

Blood Glucose Level Decrease Caused by Extracts and Fractions from *Lepechinia caulescens* in Healthy and Alloxan-diabetic Mice

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Abstract

The traditional preparation, hexane, methylene chloride, methanol and water extracts obtained from the flowers of *Lepechinia caulescens* (Labiatae) were administered to fasting healthy mice. The investigation results showed that only the traditional preparation and the water extract significantly reduce blood glucose after intraperitoneal administration ($P < 0.05$). The water extract, whose effects were in a dose-dependent manner, was macerated with methanol obtaining a precipitate (F1 fraction) and a methanol soluble fraction (F2 fraction), and both were studied in healthy mice. Methanol fraction F2 did not significantly decrease blood glucose level in this experimental model. The water fraction F1 showed significant hypoglycemic activity in healthy and mild alloxan-diabetic mice, but not in severe alloxan-diabetic mice.

Keywords: Hypoglycemic plants, anti-diabetic plants, medicinal plants, *Lepechinia caulescens*.

Introduction

In Mexico, about 150 plants are used by the population to empirically control diabetes mellitus (Alarcon-Aguilar et al., 1993), a disease that currently represents a major health problem worldwide (Committee Report, 1997). The most studied Mexican anti-diabetic plants are *Opuntia streptacantha* (cactus), *Tecoma stans* (“tronadora”), and *Psacalium decompositum* (“matarique”) (Ibañez-Camacho & Roman-Ramos, 1979; Meckes & Mellado, 1985; Frati-

Munari et al., 1989; Alarcon-Aguilar et al., 2000; Ramos et al., 2000). Furthermore, about 60 Mexican plants have been experimentally evaluated and a hypoglycemic effect has been detected in most of them (Roman-Ramos et al., 1992a; 1995; Alarcon-Aguilar et al., 1998). One of the most important of these is *Lepechinia caulescens* (Ort.) Epl., Labiatae, herbaceous popularly known as “salvia” or “bretonica”. A decoction prepared from the flowers of *L. caulescens* has been shown to decrease glycemic levels in temporally hyperglycemic rabbits and has also exhibited hypoglycemic activity in alloxan-diabetic rabbits (Roman-Ramos et al., 1991, 1992b).

Phytochemical studies have shown the presence of some compounds in *L. caulescens*, such as the abietanoid acid, as well as diterpenoid and triterpenoid acids (Delgado et al., 1992; 1994). However, there is no experimental evidence of the hypoglycemic action of these constituents.

The objectives of this study were to evaluate the hypoglycemic effect of the hexane, methylene chloride, methanol, and water extracts of *Lepechinia caulescens* in healthy mice and to determine the hypoglycemic effect of two fractions obtained from the water extract of *L. caulescens* flowers in healthy and alloxan-diabetic mice.

Materials and methods

Plant material

Herbs of *L. caulescens* were acquired from the Sonora Herbal Market at Mexico City. The identification was made, with the

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help of an expert in botany, using taxonomic rules and by means of comparisons among different herbarium samples of *L. caulescens* from MEXU-HERBARIUM (Herbarium IMSSM-Voucher Specimen 11477).

Hypoglycemic effect of traditional preparation of *Lepechinia caulescens*

The plant was prepared in the same way as described previously for *L. caulescens* (Roman-Ramos et al., 1991). Separated dried flowers (40 g) were steeped in boiling water (300 ml) for 10 min and then left to cool at room temperature. The decoctions then were filtered and directly administered to experimental animals (4 ml/kg weight or 0.102 g dried plant weight/kg body weight). A similar decoction is the common form of administration used in popular medicine.

Preparation of the extracts and fractions isolated from the water extract

The extracts were prepared as follows. *L. caulescens* flowers (1000 g) were ground and extracted three times at room temperature with hexane (3 L, 48 h). The hexane extracts were concentrated under reduced pressure and pooled, obtaining 37.10 g (yield 3.7%). Then, the plant residue was extracted three times at room temperature with methylene chloride (3 L, 48 h). The methylene chloride extracts were concentrated under reduced pressure and pooled, obtaining 34.02 g (yield 3.4%). Then, the plant residue was extracted three times at room temperature with methanol (3 L, 48 h). The methanol extracts were concentrated under reduced pressure and pooled, obtaining 56.21 g (yield 5.6%). Finally, the marc was extracted one time at room temperature with water (1 L, 24 h). The water was freeze dried, obtaining 9.2 g as residue (yield 0.92%). This material was macerated with methanol (200 ml, 24 h) obtaining a precipitate (F1 fraction = 5.5 g) and a methanol soluble fraction (F2 fraction = 3.7 g).

Experimental animals

The experimental animals were male adult mice (CD1 strain) weighing from 25 to 35 g. They were given free access to food and water. Experimental diabetes in mice, subjected to previous fasting for 18 h, was induced by intraperitoneal administration of alloxan (Rodriguez et al., 1975). The total dose of alloxan (450 mg/kg wt.) was administered in 3 injections at intervals of 48 h (150 mg/kg body weight each time). Seven days after the last administration, the animals were fasted for 18 h and blood glucose levels were determined. These animals were included in two experimental groups: a) mild alloxan-diabetic mice, whose basal glycemia ranged between 200 to 349 mg/dl; and b) severe alloxan-diabetic mice, whose basal glycemia was equal or higher than 350 mg/dl.

Biological assays

*Hypoglycemic activity of the traditional preparation obtained from *Lepechinia caulescens* flowers on fasting-blood glucose levels in healthy mice*

Healthy mice were divided into three groups of 10 animals each (I–III). Groups I and II served as controls and received isotonic saline solution (ISS) or fast action-insulin (regular insulin) as reference (0.1 U.I./kg weight). Group III received 4 ml/kg body weight of the water decoction (traditional preparation).

*Hypoglycemic activity of extracts from *Lepechinia caulescens* flowers on fasting-blood glucose levels in healthy mice*

Healthy mice were divided into six groups of 20 animals each (IV–IX). Group IV and V served as controls and received ISS or corn oil. The other four groups received 1600 mg/kg body weight of each extract. Organic extracts (hexane, methylene chloride and methanol) were dissolved in corn oil, and water extract in ISS.

The other five groups of 7 to 20 healthy mice (X–XIV) were treated under the same conditions but in these cases, only the water extract was administered at doses of 100, 200, 400, and 800 mg/kg body weight, respectively.

*Effects of the fractions isolated from *Lepechinia caulescens* flowers on fasting-blood glucose levels in healthy and alloxan-diabetic mice*

Healthy mice were divided into six groups of 8–10 animals each (XV–XX). Group XV served as control and received ISS; Groups XVI and XVII received 400 and 1000 mg/kg of F1 fraction; Groups XVIII, XIX, and XX received 400, 1000, and 1600 mg/kg body weight of F2 fraction, both dissolved in ISS.

Mild alloxan-diabetic mice were divided into two groups of 14 and 9 animals each. Group 1 served as control and received ISS; Group 2 received F1 fraction (400 mg/kg weight). Severe alloxan-diabetic mice also were divided into two groups (3 and 4) with 26 and 12 animals: Group 3 served as control and received ISS; Group 4 received 400 mg/kg body weight of F1 fraction.

In all cases the control substances, extracts, and fractions were injected intraperitoneally (4 ml/kg wt.). Blood samples were obtained from the tail vein in fasting animals ($t = 0$), and 120 and 240 min after administration substances of test. Glycemia was determined by the glucose-oxidase peroxidase method with Haemo-Glukotest 20–800 reagent strips and their evaluation was made on a Reflolux-S lightmeter (Boehringer-Mannheim).

Statistical analysis

Results were expressed as mean \pm S.E.M. The significance of the differences between the means of tests and control

studies was established by Student's *t*-test for independent samples with one tail. *P*-values < 0.05 were considered significant.

Results

The effects of *Lepechinia caulescens* on the fasting blood glucose levels of normal mice are shown in Table 1. The mean blood glucose levels of mice after intraperitoneal administration of the decoction were compared with the values in control mice which received isotonic saline solution and also with those in animals receiving 0.1 I.U./kg body weight of fast action insulin.

The basal glycemic level was 45.9 ± 2.9 mg/dl (control). There was no statistical difference between the glycemic levels of the studied groups. No differences in blood glucose were observed between the levels at times 120 and 240 min after administration of the isotonic solution, when compared with the basal values in control mice. However, after the administration of *L. caulescens*, the blood glucose levels decreased significantly at 240 min ($P < 0.05$), from control. The effect of insulin at 240 min was as high as that of *L. caulescens* ($P < 0.05$).

The extracts of hexane, methylene chloride, and methanol obtained from *L. caulescens* flowers, administered at a dose of 1600 mg/kg body weight, did not show significant hypo-

glycemic effect in healthy mice. However, the same dose of the water extract showed a significant hypoglycemic effect ($P < 0.05$). In addition, the water extract also showed a dose-dependent effect when it was administered at 100, 200, 400, and 800 mg/kg body weight (Table 2). Doses of 400 and 800 mg/kg caused a highly significant decrease of the glycemia at 120 min ($P < 0.005$).

The methanol soluble fraction (F2 fraction) did not significantly decrease blood glucose level in healthy mice (Table 3). The water soluble F1 fraction obtained from the water extract after treatment with methanol caused a significant decrease in glycemia of healthy and alloxan-diabetic mice (Tables 3 and 4). The F1 fraction (400 and 1000 mg/kg) showed a significant decrease in blood glucose levels of healthy mice at 120 and 240 min ($P < 0.05$). The F1 fraction also caused a significant decrease ($P < 0.01$) in blood glucose levels of mild diabetic mice 240 min after administration. However, in animals with severe diabetes, the F1 fraction did not show hypoglycemic activity.

Discussion

The aim of this study was to evaluate the hypoglycemic activity of *L. caulescens*, to establish a basis for the isolation of the hypoglycemic active principles, and to validate the use of these plants in diabetes mellitus control. Our results reveal

Table 1. Effect of the traditional preparation (water decoction) obtained from the flowers of *L. caulescens* on blood glucose levels in fasting healthy mice (n = 10).

Study	Dose	Blood glucose mg/dl (mean \pm S.E.M)		
		In fasting	120 min	240 min
Control (ISS)	4 ml/kg	45.9 ± 2.9	43.8 ± 1.6	42.2 ± 2.2
Insulin	0.1 U.I./kg	47.4 ± 3.0	38.7 ± 2.9	$36.4 \pm 2.3^*$
<i>Lepechinia caulescens</i>	4 ml/kg	50.3 ± 2.7	40.0 ± 1.7	$34.4 \pm 1.9^{**}$

Significantly different from the pre-value in fasting: * $P < 0.05$; ** $P < 0.01$.

Table 2. Effects of the water extract obtained from the flowers of *L. caulescens* on blood glucose levels in fasting healthy mice.

Study	n	Dose (mg/kg)	Blood glucose mg/dl (mean \pm S.E.M)		
			In fasting	120 min	240 min
Control (SSI)	20	–	52.6 ± 2.3	49.5 ± 2.3	49.6 ± 2.5
Water extract	7	100	49.4 ± 2.9	46.1 ± 5.6	$39.1 \pm 5.9^*$
	18	200	51.5 ± 3.0	54.5 ± 3.3	$37.4 \pm 2.6^{**}$
	10	400	52.5 ± 3.1	60.8 ± 4.8	$26.1 \pm 4.1^{**}$
	18	800	54.7 ± 4.0	$40.9 \pm 4.7^*$	$18.8 \pm 4.0^{**}$

Significantly different from control: * $P < 0.05$; ** $P < 0.005$.

Table 3. Effect of the F1 and F2 fractions isolated from the active water extract of *L. caulescens* flowers on blood glucose levels in fasting healthy mice.

Study	n	Dose (mg/kg)	Blood glucose mg/dl (mean \pm S.E.M)		
			In fasting	120 min	240 min
Control (SSI)	9	–	57.9 \pm 4.5	59.2 \pm 3.1	58.9 \pm 2.9
F1	9	400	53.8 \pm 3.7	47.4 \pm 4.3*	44.9 \pm 2.8**
	8	1000	55.1 \pm 1.9	31.8 \pm 4.0**	28.4 \pm 3.6**
F2	10	400	59.0 \pm 2.0	53.7 \pm 3.0	51.1 \pm 3.6
	9	1000	56.1 \pm 3.3	59.0 \pm 3.3	56.4 \pm 2.4
	8	1600	53.5 \pm 2.5	57.3 \pm 2.5	56.4 \pm 4.3

Significantly different from control: * P < 0.05; ** P < 0.005.

Table 4. Effect of F1 fraction obtained from water extract of *L. caulescens* flowers on blood glucose levels in fasting mild alloxan-diabetic mice.

Study	n	Dose (mg/kg)	Blood glucose mg/dl (mean \pm S.E.M)		
			In fasting	120 min	240 min
Control (ISS)	14	–	276.8 \pm 17.3	232.0 \pm 24.3	206.1 \pm 32.1
F1 fraction	9	400	241.3 \pm 23.6	255.8 \pm 32.5	82.4 \pm 22.2*

Significantly different from control: * P < 0.01.

that the water decoction of *L. caulescens* has a significant hypoglycemic effect in normoglycemic mice. In these animals, the hypoglycemic effect caused by insulin validates the experimental model employed. These results confirm the previously observed hypoglycemic activity of *L. caulescens* flowers water decoction in temporally hyperglycemic and alloxan-diabetic rabbits (Roman-Ramos et al., 1992b). *L. caulescens* flowers water decoction caused hypoglycemic effect in healthy and mild alloxan-diabetic rabbits but had no effect in severe alloxan diabetic rabbits. These data suggest that the *L. caulescens* active substances require the presence of functioning beta cells.

The results of this investigation show that the water extract of *L. caulescens* flowers exhibits the highest hypoglycemic effect in healthy mice compared to hexane, methylene chloride, and methanol extracts. The hypoglycemic effect of water extract was evident at doses of 100, 200, 400, 800, and 1600 mg/kg.

Di- and triterpenoid acids are the major components obtained from the organic extracts of *L. caulescens* (Delgado et al., 1992; 1994). Although there is no experimental evidence for the hypoglycemic action of these constituents to date, when the anti-diabetic properties of the organic extracts (hexane, methylene chloride and methanol) were evaluated in this research, the results were negative.

F1 fraction produced an important hypoglycemic effect in healthy and in mild diabetic mice. However, it requires the presence of functioning beta cells, because it was ineffective in severe diabetic mice.

Various plants of the Labiatae are empirically used in diabetes mellitus control, and some of them exhibit hypoglycemic effects. *Teucrium polium* Linn. caused significant reductions in blood glucose of normoglycemic and streptozotocin-hyperglycemic rats (Munir et al., 1988). *Salvia lavandulifolia* Vahl. had hypoglycemic action in healthy rats (Zarzuelo et al., 1990). *Calaminta macrostema* showed hypoglycemic activity in healthy and alloxan-diabetic mice (Pérez-Gutierrez et al., 1984). *Marrubium vulgare* Linn. and *Teucrium cubense* Jacq. reduced the glycemia in temporally hyperglycemic rabbits (Roman-Ramos et al., 1991; 1992a). However, the hypoglycemic compounds have not been identified.

In conclusion, the water extract obtained from *L. caulescens* flowers exhibits hypoglycemic activity in normoglycemic mice. The hexane, methylene chloride and methanol extracts did not show a hypoglycemic effect. The F1 fraction isolated from the water extract exhibited hypoglycemic activity in healthy and mild alloxan-diabetic mice. Chemical and pharmacological investigations should be carried out to evaluate the hypoglycemic activity in diabetic animals of the main components that can be isolated from the active F1 fraction.

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