Anti-diabetic effect of *Murraya koenigii* (L) and *Olea europaea* (L) leaf extracts on streptozotocin induced diabetic rats

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Abstract: Phytotherapy has a promising future in the management of diabetes, considered to be less toxic and free from side effects as compared to the use of synthetic drugs. The aim of the present study was to assess the antidiabetic possible of orally administered aqueous extracts of *Murraya koenigii* (ML) and *Olea europaea* (OL) leaves (100 and 200 mg/kg doses), in streptozotocin (70 mg/kg) induced diabetic rats. Metformin was used as a standard drug. Blood glucose, cholesterol, triglycerides, creatinine levels and body weight were estimated. ML and OL administration showed significant decrease ($p \geq 0.05$) in cholesterol, triglyceride, and serum glucose levels (range 55.6%-64.6%) compared to the metformin (62.7%); however, there was no significant effect on body weight and serum creatinine. Our results suggest that both the ML and OL possess a potent antihyperglycemic and hypolipidemic effect, which may be due to the presence of antioxidants such as carbazole alkaloids and polyphenols.

Keywords: Diabetes mellitus II, *Murraya koenigii* leaf, *Olea europaea* leaf, metformin, serum cholesterol.

INTRODUCTION

The global prevalence of type II diabetes mellitus (DMT2) is on the rise and at the current rate the estimates for the year 2000 through 2030 show that this global epidemic will have an increase from 171-366 million patients (Wild *et al*., 2004). The pattern of prevalence has been the same in the Gulf including Saudi Arabia. Saudi Arabia has experienced an exponential socioeconomic growth over the past few decades which have led to a sedentary and affluent lifestyle of the people in the urban society. A recent follow up epidemiological study shows an alarming increase in the prevalence of DMT2 during the past few years (Al-Daghri *et al*., 2011). This has stimulated public awareness of this endocrine disorder and the identification of risk factors associated with it.

Management of DMT2 is still a challenge to the health fraternity. Diabetes mellitus II is mainly characterized by hyperglycemia, though the metabolic disarray associated with diabetes includes dyslipidemia and a long term risk of renal dysfunctioning (Sharma *et al*., 2011). The fact that DMT2 can be delayed and prevented by a glycemic control and modifications in lifestyle (Midhet *et al*., 2011) has led to a quest for better antidiabetic medications. This in turn has triggered a search for novel phytochemicals to be used as alternative drugs in the therapeutic management of diabetes. These compounds from the plants are believed to be less toxic and free of side effects than the synthetic drugs commonly used in therapy.

Recent studies suggest that oxidative stress may contribute to the pathogenesis of diabetes counting the β-cell dysfunctioning (Baynes and Thorpe, 1999; Ceriello, 2000). Thus, a new strategy for alleviating the oxidative damage in diabetes makes use of natural antioxidants. Leaves of the plants, *Murraya koenigii* (L) and *Olea europaea* (L) used in the present study have been reported to have strong antioxidant properties. Leaves of *Murraya koenigii* (L) commonly known as "curry leaves" are widely used as condiment and spice in India and other tropical countries. Belonging to the family Rutaceae (citrus family), it has been mentioned as a treatment for diabetics in Ayurveda (Satyavati *et al*., 1999). Several studies have demonstrated the antidiabetic property of the *Murraya koenigii* leaves on diabetic animal models (Vinuthan *et al*., 2004; Kesari *et al*., 2004; Tembhurne and Sakarkar 2010). Phytochemical profile of *Murraya koenigii* leaves shows the presence of some vitamins, carbazole alkaloids, triterpenoids, mineral contents such as iron, calcium, zinc and vanadium, and phenolic compounds (Chakrabarty *et al*., 1997; Narendhirakannan *et al*., 2005). The antioxidant activity in *Murraya koenigii* is mainly attributed to carbazole alkaloids (Tachibana *et al*., 2003).

Although the main active component in olive leaf extract is oleuropein (Amro *et al*., 2002), hydroxytyrosol (Manna *et al*., 1999) which occurs naturally in olive byproducts has been proven to be a potent scavenger of free radicals too (Fragopoulou *et al*., 2007). Both oleuropein and

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hydroxytyrosol have been tested for their antioxidant activities in a series of models *in vitro* (Bouaziz, and Sayadi, 2005; Bouaziz *et al*., 2006) as well as *in vivo* (Jemai *et al*., 2008). This was further supported by a pronounced hypoglycemic and hypolipidemic effect with reduced lipid peroxidation, exhibited by these phenolic compounds in an experimental diabetic model (Jemai *et al.*, 2009).

Hence, in the present study we investigated the effect of the phyto-constituents of two plant sources both of which are dietary constituents and rich in antioxidants. The aqueous extracts of olive leaves (*Olea europaea*) and curry leaves (*Murraya koenigii*) individually, on hyperglycemia and other related markers, serum cholesterol, triglycerides and creatinine associated with the progression of diabetes, in streptozotocin (STZ)-induced diabetic rats. A comparative assessment of the plant treatments was also made with metformin, a commonly used antidiabetic drug.

MATERIALS AND METHODS

Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. Streptozotocin was used to induce diabetes (Sigma, U.S.A.). Metformin used to control diabetes (Merck Serono Middle East). Commercial kits for the estimation of serum glucose, cholesterol, trigycerides and creatinine (United Diagnostic Company, Saudi Arabia).

Plant material

Fresh leaves of *Murraya koenigii* (L.) were procured from an Indian grocery store in Riyadh city, Saudi Arabia. Leaves of Manzanillo olive (*Olea europaea* L.), the local cultivar were collected from Tabouk region of Saudi Arabia. The leaves of both plants were identified by the Department of Botany at King Saud University, Riyadh.

Preparation of extract

For each leaf extract, 100 gm of shade dried leaves were ground in an electrical grinder and dissolved in 500 ml distilled water (Gohil *et al*., 2010). The mixture was left for 24 hrs with a magnetic stirrer at room temperature. The next day the mixture was strained out in a fine sieve and the crude extract was air evaporated for 3 days. The concentrated leaf extract of each plant was then orally administered to the rats in the different treatment groups (100 mg/kg and 200 mg/kg body weight) using a syringe.

Experimental animals

Male Wistar rats weighing 300-345 gm were used. The animals were fed with standard laboratory chow and had free access to water under well ventilated conditions of 12 hrs day and 12 hrs dark cycles. The animals were acclimatized to laboratory conditions prior to the experiment.

Induction of diabetes

The rats were made to fast 12 hrs before the induction of diabetes. Thereafter they were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection the rats with fasting blood glucose higher than 200 mg/dl were considered diabetic and used for the experiment. Feeding was stopped 12 hrs before blood sampling.

Experimental design

The experimental period was 18 days. The first 5 days were for the induction of diabetes in rats and the following 13 days were the investigational period with crude aqueous extracts of curry and olive leaves which were administered separately.

There were seven groups of five rats each.

Group-1: Normal saline treated rats (Normal control-NC)
Group-2: Normal saline treated diabetic rats (Diabetic Control-DC)
Group-3: Metformin (600 mg/kg body weight) treated diabetic rats (MT)
Group-4: *Murraya koenigii* leaf extract (100 mg/kg body weight) treated diabetic rats (ML-4)
Group-5: *Murraya koenigii* leaf extract (200 mg/kg body weight) treated diabetic rats (ML-8)
Group-6: *Olea europaea* leaf extract (100 mg/kg body weight) treated diabetic rats (OL-4)
Group-7: *Olea europaea* leaf extract (200 mg/kg body weight) treated diabetic rats (OL-8)

Blood sampling and biochemical analysis

Before and after administration of the aqueous leaf extracts and metformin, rats were anaesthetized using carbon dioxide. Venous Retro orbital blood samples (Yadav *et al*., 2002) were collected in the fasting state at specific intervals (Day 0, 1, 4, 7, 10, and 13) using a glass capillary and collected in polystyrene tubes without the anticoagulant. Serum was separated by centrifugation at 3000 rpm for 10 mins after which it was tested for glucose. At the end of the experimental period (day 13) the blood samples collected were also tested for serum cholesterol, triglycerides and creatinine. Samples were stored at -20^{o}C until assayed.

STATISTICAL ANALYSIS

Results were presented as the mean standard deviation (SD). A one-way analysis variance was performed using SPSS-17 and Graphpad Prism 5 statistical software. Tukey's test was used for comparing the groups. The values were considered significantly different when the p-value was lower than 0.05.

RESULTS

Antihyperglycemic effect

The administration of aqueous extract of both the curry and olive leaves showed a significant decrease in the blood glucose level in STZ-induced diabetic rats. There was no statistical difference in the basal blood glucose levels of all groups (94-96 mg/dl) (table 1). Five days after STZ administration, blood glucose values were 2 to 3-folds higher (**p<0.005, ***p<0.0005) in all the diabetic groups in comparison to the NC group. No statistical difference was observed among the diabetic groups before the treatment. Blood glucose levels showed a significant decrease in all the treatment groups in comparison to the non diabetic rats during the xperimental period which was more pronounced from the 7th to the 13th day. After 13 days the blood glucose in all treatment groups was restored to the basal level while the diabetic control still recorded an elevated value (table 1).

The decrease in blood glucose values in the diabetic control group was however, non-significant. When compared to the blood glucose level on day 1 after induction of diabetes, the leaf extract treatments (OL-4, OL-8, ML-4, and ML-8) showed a significant decrease in blood glucose levels over the experimental period and this was comparable to the standard drug (MF). However, maximal reduction in blood glucose was seen in OL-4 (64.6 %) over the experimental period (fig. 1). Although the leaf extract treatments (OL-4, OL-8, ML-4, and ML-8) showed a significant decrease in blood glucose levels, their effect was comparable to the standard drug (MF) (table 1).

Table 1: The effect of treatment of aqueous leaf extracts of *Murraya koenigii* and *Olea europaea* on blood sugar (mg/dL) on STZ induced diabetic rats

Group	Basal	Day 1	Day 4	Day 7	Day 10	Day 13
NC	94.0± 4.5	94± 4.8	94± 5.1	95.0 ±5.0	94± 4.8	94.0± 5.1
DC	96.0± 4.6	340.6± 37.3	277.0± 42.6	259.0±2 6.7	247.0± 45.9	242.0± 36.2
MF	96.0± 4.6	261.6± 7.3	216.6± 30.5	180.8± 16.5	96.6± 3.7	97.6± 4.0
OL-4	95.4± 6.9	259.2± 54.3	228.0± 22.0	195.5± 9.7**	117.5± 21.0*	91.8± .4***
OL-8	95.4± 6.9	231.4± 66.6	220.6± 48.1	194.0± 18.6*	124.4± 41.5*	95.8± 9.0***
ML-4	95.4± 6.9	218.4± 45.5	215.6± 45.3	162.2± 27.7*	114.8± 30.7*	101.8± 21***
ML-8	95.4± 6.9	203± 15.7	199.2± 15.4	158.2± 18.4*	100.4± 18.2*	90.4± 10.3***

Values are given as mean ± SE, (n = 5); Values were significant at *p≤0.05; **p≤0.005;
***p≤0.0005 Tukey's test was used for comparing the groups. Treated groups are compared with the Diabetic Control (DC) through the experimental period.NC-Normal Control, DC-Diabetic Control, MF- Metformin, OL-4 (Olive leaf extract (100 mg/kg)), OL-8 (Olive leaf extract (200 mg/kg)), ML-4 (Curry leaf extract (100 mg/kg)), ML-8 (Curry leaf extract (200 mg/kg))

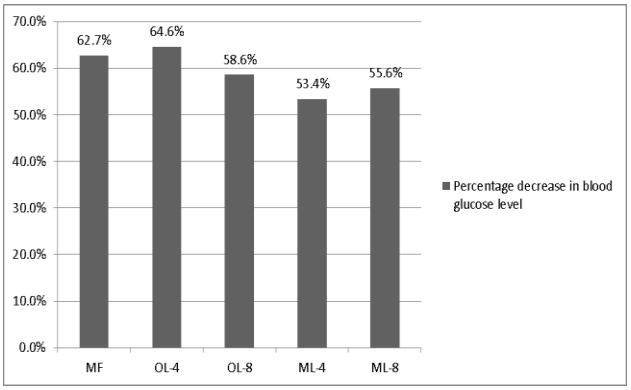

Fig. 1: Effects of *Olea europaea* and *Murraya koenigii* leaf extracts on the percentage decrease in blood glucose from day 1 to day 13 compared to DC
Groups: DC- Diabetic Control, MF- Metformin treated, OL-4 (Olive leaf extract (100mg/kg)), OL-8 (Olive leaf extract (200mg/kg)), ML-4 (Curry leaf extract (100mg/kg)), ML-8 (Curry leaf extract (200mg/kg))

Body weight in diabetic rats

Basal body weights of all experimental groups ranged from 304 to 345 g and were not significantly different. A non-significant decrease in the body weight was observed in all groups including the normal controls during the experimental period (table 2).

Table 2: The effect of treatment of aqueous leaf extracts of *Murraya koenigii* and *Olea europaea* on body weight (grams) of STZ induced diabetic rats

Group	Basal	Day 1	Day 4	Day 7	Day 10	Day 13
NC	345± 16	336± 15	334± 14	333± 14	332± 14	332± 15
DC	304± 19	303± 18	301± 17	301± 15	300± 14	300± 14
MF	305± 14	309± 12	305± 11	305± 10	304± 10	303± 10
OL-4	306± 24	299± 22	298± 22	296± 22	297± 20	297± 20
OL-8	304± 16	294± 5	293± 6	291± 7	292± 7	292± 6
ML-4	333± 21	326± 22	324± 23	321± 22	321± 22	320± 22
ML-8	328± 24	322± 22	321± 22	319± 22	318± 22	316± 22

Values are given as mean ± SE, (n = 5); Non- significant difference among the treated and diabetic control group. NC-Normal Control, DC- Diabetic Control, MF- Metformin treated, OL-4 (Olive leaf extract (100mg/kg)), OL-8 (Olive leaf extract (200mg/kg)), ML-4 (Curry leaf extract (100mg/kg)), ML-8 (Curry leaf extract (200mg/kg))

Lipid profile

Serum lipid levels were measured at the end of the

experiment (figs. 2 and 3). Separate tables provided with figs. 2, 3 and 4). The total cholesterol (TC) concentrations of diabetic control rats showed a significant increase compared with those of the normal control rats. However, rats receiving an oral administration of the leaf extracts of *Olea europaea* (OL-4, OL-8), *Murraya koenigii (*ML-4, ML-8) and metformin had significantly ($p<0.0005$) lower concentrations of TC compared with those in the diabetic control group on day 13th at the end of experimental period. The administration of the leaf extracts and the standard drug was able to restore and further decrease the total cholesterol which was statistically more pronounced ($p<0.0005$) in the groups receiving the leaf extracts.

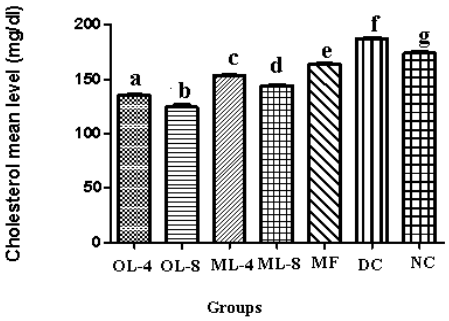

Fig. 2: Effects of *Olea europaea* and *Murraya koenigii* leaf extracts on the serum cholesterol (mg/dL).
Groups: NC-Normal Control, DC- Diabetic Control, MF-Metformin treated, OL-4 (Olive leaf extract (100mg/kg)), OL-8 (Olive leaf extract (200mg/kg)), ML-4 (Curry leaf extract (100mg/kg)), ML-8 (Curry leaf extract (200mg/kg)) Each bar represents mean ±SD from 5 rats. Bars with different letters differ significantly ($p<0.05$) compared to DC.

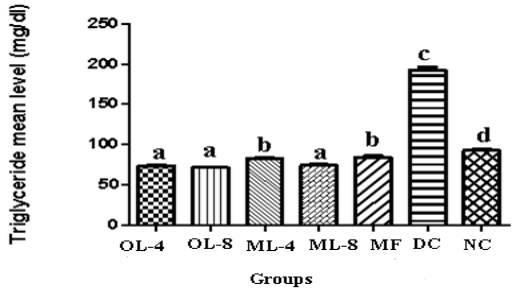

Fig. 3: Effects of *Olea europaea* and *Murraya koenigii* leaf extracts on the serum triglycerides (mg/dL).
Groups: NC-Normal Control, DC- Diabetic Control, MF-Metformin treated, OL-4 (Olive leaf extract (100mg/kg)), OL-8 (Olive leaf extract (200mg/kg)), ML-4 (Curry leaf extract (100 mg/kg)), ML-8 (Curry leaf extract (200 mg/kg)). Each bar represents mean±SD from 5 rats. Bars with different letters differ significantly ($p < 0.05$) compared to DC the triglyceride concentrations of diabetic rats showed a significant increase compared with those of the control rats. However, all the treatments (MF, OL-4, OL-8, ML-4, and ML-8) significantly ($p<0.0005$) lowered the concentrations of triglycerides as compared with those in diabetic control group and were also able to restore the normal level. All four leaf extract treatments were significantly better than the standard drug.

The higher dose of both the leaf extracts (OL-8, ML-8) was significantly better than the lower dose (OL-4, ML-4) in restoring the total cholesterol and triglycerides. The maximal effect was observed in 0L-8. Thus both the leaf extract treatments significantly corrected the hyper-cholesterolemia and hypertriglyceridemia coupled with hyperglycemia.

Creatinine

The serum creatinine levels did not show a statistical difference between all the experimental groups (fig. 4).

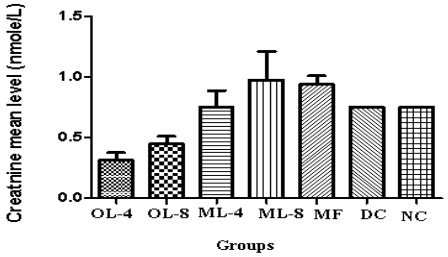

Fig 4: Effects of *Olea europaea* and *Murraya koenigii* leaf extracts on the serum creatinine (nmol/L).
Groups: NC-Normal Control, DC- Diabetic Control, MF-Metformin treated, OL-4 (Olive leaf extract (100mg/kg)), OL-8 (Olive leaf extract (200mg/kg)), ML-4 (Curry leaf extract (100mg/kg)), ML-8 (Curry leaf extract (200mg/kg)). Each bar represents mean ±SD from 5 rats.

DISCUSSION

Most phenolic and flavonoids compounds were described as having anti-oxidative action in living systems, as they act as scavengers of free radicals (Rice-Evans, 1997). Thus there is a growing trend of using plant products in therapy as they are rich in these antioxidants. The non-pharmacological management of diabetes includes an appropriate diet management. A myriad of foods like cereals, vegetables and spices have been assessed for their anti-hyperglycemic effect (Iyer, and Mani, 1990; Khan *et al*., 1997; Vats *et al*., 2002; Krawinkel and Keding, 2006; Vinod *et al*., 2011) in experimental as well as clinical studies. *Murraya koenigii* is one of the most widely acclaimed therapies for diabetes treatment. The antidiabetic effect of *Murraya koenigii* leaf extract is attributed to its insulin secretagogue effect which could be due to the stimulation of the beta cells or regeneration of beta cell functioning by alleviating the oxidative stress. Literature suggests a direct or indirect antioxidant nature of the *Murraya koenigii* leaf extract, which could be due to the free radical scavenging effect of carbazole alkaloids present in the leaves (Tachibana *et al*., 2003). Our study showed that the aqueous leaf extract of *Murraya koenigii* at both doses (ML-4 and ML-8) showed a profound anti-hyperglycemic effect on the diabetic rats which continued with duration of the treatment period. The present results are in consensus with the previous studies on the antidiabetic effect of *Murraya koenigii* leaves on alloxan induced moderately diabetic rats (Vinuthan *et al*., 2004)

and alloxan induced sub and mild-diabetic rabbits (Kesari *et al*., 2004).
Several studies support the fact that olive leaf extracts are rich in oleuropein and hydroxytyrosol and these compounds confer the antioxidant activities to the olive leaves. Thus these phenolic compounds could prove to be beneficial in the protection against metabolic diseases associated with oxidative stress such as diabetes. The use of olive leaf extract in our study demonstrated a strong antidiabetic effect on STZ diabetic rats. Both the doses (OL-4 and OL-8) significantly decreased the blood glucose level, the effect being more pronounced with the duration of the treatment. Our results are in agreement with other findings on the antihyperglycemic effect of olive leaf extract due to its antioxidative properties. It has been observed that decreased activities of hepatic antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) found in diabetic rats were restored by the use of oleuropein and hydroxytyrosol, thereby attenuating the oxidative stress associated with diabetes (Jemai *et al*., 2009; Cumaoğlu *et al*., 2011).

In the present study we used streptozotocin (70 mg/kg) which has been widely used by researchers around the world to induce experimental diabetes. The STZ diabetic animals may exhibit most of the diabetic complications through oxidative stress. A study on the induction of diabetes by streptozotocin in rats showed that in three days, a dose of 60 mg/kg streptozotocin made the pancreas swell followed by degeneration in the β-cells leading to experimental diabetes in rats (Akbarzadeh *et al*., 2007). Previous studies have established that oxidative stress characterized by high levels of oxygen free radicals is responsible for glucose oxidation and non-enzymatic glycation of proteins. This causes cytotoxicity eventually leading to the β-cell dysfunctioning and other complications associated with diabetes (Ceriello, 2000).
High blood glucose levels causes glycation of proteins like hemoglobin (Gupta *et al*., 1997) which results in the formation of reactive oxygen species leading to enhanced lipid peroxidation and subsequent cytotoxicity (Anwer *et al*., 2007). A previous study showed that the use of *Murraya koenigii* leaf extract on STZ induced diabetic rats decreased the glycosylated hemoglobin and the induction of lipid peroxidation thus supporting the potent antioxidative effect of the extract (Tembhurne and Sakarkar, 2010).

The pathophysiology of diabetes clearly defines a link between diabetes and dyslipidemia, characterized by low-high density lipoprotein and increased triglycerides (Goldberg, 2001). This explains the higher incidence of cardiovascular diseases in patients with type 2 diabetes than that of non-diabetics. The use STZ for the induction of diabetes in our experiments resulted in elevation of triglycerides and total cholesterol as compared to the normoglycemic rats as observed in other experimental diabetic model studies (Dineshkumar *et al*., 2010).

Previous literature suggests that the hypolipidemic effect of *Murraya koenigii* leaves is mainly due to the carbazole alkaloids, particularly the mahanimbine (Dineshkumar *et al*., 2010; Birari *et al*., 2010). Studies in the past have also reported a hypolipidemic effect of *Olea europaea* in diabetic rats (Fki *et al*., 2007), mainly attributed to the polyphenols, oleuropin and hydroxytyrosol in the olive by-products (Jemai *et al*., 2008; Somova *et al*., 2003). Our study further supports the hypolipidemic effect of both *Olea europaea* and *Murraya koeingii*, as the leaf extracts at both doses (OL-4, OL-8, ML-4 and ML-8) showed a pronounced hypocholesterolemic and hypotriglyceridemic effect, restoring the lipid levels which was significantly better than the standard drug.

Serum creatinine is a possible surrogate marker of skeletal muscle mass. And because skeletal muscle is one of the target tissues for insulin, skeletal muscle mass might be associated with type 2 diabetes. It has been hypothesized that a low muscle mass reflects a low serum creatinine levels in diabetics (Harita *et al*., 2009; Hjelmesath *et al*., 2010). Our study did not show any significant variation in the serum creatinine levels between the normal and the diabetic rats as well as the treated rats. This could be attributed to the short experimental period. Though, a low serum creatinine level does foreshadow the progression of diabetes type 2, however further research is needed to establish it as a biomarker for the disease.

CONCLUSIONS

A good glycemic control is the cornerstone in diabetes management. In the present study both *Murraya koeinigii* and *Olea europaea* leaves exhibited anti-hyperglycemic and hypolipidemic effects in streptozotocin-induced diabetic rats. The management of diabetes includes a combination of antihyperglycemic drug treatment with lipid-lowering effects. Thus both the leaf extracts used in our study, at two different doses (ML-4, ML-8 OL-4 and OL-8) showed a potent antidiabetic effect comparable to the synthetic drug metformin, overall being more pronounced in the *Olea* - groups (OL-4 and OL-8). These plants could be used as potential therapeutic drugs or dietary supplements for the management of diabetes type 2 and dyslipidemia associated with it. Since these plants have been used as dietary constituents since ages, their use could be potentially safe as dietary supplements. A long term study however, is imperative as plant products are slow in action than the synthetic drugs and at higher doses may also exhibit a plateau effect which would not help in diabetes management.

ACKNOWLEDGMENTS
The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding

the work through the research group project No. RGP-VPP-033.

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(Pl. use proper abbreviations in journals' name)

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