

Original Research Article

Anti-diabetic potential of *Plectranthus lanuginosus* in streptozotocin-induced diabetic rats

Mohammed H Mukhtar¹, Waleed H Almalki², Muhammad Ahmed^{2*}, Aisha Azmat³

¹Department of Biochemistry, Faculty of Medicine, ²Department of Pharmacology, Faculty of Pharmacy, ³Department of Physiology, Faculty of Medicine, Umm Al Qura University, Makkah, Saudi Arabia

*For correspondence: **Email:** hma00ahmed@hotmail.com; **Tel:** 00966-556618257

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Abstract

Purpose: To determine the antidiabetic effect of methanol extract of *Plectranthus lanuginosus* leaves in streptozotocin-induced hyperglycemic (HGD) rats.

Methods: *P. lanuginosus* leaves were collected from Saad Medhas, Al Baha, Kingdom of Saudi Arabia. After defatting with n-hexane, they were extracted in vacuo at 40 °C with 75 % methanol. Streptozotocin (50 mg kg⁻¹, i.p.) was used to induce hyperglycemia (diabetes) in the rats. The HGD rats received either standard drug (glibenclamide, 10 mg kg⁻¹, p.o.) or *Plectranthus lanuginosus* leaf methanol leaf extract (PLLM) at doses of 200 and 400 mg kg⁻¹/day, p.o. for 21 consecutive days. Blood samples were taken from the rat tails 2 h after dosing, and at 7-day intervals (i.e., 0, 7th, 14th and 21st days). The blood samples were used for measurement of fasting blood glucose (FBS), using a glucometer. On the 21st day, the rats were sacrificed via cardiac puncture. The activities of liver marker enzymes (SGPT and SGOT), and serum lipid profile (cholesterol, triglycerides, HDL and LDL) were determined using a hemolyzer.

Results: Streptozotocin treatment produced significant hyperglycemia in the rats (348.9 ± 5.6) when compared to control (79.2 ± 1.3). However, PLLM (200 and 400 mg kg⁻¹) produced significant and dose-dependent anti-diabetic (166.4 ± 5.6 and 123.86 ± 6.8 respectively) and antihyperlipidemic effects in HGD rats, at levels similar to those produced by the standard drug, glibenclamide (120.6 ± 6.4).

Conclusion: *P. lanuginosus* leaf extract possesses pronounced anti-diabetic and anti-hyperlipidemic properties which may be due to the presence of phenolic and flavonoid constituents in the plant. Therefore, the plant extract can be further developed for the management of diabetes.

Keywords: *Plectranthus lanuginosus*, Antidiabetic effect, Flavonoids, hyperglycemic (diabetic: HGD) rats

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic ailment diagnosed as FBS ≥ 7.0 mmol L⁻¹ (126 mg/dL), random blood glucose (RBS) ≥ 11.1 mmol/L

(200 mg dL⁻¹), and glycated hemoglobin (HbA_{1c}) ≥ 48 mmol/mol (≥ 6.5 DCCT %) [1]. The incidence of DM is on the increase, and it has been estimated that by the year 2030, approximately 472 million people in the world

may be affected by diabetes. The IDF 2011 report [2] indicates that Kuwait, Saudi Arabia, and Qatar are topmost countries with the preeminent percentages of DM in the Middle East and North Africa (MENA) region, with prevalence levels of 23, 24, and 23 %, respectively.

Recent pharmaceutical targets for DM management are focused on lifestyle (diet, exercise, weight loss), and use of appropriate medications. The current therapy for DM causes mild-to-severe undesirable side effects which could lead to death. Thus, attention is shifting steadily towards the folkloric system of medicinal plants for management of DM [3].

Many herbal plants and medicines have been studied for their antidiabetic and hypoglycemic effects. These plants include *Tridax procumbens* [4], *Geigeria alata* (*G. alata*) roots [5], *Acacia nilotica* pods [6]; *Balanites aegyptiaca* fruits, *Guiera senegalensis* leaves, *Hyphaene thebaica* epicarp and *Trigonella foenum-graecum* seeds [7]. *Plectranthus lanuginosus* grows in different parts of Kingdom of Saudi Arabia (KSA), especially in Al Baha. Different species of *Plectranthus* are already in use in folkloric medicine for treating disorders of the digestive system such as stomach pain, nausea, vomiting and diarrhea [8], as well as mouth and throat infections, gastritis and intestinal spasms [9]. There are no studies so far on the possible medicinal properties of *Plectranthus*. Therefore, this research was carried out to study the effect of *Plectranthus lanuginosus* on HGD rats.

EXPERIMENTAL

Collection of plant material and extraction

The whole plant material (comprising leaves and stem of *Plectranthus lanuginosus*) was collected in January 2014, from an area near Saad Medhas, Al Baha, KSA. The plant was kindly identified by Dr. Ibrahim Abd-Elhady, and a voucher specimen (no. = PLLMUQU786) was deposited at the Museum of Pharmacognosy Department, Faculty of Pharmacy, Umm Al-Qura University, Makkah-al-Mukarramah, Saudi Arabia.

Six hundred (600) grams of air-dried and powdered leaves and stems of *plectranthus lanuginosus* was defatted with *n*-hexane (2 x 1.5L) and subjected to exhaustive extraction with aqueous methanol (75 %; 3 x 3L). The combined methanol extract was concentrated to dryness *in vacuo* at 40 °C (47 g) and code-named PLLM.

Experimental animals

In-bred 2-month-old Sprague Dawley (SD) rats of both sexes (weighing 220 – 260 g) were used in the entire study. The rats were reared in the premises of the Faculty (Umm Al Qura University, Faculty of Pharmacy, Makkah, KSA) with fresh and balanced diet, and clean water was provided. Prior to STZ treatment, the rats were fasted overnight, but were permitted free access to clean drinking water. This study was executed according to the international regulations for the use of animals [10]. The experimental protocol was approved by the Animal Ethics Committee of Faculty of Medicine, Umm Al Qura University, Makkah, Saudi Arabia (ethical approval no. UQU-COP-EA#14341).

Oral acute toxicity study

Oral toxicity test (acute) for PLLM was done using fresh and healthy rats (3 rats), following the Organization of Economic Cooperation and Development (OECD) Guideline 423. For this purpose, maximum limit dose of PLLM (2 g/kg) was administered orally using gavage. Noxious and toxic signs and/or mortality were recorded during the first 2 h after dosing. Then, the animals were monitored for the next 48 h. Their body weights were recorded for 14 days. The extract was found safe at PLLM exposure levels up to a dose of 2gkg⁻¹. Thus, PLLM doses of 200 and 400 mgkg⁻¹ were selected for use in subsequent studies on the antidiabetic effect of the extract.

Anti-diabetic evaluation of PLLM

Healthy male adult rats (200 ± 10 g body weight) were randomly selected. Thirty rats were divided equally into five groups as shown in Table 1.

Rat group	Description	Treatment
I	Normal control	Distilled water
II	HGD rats (positive control)	Distilled water
III	HGD rats	Glibenclamide (10 mgkg ⁻¹)
IV	HGD rats	PLLM (200 mgkg ⁻¹)
V	HGD rats	PLLM (400 mgkg ⁻¹)

Diabetes mellitus was induced in the rats using STZ (except group 1). For induction of DM, the rats were intra-peritoneally injected freshly prepared STZ solution in cold citrate buffer, pH 4.5 [11]. After 48 h, FBS levels were measured using a Bayer Contour Blood Glucose Meter

(Germany), and rats with FBS \geq 200 mg/dL were deemed diabetic [12]. The diabetic rats (groups III to V) were given PLLM extract or standard drug (glibenclamide) using oral gavage once a day for 21 consecutive days. Blood samples were taken through the tail tips at 2 h after dosing, and subsequently at 7-day intervals i.e. at 0, 7th, 14th, and 21st days. Blood glucose level was determined in each sample with a glucometer [13]. On the 21st day, 5 - 8 ml blood sample was withdrawn directly from all experimental groups through cardiac puncture, and sera were separated [13]. The serum samples were used for estimation of various biochemical parameters, as indicated hereunder.

Biochemical analyses

Blood samples (obtained via cardiac puncture) were centrifuged at 3500 rpm for 15 mins. Liver function biomarkers: aspartate aminotransferase (SGOT) and serum glutamic pyruvic transaminase (SGPT); and lipid profile (triglycerides, total cholesterol, VLDL, and HDL-cholesterol) were assayed using HumaLyzer 3000.

Statistical analysis

Data are presented as mean \pm standard error means (SEM). For further interpretation, one-way analysis of variance (ANOVA) was done, followed by Tukey's multiple comparison tests, on Minitab 17.

RESULTS

Oral acute toxicity

The extract (PLLM) was non-toxic up to a dose of 2000 mg kg⁻¹. For acute toxicity, the physical and emotional behaviors of the rats were continuously monitored for the first 2 h, after which the rats were observed after 8 h, and then every 8 h during the next 48 h. The extract did not cause any significant changes in the animals' normal behavior, nor did it produce any mortality.

Anti-diabetic effect of PLLM

The results are presented in Table 2. The HGD rats (positive control: group II) exhibited a significant increase in FBS ($p < 0.001$) throughout the study period (21 days). The reference drug (glibenclamide, 10 mgkg⁻¹/day) and PLLM (200 and 400 mgkg⁻¹/day) produced significant and dose-dependent declines in FBS levels, relative to diabetic control rats on the 7th, 14th and 21st days. A significant anti-diabetic effect was observed after 14 consecutive days of administration of glibenclamide and PLLM (200 and 400 mgkg⁻¹). Group IV (PLLM 400 mgkg⁻¹) showed noticeable improvement in diabetic status from the first week of the drug administration onwards (Table 2). With continuous treatment till the 21st day, the high dose of PLLM (400 mgkg⁻¹) and glibenclamide (10 mgkg⁻¹) reversed the STZ-induced hyperglycemia to normal levels, as shown in Table 2. As presented in Table 3, there were significant elevations in total cholesterol, LDL and triglycerides, and a significant decrease in HDL cholesterol in the HGD rats (group II), when compared with control (group I). However, continuous treatment with PLLM (groups III and IV) and glibenclamide (group V) for 21 days produced significant alterations in lipid levels ($p < 0.05$). The SGPT and SGOT levels were also normal in PLLM- and glibenclamide-treated rats, but the diabetic control rats had significant elevations in the levels of SGPT and SGOT ($p < 0.001$).

DISCUSSION

Diabetes mellitus (DM) is the fastest growing disorder in KSA [14], with heightened possibility of high level of mortality and huge medical expenditure [15]. Diabetes increases the risk of other disorders like cardiovascular disease, neuropathy, retinopathy and nephropathy, which are the most common complication of diabetes [16]. Thus, there is need for concerted research efforts to evolve effective therapy for the disease [17].

Table 2: Effect of PLLM on blood glucose levels in STZ-induced diabetic rats

Treatment/group	Fasting blood glucose level (mg/dL)			
	0 day	7 th day	14 th day	21 st day
Control	79.6 \pm 1.28	75.28 \pm 1.0	80.26 \pm 1.56	79.21 \pm 1.3
Diabetic control	280.2 \pm 2.98**	298.19 \pm 6.15**	314.72 \pm 2.16**	348.89 \pm 5.6**
PLLM 200mgkg ⁻¹	278.2 \pm 3.78	246.6 \pm 1.29*	236.3 \pm 3.83*	166.36 \pm 5.62*
PLLM 400mgkg ⁻¹	270.93 \pm 1.67	212.36 \pm 2.56*	199.63 \pm 2.4*	123.86 \pm 6.78*
Glibenclamide (10mgkg ⁻¹)	275.36 \pm 3.65	238.62 \pm 3.38*	200.8 \pm 3.65*	120.56 \pm 6.36*

Values are presented as mean + SEM (n = 8); * $p < 0.01$, compared with HGD rats; ** $p < 0.01$, compared with control rats

Table 3: Effect of PLLM on various biochemical parameters in HGD rats

Treatment	Cholesterol (mgdL ⁻¹)	Triglyceride (mgdL ⁻¹)	HDL (mgdL ⁻¹)	LDL (mgdL ⁻¹)	SGOT (IUL ⁻¹)	SGPT (IUL ⁻¹)
Normal control	69.5±1.08	72.28±1.0	26.08±1.12	39.11±3.15	75.25±3.24	12.65±1.69
Diabetic control	115.62±0.98*	189.26±1.55*	17.65±1.26*	89.80±3.33*	119.89±5.65*	56.91±6.20*
PLLM 200mgkg ⁻¹	68.31±0.88	66.54±0.12	26.56±0.38	36.00±0.88	63.51±3.72	16.65±4.5
PLLM 400mgkg ⁻¹	65.13±0.44	63.52±1.16	28.54±0.81	38.54±7.63	65.29±4.38	15.26±2.35
Glibenclamide (10mgkg ⁻¹)	61.6±0.83	78.29±0.37	26.13±0.75	28.65±3.6	70.84±4.23	18.10±5.22

Data are presented as mean ± SEM (n = 8); *p < 0.001, compared with diabetic control rats

Alternative herbal medicines are used in different countries for DM, but most of these herbal remedies have not been scientifically investigated. It is necessary to study such medicinal plants because they might contribute to development of newer drugs/compounds for inclusion in the diabetologist's pharmacy.

Many species of *Plectranthus* are being used as alternative treatments for DM [18]. The anti-diabetic effect of PLLM was assessed in the present study because the genus *Plectranthus* is a significant, prolific and extensively used genus in folkloric medicine in southern Africa [18].

In the current work, the two PLLM doses (200 and 400 mgkg⁻¹) exhibited significant and dose-dependent blood glucose-lowering effects (anti-diabetic activity) against STZ-induced DM in rats. This anti-diabetic response in PLLM-treated rats was similar to that produced with the standard drug glibenclamide. It is known that STZ produces hyperglycemia due to its cytotoxic effect on the beta cells of the pancreas [19]. However, in STZ-treated rats, PLLM significantly reduced the blood glucose level.

In earlier studies on *Plectranthus amboinicus* using a dose of 400 mgkg⁻¹, it was suggested that the significant anti-diabetic effects in rats might be due to individual or synergistic action of flavonoids and other bioactive phytoconstituents present in *Plectranthus* [18]. Another study also revealed the antidiabetic potential of *Plectranthus esculentus* in STZ-induced hyperglycemic rats [19]

In past studies, different extracts from different species of *Plectranthus* were reported to exert anti-diabetic effects against STZ- and alloxan-induced diabetes in different animal models. The findings in the current study on the anti-diabetic effect of PLLM (200 and 400 mgkg⁻¹) are in agreement with those reported in earlier studies. Plants that contain flavonoids, terpenoids, alkaloids, and glycosides possess antidiabetic and antioxidant potential. Flavonoids rejuvenate injured pancreatic beta cells, while saponins inhibit normal glucose transport by blocking the

intestinal sodium-glucose co-transporter-1 (SGLUT-1) [20,21]. Earlier studies have reported the presence of tannins, flavonoids and saponins in *Plectranthus* [22]. Thus, the antidiabetic effect of PLLM might be due to existence of flavonoids and saponins in the extract.

Hypercholesterolemia and hyper-triglyceridemia are major complications of DM [23]. The significant fall in lipid profile (serum total cholesterol, triglycerides and LDL-cholesterol), while the concomitant increase in serum HDL-cholesterol levels in the PLLM-treated diabetic rats, are strongly indicative of the anti-hyperlipidemic potential of PLLM. Research has revealed that phenolics have considerable antioxidant potential and, by inhibiting the activities of α-glucosidase and α-amylase, they downregulate carbohydrate metabolism [24]. Furthermore, polyphenols act on cardiovascular system in DM and rectify the metabolism of lipoproteins and lipids, eventually reversing hyperlipidemia [25]. The presence of phenolic constituents in *Plectranthus* could be one of the reasons for the higher anti-diabetic and anti-hyperlipidemic actions of this plant.

CONCLUSION

The results obtained in this study indicate that PLLM exhibits anti-diabetic effect against STZ-HGD in rats. It further ameliorates hyperlipidemia in diabetic rats. These effects may be due to its contents of flavonoids, saponins and phenolic compounds. Thus, the plant has potential for development as a potential phytomedicine for the cure of DM.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All listed authors read and approved the submission of manuscript for publication. The author also declares that that manuscript data, or part thereof has not been submitted to any other journal.

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