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Original article

Evaluation of metabolic, antioxidant and anti-inflammatory effects of *Garcinia kola* on diabetic rats

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ABSTRACT

Garcinia kola (*G. kola*), is a plant characterized by its hypoglycemic properties. We recently reported our findings on the extracts of *G. kola*, in which we found that it prevented the loss of inflammation-sensible neuronal populations in streptozotocin (STZ)-induced rat models of type 1 diabetes mellitus (T1DM).

In the present study we assessed the effect of *G. kola* bioactive compounds extracted successively with water, hexane, methylene chloride, ethyl acetate, and butanol. through analyzing biochemical markers of oxidative stress, inflammation, and metabolic function in STZ-induced diabetic animals.

Animals made diabetic by a single injection with STZ (60 mg/kg, i.p.), were treated daily with either vehicle solution, insulin, or *G. kola* extracts and its fractions from the first to the 6th-week post-injection. Biochemical markers; glucose, insulin, C-peptide, neuron-specific enolase (NSE), creatinine kinase, glutathione peroxidase, malondialdehyde (MDA), resistin, soluble E-selectin (SE-Selectin), and C-reactive proteins (CRP) levels in the sera were determined in the study groups. A marked increase in blood glucose (209.26% of baseline value), and a decrease in body weight (−12.37%) were observed in diabetic control animals but not in animals treated with either insulin or *G. kola* extracts and its fractions. The sub-fraction F5, *G. kola* ethyl acetate had the highest bioactive activities, with a maintenance of blood sugar, malondialdehyde, C-peptide, E-selectin, C-reactive protein (CRP) and neuron-specific enolase (NSE) to levels and responses comparable to healthy non-diabetic vehicle group and the positive control diabetic insulin-treated group.

Our findings suggest that *G. kola* may have a strong therapeutic potential against T1DM and its microvascular complications.

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1. Introduction

Garcinia kola (*G. kola*) is an edible seed, which belongs to the family Clusiaceae/ Guttiferae that stimulates organisms' adaptation to stress by influencing multiple regulatory systems such as the immune system and neuroprotective properties also act as a general anti-bacterial agent (Akinmoladun et al., 2015; Nagayach et al., 2014). The use of *G. kola seeds* extracts is common in traditional medicine in West Africa and by African immigrants in Saudi Arabia. Experimental models of the disease showed a positive response to its hypoglycemic and antioxidant effects (Adedara and Farombi, 2014; Farombi et al., 2013; Farahna et al., 2016). These findings suggest *G. kola* as a possible therapeutic choice for diabetes complications, including diabetic encephalopathy. Type 1 diabetes mellitus (T1DM) is considered a typical cause of metabolic deregulatory, which may be the primary cause of the complications development. The plant consists of a complex mixture that

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includes: bioflavonoids, phenylatedbenzophenones, xanthones and calanolide-type coumarines (Iwu, 1993; Akintowa and Essien, 1990). Recently, it was detected that β-lactam compounds N-ethyl-2-carbethoxyazetidine (17.8%), N, N-dimethylethanolamine (15%), and isoniacinamide (9%) were expressed in phytoconstituents, ethylacetate fraction showed improvement of motor and cognitive functions in streptozotocin-induced diabetic (Paul et al., 2018 unpublished).

This study was performed on animal models induced with T1DM developing diabetic encephalopathy, serum (and plasma) samples were obtained from the experimental animals to assess the changes in the severity of diabetes mellitus and the effect on the liver by analyzing the responsible biomarkers. Indicators of metabolic functions, inflammation, and oxidative stress were investigated as well.

2. Material and methods

2.1. Preparation and characterization of G. kola fractions

2.1.1. Plant materials

Fresh seeds of *G. kola* were purchased from local market in Riyadh, Kingdom of Saudi Arabia and identified by a plant taxonomist at Qassim University, College of Agriculture. Fresh seeds were peeled to remove the outer coat, sliced and air-dried. The dried seeds were grounded to a coarse powder using an electric blender and stored in polythene bags at −20 °C until used.

2.1.2. G. kola seeds' extraction and first fractionation

Powdered material (600g) was extracted with 80% methanol (2 L for three times) with occasional shaking for 72 h. The extract was filtered using Whatman No. 2 filter paper to collect the filtrate. Methanol was removed by a rotary evaporator at 40 °C. The crude extract obtained was suspended in distilled water (H_2O) and then successively partitioned by a series of solvents namely n-hexane (n-Hex), methylene chloride (CH_2C_{l2}), ethyl acetate (EtOAc), n-butanol (n-BuOH). We dried the extract by rotary evaporation at 40 °C, while the H_2O fraction was freeze-dried.

The yield of dry extracts obtained were 2.6% (hexane fraction), 14.5% (dichloromethane fraction), 4.1% (ethyl acetate fraction), and 6.5% (water fraction). The doses administered (2x the estimated quantity in dose 100 mg/kg of crude extract) were 4.5 mg/kg (hexane fraction), 28.7 mg/kg (dichloromethane fraction), 7.7 mg/kg (ethyl acetate fraction), and 12.9 mg/kg (water fraction). Ethyl acetate fraction was fractioned using column chromatography.

2.1.3. Ethyl acetate fraction separation

A solution made of 4.09 g of ethyl acetate fraction of *G. kola* dissolved in 5 ml of HPLC grade methanol was applied to a chromatographic column and purified through exclusion chromatography on Sephadex LH-20 as stationary phase using ÄKTA purifier chromatography systems equipped with a P-900 pump, a pH/C-900 detector, a UV-900 detector, a Frac-900 fraction collector, Unicorn 5.11 work station (GE Healthcare, Uppsala, Sweden). The column (60 mm × 16 mm) filled with Sephadex LH-20 from GE Healthcare.

The Sephadex LH-20 column was equilibrated with one column volume of the eluting agent before sample loading. EtOAc residue was dissolved with a small portion of methanol (3 ml) and injected into the column. The extract was eluted with MeOH with a flow rate of 1 ml/min.

Absorbance was monitored at 280 nm with a UV/VIS spectrophotometer (Shimadzu UV 1601, Kyoto, Japan) and methanol fractions (4 ml each) were collected in test tubes Then, elutes were pooled into fractions termed F1–F5 by the elution order and the UV absorbance.

Reduced pressure was used to evaporate the solvent and produce the extracts, ("sub-fractions") were transferred into a conical flask and stored in a refrigerator (−20 °C). The extraction yields of the sub-fractions were: 0.03% (F1), 0.13% (F2), 0.04% (F3), 0.42% (F4), and 0.69% (F5). The doses administered (10-fold the estimated amount in dose 100 mg/kg of crude extract.

2.1.4. Animals

Healthy young adult male rats (N = 86, weight 24 ± 3.2 g, six months old) were obtained from the animal facility of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The study started after an acclimatization period of one week. Twelve hour - fasted animals were injected once with streptozotocin (STZ, Sigma Aldrich, USA) in citrate buffer (pH = 4.5) (60 mg/kg body weight, i.p.). Insulin insufficiency induction was considered successful if a marked increase in fasting glycemia (>2-folds) was observed one week after STZ injection. The animals were randomly divided into diabetic experimental groups, namely: disease control group, insulin-treated group and *G. kola* treated test group. Animals were housed in groups of 3 or 4 and kept in a 12/12 h dark-light cycle at 23.4 °C room temperature. Rats of test groups were treated with *G. kola* fractions (per os, daily), while disease control and healthy control animals (age- and sex-matched) received the vehicle solution (DMSO, p.o., daily). Insulin group was treated daily with insulin (0.4 UI/kg, s.c., suspension, Novo Nordisk A/S, Zürich, Switzerland). Based on our previous observation that signs of nervous system involvement are already present one month after STZ injection in laboratory rodents (Seke Etet et al., 2017), the treatment started two weeks after STZ injection and lasted two weeks for each of the three phases of the present study.

2.1.5. Collection of blood samples

All rats were 12 h fasted before analyzing the blood glucose level on day three, 15 and 30 of the experiment. During these days, 1.0 ml of blood was extracted from the *retro*-orbital plexus under light ether anesthesia. The specimens were centrifuged at 3000 rpm to separate plasma and cells. The plasma was used to estimate glucose levels.

2.1.6. Biochemical markers

Levels of biochemical markers of diabetes severity, oxidative stress and inflammation were measured in the sera and liver homogenates.

2.1.7. Markers of metabolic functions

All kits used below were provided by Cusabio Biotech Co., Ltd. www.cusabio.com or otherwise mentioned. Serum and liver homogenates were employed in most of the assays as indicated. All methods used and specifications are in the kits manuals.

2.1.8. Biochemical markers of diabetes mellitus severity

The following parameters levels were assayed for diabetes mellitus severity. The plasma glucose levels were estimated using the glucose-oxidase peroxidase (GOD-POD) method with the glucose GOD-POD kit, insulin ELISA kit, Rat Creatine kinase activity M-type were measured using ELISA kit Catalog No: CSB-EL005459RA).

2.1.9. Markers of oxidative stress

In order to assess oxidative stress related to cognitive function and investigate possible oxidative stress-induced liver-brain axis neurodegenerative, biomarkers of oxidative stress were measured in the serum as follows: Glutathione peroxidase (GPx) activity: ELISA kit (Catalog No: (CSB-E12146r) as described in (Teitze, 1969). Lipid peroxidation as revealed by malondialdehyde (MDA)

level, was determined in using thiobarbituric acid reactive substances (TBARS) method (Janero, 1990).

2.1.10. Markers of inflammation

The level of the pro-inflammatory cytokine resistin was determined using ELISA kit: Catalog No: (CSB-E06885r following the manufacturer's protocol. Human recombinant soluble E-selectin induced chemotaxis of human endothelial cells *in vitro* and was angiogenic in rat cornea was measured using Soluble E- selectin SE-selectin (Cat. No. CSB E07996r), neuron-specific enolase (NSE), ELISA kit (Catalog No: (CSB-E07963r), Rat C- reactive protein (CRP) catalog No. CSB E07922r were measured in the sera of animal groups.

3. Experimental procedures

3.1. Phase 1 of bioactivity-directed fractionation

Study of the neuroprotective properties of four sequential fractions of *G. kola* methanol extract was assessed: hexane fraction, dichloromethane fraction, ethyl acetate fraction, and the water fraction. Animal received doses of fractions equivalent to twice the content in 100 mg/kg of *G. kola* crude extract, as shown in our previous studies (Farahna et al., 2016). *G. kola* -treated diabetic animals (N = 5 per group) were compared with disease control, insulin, and vehicle groups (N = 4 per group, with two more animals added in each group in the 2 following phases of the study).

3.2. Phase 2 of bioactivity-directed fractionation

In this phase, the most active fraction was further separated in five chromatographic sub-fractions termed as F1 to F5 based on elution order. Then, the neuroprotective properties of these sub-fractions were assessed in diabetic animals (N = 5 per group) at doses equivalent to 10 times their content in 100 mg/kg of plant crude extract. *G. kola* -treated diabetic animals were also compared with disease control, insulin, and vehicle groups. The major phytocomponents of the most active sub-fraction were characterized by determining the GC–MS chromatogram of this sub-fraction and by calculating the proportions of the phytocomponents detected.

3.3. Data analysis

Comparisons were made using two-way ANOVA and LSD post-hoc test (for inter-couple difference assessment). Differences with $P < 0.05$ were considered significant. Data were presented as mean ± SD.

3.4. Eth ical approval

All procedures were approved by King Fahad Medical City (KFMC, Riyadh, Saudi Arabia) institutional review board (log 16–343, IRB registration number with OHRP/NIH IRB00010471). Ethical rules prescribed in European Union Directive 2010/63/EU for animal use in scientific research were used.

4. Results

4.1. Effect of G. kola different extracts on glucose and body weight

G. kola different extracts had an effect on Glucose and body weight, as it showed a significant increase in glycemia and a decrease in body weight of the diabetic control group compared to the vehicle non-diabetic healthy group ($P = 0.0019$ and $P < 0.0001$, respectively). Glycemia was decreased compared to diabetic control group in animals treated with *G. kola* methanol extracts and separated by liquid-liquid separation techniques as water extract ($P = 0.004$), hexane ($P = 0.0017$), dichloromethane extract ($P = 0.0018$), and ethyl acetate extract ($P = 0.0015$), or with insulin ($P = 0.0014$) (Fig. 1). The body weight increased (improved) in diabetic animals treated with all *G. kola* fractions of the methanol extract ($P < 0.001$), except water fraction ($P = 0.746$) (Fig. 2). The body weight improvement mediated by *G. kola* methanol extract was more marked in the second week of treatment. Insulin treatment decreased the glycemia and improved the body weight of diabetic animals ($P = 0.0014$ and $P = 0.0002$ vs. diabetic control group, respectively). However, treatment with insulin and *G. kola* extracts' fractions improved glycemia to values close to normal (with no significant difference compared to vehicle group), none of these treatments restored the body weight to vehicle group values ($P < 0.01$). Animals treated with ethyl acetate fraction gained weight faster than those treated with other fractions. As in the case of insulin treatment, treatment with ethyl acetate fraction raised the body weight beyond baseline value (body weight at the beginning of the experiment). Considering the observation that the treatment with the ethyl acetate fraction raised the body weight of rats and decreased the blood glucose level, the ethyl acetate was further separated and its sub-fractions were tested on rats.

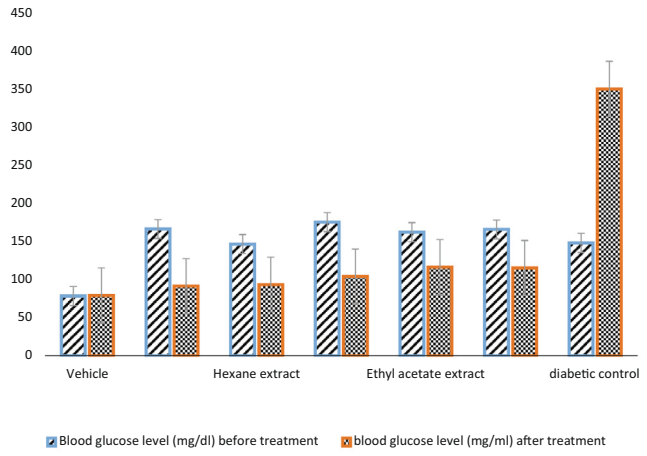

Fig. 1. Blood glucose level (mg /dL) in the study group treated with Insulin and *G. kola* different extracts compared to healthy nondiabetic control group (vehicle group).

4.2. Effect of G. kola extracts and ethyl acetate fractions on the biochemical parameters

4.2.1. Metabolic markers

Glucose level increased from the normal level to the streptozotocin (STZ) treated animals, and an inverted picture occurred with the level of the C-peptide which is usually affected by the glucose level. A drop occurred in C-peptide level with STZ-treatment while a higher level was regained on treatment with *G. kola,* while the level with insulin treatment was even higher (Figs. 2, 3 & Table 4). Creatine kinase activity M-type (CK) was used as an indicator of tissue damage in rats, the serum level of creatine kinase slightly increased in animal treated groups compared to the healthy nondiabetic group (Table 1 & Fig. 4).

4.2.2. Antioxidant markers

Glutathione peroxidase (GPX) was used to measure the antioxidant level of the blood cell and its protection against oxidant agents such as hydrogen peroxide. That is important as hyper-

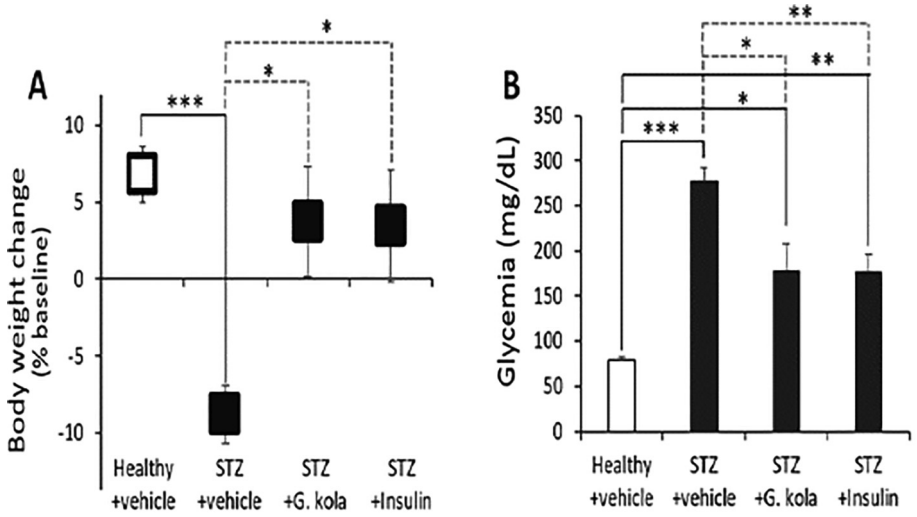

Fig. 2. (A) Changes in the body weight gain in the treated and untreated control animals. (B) Changes in the glycemia after methanol extract treatment. *P < 0.05; **P < 0.01; ***P < 0.001.

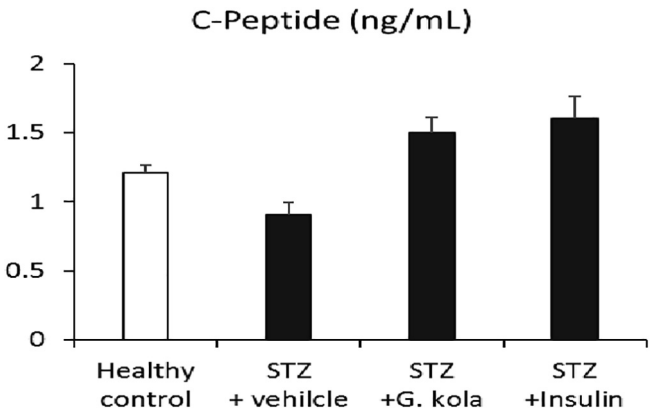

Fig. 3. Serum C- peptide level in the study groups.

glycemia induces overproduction of oxygen free radicals and hence increases protein and lipid oxidation (Teitze, 1969). The serum antioxidant level of GPX in the *G. kola* group is comparable to the normal level, yet the group treated with insulin showed a higher antioxidant level. On the other hand, *G. kola* protects cell membranes against peroxide damage more than the insulin does, the level of GPX significantly decreased in the untreated diabetic animals compared to the treated groups (Table 1).

Malondialdehyde that results from lipid peroxidation of polyunsaturated fatty acids and measured by TBARS assay in the liver indicated an increased in the level of insulin, and *G. kola* extract treated groups than the healthy and diabetic control groups (Table 2).

4.2.3. Anti-inflammatory and cytokines markers

The level of Soluble E- selectin that might be crucial to control leukocyte accumulation in inflammatory response showed significant differences between the healthy and diabetic animals P < 0.001. Where it showed a decrease in the diabetic group treated with *G. kola* extract compared to the healthy control group P < 0.05, the insulin-treated animals showed an increase compared to the healthy control animals P < 0.019 (Table 3).

Neuron-specific enolase (NSE) is a potential biomarker of diabetic retinopathy and neurovascular diseases. The protection done by insulin against the tissue damage of diabetes is a little higher than that done by *G. kola* as the level of NSE increase in the serum of animals treated with *G. kola* compared to diabetic control group (**Tables 1 & 2**). The adipocyte-hormone, resistin, could contribute to retinopathy, nephropathy, neuropathy, and cardiopathy of diabetic complications. The level of resistin in the serum of study groups is shown in (Table 1). It indicates that *G. kola* is more effective in combating the effect of resistin than insulin.

C-reactive protein (CRP) is an inflammation indicator substance produced by the liver. The level of CRP decreased significantly in the diabetic control group while increased in the insulin-treated group where there was no significant difference in *G. kola* treated group compared to the healthy control group (Table 4).

5. Discussion

Garcinia kola is a traditional food and medicinal plant with centuries of human use. Empirical evidence and emerging research suggest that it is safe and beneficial, especially when used in traditional dosages for traditional indications in traditional populations. It displays a wide range of traditional uses and promises a similar

Table 1
Serum Levels of some Biochemical markers in Healthy Control Rats, Diabetic Rats and those treated with Insulin and *G. kola.*

Biochemical Marker	Animal Group			
	Control (n = 4)	Diabetic (n = 4)	Insulin (n = 4)	*G. kola* (n = 5)
Mean ± (SD)				
Resistin (pg/ml)	9.80 ± 4.55	9.88 ± 7.50	6.78 ± 3.30	8.19 ± 9.97
NSE (ng/ml)	4.36 ± 3.21	6.32 ± 5.74	3.08 ± 2.35	7.30 ± 3.97
GPX (U/ml)	1.05 ± 0.42	1.00 ± 0.55	1.75 ± 0.81	1.15 ± 0.71
CK-M (mU/ml)	55.80 ± 37.35	83.70 ± 55.43	87.81 ± 40.50	92.13 ± 80.88
C- peptide (ng/ml)	1.23 ± 0.25	1.10 ± 0.27	1.26 ± 0.53	1.44 ± 0.18

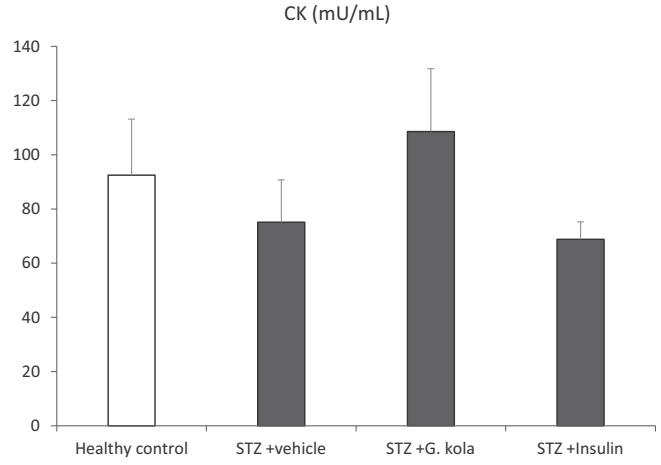

Fig. 4. Serum creatinine Kinase (M-type CK) level (mU/ml) in the study groups.

scope for modern and clinical use as new research progresses (Iwu, 1993; Plowden, 1972; Adesuyi et al., 2012; Esomonu et al., 2005; Braide and Vitrotio, 1989). The need for further research in chemical, pharmacological, *in-vitro*, animal and human research is clear, as is the need to capture apparently rare toxicity data. If developed as a concentrated or extracted product and used for needed but non-traditional indications, more meticulous study is needed; this unique 'bittercola' promises to deliver significant benefits to some common human ailments, and merits worldwide attention and information dissemination.

Hyperglycemia and a marked drop in body weight were observed in diabetic control animals during the study following injection with STZ. As with insulin, daily treatment with *G. kola* extract and its fractions allowed the diabetic rats to keep their body weight increase and glycemia decreased comparable to non-diabetic animals. Animals treated with ethyl acetate fraction gained weight faster than those treated with other fractions, as insulin treatment, treatment with ethyl acetate fraction raised the body weight beyond baseline value (body weight at the beginning of the experiment).

The serum antioxidant level of Glutathione oxidase (GPX) in the *G. kola* group is comparable to the normal level, yet the group treated with insulin showed a higher antioxidant level. In the study, we observed a significant increase in GPX and malondialdehyde (MDA) levels in insulin, and *G. kola* treated extracts compared to the diabetic control. Oxidative stress which usually results from excessive production of reactive oxygen species (ROS) or diminished activity of antioxidants have been implicated as a major contributor to the etiology of severe pathologies, including diabetes.

Table 2
Liver levels of some biochemical parameter in healthy control rats, diabetic rats and those treated with insulin, *G. kola*.

Groups	Control	Diabetic	Insulin	*G. kola*
Mean ± (SD)				
Resistin (pg/ml)	2.38 ± 7.12	9.03 ± 2.27	6.68 ± 3.69	6.55 ± 5.93
NSE (ng/mg)	2.99 ± 2.33	4.34 ± 1.96	2.63 ± 1.31	4.94 ± 2.69
GPX (U/ml)	1.19 ± 0.48	0.84 ± 0.37	1.57 ± 0.47	1.00 ± 0.3
CK-M (mU/ml)	67.38 ± 50.71	80.77 ± 32.03	72.55 ± 18.71	121.32 ± 61.58
C-Peptide (ng/ml)	1.39 ± 0.36	1.11 ± 0.26	1.41 ± 0.49	1.52 ± 0.39
MDA (ng/ml)	5.50 ± 10.29	8.15 ± 1.75	30.84 ± 5.19	23.24 ± 12.44

Table 3
Biochemical markers level in treated groups compared to the normal subjects (Normal + vehicle) (mean + SD) P value ≤ 0.05 consider significant. F. = Fraction.

Biochemical markers	AnimalsGroup							
	Normal + vehicle	Diabetic + vehicle	Diabetic + Insulin	Diabetic + *G. kola* F.4	Diabetic + *G. kola* F.5	Diabetic + *G. kola* F.4 dil. 1/20	Diabetic + *G. kola* F.4 dil. 1/10	Diabetic + *G. kola* F.4 dil. 1/4	Diabetic + *G. kola* F.4 dil. 1/2
E- selectin (ng/ml)	1442.0 ± 43.9	1156.8 ± 40.9	1653.0 ± 58.4	1271.0 ± 72.3	1274.25 ± 60.0	1383.33 ± 62.28	1239.0 ± 140.3	1379.5 ± 125.9	1195.6 ± 50.7
P- value		0.001	0.019	0.070	0.050	0.467	0.113	0.597	0.011
CRP (ng/ml)	133.03 ± 7.9	95.04 ± 4.50	167.4 ± 8.27	152.66 ± 16.18	117.55 ± 5.62	158.06 ± 7.1	332.06 ± 118.9	185.5 ± 43.25	112.7 ± 9.4
P- value		0.004	0.021	0.253	0.194	0.087	0.039	0.179	0.168
C- peptide (ng/ml)	16.53 ± 1.65	14.920 + 0.66	13.375 ± 1.75	11.36 ± 2.7	8.025 ± 0.79	16.57 ± 0.3	12.6 ± 2.16	12.9 ± 0.98	15.5 ± 0.3
P- value		0.424	0.240	0.129	0.004	0.985	0.211	0.139	0.703

Table 4
Biochemical markers level in the treated group compared to the untreated diabetic group (Diabetic + vehicle) (mean + SD) P value ≤ 0.05 consider significant. F. = Fraction.

Biochemical markers	Animal Group							
	Diabetic + vehicle	Diabetic + Insulin	Diabetic + *G. kola* F. 4	Diabetic + *G. kola* F.-5	Diabetic + *G. kola* F.4 dil. 1/20	Diabetic + *G. kola* F.4 dil. 1/10	Diabetic + *G. kola* F.4 dil. 1/4	Diabetic + *G. kola* F. 4 dil. 1/2
E- selectin (ng/ml)	1156.8 ± 40.9	1653.0 ± 8.4	1271.0 ± 72.3	1274.25 ± 60.0	1383.33 ± 62.28	1239.0 ± 140.3	1379.5 ± 125.9	1195.6 ± 50.7
P- value		0.001	0.184	0.139	0.019	0.506	0.105	0.578
CRP (ng/ml)	95.04 ± 4.50	167.4 ± 8.2	152.66 ± 16.18	117.55 ± 5.62	158.06 ± 7.1	332.06 ± 118.9	185.5 ± 43.25	112.7 ± 9.4
P- value		0.001	0.005	0.016	0.001	0.035	0.50	0.102
C- peptide (ng/ml)	14.9 ± 0.66	13.3 ± 1.7	11.36 ± 2.7	8.025 ± 0.79	16.57 ± 0.3	12.6 ± 2.16	12.9 ± 0.98	15.5 ± 0.3
P- value		0.399	0.154	0.001	0.079	0.261	0.124	0.511

Moreover, increasing evidence shows that excess ROS acts as negative regulators of insulin signaling leading to insulin resistance, a known metabolic abnormality associated with diabetes (Hoffman, 2009). Oxidative stress has been reported as a key role and common event in the pathogenesis of secondary diabetic complications (Rains and Jain, 2011). ROS may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehydes that will damage cell membranes.

The level of Soluble E- selectin that might be crucial to control leukocyte accumulation in inflammatory responses (Ley, 2003). Significant difference was found between the healthy and diabetic animal $P < 0.001$. Also, a significant decrease in diabetic treated with *G. kola* extract compared to the healthy control group $P < 0.05$. Neuron-specific enolase (NSE) is a potential biomarker of diabetic retinopathy and neuro-vascular disease (Kaiser et al., 1989; Li et al., 2013). The insulin protection against the tissue damage of diabetes is a little higher than the *G. kola* as the level of NSE increased in the serum and liver of animals treated with *G. kola* compared to diabetic control group. The adipocyte-hormone, resistin, could contribute to retinopathy, nephropathy, neuropathy, and cardiopathy of diabetic complications. The level of resistin in the serum of untreated diabetic group was higher than those treated with insulin and *G. kola*. It seems that G. kola is as effective as insulin in combating the effect of resistin on diabetes mellitus.

The level of C-reactive protein that produced by the liver in response to inflammation (Black et al., 2004) was decreased significantly in the diabetic control group while it was increased in the insulin-treated group while there was no significant difference in *G. kola* treated group compared to the healthy group. The biochemical markers for metabolic, anti-inflammatory and antioxidant for animal treated by *G. kola* showed an improvement for many of these markers indicating that the *G. kola* is a potential candidate for therapeutic uses. Purification of the active ingredient can increase the hypoglycemic response and combat the complications of diabetes effectively. The results we observed on our experimental models indicate that T1DM-like functional alterations are affected by neuroinflammation and neuronal loss. Early treatment with *G. kola* proved to prevent such alterations which confirms the neuroprotective properties of the plant and warrant further mechanistic studies.

Given the extent of the wide use of *G. kola* as herbal remedies and food supplements worldwide, comprehensive quantitative analysis for monitoring the quality of these products is essential. GC–MS chromatogram of ethyl acetate sub-fraction F5 of *G. kola* revealed that the most phytoconstituents were the molecules of β-lactam family N-ethyl-2-carbethoxyazetidine, N, N-dimethylethanolamine (DMEA), and isoniacinamide.

Further research in chemical, pharmacological, in-vitro, animal and human research is needed to clarify if any rare toxicity data. Conducting more studies on this topic is necessary especially if the *G. kola* is going to be used in concentrated extracts. *G. kola* represents an excellent opportunity for retailers and manufacturers, looking for something new to stimulate a sluggish dietary-supplements market.

Declaration of Competing Interest

Authors declare no competing financial interest.

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