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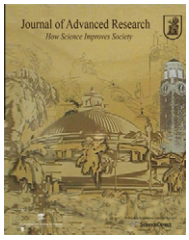

ORIGINAL ARTICLE

Antihyperglycemic and hypolipidaemic effects of the methanolic extract of Saudi mistletoe (*Viscum schimperi* Engl.)

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KEYWORDS

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Abstract The current study evaluated the antihyperglycemic and hypolipidaemic effects of methanolic extract of *Viscum schimperi* Engl. whole parts in streptozotocin (STZ)-induced diabetic rats. The antihyperglycemic activity was evaluated by measuring the fasting blood glucose level (BGL) and by applying the oral glucose tolerance test (OGTT) in diabetic rats. In addition, the effect of the extract on blood plasma insulin was measured as well as its effect on tissue glycogen contents in muscle and liver. The hypolipidaemic effect was evaluated by assaying triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C). Diabetic male Wistar rats of a same age group were treated orally once a day for 4 weeks with a dose of 500 mg/kg bw of methanolic extract. Fasting BGL was measured on the 7th, 14th, 21st and 28th days, while plasma insulin levels were measured at the end of the 28th day. Maximum reduction in BGL of 37% was observed at the 4th week. Furthermore, there was

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a significant increase in plasma insulin, by 321.6%. The hypolipidaemic effect was demonstrated by significant reductions in plasma TC (32.6%), in TG (32.2%) and in LDL-C (27.2%); and an increase in HDL-C of 171.5%. The present data suggest that *V. schimperi* has both antihyperglycemic and hypolipidaemic effects with high insulin-secreting activity.

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Introduction

In recent years, developed nations have witnessed an explosive increase in the prevalence of diabetes mellitus (DM), predominantly related to lifestyle changes and the resulting surge in obesity [1]. Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins [2]. This metabolic disorder is due to deficiency in insulin secretion or resistance to insulin action or both [3]. Dyslipidemia or hyperlipidemia is also involved in the development of cardiovascular complications in diabetes, which are the major causes of morbidity and mortality [4]. Longstanding diabetes, poor glycemic control, hypertension and dyslipidemia can lead to the development of diabetic vascular complications [5].

Renewed attention to alternative medicines and natural therapies has channeled research interest towards traditional herbal medicine. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown [6]. Extracts of the European mistletoe (*Viscum album* L.) have been used for several decades against a variety of diseases. A tea prepared from the leaves of mistletoe is used traditionally to treat diabetes in the West Indies [7]. *V. album* has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice [8]. Mistletoes have shown the ability to lower blood pressure, slow the heart beat, stimulate the immune system, relax spasms and exert sedative, diuretic and anti-cancer effects [9]. They have also shown insulin-secreting activity [10]. From *Viscum* extracts, different components with cytotoxic, immunoactive, and tumour-inhibiting properties have been identified, including the three types of ribosome-inactivating proteins, mistletoe lectins I–III, class 3 thionins (viscotoxins A1, A2, A3, B, 1-Ps), polysaccharides, oligosaccharides and alkaloids [11].

In a continuation of our interest in the chemical composition and biological activity of Saudi mistletoe, *Viscum schimperi* Engl., we investigated it for its antidiabetic and hypolipidaemic activities in streptozotocin (STZ)-induced diabetic rats.

Material and methods

Plant material and extraction

The aerial parts of *V. schimperi* Engl. were collected from the Al-Sheffa area, Al-Taif governorate, Saudi Arabia, in March 2008 and were dried in shade. The plant was identified by Dr. Farag A. Al-Ghamdi, Department of Biology, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia. A specimen (*V. schimperi* Engl. #VS1167) was deposited in the herbarium of the College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The powdered plant material (500 g) was extracted with methanol using Ultraturrax T25 homogeniser (Janke & Kunkel, IKA Labortechnik, Stauten, Germany) (3×2000 ml). The vehicle was distilled off under reduced pressure and the dried methanolic extract was kept at 4 °C.

Chemicals

Streptozotocin (STZ) and α-D-glucose were purchased from Sigma–Aldrich (St. Louis, MO, USA). Carboxymethylcellulose sodium (CMC-Na) was purchased from Acros Organics (NJ, USA), while heparin sodium was purchased from Merck (Dramstadt, Germany). Insulin kit (Coat-A-Count Insulin) was purchased from Siemens Medical Solutions Diagnostics (Los Angeles, USA). All other biodiagnostic kits were purchased from Diagnostic and Research Reagents (Giza, Egypt).

Animals

Male Wistar rats, weighing 150–200 g, were used in this study in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia, (which accord with the NIH guidelines). Animals were purchased from the animal house of King Fahed Medical Research Center, King Abdulaziz University. They were housed for 2 days under standard conditions (well ventilated, temperature 22 ± 2 °C, relative humidity 50–60% and 12 h day and night cycle). Food consisted of normal rat chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed from 8 to 10 a.m.

All the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee.

Acute toxicity study

Twenty-four adult male Wistar rats were used and divided into four groups receiving *V. shimperi* extract at increasing doses of 100, 250, 500 and 1000 mg/kg daily for a period of 3 weeks (six rats were used for each dose). The animals were observed during the first hour continuously and then every hour for 6 h, then after 12 and 24 h, and finally after every 24 h up to 3 weeks, for any physical signs of toxicity such as writhing, gasping, palpitation and decreased respiratory rate or mortality.

Induction of diabetes and experimental design

Diabetes was induced by an ip injection of a fresh solution of a single dose of STZ (in 0.1 M sodium citrate buffer, pH 4.5) in a dose of 55 mg/kg body weight, to overnight fasting rats [12]. Ten days after STZ administration, rats with fasting BGL

between 20 and 30 mmol/l (360–540 mg/dl) were selected and divided into three groups (groups II–IV).

Thirty-two rats were randomly divided into four groups, eight animals in each group:

- *Group I (normal control)* received an equivalent volume of the 0.1 M sodium citrate buffer.
- *Group II (diabetic group)* received a single daily dose of 1% CMC-Na starting at the 11th day, and this group served as the −ve control group.
- *Group III (glibenclamide group)* diabetic rats received a single daily dose of glibenclamide in a dose of 5 mg/kg starting at the 11th day and served as the +ve control group.
- *Group IV (V. schimperi group)* diabetic rats received the plant extract of *V. schimperi* in a dose of 500 mg/kg body - weight each as a single daily dose starting at the 11th day. The dosage of the extract was determined at 500 mg/kg/day [13] from a preliminary short-term pilot study in our laboratory using a range of variable doses.

Vehicle, glibenclamide and plant extract were given orally by gavage as single daily treatments for 4 weeks. Blood samples were collected from the tail vein and fasting BGL of the overnight fasted animals was measured before and at days 7, 14, 21 and 28 from the treatment.

At the end of the experiment, blood samples were withdrawn from the orbital sinus and animals were sacrificed by cervical dislocation under light ether anesthesia for separation of the liver. Livers were dissected out and kept in liquid nitrogen. Liver and muscle tissues were excised, blotted, weighed and stored at −80 °C for assay of glycogen content.

Estimation of plasma insulin level

At the end of the 28th day, 3 h after the last dose of the vehicle, glibenclamide or the extract, blood samples were withdrawn from the orbital sinus of rats under light ether anesthesia into heparinized tubes. Samples were centrifuged at 3500*g* for 15 min for separation of plasma. Plasma samples were separated and kept at −20 °C for analysis when required. Insulin concentrations were determined by radioimmunoassay procedure using insulin kits.

Estimation of plasma glucose and tissue glycogen

Fasting plasma glucose was estimated using glucose oxidase peroxidase method [14]. Tissue glycogen was estimated by the method of Morales et al. (1973) [15].

Estimation of plasma lipid profile

Blood lipids were determined using spectrophotometric assay techniques. Plasma of normal (group I), diabetic control (group II) and diabetic-treated (groups III and IV) rats were used for the determination of plasma TC, TG, LDL-C and HDL-C.

Oral glucose tolerance test (OGTT)

Twenty-four male Wistar rats were divided into four groups (six rats in each group) as shown in the experimental design. At the end of the 28th day, 3 h after the last dose of the vehicle, glibenclamide or the extract, blood samples were withdrawn from the tail vein of overnight fasting rats and BGL was determined, indicating zero time of the test. Glucose solution (50%) in a dose of 2.5 g/kg was given orally [16]. Blood samples were taken at 15, 30, 60, 90 and 120 min after glucose loading and BGL were determined at these time intervals using One Touch Ultra. Curves of BGL (mg/dl) versus the time intervals (min) were constructed and the area under the curve (AUC) was calculated by the trapezoidal method. The AUCs of the curves of each group were compared and tested for significance against the control–diabetic group, to represent the glucose tissue utilization.

Statistical analysis

Data are expressed as mean ± standard error (SE) of mean. Unless otherwise indicated, statistical analyses were performed using one-way analysis of variance (ANOVA). If the overall *F*-value was found statistically significant ($P < 0.05$), further comparisons among groups were made according to *post hoc* Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

Results

Toxicity study

No deaths were reported in the rats treated with different doses of *V. schimperi* and rats did not show any apparent physical signs of drug-induced toxicity during the whole experimental period.

Effect of extract on plasma glucose level

STZ-diabetic rats showed significant increases in the levels of plasma glucose when compared to normal rats. Glibenclamide treatment of diabetic rats showed significant reduction in plasma glucose level at the 21st day of treatment compared to before treatment. On the other hand, po administration of *V. schimperi* showed highly significant reduction in plasma glucose levels starting at the 14th day of treatment compared to before treatment (Table 1).

Effect of extract on plasma insulin level

The induction of diabetes significantly reduced the plasma insulin level, by 56.9% (Table 2). After 4 weeks of po administration of glibenclamide, a significant increase in plasma insulin level, by 272.7% of the diabetic control, was produced. However, treatment of diabetic rats with *V. schimperi* extract significantly increased the insulin blood level compared to the diabetic rats, by 321.6% (Table 2).

Effect of extract on muscle and liver glycogen

Table 2 shows the levels of muscle and liver glycogen in normal diabetic and treated rats. There was a decrease in the muscle glycogen (79.2%) and liver glycogen (57.3%) content of diabetic rats when compared to normal rats. Glibenclamide treatment significantly increased both muscle and liver glycogen, by

Table 1 Effect of treatment with *V. schimpe* extract (500 mg/kg/day for 4 weeks) on the blood glucose levels of diabetic rats.

Groups	Blood glucose level (mg/dl)				
	Before treatment	After treatment			
		7th day	14th day	21st day	28th day
Normal control	103 ± 3.8	102 ± 2.9	102 ± 3.6	98 ± 5.7	111 ± 7.5
Diabetic group	394 ± 27.1	406 ± 22.7	433 ± 17.0	470 ± 31.8	494. ± 31.2
Glibenclamide group	416 ± 18.7	456 ± 19.6	443 ± 22.7	376 ± 21.7[a]	335 ± 25.6[a]
V. schimperi group	425 ± 29.4	485 ± 3.0	395 ± 19.0[a]	345 ± 30.4[a]	327 ± 23.7[a]

The values are expressed as the mean ± SE of the mean of eight rats in each group.
[a] Statistically significant from corresponding before treatment value at $P < 0.05$.

Table 2 Effects of oral administration of *V. schimperi* extract (500 mg/kg/day for 4 weeks) on plasma insulin level, muscle glycogen and liver glycogen in diabetic rats.

Groups	Insulin (μIU/ml)	Muscle glycogen (mg/g tissue)	Liver glycogen (mg/g tissue)
Normal control	7.48 ± 1.32	10.12 ± 0.9	56.43 ± 5.4
Diabetic group	3.22 ± 0.387[a]	2.10 ± 0.2[a]	24.12 ± 2.3[a]
Glibenclamide group	12.47 ± 2.39[b]	7.41 ± 0.6[b]	49.13 ± 4.2[b]
V. schimperi group	13.61 ± 1.72[b]	8.83 ± 0.8[b]	50.13 ± 5.2[b]

The values are expressed as the mean ± SE of the mean of eight rats in each group.
[a] Significantly different from the values of the normal rats at $P < 0.05$.
[b] Significantly different from the control values of diabetic rats at $P < 0.05$.

71.7% and 50.9%, respectively. When *V. schimperi* was administered to diabetic rats for 28 days, the muscle and liver glycogen contents increased significantly (by 76.2% and 51.9%, respectively).

Effect of extract on plasma lipid profile

Diabetic rats showed significant increase in the blood levels of TC, TG and LDL-C (Table 3). The recorded increases were 90.9%, 30.3% and 114.3%, respectively, of the values of the normal rats. On the other hand, HDL-C was significantly reduced, by 32.4% of the value of the normal rats. Oral administration of glibenclamide significantly decreased the blood levels of TC and LDL-C, by 25.7% and 33.5% of the diabetic rats, respectively. However, the level of HDL-C was significantly increased, by 103.2%. Glibenclamide did not significantly change the blood level of TG. Treatment with *V. schimperi* extract significantly decreased the blood levels of TC, TG and LDL-C, by 32.6%, 32.2% and 27.2%, respectively, of diabetic rats; however, the blood level of HDL-C was significantly increased, by 171.5%.

Effect of extract on OGTT

The blood levels of glucose in control, diabetic group, diabetic treated with glibenclamide or plant extract groups, demonstrated a significant change in BGL after po loading with 50% glucose solution (Fig. 1). The rats of the diabetic group had a significant elevation in BGL throughout the total measurement period (120 min) with respect to normal control (Fig. 1); also it did not come back to the initial value (0 min level) even at the end of the period tested (120 min).

Treatment of diabetic rats with glibenclamide induced a significant reduction (15%) in the AUC relative to the diabetic control group (Fig. 1). However, treatment of the diabetic rats with *V. schimperi* extract, produced a significant reduction of the AUC, by 22% (Fig. 1).

Discussion

Conventional therapies for diabetes have many side effects and high rate of secondary failure. On the other hand herbal extracts are expected to have similar efficacy with fewer side effects than conventional drugs [17]. Nowadays, more than 1200 plant species are used to treat symptoms of diabetes mellitus. The hypoglycemic property of almost 50% of these traditionally consumed medicines has been experimentally tested [18]. *V. schimperi* belongs to the Viscaceae family and members of genus *Viscum* are traditional hypoglycemic herbs [19]. The present investigation reports the antihyperglycemic effect of the methanolic extract of *V. schimperi* on STZ-induced diabetic rats.

STZ injection resulted in diabetes mellitus close to that of humans, which is probably due to the destruction of β-cells of islets of Langerhans as proposed by many authors [20–22]. This effect is being depicted in our study by elevation of BGL and decreased insulin levels in STZ-induced diabetic rats. The increased levels of plasma glucose in diabetic rats were lowered by the administration of *V. schimperi*. The reduced glucose levels suggested that *V. schimperi* might exert an insulin-like effect on peripheral tissues. Our study indicated that the hypoglycemic effect of *V. schimperi* is caused by stimulation and potentiation of insulin release from the remnant existing beta cells of islets of Langerhans. It caused a large increase of plasma insulin in STZ-treated rats. This effect of *V. schimperi* seems to be similar to that of glibenclamide which induces insulin exocytosis from beta cells as proposed by Tian et al. (1998) [23]. Gray and Flatt (1999) [10], demonstrated the

Table 3 Effects of oral administration of plant extracts (500 mg/kg) on total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) of diabetic rats.

Treatment	TC (mg/dl)	% Change	TG (mg/dl)	% Change	HDL-C (mg/dl)	% Change	LDL-C (mg/dl)	% Change
Normal control	125.71 ± 4.04		87 ± 2.52		29.57 ± 5.47		78.70 ± 4.58	
Diabetic control	239.99 ± 21.53[a]	+90.9	113.33 ± 11.15[a]	+30.3	20.56 ± 3.09[a]	−32.4	168.68 ± 8.75[a]	+114.3
Glibenclamide 5 mg	178.28 ± 15.05[b]	−25.7	121.6 ± 14.62	+7.3	41.84 ± 7.15[b]	+103.2	112.10 ± 8.02[b]	−33.5
Viscum schimperi	161.90 ± 10.08[b]	−32.6	76.80 ± 3.88[b]	−32.2	55.82 ± 7.38[b]	+171.5	122.50 ± 10.85[b]	−27.2

The values are expressed as the mean ± SE of the mean of eight rats in each group.
[a] Significantly different from the values of the normal rats at $P < 0.05$.
[b] Significantly different from the control values of diabetic rats at $P < 0.05$.

presence of insulin-releasing natural products, including lectins, in *V. album*, which may contribute to the reported antidiabetic property of the plant. The ability of lectins isolated from mushrooms (*Agaricus campestris, Agaricus bisporus*) to enhance insulin release by isolated rat islets of Langerhans has been documented [24–26]. Moreover, the antidiabetic properties of the African mistletoe (*Loranthus bengwengis*) were investigated in streptozotocin-diabetic rats and found to be highly dependent on the host plant species [27]. However, Onal et al. (2005) [28] demonstrated that *V. album* has an antidiabetic activity. This effect may be due to the inhibition of α-glucosidase activity and glucose absorption as *V. album* contains lectins, misteloe lectin I, II, III, viscotoxins and cyclitols.

Glycogen synthesis in the rat liver and skeletal muscles was impaired during diabetes [29]; hence the glycogen content of skeletal muscle and liver were markedly decreased in diabetes [30]. Results of the present study showed an increase in skeletal muscle and liver glycogen content in STZ-diabetic rats after po administration of *V. schimperi*. This prevention of glycogen depletion in both liver and muscles is possibly due to stimulation of insulin release from beta cells [31]. The effect of *V. schimperi* extract on glucose tolerance and tissue utilization of glucose was studied in diabetic rats using the OGTT. OGTT was reported to measure the rate of tissue uptake and utilization of glucose. Diabetic control rats showed a significant increase in the AUC of the glucose concentration after po glucose loading. This effect may be due to the reduction of glucose tissue utilization and increased hepatic glucose production as a result of decreased insulin secretion. The partial or complete destruction of pancreatic β-cells in STZ-diabetic rats is the main effect [32]. *V. schimperi* extract produced significant reduction of the AUC of the diabetic control rats. These results revealed that the *V. schimperi* extract induced an increase in glucose utilization and glucose tolerance by the body tissues of diabetic rats.

It is well known that in uncontrolled type 2 diabetes mellitus, TC, LDL-C, VLDL-C and TG increase, while the HDL levels decline contributing to secondary complications [33,34]. The abnormally high concentrations of serum lipids in the diabetic subjects are due mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the

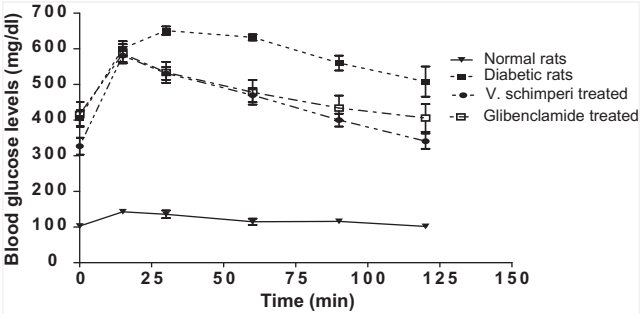

Fig. 1 Effect of *V. schimperi* extract (500 mg/kg) on the fasting blood glucose level of diabetic rats during OGTT. The values are expressed as the mean ± SE of the mean of six rats in each group.

metabolism of cholesterol rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in an increased production of LDL-C particle [35]. The administration of *V. schimperi* increased the level of serum HDL-C and decreased the levels of TC, TG and LDL-C, which could be secondary to a partially restored beta-cell function with increased insulin levels.

Conclusion

This study indicates that *V. schimperi* has both hypoglycemic effect and antidyslipidemic activity. The possible mechanism by which *V. schimperi* brings about its antidiabetic action may be through stimulation of insulin release from the remnant pancreatic β-cells. Considering the *V. schimperi* effect on lipid profile, it may be a potential hypolipidaemic agent, which will be a great advantage in treating diabetic conditions associated with atherosclerosis or hyperlipidemia.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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