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Hypoglycemic Potentials and Toxicity studies of *Garcinia kola* Seed Tincture in Alloxan-Induced Diabetic Rats

N. C. Wokne-Eze[1*], T. G. Davies[1] and E. S. Bartimaeus[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author ESB designed the study and Author TGD wrote the protocol, and wrote the first draft of the manuscript. Author NCW managed the analyses of the study. And the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate the hypoglycemic potentials and toxicity studies of *Garcinia kola* seed tincture in Alloxan-induced Diabetic Rats.
Study Design: This study is an interventional study.
Place and Duration of Study: A total of fifty one (51) adult wister rats weighing 180 – 200g were used. The rats weighed and grouped into 6 groups of 6 rats each. Group 1 (negative control) were placed on normal diet, while groups 2 to 6 were administered with 150mg/kg alloxan(Sigma-Aldrich) to achieve the animal model of Type 2 diabetes mellitus. Glibenclamide was used for this study. Treatments were given according to the groupings by means of oral gavage for a period of 21 days. At the end of the treatments, the rats were anaesthetized and blood samples collected for biochemical analysis. The heart, liver and kidneys were also harvested for histological analysis. Fasting plasma glucose (FPG) was estimated using the glucose oxidase method. Enzymatic method was used for the determination of total cholesterol (TC), triglyceride (TG), and high density lipoprotein cholesterol (HDL-C), whereas low density lipoprotein cholesterol (LDL-C) was calculated from the friedewald equation. Reitman-Frankel method was used for the determination of liver transaminases, while phenolophthalein method was used for the determination of alkaline

**Corresponding author: E-mail: tak2.newman@gmail.com;*

phosphatase. The heart, liver and kidney sections were stained with the standard haemotoxylin and eosin staining technique.

Results: The results showed significantly higher mean FPG levels ($p<0.05$) in all groups that received alloxan, as compared to the negative control. Extract of *Garcinia kola* combined with glibenclamide produced significant blood glucose-lowering effects in diabetic rats ($p<0.05$) when compared to the positive control group, revealing a potentiating drug interaction between the extract and glibenclamide. Troponin showed no significant difference ($p>0.05$) in all groups when compared to the positive control. There was a significant elevation ($p<0.05$) of total cholesterol, triglyceride and LDL-C in all groups induced with diabetes when compared to the negative control. HDL-C however, was lowered significantly ($p>0.05$) in all groups when compared to the negative control. Liver enzyme levels were significantly higher in diabetic control compared to negative control, except in groups 4 (glibenclamide), and 6 (alcohol). Histological analysis of the heart showed normal branching muscle fibres and peripherally placed nuclei in all groups. Liver sections showed normal histoarchitecture in the negative control compared to the diabetic control which had sinusoids filled with inflammatory cells. The treatment groups showed nearly normal liver architecture with hepatic artery and portal vein.

Conclusion: In conclusion, the combined treatment with the tincture and glibenclamide showed hepatoprotective potentials in addition to its hypoglycaemic effects.

Keywords: Hypoglycemic potentials; toxicity studies; Garcinia kola seed tincture; alloxan-induced diabetic rats.

1. INTRODUCTION

With the advent of a paradigm shift on the approach of treatment and management of diseases such Diabetes Mellitus, from the application of synthetic drugs to natural plant remedies. Various plants and herbal extracts which have been discovered to serve as precursors for drug compounds are now being patronized as an enhancer for organs and body system performance in man and animals [1]. Sometimes the aim is to lower blood concentrations or activities of some disease markers in order to ameliorate health conditions. An illustration can be drawn from the use of *Garcinia kola* seed (hereafter referred to as *G. kola* seed) to lower blood cholesterol level in animals with hypercholesterolemia [2]. *G. kola* plant is fruit-bearing plant that belongs to the family Guttiferae. The plant is found mainly in the tropical rain forest region of West and Central Africa. It is acclaimed traditionally that every part of the plant is of therapeutic importance. *G. kola* seed otherwise called “bitter kola” owing to its bitter taste has been shown to possess anti-inflammatory, antidibetic, antioxidant and anti-hepatotoxic activities [2]. Phytochemical analysis of *G. kola* seed have shown that the plant contains a variety of phytochemicals such as, flavonoids, saponins, and cardiac glycosides. The flavonoid component has been shown to be responsible for most of the biological activities of *G. kola* seed including its potential to reduce blood glucose concentration in diabetic animals [3]. It is in the light of the possibility of *G. kola* seed to exert an effect on blood glucose concentration, and the fact that herbal doctors often subscribe to the use and administration of herbal preparations to their patients without due cognisance to possible adverse effect that this study was undertaken to investigate the hypoglycaemic potential and toxicity of *G. kola* seed tincture in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *G. kola* seeds were purchased from the local market in Port Harcourt, Nigeria during December, 2019. The outer testa of each seed was peeled off and the seed chopped into small pieces. Suspension of 200g dry pieces in 500cl of 50% ethanol for twenty-one (21) days produced the tincture used for the study.

2.2 Experimental Animals

This study was carried out with a total of fifty one (51) Wister rats weighing 180 – 200g. The animals were weighed and housed in a wire gauze cage and allowed to acclimatize for two weeks. After acclimatization the rats were randomly divided into six (6) groups of five (6) rats each.

2.3 Study Design

This study is an interventional study. The rats were weighed and grouped into 6 groups of 6 rats each. Group 1 (negative control) were placed on a normal diet, while groups 2 to 6 were placed on a high fat diet (HFD) prior to induction with alloxan, to achieve the animal model of type 2 diabetes mellitus. Treatments (*Garcinia kola* seed tincture, glibenclamide and alcohol) were given according to the groupings by means of oral gavage for a period of 3 weeks as shown below.

2.3.1 Acute toxicity study

Acute toxicity was done using the Lorke's method.

This method has two phases.

Phase 1

The first phase required the use of nine (9) animals, which were divided into three groups (group 1, 2 and 3) of three animals each, and housed in separate cages. Each group of animals were administered different doses (10mg/kg, 100mg/kg and 1000mg/kg) of *Garcinia kola* seed tincture. The animals were then observed for 24 hours to monitor sign of toxicity as well as mortality.

Phase 2

This phase involved the use of three animals, divided into three groups of one animal each. Higher doses (1500, 2900 and 5000mg/kg) of the tincture were administered to the animals and then placed under observation for another 24 hours for any sign of toxicity and mortality.

The remaining rats were divided into six groups of five rats each as shown below:

Group 1: This group served as the control group. The animals in this group were not induced with alloxan and were not given any treatment. They were only allowed unrestricted access to normal feed and water all through the 21 days period of study.

Group 2: The animals in this group served as positive control. They were induced with alloxan and confirmed diabetic, but were not given any form of treatment.

Group 3: The animals in this group were induced with alloxan, confirmed diabetic and were treated with the G. *kola* seed tincture.

Group 4: The animals were induced with alloxan, confirmed diabetic and were treated with glibenclamide, a standard anti-diabeic drug.

Group 5: The animals were induced with alloxan, confirmed diabetic and were treated with a combination of the tincture and glibencclamide.

Group 6: The animals were induced with alloxan, confirmed diabetic and were treated with 50% ethanol.

2.4 Reagent Preparations and Calculations

2.4.1 Preparation of *Garcinia kola* tincture

The tincture preparations were administered through the oral route after appropriate calculations of doses. The dose calculation was made as shown below using the method as described by Ofor et al. [4].

> Standard dose = 400 mg/kg, that is; 10ml of the tincture was given to 1 kg or 1000 g rat.

So if 1000 g rat takes 400 mg of tincture

> 200g rat = Xmg of extract?
>
> Xmg = 200 g x 400 mg / 1000 g = 80m g of tincture.

Therefore, 2 ml (80 mg) of the tincture was given to all rats in the group weighing 200 g. (This was done for all groups of animals with their body weight taken into consideration).

2.4.2 Glibenclamide

The administered dosage was extrapolated from human doses as shown below.

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

Rat dose (mg/kg) = Human dose x 5 [5]

= 10 x 0.018 x 5

= 0.9mg/kg.

This dose was administered mg per kg body weight to the rats.

2.4.3 Preparation of alloxan monohydrate

For the preparation of alloxan for the induction of diabetes, 750 mg of alloxan in a dark environment was dissolved in 10mls of normal saline (0.9%) to yield 75mg/ml with a pink to light purple coloration. This was done diligently because the volume to be administered to the rats is very important, as the higher the volume used, the least likelihood of success.

Standard dose for diabetes induction is = 150 mg/kg

Average weight of the rats is = 140 g.

So if 150 mg is given to 1 kg or 1000 g rat as;

150 mg = 1000 g rat

X mg = 140 g

X mg = 150 x 140 / 1000 = 21 mg/140 g rat.

Therefore, 21mg of alloxan was given to all rats in the group weighing 140g (this was done for all groups of animals taking their weights into consideration).

2.5 Induction of Experimental Diabetes

Prior to alloxan administration experimental animals were allowed to fast for 8 hours. After an 8 hour fast 150mg/kg of alloxan was administered intraperitoneally holding the animal upwards. After administration, experimental animals were restricted access to any form of feed and water for 30 minutes and then given oral administration of 5ml of 10%(50 g/500ml) dextrose and 5%(25 g/500ml) dextrose solution in drinking water to counter alloxan induced hypoglycaemic toxicity due to insulin leakage from damaged b-cells. After 8 hours the diabetic state was determined in blood from the tail by the glucose oxidase method using a potable glucometer (GlucoDr.). After intraperitoneal administration of alloxan, all animals became diabetic with fasting glucose levels ranging between 17 to 25 mmol/L. Diabetes was also confirmed by polyuria and polydipsia in the rats.

2.6 Sample Collection and Analysis

2.6.1 Sample collection

At the end of the treatments, after a 6 hour fast, the rats were anaesthetized with chloroform, and blood samples were obtained using a sterile syringe by cardiac puncture. This was done in line with the National Institutes of Health (NIH) and the Animal Models of Diabetic Complications Consortium (AMDCC) protocol, on fasting of laboratory animals. 2ml of blood was put in fluoride oxalate container for the determination of fasting plasma glucose (FPG), another 2ml of blood was put in EDTA container for the determination of troponin I, and then 5ml of blood was put in a plain container for the determination of liver enzymes and lipid profile and renal function parameters. The samples were mixed with the anticoagulant in the container and the determination of FPG was done within 6 hours of sample collection. The samples in the plain bottle were separated within an hour of sample collection using a centrifuge. The serum samples were then stored frozen at -20oC, until the time for determination of other parameters.

2.6.2 Sample analysis

All reagents used for this study were commercially purchased and the manufacturer's standard operating procedures were strictly followed. Quality control (QC) samples were run in each batch of the biochemical analysis.

2.6.2.1 Determination of fasting plasma glucose (FPG)

Fasting plasma glucose was estimated quantitatively using the glucose method (as described by Randox laboratories limited (United Kingdom). Glucose oxidase (GOD) catalyses the oxidation of glucose to give hydrogen peroxide (H_2O_2) and gluconic acid. The hydrogen peroxide formed reacts under catalysis of peroxidase (POD), with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator whose absorbance is directly proportional to the concentration of glucose.

2.6.2.2 Determination of total cholesterol (TC)

TC was measured quantitatively by enzyme method, as described by Randox laboratories limited (United Kingdom). The Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The amount of colour formed is directly proportional to the concentration of cholesterol.

2.6.2.3 Determination of high density lipoprotein cholesterol (HDL-C)

HDL-C was measured quantitatively by enzymatic method, as described by Randox laboratories limited (United Kingdom). Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined by enzyme method.

2.6.2.3 Determination of triglycerides (Trigs)

Triglycerides were measured quantitatively by enzymes method, as described by Randox laboratories limited (United Kingdom). Triglycerides are determined after enzymes hydrolysis with lipase. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase.

2.6.2.4 Determination of low density cholesterol (LDL-C)

LDL cholesterol was calculated from the Friedwald's equation.

2.6.2.5 Determination of alanine aminotransferase (ALT)

ALT was estimated quantitatively using the Reitman-Frankel method as described by Randox laboratories limited (United Kingdom). Alanine aminotransferase (ALT) catalyses the transfer of amino group from alanine to ketoglutarate, forming pyruvate and glutamate The pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-Dinitrophenylhydrazone which in an alkaline medium gives a red-brown colour. The absorbance of the colour is directly proportional to the concentration of the eenzyme.

2.6.2.6 Determination *of aspartate aminotransferase (AST)*

AST was estimated quantitatively using the Reitman-Frankel method as described by Randox Laboratories Limited (United Kingdom). Aspartate aminotransferase (AST) catalyses the transfer of amino group from aspartate to ketoglutarate, forming oxaloacetate and glutamate. Oxaloactate reacts with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-Dinitrophenylhydrazone which in an alkaline medium gives a red-brown colour. The absorbance of the colour is directly proportional to the concentration of the enzyme.

2.6.2.7 Determination of alkaline phosphatase (ALP)

ALP was estimated quantitatively using the colorimetric phenolphthalein as modified by Teco Diagnostics (USA). Alkaline phosphatase (ALP) catalyzes the hydrolysis of the colourless organic phosphate ester substrate, p-Nitrophenylphosphate, to the yellow coloured product p-Nitrophenol and phosphate. The absorbance of the coloured product is directly proportional to the concentration of the enzyme.

2.6.2.8 Protein determination

Protein content of serum was determined using the Biuret method as described by Michael Lubran in 1978.

2.7 Statistical Analysis

The data were statistically analyzed using Graph Pad Prism version 5.03. Comparison of levels of significance between control and experimental groups were done using Analysis of Variance (ANOVA) with Tukey's Post hoc test. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

In the phase 1 acute toxicity study, at the end of 24 hours, there were no sign of toxicity or mortality recorded (Table 1), thus the need to proceed with phase 2.

After 24 hours of observation, all graded doses of the ethanolic tincture of *Garcinia kola* seed administered to the animals showed no signs of toxicity and no mortality was recorded (Table 1). Therefore, the ethanolic tincture of *Garcinia kola* seed was found to be safe up to 5000mg/kg body weight in rat models.

The observation in this study was in line with previous research finding, in that Fasting Plasma Glucose (FPG) levels, after 8 hours of alloxan-induced diabetes in untreated rats (Negative control, Group 2) was significantly higher than in non-diabetic rats (Positive control, Group 1),

Table 1. Acute toxicity studies using the Lorke's method

Groups	No. of rats	Dose (mg/kg)	Clinical signs	Mortality
Phase 1				
1	3	10	None	Zero
2	3	100	None	Zero
3	3	1000	None	Zero
Phase 2				
1	1	1500	None	Zero
2	1	2900	None	Zero
3	1	5000	None	Zero

(Table 3). This observed increase in FPG levels have also been reported in diabetic untreated rats [6,2]. Alloxan exerts a damaging effect and causes death of pancreatic islet cells in experimental animal models, resulting diabetes hence decreasing or stopping insulin secretion. The cytotoxic action of alloxan is mediated by a number of factors such as; reactive oxygen species, alloxan itself and dialuric acid, the products of alloxan reduction, generates a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, hence precipitating experimental diabetes mellitus [7]. Oral administration of *Garcinia kola* seed (G. *kola* Seed) tincture significantly ($p<0.05$) reduced the increased glucose level in diabetic animals. (Table 2) A similar observation with oral administration of *Garcinia kola* seed powder (GKP) was report by Iwu et al. [2].

The reduction of glucose concentration could partly be that kolaviron, the biflavonoid complex of *Garcinia kola* promotes entry of glucose into cells, stimulates glycolytic enzymes and glycogenic enzymes or inhibits the glucose 6-phosphatase in the liver and subsequently reducing the release of glucose in blood. Naringenin another flavonoid component similar to kolaviron of *Garcinia kola*, has also been implicated in eliciting its hypoglycaemic effect by an extra-hepatic action, possibly by stimulating glucose utilization in extra hepatic tissues or increasing the expression of insulin receptors in the liver plasma membranes [8]. This study also revealed that oral administration of *G. Kola* seed tincture together with glibenclamide (group 5) caused an even more significant ($p < 0.05$) decrease in fasting plasma glucose level in alloxan-induced diabetic rats. This reduction suggests that glibenclamide exerts a potentiating effect on *Garcinia kola* seed tincture in lowering blood glucose levels.

Hyperlipidemia has been established as one of the major complications of diabetes mellitus [9] and this is mediated by hyperglycemia which is characterized by increased levels of cholesterol, triglycerides and phospholipids and changes in lipoproteins [10]. Diabetes is also often associated with increased dyslipidaemia [11].

At the end of the 21 day period of this study, it was observed that serum total cholesterol, triglyceride and LDL-cholesterol of alloxan-induced untreated diabetic rats (Positive control, group 2) were significantly increased when compared to the non-diabetic negative control (group 1). While the serum HDL-cholesterol of untreated diabetic rats were significantly reduced when compared to non-diabetic negative control (Table 3). The observation is consistent with the reports of previous researchers [12]. The abnormally high concentration of serum lipids was mainly due to increase in the mobilization of Free Fatty Acids (FFA) from peripheral tissue due to activation of the hormone sensitive lipase during insulin insufficiency. Lack of insulin in diabetes is also known to be associated with increased synthesis of cholesterol [12]. Insulin resistance in diabetic rats could increase the hepatic uptake of fatty acids released by lipolysis of adipose tissue, the intra-hepatic synthesis of triglycerides and the over production and secretion of VLDL particle that, in turn, leads to increased plasma levels of TG. HDL-cholesterol is the smallest of the lipoprotein species containing approximately 20% cholesterol ester and very little triglyceride. It is strongly and independently related to coronary heart disease (CHD). Unlike LDL, the relationship is inverse, being that a low HDL is an important indicator of CHD and high HDL level protects the body against CHD. The fact that oral administration of *G. kola* seed tincture caused a significant increase ($p<0.05$) in serum HDL-cholesterol

concentration suggests that the tincture may possess protective role against coronary heart diseases. This protective role can be explained by its ability to counteract the effect of LDL-cholesterol and promote the reverse cholesterol transport pathway by inducing an efflux of excess cellular cholesterol.

Decreased turnover has also been implicated in diabetes mellitus. Some authors have published that non-enzymatic glycosylation of HDL rapidly increases its catabolism and same mechanism has been established to be involved in the low level of HDL observed in alloxan-induced diabetic rat in this. In the present study, after the 21-day period, oral administration of G. *kola* seed tincture to diabetic rats reduced significantly ($p<0.05$) the serum total cholesterol, triglyceride and LDL-cholesterol, in the diabetic treated group 3 compared to the positive control (group 2).

Table 2. Fasting plasma glucose levels of research animals before and after induction of diabetes with graded doses of Alloxan

Groups	FPG (mmol/L) before induction	FPG (mmol/L) after induction
Group 1Alloxan(100mg/kg)n = 3	6.53 ± 1.36	8.67 ± 0.55
Group 2 Alloxan(150mg/kg)n = 3	5.47 ± 0.65	20.57 ± 3.00
Group 3 Alloxan(200mg/kg)n = 3	6.53 ± 0.67	22.13 ± 2.88
p-value	0.4316	< 0.0001
F-value	1.054	20.03
Summary	NS	S

Values are expressed as mean +/- SD; S – Significant; NS – Not significant; n - Number of samples

Table 3. Effect of treatment on FPG, troponin I and lipid profile parameters

Groups	FPG (mmol/L)	Troponin I (ng/mL)	TC (mmol/L)	Trigs (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Group 1 (Negcont)n= 6	7.13±0.31[b]	0.103±0.01[b]	2.04±0.62[b]	0.62±0.18[b]	1.27±0.15[b]	0.49±0.08[b]
Group 2 (Pos.cont)n6	19.1±2.79[a]	0.39±0.16[a]	4.83±0.31[a]	2.97±0.04[a]	0.43±0.18[a]	3.05±0.27[a]
Group 3 (Tincture)n= 6	9.1±3.10[b]	0.15±0.06	2.03±0.25[b]	0.9±0.14[b]	0.66±0.14[a]	0.99±0.34[b]
Group 4 (Gli)n=6	17.03±2.63[af]	0.15±0.06	2.31±0.12[b]	1.22±0.12[a] [b]	0.60±0.11[a]	1.16±029[ab]
Group 5 (Tinc + Gli) n=6	7.1±1.52[bh]	0.29±0.14	2.14±0.07[b]	0.96±0.12[b]	0.72±0.04[a]	0.98±0.03[b]
Group 6 (Alcohol) n=6	16.67±2.18[a]	0.16±0.06	3.98±0.21[a]	2.55±0.14[a]	0.41±0.03[a]	2.41±0.13[a]
p-value	< 0.0001	0.0219	< 0.0001	< 0.0001	0.0122	< 0.0001
F-value	15.21	4.049	54.19	19.07	6.213	33.91
Summary	S	S	S	S	S	S

Values are expressed as mean +/- SD; Gli – Glibenclamide; n - Number of samples; S – Significant; NS – not significant; a - significant difference versus Group 1 (Neg. control);[b]- significant difference versus Group 2 (Pos. control);[c] –significant difference between, groups 3 vs 4;[d] –significant difference between, groups 3 vs 5;[e] – significant difference between, groups 3 vs 6;[f] –significant difference between, groups 4vs 5;[g] –significant difference between, groups 4vs 6;[h] –significant difference between, groups 5vs 6

Table 3b. Effect of treatment on very low density lipoprotein cholesterol (VLDL), atherogenic index of plasma (AIP), cardiac risk ratio (CRR) and atherogenic coefficient (AC)

Groups	VLDL(mmol/L)	AIP	CRR	AC
Group 1 (Neg.cont) n=6	0.28±0.08	0.014±0.04[b]	4.55±1.95[b]	3.88±1.52[b]
Group 2 (Pos.cont) n=6	0.25±0.02	0.48±0.18[a]	9.97±11.32[a]	7.97±14.45[a]
Group 3 (Extract) n=6	0.21±0.24	0.23±0.13	2.95±1.09[b]	1.95±0.59[b]
Group 4 (Gli) n=6	0.23±0.06	0.11±0.05[b]	3.51±1.06[b]	2.51±1.06[b]
Group 5 (Extract + Gli) n=6	0.20±0.05	0.06±0.02[b]	2.99±0.08[b]	1.99±0.08[b]
Group 6 (Alcohol) n=6	0.29±0.06	0.08±0.02[b]	2.46±0,42[b]	1.46±0.42[b]
p-value	0.4834	0.0011b	0.0013	0.0013
F-value	0.9519	8.318	8.383	8.391
Summary	NS	S	S	S

Values are expressed as mean +/- SD; Gli – Glibenclamide; n - Number of samples; S – Significant; NS – not significant; Values without superscript shows no significant difference versus Groups1 and 2; a - significant difference versus Group 1 (Neg. control); [b]- significant difference versus Group 2 (Pos. control); [c] –significant difference between, groups 3 vs 4; [d] –significant difference between, groups 3 vs 5; [e] –significant difference between, groups 3 vs 6; [f] –significant difference between, groups 4vs 5

Table 4. Effect of treatment on renal function parameters

Groups	Creatinine (µmol/L)	Urea (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)
Group 1 (Negcont)n=6	41.33±2.15[b]	2.73±0.47[b]	134.3±5.69	2.90±0.50[b]	16.0±3.61[b]
Group 2 (Poscont) n=6	78.33±3.08[a]	10.10±0.26[a]	133.0±6.56	8.87±0.68[a]	26.67±4.16[a]
Group 3 (Extract) n=6	42.0±3.61[be]	3.73±0.91[be]	135.7±2.52	7.27±0.40[abd]	16.50±3.62[b]
Group 4 (Gli) n=6	40.0±4.58[bg]	3.80±0.46[bg]	140.7±5.86	7.10±0.30[abf]	16.70±4.16[b]
Group 5 (Extr+Gli) n=6	45.56±4.12[bh]	2.90±0.72[bh]	140.0±4.0	5.47±0.65[abh]	12.0±2.0[b]
Group 6 (Alcohol) n=6	66.34±5.26[a]	14.17±0.59[a]	138.3±2.08	7.60±0.2[a]	20.3±2.52
p-value	21.96	5.668	0.3281	< 0.0001	0.0040
F-value	< 0.0001	0.0144	1.297	57.60	6.405
Summary	S	S	NS	S	S

Values are expressed as mean +/- SD; Gli – Glibenclamide; n - Number of samples; S – Significant; NS – not significant; Values without superscript shows no significant difference versus Groups1 and 2; a - significant difference versus Group 1 (Neg. control); [b]- significant difference versus Group 2 (Pos. control); [c] –significant difference between, groups 3 vs 4; [d] –significant difference between, groups 3 vs 5; [e] –significant difference between, groups 3 vs 6; [f] –significant difference between, groups 4vs 5; [g] –significant difference between, groups 4vs 6; [h] –significant difference between, groups 5vs 6

Because cardiovascular assessment is a core aspect of this research, there was the need to evaluate VLDL-C, and some artherogenic indices such; Atherogenic index of plasma (AIP); log (TG/HDL-c), Cardiac risk ratio (CRR) (Table 3b); (TC/HDL-c), and Atherogenic coefficient (AC); (TC-HDL-c/HDL-c). VLDL contains the highest amount of triglycerides, because it helps cholesterol build up on the walls of the arteries. The atherogenic indices are mathematical relationships between TC, TG, LDL-c and HDL-c that have been successfully used as markers of assessing atherosclerosis development and extent VLDL of CHDs [13]. There was no significant difference in the values of -C in all groups when compared to the negative control and positive control groups. The values of VLDL in this result were all within the normal range (0.1 - 1.7 mmol/L). HDL-c/TC ratio greater than 0.3 and LDL-c/HDL-c ratio less than 2.3 indicate a reduced risk of peripheral arterial disease [14]. However, AIP has been considered the most accurate in determining the extent of atherosclerosis and the risk of myocardial infarction [15].

It has been suggested that log (TG/HDL-c) values of -0.3 to 0.1 are associated with low risk, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular disease risk [15]. According to these ranges the most important atherogenic risk predictor being atherogenic index of plasma

was within 0.08 =/- 0.02 – 0.23=/- 0.13 in all treatment groups suggesting that diabetic rats administered with 500mg/kg body weight of *G. kola* seed tincture are at medium risk of developing cardiovascular disease. This finding suggests an anti-atherogenic abilities of *Garcinia kola* seed tincture, against the development of cardiovascular disease.

The kidney plays a leading role in maintenance of body homeostasis by reabsorbing important material and excreting waste products [16]. The function of the kidney is mainly assessed by the levels of urea and creatinine in the blood; creatinine being the most specific, as its levels in the blood is independent of diet and physical activity. Whereas, urea being the main end product of protein catabolism, varies directly with protein intake and inversely with the rate of excretion. Renal diseases which alters the integrity of the glomerular filtration lead to urea retention resulting in decrease in urea seen in severe liver disease and destruction of cells leading to impairment of the urea cycle [17]. Creatinine is a waste product formed in muscle by creatine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment. Evidence from this study (Table 4) that oral administration of *Garcinia kola* tincture (Group 3) caused a significant ($p < 0.05$) decrease in urea and creatinine levels compared to the diabetic group (Positive control, group 2). This reduction in urea and creatinine levels suggests that the tincture exerts a protective effect on the kidneys.

The liver an important homeostatic organ in the body. The degree of liver damage caused by toxic substances can be assessed by the determination of activities of biochemical markers of liver function such as activities of AST, ALT and ALP [18]. The enzyme ALP is located in the cytoplasm and is released into the circulation after cellular damage. ALT and AST are also enzymes released when injury involves organelles such as liver mitochondria [18]. Elevation of the activities of these enzymes can be indicative of cellular leakage and loss of functional integrity of hepatic cell membrane.

This study also investigated the effect of *Garcinia kola* tincture on the activity of these liver enzymes after 21 days of oral administration. In this study (Table 5), administration of the tincture (group 3) caused a significant ($p < 0.05$) decrease in the activities of ALT, AST and ALP. This reflects the organ-protective effect of *Garcinia kola* seed tincture on the liver. Tissue micrograph also shows that the histology of the liver of rats administered *Garcinia kola* seed tincture was not affected. Deleterious effect of hyperglycemia in the liver, observed in this study is evidenced by a significant ($p < 0.05$) serum

Table 5. Effect of treatment on liver function parameters

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/dl)	Bilirubin
Group 1 (Negcont)n=6	6.0±1.0[b]	50.67±3.22[b]	21.67±2.08[b]	51.0±9.64	7.67±2.52
Group 2 (Poscont)n=6	31.67±8.51[a]	146.0±8.19[a]	193.3±11.97[a]	52.0±5.43	11.0±2.65
Group 3 (Tincture) n=6	10.0±3.61[be]	59.0±9.80[bce]	153.7±13.58[abcde]	51.23±5.13[b]	8.27±1.16[b]
Group 4 (Gli) n=6	21.0±2.65	101.0±5.29[abfg]	58.67±10.97[bg]	62.33±3.51	9.67±2.52
Group 5 (Ext+Gli) n=6	11.45±2.34[bh]	55.33±7.71[bh]	70.0±9.98[abh]	58.67±3.22	9.0±2.13
Group 6 (Alcohol) n=6	37.67 ± 9.14[a]	147.7±11.06[a]	212.3±15.57[b]	57.33±7.64	12.0±2.65
p-value	0.0002	< 0.0001	< 0.0001	0.7972	0.3090
F-value	13.21	68.06	96.92	3.916	1.350
Summary	S	S	S	NS	NS

Values are expressed as mean +/- SD; Gli – Glibenclamide; n - Number of samples; S – Significant; NS – not significant; Values without superscript shows no significant difference versus Groups1 and 2; a - significant difference versus Group 1 (Neg. control); [b]- significant difference versus Group 2 (Pos. control); [c] –significant difference between, groups 3 vs 4; [d] –significant difference between, groups 3 vs 5; [e] –significant difference between, groups 3 vs 6; [f] –significant difference between, groups 4vs 5; [g] –significant difference between, groups 4vs 6; [h] –significant difference between, groups 5vs 6

elevation of liver enzyme activities of diabetic rats (positive control, group 2) when compared to the non-diabetic rats (Negative control, group 1). The hepatoprotective effect of G. *kola* seed tincture is demonstrated by the significant reduction of serum levels of ALT and AST in the diabetic treated rats, as shown in Table 5.

4. CONCLUSION

The findings of this study revealed that alloxan at 150mg/kg induced a hyperglycaemic state in the test animals and that *Garcinia kola* tincture has hypoglycaemic potentials as it significantly reduced the blood glucose level of alloxan-induced diabetic animals. Also discovered in this study is that a combination of the tincture and a standard antidiabetic drug, glibenclamide had a potentiating effect on blood glucose levels, this combination showed a significantly lower level of plasma glucose. This study also confirmed that cardiac, renal and hepatic function indices were significantly elevated during alloxan-induced diabetes, and that oral administration of *Garcinia kola* tincture significantly reduced the levels of some of the indices.

CONSENT

Not applicable.

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.

DISCLAIMER

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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