or abnormal liver test results. In: Goldman L, Ausiello D, eds. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier, Chap. 150. 2007.
59. 59. Liu Y, Li P, Lu J, Xiong W, Oger J, Tetzlaff W, Cynader M. "Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis". *J. Immunol*. 2008; 181 (3): 1887–97.
60. Lewis GF, Carpentier A, Khosrow A, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and Type 2 diabetes. *Endocr Rev*, 2002; 23: 201-292.
61. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL. Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ab/ab mice. Mol Cell 2000; 6: 77-86.
62. Chapatwala K, Boykin MA, Rajanna B. Effects of intraperitoneally injected cadmium on renal and hepatic glycogenic enzymes in the rat. Drug. Chem.Toxicol. 1982; 5:305-317.
63. Shahjahan M, Sabitha KE, Mallika J, Shyamala-Devi CS. Effect of *Solanum trilobatum* against carbon tetrachloride-induced hepatic damage in albino rats. *Indian J. Med. Res.* 2004; 120: 194-198.