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Endogenous Lebanese Plants Treating Diabetes and Related Complications

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Abstract

Diabetes mellitus and its related complications is becoming a serious threat to public health in all parts of the world. The treatment and control of diabetes mainly depend on the synthetic agents, but the fact is that it has never reported total recovery from diabetes. Natural medicines mainly originated in herbs, the traditional Lebanese medicine performed a good experimental practice and is showing a bright future in the therapy of diabetes mellitus and its complications. Based on a large number of Ethnopharmacological research work, numerous bioactive compounds have been found in Lebanese medicinal plants for diabetes. This work presents three natural medicines with regards to their anti-diabetic active principles and/or pharmacological test results, which are commonly used in the traditional Lebanese medical system and have demonstrated experimental anti-diabetic effectiveness. It is highly significant to pay attention to traditional Lebanese natural medicines for treatment and control of diabetes mellitus and its complications.

Keywords: Phytomedicine; *Diabetes mellitus*; *Salvia libanotica fruticosa*, *Centaurea horrida*; *Hordeum spontaneum*; Antioxidant; Diabetic neuropathy

Abbreviations: DM: Diabetes Mellitus; DMSO: Dimethyl Sulfoxide; NC: No Change; SLF: Salvia Libanotica Fruticosa; CH: Centaurea Horrida; HS: Hordeum Spontaneum IP: Intraperitoneal Injection; ROS: Reactive Oxygen Species; CAT: Catalase

Introduction

Diabetes mellitus (DM) is a major endocrine disorder. Worldwide projections predict that more than 300 million people will suffer from diabetes by the year 2025 and that its management will be expensive [1,2]. Numerous studies have been shown that oxidative stress, mediated mainly by hyperglycemia induced generation of free radicals, contributes to the development and progression of diabetes and its complications [3]. Diabetic neuropathy, which is one of the most frequent long-term complications of diabetes mellitus, is frequently accompanied with inferior quality of life [4]. This complication happens in about one quarter of diabetic patients [5]. Painful diabetic neuropathy is combined with symptoms and signs such as burning, tingling or lancing type of spontaneous pain, allodynia and hyperalgesia [6].

The use of endogenous natural plants with therapeutic properties is as ancient as human civilization and, for a long time, plant products were the main sources of drugs [7]. Many plant species are known in folk medicine of different cultures and are used for their hypoglycemic properties [8]. Salvia, *Salvia libanotica fruticosa* (Lamiaceae) is an evergreen shrub growing in many areas in the world, especially, in Lebanon. Salvia is used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world [9]. *Salvia fruticosa* M. leaves have a folk reputation in the eastern Mediterranean region as a hypoglycemic agent. *Salvia fruticosa* M. leaves caused a statistically significant reduction in blood glucose levels in alloxan hyperglycemic rabbits [10]. *Salvia libanotica fruticosa* roots were not reported before to neither have hypoglycemic activity nor manage diabetic neuropathy.

Centaurea is a large genus (Asteraceae) distributed mainly in the Mediterranean area, southwest Asia, North America, south America and in Iran [11]. *Centaurea horrida* grows in coastal habitats on rocks and seas and is commonly known in Turkey, in Lebanon and in other Mediterranean regions [12]. Many *Centaurea* species have been used in folk medicine for many purposes as diuretic, bitter tonic, digestive and emmenagogue [13]. In addition; they were used traditionally to cure various ailments such as diarrhea, hypertension and as antibacterial, antitumor and diabetes agents [14]. It was reported that the constituents of *Centaurea aspera* causing hypoglycemia in rats and mice appeared to be localized primarily in leaf [15]. Extracts of leaves and flowers of *Centaurea corubionensis* reduced sugar levels in rats with glucose-induced hyperglycemia, but had no effect on alloxan diabetic animals [16]. It was not reported before that *Centaurea horrida* herb and roots neither have hypoglycemic activity nor manage diabetic neuropathy.

Wild barley, *Hordeum spontaneum* K. (Poaceae), are collected from Mediterranean region [17]. Barely is an important cereal food all over the world. It is abundantly used in Asia, Africa, and is also cultivated in Europe, America and Australia. Barely is used as feed for animals as well as food for human consumption. Certain new applications of barely are under investigation for new value added products. In recent years, the importance of barley grains as a nutraceutical ingredient has increased since their high contents of soluble fibers, especially as a rich source of β-glucan. The most nutritional benefit of β-glucan in foods is flattening of postprandial blood glucose and insulin rises [18]. *Hordeum spontaneum* grains were not previously reported to neither have hypoglycemic activity nor manage diabetic neuropathy.

In the current study, DM was provoked using alloxan. Alloxan is a beta-cytotoxin induces "chemical diabetes" (alloxination) in an experimental animals by harming β-cells of the pancreas leading to decrease the consumption of glucose by the tissue [19]. It is well established that sulphonylureas, like glibenclamide, produce hypoglycemia by increasing the secretion of insulin from pancreas. Sulphonylureas are active in some mild cases of alloxan-induced diabetes, but they are inactive in intense alloxan diabetes [20]. Glibenclamide was utilized in this study as the hypoglycemic positive control agent.

Accordingly, the aim of the present work involves the study of possible hypoglycemic and diabetic neuropathy management by

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endogenous Lebanese plants, namely, *Salvia libanotica fruticosa* roots, *Centaurea horrida* herb and roots and *Hordeum spontaneum* grains.

Materials and Methods

Plant material

Dried *Salvia libanotica fruticosa* roots, *Centaurea horrida* herb and roots and *Hordeum spontaneum* grains were purchased commercially from Ibn-Al-Nafess herbalist, Beirut, Lebanon. They were authenticated with a reference sample and a dried specimen was deposited in the Pharmaceutical Sciences Department, Faculty of Pharmacy, and Beirut Arab University (BAU).

Preparation of plant extracts

The dried plant materials were separately dry ground using TCM grinder (TCM, China). All plant materials fine powders were extracted using 2500 ml of 80% ethanol and were stirred for 24 hrs in their ethanol liquors. During which the flasks were covered by aluminum foil to prevent the light damage. After 24 hrs, the extracts were double filtered through a porcelain funnel using 20-25 μM filters. The filtered extracts (except, *Centaurea horrida* herb and roots) were well dried using Rotavap (Buchi, Germany) at temperature 40°C under vacuum [21]. *Centaurea horrida* herb and root extract was spray dried using Labplant Spray Dryer™ (Labplant, UK).

Animals

Before experimentation, Male Swiss-Webster mice weight from 18-22 gm and age between 18 and 24 weeks (Faculty of Pharmacy, Beirut Arab University (BAU), were housed for one week. The environment composed of standard mice cages with a 12-h light/dark cycle. The temperature was 22 ± 1°C, animals had an open access to water and standard laboratory pellets (20% proteins, 5% fats, and 1% multivitamins [21,22]. The mice were kept in those conditions for a 7-day period of adaptation prior to the start of the experiment. Sixteen hours before the experiments, they were fasted overnight, but permitted free access to water. All animal care and experiments were performed in accordance with animal experiment legislation and with approval of the local ethics commission.

Diabetes induction

Experimental diabetes was induced by i.p. injection of freshly prepared alloxan (Sigma-Aldrich, Germany) dissolved in sterile cold saline (0.9%) every 48-h for three times at a dose of 180 mg/kg. Fasting glucose levels in the blood samples obtained from the tail of each mouse 72 h after the last alloxan injection were measured with glucose strips test meter. The mice with blood glucose level more than 200 mg/dL were considered to be diabetic and were used in the experiments.

Acute effect of plant extracts in alloxan-induced diabetic mice

The diabetic mice were divided, for each extract, into 5 groups of seven animals each. Group I received only vehicle, sterile cold saline (0.9%), i.p. and served as control. Group II received glibenclamide as reference drug (5 mg/kg, i.p.) dissolved in DMSO. The plant extracts, dissolved in vehicle and was administered one extract at a time, at the doses of 12.5, 25 and 50 mg/kg (except *Centaurea horrida* herb and root extract dose was 25, 50 and 100 mg/kg) i.p. to the animals of group III, IV and V, respectively. Blood samples were collected from the tail just prior to and at 1, 2 and 6 h after dosing. Blood glucose and body weight were determined.

Subacute effect of the plant extracts in alloxan-induced diabetic mice

The action of plant extracts were also tested during a longer duration of treatment. The mice were divided into groups containing healthy and diabetic animals. Group I (healthy mice, n = 7) received only vehicle i.p. for 7 days and served as control [23]. The diabetic mice were divided into five groups (II– VI) of seven animals each. Group II received only vehicle, sterile cold saline (0.9%), i.p. for 7 days and served as diabetic control. Group III received glibenclamide as reference drug (5 mg/kg, i.p.) dissolved in DMSO for 7 days (positive control). The plant extracts, dissolved in vehicle, was administered at the doses of 12.5, 25 and 50 mg/kg (except *Centaurea horrida* herb and root extract doses were 25, 50 and 100 mg/kg) i.p. to the animals of group IV, V and VI, respectively. Blood samples were collected from the tail at 1st, 3rd, 5th, and 8th days after each treatment. Blood glucose and antioxidant enzyme (catalase) levels were measured. The body weights of the animals were also recorded at the same day.

Determination of blood glucose concentration

Blood glucose concentration was determined in blood by Accu-chek Active™ glucose strips in Accu-chek Active™ Test Meter (Roche, USA). The glucose levels were expressed as mg/dl.

Assessment of *in vivo* antioxidant activity

Catalase (CAT) activity was determined in serum using the modified method described before in literature [24]. Briefly, the reaction mixture at 30°C contains 5 μL of serum and 395 μL of phosphate buffer, pH 7.0. At zero time, the reaction is started by the addition of 200 μL of 38 mmol/L of H_2O_2 (in phosphate buffer), and the absorbance at 240 nm is monitored for 5 mm in a JASCO spectrophotometer (JASCO, Japan). Serum activity CAT activity was expressed as kU/l.

Hot plate test

For assessment of management of diabetic neuropathy, hot plate analgesia meter (Ugo Basile, Italy) was used. The animals were located one at a time on a hot plate that is maintained at a temperature of 55 ± 0.1°C. Response latency either to jump or a hindpaw lick was measured by means of an electronic timer. To prevent tissue damage, a cut-off time of 30 s was done [25].

Tail flick test

Tail-flick test was done on Tail-flick apparatus (Hugo Sachs Elektronik, Germany) was also used to assess the management of diabetic neuropathy. Briefly, a beam of light was focused on the dorsal surface of the mouse tail and the time until the tail flicked was recorded. The tail-withdrawal latency, time from onset of the radiant heat to the withdrawal of the tail, was detected with a timer. The light intensity in the apparatus was set such that the baseline tail-withdrawal latencies were ca. 5.6 s in all mice. A cut-off time of 10 s was done in order to prevent tissue damage [25].

Statistical analysis

All values were presented as means ± S.E.M. Statistical differences between the treatments and the controls were tested by two-way ANOVA using the "OriginPro" statistic computer program. A difference in the mean values of $p<0.05$ or less was regarded to be statistically significant.

Results and Discussions

Acute effect of the plant extracts in alloxan-induced diabetic mice

Acute effects of various doses of the different plant extracts in diabetic animals were studied using alloxan-diabetic mice. Glibenclamide prevented the drastic increase of blood glucose 1h after the glucose loading and reduced the level 2 and 6 h after the glucose loading.

Salvia libanotica fruticosa root extract (SLF) at all doses (12.5, 25 and 50 mg/kg) showed a significant effect, with blood glucose levels dropped to 50.3, 39.4 and 48.1%, respectively from that of control after 6 h of glucose administration (Table 1). It therefore appeared that 12.5 mg/kg of the SLF extract is the most effective SLF dose on blood glucose of hyperglycemic mice.

Moreover, *Centaurea horrida* herb and root spray dried methanolic extract (CH) at all doses (25, 50 and 100 mg/kg) showed a significant effect, with blood glucose levels dropped to 28.1, 31.2 and 49.0%, respectively from that of control after 6 h of glucose administration (Table 2). It therefore appeared that 100 mg/kg of the extract of CH is the most effective CH dose on acute blood glucose of hyperglycemic mice.

Furthermore, *Hordeum spontaneum* grain methanolic extract (HS) at all doses (12.5, 25 and 50 mg/kg) showed a significant effect, with blood glucose levels dropped to 33.3, 36.6 and 41.3%, respectively from that of control after 6 h of glucose administration (Table 3). It therefore appeared that 50 mg/kg of the HS extract is the most effective HS dose on blood glucose of hyperglycemic mice.

It could be concluded that 100 mg/kg of CH extract is the most effective acute hypoglycemic dose of the endogenous Lebanese medicinal plants under investigation.

Subacute effect of various plant extracts on alloxan-induced diabetic mice

In order to determine the subacute effects, three doses of each extract were administered throughout 8 days consecutively. The blood glucose level of each animal was monitored on 1st, 3rd, 5th and 8th days after the administration of the test samples. As shown in Tables 4-6 the blood glucose levels of diabetic control mice were significantly higher than those of the control mice during the experiment period.

In glucose-hyperglycemic mice, SLF at all doses (12.5, 25 and 50 mg/kg) showed a significant effect, with blood glucose levels dropping to 36.7, 34.1 and 33.6%, respectively from that of diabetic control on the 8th day. The highest reduction in blood glucose with SLF was observed with a dose of 50 mg/kg. It was more potent 7.1, 8.4 and 50.2% than that of 12.5, 25 mg/kg doses and glibenclamide respectively on the 8th day (Table 4).

Additionally, in glucose-hyperglycemic mice, CH at all doses (25, 50 and 100 mg/kg) showed a significant effect, with blood glucose levels

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		0 hr	0 hr	2 hr	6 hr
Diabetic control	__	201.29 ± 5.60	210.43 ± 4.50	213.71 ± 9.70	209.05 ± 7.30
Glibenclamide	5	219.20 ± 3.70	222.14 ± 1.80	158.24 ± 2.10	129.14 ± 2.40**
Salvia libanotica fruticosa	12.5	209.56 ± 4.40	193.33 ± 4.20	191.57 ± 2.50	103.89 ± 4.20*
Salvia libanotica fruticosa	25	213.66 ± 3.80	171.54 ± 4.20	221.43 ± 3.70	126.77 ± 3.40*
Salvia libanotica fruticosa	50	217.44 ± 3.20	154.56 ± 1.80	106.99 ± 2.90*	108.56 ± 1.80

S.E.M.: mean standard error
* $p<0.05$ significant from the control animals.
** $p<0.01$ significant from the control animals.

Table 1. Acute effect of *Salvia libanotica fruticosa* roots methanolic extract on blood glucose.

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		0 hr	0 hr	2 hr	6 hr
Diabetic control	__	201.29 ± 5.60	210.43 ± 4.50	213.71 ± 9.70	209.05 ± 7.30
Glibenclamide	5	219.20 ± 3.70	222.14 ± 1.80	158.24 ± 2.10	129.14 ± 2.40**
Centaurea horrida	25	211.65 ± 3.30	202.22 ± 3.30	170.77 ± 3.40	150.33 ± 3.10*
Centaurea horrida	50	214.44 ± 2.90	239.87 ± 3.10	154.33 ± 3.20	143.77 ± 2.90*
Centaurea horrida	100	219.33 ± 3.10	154.56 ± 1.80	106.99 ± 2.90	106.56 ± 1.80*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.

Table 2. Acute effect of *Centaurea horrida* herb and root spray dried methanolic extract on blood glucose.

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		0 hr	0 hr	2 hr	6 hr
Diabetic control	__	201.29 ± 5.60	210.43 ± 4.50	213.71 ± 9.70	209.05 ± 7.30
Glibenclamide	5	219.20 ± 3.70	222.14 ± 1.80	158.24 ± 2.10	129.14 ± 2.40**
Hordeum spontaneum	12.5	112.13 ± 1.10	231.79 ± 1.20	164.35 ± 1.40	139.43 ± 3.20*
Hordeum spontaneum	25	109.34 ± 2.60	202.46 ± 2.40	278.67 ± 2.10	132.56 ± 2.40*
Hordeum spontaneum	50	107.04 ± 2.30	218.67 ± 1.70	390.87 ± 5.30	122.74 ± 2.80*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.

Table 3. Acute effect of *Hordeum spontaneum* grains methanolic extract on blood glucose.

dropping to 49.6, 37.6 and 33.8%, respectively from that of diabetic control on the 8th day. The highest reduction in blood glucose with CH was observed with a dose of 100 mg/kg. It was more potent 24.2, 31.9 and 63.2% than that of 25, 50 mg/kg doses and glibenclamide respectively on the 8th day (Table 5).

Nevertheless, in glucose-hyperglycemic mice, HS at all doses (12.5, 25 and 50 mg/kg) showed a significant effect, with blood glucose levels dropping to 9.6, 27.1 and 35.4%, respectively from that of diabetic control on the 8th day. The highest reduction in blood glucose with HS was observed with a dose of 50 mg/kg. It was more potent 72.9, 23.4 and 48.4% than that of 12.5, 25 mg/kg doses and glibenclamide respectively on the 8th day (Table 6).

It could be concluded that 100 mg/kg of CH extract is the most effective subacute hypoglycemic dose of the medicinal plants under investigation.

During the subacute administration, mice treated with various doses of SLF, CH and HS extracts and glibenclamide were also monitored for changes in weight (Tables 7-9). The SLF extract showed 5.2, 7.9 and 19.5% increase in body weight at all doses, 12.5, 25 and 50 mg/kg respectively on the 8th day (Table 7). The CH extract showed 1.3, 1.6 and 4.5% increase in body weight at all doses, 25, 50 and 100 mg/kg respectively on the 8th day (Table 8). The HS extract showed 1.5, 4.9 and 7.5% increase in body weight at all doses, 12.5, 25 and 50 mg/kg respectively on the 8th day (Table 9).

It could be suggested that 100 mg/kg of SLF extract had the most effective subacute rise in weight on the 8th day of treatment with the medicinal plants under investigation.

In order to evaluate *in vivo* antioxidant effect of the various extracts, CAT level in serum of each mouse was monitored on 1st, 3rd, 5th and 8th days after the administration of the test samples.

As shown in Table 10, diabetic mice treated at all doses, 12.5, 25 and 50 mg/kg the SLF extract had a gradual rise in serum CAT activity to reach a significant difference on 5th (8.0, 13.3 and 20.1% respectively) and 8th day (6.3, 12.4 and 22.3% respectively) as compared with diabetic control mice.

Table 11, show that diabetic mice treated at all doses, 25, 50 and 100 mg/kg the CH extract had a gradual rise in serum CAT activity to reach a significant difference on 5th (10.5, 21.5 and 21.9% respectively) and 8th day (13.7, 21.0 and 24.9% respectively) as compared with diabetic control mice.

Likewise, diabetic mice, as shown in Table 12, treated at all doses, 12.5, 25 and 50 mg/kg the HS extract had a gradual rise in serum CAT activity to reach a significant difference on 5th (6.4, 16.5 and 19.1% respectively) and 8th day (7.0, 12.0 and 16.1% respectively) as compared with diabetic control mice. Therefore, 100 mg/kg of CH extract had the most effective subacute rise in serum CAT activity dose on the 8th day of treatment with the medicinal plants under investigation.

It has been found that CH 100 mg/kg is the most effective doses in all groups. This dose has more significant effect on blood glucose level compared to that of the synthetic drug, glibenclamide, as the acute and subacute antihyperglycemic was more potent and prolonged than those of glibenclamide.

Currently, much attention has been focused on the role of oxidative stress. It has been suggested that oxidative stress may comprise the key and common events in the pathogenesis of different diabetic complications [26].

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		1st day	3rd day	5th day	8th day
Control	__	106.50 ± 2.50	108.70 ± 3.60	107.36 ± 3.20	116.11 ± 4.70
Diabetic control[a]	__	201.29 ± 5.60***	210.43 ± 4.50***	213.71 ± 9.70***	209.05 ± 7.30***
Glibenclamide[b]	5	185.20 ± 3.70	178.03 ± 2.90	160.04 ± 2.40**	170.87 ± 3.10
Salvia libanotica fruticosa[b]	12.5	149.34 ± 3.60	140.55± 3.70	140.88 ± 3.90	138.77 ± 3.33**
Salvia libanotica fruticosa[b]	25	143.77 ± 2.90	139.88 ± 3.10	144.84 ± 3.40	137.67 ± 2.20**
Salvia libanotica fruticosa[b]	50	162.48 ± 2.60	168.78± 3.80	134.55 ± 2.40	132.24 ± 4.20**

S.E.M.: mean standard error
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 4. Subacute effect of *Salvia libanotica fruticosa* roots methanolic extract on blood glucose.

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		1st day	3rd day	5th day	8th day
Control	__	106.50 ± 2.50	108.70 ± 3.60	107.36 ± 3.20	116 ± 4.70
Diabetic control[a]	__	201.29 ± 5.60***	210.43 ± 4.50***	213.71 ± 9.70***	209.05 ± 7.30***
Glibenclamide[b]	5	185.20 ± 3.70	178.03 ± 2.90	160.04 ± 2.40**	170.87 ± 3.10
Centaurea horrida[b]	25	150.44 ± 3.40	142.69 ± 3.50	140.98 ± 3.60	138.33 ± 3.60*
Centaurea horrida[b]	50	143.77 ± 2.90	132.88 ± 3.10	131.48 ± 3.60	130.55 ± 2.40*
Centaurea horrida[b]	100	121.84 ± 2.40	108.56 ± 1.80	106.66 ± 1.90	105.42 ± 1.60*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 5. Subacute effect of *Centaurea horrida* herb and root methanolic extract on blood glucose.

Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)			
		1st day	3rd day	5th day	8th day
Control	__	106.50 ± 2.50	108.70 ± 3.60	107.36 ± 3.20	116 ± 4.70
Diabetic control[a]	__	201.29 ± 5.60***	210.43 ± 4.50***	213.71 ± 9.70***	209.05 ± 7.30***
Glibenclamide[b]	5	185.20 ± 3.70	178.03 ± 2.90	160.04 ± 2.40**	170.87 ± 3.10
Hordeum spontaneum [b]	12.5	194.21 ± 1.30	192.89 ± 2.70	190.45 ± 3.90	188.89 ± 3.40*
Hordeum spontaneum [b]	25	165.34 ± 2.70	153.88 ± 2.00	154.77 ± 2.40	152.33 ± 2.90*
Hordeum spontaneum [b]	50	147.12 ± 1.50	140.76 ± 2.10	137.89 ± 2.60	135.13 ± 1.80**

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 6. Subacute effect of *Hordeum spontaneum* methanolic extract on blood glucose.

		1st day	3rd day	5th day	8th day
Control	__	24.40 ± 0.50	24.50 ± 0.60 (0.4%)	24.66 ± 0.97 (1.1%)	25.12 ± 0.70(3.0%)
Diabetic control[a]	__	25.18 ± 0.70	25.60 ± 0.20 (1.7%)	26.65 ± 0.80 (5.8%)	27.19 ± 0.50(8.0%)
Glibenclamide[b]	5	21.40 ± 0.70	26.67 ± 1.70 (24.6%)	27.04 ± 0.40 (26.4%)	28.87 ± 1.10 (34.9%)*
Salvia libanotica fruticosa[b]	12.5	20.00 ± 2.60	20.50 ± 2.50 (2.4%)	20.50 ± 2.90 (2.4%)	21.10 ± 2.50 (5.2%)*
Salvia libanotica fruticosa[b]	25	22.10 ± 1.40	23.10 ± 1.90 (4.3%)	23.50 ± 1.50 (6.0%)	24.00 ± 1.50 (7.9%)*
Salvia libanotica fruticosa[b]	50	19.55 ± 2.80	21.22 ± 2.10 (7.9%)	22.18 ± 2.50 (11.9%)	24.30 ± 2.40 (19.5%)*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 7. Subacute effect of *Salvia libanotica fruticosa* roots methanolic extract on body weights in alloxan-induced diabetic mice.

Group	Dose (mg/kg)	Mean body weight ± S.E.M. (gm) (% increase from the initial weight)			
		1st day	3rd day	5th day	8th day
Control	__	24.40 ± 0.50	24.50 ± 0.60 (0.4%)	24.66 ± 0.97 (1.1%)	25.12 ± 0.70 (3.0%)
Diabetic control[a]	__	25.18 ± 0.70	25.60 ± 0.20 (1.7%)	26.65 ± 0.80 (5.8%)	27.19 ± 0.50 (8.0%)
Glibenclamide[b]	5	21.40 ± 0.70	26.67 ± 1.70 (24.6%)	27.04 ± 0.40 (26.4%)	28.87 ± 1.10 (34.9%)*
Centaurea horrida[b]	25	22.00 ± 2.80	22.10 ± 2.90 (0.5%)	22.00 ± 3.00 (NC[c])	22.30 ± 2.50 (1.3%)
Centaurea horrida[b]	50	31.10 ± 1.90	31.00 ± 1.80 (NC[c])	31.50 ± 1.70 (1.6%)	31.60 ± 1.50 (1.6%)
Centaurea horrida[b]	100	30.55 ± 2.90	31.22 ± 3.10 (2.2%)	31.18 ± 2.50 (2.0%)	32.00 ± 2.60 (4.5%)*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.
[c] NC: No change.

Table 8. Subacute effect of *Centaurea horrida* herb and root methanolic extract on body weights in alloxan induced diabetic mice.

Group	Dose (mg/kg)	Mean body weight ± S.E.M. (gm) (% increase from the initial weight)			
		1st day	3rd day	5th day	8th day
Control	__	24.40 ± 0.50	24.50 ± 0.60 (0.4%)	24.66 ± 0.97 (1.1%)	25.12 ± 0.70 (3.0%)
Diabetic control[a]	__	25.18 ± 0.70	25.60 ± 0.20 (1.7%)	26.65 ± 0.80 (5.8%)	27.19 ± 0.50 (8.0%)
Glibenclamide[b]	5	21.40 ± 0.70	26.67 ± 1.70 (24.6%)	27.04 ± 0.40 (26.4%)	28.87 ± 1.10 (34.9%)*
Hordeum spontaneum [b]	12.5	25.87 ± 0.90	25.12 ± 0.60 (-2.8%)	26.13 ± 0.70 (1.0%)	26.26 ± 0.50 (1.5%)
Hordeum spontaneum [b]	25	24.25 ± 0.70	24.75 ± 0.40 (2.1%)	25.07 ± 0.20 (3.4%)	25.43 ± 0.80 (4.9%)*
Hordeum spontaneum [b]	50	25.36 ± 0.70	26.24 ± 0.50 (3.5%)	26.85 ± 0.80 (5.9%)	27.25 ± 1.10 (7.5%)*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 9. Subacute effect of *Hordeum spontaneum* methanolic extract on body weights in alloxan-induced **diabetic mice.**

SLF, CH and HS extracts showed a significant increase in body weight, as an evidence of alleviating of hyperglycemia, as demonstrated before with pharmacotherapies used in management of DM [27].

In our study, the activity of CAT decreased in diabetic mice as reported earlier [26,28] which could be due to inactivation caused by alloxan-generated ROS. Long-term treatment of DM with all doses, especially with the highest dose of the SLF, CH and HS extracts had reversed the activities of this enzymatic antioxidant, which might be due to lessened oxidative stress as evidenced by the elevation in CAT activity.

The mechanism of the hypoglycemic activity of SLF, CH and HS extracts might be independent from insulin secretion. These extracts might inhibit the endogenous glucose production or by the inhibition of intestinal glucose absorption and might play a role in controlling dietary glucose uptake in the small intestinal track [23].

Plant extracts improved neurological function in the diabetic mouse

Impairment of peripheral nerve conduction is a key indicator for diabetic patients having peripheral neuropathy [29,30]. We then examined the effect of various plant extracts treatment on sensory function by measuring the thermal latency with tail flick and hot plate tests. Treatment of the alloxan treated diabetic mice with SLF markedly improved the thermal latency (Figure 1 and 2).

Diabetic mice exhibited transient hyperalgesic response in thermal tests. On the 8th week after alloxan injection, treatment with all doses of SLF, hot-plate latency markedly improved by 29.3, 33.2 and 36.6% in all SLF doses, 12.5, 25 and 50 mg/kg respectively, compared to vehicle treated group (Figure 1).

Nevertheless, on the 8th week after alloxan injection, treatment with all doses of SLF, tail-flick latency markedly improved by 29.9, 34.8 and 61.6% in all SLF doses, 12.5, 25 and 50 mg/kg respectively, compared to vehicle treated group (Figure 2).

Treatment of the alloxan treated diabetic mice with CH markedly improved the thermal latency (Figure 3 and 4).

On the 8th week after alloxan injection, treatment with all doses of CH, hot-plate latency markedly improved by 23.8, 30.3 and 45.8% in all CH doses, 25, 50 and 100 mg/kg respectively, compared to vehicle treated group (Figure 3).

Group	Dose (mg/kg)	Catalase level ± S.E.M. (kU/l)			
		1st day	3rd day	5th day	8th day
Control	__	39.50 ± 1.50	40.10 ± 1.60	39.36 ± 1.20	40.12 ± 1.70
Diabetic control[a]	__	20.17 ± 1.60***	19.43 ± 1.30***	22.51 ± 1.90***	24.05 ± 1.40***
Glibenclamide[b]	5	21.10 ± 1.70	23.5 ± 1.70	30.04 ± 1.40	30.87 ± 1.00**
Salvia libanotica fruticosa [b]	12.5	20.33 ± 1.40	23.78 ± 2.60	24.45 ± 1.50*	25.66 ± 1.50*
Salvia libanotica fruticosa [b]	25	19.73 ± 1.40	24.77 ± 1.30	25.97 ± 2.70*	27.44 ± 2.70*
Salvia libanotica fruticosa [b]	50	20.65 ± 1.60	26.38 ± 1.50	28.17 ± 1.30*	30.95 ± 1.10*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 10. *In vivo* assessment of the antioxidant activity of *Salvia libanotica fruticosa* methanolic extract using catalase levels in serum of alloxan-induced diabetic mice.

Group	Dose (mg/kg)	Catalase level ± S.E.M. (kU/l)			
		1st day	3rd day	5th day	8th day
Control	__	39.50 ± 1.50	40.10 ± 1.60	39.36 ± 1.20	40.12 ± 1.70
Diabetic control[a]	__	20.17 ± 1.60***	19.43 ± 1.30***	22.51 ± 1.90***	24.05 ± 1.40***
Glibenclamide[b]	5	21.10 ± 1.70	23.5 ± 1.70	30.04 ± 1.40	30.87 ± 1.00**
Centaurea horrida [b]	25	19.45 ± 1.80	24.97 ± 2.50	25.14 ± 1.80*	27.87 ± 1.10*
Centaurea horrida [b]	50	21.37 ± 1.70	27.43 ± 1.90	28.66 ± 2.40*	30.43 ± 2.60*
Centaurea horrida [b]	100	20.65 ± 1.50	28.38 ± 1.50	28.81 ± 1.80*	32.01 ± 1.10*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 11. *In vivo* assessment of the antioxidant activity of *Centaurea horrida* methanolic extract using catalase levels in serum of alloxan-induced diabetic mice.

Group	Dose (mg/kg)	Catalase level ± S.E.M. (kU/l)			
		1st day	3rd day	5th day	8th day
Control	__	39.50 ± 1.50	40.10 ± 1.60	39.36 ± 1.20	40.12 ± 1.70
Diabetic control[a]	__	20.17 ± 1.60***	19.43 ± 1.30***	22.51 ± 1.90***	24.05 ± 1.40***
Glibenclamide[b]	5	21.10 ± 1.70	23.5 ± 1.70	30.04 ± 1.40	30.87 ± 1.00**
Hordeum spontaneum [b]	12.5	21.33 ± 1.40	23.89 ± 2.70	24.04 ± 1.40*	25.87 ± 1.10*
Hordeum spontaneum [b]	25	20.73 ± 1.40	26.34 ± 1.60	26.97 ± 2.30*	27.33 ± 2.80*
Hordeum spontaneum [b]	50	21.55 ± 1.60	27.10 ± 1.40	27.81 ± 1.80*	28.65 ± 1.30*

S.E.M.: mean standard error
* $p < 0.05$ significant from the control animals.
** $p < 0.01$ significant from the control animals.
*** $p < 0.001$ significant from the control animals.
[a] Compared to vehicle control.
[b] Compared to diabetic control.

Table 12. *In vivo* assessment of the antioxidant activity of *Hordeum spontaneum* grains methanolic extract using catalase levels in serum of alloxan-induced diabetic mice.

Nevertheless, on the 8th week after alloxan injection, treatment with all doses of CH, tail-flick latency markedly improved by 10.4, 25.9 and 32.8% in all CH doses, 25, 50 and 100 mg/kg respectively, compared to vehicle treated group (Figure 2).

Treatment of the alloxan treated diabetic mice with HS somewhat improved the thermal latency (Figure 5 and 6).

On the 8th week after alloxan injection, treatment with all doses of HS, hot-plate latency markedly improved by 9.6, 18.7 and 25.0% in all HS doses 12.5, 25 and 50 mg/kg respectively, compared to vehicle treated group (Figure 5).

Furthermore, on the 8th week after alloxan injection, treatment with all doses of HS, tail-flick latency markedly improved by 2.3, 17.3 and 28.3% in all HS doses, 12.5, 25 and 50 mg/kg respectively, compared to vehicle treated group (Figure 6).

These data suggest that SLF, CH and HS improve peripheral nerve function in the diabetic mouse. Our work highlight that the

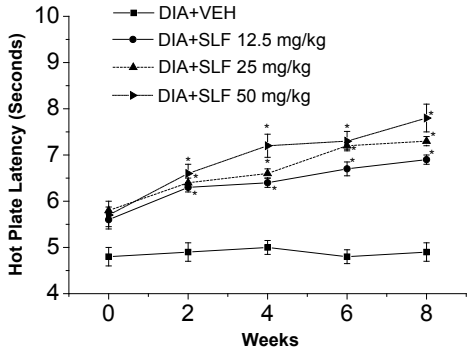

Figure 1: Effect of *Salvia Libanotica Fruticosa* (SLF) on the hot plate latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ SLF 12.5 mg/kg: : diabetic animals treated with SLF 12.5 mg/kg. (Up triangles and dashed line) DIA+ SLF 25 mg/kg: diabetic animals treated with SLF 25 mg/kg. (Right triangles and dashed-dotted line) DIA+ SLF 50 mg/kg: diabetic animals treated with SLF 50 mg/kg. Data are expressed in mean ± S.E.M. * P < 0 .05 is compared with control.

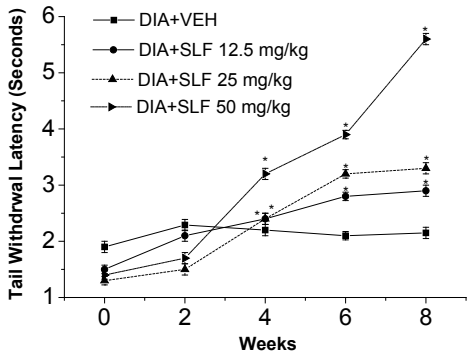

Figure 2: Effect of *Salvia Libanotica Fruticosa* root extract (SLF) on the tail withdrawal latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ SLF 12.5 mg/kg: : diabetic animals treated with SLF 12.5 mg/kg. (Up triangles and dashed line) DIA+ SLF 25 mg/kg: diabetic animals treated with SLF 25 mg/kg. (Right triangles and dashed-dotted line) DIA+ SLF 50 mg/kg: diabetic animals treated with SLF 50 mg/kg. Data are expressed in mean ± S.E.M. * P < 0 .05 is compared with control.

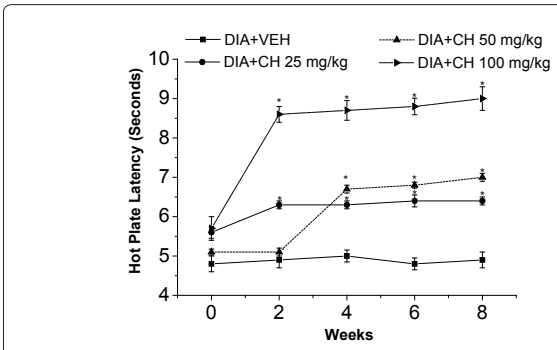

Figure 3: Effect of *Centaurea horrida* (CH) on the hot plate latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ CH 25 mg/kg: diabetic animals treated with CH 25 mg/kg. (Up triangles and dashed line) DIA+ CH 50 mg/kg: diabetic animals treated with CH 50 mg/kg. (Right triangles and dashed-dotted line) DIA+ CH 100 mg/kg: diabetic animals treated with CH 100 mg/kg. Data are expressed in mean ± S.E.M. * P < 0 .05 is compared with control.

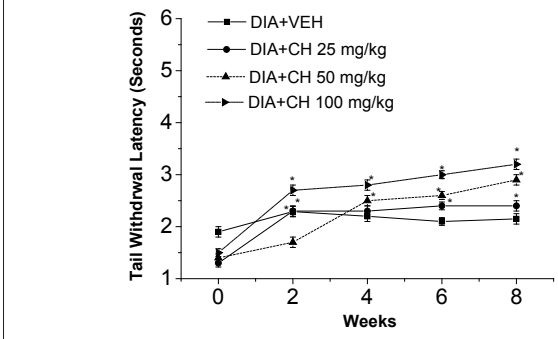

Figure 4: Effect of *Centaurea horrida* herb and root extract (CH) on the tail withdrawal latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ CH 25 mg/kg: diabetic animals treated with CH 25 mg/kg. (Up triangles and dashed line) DIA+ CH 50 mg/kg: : diabetic animals treated with CH 50 mg/kg. (Right triangles and dashed-dotted line) DIA+ CH 100 mg/kg: diabetic animals treated with CH 100 mg/kg. Data are expressed in mean ± S.E.M. * P < 0 .05 is compared with control.

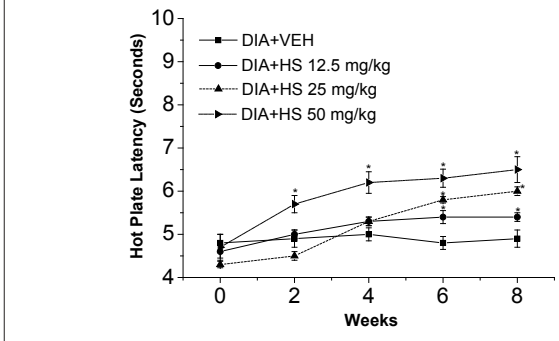

Figure 5: Effect of *Hordeum spontaneum* grain extract (HS) on the tail withdrawal latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ HS 12.5 mg/kg: diabetic animals treated with HS 12.5 mg/kg. (Up triangles and dashed line) DIA+ HS 25 mg/kg: diabetic animals treated with HS 25 mg/kg. (Right triangles and dashed-dotted line) DIA+ HS 50 mg/kg: diabetic animals treated with HS 50 mg/kg. Data are expressed in mean ± S.E.M. * P<0.05 is compared with control.

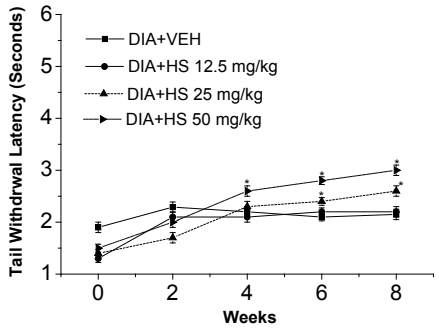

Figure 6: Effect of *Hordeum spontaneum* grain extract (HS) on the tail withdrawal latency in alloxan treated mice. (Closed squares and straight line) DIA+ VEH: diabetic animals treated with vehicle as control. (Open circles and straight line) DIA+ HS 12.5 mg/kg: diabetic animals treated with HS 12.5 mg/kg. (Up triangles and dashed line) DIA+ HS 25 mg/kg: diabetic animals treated with HS 25 mg/kg. (Right triangles and dashed-dotted line) DIA+ HS 50 mg/kg: : diabetic animals treated with HS 50 mg/kg. Data are expressed in mean ± S.E.M. * P < 0 .05 is compared with control.

improvement of catalase activity might be an indicator for improvement of the peripheral nerve function in the diabetic mouse as well as management of hyperglycemia.

In conclusion, Systemic administration of SLF, CH and HS extracts alleviated hyperalgesia in pain conditions. Our findings provide clinicians promising drugs for the management of the symptoms of diabetic neuropathy.

The present study indicated that endogenous Lebanese plants, explicitly, *Salvia libanotica fruticosa* roots, *Centaurea horrida* herb and roots and *Hordeum spontaneum* grains extracts exerted remarkable hypoglycemic activity and improved peripheral nerve function which will pave the way for management of diabetes and its related complications especially, diabetic neuropathy.

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