

Antihyperglycemic Activities of Extracts of the Mistletoes *Plicosepalus acaciae* and *P. curviflorus* in Comparison to their Solid Lipid Nanoparticle Suspension Formulations

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The antihyperglycemic activity of the extracts and preparations of solid lipid nanoparticle suspensions of two mistletoes growing in Saudi Arabia, *Plicosepalus acaciae* and *P. curviflorus*, as well as their possible antioxidant effect were investigated in a type 2 diabetic animal model. Type 2 diabetes was induced in adult male Wistar rats by a high-fat diet followed by injection of streptozotocin (STZ). The diabetic rats were treated in parallel with pioglitazone hydrochloride (PIO), non-toxic extracts of *P. acaciae* and *P. curviflorus*, as well as three different solid lipid nanoparticle (SLN) suspension formulations prepared from each of the two extracts. Blood glucose level, insulin resistance, oxidative stress parameters, and antioxidant markers were determined. The total extracts of *P. acaciae* and *P. curviflorus* as well as the SLN formulations exhibited a significant blood glucose-lowering effect associated with antioxidant effects in the diabetic rats. The SLN preparation with the highest lipid content gave the best result. Reduction of hyperglycemia and insulin resistance in the diabetic rats was, at least partly, due to the antioxidant activities of the extracts and their SLN formulations.

Key words: Antihyperglycemic, *Plicosepalus*, Nanosuspension Formulation

Introduction

Mistletoe is the common name of hemi-parasitic plants belonging to several families of the order Santalales, the Loranthaceae being the largest of them with approximately 70 genera and 800 species around the world (Leitão *et al.*, 2013). While mistletoes are known all over the world to cause damage to their host plants, they have medicinal value in the treatment of various ailments including diabetes (Ibrahim and Ayo-dele, 2013; Ogunmefun *et al.*, 2013). Previous investigations on related species of the genus *Plicosepa-*

lus (previously known as *Loranthus*) revealed their efficacy as antidiabetic drugs (Osadebe *et al.*, 2004; Obatomi *et al.*, 1994). In present studies we selected two species of this genus for the evaluation of their antidiabetic activities, *i. e.* *P. acaciae* and *P. curviflorus* that are widely distributed in Saudi Arabia and commonly used in traditional medicine for the treatment of diabetes (Al-Taweel *et al.*, 2012; Waly *et al.*, 2012). Type 2 diabetes can be induced in rats by combining a high-fat diet (HFD) with a low dose of streptozotocin (STZ) (Srinivasan *et al.*, 2004). There is evidence that reactive oxygen species (ROS), generated by glucose

autoxidation, play a critical role in the cytotoxicity of STZ (Matsumoto *et al.*, 2003). Synthetic and natural radical scavengers have been proposed for the prevention and treatment of oxidative damage in pathological states caused by ROS (Kucharska *et al.*, 2004). A solid lipid nanoparticle (SLN) suspension may be considered an aqueous colloidal dispersion, in which a matrix of a solid biodegradable lipid is formed (Swathi *et al.*, 2010). It has the advantage of being physically more stable than traditional colloidal systems, of being able to incorporate labile drugs (hydrophilic or lipophilic) with protection against degradation, and of possessing, in most cases, a controlled release action while being well tolerated (Sarathchandiran, 2012; Rohit and Pal, 2013; Shah *et al.*, 2012). Glyceryl behenate (Compritol® 888 ATO) is a hydrophobic fatty acid ester that has been used in sustained release tablet formulations as lubricant, coating or binder (Roberts *et al.*, 2012). It has also been used in the preparation of SLN formulations (Fouad *et al.*, 2011; Dodiya *et al.*, 2011). Different routes of administration of SLN have been adopted including parenteral, oral, dermal, ocular, pulmonary, and rectal (Mulla *et al.*, 2010). The aim of the present study was to investigate the antihyperglycemic activity of the extracts and preparations of SLN suspensions of *P. acaciae* and *P. curviflorus*, as well as to evaluate their potential antioxidant effect in a type 2 diabetic animal model.

Experimental

Plant collection and preparation of extracts

Whole plants of *P. acaciae* (Zucc.) Wiens & Polhill and *P. curviflorus* (Oliv.) Tiegh. were collected during March 2010 from Al'Ula (also named Al Ola) in northwestern Saudi Arabia and from Abha, situated at 2200 meters above sea level in the fertile mountains of southwestern Saudi Arabia, respectively. The plants

were identified by Dr. Nahed Morad, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. A voucher sample was deposited at the Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, under the registration code "2010-PA1" for *P. acaciae* and "2010-PC1" for *P. curviflorus*. The plant material was air-dried and finely powdered (1 kg of each species), followed by maceration with methanol (3×3000 ml) at room temperature for one week. The total methanolic extract from each species was concentrated under vacuum and kept at 4 °C.

Chemicals

Sodium dodecyl sulfate (SDS) was purchased from Sigma Aldrich (St. Louis, MO, USA). All organic solvents used were of analytical reagent grade. Compritol 888 ATO was obtained from Gattefosse Corporation (Paramus, NJ, USA).

Preparation of SLN suspensions

SLN suspensions were prepared by the emulsion-solvent evaporation method with slight modification as documented in Table I (Rahman *et al.*, 2006). The calculated amounts of the extracts of *P. acaciae* (A) and *P. curviflorus* (C) to produce 25 mg extract/ml suspension, in addition to Compritol 888 ATO, were dissolved in 10 ml of chloroform and added dropwise to 20 ml of an aqueous SDS solution (0.05 % w/v) while homogenizing at 6000 rpm with a probe type homogenizer PowerGen 125 (Fisher Scientific, Waltham, MA, USA) for 10 min. The suspension formed was mechanically stirred at 1000 rpm for 2 h to evaporate the organic solvent. An 1-ml sample was withdrawn for measurement of the size distribution using the Zetasizer NanoZS (Malvern Instruments, Westborough, MA, USA). The selected SLN suspension was examined by transmission electron microscopy with a JEOL JEM-100CX microscope (JEOL, Tokyo, Japan).

Table I. Formulations of SLN suspensions.

Ingredient	A1	A2	A3	C1	C2	C3
<i>P. acaciae</i> extract [mg]	500	500	500	–	–	–
<i>P. curviflorus</i> extract [mg]	–	–	–	500	500	500
Compritol 888 ATO [mg]	200	350	500	200	350	500
Sodium dodecyl sulfate (SDS) [mg]	10	10	10	10	10	10
Chloroform [ml]	10	10	10	10	10	10
Total volume of suspension after addition of distilled water [ml]	20	20	20	20	20	20

A, extract of *P. acaciae*; C, extract of *P. curviflorus*.

Animals

One hundred thirty four adult male Wistar rats, weighing 200–250 g, were used in this study. They were housed in cages under a conditioned atmosphere at 23–25 °C and (55 ± 15)% humidity. The rats were maintained at a 12-h light/12-h dark cycle throughout the experiment and were allowed to acclimatize for 1 week. The animal experiments had been approved by the responsible committee in the Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.

Experimental induction of type 2 diabetes by a high-fat diet and STZ

The rats were allocated into two dietary regimens; 10 rats were fed normal pellet diet (NPD; 12% calories as fat), and 100 rats were fed high-fat diet (HFD; 58% calories as fat) (Reed *et al.*, 2000). The composition of HFD was as described by Srinivasan *et al.* (2004). After two weeks of dietary manipulation, the 100 rats that had been fed HFD were injected intraperitoneally (i.p.) with 35 mg/kg body weight (BW) STZ (Srinivasan *et al.*, 2005) dissolved in 0.1 M disodium citrate buffer (pH 4.5). Ten d after STZ administration, blood samples were obtained from the tail tip, and glucose levels were determined by the glucometer method (Accutrend Alpha; Boehringer, Mannheim, Germany). Only rats with fasting blood glucose levels of 250–300 mg/dl were employed in the study (Algandaby *et al.*, 2010).

Experimental design

Diabetic rats were administered orally pioglitazone hydrochloride (PIO) (Sigma Chemical Company, Cairo, Egypt), the extracts and SLN formulations (Table I) of *P. acaciae* and *P. curviflorus* once daily for 4 weeks, starting from the 11th day after induction of diabetes. Rats were randomly allocated into 11 groups of 10 animals each:

- Group 1: non-diabetic rats, fed NPD, received only a single i.p. injection of citrate buffer (1 ml/kg BW) and served as normal control group.
- Group 2: diabetic rats received only vehicle and served as diabetic control group.
- Group 3: diabetic rats treated with PIO at a dose of 20 mg/kg BW (Dong *et al.*, 2004).
- Group 4: diabetic rats treated with 250 mg/kg BW *P. acaciae* extract.

Group 5: diabetic rats treated with 250 mg/kg BW SLN A1.

Group 6: diabetic rats treated with 250 mg/kg BW SLN A2.

Group 7: diabetic rats treated with 250 mg/kg BW SLN A3.

Group 8: diabetic rats treated with 250 mg/kg BW *P. curviflorus* extract.

Group 9: diabetic rats treated with 250 mg/kg BW SLN C1.

Group 10: diabetic rats treated with 250 mg/kg BW SLN C2.

Group 11: diabetic rats treated with 250 mg/kg BW SLN C3.

Toxicity study

The toxicity of *P. acaciae* and *P. curviflorus* extracts was tested using four doses; 250, 500, 1000, and 2000 mg/kg BW (six rats for each dose). Six control rats without any treatment were kept under the same conditions. The animals were observed continuously during the first hour, and then every hour for the following time intervals (6, 12, and 24 h), and then every 24 h for 3 weeks, to detect any physical signs of toxicity such as writhing, gasping, salivation, diarrhea, cyanosis, pupil size, any nervous manifestations, or mortality.

Blood sampling and biochemical analysis

Rats were fasted overnight after the termination of an experiment. Blood samples were collected by cardiac puncture. Whole blood was subjected to the glycated hemoglobin (Hb_{A1c}) assay using a kit from Stanbio (San Antonio, TX, USA). The remaining blood was centrifuged for 15 min at 2000 × *g* 30 min after collection and stored at –80 °C until assayed. A kit from Spinreact (Girona, Spain) was used to determine the serum glucose level enzymatically.

Enzyme-linked immunosorbent assay (ELISA) for insulin

Serum insulin was determined using the rat insulin ultrasensitive ELISA kit (Crystal Chem, Downers Grove, IL, USA) and an ELISA reader.

Calculation of insulin resistance

Insulin resistance was determined using the homeostasis model assessment index for insulin resis-

tance (HOMA-IR) according to the following formula: $\text{HOMA-IR} = [\text{fasting glucose (mg/dl)} \cdot \text{fasting insulin (mU/ml)}] / 405$ (Matthews *et al.*, 1985). The revised quantitative insulin sensitivity check index (R-QUICKI = $1 / [\log \text{fasting insulin (mU/ml)} + \log \text{fasting glucose (mg/dl)}]$) was used to assess the insulin sensitivity (Katz *et al.*, 2000).

Determination of malondialdehyde (MDA) and antioxidant markers in pancreas

The pancreas was isolated and weighed, and 100 mg were homogenized in phosphate-buffered saline (pH 7.4) using a Teflon homogenizer (Cole-Parmer, Vernon Hills, IL, USA). The homogenate was sonicated and centrifuged at $2000 \times g$ for 10 min. The supernatant was kept at -80°C until the spectrophotometric analysis of the MDA (Preuss *et al.* 1998), and reduced glutathione (GSH) (Ellman, 1970) levels, as well as of the activities of superoxide dismutase (SOD) (Marklund, 1992) and catalase (CATA) (Aebi, 1984).

Determination of liver enzymatic activities

A commercial kit from BioMed Diagnostics (White City, OR, USA) was used for the spectrophotometric determination of the activities of alanine aminotrans-

ferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Measurement of total protein

Total protein was determined by a colorimetric method using a kit from Diamond Diagnostics (Cairo, Egypt) according to the manufacturer's protocol.

Statistical analysis

All data were expressed as mean \pm standard deviation (SD) and analysed using the Statistical Package of Social Sciences (SPSS) program version 16. For all parameters, comparisons among groups were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (Katz, 2006). All *P* values reported are two-tailed, and *P* < 0.05 was considered significant.

Results

Toxicity study

The *P. acaciae* and *P. curviflorus* extracts were not toxic at doses up to 2 g/kg BW. No signs of toxicity during the entire experimental period and no deaths were reported.

Table II. Effect of *P. acaciae* and *P. curviflorus* extracts and their formulations on fasting blood glucose level, glycated hemoglobin, serum insulin level, calculated HOMA-IR, and R-QUICKI in diabetic rats.

Group	Glucose [mg/dl]	HbA _{1c} (%)	Insulin [mU/ml]	HOMA-IR	R-QUICKI
Normal	105.0 \pm 6.0	4.2 \pm 0.26	13.5 \pm 1.30	3.5 \pm 0.21	3.2 \pm 0.026
Diabetic	310.0 \pm 10.0*	11.1 \pm 0.85*	12.1 \pm 0.15	9.3 \pm 0.19*	2.8 \pm 0.007*
Diabetic + PIO	172.7 \pm 4.5 [#]	6.3 \pm 0.15 [#]	12.3 \pm 0.23	5.2 \pm 0.15 [#]	3.0 \pm 0.012 [#]
Diabetic + <i>P. acaciae</i> extract	208.3 \pm 4.6 [#]	7.3 \pm 0.25 [#]	12.3 \pm 0.26	6.3 \pm 0.35 [#]	2.9 \pm 0.021 [#]
Diabetic + SLN A1	170.0 \pm 5.0 [#]	6.4 \pm 0.2 [#]	12.2 \pm 0.30	5.1 \pm 0.1 [#]	3.0 \pm 0.008 [#]
Diabetic + SLN A2	199.0 \pm 11.5 [#]	7.0 \pm 2.1 [#]	12.4 \pm 0.11	6.1 \pm 0.6 [#]	3.0 \pm 0.035 [#]
Diabetic + SLN A3	145.0 \pm 2.0 ^{#£\$}	5.9 \pm 0.7 ^{#£\$}	12.1 \pm 0.25	4.3 \pm 0.5 ^{#£\$}	3.1 \pm 0.044 ^{#£\$}
Diabetic + <i>P. curviflorus</i> extract	203.0 \pm 9.8 [#]	7.2 \pm 0.3 [#]	12.4 \pm 0.21	6.2 \pm 0.3 [#]	2.9 \pm 0.019 [#]
Diabetic + SLN C1	161.7 \pm 10.4 [#]	6.3 \pm 1.5 [#]	12.2 \pm 0.22	4.9 \pm 0.4 [#]	3.0 \pm 0.033 [#]
Diabetic + SLN C2	197.0 \pm 2.2 [#]	7.1 \pm 2.2 [#]	12.3 \pm 0.13	5.9 \pm 0.1 [#]	3.0 \pm 0.007 [#]
Diabetic + SLN C3	148.3 \pm 2.2 ^{#£¥}	5.8 \pm 0.2 ^{#£¥}	12.2 \pm 0.30	4.5 \pm 0.4 ^{#£¥}	3.1 \pm 0.040 ^{#£¥}

HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostasis model assessment index for insulin resistance; R-QUICKI, revised quantitative insulin sensitivity check index; PIO, pioglitazone.

Rats were treated for 4 weeks. Results are expressed as mean \pm SD (*n* = 10) and were analysed using one-way ANOVA followed by Bonferroni's post hoc test.

* *P* < 0.05 compared to the normal control group.

[#] *P* < 0.05 compared to the diabetic control group.

£ *P* < 0.05 compared to the diabetic group that received PIO.

\$ *P* < 0.05 compared to the diabetic group that received *P. acaciae* extract.

¥ *P* < 0.05 compared to the diabetic group that received *P. curviflorus* extract.

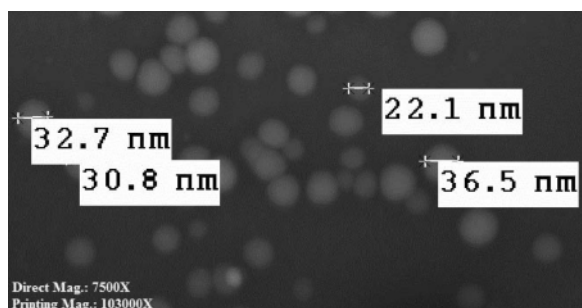


Fig. 1. Representative TEM photograph of SLN suspension, SLN formula (A3).

Effects of extracts and SLN formulations of P. acaciae and P. curviflorus on blood glucose level, glycated hemoglobin, and insulin resistance

Injection of STZ after two weeks of HFD induced a significant ($P < 0.05$) increase in the blood glucose and glycated hemoglobin levels compared to the normal control group (Table II). Although the serum insulin level did not change significantly in the diabetic rats, the calculated HOMA-IR was significantly increased, while the R-QUICKI was significantly decreased as compared to the normal control rats ($P < 0.05$; Table II). Oral administration of PIO, *P. acaciae* and *P. curviflorus* extracts, respectively, and SLN formulations resulted in a significant reduction in the blood glucose and glycated hemoglobin levels as well

as insulin resistance in comparison with the diabetic control group. The effects of the two extracts and their SLN formulations (A1, A2, A3, C1, C2, and C3) was comparable to that of the beneficial effect of PIO. Table I shows the composition of the SLN formulations which differed in the ratio of lipid added. The amount of dispersing agent, SDS, was kept constant in all formulations. The effects of the SLN formulations containing the highest lipid proportion (A3 and C3) showed the most beneficial effect in rats when compared to those of the extracts alone. Particle sizes of the formed SLN suspensions were all in the range from 22 to 70 nm. However, most spherical and uniformly structured particles were seen at an increased lipid ratio (Fig. 1).

Effect of extracts and SLN formulations of P. acaciae and P. curviflorus on pancreatic levels of MDA and antioxidant markers

In diabetic control rats, there was a significant reduction in components of the cellular antioxidant defence system. The pancreatic MDA level was significantly increased, while GSH levels and activities of SOD and CATA were significantly reduced ($P < 0.05$; Table III). Oral administration of PIO to diabetic rats caused a significant decrease in the MDA level; while it caused significant increases in the pancreatic GSH, SOD, and CATA levels ($P < 0.05$; Table III). The effects of the *P. acaciae* and *P. curviflorus* extracts and

Table III. Effect of *P. acaciae* and *P. curviflorus* extracts and their formulations on pancreatic MDA and antioxidant markers.

Group	MDA [nmol/mg protein]	GSH [nmol/mg protein]	SOD [U/mg protein]	CATA [U/mg protein]
Normal	31.7 ± 0.31	69.2 ± 1.08	65.0 ± 1.00	9.0 ± 0.53
Diabetic	90.2 ± 0.70*	23.0 ± 1.00*	27.2 ± 1.04*	4.1 ± 0.17*
Diabetic + PIO	35.5 ± 0.52 [#]	54.3 ± 1.53 [#]	57.2 ± 1.04 [#]	7.1 ± 0.23 [#]
Diabetic + <i>P. acaciae</i> extract	47.0 ± 1.00 [#]	50.7 ± 2.08 [#]	46.0 ± 1.00 [#]	6.2 ± 0.21 [#]
Diabetic + SLN A1	46.6 ± 0.55 [#]	52.0 ± 2.00 [#]	48.7 ± 1.53 [#]	6.4 ± 0.12 [#]
Diabetic + SLN A2	49.0 ± 1.00 [#]	47.7 ± 1.53 [#]	47.3 ± 1.53 [#]	5.8 ± 0.15 [#]
Diabetic + SLN A3	39.0 ± 0.30 [#] ^{\$}	55.3 ± 0.58 [#] ^{\$}	55.0 ± 1.00 [#] ^{\$}	7.2 ± 0.15 [#] ^{\$}
Diabetic + <i>P. curviflorus</i> extract	51.3 ± 1.53 [#]	49.3 ± 1.15 [#]	45.7 ± 2.08 [#]	6.2 ± 0.21 [#]
Diabetic + SLN C1	46.3 ± 0.52 [#]	51.0 ± 1.00 [#]	47.7 ± 1.53 [#]	5.6 ± 0.17 [#]
Diabetic + SLN C2	50.3 ± 1.53 [#]	46.3 ± 2.08 [#]	43.7 ± 0.58 [#]	5.3 ± 0.64 [#]
Diabetic + SLN C3	38.3 ± 1.19 [#] [¥]	55.7 ± 1.53 [#] [¥]	56.3 ± 2.08 [#] [¥]	7.0 ± 0.10 [#] [¥]

MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CATA, catalase; PIO, pioglitazone.

Rats were treated for 4 weeks. Results are expressed as mean ± SD ($n = 10$) and were analysed using one-way ANOVA followed by Bonferroni's post hoc test.

* $P < 0.05$ compared to the normal control group.

[#] $P < 0.05$ compared to the diabetic control group.

^{\$} $P < 0.05$ compared to the diabetic group that received *P. acaciae* extract.

[¥] $P < 0.05$ compared to the diabetic group that received *P. curviflorus* extract.

their SLN formulations were comparable to those of PIO. The highest beneficial effect was shown in rats that had received A3 and C3 formulations.

Effect of extracts and SLN formulations of P. acaciae and P. curviflorus on liver enzyme activities and total protein

Feeding of HFD for two weeks followed by a low dose of STZ resulted in a non-significant increase in the serum levels of ALT, AST, and ALP compared to normal control rats at the end of the study. The *P. acaciae* and *P. curviflorus* extracts and their SLN formulations did not affect these parameters significantly. The two extracts and their formulations did not exert a significant effect on the total protein level.

Discussion

Our work was designed to evaluate the extracts of *P. acaciae* and *P. curviflorus* and their nanoparticle suspension formulations as antihyperglycemic agents as well as their effects on pancreatic oxidative stress in HFD- and STZ-induced type 2 diabetic rats. No significant differences in the serum levels of ALT, AST, ALP, and total protein were observed in all studied groups in comparison to the normal control group. Therefore, we conclude that these extracts are safe to use and do not have hepatocellular or renal damaging effects in diabetic rats.

Our data indicate that a combination of HFD followed by a low dose of STZ resulted in a significant increase in the blood glucose level and insulin resistance in a manner reported in the literature (Hininger-Favier *et al.*, 2009).

ROS are a major factor in the onset and the development of diabetes. Furthermore, ROS have been shown to inactivate the signal pathway between the insulin receptor and the glucose transporter system. ROS generation acts as a mediator of insulin resistance and β -cell dysfunction. Therefore, antioxidants might be beneficial in the treatment of diabetes (Evans *et al.*, 2003).

Administration of PIO for four weeks reduced the blood glucose level and alleviated the insulin resistance to some extent, indicating its antihyperglycemic activity without significantly altering the insulin level. This can be explained by the fact that the drug improves the whole body insulin sensitivity rather than stimulating the β -cell insulin secretion (Srinivasan *et al.*, 2005). Moreover, the antioxidant effect of PIO can improve the β -cell insulin resistance (Iida *et al.*, 2003).

Administration of *P. acaciae* and *P. curviflorus* extracts and their SLN formulations significantly lowered the level of blood glucose in diabetic rats. This is in agreement with the traditional use of *Plicosepalus* in folk medicine as an antihyperglycemic agent (Wahab *et al.*, 2010; Ibrahim and Ayodele, 2013; Ogunmefun *et al.*, 2013). The antihyperglycemic effects of *P. acaciae* and *P. curviflorus* extracts confirm the results of Osadebe *et al.* (2004), who reported that a methanolic extract of *P. micranthus* (previously named *Loranthus micranthus*) has an antidiabetic effect.

The *P. acaciae* and *P. curviflorus* extracts also had a marked effect on antioxidant enzymes. These effects confirm the results of Bamane *et al.* (2012) who demonstrated that extracts of *P. acaciae* and *P. curviflorus* contain significant antioxidant activity. Antioxidants have a potential effect in the prevention and treatment of diabetes mellitus (Sadi and Güray, 2009).

A number of phenolic compounds, including catechin, quercetin, rutin, gallic acid, methyl gallate, and a new flavanocoumarin, named loranthin, have been identified in *P. acaciae* and found to have high radical scavenging activities (Badr *et al.*, 2013). Likewise, a number of phenolic compounds including two new flavane gallates, catechin, and quercetin, in addition to the known triterpenes and phytosterols lupéol, pomolic acid, β -sitosterol, and β -sitosterol 3-*O*- β -D-glucopyranoside were identified in *P. curviflorus* (Al-Taweel *et al.*, 2012), and the flavane gallates were found to possess hypoglycemic activity (Al-Taweel *et al.*, 2012). These and our results thus provide a scientific basis for the use of these plant extracts as traditional medicines in the treatment of diabetes mellitus.

In our work, we also aimed at preparing these extracts in a pharmaceutical dosage form as a preliminary step for drug design; accordingly, three different preparations were designed for each extract. It was noticed that formulas A3 and C3 provoked a significant decrease in the blood glucose level, glycated hemoglobin, and insulin resistance in comparison with the PIO group and with the group of rats that received *P. acaciae* and *P. curviflorus* extracts. These findings could be explained on several bases, including; the fact that increasing the lipid content in A3 and C3 compared to the other concentrations used may have enhanced the process of permeation and absorption of the incorporated extracts (Charman *et al.*, 1997). On the other hand, it has been reported that lipid nanoparticles in general exhibit properties of bioadhesion to the gastric mucosa due to their small size, and this would probably extend their retention time (Luo *et al.*, 2006),

as they release the extract gradually with a lower local concentration. The released extract will then be dispersed in the gastric fluid and dissolved in the form of solubilized surfactant micelles ready for the absorption process in a gradual slow controlled rate (Zhao *et al.*, 2012). Moreover, SLN have proven in previous studies to have a positive effect on increasing the activity of some water-soluble drugs (Shah *et al.*, 2012). It was also reported that SLN could potentially be exploited as a delivery system with improved drug entrapment efficiency and controlled drug release for water-soluble

active compounds (Gandomi *et al.*, 2012; Rohit and Pal, 2013).

Further studies are needed to illustrate the details of the mechanism(s) by which *P. acaciae* and *P. curviflorus* extracts normalize the glucose level and to evaluate the bioavailability of these extracts.

Our results confirm and justify the traditional use of *P. acaciae* and *P. curviflorus* extracts as antidiabetic agents. SLN formulations with high lipid content possess better antihyperglycemic and antioxidant activities.

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