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Ethnopharmacological communication

Anti-hyperglycemic effect of *Opuntia streptacantha* LemAdolfo Andrade-Cetto^{a,*}, Helmut Wiedenfeld^b^a Laboratorio de Etnofarmacología, Facultad de Ciencias, Universidad Nacional Autónoma de México, 04511 México, DF, Mexico^b University of Bonn, Pharmaceutical Institute, An der Immenburg 4, D-53121 Bonn, Germany

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ABSTRACT

Aim of the study: This experiment studied two extracts of *Opuntia streptacantha*, a plant used by the Mexican population to treat type 2 diabetes, in different assays to contribute to the understanding of the hypoglycemic mechanism of this plant.

Materials and methods: Two different extracts were prepared and tested: the first extract was a filtrate of the traditional liquefied extract (LE) preparation of the cladode; and the second filtrate extract (FE) is a filtered sample of the first. Both extracts contained a newly identified compound for *Opuntia* (4-hydroxy)-phenyl acetic acid derivate, they were tested on streptozotocin (STZ)-diabetic rats in a series of two tests. The first test was performed to confirm if STZ-diabetic rats presented a hypoglycemic effect after administration of the extracts (LE 135 mg/kg and FE 27 mg/kg). In the second experiment, the extracts were administered before an oral glucose tolerance test (OGTT) to confirm if they have an anti-hyperglycemic effect (LE 135 mg/kg, FE 12 and 27 mg/kg).

Results: The extracts administered to STZ-diabetic rats did not produce a significant hypoglycemic effect compared to the control group, while the same extracts administered before an OGTT produced an anti-hyperglycemic effect compared to the control group.

Conclusions: The filtered, traditional LE of the cladode of *Opuntia streptacantha* produces an anti-hyperglycemic effect when administered before a glucose challenge, and this anti-hyperglycemic effect is maintained after filtering the extract.

Administration of both plants can improve glycemic control by blocking the hepatic glucose output, especially in the fasting state. These data support the traditional use of the plants as “agua de uso”, a cold infusion of the plant consumed over the course of a day.

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1. Introduction

The term “diabetes mellitus” is used to refer to a metabolic disorder of multiple etiologies in which chronic hyperglycemia is caused by defects in either the secretion or action of insulin or alterations in both of these processes. This results in disturbances in the metabolism of carbohydrates, fats and proteins. Type 2 diabetes is a particular form of diabetes caused either predominantly by insulin resistance with a relative deficiency of insulin or predominantly by impaired insulin secretion that may or may not be accompanied by insulin resistance (WHO, 1999; Inzucchi, 2003).

Type 2 diabetes is one of the most prevalent health problems in Mexico (SSA, 2010), and common treatment options include a wide variety of both medicinal products and health food plants (Andrade-Cetto and Heinrich, 2005). In Mexico, over 306 plant

species from 235 genera and 93 families have been reported to treat diabetes.

Opuntia streptacantha Lemaire (Cactaceae), known as Nopal in Mexico, is a plant with anti-hyperglycemic effects used to treat type 2 diabetes. The traditional use came from the ancient Mexican tribes, as documented in the Florentine codex S. XVI and by F. Hernandez. The cladode of the plant is traditionally used to treat gastritis, intestinal colic and ulcers (Argueta, 1994), while recent clinical studies led by Shane-McWhorter (2001, 2005), Shapiro and Gong (2002), Yeh et al. (2003), and Najm and Desiree (2010), report the effects of this plant on type 2 diabetic patients. In a recent study with OpunDia™, a combination of extracts from cladodes and fruit skins of *Opuntia ficus-indica*, the acute blood glucose lowering effects on type 2 diabetic patients were shown only after an OGTT (Godard et al., 2010), but there has yet to be a clear, established mechanism of action for this plant (Najm and Desiree, 2010).

2. Aim of the study

The aim of our study was test two extracts of *Opuntia streptacantha* in different assays helping to understand the hypoglycemic properties of the plant.

Abbreviations: OGTT, oral glucose tolerance test; LE, liquefied extract; FE, filtrate extract; STZ, streptozotocin.

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For the first assay, the extracts were administered to STZ-diabetic rats to demonstrate whether the extracts show a hypoglycemic effect. During the second test, the extracts were administered before an oral glucose tolerance test (OGTT) to illustrate if the extracts have an anti-hyperglycemic effect. Both experiments were performed with two extract samples: the first was prepared in the traditional way to produce a liquefied extract (LE); and the second was a filtrate of the first extract to create a filtrate extract (FE) containing a newly isolated compound.

3. Materials and methods

3.1. Plant extracts

An ethnobotanical survey was conducted on type 2 diabetic patients and plant sellers in markets of Mexico City (Central Mexico) to confirm how the plant is traditionally used.

Fresh cladodes from *Opuntia streptacantha* were collected at Milpa Alta, D.F., Mexico, in September 2007. Their identity was confirmed and voucher specimens were deposited at the IMSS Herbarium in Mexico City (IMSSM15048). Two extracts were prepared: for the first extract, the spines were first mechanically removed from the cladodes (70 g), then washed with water and liquefied, and finally lyophilized and stored at -80°C for future use, final yield 8.7 g; for the second preparation, the LE was filtered to a filtrate extract (FE) through a C18 filter, then lyophilized and stored at -80°C for future use, final yield 1.7 g.

3.2. Isolation of the compound

The FE was dissolved in ethanol–water 7:3 and the solution was loaded into a 100×2 cm Polygoprep 60–30 C₁₈ (Macherey & Nagel, Düren, Germany) flash-column and eluted with H₂O/MeOH/acetonitrile 70:15:15 at a rate of 4 ml/min. The eluted solvent was collected in 5 ml fractions, monitored by UV-detection and controlled by HPLC. The fractions were applicated on a Nucleosil 60–30 C₁₈ (Macherey & Nagel, Düren, Germany) column and eluted with H₂O/MeOH/AcCN 70:15:15, 4 ml/min monitored by DAD-HPLC, Beckman System Gold with 32 Karat software.

3.3. Induction of diabetes in experimental animals

Five-day-old Wistar rats (weighing 10–12 g) received 90 mg/kg IP injections of STZ (Sigma, No. 242-646-8) in acetate buffer 0.1 M, pH 4.5. Non-diabetic control group received only IP injections of buffer.

At four weeks of age, the rats were separated from their mothers and acclimatized with free access to food and water in an air conditioned room (23 °C with 55% humidity) under a 12 h light:dark cycle at the Bioterium of the Science School, UNAM.

After 12 weeks, diabetes was identified by symptoms of polydipsia and polyuria and confirmed by measuring fasting plasma glucose (FPG) levels. Male and female rats with FPG levels >150 mg/dl were included in the study.

The animals were handled according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (OACU, 1996). All methods used in this study were approved by the Internal Council of the “Facultad de Ciencias”, Universidad Nacional Autónoma de México.

3.4. Administration of plant extracts administration in n5STZ-rats

Animals were classified into five groups, each consisting of 11 rats: one non-diabetic group (1) and four diabetic groups (2–5). Groups 1 and 2 both received 1.5 ml of physiological NaCl-solution (vehicle), serving as the non-diabetic and diabetic control groups,

respectively. Groups 3–5 received the following doses per body weight (BW): the standard oral hypoglycemic agent glibenclamide (3 mg/kg); *Opuntia streptacantha* LE (135 mg/kg, BW), and *Opuntia streptacantha* FE (27 mg/kg = 135 mg/kg LE before filtration, BW), respectively. All hypoglycemic agents were administered to Groups 3–5 in the same vehicle as those given to Groups 1 and 2. The extracts were re-dissolved in a vehicle of 1.5 ml of physiological NaCl-solution and administered orally using a cannula.

3.5. Administration of plant extracts administration in maltose-loaded n5STZ-rats

The experimental animals were classified into five groups (1–5), each of them with eleven rats. Group 1 served as the non-diabetic control and received 1.5 ml of physiological NaCl-solution (vehicle), while Group 2 was the diabetic control and also received 1.5 ml of physiological NaCl-solution. The rats of Group 3 were treated with a standard oral hypoglycemic agent, acarbose, with a dose of (3 g/kg BW). Groups 4–6 were treated with the plant extracts: Group 4 received *Opuntia streptacantha* LE (135 mg/kg BW), Group 5 received *Opuntia streptacantha* FE (12 mg/kg) and Group 6 received *Opuntia streptacantha* FE (27 mg/kg = 135 mg/kg LE before filtration BW).

Test samples, control drug or vehicle were given orally to 8 h fasted rats, 5 min before the administration of maltose 3 g/kg, the extracts were dissolved in 1.5 ml of physiological NaCl-solution and administered orally by a cannula.

3.6. Collection of blood and determination of blood glucose

Blood samples were obtained from the tail vein according to procedures outlined in the Institutional Animal Care and Use Committee Guideline 9 (3/10/99) (IACUC, 1999). During the first experiment, blood was collected 5 min before the oral administration of the extracts or the vehicle (T0) and at times T30, T60 and T90 min thereafter. For the second experiment, blood was collected at times T0, T60, T120 and T180 min thereafter.

The glucose concentration was measured in plasma serum with Reflotron equipment (each assay used 32 μl of blood) and confirmed by Accutrend GC equipments (Roche). The animals were handled according to the Office of Animal Care and Use of the National Institute of Health Guide for the Care and Use of Laboratory Animals (OACU, 1996). Bioethical allowance; all methods used in this study were approved by the Internal Council of the “Facultad de Ciencias”, Universidad Nacional Autónoma de México.

3.7. Statistical analysis

The data were statistically analyzed by one-way ANOVA followed by a Tukey's test. The plasma glucose levels were expressed as the mean (S.E.M.).

4. Results

4.1. Ethnobotany

After interviewing diabetic patients and plant sellers, we confirmed that the plant is traditionally used to treat diabetes in the form of a blended shake made from young cladodes. In preparation, the spines (leaves) are first removed mechanically and the cladode is then washed and cut. The cut pieces are then combined with water and liquefied and the blended shake is consumed before breakfast.

We also confirmed that while the liquefied blend of Nopal is used as a treatment, the broiled form is typically consumed as food, not as medicine.

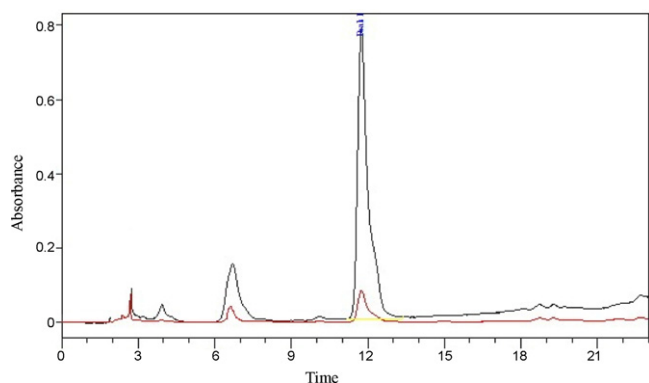


Fig. 1. HPLC comparison of both *Opuntia* extracts at 220 nm. Upper line, FE; lower line, LE.

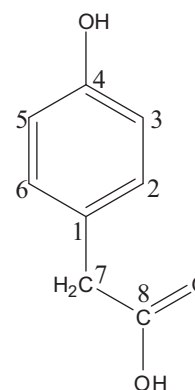


Fig. 2. (4-Hydroxy)-phenyl acetic acid.

4.2. Compound

After comparing the extracts by HPLC DAAD, we can say that the soluble part of both extracts is similar (Fig. 1).

We isolated a (4-hydroxy)-phenyl acetic acid derivate, which has not been previously reported on *Opuntia* the structure, was identified by NMR as shown in Fig. 2. (500 MHz; Bruker; d_6 -DMSO; δ =ppm; J in Hz): 1H: 9.18 (1H, s, OH-4); 8.30 (1H, s, OH-8); 6.97 (2H, d, $J=8$, H-2/6); 6.63 (2H, d, $J=8$, H-3/5); 2.75 (2H; d, $J=4$, H2-7); 13C: 171.85 (C-8); 156.04 (C-4); 131.49 (C-2/6); 126.55 (C-1); 114.68 (C-3/5); 43.62 (C-7).

4.3. Effect of the extracts on STZ-diabetic rats

The data presented in Table 1 show that neither of the tested extracts of *Opuntia* produced a hypoglycemic effect and the diabetic control group presented significantly higher glucose levels with respect to the non-diabetic control group. The group treated with the standard hypoglycemic agent glibenclamide produced a hypoglycemic effect beginning at time-point T60.

Table 1
Effect of the oral administration of *Opuntia* extracts and control drug over the plasma glucose concentration in *n*-STZ-diabetic rats.

Treatments	Plasma glucose levels (mg/dl) \pm standard error			
	T0	T60	T120	T180
Control (+) no diabetic	107 \pm 3 ^a	104 \pm 4 ^a	114 \pm 4 ^a	115 \pm 4 ^a
Control (-) (vehicle)	162 \pm 7 ^{a,1}	155 \pm 6 ^{a,1}	155 \pm 5 ^{a,1}	157 \pm 4 ^{a,1}
Glibenclamide (3 mg/kg bw)	158 \pm 4 ^{a,1}	136 \pm 6 ^{b,2}	131 \pm 3 ^{b,2}	130 \pm 3 ^{b,2}
<i>Opuntia streptacantha</i> (135 mg/kg bw)	158 \pm 4 ^{a,1}	167 \pm 10 ^{a,1}	172 \pm 10 ^{a,1}	164 \pm 4 ^{a,1}
<i>Opuntia streptacantha</i> FE (27 mg/kg bw)	152 \pm 4 ^{a,1}	163 \pm 6 ^{a,1}	160 \pm 8 ^{a,1}	159 \pm 6 ^{a,1}

The values represent the mean \pm SE. Different letters in the same row indicate statistical differences compared to time 0. Different numbers in the same column indicate statistical differences against the control group (diabetic control group compared against non-diabetic control). Significance is defined to be at least $p < 0.05$.

Table 2
Effect of the oral administration of *Opuntia* extracts and control drug over the plasma glucose concentration on maltose loaded *n*-STZ-diabetic rats.

Treatments	Plasma glucose levels (mg/dl) \pm standard error			
	T0	T30	T60	T190
Control (+) no diabetic	100 \pm 5 ^a	133 \pm 7 ^b	129 \pm 6 ^b	133 \pm 6 ^b
Control (-) (vehicle)	155 \pm 6 ^{a,1}	242 \pm 12 ^{b,1}	213 \pm 16 ^{b,1}	194 \pm 14 ^{b,1}
Acarbose (3 g/kg bw)	150 \pm 6 ^{a,1}	179 \pm 11 ^{a,2}	169 \pm 6 ^{a,2}	158 \pm 6 ^{a,2}
<i>Opuntia streptacantha</i> (135 mg/kg bw)	150 \pm 2 ^{a,1}	152 \pm 4 ^{a,2}	150 \pm 6 ^{a,2}	140 \pm 3 ^{a,2}
<i>Opuntia streptacantha</i> FE (12 mg/kg bw)	147 \pm 3 ^{a,1}	157 \pm 5 ^{a,2}	150 \pm 7 ^{a,2}	141 \pm 2 ^{a,2}
<i>Opuntia streptacantha</i> FE (27 mg/kg bw)	150 \pm 2 ^{a,1}	152 \pm 4 ^{a,2}	150 \pm 6 ^{a,2}	140 \pm 3 ^{a,2}

The values represent the mean \pm SE. Different letters in the same row indicate statistical differences compared to time 0. Different numbers in the same column indicate statistical differences against the control group (diabetic control group is compared against non-diabetic control). Significance is defined to be at least $p < 0.05$.

4.4. Effect of the extracts on maltose-loaded STZ-rats

The data presented in Table 2 show that the diabetic animals also have significantly higher glucose levels than the non-diabetic group. The standard anti-hyperglycemic agent acarbose inhibited the glucose peak following time-point T30. *Opuntia* extracts similarly inhibited the glucose peak after time-point T30 comparable to acarbose.

5. Discussion

The aim of our study was to test two extracts of *Opuntia streptacantha* in different assays to contribute to the understanding of the hypoglycemic mechanism of this plant. The results of our study confirm that the plant extracts produce an anti-hyperglycemic effect and this effect is maintained after filtering the extract. Previous reports suggest that the anti-hyperglycemic effect of Nopal may be due to its fiber and pectin content, which may decrease carbohydrate absorption (Shane-McWhorter, 2005). Furthermore, studies exploring other dosage forms of Nopal, such as capsules or juice, did not demonstrate any significant changes in serum glucose

level (Najm and Desiree, 2010). The results presented in this study confirm that the anti-hyperglycemic effect is independent of the fiber or mucilage content, demonstrated by testing the FE, which contains nor fibers or pectine. This experiment also explains that using the juice alone did not result in a hypoglycemic effect, but it is effective as an anti-hyperglycemic agent, comparable to the acarbose.

This study also newly identified one of the compounds present in this extract as (4-hydroxy)-phenyl acetic acid.

The blended shake extract created from the cladode of *Opuntia streptacantha* produces an anti-hyperglycemic effect administered after a glucose challenge, and this effect is maintained after filtering the extract. Our results proves the efficacy of *Opuntia streptacantha* for the control of blood sugar levels, because the here tested doses are related to the traditional use of the plant, this study support the traditional use of Nopal in Central Mexico in the treatment of type 2 diabetes.

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