Original Article

Antidiabetic and Antiradical Effects of *Garcinia kola* Seeds in Dexamethasone-Induced Hyperglycemic Rats

Abstract

Background: In traditional medicine, the maceration of seeds of *Garcinia kola* (GK) is used to treat various diseases including diabetes. In traditional pharmacopoeia, GK seeds are used to strengthen the immune system and as a stimulant and aphrodisiac. **Aims:** This study aimed to evaluate the antidiabetic free radical scavenging effects of the hydroalcoholic extract of GK seeds (HAEGS) in a dexamethasone-induced hyperglycemic (DexIH) rat model. **Settings and Design:** This study was an interventional study. **Subjects and Methods:** Here using *in vivo* model, we assessed some pharmacological properties of HAEGS in DexIH rat. Hypoglycemia, antihyperglycemia, spasmolytic and laxative activities were also evaluated in DexIH. *In vitro* study assessed antiradical activity. The HAEGS was obtained by decoction introducing 250 g with water–ethanol mixture (30:70). The plant extract was administered to the animals at doses of 50 (GK50) and 100 (GK100) mg/kg body weight. All animal experiments were in accordance with ARRIVE guidelines and were performed in accordance with the scientific procedures of UK Animals. Antiradical activity of GK was assessed *in vitro* by inhibition of the activity of 2,2-diphenyl-1-picrylhydrazyl. **Statistical Analysis Used:** Statistical analysis was performed using GraphPad Prism 5.03 software, and *P* values less than 0.05 were considered statistically significant. **Results:** At doses 50 and 100 mg/kg, GK significantly ($P < 0.001$) regulated DexIH after two weeks of treatment compared to the normoglycemic control and hyperglycemic rats. The extract at both doses significantly ($P < 0.001$) inhibited the spasmolytic activity in both normoglycemic and hyperglycemic rats compared to Imodium®. In rats DexIH rats, only dose 100 mg/kg significantly ($P < 0.05$) increased laxative effects when compared to the negative control. *In vitro* antiradical activity of GK revealed vitamin C-like antiradical activity. **Conclusions:** This study justifies the traditional use of GK seeds as an antidiabetic.

Keywords: *2,2-diphenyl-1-picrylhydrazyl scavenging, dexamethasone, Garcinia kola, intestinal transit, oral glucose tolerance test, rat*

Barnabé Lucien Nkono Ya Nkono, Ablassé Rouamba[1], Mc Jesus Kinyok[2], Jean Guy Stéphane Omokolo, Balthazar Tchouanka Tcheudi, Benjamin Arnaud Tigui, Paul D. Djomeni Dzeufiet[3], Sélestin Dongmo Sokeng[4], Pierre Kamtchouing[3]

Department of Biological Sciences, Laboratory of Animal Physiology, Higher Teacher Training College, University of Yaoundé I, [2]Department of Chemistry, Higher Teacher Training College, University of Yaoundé I, Yaoundé, [3]Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, Yaoundé, [4]Department of Biological Sciences, Faculty of Sciences, University of Ngaoundéré, Ngaoundéré, Cameroon, [1]Laboratory of Applied Biochemistry and Chemistry, University Joseph KIZerbo, Joseph KIZerbo, Burkina Faso

Submitted: 16-Mar-2022
Revised: 02-Jun-2022
Accepted: 10-Jun-2022
Published: 26-Jul-2022

Address for correspondence:
Dr. Barnabé Lucien Nkono Ya Nkono,
Department of Biological Sciences, Laboratory of Animal Physiology, Higher Teacher Training College, University of Yaounde I, 8201 Yaounde, Cameroon.
E-mail: luciennkono@gmail.com

Access this article online

Website: www.ijabmr.org

DOI: 10.4103/ijabmr.ijabmr_199_22

Quick Response Code:

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defect in insulin secretion, insulin action, or both.[1] Insufficient insulin secretion and insulin resistance are the basic pathologic characteristics of type 2 diabetes mellitus (T2DM).[2] In general, diabetes sets in following a metabolic disorder also called metabolic syndrome, which is manifested by frequent headaches, fatigue, dizziness, visual disturbances, frequent urges to urinate, and drowsiness among others. Oxidative stress is closely associated with diabetes mellitus, particularly T2DM, where it very often causes micro- and macroangiopathies. Oxidative stress has been known to cause damage to the cells, tissues, even to organs by impairing important biomolecules and cells.[3] Production of high levels of reactive oxygen species (ROS) causes a significant decrease in antioxidant defense mechanisms, leading to protein, lipid and DNA damage, and subsequent disruption of cellular functions and cell death, but at lower levels induce subtle changes in intracellular signaling pathways.[4]

Many plants are traditionally considered antidiabetic or are involved in treating the complications of diabetes. *Garcinia kola* (GK) seeds, stem bark, and roots are part of these plants heavily used in traditional pharmacopoeia in the treatment of diabetes mellitus.[5-7] The seeds of GK are bitter, they are used as a stimulant and aphrodisiac, and are generally chewed like kola nuts all day long and without moderation largely by the elderly. Other

How to cite this article: Nkono Ya Nkono BL, Rouamba A, Kinyok McJ, Omokolo JGS, Tcheudi BT, Tigui BA, *et al.* Antidiabetic and antiradical effects of *Garcinia kola* seeds in dexamethasone-induced hyperglycemic rats. Int J App Basic Med Res 2022;12:203-10.

medicinal use of *Garcinia kola* (GK) seeds intervene in cold symptoms, antimicrobial, diarrhea, dysentery, liver disorders, poison antidote, and sickle cell.[8]

Recently, it was reported that the reduced glucose concentration could be due to the activity of bitter kola seed extract against inflammation and ROS of free radicals on the pancreatic beta cells.[9] It has also been reported that GK seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. GK seed administration significantly ameliorated hyperglycemia-mediated damage by decreasing the blood glucose level (72.8% and 84.6% on the 7th and 14th posttreatment days, respectively).[10] It has been reported in many investigations that bioactive fractions of different medicinal plants having free radical scavenging and antioxidant are used in many diseases such as cancer, tissue inflammatory, and cardiovascular disease.[11-14]

Therefore, this study aimed to evaluate the *in vivo* antidiabetic and *in vitro* free radical scavenging effects of hydroalcoholic extract of GK seeds (HAEGS) in a dexamethasone-induced hyperglycemic rat model.

Subjects and Methods

Place and duration

This study was an interventional study; the *in vivo* experimental aspect of this study was conducted at the Laboratory of Animal Physiology between May and July 2020. The *in vitro* experimental study was carried out at the Applied Biochemistry and Chemistry Laboratory, during the same period.

Chemicals and drugs

Chemicals were from analytical grade: Dexamethasone sodium phosphate (Rotexmedica®, Panpharma, Germany), D-(+)-glucose (Sigma Aldrich, Germany), glibenclamide (Daonil®, Sanofi, Pari-France), loperamide hydrochloride (Imodium®, Jasen, street Camille Desmoulins-France), sodium picosulfate (Fructine®, DB Pharma, Château Neuf-France), methanol (Prolabo Paris, France). 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (Vitamin C), aluminum trichloride ($AlCl_3$), quercetin, and dimethyl sulfoxide (DMSO) were purchased by Sigma–Aldrich (St. Louis-USA).

Plant materials

The decoction of the hydroalcoholic (30% water and 70% ethanol) extract of GK seeds was obtained by introducing 250 g of powder into an Erlenmayer flask by supplementing the total volume to 1 L with water–ethanol mixture (30:70) and then brought to boil at 80°C for 15 min. After cooling and filtration with Whatman paper No. 4, the filtrate was concentrated with Rotavapor. The extract was reconstituted in 100% DMSO solution for purification and filtered through a 0.45 µm diameter microporous filter and then prepared at 1% by dissolving in distilled water at concentrations of 5 mg/mL and 10 mg/mL for administration to animals at doses of 50 and 100 mg/kg.

Study design and population

Animals

All animal experiments were in accordance with ARRIVE guidelines and were performed in accordance with the scientific procedures of UK Animals.[15]

To evaluate antidiabetic properties, 30 rats of Wistar strain were used. The animals were divided into 5 groups of 6 rats each (the sixth animal was used for making histological sections). Glibenclamide 2.5 mg/kg was used as a positive control. To evaluate the effect of GK on intestinal transit in normoglycemic and hyperglycemic rats, we used 48 rats. Each group consisted of 3 normoglycemic rats and 3 hyperglycemic rats. Imodium® and Fructin® were used as positive controls, respectively, for the evaluation of the spasmolytic and laxative effect of GK. All the animal experiments were carried out in accordance with the guidelines of Cameroon National Ethics committee (Ref. FWIRB 00001954).

In vivo study

Induction of hyperglycemia

Induction of hyperglycemia was done as previously reported.[16] Briefly, hyperglycemia was induced in rats by repeated administration of dexamethasone sodium phosphate (Rotexmedica®, Germany) for 3 days at a dose of 4 mg/kg body weight intravenously once daily between 7 a. m. and 8 a. m. After administering the last dose of dexamethasone, the animals were subjected to a 12-hour food fast, then the fasting blood glucose levels were measured using a OneTouch Ultra brand glucometer. The animals which had a fasting blood glucose level greater than 120 mg/dl were selected for carrying out our experiments.

Grouping of experimental animals

The rats were divided into five groups of six animals each for blood glucose level tests as following:

- Group 1: Negative fasting control group (NFC), normoglycemic animals that did not receive oral glucose overload at any time during the entire duration of the test./negative normal control (NNC), normoglycemic animals having undergone oral glucose overload. The NFC and NNC animals did not receive any treatment but rather received orally a solution of 1% DMSO (10 ml/kg) which served as vehicle.
- Group 2: NDC: Negative dexamethasone-induced hyperglycemic rats control group (NDC). The animals of the NDC group were the hyperglycemic animals and received no treatment other than the vehicle solution of

1% DMSO (10 ml/kg, p.o).
- Group 3: GK50: Dexamethasone-induced hyperglycemic rats treated with GK 50 mg/kg (*p. o*)
- Group 4: GK100: Dexamethasone-induced hyperglycemic rats treated with GK 100 mg/kg (*p. o*)
- Group 5: Glib2.5: Dexamethasone-induced hyperglycemic rats treated with glibenclamide at a dose of 2.5 mg/kg (*p. o*).

For the spasmolytic and laxative tests, the animals were divided as follows:

- Group 1: Negative control normoglycemic and negative control hyperglycemic animals given 9% NaCl solution, *p. o* (10 ml/kg)
- Group 2: Normoglycemic and DexIH animals as positive control group received Imodium® (2 mg/kg, *p. o*) or Fructine® (5 mg/kg, *p. o*).
- Group 3 and 4: Normoglycemic and hyperglycemic animals treated with respective doses of 50 and 100 mg/kg of GK (*p. o*).

Treatment of animals with hydroalcoholic extract of Garcinia kola seeds

The animals received HAEGS orally once daily for 14 days at doses of 50 and 100 mg/kg, which are minimal therapeutic doses. These doses were estimated after screening based on the therapeutic dose used in traditional medicine.

Oral glucose tolerance test

To carry out this test, the animals were fasted on food and not on water for a minimum of 12 hours, and then the fasting blood glucose level was measured. The OGTT was performed in normoglycemic animals on the first day before induction of hyperglycemia and on the fourteenth day in hyperglycemic-induced dexamethasone rats after a 14-day pretreatment with plant extract and different treatments. Blood glucose level was measured at minute zero using a OneTouch Ultra brand glucometer from the tail puncture of the rats, and the various treatments were immediately administered to the animals by gastric intubation. Thirty minutes after the administration of the various treatments, all groups of animals received D-glucose at a dose of 3 g/kg of body weight orally. Blood glucose level was then measured at 30, 60, 90, and 120 min after glucose load.

Evaluation of gastrointestinal motility

30 min after oral administration of fructine, Immodium and HAEGS at two test groups (50 and 100 mg/kg respectively), animals received 10% charcoal suspension (20 ml/kg, *p.o*). Twenty minutes after oral gavage of the 10% charcoal suspension, the animals were sacrificed after anesthesia with ketamine (50 mg/kg). The small intestine was then completely removed from each animal after performing a midline laparotomy by sectioning from the pylorus to the cecum when avoiding pulling it. The total length of the small intestine (TLSI), as well as the length travelled by the charcoal suspension (LTCS), was measured.

The determination of the spasmolytic and laxative effects (%) was made by calculation as previously described[17] using equations (1) and (2) as follows:

$$Spasmolytic\ effect(\%) = 100 - \left[\frac{LTCS}{TLSI}\right] X\ 100 \quad (1)$$

$$Laxative\ effect(\%) = \left[\frac{LTCS}{TLSI}\right] X\ 100 \quad (2)$$

In vitro study

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Antiradical activity of GK seeds was assessed *in vitro* by inhibition of the activity of DPPH. The DPPH radical scavenging assay was performed as previously described.[18] The DPPH radical is a stable purple-colored radical. It discolors in the presence of an antioxidant compound, which reduces it to a colorless nonradical molecule. The discoloration of the DPPH radical in the presence of the antioxidant compound is monitored using a spectrophotometer at 517 nm.

In a 96-well plate, 100 µL of solution of the DPPH radical dissolved in methanol (20 µg/mL) was mixed with 100 µL of plant extract dissolved in methanol at different concentrations (250 µg/mL – 3.90625 µg/mL). A well without plant extract served as a control and contained only 100 µL of methanol and 100 µL of the DPPH solution. The plate was incubated for 15 min protected from light, and the optical density (OD) was measured at 517 nm using an Epoch brand spectrophotometer. The experiment was carried out in triplicate and the percentage radical scavenging activity (RSA) was calculated according to the formula as follows.

$$RSA(\%) = \left[\frac{(control - sample)}{control}\right] X\ 100 \quad (3)$$

Ascorbic acid was used as a positive control and the concentration of 25 µg/mL showed 86.74% radical scavenging activity.

Total flavonoid content

To determine total flavonoid content in the sample of HAEGS, the aluminum chloride colorimetric method was used.[19] A volume of 100 µL of 2% $AlCl_3$ dissolved in methanol was mixed with an equal volume of extract diluted in methanol (initial concentration 1 mg/mL). Absorbance was measured after 10 min incubation at 37°C away from light at a wavelength of 415 nm against a blank. The blank consisted of a mixture of 100 µL of 2% AlCl3 and 100 µL of methanol.

Quercetin was used as a reference compound for establishing the standard curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, and

then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200 μg/mL). The experiment was repeated in triplicate and the results were expressed in mg quercetin equivalent (QE) per mass of dry extract (mg QE/mass of extract). The concentration of total flavonoid content in the test samples was calculated from the calibration plot (Y = 0.02X + 0.0116, R^2 = 0.9997).

Statistical analysis

All the tests were performed as individual triplicate experiment (for *in vitro* tests as well as for the evaluation of intestinal transit) and quintuple experiment (for *in vivo* evaluation of hypoglycemic and antihyperglycemic properties). The data were analyzed with the GraphPad Prism software version 5.0.3. All the data were expressed as mean ± standard error of mean (n = number of experiments). The statistical analyses were obtained by the analysis of variance, followed by the Bonferroni's posttest where necessary. The confidence interval was set at 95% with a significance threshold of less than 5% ($P < 0.05$).

Results

Effect of hydroalcoholic extract of *Garcinia kola* seeds on oral glucose tolerance test

Figure 1 shows the effect of HAEGS on OGTT in normoglycemic and dexamethasone-induced hyperglycemic (DexIH) rats.

After two weeks of treatment, all groups of animals recovered a fasting blood sugar level (12 h) around normal values [Figure 1c] with the highest fasting blood level in the DexIH negative control group NDC (81.16 ± 2.72 mg/dL) who received exclusively dexamethasone, followed by the glycaemia of the normal negative control (NNC) group (73.00 ± 1.03 mg/dL). Compared to the NNC and NDC groups after two weeks of treatment, taking fasting blood glucose (12 h) revealed that HAEGS had a nonsignificant hypoglycemic effect ($P > 0.05$) at the two doses tested with a greater effect at the dose of 50 mg/kg (68.33 ± 1.58 mg/dL) compared to the dose of 100 mg/kg (72.66 ± 1.14 mg/dL).

To assess glucose intolerance on normoglycemic and DexIH rats, OGTT was performed. Figure 1a shows that different treatments of HAEGS non-significantly prevented postprandial glycemia peak in normoglycemic rats when compared to the NNC group. This result is similar to that of the positive control group (Glib2.5) that received glibenclamide at a dose of 2.5 mg/kg [Figure 1b].

In DexIH rats, only dose 100 mg/kg of HAEGS significantly prevented the postprandial glycemia peak when compared to NNC and NNC groups [Figure 1c]. In the positive control group, glibenclamide reduced postprandial glycemia highly significantly ($P < 0.001$) only at the 2nd h after oral glucose overload compared to the NNC and NDC groups [Figure 1d].

Effect of HAEGS on intestinal transit

The effects of HAEGS on intestinal transit are shown in Figure 2. In normoglycemic rats, the plant extract inhibited spasmolytic activity (P<0.001) in a dose-response manner (30.84 ± 8.19% and 23.88 ± 2.64% at 50 and 100 mg/kg, respectively) when compared to the positive control group that received the reference drug (Imodium). Compared to the

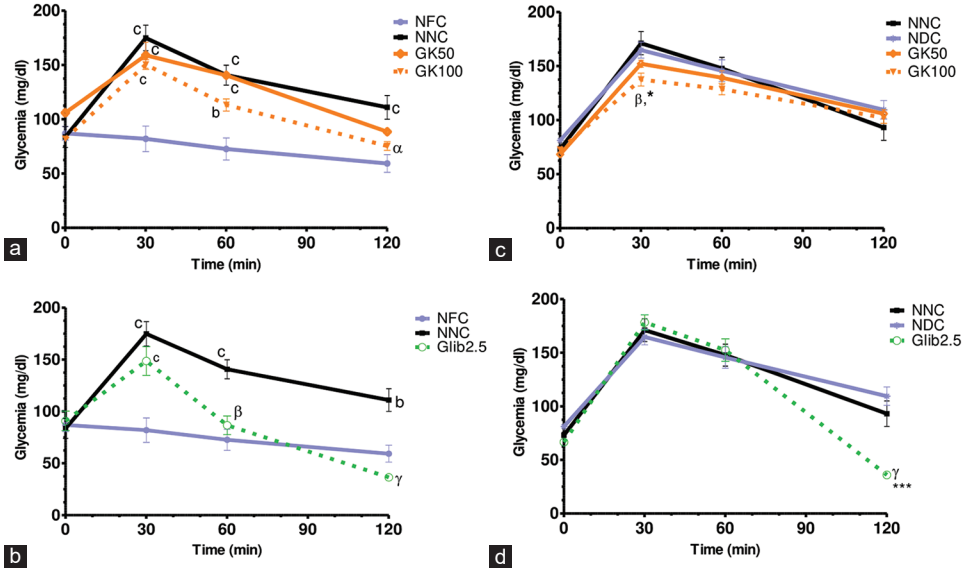

Figure 1: Effect of *Garcinia kola* on OGTT in normal (a and b) and dexamethasone-induced hyperglycemic rats on the 14th day of treatment (c and d). Data are presented as mean ± ESM of five animals per group. NFC: Negative fast control; NNC: Negative normal control; NDC: Negative Dexamethasone control; GK50: *Garcinia kola* 50 mg/kg; GK100: *Garcinia kola* 100 mg/kg; Glib2.5: Glibenclamide 2.5 mg/kg. b: ($P < 0.01$), c: ($P < 0.001$) significant from NFC group. α: ($P < 0.05$), β: ($P < 0.01$), ɣ: ($P < 0.001$) significant from NNC group. *($P < 0.05$), *($P < 0.001$) significant from NDC group. OGTT: Oral glucose tolerance test: ESM: Error Standard Mean**

negative control group that received the vehicle, it should be noted that only the 100 mg/kg dose of HAEGS induced a significant spasmolytic effect ($P < 0.05$) only in DexIH rats (37.82 ± 2.22% and 18.26 ± 0.51%, respectively, for the group that received the vehicle and the group that received the HAEGS at the dose of 100 mg/kg).

With regard to the evaluation of the laxative effect of HAEGS, no significant difference ($P > 0.05$) was observed in normoglycemic animals compared to the positive control groups that received Fructine® and the negative control that received the vehicle [Figure 2b]. On the other hand, in DexIH rats, only dose 100 mg/kg significantly ($P < 0.05$) increased laxative effects when compared to the negative control. The potentiation of this effect was dose related because no significant difference ($P > 0.05$) was observed in both normoglycemic and hyperglycemic rats in comparison to the reference laxative drug Fructin® [Figure 2b]. It should also be noted that only the 100 mg/kg dose of HAEGS induced in DexIH rats a significant increase ($P < 0.05$) in the laxative effect (81.74 ± 0.51%) compared to the negative control group (62.20 ± 2.22%) [Figure 2b].

Effect of hydroalcoholic extract of *Garcinia kola* seeds on 2,2-diphenyl-1-picrylhydrazyl free radical scavenging

In vitro antiradical activity of HAEGS revealed vitamin C-like antiradical activity [Figure 3]. The lowest concentration tested on DPPH radical scavenging had a percentage of 6.87 ± 0.56% for HAEGS (3.90625 µg/mL) and 17.96 ± 3.06% for Vitamin C (0.39062 µg/mL), which showed a highly significant difference ($P < 0.001$). On the other hand, six other concentrations were tested and the last two concentrations tested of HAEGS did not show any significant difference compared to Vitamin C ($P > 0.05$). It should be noted that the concentration of Vitamin C corresponds to one-tenth of that of the HAEGS. The highest concentration of HAEGS (250 µg/mL) had a percentage of DPPH radical scavenging similar to that of Vitamin C (25 µg/mL), notably 80.79 ± 0.54% and 86.74 ± 0.71%, respectively, for the HAEGS and Vitamin C.

Total flavonoid content

Total flavonoid content of HAEGS was 2.94 ± 0.33 mg QE/100 mg extract.

Histopathological examination of the pancreatic tissues

Figure 4 shows a photomicrograph of pancreatic tissue from normoglycemic and DexIH rats that received different treatments.

The histological analysis showed, in the normal control [Figure 4a], a normal structuring of the pancreatic parenchyma. Compared to the normal group, the animals of the negative control group [Figure 4b] showed several histopathological alterations, in particular hypertrophy of the islets of Langerhans. The batches treated with the reference drug [Figure 4c], just like those receiving the extract at different doses [Figure 4d and e], presented a restructuring of the pancreatic tissue close to that of the normal control. Indeed, it appears from the histopathological study of pancreas that extract restored the histological integrity of these organs initially altered by dexamethasone.

Discussion

Dexamethasone is a synthetic glucocorticoid, which has a high affinity for the glucocorticoid receptor. It has been used to rapidly generate insulin resistance in rodents in a relatively short period of time.[20] It was reported that the acute high doses of dexamethasone (4, 8, and 16 mg/kg) for 6 days caused hepatic steatosis and showed mild-to-moderate arteriosclerosis in the aorta. These changes may be secondary consequences of insulin resistance.[21] The present study reported that treatment with dexamethasone intravenously at a dose of 4 mg/kg for three consecutive days induced postprandial hyperglycemia in Wistar rats and induced pancreatic lesions in the pancreatic parenchyma of untreated rats. This study revealed that HAEGS had hypoglycemic activity after two weeks of treatment of DexIH rats. The OGTT also showed that HAEGS prevents the postprandial blood

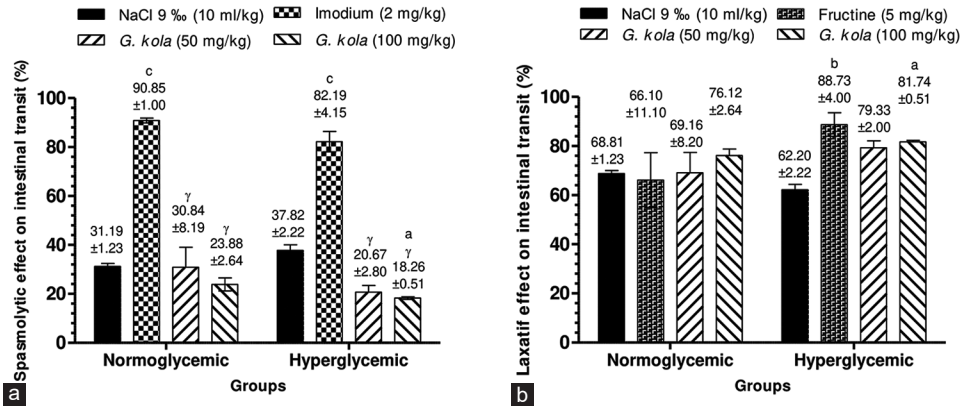

Figure 2: Effects of *Garcinia kola* seeds on intestinal transit. Values are means ± ESM, n = 3.(a): spasmolytic effect.(b): laxative effect. a: (*P < 0.05*), b: (*P < 0.01*), c: (*P < 0.001)* significant from NaCl group. ɣ: (*P < 0.001*) significant from positive control group Imodium. *P > 0.05*; no significant from positive control group Fructine

glucose level spike in both normoglycemic rats ($P > 0.05$) and DexIH rats ($P < 0.05$). It was observed in the DexIH rats after fourteen days of treatment with HAEGS at a dose of 100 mg/kg of body weight that the plant extract has significantly prevented the postprandial peak in blood glucose level 30 min after oral glucose overload. Intuitively, health-care providers believe that the best metabolic status corresponds to persons who concomitantly exhibit low mean glucose concentrations (HbA1c approximately at 7% or below) and small glucose variability, i.e., the least exposures to chronic hyperglycemia and acute glucose swings from peaks.[22] Experimental studies have shown

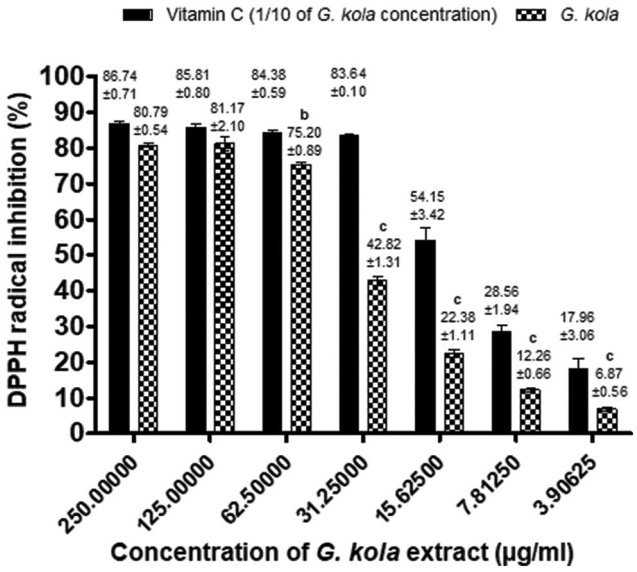

Figure 3: Antiradical activity of *Garcinia kola* seeds. Values are means ± ESM, *n* = 3. b: (*P < 0.01*), c: (*P < 0.001*) significant from Vitamin C

that dexamethasone produces fatty accumulation in the liver parenchyma, which results in fatty liver disease. Steatosis is the first stage of nonalcoholic fatty liver syndrome and is the hepatic manifestation of insulin resistance.[21,23,24]

A spasmolytic substance partially inhibits intestinal peristalsis, while a laxative substance accelerates intestinal transit. Administered to rats, the spasmolytic substance (Imodium® 2 mg/kg) opposes the progression of the food bolus, unlike the laxative substance (Fructine® 5 mg/kg), which acts in synergy with the sense of peristaltic movements of the small intestine. The extract at both doses significantly ($P < 0.001$) inhibited the spasmolytic activity in both normoglycemic and hyperglycemic rats compared to Imodium®. In rats made hyperglycemic, only dose 100 mg/kg significantly ($P < 0.05$) increased laxative effects when compared to the negative control. This suggests that the extract may contain compounds that exert the same effects as acetylcholine. Acetylcholine increases gastrointestinal contraction by stimulating muscarinic receptors in a process mediated by G-protein, phospholipase C, and inositol triphosphate.[25] The increase in small intestine contractility might be due to the stimulatory influence of some phytochemical components of GK on intestinal muscarinic receptor.[17] The quantitative phytochemical screening of GK seeds (g/100 g) was reported and revealed phenols 0.147 ± 0.00, saponins 2.471 ± 0.00, alkaloids 0.647 ± 0.20, flavonoids 2.041 ± 0.30, and glycosides 3.421 ± 0.00.[26]

In vitro antiradical activity of GK revealed vitamin C-like antiradical activity.

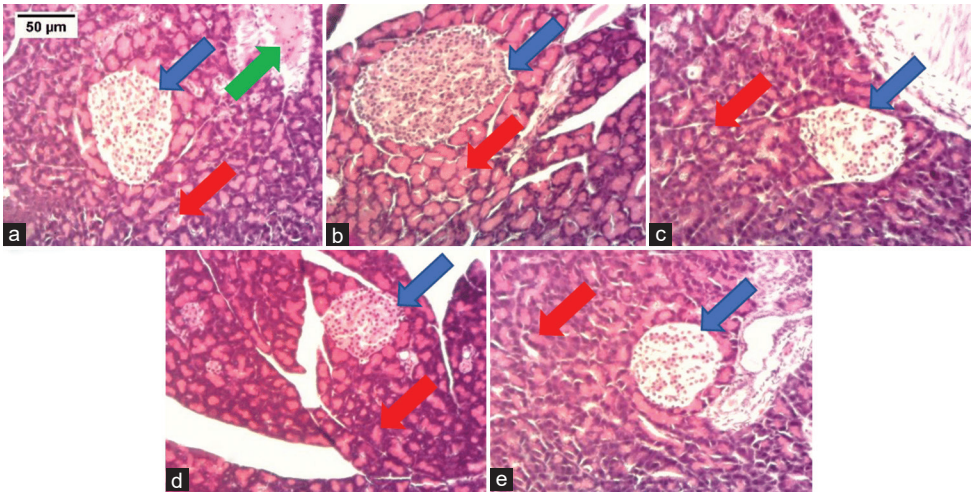

Figure 4: Microphotograph of pancreatic tissue (H and E, ×100) of dexamethasone-induced hyperglycemic rats treated with hydroalcoholic extract of *Garcinia kola* seeds (50 and 100 mg/kg b. w.) for 14 days. (a) NNC: Negative normal control (normoglycemic group): Histological analysis showed normal pancreatic structure in the normal control group with high number of ß-cells (the blue arrows show the endocrine pancreas which contains the pancreatic islets of Langerhans including the beta cells, the red arrows show the exocrine pancreas (acini), and the green arrow shows the pancreatic artery). (b) NDC: Negative dexamethasone control: Compared to normal control group, the animals in the negative control group showed hypertrophy of the islets of Langerhans. (c) Glib2.5: Positive dexamethasone control; (d and e) GK50 & GK100: Experimental groups which received the extract at the respective doses of 50 and 100 mg/kg. The groups treated with the reference substance, like those receiving the extract at different doses, presented a restructuring of these organs, close to that of the normal control

Free radical and reactive oxygen species (ROS) are basically the main cause of several disorders in humans that are generated as an imbalance between formation and neutralization of pro-oxidants, resulting in oxidative stress.[27] The results imply that the radical scavenging activity of the extracts may be attributed to their proton-donating abilities.[28] It has been established that the flavonoids of plant extracts are part of one of the most important hydrogen-donating groups.[29] The flavonoids present in HAEGS may be responsible for the antihyperglycemic, laxative, and the scavenging activity against DPPH effects observed *in vivo* and *in vitro,* respectively.[30-32] These pharmacological effects of GK seeds could be attributed to numerous flavonoids, as plant extract revealed a total flavonoid 2.94 ± 0.33 mgEq/100 mg extract. Several studies demonstrated the involvement of flavonoids in the regulation of blood sugar and diabetes complications.[6,33-36]

Histopathological examination of the pancreas of untreated DexIH rats revealed several histopathological alterations, in particular hypertrophy of the islets of Langerhans. This abnormality was less in the groups of animals treated with HAEGS at the two doses tested, which had a structuring of the pancreatic tissue close to that of the normal control. It has been reported that the treatment with higher doses of dexamethasone intraperitoneally produced fatty accumulation in liver parenchyma which resulted in hepatic steatosis.[21] The mechanism responsible for the development of steatosis might be due to break down of lipids into fatty acids (lipolysis), decreased sensitivity to insulin, and deposition of fat in liver tissue.[37,38] Recent studies have suggested that hyperinsulinemia, chronic inflammation, and hyperglycemia, all commonly seen in diabetics, can lead to increased tumor growth; the underlying molecular mechanisms of this association are not fully understood.[39]

Conclusions

From this study, we can conclude that these results suggest that the HAEGS regulates blood glucose levels, possesses laxative and anti-free radical properties, which justifies its traditional use in Sub-Saharan Africa. However, further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

Ethical clearance

All the animals experiments were carried out in accordance whith the guidelines of Cameroon National Ethics committe (Ref. FWIRB 00001954).

Financial support and sponsorship

Ministry of Higher Education, Cameroon and University Joseph Ki-Zerbo, Burkina Faso. The authors would like to thank the Ministry of Higher Education, which financed the *in vivo* experimentation of this study through the research modernization grant. The authors would also like to thank Laboratory of Biochemistry and Applied Chemistry, University Joseph Ki-Zerbo, Burkina Faso, for carrying out the *in vitro* experiments of this study.

Conflicts of interest

There are no conflicts of interest.

References

1. Abdel-Moneim LA, Fayez H. A review on medication of diabetes mellitus and antidiabetic medicinal plants. Int J Bioassays 2015;4:4002-13.
2. Pang GM, Li FX, Yan Y, Zhang Y, Kong LL, Zhu P, *et al.* Herbal medicine in the treatment of patients with type 2 diabetes mellitus. Chin Med J (Engl) 2019;132:78-85.
3. Obisike UA, Boisa N, Nwachuku EO. Pomegranate seed extract : A strong antioxidant against benign prostatic hyperplasia induced oxidative stress in albino Wistar rats. J Cancer Tumor Int 2021;11:50-60.
4. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. Heart 2007;93:903-7.
5. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. J Pharm Pharmacol 1990;42:290-2.
6. Adaramoye OA, Adeyemi EO. Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin-induced diabetes mellitus rats. J Pharm Pharmacol 2006;58:121-8.
7. Antia BS, Pansanit A, Ekpa OD, Ekpe UJ, Mahidol C, Kittakoop P. Alpha-glucosidase inhibitory, aromatase inhibitory, and antiplasmodial activities of a biflavonoid GB1 from *Garcinia kola* stem bark. Planta Med 2010;76:276-7.
8. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J Ethnopharmacol 2014;155:857-924.
9. Etukudoh NS, Uchejeso OM, Etim II. Bitter kola (*Garcinia kola*) as herbal remedy for diabetes. Curr Top Med Med Res 2021;12:61-8.
10. Adedara IA, Awogbindin IO, Anamelechi JP, Farombi EO. *Garcinia kola* seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. Pharm Biol 2015;53:695-704.
11. Langley-Evans SC. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. Int J Food Sci Nutr 2000;51:181-8.
12. Amarowicz R, Troszyńska A, Shahidi F. Antioxidant activity of almond seed extract and its fractions. J Food Lipids 2005;12:344-58.
13. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-20S.
14. Alasalvar C, Karamać M, Amarowicz R, Shahidi F. Antioxidant and antiradical activities in extracts of hazelnut kernel (Corylus avellana L.) and hazelnut green leafy cover. J Agric Food Chem 2006;54:4826-32.
15. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 2020;18:e3000410.

16. Nkono Ya Nkono BL, Sokeng Dongmo S, Dzeufiet Djomeni PD, Kamtchouing P. Antihyperglycemic and antioxydant properties of Alstonia boonei De Wild. (Apocynaceae) stem bark aqueous extract in dexamethasone-induced hyperglycemic rats. Int J Diabetes Res 2014;3:27-35.
17. Hamid I, Janbaz KH. Investigation of the laxative, spasmolytic and prokinetic properties of aqueous methanol extract of Buxus sempervirens Linn (Buxaceae). Trop J Pharm Res 2017;16:1865-72.
18. Rouamba A, Ouédraogo V, Compaoré E, Compaoré M, Kiendrebeogo M. Free radical scavenging capacity and anti-biofilm potentiality of six wild edible fruits from Burkina Faso. Int J Curr Microbiol Appl Sci 2018;7:2085-93.
19. Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, *et al.* Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evid Based Complement Alternat Med 2014;2014:253875.
20. Gounarides JS, Korach-André M, Killary K, Argentieri G, Turner O, Laurent D. Effect of dexamethasone on glucose tolerance and fat metabolism in a diet-induced obesity mouse model. Endocrinology 2008;149:758-66.
21. Kumar VH, Nayak IMN, Huilgol SV, Yendigeri SM, Narendar K, Rajasekhar CH. Dose dependent hepatic and endothelial changes in rats treated with dexamethasone. J Clin Diagn Res 2015;9:F08-10.
22. Monnier L, Colette C, Owens D. The glycemic triumvirate and diabetic complications: Is the whole greater than the sum of its component parts? Diabetes Res Clin Pract 2012;95:303-11.
23. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, *et al*. Nonalcoholic fatty liver disease. Metab Med Surg 2014;50:115-32.
24. Carbone DL, Zuloaga DG, Hiroi R, Foradori CD, Legare ME, Handa RJ. Prenatal dexamethasone exposure potentiates diet-induced hepatosteatosis and decreases plasma IGF-I in a sex-specific fashion. Endocrinology 2012;153:295-306.
25. Mandl P, Kiss JP. Role of presynaptic nicotinic acetylcholine receptors in the regulation of gastrointestinal motility. Brain Res Bull 2007;72:194-200.
26. Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AG. Nutritional and phytochemical screening of *Garcinia kola*. Adv J Food Sci Technol 2012;4:9-14.
27. Gangwar M, Gautam MK, Sharma AK, Tripathi YB, Goel RK, Nath G. Antioxidant capacity and radical scavenging effect of polyphenol rich Mallotus philippenensis fruit extract on human erythrocytes: An *in vitro* study. ScientificWorldJournal 2014;2014:279451.
28. De Almeida Melo E, Mancini Filho J, Barbosa Guerra N. Characterization of antioxidant compounds in aqueous coriander extract (Coriandrum sativum L.). LWT Food Sci Technol 2005;38:15-19.
29. Nickavar B, Kamalinejad M, Haj-Yahya M, Shafaghi B. Comparison of the free radical scavenging activity of six Iranian Achillea species. Pharm Biol 2006;44:208-12.
30. Deepak K, Nageswara RR, Surekha C. Role of antidiabetic compounds on glucose metabolism – A special focus on medicinal plant : Salacia sps. Med Chem (Los Angeles) 2014;4:373-81.
31. Ayepola OR, Chegou NN, Brooks NL, Oguntibeju OO. Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complement Altern Med 2013;13:363.
32. Wang KJ, Zhao JL. Corn silk (Zea mays L.), a source of natural antioxidants with α-amylase, α-glucosidase, advanced glycation and diabetic nephropathy inhibitory activities. Biomed Pharmacother 2019;110:510-7.
33. Okwuosa CN, Unekwe PC, Achukwu PU, Udeani TK, Ogidi UH. Glucose and triglyceride lowering activity of Pterocarpus santaniloides leaf extracts against dexamethasone induced hyperlipidemia and insulin resistance in rats. African J Biotechnol 2011;10:9415-20.
34. Patil R, Patil R, Ahirwar B, Ahirwar D. Current status of Indian medicinal plants with antidiabetic potential : A review. Asian Pacific J Trop Biomed 2011:S291-8.
35. Adaramoye OA. Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats. Afr Health Sci 2012;12:498-506.
36. Mahmoud AM, Ahmed OM, Ashour MB, Abdel-Moneim A. *In vivo* and *in vitro* antidiabetic effects of citrus flavonoids; a study on the mechanism of action. Int J Diabetes Dev Ctries 2015;35:250-63.
37. Safaei N, Shomali T, Taherianfard M. Niacin ameliorates lipid disturbances due to glucocorticoid administration in rats. Iran J Basic Med Sci 2012;15:997-1002.
38. Pezzilli R, Calculli L. Pancreatic steatosis: Is it related to either obesity or diabetes mellitus? World J Diabetes 2014;5:415-9.
39. Ryu TY, Park J, Scherer PE. Hyperglycemia as a risk factor for cancer progression. Diabetes Metab J 2014;38:330-6.