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Full Length Research Paper

Effects of an ethanolic extract of *Garcinia kola* on glucose and lipid levels in streptozotocin induced diabetic rats

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The effect of *Garcinia kola* on glucose and lipid levels in streptozotocin-induced diabetic rats was investigated. Ethanolic extract of *G. kola* was prepared and used for animal treatments. Diabetes was induced by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight). Acute effects of *G. kola* on glucose were investigated by giving a single dose of distilled water or 300 mg/kg *G. kola* extract or metformin 300 mg/kg. Glucose levels were measured 2, 4 and 6 h after treatment. To investigate the long term effects, animals were treated daily for four weeks with either distilled water (controls) or 300 mg/kg *G. kola* extract. At termination, serum glucose, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels were measured. There was no significant difference (P>0.05) in single dose glucose levels, long term HDL levels and body weights compared to the controls. However, in the four week treated rats, glucose (mmol/L) was significantly lower (16.2±2.9; P<0.05) than in the controls (21.6±3.6) and the LDL levels were significantly decreased by 66% in the treated group compared to controls (P<0.01; 86.8±18.2 versus 29.8±10.9). This confirms the hypoglycaemic and especially the hypolipidemic effects of *G. kola* in a diabetic rat model.

Key words: *G. kola*, ethanolic extract, hyperglycemia, low density lipoprotein (LDL), high density lipoprotein (HDL), streptozotocin,

INTRODUCTION

There is growing concern that diabetes and obesity (diabesity) will reach epidemic proportions, affecting the developing world in Asia and Africa more than the developed world (Amos et al., 1997; Rheeder, 2006). Type 2 diabetes mellitus is the more common form of diabetes affecting over 200 million people worldwide (Emerson et al., 2009). Type 2 diabetes is characterized by a dual pathogenesis of insulin resistance and impaired insulin secretion leading to hyperglycaemia usually associated with dyslipidemia, a risk factor for cardiovascular disease and stroke (Gong et al., 2009; Carmena, 2005). As such, in current management of diabetes, statins are included to control the dyslipidemia. Before the advent of current treatment methods, plants were used in the treatment of diabetes mellitus. Some of these plants are continually used for the management of diabetes today. Several plants have been shown to have glucose lowering (Amrani et al., 2009; Aguilar-Santamaria et al., 2009), lipid lowering (Goyal and Grewal, 2003; Hilaly et al., 2006; Chahlia, 2009) effects or both. A plant recommended traditionally for treatment of diabetes is the seed of *Garcinia kola* (Family: Guttiferae, sub-family: Clusoideae), a plant cultivated in West and Central Africa. Commonly known as bitter kola, this nut is served as refreshment, medicinally used for the

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treatment of abdominal pain, cough, laryngitis, liver disease infections and erectile problems (Irvine, 1961; Odebunmi et al., 2009; Njume et al., 2011). Traditionally, it is said that regular consumption of this nut lowers blood glucose levels and improves the complications of diabetes mellitus (Esomonu et al., 2005). The seed is chewed whole and one to three whole seeds may be taken a day. Phytochemical studies of the seeds revealed the presence of biflavonoids, xanthones, triterpenes, cycoartenols and benzophenones (Adaramoye et al., 2005). Kolaviron, consisting of the main bioactive components: biflavanones and kolaflavanones is the predominant constituent of *G. kola*. Kolaviron components account for most of the seed's biological activities (Adaramoye and Adeyemi, 2006; Terashima et al., 2002). Although, kolaviron has been shown to have glucose and lipid lowering effects (Adaramoye et al., 2005; Adaramoye and Adeyemi, 2006), there has been no experimental work demonstrating effects of the crude extract of *G. kola* on glucose and lipids in a streptozotocin-induced diabetic model. Streptozotocin destroys pancreatic beta cells via oxidative stress thus inducing experimental diabetes. Metformin is a hypoglycemic biguanide diabetic medication which acts via increased peripheral tissue glucose uptake (Klip and Leiter, 1990). This forms a good positive control in hypoglycaemic studies. Therefore, the aim of this research work was to investigate the effects of *G. kola* on glucose levels and lipid profiles in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Preparation of extract of *G. kola*

The *G. kola* seeds were bought at a local market in Cameroon and were authenticated at the herbarium of the Department of Botany, University of Cameroon where a voucher specimen already exists. Extract was prepared using the method of Adaramoye (2010) with modifications. The seeds were peeled, cut into small pieces and then crushed using an electric household blender. The paste was dried in a fan oven at 35°C and then extracted twice with 70% ethanol at room temperature with continuous agitation. Ethanol was removed using rotary evaporator (Buchi RE111) under reduced pressure at 60°C. Water was removed by freeze drier (Modulyo Edwards) for 12 h. From approximately 1200 g of *G. kola* seeds, 564 g of solid matter was obtained giving a yield of 47%. The extract solid was stored at -70°C until used for bioassays. The dried extract was reconstituted in distilled water for animal oral treatments.

Animals

Wistar strain albino rats (120 to 160 g) and mice for toxicity studies were supplied by Shalom Labs (Durban). Animals were housed in the Department of Physiology animal holding facility and maintained at a temperature of 24 to 28°C, in a 12:12 h light:dark cycle, with water and pellet food (Epol-SA) *ad libitum*. The study was approved by the ethical committee of the Faculty of Health Sciences and Walter Sisulu University; clearance number 0023/009.

Induction of diabetes

After two weeks of acclimatization in the holding facility, diabetes was induced in rats (n=16) by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (40 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) into overnight-fasted rats. Rats were given 5% glucose solution in place of water on the first night to counter STZ induced hypoglycaemia due to insulin leakage from damaged β cells. After one week, fasting glucose levels were determined in blood from the tail by the glucose oxidase method using a portable glucometer (Accutrend Plus®). All animals developed diabetes. A blood glucose level ≥ 20 mmol/L was considered diabetic and used for *G. kola* treatments. All treatments were given orally by gavage using a 16 G feeding needle.

Animal treatments

Single dose effects on blood glucose levels

Single dose effects were determined in animals fasted overnight. This was done by determining glucose levels via tail vein (time 0) and giving a single oral dose of water (controls n=8), 300 mg/kg metformin (positive controls n=7) or 300 mg/kg *G. kola* (treated n=8). Glucose levels were then determined at 2, 4 and 6 h post-treatment by the glucose oxidase method using a portable glucometer (Accutrend Plus®). Metformin treatment (300 mg/kg) was used as positive hypoglycaemic control.

Long term treatment

For long term effects of *G. kola* treatment, diabetic rats were orally treated daily with either distilled water (controls n=8) or 300 mg/kg *G. kola* extract (n=8) for a total of four weeks. Comparison was made between the *G. kola* treated and untreated group. Blood glucose levels were measured weekly from the tail vein by glucometer. On the final day, animals were weighed and glucose levels were determined by glucometer. Animals were deeply anaesthetized with sodium pentobarbital (65 mg/kg IP) and blood was collected by cardiac puncture into plain dry tubes. After clotting, the blood was centrifuged and serum collected and stored at -70°C until biochemical tests.

Lipid profiles

HDL and LDL concentrations were determined using a commercial direct colorimetric ELISA method as per manufacturer's instructions (Latest Diagnostics, Brazil). Values were reported in mg/dL.

Acute toxicity studies

Acute toxicity was determined in healthy adult albino mice of either sex as previously described by Asare et al. (2011). Two groups of five mice/group received single oral dose of *G. kola* extract at 1200 and 2400 mg/kg body weight. The animals were observed continuously for 1 h, then hourly for the next 4 h, intermittently over the next 48 h and at least once a day for two weeks. Physical manifestations of toxicity such as writhing, gasping, salivation, hyperactivity, drowsiness and mortality were recorded during the experimental period.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM).

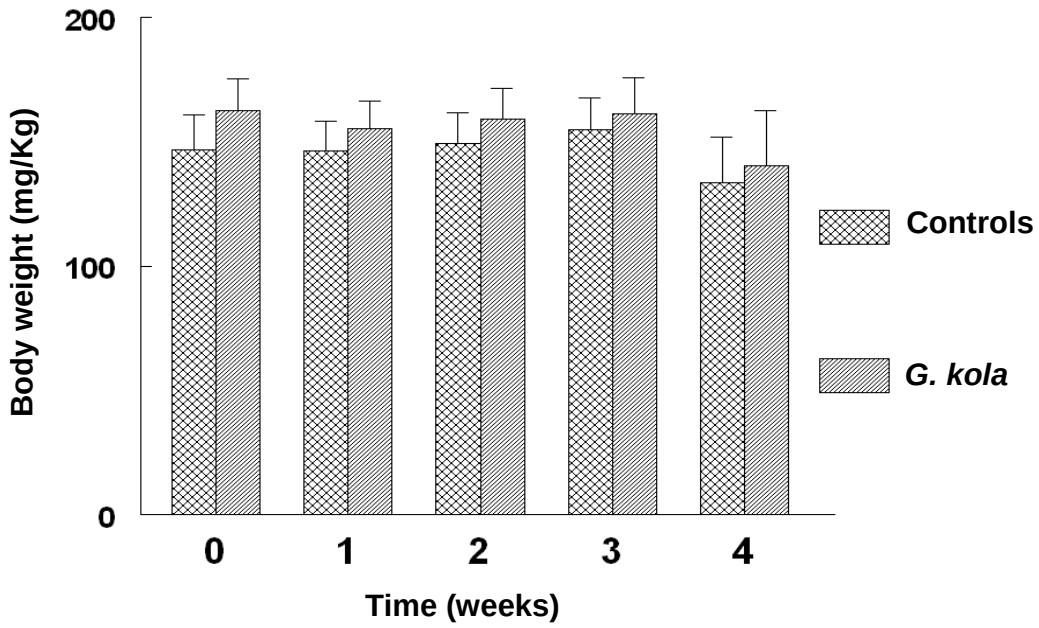

Figure 1. Effect of four week daily oral treatment with distilled water (controls) or *G. kola* (300 mg/kg) on body weights in diabetic rats. Body weights were similar between groups.

Statistical analysis was done using GraphPad InStat Version 3 and data was compared using the student t-test or ANOVA. Values were considered significantly different when $P<0.05$.

RESULTS

Induction of diabetes mellitus

After intraperitoneal administration of streptozotocin, all animals became diabetic with fasting glucose levels' ranging between 23 to > 33 mmol/L. Diabetes was also confirmed by glycosuria, polyuria and polydipsia in the rats.

Body weights

The mean body weights of both *G. kola* treated and control groups during the four weeks duration are shown in Figure 1. There was no significant difference ($P>0.05$) in the body weights of the treated group compared to the control group throughout the four week treatment period.

Single dose treatment: Effect on glucose levels

G. kola treatment had no significant effect ($P>0.05$) on blood glucose levels over the 6 h post treatment. Blood glucose levels after a single oral dose of *G. kola* extract at a dose of 300 mg/kg are shown in Figure 2. Metformin showed a significant decrease in glucose levels over time compared to the controls and *G. kola* treated animals.

Long term treatment: Effect on blood glucose levels

Treatment with *G. kola* for four weeks with weekly blood glucose level measurements showed no significant difference in glucose levels up to the end of the third week. However, there was a significant decrease ($P<0.05$) in glucose levels after four weeks of treatment compared to the controls (Figure 3).

Long term treatment: Effect on serum lipid levels

G. kola treatment resulted in a highly significant ($P<0.01$) decrease in LDL levels, a 66% decrease compared to the untreated controls (Figure 4). However, there was no difference ($P>0.05$) in HDL levels after a 4 week treatment period (Figure 5).

Acute toxicity studies

Toxicity studies carried out using oral doses of 1200 and 2400 mg/kg *G. kola* extract did not cause any obvious signs of toxicity as assessed by behavioral changes by the experimental mice. Mice looked active and healthy after two weeks of observation.

DISCUSSION

The streptozotocin induced diabetes rat model is valuable for the study of both type I and II diabetes (Szkudelsk, 2001). In addition to hyperglycaemia, this model also

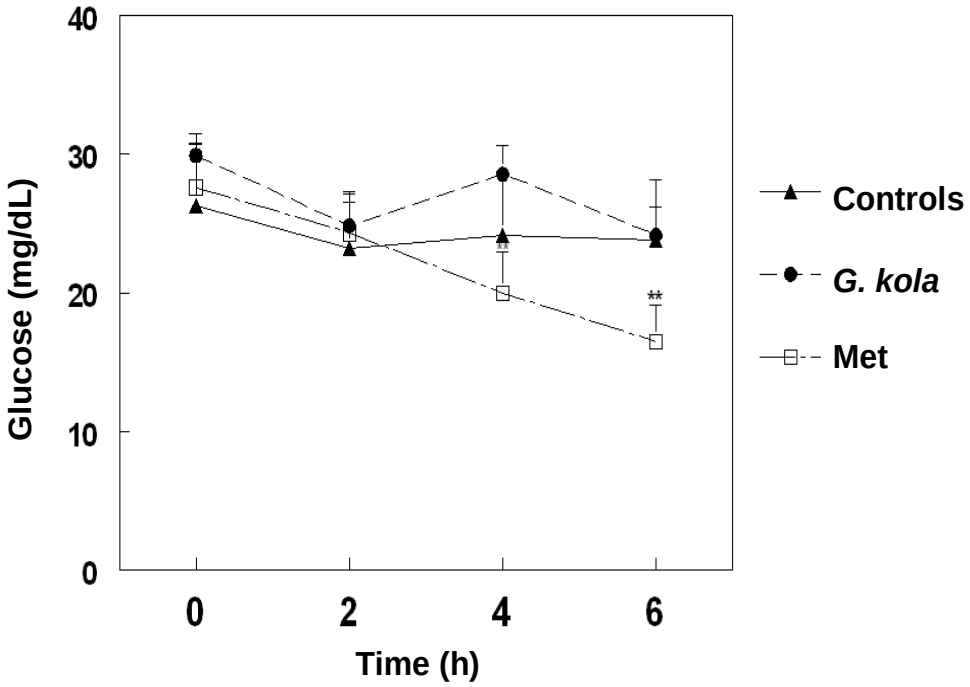

Figure 2. Single dose effect of 300 mg/kg *G. kola* on blood glucose levels in diabetic rats. After giving a single dose of *G. kola* (n=8), distilled water (controls; n=8) or metformin (Met; 300 mg/kg; n=7), blood glucose levels were measured at 2, 4 and 6 h post treatment. Values are mean ± SEM. ** P<0.01 versus controls.

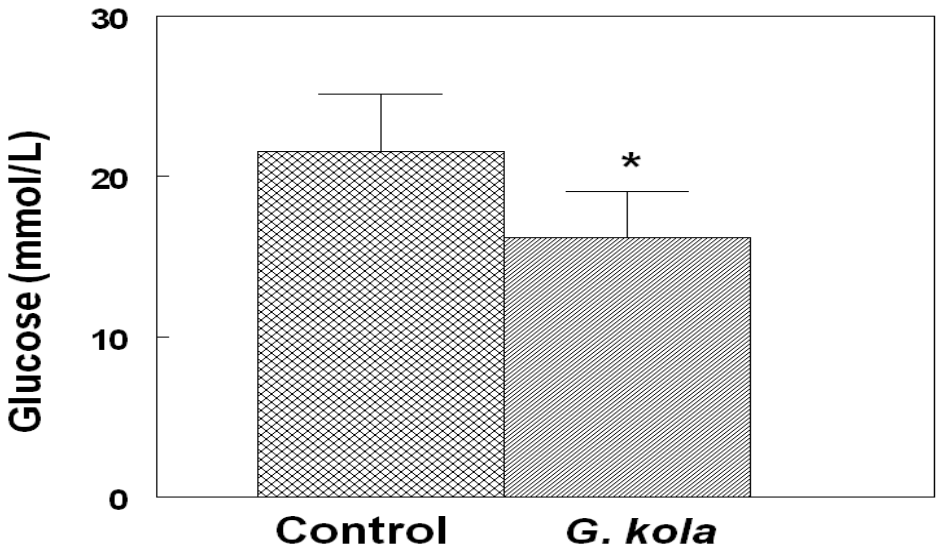

Figure 3. Effect of four weeks treatment with *G. kola* on blood glucose levels of diabetic rats. Values are mean ± SEM; * P<0.05 versus controls. *G. kola* treated group n=8; controls n=8.

exhibits diabetic complications including dyslipidaemia (Sharma et al., 1997). We used this rat model to investigate acute and subacute (four weeks) effects of a crude ethanolic extract of *G. kola* on glucose, LDL and HDL levels in streptozotocin induced diabetic rats. The results show that, at a dose of 300 mg/kg, *G. kola* had no significant acute effects on glucose levels. However, after four weeks of treatment, *G. kola* had effective hypoglycaemic and LDL lowering effects with no effect on HDL levels.

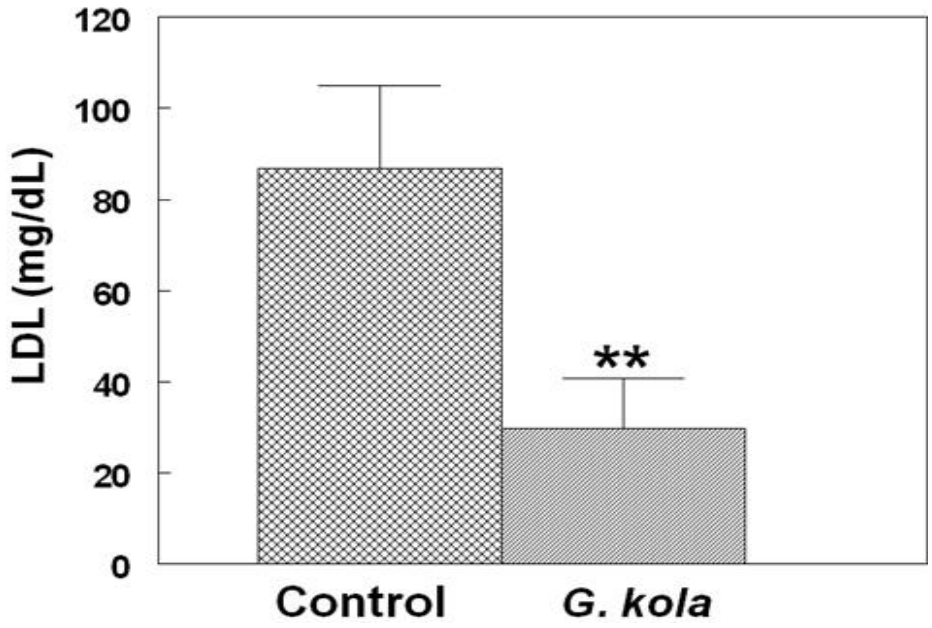

Figure 4. Effect of four weeks treatment with *G. kola* on serum LDL levels of diabetic rats. Values are mean ± SEM; ** P<0.01 versus controls. *G. kola* treated group n=8; controls n=8.

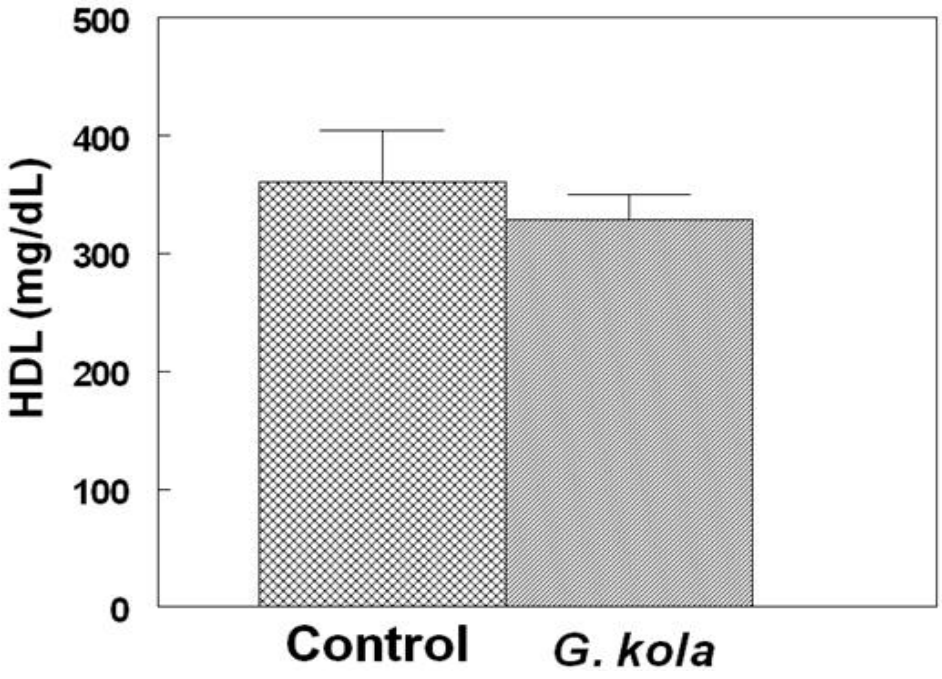

Figure 5. Effect of four weeks treatment with 300 mg/kg *G. kola* extract on serum HDL levels of diabetic rats. Values are mean ± SEM; *G. kola* treated group n=8; controls n=8.

Several studies have demonstrated that a variety of plant extracts effectively lower glucose and lipid levels in streptozotocin-induced diabetic animals (Kim et al., 2006; Aguilar-Santamaria et al., 2009; Lu et al., 2009; Gupta et al., 2009). At a dose of 300 mg/kg, *G. kola* had no acute effect after 6 h treatment on glucose levels. However, after a four week treatment period, glucose levels were lower in the treated diabetic rats compared to the untreated. These data imply that, daily chewing of at least three *G. kola* nuts by diabetic patients may assist with

long term regulation of glucose levels but not as an acute effect. Mechanisms by which plant extracts may act as hypoglycaemic agents include inhibition of hepatic glucose production, inhibition of intestinal absorption and stimulation of glucose transport to correct insulin resistance (Ju et al., 2008). *G. kola* may be acting via one of these mechanisms, probably via induction of protein synthesis, although other mechanisms cannot be ruled out.

In diabetes, hyperglycemia is further complicated by dyslipidemia characterized by increase in total cholesterol (TC), LDL, very low density lipoproteins (VLDL), triglycerides (TG) and a fall in HDL (Taskinen, 2005; Gadi and Samaha, 2007). This predisposes diabetic patients to the development of atherosclerosis, coronary insufficiency and myocardial infarction (Tang et al., 2004; Movahedian et al., 2010). As such, ideal treatment for diabetes should, in addition to glycemic control, include control of the dyslipidemia (Hilaly et al., 2006). The most significant finding in this study was the effect of *G. kola* treatment on LDL levels. There was a highly significant ($P<0.01$) reduction in LDL levels (66% decrease) in the treated animals compared to the controls. The two thirds decrease in LDL levels in treated animals compared to controls implies that *G. kola* lowers atherosclerotic lipids. Although, treatment with *G. kola* was highly effective in reducing LDL levels by 66%, but there was no effect on HDL levels which remained similar to controls. The mechanism(s) of the hypolipidemic actions of *G. kola* could be mediated by reduced activity of cholesterol biosynthetic enzymes or reduced lipolysis due to improved insulin secretion and/or action (Pepato et al., 2005; Zhou et al., 2009; Movahedian et al., 2010). This result is in agreement with the finding of Adaramoye et al. (2005), but using kolaviron treatment on hypercholesterolaemic rats. Cholesteryl ester transfer protein (CETP) transfers cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins and plays an important role in regulating the concentration and composition of HDL (Arai et al., 2011; Redondo et al., 2011). Thus, *G. kola* treatment may not affect CETP activity, hence the unchanged HDL levels observed after four weeks of treatment.

Acute toxicity tests showed no adverse effects or mortality in mice at a dose of 2400 mg/kg, eight times the test dose in this study. This confirms that *G. kola* is safe to use, although biochemical studies for organ function and chronic treatment studies may be necessary to determine LD_{50} and thus confirm safety. However, *G. kola* has been used for centuries as a food product in West and Central Africa.

Conclusion

In conclusion, the data obtained from this study shows that *G. kola* possesses both hypoglycaemic and hypolipidemic effects after a 4 week treatment period. Therefore, using *G. kola* as a diet supplement may be beneficial in diabetes, especially by lowering LDL cholesterol thus protecting against cardiovascular disease. Traditional use of *G. kola* by diabetics to lower glucose levels and reducing complications of diabetes is thus validated. Further studies on the effects of *G. kola* on other complications of diabetes mellitus, such as erectile dysfunction, may be useful.

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