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The effects of gavage treatment with *Garcinia kola* seeds on biochemical markers of liver functionality in diabetic rats

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ABSTRACT

The effects of gavage treatment with Garcinia kola seeds on biochemical markers of liver functionality in diabetic rats were evaluated. A total of thirty (30) male albino wistar rats weighing between 240 – 250g were divide into six (6) groups of five (5) animals per group (n=5). The first two groups of rats; the non-diabetic control and non-diabetic treated groups received by gavage normal saline and 600mg/kg b.w of Garcinia kola seed powder suspended in normal saline (GKP) respectively. The last four groups which were made diabetic by intraperitoneal injection of freshly prepared alloxan monohydrate had one diabetic control group that received normal saline and three (3) diabetic treated groups which got 300mg/kg, 600mg/kg and 900mg/kg of GKP respectively. GKP was administered twice daily for 21 days. At the end of the treatment period, the rats were sacrificed and blood collected through cardiac puncture from where serum was obtained for biochemical analysis. GKP treatment significantly attenuated serum glucose, aspartate transaminase, alanine transaminase, alkaline phosphatase, and urea levels of diabetic rats. It also significantly increased albumin concentration at a high dosage while significantly reducing the relative liver weight of diabetic rats. These findings therefore suggest that GKP is not only hypoglycaemic but also has the capacity to protect against liver damage secondary to diabetes mellitus.

Keywords: *Garcinia kola*, Diabetes mellitus, Hypoglycaemic, Liver functionality.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterised by inappropriate hyperglycaemia caused by relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million people in the Unites States and as many as 200 million worldwide. The primary defect in fuel metabolism results in widespread, multi-organ complications that ultimately encompass virtually every system of the body [1]. Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modifications in liver enzyme levels [2].

The negative impact of diabetes on the retinal, renal, nervous and cardiovascular systems is well established [3], [4], yet little is known about its effects on the liver. It has recently been realised that hepatic dysregulation in the setting of obesity is marked by oxidative stress and steatosis related to insulin resistance [5]. The ensuing effect is non-alcoholic liver disease, a common spectrum of diseases ranging from simple steatosis (fatty infilteration) to inflammatory steatohepatitis to possible long term injury (fibrosis and cirrhosis) and eventual liver failure [6)]. Recent evidence has even suggested that elevated levels of transaminases may be a marker of future risk of diabetics [7].

Herbal medicine has been used as an antidiabetic therapy alone, along with insulin or other synthetic oral hypoglcemic agents [8]. The use of synthetic agents, on the other hand, has shown several undesirable side effects and has failed to correct the fundamental biochemical lesion and diabetic complications [9].

One of such herbal practices that have shown promise in treatment of diabetes mellitus and its complications is the use of *Garcinia kola* seed. *Garcinia kola* (Heckel) is commonly called bitter kola. It is an angiospermae, belonging to the family of Guttiferae. *Garcinia kola* is a highly valued ingredient in African ethnomedicine because of its varied and numerous uses which are social and medicinal; thus making the plant an essential component in folk medicine [10]. The seeds have been proven to possess numerous physiological and pharmacological effects which include hepatoprotective effects [11], [12]; anti-inflammatory [13], antioxidant [14] antifertility effect [15], haematological effects [16], anticancer effect [17].

The objective of the present study was to evaluate the effects of gavage treatment with *Garcinia kola* seed powder on serum glucose and biochemical markers of liver functionality in diabetic rats.

MATERIALS AND METHODS

Plant Material

Fresh *Garcinia Kola* seeds were purchased from Watt Market, Calabar, Nigeria in September 2010. They were identified and authenticated by Mr Frank Apejoye, a botanist at the Botany Department of the University of Calabar where voucher samples (No. 176) were kept in the herbarium of Botany Department for reference purposes.

Preparation of seed samples

The outer testa of each *Garcinia kola* seed was removed washed and air dried for about 24 hours. Each seed was cut into small pellets with kitchen knife and the resulting pellets were subsequently dried in and electric oven for 12 hours at 40^0c. The dry seed pellets were ground to fine powder using manual grinder and then sieved with 10 micrometre sieve. The resulting powder aliquots were used for phytochemical analysis and the remaining reconstituted with normal saline to obtain suspensions of appropriate concentration for oral administration.

Phytochemical Analysis

A portion of the powder was subjected to phytochemical analysis using [18] and [19] methods to test for alkaloids, tannins, flavonoids, saponins, and cardiac glycosides. The intensity of the colouration determines the abundance of the compound.

Acute toxicity studies

Acute toxicity test was done using probit method. 35 male mice weighing between 24.8 – 25.5g were used. The test was carried out by single oral administration of GKP in normal saline at doses of 0, 100, 500, 1500, 3000, 5000 and 6000 mg/kg to different groups of mice (5 mice per group). Mortality and general behaviour was observed continuously for one, three and intermittently for the next six hours and again at 24 and 48 hours. The parameters observed were gross behavioural changes, grooming, alertness sedation, loss of righting reflex, tremors and convulsions. The LD_{50} of *Garcinia kola* seed powder was found to be 6741.43mg/kg and doses up to 900mg/kg body weight were found to be safe. All doses used in this study were carefully chosen to exclude the lethal dose.

Laboratory Animals

Thirty male albino rats weighing about 240-250g were purchased from the animal unit of the department of pharmacology, University of Calabar. The animals were kept in cages to acclimatize with conditions of the animal housing facility with ambient temperature 26-28^0C and adequate ventilation for two weeks and fed with standard growers mash (Vita Feeds Nig. LTD) and clean water *ad libitum.* They were used in accordance with National Institute of Health (NIH) Guide for the care and use of laboratory Animals [20].

Induction of Diabetes Mellitus

A single dose freshly prepared alloxan monohydrate (Sigma, St Lois MO, USA) in normal saline at a dose 150mg/kg body weight [21] was injected intra-peritoneally into the rats. Blood samples collected by tail vein tapping were monitored for glucose levels, using a glucometer. After 72 hours rats that had blood glucose level above 200mg/dl were considered diabetic and selected for the study.

Experimental Design

The thirty male albino rats were divided into six groups of five rats per group. Animals in all groups received, by gavage, the following:-

- Non-diabetic control received normal saline (0.5ml/kg)

- Non-diabetic treated group received 600mg/kg of *Garcinia kola* seed (GKP)
- Diabetic control received normal saline (0.5ml/kg)
- Diabetic treated I received 300mg/kg of GKP
- Diabetic treated II received 600mg/kg of GKP
- Diabetic treated III received 900mg/kg of GKP

Garcinia kola suspension was administered to all the test groups twice daily for every 12h (6.00am and 6.00pm) for 21 days. The blood glucose and body weight changes were strictly monitored every three days during the period.

Collection and analysis of samples

At the end of the 21-day period, the animals were fasted for 12 hours, anaesthetized with chloroform and then sacrificed and blood collected by cardiac puncture. The blood collected was emptied into non heparinised sample tubes and allowed to clot for two hours and later centrifuged at 3000rpm for 10minutes, after which serum was recovered for analysis. The liver was surgically removed and was immediately blotted using filter paper to remove traces of blood and then weighed with digital analytical balance.

Serum glucose level was estimated by GOD-PAP method based on [22], total proteins was determined by Biuret method based [23], albumin was estimated based on [24], alanine amino transaminase (ALT) and aspartate transaminase activities were determined based on [25], urea was estimated by urease Berthelot method based on [26] and alkaline phosphatase activity was quantified using fortress assay kit.

Statistical Analysis

Data were collected and analysed by ANOVA using Statistical Package for Social Science (SPSS) software for windows and post hoc testing was performed for inter-group comparison using the Least Significant Difference (LSD). All data were expressed as Means ± Standard error of the mean (SEM). P Value<0.05, 0.01 and 0.001 were considered significant.

RESULTS

Phytochemical studies of *Garcinia kola* seed

Phytochemical screening was carried out on *Garcinia kola* seed powder. *G. kola* was found to contain glycosides, flavonoids, tannins and saponins, while alkaloids were not detected as shown in table 1.

Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on serum glucose of diabetic and non-diabetic rats.

The effects of treatment on serum glucose level are depicted in table 2. Serum glucose concentration of diabetic control group was significantly increased (P< 0.001) compared to the non-diabetic control. Treatment with different doses of GKP significantly (P< 0.001) attenuated this increase

Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on the activity of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phoshatase (ALP) in serum of diabetic and non-diabetic rats.

The effects of treatment on serum markers of liver injury which include aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatise (ALT), are shown in table 3. Aminotransferases (AST and ALT) and alkaline phosphatase activities were significantly raised (P<0.05, 0.001) in diabetic controls relative to the non-diabetic controls. Treatment with different doses of GKP significantly (P<0.001) reduced the levels of AST and ALT and ALP in a dose-dependent manner, with the highest dose producing the most favourable reductions compared to the diabetic control.

Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on total protein (TP), albumin (ALB), and urea in serum of diabetic and non-diabetic rats.

The effects of GKP treatment on serum markers of liver synthetic function are shown in table 4. There was no significant difference at the levels of total protein and albumin across all groups except for the groups that received the highest dose of GKP whose albumin concentration was significantly (P<0.05) increased compared to the diabetic control. The urea concentration of diabetic control rats was also significantly (P< 0.05 and 0.001) raised compared to the non-diabetic controls, however treatment with graded doses of GKP reduced the increases, significantly (P< 0.001) compared to the diabetic control.

Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on body and liver weight of diabetic and non-diabetic rats.

The weight of animals after three weeks of GKP administration were obtained and compared with the initial body weight. The differences in weight were used to determine increases in weight and growth rates during the period. The results obtained in table 5 shows that the diabetic control animals progressively lost weight over the 21-day period where as the non-diabetic control and non-diabetic treated groups and diabetic treated groups progressively gained weight across same period. There was a significant (P<0.01, 0.001, 0.05) decrease both in growth rate and body weight gain of diabetic control animals compared to the non-diabetic control. Treatment with GKP showed a dose dependent significant increase (P< 0.001) in body weight and growth rate of diabetic treated rats compared to the diabetic control. The final body weight of non-diabetic treated group showed a significant (P < 0.001) increase when compared to that of the non-diabetic control group.

There was an insignificant increase in the absolute wet liver weight of diabetic control. The highest doses (600mg/kg and 900mg/kg) mitigated these increases insignificantly as well when compared to the diabetic control. However, the relative liver weight of diabetic control animals was significantly increased (P<0.01) compared to non-diabetic control group. Treatment with GKP showed a significant (P<0.01, 0.05) dose related reduction in the relative liver weight of diabetic treated groups except the group that received the lowest dose where the reduction was not significant.

TABLE 1: Phytochemical studies of *Garcinia kola* seed powder

Components	Presence
Alkaloids	–
Glycoside	++
Flavonoids	++
Tannins	+
Saponins	++

(+): Trace amount present; (++): Abundant amount present; (-): No amount present

TABLE 2: Effects of gavage treatment with *Garcinia kola* seed on serum glucose profile of diabetic and non-diabetic rats

Treatment	serum glucose concentration (mg/dl)
Non-diabetic control (GKP, 0mg/kg)	71.51±2.95
Non-diabetic treated (GKP, 600mg/kg)	66.96±4.87
Diabetic control (GKP, 0mg/kg)	367.07±16.21***
Diabetic treated I (GKP, 300mg/kg)	134.38±4.63***, c
Diabetic treated II (GKP, 600mg/kg)	81.50±17.72c
Diabetic treated III (GKP, 900mg/kg)	71.65±4.30c

****significantly different from non-diabetic controls (P< 0.001)*
c significantly different from diabetic control (P< 0.001)

TABLE 3: Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on the activity of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phoshatase (ALP) in serum of diabetic and non-diabetic rats.

Treatment	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Non diabetic control (GKP, 0mg/kg)	36.52 ±1.99	5.75 ±0.20	151.84 ±3.18
Non diabetic treated `(GKP, 600mg/kg)	39.25 ±1.51	4.58 ±0.30	144.96 ±1.89*
Diabetic control (GKP, 0mg/kg)	133.49 ±2.52***	35.20 ±1.58***	190.13 1.08***
Diabetic treated I (GKP, 300mg/kg)	96.17 ±2.75***, c	29.39 ±0.67***, c	190.54 ±1.48***
Diabetic treated II (GKP, 600mg/kg)	67.68 ±1.54***, c	18.25 ±0.70***, c	188.04 ±1.46***
Diabetic treated III (GKP, 900mg/kg)	48.87 ±2.42*, c	12.14 ±0.50***, c	155.34 ±2.06 c

****significantly different from non-diabetic controls (P< 0.001,)*
** significantly different from non-diabetic control (P< 0.05,)*
a significantly different from diabetic control (P< 0.05,)
c significantly different from diabetic control (P< 0.001,).

TABLE 4: Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on total protein (TP), albumin (ALB), and urea in serum of diabetic and non-diabetic rats.

Treatment	Total protein(g/L)	Albumin(g/L)	Urea(mg/dL)
Non diabetic control (GKP, 0mg/kg)	7.61 ±0.20	3.88 ±0.21	44.90 ±2.17
Non diabetic treated (GKP, 600mg/kg)	7.50 ±0.21	4.09 ±0.13	34.46 ±1.72*
Diabetic control (GKP, 0mg/kg)	7.31 ±0.36	3.65 ±0.27	121.97 ±1.05***
Diabetic treated I (GKP, 300mg/kg)	7.65 ±0.20	3.85 ±0.09	101.97 ±4.42***, c
Diabetic treated II (GKP, 600mg/kg)	7.82 ±0.26	3.78 ±0.07	80.46 ±1.63***, c
Diabetic treated III (GKP, 900mg/kg)	7.77 ±0.34	4.20 ±0.09 a	54.17 ±1.93*, c

****significantly different from non diabetic controls (P< 0.001)*
** significantly different from non diabetic control (P< 0.05)*
a significantly different from diabetic control (P< 0.05)
c significantly different from diabetic control (P< 0.001)

TABLE 5: Effects of gavage treatment with *Garcinia kola* seed powder (GKP) on body and liver weight of diabetic and non-diabetic rats

Treatment	Absolute liver weight(g)	Relative liver weight(%)	Initial body weight(g)	Final Body weight(g)	Body Weight change(g)	Growth rate (g)
Non diabetic control (GKP, 0mg/kg)	6.10 ±0.11	2.39 ±0.05	245.04 ±1.91	255.10 ±1.54	10.06 ±2.10	0.48 ±0.10
Non diabetic treated (GKP, 600mg/kg)	6.16 ±0.12	2.35 ±0.03	246.74 ±2.36	261.94 ±1.89**	15.20 ±0.50	0.72 ±0.02
Diabetic control (GKP, 0mg/kg)	6.50 ±0.22	3.07 ±0.10**	245.92 ±3.50	211.74 ±1.02***	-34.18 ±3.62***	-1.63 ±0.17***
Diabetic treated I (GKP, 300mg/kg)	6.90 ±0.17	2.76 ±0.07	246.80 ±1.56	250.42 ±0.83*, c	3.62 ±1.04*, c	0.17 ±0.05*, c
Diabetic treated II (GKP, 600mg/kg)	6.60 ±0.67	2.62 ±0.26a	245.88 ±2.17	251.42 ±0.76 c	5.54 ±1.59c	0.26 ±0.08c
Diabetic treated III (GKP, 900mg/kg)	6.20 ±0.39	2.38 ±0.14c	246.80 ±2.30	259.96 ±1.60*, c	13.16 ±1.89c	0.63 ±0.09c

**significantly different from non-diabetic control (P< 0.05)*
*** significantly different from non-diabetic control (P< 0.01)*
**** significantly different from non-diabetic control (P< 0.001)*
a significantly different from diabetic control (P< 0.05)
c significantly different from diabetic control (P< 0.001).

DISCUSSION

The study sought to know the effects of gavage treatment with *Garcinia kola* seeds on biochemical markers of liver functionality in diabetic rats. Result of the phytochemical analysis of GKP showed the presence flavonoids, tannins, glycosides and saponins, this finding agrees with [10] who reported the presence of these phytoconstituents in addition to alkaloid which was not detected in present study.

Alloxan causes damage and death of pancreatic islet cells in experimental animal models, decreasing insulin secretion and hence precipitating diabetic mellitus. The cytotoxic action of alloxan is mediated by reactive oxygen species (ROS), alloxan and the products of its reduction, dialuric acid; establish a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide. Then highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, thus resulting to experimental diabetes mellitus [27].

At the end of the 21-day treatment period, the serum glucose concentration of diabetic control rats was significantly increased compared to the non-diabetic controls and non-diabetic treated group. This same observation has been previously reported in alloxan diabetic animal models [28]. Oral administration of different doses of *Garcinia kola* seed powder attenuated significantly the hitherto elevated serum glucose level across the diabetic treated groups and this was similar to what was observed by [29]. Flavonoids one of the major phytoconstituents of *Garcinia kola* seed have variously been implicated in the reduction of glucose levels in experimental animal models. However, it is noted that the hypoglycaemic effect of GKP is not only attributable to the flavonoid content alone as other phytochemical compounds (saponin, tannins, glycosides) present in it have various hypoglycaemic effects [30]. The possible mechanism of action of GKP could be that it reverses the catabolic features of insulin deficiency, decrease the release of glucagon or increase the secretion of insulin, stimulate directly glycolysis in peripheral tissues, increase glucose removal from blood or reduce glucose absorption from gastrointestinal tract [31].

The liver is an important insulin dependent tissue, which plays a major role in glucose and lipid homeostasis and is severely affected during diabetes [32]. The extent of liver damage caused by hepatotoxic agents is often assessed by the determination of concentration or activities of biochemical markers of liver functionality, such as total bilirubin concentration and activities of AST, ALT and ALP [33]. The enzyme ALP is located in the cytoplasm and will be released into circulation after cellular damage [34]. In addition, soluble enzymes ALT and AST are released when injury involves organelles such as mitochondria of the liver [35]. [36] also reported that liver injury involves both plasma membrane and organelle damage in liver of an experimentally induced diabetic rat. [37] also reported that elevated activities of these enzymes were indicative of cellular leakage and loss of the functional integrity of the hepatic cell membrane.

In the present study, the activities of ALT, AST and ALP of diabetic control animals were significantly increased when compared to non-diabetic controls. This is in line with previous reports in which alloxan diabetic animals showed high activities of these enzymes [38]; [39]. Other authors have reported increased levels of these enzymes in STZ diabetic rats [40]; [41]. The increase in the activity of hepatic enzymes in diabetes is due to hepatocellular damage [42]. Diabetic liver damage and dysfunction has variously been attributed to increased production of ROS, increased lipid peroxidation and decrease in GSH content and cellular antioxidant defence mechanism [40].

Oral administration of GKP for 21 days reduced the activities of these enzymes in a dose dependent-manner compared to the diabetic control, with the highest reduction observed at the highest dose of GKP. This reduction in serum enzymes confirms the hepatoprotective effect of GKP against hepatotoxic agents [43], [11]. *Garcinia kola* is known to be rich in phytoconstituents, particularly the flavonoids [10]. Flavonoids have antioxidant, free radical scavenging and antiliperoxidative properties and are known to exhibit anti-hepatotoxic effects [44]; [45]. The mechanism of hepatoprotective effects of these potent antioxidants (flavonoids) could be by: enhancing the enzymes responsible for antioxidant activity, scavenging free radicals responsible for cell damage or induction of regeneration of liver cells [36].

The liver synthesizes about 90% of all plasma proteins including, albumin [46]. Total proteins and albumin concentrations in alloxan-diabetic rats were reduced when compared to normal controls and non-diabetic treated control. This is in consonance with previous reports [21]; [47]. However, this reduction was not significant. Treatment with various doses of GKP showed a dose-dependent increase in serum total protein which also was not significant. The serum albumin concentration was also increased in a dose-dependent fashion, but was only significant at a dose of 900mg/kg of GKP compared to the diabetic control

Serum urea concentration of diabetic control was significantly increased compared to the normal control. This observation has been previously reported [38]; [48]. The possible explanation of this observation is that, since diabetes mellitus is characterized by deranged metabolism of biomolecules including proteins, it could be that body proteins were increasingly catabolized in the liver, hence an increase in urea concentration and in this way increasing the burden of the kidney in excretion. Treatment with GKP significantly attenuated this increase in a dose-dependent pattern. GKP significantly reduced the urea concentration of the non-diabetic treated group when compared to the non-diabetic control. What this therefore implies is that the drug (GKP) restored the protein metabolism to near normal at the highest dose. Similar studies with plants and its extracts which contain similar phytoconstituents as GKP have been reported to also reduce the diabetic induced increase in urea concentration as observed in this study [36].

Diabetic hyperglycaemia causes a marked reduction in body weight of experimental animals, [48], [47]. This was the case in this work, where it was observed that diabetic untreated group progressively lost body weight over the 21-day period of the experiment. The probable reason for this reduction in weight of diabetic control group is that diabetes engenders poor glycaemic control and since insulin (which facilitates tissue utilization of glucose) is absent,

glucose therefore becomes unavailable for building up processes and hence tissue wasting ensures with concomitant loss of body weight [49]. Another probable explanation for diabetic tissue damage and hence loss of body weight is that, diabetes mellitus induced by alloxan furnishes ROS which are potent free radicals that inflict damage to the tissue and also attacking body building materials particularly proteins.

The body weight gain/growth rate of the non-diabetic control group and non-diabetic treated group was higher than the diabetic treated groups while the diabetic control group was the least. This is because insulin was available in non-diabetic control and non-diabetic treated groups and hence promotes growth and facilities protein metabolism [46]. There was a dose-dependent increase in body weight, growth rate/weight gain of the diabetic treated groups. This of course signifies improved glycaemic control due to the administration of *Garcinia kola* seed powder. This same observation was made by [50] who reported that ethanolic extract of *Garcinia kola* in *C. Gariepinus* broodstock of fish improved weight and growth rate.

Liver hypertrophy in diabetic rats is mainly due to excessive fat deposition in the liver [51], [52]. The absolute liver weight showed no significant difference across all groups. [47] corroborated this finding where *Vernonia amygdalina* administered to diabetic rats showed no significant difference in the wet liver weight of both diabetic and non-diabetic animals for 21 days. This observation was contrasted by [51] whose diabetic model was STZ. They reported a significant liver weight increase in STZ diabetic rat when compared to normal control, although the treatment attenuated the increase. However, the relative liver weight of diabetic control was significantly different when compared to the non-diabetic control group, whereas GKP in diabetic treated group (600mg/kg of GKP) and diabetic treated group (900mg/kg of GKP) attenuated significantly this increase.

CONCLUSION

Reduction in serum glucose of diabetic rats and restoration to normal levels, serum markers of liver functionality showed that *Garcinia kola* seeds possess anti-diabetic properties with a strong potential to protect against diabetic induced liver damage and dysfunction.

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