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# Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia Kola* in streptozotocin-induced diabetes mellitus rats

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## Abstract

In the search for natural hypoglycaemic agents as alternatives to synthetic ones that are expensive and not easily accessible, and to justify the use of *Garcinia kola* seeds in traditional African medicine to treat diabetes, the hypoglycaemic and hypolipidaemic effects of fractions from kolaviron (KV) (a *Garcinia kola* seed extract) were investigated in normal and streptozotocin (STZ)-diabetic rats. KV, a biflavonoid complex from *Garcinia kola* seed, was separated by thin-layer chromatography into three fractions; Fraction I (FI), Fraction II (FII) and Fraction III (FIII) with RF values of 0.48, 0.71 and 0.76, respectively. In normoglycaemic rats, KV, FI and FII administered at a dose of 100 mg $kg^{-1}$ body weight elicited significant ($P < 0.05$) hypoglycaemic activity within 4 h of oral administration. Precisely, KV, FI and FII decreased blood glucose levels of normoglycaemic rats by 66%, 50% and 61%, respectively, when compared with controls 30 min after oral administration of the extracts. In hyperglycaemic rats, KV, FI and FII significantly ($P < 0.05$) reduced blood sugar levels in STZ-diabetic rats within 4 h of oral administration. Furthermore, KV alone produced a significant ($P < 0.05$) anti-diabetic effect from day 3 to day 7 of oral intubation of STZ-diabetic rats. In addition, the extracts showed favourable effect on the plasma lipid profile of STZ-diabetic rats, and also decreased significantly ($P < 0.05$) the STZ-induced increase in the activity of microsomal glucose-6-phosphatase and lipid peroxidation (LPO) products. This study confirms the anti-diabetic and hypolipidaemic effects of KV in STZ-diabetic rats. These observed effects of KV are attributed to two of its fractions, FI and FII, with RF values of 0.48 and 0.71, respectively.

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## Introduction

The pathogenesis of diabetes mellitus and its management by the oral administration of hypoglycaemic agents has stimulated great interest in recent years. Only two groups of oral hypoglycaemic agents are available for clinical use – sulphonylureas and biguanides (Al-Awaidi et al 1985; Mariam et al 1996). Although insulin has become one of the most important therapeutic agents known to reduce plasma glucose levels of diabetes, efforts continue to find insulin substitutes from synthetic or plant sources (Choi et al 1991). Over 150 plant extracts and some of their active principles, including flavonoids, tannin, alkaloids, etc., are used for the treatment of diabetes (Erenmemisoglu et al 1995). For instance, *Cyamopsis tetragonoloba* (guar gum) has been shown to elicit hypoglycaemic and hypolipidaemic effects in normal and alloxan-diabetic guinea-pigs (Srivastava et al 1987). Likewise, the hypoglycaemic effects of green tea, *Phyllanthus sellowianus*, *Cogniauxia podoleana* and several others have been documented (Hnatyszyn et al 2002; Sabu et al 2002; Diatewa et al 2004; Yokozawa et al 2005).

*Garcinia kola* seeds (Family: Guttiferae, Sub-family: Clusoideae) are eaten as a refreshing pastime in West and Central Africa, and are known to contain a high content of biflavonoid compounds (Iwu & Igboko 1982). Seeds of *Garcinia kola* are known to have a general antidotal effect in folk medicine in Africa and have been used for the treatment of catarrh, abdominal colicky pain, laryngitis, diabetes and liver disorders (Irvine 1961). Kolaviron, a biflavonoid complex extracted from the *kola* seed, contains *Garcinia* biflavanone (GB) 1, GB 2 and kolaflavanone in an approximate ratio of 2:2:1 (Cotterhill et al 1978). Kolaviron has been reported to modulate the hepatotoxicity of carbon tetrachloride, galactosamine, amanita toxin, paracetamol, thioacetamide and 2-acetylaminofluorene in various experimental

animal models (Iwu 1985; Iwu et al 1987; Akintonwa & Essien 1990; Farombi et al 2000, 2005). Recently, Adaramoye et al (2005) reported the anti-atherogenic effect of kolaviron in both normal and hypercholesterolaemic rats. Likewise, kolaviron has been reported to produce a hypoglycaemic effect in normal and alloxan diabetic rabbits (Iwu et al 1990). It was also reported that kolaviron inhibited rat lens aldose reductase activity and may therefore prevent cataract formation in galactosaemic and diabetic animals (Kinoshita 1974). Since kolaviron is a biflavonoid complex of three structurally related compounds (Cotterhill et al 1978), this investigation was undertaken to determine which fractions of kolaviron are responsible for the observed hypoglycaemic effect in normal and diabetic animals, and to justify its use in traditional herbal medicine in Africa. The hypolipidaemic effect of kolaviron and its fractions in streptozotocin (STZ)-diabetic rats was additionally studied.

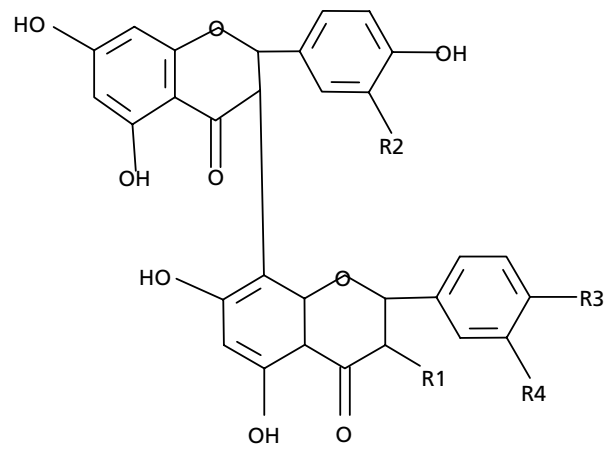
## Materials and Methods

### Preparation of extracts of *Garcinia kola* seeds

*Garcinia kola* seeds were obtained commercially in Ibadan, Nigeria, and certified at the herbarium in the Department of Botany, University of Ibadan, Nigeria. A voucher specimen of *Garcinia kola* seeds already exists in this herbarium. Peeled seeds (3 kg) were sliced, pulverized with an electric blender and air-dried in the laboratory (25–28°C). Extraction of kolaviron was achieved by the method of Iwu et al (1990). Briefly, powdered seeds were extracted with light petroleum ether (bp 40–60°C) in a Soxhlet extractor for 24 h. The defatted, dried marc was repacked and re-extracted with methanol. The methanolic extract was concentrated under reduced pressure and diluted to twice its volume with distilled water and extracted with ethyl acetate (6 × 250 mL). The concentrated ethyl acetate fraction gave a yellow solid known as kolaviron with a percentage yield of 6.0 (Figure 1). Kolaviron is a complex of biflavonoids from *Garcinia kola* plants and was first isolated and purified by Cotterhill et al (1978). Kolaviron was separated by thin-layer chromatography (TLC) using Silica gel GF 254-coated plates and a solvent mixture of methanol and chloroform in ratio of 1:4 v/v. The separation revealed the presence of three main bands that were viewed under UV light at wavelength 254 nm. The fractions, designated as FI, FII and FIII with RF values of 0.48, 0.71 and 0.76, respectively, were collected into separate beakers and then eluted with methanol. The methanolic portions of these fractions were freeze-dried to obtain the fractions, FI, FII and FIII.

### Animals

Male Wistar rats, 200–210 g, were used for the study. The rats were 11–12 weeks of age at the time of this study. They were bred and housed in the Central Animal House, Faculty of Medicine and Health Sciences, UAE University, Al Ain, UAE, which was well ventilated with a 12-h light–dark cycle. They were fed on normal laboratory chow and allowed free access to water for two weeks before the commencement and




| | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| GB1 | OH | H | OH | H |
| GB2 | OH | H | OH | OH |
| Kolaflavanone | OH | H | OMe | OH |

**Figure 1** Structure of kolaviron.

during the period of the experiment. The animal care ethics committee of Faculty of Medicine and Health Sciences, UAE University Al Ain, UAE approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH) (NIH publication 85-23, 1985).

### Effect of extracts on normoglycaemic rats

The procedure described by Sharma et al (1997) was used. Rats were fasted overnight for 18 h and were divided into five groups (A–E) of five rats each. Groups A, B, C and D received 0.3 mL of kolaviron, FI, FII and FIII, respectively (100 mg $kg^{-1}$, p.o.). The control group (E) received 0.3 mL of DMSO (dimethyl sulfoxide), the drug vehicle. The blood glucose levels per rat in all groups were estimated just before extract administration and then 30, 60 and 90–240 min after administration.

### Effect of extracts on STZ-diabetic rats

Another set of Male Wistar rats, 180–200 g, about 11–12 weeks of age, were fasted for 18 h and made hyperglycaemic by a single intraperitoneal injection of STZ (Sigma, USA) dissolved in 0.05 M of citrate buffer (pH 4.3), at a dose of 65 mg $kg^{-1}$ (Ganda et al 1976). The blood glucose levels of these rats were estimated 72 h after STZ administration, and moderately STZ-diabetic rats having blood glucose level above 250 mg $dL^{-1}$ were selected and divided into seven groups of five rats each. One group received insulin

(0.1 mL kg$^{-1}$, s.c.) and a control group received 0.3 mL DMSO orally. Other groups received 0.3 mL of the extracts (KV, FI, FII and FIII) at an oral dose of 100 mg kg$^{-1}$. The last STZ group was kept as a negative control and was given feed and water only. Blood glucose levels were estimated in STZ-diabetic rats before treatment with the extracts (Dose 0), and 30, 60 and 90–240 min after the treatment. Extracts were administered for 14 consecutive days to the STZ-diabetic rats. Before the treatment (Dose 0) and after the treatment (Doses 3, 5 and 7), plasma glucose levels were estimated using the glucose oxidase method (Sharma et al 1997). Rats were fasted overnight at the end of the 14$^{th}$ dose and sacrificed by cervical dislocation. Visceral organs were obtained by dissection and immediately weighed, while blood was collected from the inferior vena cava into heparinized tubes.

### Preparation of plasma

The blood collected in the heparinized tube was then centrifuged at 3000 *g* for 10 min to obtain plasma, and was used for the estimation of glucose and lipid profile of the rats.

### Preparation of microsomes

Liver samples were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. The liver samples were homogenized in 4 volumes of 5 mM phosphate buffer, pH 7.4, and then centrifuged at 10 000 *g* for 15 min to obtain the post-mitochondrial supernatant fraction. Microsomes were pelleted by subsequent centrifugation at 100 000 *g* for 90 min. Microsomes were re-suspended in 0.25 M sucrose solution and stored at −80°C until use.

### Assay methods

*Protein determination*
The protein content of plasma and microsomes were determined according to the method of Lowry et al (1951) using bovine serum albumin as a standard.

*Glucose-6-phosphatase determination*
Microsomal glucose-6-phosphatase activity was assayed by measuring the rate of inorganic phosphate liberated at 660 nm, according to the method of Koide & Oda (1959).

*Glucose determination*
Plasma glucose levels were determined by the glucose oxidase method of Sharma et al (1997).

*Lipid peroxidation (LPO) determination*
LPO in the microsomes and plasma was assayed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, as described by Walls et al (1976) and then expressed in terms of malondialdehyde (MDA) formed per mg protein.

*Determination of triglyceride and cholesterol levels*
Plasma triglyceride and cholesterol levels were assayed using commercial diagnostic kits (Boehringer Mannheim).

*Phospholipid determination*
Plasma phospholipid levels were assayed by the spectrophotometric method of Chen et al (1956).

*Determination of HDL-cholesterol*
Lipoproteins very-low-density lipoprotein (VLDL)- and low-density lipoprotein (LDL)-cholesterol were precipitated by addition of phosphotungstic acid and magnesium chloride to plasma. After centrifugation, the clear supernatant, which contained the high-density lipoprotein (HDL)-cholesterol, was assayed with Boehringer Mannheim (GmbH) diagnostic kit.

*LDL-cholesterol determination*
The LDL-cholesterol was calculated using the formula of Friedewald et al (1972).

### Statistical analysis

The results are presented as means ± s.d. of 5 rats per group. Data were analysed using one-way analysis of variance followed by the post-hoc Duncan multiple range test for analysis of biochemical data using Spss (10.0) statistical software. Values were considered statistically significant when $P < 0.05$.

## Results

Table 1 shows the effect of kolaviron and its fractions on body weight and weight of visceral organs of STZ-diabetic rats. As expected, the final body weight of STZ-diabetic rats decreased significantly when compared with their initial body weight. The decrease in body weight was highest for untreated STZ-diabetic rats when compared with extract-pretreated groups. Table 2 depicts the effect of kolaviron and its fractions on the fasting glucose levels of normoglycaemic rats. Significant hypoglycaemic effects of kolaviron, FI and FII were observed 30–240 min after oral administration of extracts at a dose of 100 mg kg$^{-1}$ ($P < 0.05$). In contrast, there was no significant difference ($P > 0.05$) in blood glucose levels of FIII-pretreated rats at 30–240 min after administration when compared with the baseline value.

Table 3 shows the effect of kolaviron and its fractions on the fasting glucose levels of STZ-diabetic rats. In hyperglycaemic rats, kolaviron, FI and FII exhibited a significant hypoglycaemic effect 30 min after oral administration ($P < 0.05$). As seen in Table 4, kolaviron exhibited a significant hypoglycaemic effect at Doses 3, 5 and 7 when compared with Dose 0 ($P < 0.05$). However, there were no significant differences ($P > 0.05$) in the fasting glucose levels of FI-pretreated STZ-diabetic rats at Dose 3 or 5 when compared with Dose 0 within the group. However, at these doses FI-pretreated diabetic rats had significantly lowered fasting glucose levels when compared with the STZ-only group ($P < 0.05$), across the group. Furthermore, the hypoglycaemic effect of kolaviron was statistically similar to insulin-treated STZ-diabetic rats at Doses 3, 5 and 7.

Figure 2 shows the effect of kolaviron and its fractions on the lipid profile and microsomal glucose-6-phoshphatase

**Table 1** The effect of kolaviron and its fractions on body weight, visceral organ weight and relative weight of streptozotocin-diabetic rats

| Group | Initial body weight (g) | Final body weight (g) | Change in body weight (g) | Kidneys (g) | Pancreas (g) | Heart (g) | Liver (g) | Relative weight of liver (as % of body weight) |
|---|---|---|---|---|---|---|---|---|
| STZ + KV | 212.5 ± 4.1 | 200.2 ± 9.9 | −12.3 ± 1.3 | 1.48 ± 0.14 | 0.38 ± 0.02 | 0.52 ± 0.03 | 7.5 ± 0.2 | 0.04 ± 0.01 |
| STZ + FI | 211.8 ± 3.9 | 199.4 ± 7.2 | −12.4 ± 1.6 | 1.52 ± 0.04 | 0.35 ± 0.04 | 0.58 ± 0.07 | 7.7 ± 1.3 | 0.04 ± 0.01 |
| STZ + FII | 204.0 ± 5.8 | 201.2 ± 15.8 | −2.8 ± 0.4 | 1.86 ± 0.05 | 0.35 ± 0.03 | 0.63 ± 0.03 | 8.9 ± 0.8 | 0.04 ± 0.01 |
| STZ + FIII | 211.7 ± 6.7 | 207 ± 13.7 | −4.7 ± 0.9 | 1.58 ± 0.07 | 0.42 ± 0.04 | 0.59 ± 0.03 | 8.1 ± 0.5 | 0.04 ± 0.03 |
| STZ only | 206.5 ± 2.6 | 189.9 ± 0.8 | −16.6 ± 3.4 | 1.98 ± 0.07 | 0.39 ± 0.06 | 0.56 ± 0.02 | 8.8 ± 0.5 | 0.05 ± 0.02 |
| STZ + DMSO | 207.0 ± 3.5 | 201.6 ± 11.9 | −5.4 ± 1.1 | 1.65 ± 0.08 | 0.45 ± 0.06 | 0.57 ± 0.04 | 8.3 ± 0.3 | 0.04 ± 0.01 |
| STZ + insulin | 210.3 ± 2.8 | 213.9 ± 2.3 | −3.6 ± 0.6 | 1.99 ± 0.13 | 0.45 ± 0.04 | 0.56 ± 0.05 | 8.8 ± 0.7 | 0.04 ± 0.01 |

The results are the means ± s.d. of five rats in each group. KV, kolaviron; FI, Fraction 1; FII, Fraction II; FIII, Fraction III; STZ, streptozotocin; DMSO, dimethyl sulfoxide.

**Table 2** The effect of kolaviron and its fractions on fasting blood glucose levels in normoglycaemic rats

| Time (min) | Blood glucose level (mg $dL^{-1}$) | | | | |
|---|---|---|---|---|---|
| | KV | FI | FII | FIII | Control (DMSO) |
| 0 | 62.80 ± 0.13 | 47.20 ± 0.57 | 48.50 ± 0.06 | 48.45 ± 0.10 | 63.20 ± 0.25 |
| 30 | 66.25 ± 0.29* | 44.25 ± 0.41* | 33.15 ± 0.35* | 48.00 ± 0.16 | 63.80 ± 0.38 |
| 60 | 23.70 ± 0.06* | 43.15 ± 0.54* | 34.2 ± 0.38* | 48.80 ± 0.76 | 63.55 ± 0.73 |
| 90 | 23.55 ± 0.10* | 46.35 ± 0.29* | 31.45 ± 0.66* | 47.85 ± 0.35 | 61.50 ± 0.76 |
| 120 | 20.85 ± 0.03* | 30.80 ± 0.25* | 30.66 ± 0.16* | 48.80 ± 0.51 | 61.15 ± 0.29 |
| 150 | 23.15 ± 0.35* | 31.70 ± 0.06* | 26.70 ± 0.13* | 47.80 ± 1.77 | 60.65 ± 0.60 |
| 180 | 23.55 ± 0.10* | 29.15 ± 0.47* | 33.77 ± 0.74* | 47.55 ± 0.73 | 61.10 ± 0.19 |
| 210 | 22.75 ± 0.03* | 31.95 ± 0.29* | 31.80 ± 0.06* | 49.70 ± 0.70 | 62.60 ± 0.06 |
| 240 | 23.50 ± 0.57* | 33.57 ± 0.36* | 33.11 ± 0.44* | 47.65 ± 0.03 | 63.35 ± 0.72 |

The results are the means ± s.d. of five rats in each group. KV, kolaviron; FI, Fraction 1; FII, Fraction II; FIII, Fraction III; DMSO, dimethyl sulfoxide. *$P < 0.05$, compared with values at time t = 0.

**Table 3** The influence of kolaviron and its fractions on fasting glucose levels of streptozotocin-diabetic rats

| Time (min) | Blood glucose level (mg $dL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Insulin-treated | KV | FI | FII | FIII | Control |
| 0 | 273.3 ± 9.7 | 275.3 ± 5.4 | 254.7 ± 16.3 | 262.0 ± 7.4 | 281.4 ± 9.3 | 297.5 ± 0.5 |
| 30 | 52.7 ± 3.5* | 95.5 ± 10.6* | 67.3 ± 2.5* | 87.6 ± 8.3* | 275.1 ± 7.5 | 305.0 ± 9.8 |
| 60 | 38.0 ± 10.8* | 117.5 ± 10.5* | 92.3 ± 2.5* | 103.5 ± 3.8* | 269.4 ± 10.3 | 299.0 ± 1.0 |
| 90 | 25.3 ± 1.5* | 144.0 ± 8.8* | 105.0 ± 3.0* | 141.0 ± 2.6* | 286.0 ± 11.7 | 302.0 ± 3.0 |
| 150 | 28.7 ± 2.5* | 154.0 ± 46.3* | 103.0 ± 1.7* | 145.8 ± 4.4* | 270.8 ± 15.4 | 302.5 ± 7.5 |
| 210 | 31.0 ± 4.1* | 167.8 ± 33.8* | 133.7 ± 2.1* | 161.5 ± 2.7* | 276.9 ± 13.2 | 312.5 ± 7.5 |
| 240 | 29.1 ± 6.3* | 194.8 ± 39.9* | 138.3 ± 1.5* | 201.3 ± 3.6* | 283.6 ± 8.9 | 298.0 ± 3.0 |

The results are the means ± s.d. of five rats in each group. KV, kolaviron; FI, Fraction 1; FII, Fraction II; FIII, Fraction III; DMSO, dimethyl sulfoxide. *$P < 0.05$, compared with values at time t = 0.

activity of STZ-diabetic rats following administration of extracts for 14 consecutive days. Kolaviron, FI and FII administration to STZ-diabetic rats significantly ameliorated the elevated levels of microsomal glucose-6-phosphatase caused by STZ intoxication ($P < 0.05$). However, there were no significant differences in plasma HDL-cholesterol and phospholipid levels of extract-pretreated STZ-diabetic rats when compared with the STZ-only group ($P > 0.05$). The administration of kolaviron, FI, FII and FIII to STZ-diabetic rats significantly lowered plasma triglyceride levels when

**Table 4** The hypoglycaemic effect of kolaviron and its fractions administered for seven consecutive days on fasting glucose levels of streptozotocin-diabetic rats

| Group | Plasma glucose level (mg $dL^{-1}$) | | | |
|---|---|---|---|---|
| | Dose 0 | Dose 3 | Dose 5 | Dose 7 |
| STZ + KV | 275.3 ± 5.4 | 131.7 ± 4.1* | 204.3 ± 8.4* | 216.0 ± 8.0* |
| STZ + FI | 254.7 ± 16.3 | 246.7 ± 4.8 | 243.7 ± 6.3 | 327.0 ± 10.5** |
| STZ + FII | 262.0 ± 7.4 | 311.1 ± 11.7** | 313.7 ± 11.9** | 291.7 ± 6.6** |
| STZ + FIII | 285.3 ± 8.7 | 296.7 ± 11.4 | 301.2 ± 9.4 | 298.4 ± 13.7 |
| STZ + Insulin | 273.3 ± 9.7 | 196.7 ± 14.3* | 195.7 ± 9.1* | 198.3 ± 8.0* |
| Control | 297.5 ± 0.5 | 302.0 ± 13.2 | 287.0 ± 11.0 | 304.0 ± 4.1 |
| STZ only | 304.7 ± 8.5 | 308.4 ± 10.9 | 332.0 ± 8.1 | 327.3 ± 5.3 |

The results are the means ± s.d. of five rats in each group. KV, kolaviron; FI, Fraction 1; FII, Fraction II; FIII, Fraction III; STZ, streptozotocin; DMSO, dimethyl sulfoxide. *$P < 0.05$, compared with values at dose 0; **significantly higher than values at dose 0 ($P < 0.05$).

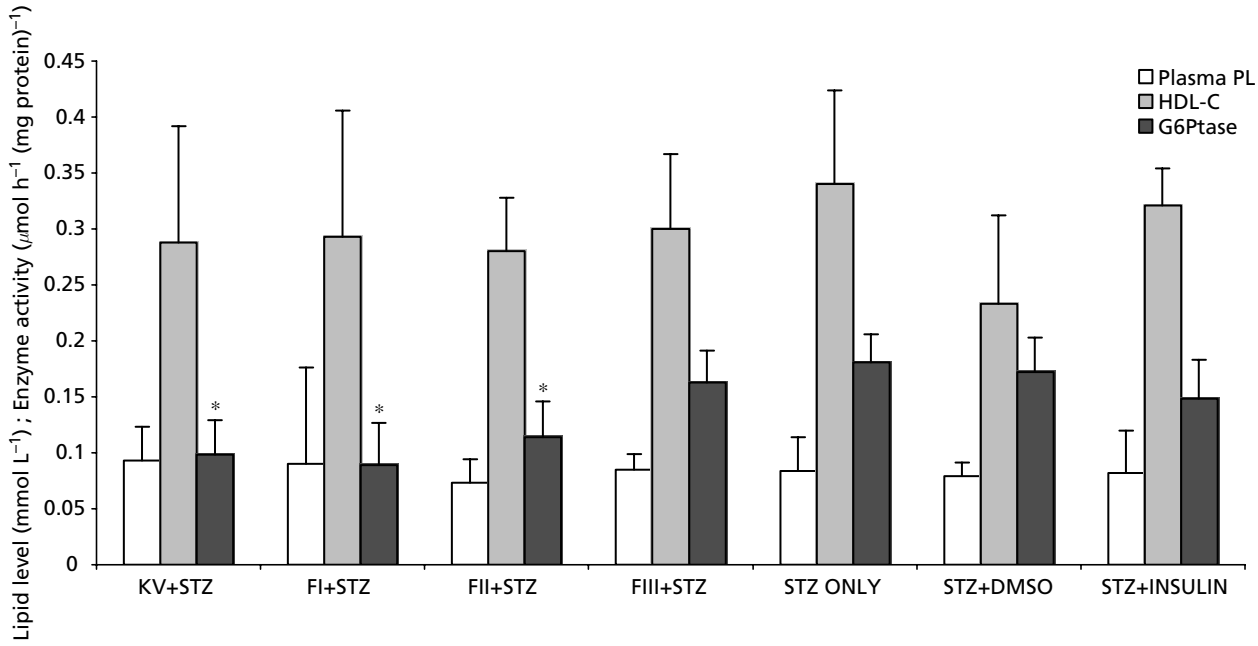



**Figure 2** The effect of kolaviron (KV) and its fractions on plasma phospholipids (PL), HDL-cholesterol (HDL-C) and microsomal glucose-6-phosphatase (G6Ptase) levels in STZ-diabetic rats.

compared with untreated STZ-diabetic rats ($P < 0.05$) (Figure 3). Precisely, kolaviron, FI, FII and FIII administered to STZ-diabetic rats decreased plasma triglycerides by 62%, 36%, 37% and 30%, respectively, over the STZ-only group. Furthermore, kolaviron, FI and FII administration significantly lowered plasma total cholesterol and LDL-cholesterol levels of STZ-diabetic rats by well over 40% when compared with the STZ-only group ($P < 0.05$). In the same vein, kolaviron, FI and FII given to STZ-diabetic rats inhibited microsomal lipid peroxidation by 37%, 40% and 29%, respectively, when compared with the STZ-only group, while kolaviron and FI decreased plasma lipid peroxidation by 22% and 46%, respectively (Figure 3).

## Discussion

STZ-induced experimental diabetes is a valuable model for induction of type I diabetes. Further, STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunction, through oxidative stress (Ozturk et al 1996). Several authors have reported the hypoglycaemic and hypolipidaemic activity of different plant extracts (Akhani et al 2004; Rajasekaran et al 2005a; Sezik et al 2005). In this study, kolaviron and its fractions (FI and FII) exhibited hypoglycaemic activity in fasted normoglycaemic rats. Like-

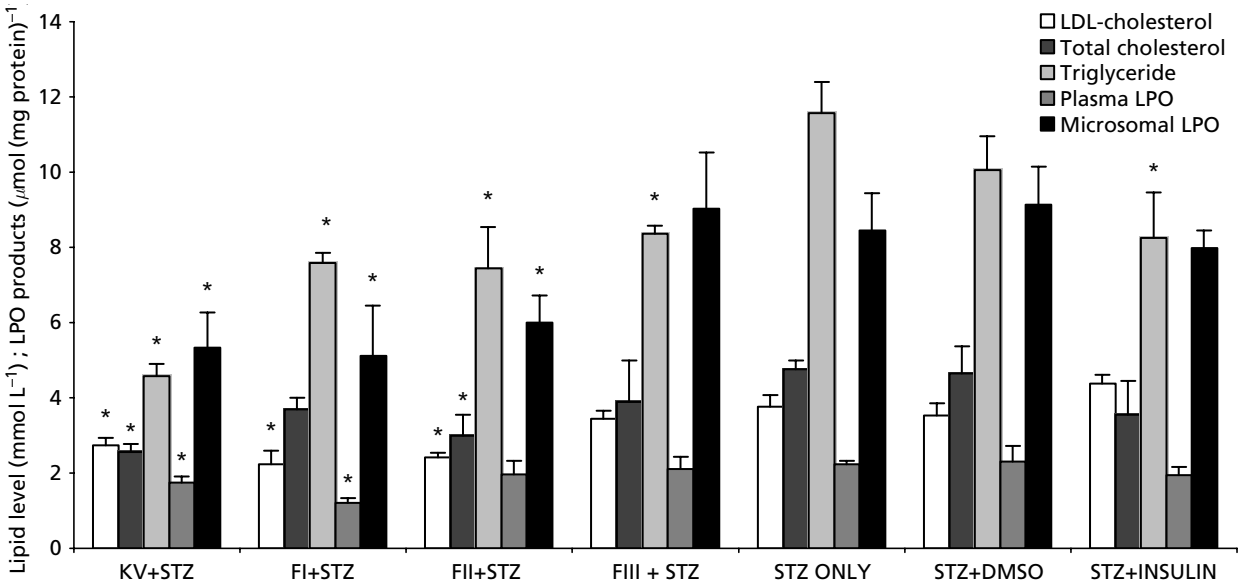



**Figure 3** The influence of kolaviron and its fractions on plasma total cholesterol, LDL-cholesterol, triglyceride and lipid peroxidation (LPO) levels in STZ-diabetic rats. Values are means ± s.d. of five rats. KV, kolaviron; FI, Fraction I; FII, Fraction II; FIII, Fraction III; STZ, streptozotocin; DMSO, dimethyl sulfoxide. *$P < 0.05$, compared with STZ-only.

wise, kolaviron, FI and FII produce significant plasma glucose-lowering effect in STZ-diabetic rats from 30 to 240 min after the administration of extract, and on days 3, 5 and 7 (for kolaviron alone). The significant hypoglycaemic effect of extracts on normal rats may be indicative that kolaviron, FI and FII exert their effect by both direct and indirect mechanisms (Iwu et al 1990). If the extracts acted only as indirect hypoglycaemic agents, no effect would be observed when they were administered to STZ-diabetic rats, since STZ administered at a dose of 65 mg kg$^{-1}$ should have caused severe destruction of $\beta$-cells of the pancreas (Sharma et al 1997). The likely indirect mechanism suggests that kolaviron, FI and FII probably act by stimulating the few surviving $\beta$-cells to release more insulin rather than by aiding the regeneration of necrotic $\beta$-cells of the pancreas. The results of this study suggest that the hypoglycaemic effect of kolaviron is due to two of its fractions – FI and FII, and not FIII. Furthermore, administration of these extracts for seven consecutive days to STZ-diabetic rats revealed that only kolaviron exhibits potent hypoglycaemic property.

Clinical and experimental evidence suggests that free-radical-mediated oxidative processes are involved in the pathogenesis of diabetic complications (Cai & Kang 2001). An increase in the production of free radicals can result in hyperglycaemia-induced enhancement in glucose autoxidation, protein glycation, and subsequent oxidative degradation of glycated proteins (Singal et al 2001), since cellular defence mechanisms, such as antioxidant materials and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), play a fundamental role in protecting the cell against reactive free radicals and other oxidant species. It is possible that a loss of these factors occurs in diabetes and thus promotes the occurrence of oxidative stress. There is growing evidence that oxidative stress is implicated in cardiac dysfunction, leading to heart failure in diabetes (Somogyi et al 2005). It has been reported that over 75% of early deaths in diabetes are related to coronary artery disease caused by abnormal lipid metabolism, which often leads to altered lipid profile of the victim (Tattersalt 1995). Other long-term complications of diabetes include retinopathy, nephropathy and neuropathy (Nathan 1995). In this study, kolaviron, FI and FII administered for two consecutive weeks altered significantly the lipid profile of the STZ-diabetic rats when compared with controls. STZ-diabetic rats had significantly higher plasma triglyceride, LDL-cholesterol and total cholesterol levels when compared with extract-pretreated groups ($P < 0.05$). This result is consistent with the findings of Shunkla et al (2000) who reported elevated levels of total serum cholesterol, LDL-cholesterol and VLDL-cholesterol in alloxan-diabetic rabbits. In their study, these biochemical indices were significantly reduced in both diabetic and subdiabetic rabbits following administration of water extract of *Ficus bengalensis*.

LPO is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to LPO. LPO will, in turn, result in the elevated production of free radicals (Levy et al 1999). Lipid peroxide-mediated damage has been observed in the development of type I and type II diabetes mellitus. Insulin secretion is also closely associated with lipoxygenase-derived peroxides (Walsh & Pek 1984). Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled LPO leading to cellular infiltration and islet cell damage in type I diabetes (Metz 1984). In agreement with previous studies that have used the TBARS assay as an index of LPO (Bastar et al 1998; Kakkar et al 1998), we found an increase in TBARS levels in both plasma and microsomes of STZ-diabetic rats. However, the magnitude of the increase in TBARS levels in

the liver was greater than in plasma. The increased LPO in the tissues of diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and hydroperoxides in the liver, pancreas and kidney (Stanely et al 2001; Rajasekaran et al 2005b). In this study, kolaviron-, FI- and FII-pretreated STZ-diabetic rats had significantly lowered TBARS levels when compared with untreated STZ-rats ($P < 0.05$). The ability of kolaviron, FI and FII to reduce the TBARS levels further confirmed the antioxidant property of *Garcinia kola* extracts in-vivo (Farombi 2000).

It is known that in diabetes the levels of hepatic glucose metabolic enzymes, especially glucose-6-phosphatase and fructose-1,6-bisphosphatase, are adversely affected (Shieh et al 2004), and the process of gluconeogenesis is much more favoured than glycolysis. It is therefore expected that a potent antidiabetic agent such as diasulin (Pari & Saravanan 2004) or *Boerhaavia diffusa* (Pari & Amarnath-Satheesh 2004) would decreased the flux through the gluconeogenic pathway by reducing the activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase. In this study, kolaviron, FI and FII pretreatments significantly decreased the activity of microsomal glucose-6-phosphatase in STZ-diabetic rats. The extract-induced reduction in enzyme activity may lead to a decrease in flux through the gluconeogenic pathway and thus place a lower demand on pancreatic insulin than in the untreated STZ-diabetic rats.

## Conclusions

The hypoglycaemic effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds, is attributed to two of its fractions (FI and FII). These fractions decreased the plasma glucose levels in STZ-diabetic rats, thereby preventing excessive formation of free radicals in other tissues, especially in the liver. Our results also draw attention to the possibility that kolaviron and its fractions (FI and FII) may play a role in alleviating complications of diabetes, such as cardiac dysfunction, by decreasing the levels of LDL-cholesterol and triglyceride in diabetes.

However, other studies are warranted to investigate the effect of kolaviron and its fractions on the activity of antioxidant enzymes in tissues of diabetic animals and their possible mechanism of action.

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