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# ANTIDIABETIC EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* LEAVES ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Antihyperglycemic, antioxidant, pancreas, *G. kola* leaves, glucose-6-phosphate dehydrogenase

ABSTRACT

The leaf of *Garcinia kola* is an ethnomedicinal leaf used in the treatment of diseases. This present research sought to investigate the antidiabetic effect of methanolic leaf extract of *Garcinia kola* on streptozotocin-induced diabetic rats. Forty-two male albino Wistar rats were divided into seven groups of six animals each: vehicle (distilled water); leaf exract only (100 and 200mg/kg respectively); untreated diabetic rats; treated diabetic rats (extract: 100 and 200mg/kg respectively, glibenclaimide: 0.5 mg/kg). The extract was orally administerd to the diabetic rats for fourteen consecutive days. A Significant decrease in hyperglycemia, active regeneration of the beta cells of the islets of Langerhans, restoration of pancreatic architecture, increased level of glutathione and increased activities of glucose-6-phosphate dehydrogenase and antioxidant enzyme were observed in the extract treated diabetic rats compared with the untreated diabetic rats. The leaf extract compared favourably with the standard antidiabetic drug, glibenclaimide. This result suggests that the leaf extract of *Garcinia kola* possesses antidiabetic activity possibly through antioxidant mechanism. This may have implication in herbal medicine.

**INTRODUCTION**

Diabetes mellitus is one of the leading causes of death worldwide. It is characterized by hyperglycemia (high blood sugar level). Hyperglycemia inhibits the activity of glucose-6-phosphate dehydrogenase, leading to increased generation of reactive oxygen species and beta-cell apoptosis [1], [2]. The underlying cause of hyperglycemia is insulin deficiency (caused by defective pancreatic beta cells) or insulin ineffectiveness. Reversing hyperglycemia is the major target of antidiabetes interventions [3].

Diabetes prevalence has been rising more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. An estimated 1.6 million deaths were directly caused by diabetes in 2016 [3]. Given the side effects of clinical antidiabetes medications, research on medicinal plants as possible supplement / alternative to antidiabetes medication is been explored. A reduction in hyperglycemia after the administration of crude extracts of medicinal leaves to diabetic rats has been reported [4], [5].

*Garcinia kola* (Heckel) plant, commonly called bitter kola is widely known for the medicinal properties of its seeds, root, stem, bark, fruit and leaves. The leaves of *Garcinia kola* have been reported to possess medicinal properties. This leaf is available all the year round, unlike the seeds which are seasonal. Previous studies have shown that *G. kola* leaves have a high content of flavonoids and polyphenols including other phytochemicals [6], [7]. This present research sought to investigate the antidiabetic potential of *Garcinia kola* leaves.

## MATERIALS AND METHODS

### Chemicals

Streptozotocin (STZ), glibenclamide and glutathione (GSH) were purchased from Sigma-Aldrich Corporation (London, UK). Assay kits for total protein, lactate dehydrogenase and glucose-6-phosphate dehydrogenase activities were purchased from Randox Laboratories Ltd (Crumlin, UK). All other chemicals where of analytical grade and were obtained from British Drug Houses Ltd (Poole, UK).

### Plant Material

The leaves of *Garcinia kola* were collected from a farm at Aba Oyo, Akure, Nigeria in the month of November. They were authenticated at the Herbarium of Botany Department, Obafemi Awolowo University, Ile – Ife, Nigeria by Mr Ademoriyo. A voucher specimen with voucher number IFE17348 was prepared and deposited at the herbarium.

### Methanolic Extraction of *Garcinia kola* Leaves

Extraction was carried out according to the method previously described by RajeswaraReddy et al. [8]. Briefly, *Garcinia kola* leaves were washed clean, air-dried and pulverized. The pulverized leaves (500 g) were macerated in 6.5 litres of 80% methanol (Sigma-Aldrich Corporation, London, UK) at 25$^{o}$C with constant agitation for 72 hours. The mixture was filtered and the filtrate was evaporated to dryness using a rotary evaporator and a freeze dryer. The powdered crude extract was kept in a refrigerator at 4°C until further use.

### Phytochemical screening

A small portion of *Garcinia kola* leaf extract (GKLE) was subjected to phytochemical screening using Harbourne [9] and Trease and Evans [10] method to test for the presence of flavonoids, tannins, saponins, alkaloids, anthraquinones, cardiac glycoside and terpenoids.

### Animals

Forty-two male albino Wistar rats (200 $\pm$ 10 g, aged 50-55 days) purchased from the central animal house of the University of Ibadan were used for this study. The animals were kept under standard conditions of 12 hours light : 12 hours dark cycle (room temperature 25 – 28$^{o}$C and relative humidity 70 – 80%). The animals were acclimatized to laboratorty conditions and were provided with standard commercial rat chow pellets and tap water *ad libitum*. They were handled in accordance with the international guide for the care and use of laboratory animals.

### Experimental design

The forty-two albino rats were divided into seven groups of six animals each: Group I: Vehicle (distilled water); Group II: GKLE (100 mg/kg b. wt); Group III: GKLE (200 mg/kg b. wt); Groups IV: Diabetic rats (no treatment); Groups V: GKLE (100 mg/kg b. wt) treated

diabetic rats; Groups VI: GKLE treated (200 mg/kg b. wt) diabetic rats; Groups VII: Glibenclaimide (0.5 mg/kg b.wt.) treated diabetic rats; b. wt = body weight [11].

## Diabetes induction

Diabetes was induced by intraperitoneally administering a freshly prepared solution of STZ (65 mg/kg b. wt) in ice-cold 0.1 M citrate buffer, pH 4.5, as a single dose to overnight fasted Wistar rats after baseline glucose estimation. Seventy-two hours after STZ injection, Wistar rats with blood glucose level $\geq$ 250 mg/dl were considered diabetic and treated for fourteen consecutive days [12].

## Blood glucose estimation

Fasting blood glucose concentration of the rats was monitored on day zero, seven and fourteenth of treatment using Accu Chek compact glucometer (Roche, Madrid, Spain). Blood was drawn from overnight fasted rats by tail venipuncture.

## Sacrifice

The animals were fasted overnight and sacrificed by cervical dislocation at the end of the experimental period. Blood drawn through cardiac puncture was collected into plain serum bottles. The liver and pancreas of each rat were excised, washed in 1.15% KCl solution, blotted dry and weighed [12].

## Histopathological examination and tissue preparation

The pancreas was fixed in 10% formalin and cut into four sections. Each section was stained with haematoxylin and eosin stain for histological examination [13], while the liver was homogenized in a saline solution (0.9%) and centrifuged at 9000 rpm for 10 min at 4$^{o}$C using a cold centrifuge. The supernatant was stored at 4$^{o}$C and was used for biochemical assay [6].

## Biochemical assay

Reduced glutathione concentration in the liver was determined by a method previously described by Ellman [14]. Hepatic glutathione peroxidase activity was measured according to a previously described method [15]. The activities of hepatic lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) as well as total protein content were assessed using commercially available assay kits (Randox kits, 55 Diamond Road, Crumlin, County Antrim, Northern Ireland, United Kingdom).

## Statistical analysis

Values were expressed as mean ± SEM of five animals. One Way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test was used to estimate differences between multiple groups where appropriate. The significance level was set at $p < 0.05$.

# RESULTS

## Phytochemical screening

Table 1 shows that flavonoids is highly present in *Garcinia kola* leaf extract (GKLE)

**Table 1: Phytochemical constituents of GKLE**

| Phytochemicals | | | | | | |
|---|---|---|---|---|---|---|
| Flavonoid | Saponins | Alkaloids | Tannins | Cardiac glycosides | Terpenoids | Anthraquinones |
| +++ | + | + | ++ | - | + | ++ |

+ ++ = highly present; ++ = moderately present; + = present - = absent

## Antihyperglycemic activity of GKLE on STZ-induced diabetic rats

Table 2, shows a significant ($p < 0.05$) reduction in hyperglycemia in the extract treated diabetic rats compared with the untreated group.

**Table 2 Antihyperglycemic activity of GKLE on STZ-induced diabetic rats**

| | Blood glucose concentration (mg/dl) | | |
|---|---|---|---|
| Groups | Day 0 | Day 7 | Day 14 |
| I | 95.27 ± 0.16$^{f}$ | 95.07 ± 0.54$^{f}$ | 95.08 ± 0.08$^{f}$ |
| II | 94.97 ± 0.29$^{f}$ | 94.45 ± 0.06$^{f}$ | 94.31 ± 0.18$^{f}$ |
| III | 92.20 ± 0.28$^{b}$ | 91.86 ± 0.40$^{e}$ | 91.51 ± 1.89$^{c}$ |
| IV | 299.41 ± 10.90$^{a}$ | 380.35 ± 21.01$^{h}$ | 428.15 ± 12.68$^{n}$ |
| V | 319.39 ± 18.19$^{i}$ | 160.80 ± 5.44$^{o}$ | 96.10 ± 0.48$^{f}$ |
| VI | 342.07 ± 16.32$^{k}$ | 117.04 ± 3.88$^{j}$ | 91.71 ± 0.72$^{c}$ |
| VII | 339.60 ± 18.85$^{k}$ | 180.04 ± 9.00$^{d}$ | 120.63 ± 3.00$^{i}$ |

Result is presented as mean ± SEM of five independent experiments, each performed in triplicates. Values with different superscripts in a column are significantly different ($p < 0.05$). Group I: Vehicle (distilled water); Group II: GKLE (100 mg/kg b. wt); Group III: GKLE (200 mg/kg b. wt); Groups IV: Diabetic rats (no treatment); Groups V: GKLE (100 mg/kg b. wt) treated diabetic rats; Groups VI: GKLE treated (200 mg/kg b. wt) diabetic rats; Groups VII: Glibenclaimide (0.5 mg/kg b.wt.) treated diabetic rats; b. wt = body weight; GKLE = *Garcinia kola* leaf extract.

## Effect of GKLE on the activities of G-6-PDH and LDH in STZ-induced diabetic rats

Effect of the extract on the activities of carbohydrate metabolizing enzymes is shown in Figure 1. A significant ($p < 0.005$) increase in the activity of hepatic G-6-PDH was found in the extract treated diabetic rats compared with the untreated diabetic rats, while a non-significant increase in the activity of hepatic LDH was found in the extract treated diabetic rats compared with the untreated diabetic rats.

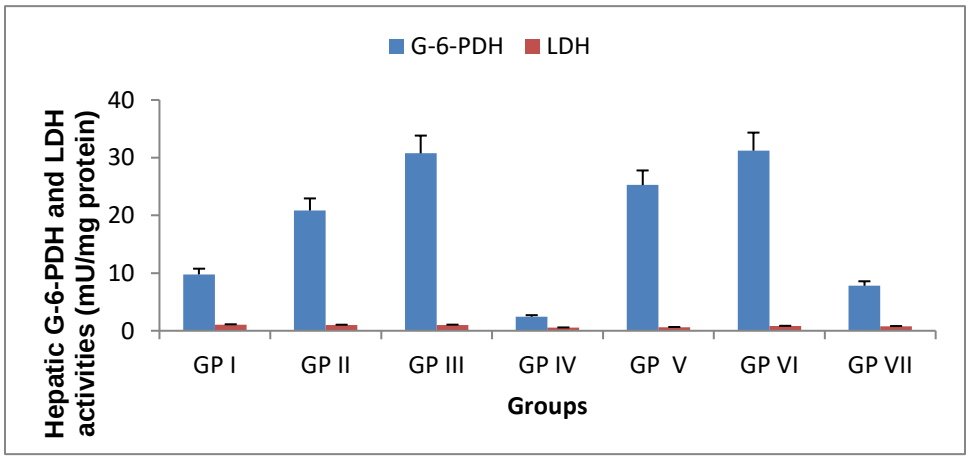


**Figure 1**: **Effect of GKLE on the activity G-6-PDH in STZ-induced in diabetic rats.**

Result is presented as mean ± SEM of five independent experiments, each performed in triplicates. Group I: Vehicle (distilled water); Group II: GKLE (100 mg/kg b. wt); Group III: GKLE (200 mg/kg b. wt); Groups IV: Diabetic rats (no treatment); Groups V: GKLE (100 mg/kg b. wt) treated diabetic rats; Groups VI: GKLE treated (200 mg/kg b. wt) diabetic rats; Groups VII: Glibenclaimide (0.5 mg/kg b.wt.) treated diabetic rats; b. wt = body weight; GKLE = *Garcinia kola* leaf extract.

## Antioxidant effects of GKLE on STZ-induced diabetic rats

Clearly shown in Tables 3 and Figure 3 are the antioxidant effects of GKLE on STZ–induced diabetic rats. GKLE significantly ($p < 0.05$) increased the the level of glutathione and the activity of glutathione peroxidase (GPx).

**Table 3. Effect of GKLE on hepatic GSH concentration in STZ–induced diabetic rats**

| GSH (nmol/mg protein) | | | | | | |
|---|---|---|---|---|---|---|
| Groups | | | | | | |
| I | II | III | IV | V | VI | VII |
| 4.96 ± 0.05[a] | 42.21 ± 2.95[b] | 56.32 ± 0.36[c] | 0.42 ± 0.07[d] | 40.00 ± 2.90[b] | 57.74 ± 0.10[e] | 3.95 ± 0.85[g] |

Result is presented as mean ± SEM of five independent experiments, each performed in triplicates. Values with different superscripts are significantly different ($p < 0.05$). Group I: Vehicle (distilled water); Group II: GKLE (100 mg/kg b. wt); Group III: GKLE (200 mg/kg b. wt); Groups IV: Diabetic rats (no treatment); Groups V: GKLE (100 mg/kg b. wt) treated diabetic rats; Groups VI: GKLE treated (200 mg/kg b. wt) diabetic rats; Groups VII: Glibenclaimide (0.5 mg/kg b.wt.) treated diabetic rats; b. wt = body weight; GKLE = *Garcinia kola* leaf extract.

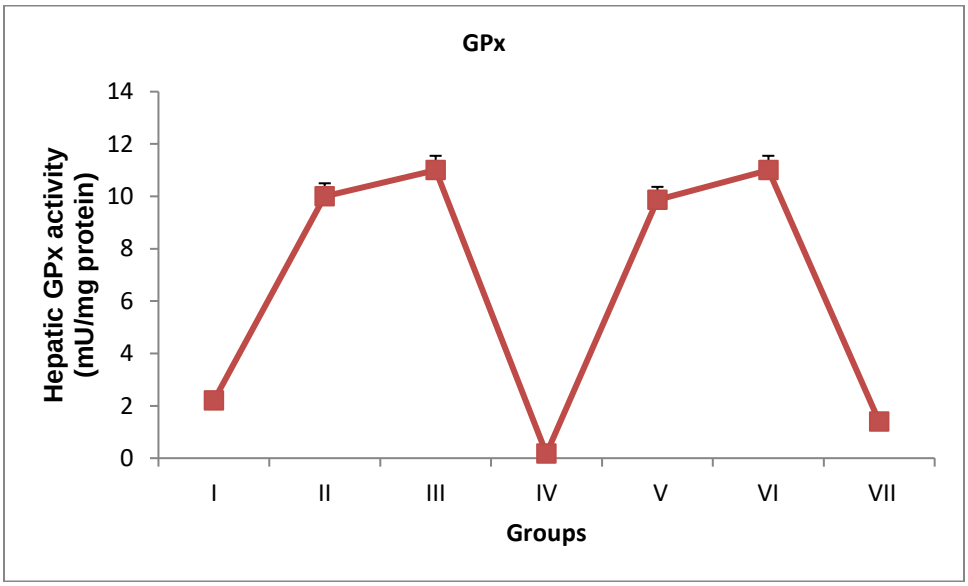


**Figure 2**: **Effect of GKLE on the activity of glutathione peroxidase (GPx) in STZ-induced in diabetic rats.**

Result is presented as mean ± SEM of five independent experiments, each performed in triplicates. Group I: Vehicle (distilled water); Group II: GKLE (100 mg/kg b. wt); Group III: GKLE (200 mg/kg b. wt); Groups IV: Diabetic rats (no treatment); Groups V: GKLE (100 mg/kg b. wt) treated diabetic rats; Groups VI: GKLE treated (200 mg/kg b. wt) diabetic rats; Groups VII: Glibenclaimide (0.5 mg/kg b.wt.) treated diabetic rats; b. wt = body weight; GKLE = *Garcinia kola* leaf extract.

## Histopathological examination of the pancreas of experimental rats

Histopathological examination of the pancreas of extract treated diabetic rats revealed active regeneration of clusters of beta cells of the islets of Langerhans, restoration of the architecture of the pancreas and no visible lesion (Figures 3C and D) compared with the untreated diabetic group (Figure 3B) which showed extensive necrosis and architectural degeneration.

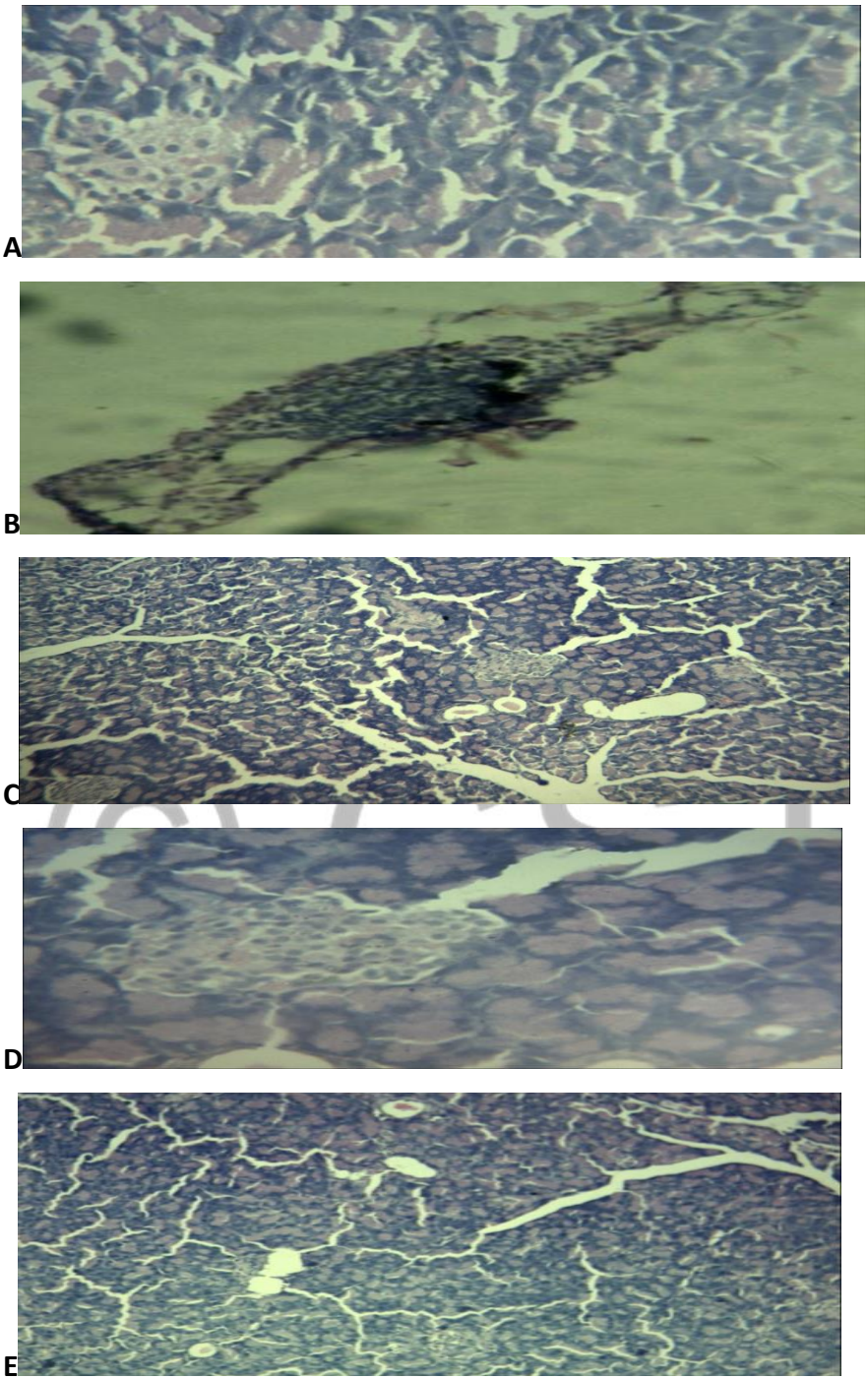


**Figure 3: Histopathological photomicrographs of heamatoxylin-eosin staining of the pancreatic tissues of experimental rats (x200 magnification).**

**(**A) normal rats showing normal acini cells and a cluster of the islets of Langerhans; (B) STZ-induced diabetic rat showing severe necrosis and degeneration of pancreatic architechture; (C) 100 mg/kg GKLE treated diabetic rat showing regenerating acini cells and small clusters of the islets of Langerhans with beta cells; (D) 200 mg/kg GKLE treated diabetic rat showing normal acini cells and a large cluster of the islets of Langerhans with beta cells; (E) 0.5 mg/kg Glibenclaimide treated diabetic rat showing regenerating acini cells and a small cluster of islets of Langerhans with beta cells. GKLE = *Garcinia kola* leaf extract.

## Discussion

Plants have been used over the ages to cure diseases. Hyperglycemia has been reported to impair G-6-PDH activity leading to increased level of reactive oxygen species (ROS) and decreased cell survival [1], [2]. This study however reported a decrease in hyperglycemia and an increase in the activity of G-6-PDH in the extract treated diabetic rats compared with the untreated group. Similarly, the antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, has been reported in rats [16]. This indicates that the antidiabetic potency of *G. kola* plant lies not only in the seeds but in the leaves also. G-6-PDH catalyzes the reaction that releases NADPH for the regeneration of reduced glutathione [17], [2]. Thus, an increase in the activity of G-6-PDH would lead to an increase in the level of glutathione. This might explain why there was a dramatic increase in both reduced glutathione and the activity of glutathione peroxidase in the extract treated diabetic rats.

The increase in antioxidant activity found in the extract treated diabetic rats may have led to the observed active regeneration of the beta cells of the pancreas and the subsequent reduction in hyperglycemia. Flavonoids and polyphenols in plants have long been known to mimic the activity of antioxidants [18]. This study found a high level of flavonoids in GKLE. This might be responsible for the high antioxidant activity found in the extract treated diabetic rats. Previous studies have reported the important role played by plants in the reduction of hyperglycemia and oxidative stress in the pancreatic beta cells of STZ-induced diabetic rats [16], [19]. It is interesting to note that the leaf extract of *Garcinia kola* compared favourably in a dose dependent manner with the standard antidiabetic drug, glibenclaimide.

## Conclusion

This research provides information on the antidiabetic potential of the leaves of *Garcinia kola* in STZ-induced diabetic rats. This may have implications in alternative medicine.

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