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Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. in Wistar rats

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Abstract
The present study investigated the hypoglycemic, hypolipidemic and cardioprotective effects of 100 - 400 mg/kg/day/oral route of the aqueous seed extract of *Carica papaya* Linn (*CP*E) in normal male Wistar rats for 30 days. The rats, weighing between 120 and 150 g which were divided into groups I - V of six rats each and were orally administered with 10 ml/kg/day of distilled water, 0.1 mg/kg/day of glibenclamide, 100, 200, and 400 mg/kg/day of extract respectively, for 30 days. In addition, the acute oral toxicity and phytochemical analyses of the extract were conducted. On day 31, after an overnight fast, blood samples were obtained by cardiac puncture under inhaled diethyl ether anesthesia for the determination of the fasting blood glucose (FBS), serum triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), and high density cholesterol (HDL-c). The atherogenic (AI) and coronary artery (CAI) indices were also calculated. Results showed that *CP*E significantly and progressively ($p<0.05$, $p<0.01$ and $p<0.001$) lowered the FBS, TG, TC, LDL-c, and VLDL-c dose-dependently, while significantly ($p<0.05$, $p<0.01$, $p<0.001$) causing dose-related elevation in HDL-c concentration when compared to the untreated control and glibenclamide treated rats. The extract also significantly ($p<0.05$, $p<0.01$ and $p<0.001$) lowered the AI and CAI indices dose-dependently. The acute oral toxicity showed the extract to be safe. Phytochemical analyses revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, anthocyanosides and reducing sugars. Thus, lending support to its folkloric use in the management of suspected type 2 diabetic patients.

Keywords: *Carica papaya* Linn; Aqueous seed extract; Hypoglycemia; Hypolipidemia; Atherogenic index; Coronary artery index.

Introduction
The quest for the scientific understanding of the etiopathogenesis of diabetes mellitus (DM) and the ultimate development of definitive curative and/or prophylactic options in its management have stimulated great scientific research interest in recent years (Shafrir, 1997; Bailey, 2001). Drug management of DM without associated untoward effect has also remained a challenge for orthodox medical practice. This has necessitated exploration and screening of medicinal plants with acclaimed therapeutic efficacies in DM management as recommended by the WHO Expert Committee on DM (WHO, 1980; WHO, 2002). Of the array of medicinal plants with oral hypoglycemic potentials are the dry seeds of the unripe fruits of *Carica papaya*. Ethno-botanical survey conducted by us have indicated the seeds are reputed for the management of various human and veterinary diseases including abdominal discomfort, pain, malaria, diabetes, obesity, infections and oral drug poisonings by the Yoruba herbalists (South-West Nigeria).

Carica papaya Linn. (family: Caricaceae) is a tropical tree which is native to the tropics of the Americas but now widely cultivated in other tropical regions of the world, for its edible melon-like fruit, which is available throughout the year (Banerjee *et al*., 2006). *Carica papaya* tree is an erect, fast-growing tree measuring 7 - 8 m tall, with copious latex and trunk of about 20 cm in diameter (Duke, 1984). Its leaves are soft, lobulated (radiating like the fingers of the hand), clustered near top of plant, long-petiolated and measuring up to 80 cm long (Kapanadze and Khasaya, 1988). Its fruit is a large yellow to greenish-orange berry, oblong to nearly globose or pyriform, about 7.5 cm long and bitter in wild types, up to 45 cm long with flesh 2.5 - 5 cm thick, sweet, juicy and of orange color in cultivars (Kapanadze and Khasaya, 1988). In Nigeria, where the ripe fruit is cooked as soup with melon seeds and other spices, it is locally known as *Ibepe*, *Gwanda* and *Okwere* in Yoruba, Hausa and Igbo languages, respectively (Gill, 1992). Phytochemical studies have shown *Carica papaya* to contain alkaloids, carpain,

nicotine, flavonols, tannins, and terpinenes as well as enzymes such as papain and chymopapain (Brocklehurst *et al.*, 1985; Tona *et al*., 1998).

Different parts of the plant are employed in the treatment of different human and veterinary diseases in various parts of the world. For example in Asian folk medicine, the latex is employed as an abortificant, antiseptic for wound dressing and as a cure for dyspepsia (Reeds, 1976) while in Africa the root infusion is reputed for treating venereal diseases, piles and yaws (Duke, 1978). In Cuba, the latex is used in the treatment of psoriasis, ringworm and cancerous growth (Duke, 1984). Its fruit and seed extracts have been reported of possessing pronounced bactericidal activity against *Staphylococcus aureus, Bacillus cereus, Escherischia coli, Pseudomonas aeruginosa,* and *Shigella flexneri* (Emeruwa, 1982). The pulverized seeds are also documented to possess anti-parasitic activities against *Entamoeba histolytica*, and *Dirofilaria immitis* infections (Suhaila *et al.*, 1994; Tona *et al*., 1998). The plant extracts are also reported to have sedative and muscle relaxant (Gupta *et al.*, 1990), reversible antifertility (Harsha and Chinoy, 1996; Chinoy *et al*., 1997) and purgative properties (Akah *et al*., 1997). While several independent animal (Olagunju *et al*., 1995) and human (Olapade, 1995; Salau *et al*., 2003) studies have reported the antidiabetic effects of the unripe mature fruits of *Carica papaya*, there is a dearth of reports on the hypoglycemic and/or antidiabetic effects of the plant seed despite its extensive and historical use in the traditional management of diabetes and obesity. Seeds boiled with milk are believed to be a powerful abortificant agent and a remedy for diabetes (Gill, 1992). Also, one to two measures of table spoon full of the pulverized pawpaw seeds dissolved in 250 ml of hot water for about half an hour is drunk twice daily for several weeks in the traditional management of diabetes and obesity in the adult as revealed by ethno-botanical survey carried out by our research team. Despite the wide and historical use of the dry seeds of *Carica papaya* in the traditional management of suspected diabetic and obese patients by the Yoruba herbalists (South-West Nigeria), the scientific validation of its use in the management of diabetes mellitus, obesity and dyslipidemia are lacking. In view of this, the current preliminary study was designed to evaluate the hypoglycemic, hypolipidemic and cardioprotective potentials of the seed extract in normal Wistar rats for 30 days. The chosen dose range of 100 - 400 mg/kg/day of *CP*E for the study were based on the active pharmacological dose range obtained from the orientation study earlier conducted.

Materials and Methods

Plant materials and the aqueous extraction procedure

Six unripe, mature fruits of *Carica papaya* were collected from a cultivated Pawpaw Plantation at Oke-Afa, Isolo, Lagos, Nigeria, in the first week of February, 2008. Plant identification and authentication had earlier been done by Olagunju *et al*. (1995). The pawpaw fruits were cut into pieces and the wet seeds separated out. These were then gently but thoroughly washed in tap water for two times and completely air-dried at room temperature for 4 weeks. The dry seeds were pulverized into fine powder using domestic mixer grinder (Kanchan Tycoon®, Kanchan International Limited Unit III, Daman, India).

The powdered sample (25 g) was boiled in hot water for 30 minutes after which it was filtered using a piece of clean white cotton gauze. The filtrate was evaporated to complete dryness at 40 ºC, producing a fine, aromatic, and chocolate color solid residue [yield: 25% (w/w)]. The dry residue was weighed and stored in air- and water-proof containers kept in a refrigerator at 4 ºC. From this stock, fresh preparation was made whenever required.

Phytochemical analyses

The presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars in the extract were tested for using simple and standard qualitative methods earlier described by Trease and Evans (1989) and Sofowora (1993).

Acute oral toxicity testing

The acute oral toxicity study was conducted using the computer-guided OECD Guidelines on Acute Oral Toxicity Testing method (AOT425 StatPgm) at a limit oral dose of 2000 mg/kg of the extract as adopted by Adeneye *et al*. (2006).

Experimental animals and their management

Young adult male Wistar rats, weighing between 120 and 150 g were used for this study. The rats were obtained from the Animal Colony of Zoology Department of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria and

allowed 2 weeks of acclimatization under standard laboratory conditions. The rats were maintained on standard rat feed and potable water *ad libitum*. The experimental rats were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research (1984).

Drug treatment

Rats which were fasted overnight for 12 - 14 hours were randomly divided into 5 groups of 6 rats per group. Group I served as the untreated control group and was orally treated with single, daily oral dose of 10 ml/kg/day of distilled water. Groups II - V that served as the treatment (experimental) groups were gastro-gavaged with 0.1 mg/kg/day of glibenclamide (Daonil®, Hoechst Marion Roussel Limited, Mumbai, India), 100 mg/kg/day, 200 mg/kg/day and 400 mg/kg/day of *CP*E, respectively, for 30 days.

Effect of extract on average body weight of rats

On day 1 and 31 respectively, the rat weights were taken with Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland) and the difference in weight from the initial weight per group was calculated.

Blood collection and bioassays

Prior to termination of the experiment on day 31, the rats were fasted overnight and distilled water was made available *ad libitum*. The rats were sacrificed and blood samples collected by cardiac puncture under halothane anesthesia. FBS was determined using One Touch Basic Blood Glucose Monitoring System (LifeScan Inc., Milpitas, California, U.S.A.). Total plasma cholesterol, triglycerides (TG), high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) concentrations were determined by enzymatic assay method using analytical kits (Biolabo SA, Maizy, France) while very low density lipoprotein-cholesterol (VLDL-c) was calculated by deduction of the sum of the cholesterol fractions from the total cholesterol serum concentration as described by Salau *et al*. (2003). Atherogenic Index was calculated using the formula of Abbot *et al*. (1988), and Coronary Risk Index (CRI) was obtained by the method of Alladi *et al*. (1989).

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL-cholesterol}}{\text{HDL-cholesterol}}$$

$$\text{Coronary Risk Index (CRI)} = \frac{\text{Total cholesterol}}{\text{HDL-cholesterol}}$$

Data analysis

Results were presented as mean ± S.D. for body weights while data for biochemical values were expressed as mean ± S.E.M. of six observations and statistically analyzed using two-way analysis of variance on statistical computer software program, SYSTAT 10.6. Post hoc test was conducted using Student-Newman-Keuls test and results were considered significant when the *p* value was less than 0.05, 0.01, and 0.001.

Results

Acute oral toxicity result

Table 1 shows the sequence and result of acute oral treatment of rats with 2000 mg/kg of the seed extract. As shown in the table, the extract did not induce mortality in any of the five sequentially treated rats. The extract induced decreased locomotor activity within the first hour of administration and its delayed behavioral toxicity includes hair loss and progressive weight loss. However, the computer-generated LD_{50} estimate was shown to be greater than 2000 mg/kg body weight/oral route.

Table 1: Sequence, result and effect of *CP*E on the body weight and the limit dose test of Up-and-Down procedure of *CP*E in rats.

Test sequence	Animal ID	Dose (mg/kg)	Body weight (g) on Day 0	Day 7	Day 14	Short term outcome	Long term outcome
1	01	2000	145	125	110	survival	survival
2	02	2000	140	120	109	survival	survival
3	03	2000	133	125	98	survival	survival
4	04	2000	130	116	94	survival	survival
5	05	2000	125	108	90	survival	survival

(ID = Identification number)

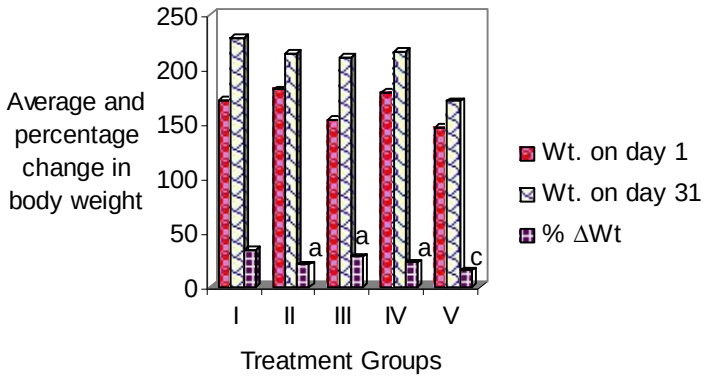

Figure 1: Effect of 0.1 mg/kg/day of glibenclamide, 100 – 400 mg/kg/day of *CP*E on the average body weight of the treated rats.

[a] and [c] represent significant decreases at $p<0.05$, $p<0.001$ when compared to untreated (group I) rats

Group I = 10 ml/kg/day of distilled water
Group II = 0.1 mg/kg/day of glibenclamide
Group III = 100 mg/kg/day of *CP*E
Group IV = 200 mg/kg/day of *CP*E
Group V = 400 mg/kg/day of *CP*E

Effect of CPE on average body weight of treated rats

Figure 1 shows the effect of glibenclamide and graded oral doses of *CP*E on the pattern of weight gain in rats. As shown in the figure, oral treatments with glibenclamide and extract significantly ($p<0.05$, $p<0.001$) reduced the pattern of weight gain in dose-related fashion.

Effect of CPE on fasting blood glucose (FBS)

Figure 2 depicts effects of 30 days of oral treatments with 10 ml/kg/day of distilled water, 0.1 mg/kg/day of glibenclamide and 100 - 400 mg/kg/day of *CP*E on the fasting blood glucose. As shown in the figure, oral treatment of rats with glibenclamide and graded oral doses of *CP*E caused significant ($p<0.05$, $p<0.01$, $p<0.001$) and progressive reductions in the FBS of rats. However, the reduction in FBS concentrations by 200mg/kg/day of *CP*E was comparable to 0.1 mg/kg/day of glibenclamide but was significantly higher at 400 mg/kg/day of *CP*E than that of glibenclamide (Figure 2).

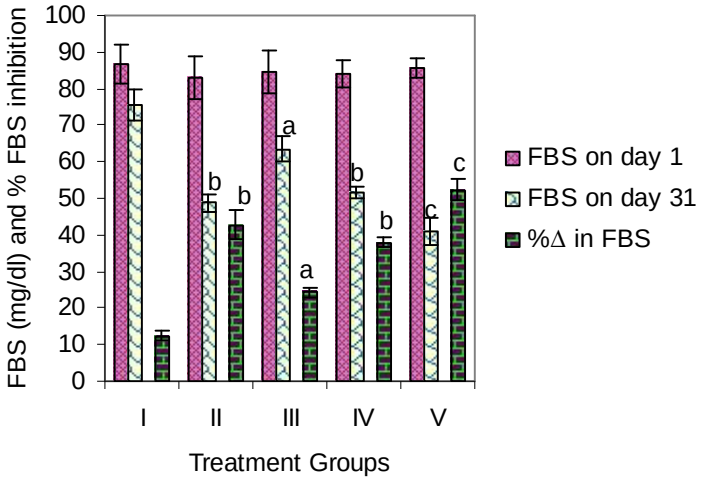

Figure 2: Effect of 0.1 mg/kg/day of glibenclamide, 100 – 400 mg/kg/day of *CP*E on the fasting blood glucose concentrations (FBS) and percentage FBS inhibition (%Δ) on days 1 and 31 in the treated rats.

Group I = 10 ml/kg/day of distilled water
Group II = 0.1 mg/kg/day of glibenclamide
Group III = 100 mg/kg/day of *CP*E
Group IV = 200 mg/kg/day of *CP*E
Group V = 400 mg/kg/day of *CP*E
FBS = fasting blood glucose
%Δ = percentage inhibition in FBS

Effect of CPE on serum lipids, AI and CRI

Similarly, repeated oral administration of glibenclamide and graded oral doses of *CP*E caused significant ($p<0.05$, $p<0.01$ and $p<0.001$) and dose-related reductions in the serum concentrations of TG, TC, LDL-c and VLDL-c but caused the reverse effect on the serum concentration of HDL-c (Table 2). The extract also caused significant ($p<0.05$, $p<0.01$ and $p<0.001$) and progressive reductions in AI and CRI values.

Result of phytochemical analyses

Phytochemical analyses of *CP*E showed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, anthocyanosides and reducing sugars while cardiac glycosides, phlobatinnin were absent.

Table 2: Effect of 30 days of oral administration of 10 ml/kg/day distilled water, 0.1 mg/kg/day glibenclamide and 100 – 400 mg/kg/day of *CP*E on the serum lipids, atherogenic index and coronary index in rats.

	I	II	III	IV	V
TG (mg/dl)	100.5 ± 1.6	82.3 ± 9.5[d]	73.7 ± 4.8[d]	68.3 ± 2.8[e]	50.3 ± 7.7[f]
TC (mg/dl)	100.0 ± 3.1	88.5 ± 4.4[d]	81.3 ± 3.9[d]	71.0 ± 1.0[e]	63.7 ± 2.3[e]
HDL-c (mg/dl)	23.0 ± 0.5	27.2 ± 1.2	37.8 ± 1.8[b]	39.0 ± 2.8[b]	43.0 ± 0.6[b]
LDL-c (mg/dl)	56.9 ± 1.5	51.4 ± 2.6[d]	32.1 ± 3.7[d]	19.7 ± 1.5[f]	11.9 ± 1.0[f]
VLDL-c (mg/dl)	19.1 ± 0.8	11.1 ± 1.8[d]	14.5 ± 1.1[d]	12.3 ± 1.4[d]	8.7 ± 1.8[e]
AI	2.5 ± 0.0	2.0 ± 0.1[d]	0.9 ± 0.1[d]	0.5 ± 0.1[e]	0.3 ± 0.0[f]
CRI	4.4 ± 0.1	3.3 ± 0.1[d]	2.2 ± 0.2[e]	1.9 ± 0.1[f]	1.5 ± 0.1[f]

[b] represents significant rise at p<0.01 while [d, e] and [f] represent reduction at p<0.05, p<0.01 and p<0.001, respectively, when compared to untreated control (group I) rats.

Discussion

Diabetes mellitus remains the most common chronic disorder of carbohydrate, fat and protein metabolism. It is characterized by chronic and persistent hyperglycemia (fasting blood glucose concentration greater than 140 mg/dl taken on at least two separate occasions), degenerative vascular changes and neuropathy due to complete or partial insulin secretion or insulin resistance (Murray and Pizzorno, 1998). Apart from hyperglycemia, diabetes mellitus is accompanied by hypercholesterolemia, hyperlipidemia and hepatic steatosis (Harris and Crabb, 1982). The hypercholesterolemia is a consequence of accelerated fatty acid oxidation to *acetyl CoA* which is the primary substrate for cholesterol synthesis (West *et al*., 1966). Similarly, the hyperlipidemia associated with diabetes mellitus results from accelerated *de novo* hepatic biosynthesis and release of VLDL-c without a corresponding increase in the rate of clearance from the blood by the lipoprotein lipase whose activity is dependent on high insulin: glucagon ratio (Harris and Crabbs, 1982).

The treatment strategies of diabetes mellitus include nutritional therapy, insulin injection, treatments with the various classes of oral hypoglycemic agents which could be synthetic or of herbal origin (WHO, 1985) and/or combination of any of these strategies. However, in the African herbal management of DM, varieties of medicinal plants are employed, some of which have been widely investigated and reported. Some of the plants and their parts include leaves and roots of *Gongronema latifolium* (Ugochukwu *et al*., 2003; Nwanjo *et al*., 2006), unripe, mature pawpaw fruit (Olagunju *et al*., 1995), leaves and seeds of *Phyllanthus amarus* (Adeneye *et al*., 2006), aqueous and ethanol stem bark extracts of *Musanga cecropioides* (Adeneye *et al*., 2007), *Carica papaya* soaked with the stem bark of *Enantia chlorantia* in water (Olapade, 1995), fresh leaves ethanol extract of *Morinda lucida* Benth (Adeneye and Agbaje, 2008), aqueous and methanol extracts of *Anarcardium occidentale* L. (Sokeng *et al*., 2001; Sokeng *et al*., 2007), alcoholic extract of the aerial parts of

Barleria lupilina (Suba *et al*., 2004), to list just a few.

In the present study, the hypoglycemic and hypolipidemic effects of the aqueous seed extract of *Carica papaya* were investigated in normal male rats for 30 days as a way of validating its traditional use. The study showed that oral treatment with 0.1 mg/kg/day of glibenclamide, and 100 - 400 mg/kg/day of *CP*E induced significant, steady and progressive hypoglycemia and hypolipidemia as the dose of the extract increased.

Glibenclamide, a prototype of the second generation sulfonyurea class of the oral hypoglycemic agents, is known to mediate its hypoglycemic effect by stimulating insulin release from the pancreatic β cells, reducing hepatic insulin clearance, stimulating the release of somatostatin and suppressing the secretion of glucagon (Davis and Granner, 2001). Sulfonyureas have also been shown to suppress hepatic gluconeogenesis (Blumenthal, 1977). Comparing the results of glibenclamide with those obtained for *CP*E in this study, it appears the latter may be eliciting its hypoglycemic effect either through induction of hyperinsulinemia or enhancement of peripheral glucose utilization. The observed significant reduction in the serum concentrations of triglycerides, total cholesterol and cholesterol fractions could also be due to depressed hepatic gluconeogenesis by *CP*E, although this claim remains a speculation until it is subjected to further scientific validation by the key enzymes regulating this pathway. A positive relationship between gluconeogenesis and lipogenesis has been well documented in literature (Harris and Crabbs, 1982). Any drug that interferes with gluconeogenesis has also been reported to also interfere with lipogenesis. From the foregoing, it is possible for *CP*E to be inducing its hypoglycemic and hypolipidemic effects via this common pathway. However, this also remains a speculation. In a previous study, *papaya* juice was reported to reduce total cholesterol and triglycerides in high-fat diet fed rats by interfering with their biosynthesis (Banerjee *et al*., 2006). This further reinforces the speculation that *CP*E to be inducing hypocholesterolemia and hypolipidemia via similar mechanism.

The results of the phytochemical analysis of *CP*E showed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, anthocyanosides and reducing sugars. Previous studies have reported some of these phytocomponents to elicit a wide range of biological activities which include hypoglycemic, hypolipidemia, hypoazotemia among others (Oladele *et al*., 1995). Specifically, saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids (Topping *et al*., 1980). In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia (Kritchevsky, 1977; Potter *et al*., 1979). Equally literature has reported the hypoglycemic and hypolipidemic effects of flavonoids, alkaloids and tannins (Oladele *et al*., 1995). The presence of these phytocomponents in the extract in high concentrations could account for these observed biological effects, particularly hypoglycemic and hypolipidemic effects. Again, this hypothesis would require experimental validation.

Another observation arising from this study is the effect of the extract on the average weight pattern in the treated rats. The pattern of weight gain decreases with progressive increasing dose of the extract when compared with untreated control rats. This extract can therefore be used not only to control glucose homeostasis in diabetes but to control dyslipidemia and obesity alike. It is well established that there is a strong link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease (Modan *et al*., 1985; NCEP, 1990). Previous human studies have shown *papaya* to slow down the heart beat, and reduce blood pressure (Gupta *et al*., 1990). Our preliminary results from this study indicate that this seed extract could be useful in the management of Syndrome X, which is a consortium of type 2 diabetes mellitus, obesity, dyslipidemia and hypertension.

Another observation drawn from this study is the relative oral safety of the extract at the dose of 2000 mg/kg. According to Clarke and Clarke (1977), any compound or drug with the oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe. Arising from this documented fact, *CP*E at an oral dose of 2000 mg/kg could be considered relatively safe on acute oral exposure.

In conclusion, this preliminary study has been able to demonstrate the hypoglycemic, hypolipidemic and cardioprotective potentials of *CP*E in normal rats. Further evaluation of the extract in the diabetic and hyperlipidemic models is currently underway.

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Competing interests statement
None declared.

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