



## Effect of *Opuntia streptacantha* Lem. on alpha-glucosidase activity

Jaime Becerra-Jiménez<sup>a</sup>, Adolfo Andrade-Cetto<sup>b,\*</sup>

<sup>a</sup> Pharmaceutical Institute of the University of Bonn, An der Immenburg 4, D-53121, Germany

<sup>b</sup> Laboratorio de Etnofarmacología, Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, México DF, Mexico

### ARTICLE INFO

#### Article history:

Received 5 October 2011  
Received in revised form 7 November 2011  
Accepted 19 November 2011  
Available online 1 December 2011

#### Keywords:

*Opuntia streptacantha*  
Type 2 diabetes  
Alpha glucosidase inhibitors  
n-Stz diabetic rat  
Medicinal plant

### ABSTRACT

**Ethnopharmacological relevance:** In Mexico, *Opuntia streptacantha* is used as part of a traditional medicine in the treatment of diabetes mellitus. Several studies have reported an anti-hyperglycemic effect from the plant, but none have focused on finding a mechanism of action to explain these results. This paper focuses on one of the most recurrent hypotheses for the effect of *O. streptacantha*: the mechanism of action is the hydrolysis of disaccharides, either by enzymatically inhibiting the alpha glucosidase inhibitors (AGIs) or mechanically acting as a barrier.

**Materials and methods:** To test our hypothesis, three different experiments were performed. A maltose tolerance test was carried out using the total extract (TE) and the juice of the plant. Six different extracts were tested in a free-enzyme assay, four of which were aqueous extracts varying according to the procedure of removing the insoluble particles in an attempt to reduce the loss of the water-soluble compounds. The main component of the total extract, a derivative of (4-hydroxy)-phenyl acetic acid, was also tested. For both the maltose tolerance test and the free-enzyme assay, acarbose was used as a control drug. Finally, an everted small intestine assay was also performed.

**Results and conclusions:** The maltose tolerance test confirmed that an anti-hyperglycemic effect occurs at doses of 100 mg/kg TE and 4 mL/kg juice. No inhibition on AGIs was observed in the free-enzyme assay, and neither a mechanic nor enzymatic effect on disaccharide hydrolysis was observed in the everted intestine assay.

**Conclusions:** The anti-hyperglycemic effect of *O. streptacantha* was confirmed, and the results presented here contribute to the understanding that this effect is not due to the action on alpha-glucosidases or related to the intestinal hydrolysis of disaccharides.

© 2011 Elsevier Ireland Ltd. All rights reserved.

### 1. Introduction

Diabetes mellitus is defined as hyperglycemia associated with the absence or inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action. Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. An individual may present more resistance or more beta-cell deficiencies, and these abnormalities can range from mild to severe. The impaired insulin action affects lipid metabolism, resulting in increased free fatty acid flux and triglyceride levels and low levels of high-density lipoprotein (HDL) (Expert Committee, 2003).

In Mexico, it has been estimated that approximately 500 species of medicinal plants are used to treat type 2 diabetes. One of these plants is *Opuntia streptacantha* Lem. (Cactaceae), commonly known as “Nopal”, which has traditional uses by the pre-Columbian tribes in Mexico documented by the Florentine codex, a document elaborated in the S. XVI. Later (S. XVII), Hernandez refers to the use of the cladode in the treatment of gastritis, intestinal colic and ulcers (Argueta, 1994).

In the late 1980s and in the beginning of the 1990s, one group conducted several clinical studies on humans using broiled plant stems from *O. streptacantha* Lem. (Frati-Munari et al., 1989) and found an anti-hyperglycemic peak-effect 3 h after administration, with sustained action after 6 h. Recent clinical studies led by Najm and Desiree (2010), Shane-Mc Whorter (2001, 2005), Shapiro and Gong (2002), and Yeh et al. (2003) report a positive effect of this plant on type 2 diabetic patients. In a recent study with OpunDia™, a commercial preparation made from cladode extracts and fruit skins of *Opuntia ficus-indica*, the acute blood glucose lowering effect on type 2 diabetic patients was obtained only after an oral glucose tolerance test (OGTT) (Godard et al., 2010).

Abbreviations: AGIs, alpha glucosidase inhibitors; bw, body weight; 4-NPGP, 4-nitrophenol glucopyranoside.

\* Corresponding author at: Apartado Postal 70-359, Coyoacan, México DF, Mexico. Tel.: +52 555 622 4834; fax: +52 555 622 4828.

E-mail addresses: [aac@ciencias.unam.mx](mailto:aac@ciencias.unam.mx), [aac@ciencias.unam.mx](mailto:aac@ciencias.unam.mx) (A. Andrade-Cetto).

We previously reported that the plant is traditionally used to treat diabetes in the form of a blended shake made from young cladodes. For its preparation, the spines (leaves) are mechanically removed, and the cladodes are washed and cut into pieces, which are then liquefied; the resulting shake is consumed before breakfast. In the same study we reported a (4-hydroxy)-phenyl acetic acid derivative as a component of *Opuntia* (Andrade-Cetto and Wiedenfeld, 2011). Although many *Opuntia* studies have been performed, a clear mechanism of action for this plant has not yet been established (Najm and Desiree, 2010).

Several hypotheses have been formulated about the putative mechanism of action. Shane-Mc Whorter (2001) infers that the fiber content may affect the intestinal uptake of glucose, while Zareba et al. (2005) proposes that the mechanism of hypoglycemic action is partly due to the plant's highly soluble fiber and pectin content, which may affect intestinal glucose uptake.

Acarbose and miglitol, compounds used in the diabetes therapy, are competitive inhibitors of the intestinal alpha-glucosidases and reduce post-meal glucose excursions by delaying the digestion and absorption of starches and disaccharides. Complex starches, oligosaccharides, and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic amylase and glucosidases, which are attached to the brush border of the intestinal cells (Nolte, 2009).

## 2. Aim of the study

In this study, we evaluated the effect of *O. streptacantha* cladodes on the intestinal hydrolysis of disaccharides.

## 3. Materials and methods

### 3.1. Extracts from *Opuntia streptacantha*

Plant material was collected in the community of San Pablo Oztotepec in Mexico City, and a herbarium exemplar was deposited into the IMSS-Herbarium under the number 15040.

A maltose tolerance test (MTT) was carried out using the, traditionally used, total extract (TE) prepared as previously reported (Andrade-Cetto and Wiedenfeld, 2011). Spineless cladodes of *O. streptacantha* (100 g) were washed, homogenized and lyophilized and stored at  $-80^{\circ}\text{C}$  until further use; the final yield was 12.4%. In addition, for this study, to TE, a juice (J) of the plant was also tested in this model. The juice was prepared by pressing young fresh spineless cladodes in a hydraulic press (Hafico<sup>®</sup>) at 150 kg/cm<sup>2</sup>. The resulting juice was lyophilized and stored at  $-80^{\circ}\text{C}$ .

For the free-enzyme assay, 6 different extracts were tested, 4 of which were aqueous extracts varying by the procedure of removing the insoluble particles in an attempt to reduce the loss of the water-soluble compounds. For their preparation, 6 g of TE were extracted in 100 mL of water by stirring for 2 h at room temperature; the non-soluble particles were then removed by decantation (AE), by centrifugation at 4000 rpm/10 min (CE) or by filtration with diatomaceous earth and a vacuum (FE). The aqueous extract (AE) without pH adjustment was also tested; the final pH of this extract was 5 (AE5).

The previously isolated (4-hydroxy)-phenyl acetic acid derivative (Andrade-Cetto and Wiedenfeld, 2011) was also tested because it was the main component in the AE. Two additional extracts that resembled the conditions in this compound was isolated were also tested. A water-ethanol (EW) extract was prepared by extracting 6 g of TE in an 80/20 solution of water/ethanol, respectively, for 2 h at room temperature and clarified via filtration with diatomaceous earth and a vacuum. The second extract was

prepared in the same fashion except that the water was replaced with a 10 mM solution of phosphoric acid (PhE). For the assay, all extracts and the positive control acarbose were dissolved in 0.1 M phosphate buffer, and the pH was adjusted to 6.8.

### 3.2. Animals and induction of experimental diabetes

Five-day-old Wistar rats (each weighing 10–12 g) were obtained from the Bioterium of the Science School (UNAM) and given intraperitoneal (i.p.) injections of 90 mg/kg of streptozotocin (STZ, Sigma, No. 242-646-8) dissolved in 0.1 M acetate buffer, pH 4.5. The non-diabetic control group received i.p. injections of buffer only. At 4 weeks, 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. After 12 weeks, animals with fasting glucose values over 60 mg/dL were selected as diabetic individuals.

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

### 3.3. Maltose tolerance test

Groups 1 and 2 ( $n = 11$ ) received either physiological solution as a negative control or acarbose (3 g/kg) as a positive control, respectively. Diabetic animals were randomly divided into 4 groups ( $n = 11$ ). Groups 3 (physiological solution) and 4 (acarbose, 3 mg/kg) also served as the negative and positive controls, respectively. Groups 5 and 6 corresponded to treatments of *O. streptacantha* total extract (100 mg/kg) and *O. streptacantha* juice (4 mL/kg), respectively. Prior to the administration of any compounds, blood samples were obtained from every group ( $T_0$ ). Treatments were administered, and a load of 3 g/kg of maltose was given 5 min later. Blood was collected at 30 min ( $T_{30}$ ), 60 min ( $T_{60}$ ), and 90 min ( $T_{90}$ ) after drug administration from the tail vein in accordance to the procedures outlined in the Institutional Animal Care and Use Committee Guideline 9 (IACUC, 1999). Plasma glucose concentration was measured using an Accu-trend GC glucometer (Roche) with its corresponding glucose-test strips.

### 3.4. Crude small intestinal extract

Crude rat intestinal extract was prepared according to the method described by Nishioka et al. (1998) with slight modifications. Small intestines from 6 Wistar rats were dissected and washed twice with physiological saline solution (0.9% NaCl), followed by a wash with 0.1 M potassium phosphate buffer (pH 7) with 5 mM EDTA. From the washed small intestine, the mucosa was scraped and homogenized in the same potassium phosphate buffer and then centrifuged at  $21,000 \times g$  for 1 h. The precipitate was dissolved and stirred for 30 min in 0.1 M potassium phosphate buffer (pH 7.0) containing 1% Triton X-100 before being centrifuged at  $32,000 \times g$  for 60 min. The supernatant was dialyzed in 0.01 M potassium phosphate buffer (pH 7.0) for 24 h. The content of the membranes was lyophilized and stored at  $4^{\circ}\text{C}$  until further use.

### 3.5. Free-enzyme assay

Enzyme activity was measured following the procedure previously described by Matsui et al. (1999) with slight modifications. AGH activity was quantified by the amount of p-nitrophenol released from p-nitrophenyl-alpha-D-glucopyranoside into 0.1 M sodium phosphate buffer (pH 6.8). Each assay was performed in

**Table 1**  
Effects of the oral administration of plant extracts and control drug on the plasma glucose concentration of maltose-loaded n-stz diabetic rats.

Treatment	Plasma glucose levels (mg/dL)			
	$T_0$	$T_{30}$	$T_{60}$	$T_{90}$
Non diabetic control (–)	123 ± 3	144 ± 4 <sup>a</sup>	138 ± 4 <sup>a</sup>	132 ± 2
Non diabetic control (+)				
Acarbose (3 mg/kg bw)	122 ± 2	136 ± 4 <sup>a</sup>	129 ± 4 <sup>a</sup>	128 ± 3 <sup>1</sup>
Diabetic control (–)	154 ± 3 <sup>1</sup>	209 ± 8 <sup>a,1</sup>	220 ± 17 <sup>a,1</sup>	290 ± 19 <sup>a,1</sup>
Diabetic control (+)				
Acarbose (3 mg/kg bw)	153 ± 3	171 ± 4 <sup>a,1</sup>	163 ± 5 <sup>1</sup>	161 ± 5 <sup>1</sup>
<i>Opuntia streptacantha</i> (TE)	156 ± 2	176 ± 5 <sup>a,1</sup>	153 ± 5 <sup>1</sup>	141 ± 4 <sup>1</sup>
<i>Opuntia streptacantha</i> (J)	154 ± 3	162 ± 4 <sup>a,1</sup>	157 ± 5 <sup>1</sup>	145 ± 4 <sup>1</sup>

Letter (a) in the same row indicates statistical differences ( $p < 0.05$ ) as compared to time 0. Number (1) in the same column, indicates statistical differences compared to control groups (the diabetic control group is compared to a non-diabetic control). Values are expressed as mean ± SE,  $n = 11$ .

the aforementioned buffer with 2 mM p-NPGP and 0.1 U from crude small intestinal extract. The experimental extracts and control (Acarbose, Bayer) were assayed with varying concentrations of the extracts (0.2–20,000 µg/mL). The reaction was tracked for 600 s, and readings were taken every 15 s at a wavelength of 405 nm with a Beckman Coulter Spectrophotometer Model DU-640.

### 3.6. Everted small intestine assay

The method was adapted from Lee et al. (1998). Small intestines from Wistar rats were dissected and washed with a Krebs–Ringer solution (pH 7.3). Sections of intestine (3 cm) were everted, ligated at their extremities and filled with the same saline Krebs–Ringer solution. The sacks were incubated in Krebs–Ringer solution containing the appropriate experimental treatments, 0.5 mM phlorizine (to measure the alpha-glucosidase activity while blocking sugar transport) and sucrose as a substrate. The glucose released into the medium was measured using a glucose oxidase test kit (Spinreact). For this experiment it was decided to test a single concentration of 200 mg since this was the maximal amount in the free-enzyme test.

## 4. Results

### 4.1. Maltose tolerance test

The results of the maltose tolerance experiment are presented in Table 1. After the administration of maltose to normal and

diabetic-induced rats, the blood-glucose levels rose in both groups after 30 min. This elevation is statistically significant compared with the levels at time 0 for each group. At 120 min, the blood-glucose levels of the non-diabetic group returned to the basal values, while the glucose levels remained increased in diabetic rats. The administration of acarbose to diabetic and non-diabetic rats inhibited the glucose peak after 60 min in both groups; these results were statistically significant compared with their corresponding controls at each time point and to the respective values at  $T_0$ . Similar results were observed in the groups treated with the total extract of *Opuntia* and with juice, where both prevent the glucose peak 90 min after administration.

### 4.2. Free-enzyme assay

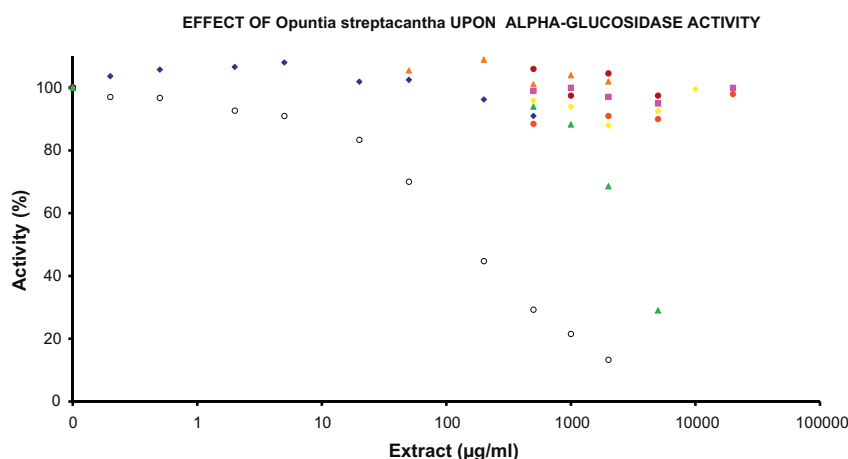
Fig. 1 shows that all extracts and the (4-hydroxy)-phenyl acetic acid exerted no prominent inhibition on enzymatic activity at concentrations as high as 20 mg/mL. Neither the clarification methods (i.e., decantation, centrifugation or filtration) nor the extraction methods (i.e., water, water/ethanol or phosphoric acid/ethanol) resulted in a significant difference on the extract activity. The phosphoric acid/ethanol extract produced the maximal amount of inhibition, with a reduction of 12% in enzymatic activity at a concentration of 2 mg/mL in contrast to the positive control acarbose with an  $IC_{50}$  of 128 µg/mL.

### 4.3. Everted small intestine assay

In this model, a single concentration of 20 mg/mL of the TE was tested. Each gut segment served as its own control, meaning that the amount of hydrolyzed sucrose was measured in the presence and in the absence of the TE. The total amount of glucose liberated after 20 min of incubation in the absence of the TE is referred to as 100% of activity, while the glucose liberated by the same gut segment in the presence of the TE is referred to as a percentage of the total activity. The activity in the presence of the TE was  $98 \pm 3\%$  ( $n = 6$ ), suggesting that the TE of *Opuntia* did not inhibit alpha-glucosidase activity.

## 5. Discussion

The results of the maltose tolerance test confirmed that the total extract and the juice of the plant produced an anti-hyperglycemic effect. The inhibition of the glucose peak exerted by both extracts is comparable to treatment with the positive control acarbose. These



**Fig. 1.** Effect of *O. streptacantha* on alpha-glucosidases activity. (□) Aqueous extract (AE); (▢) centrifugated extract (CE); (●) filtrated extract (FE); (●) water–ethanol extract (EW); (▣) ethanol–phosphoric acid extract (PhE); (○) acarbose; (◻) Aqueous extract pH 5(AE5); and (◻) (4-hydroxy)-phenyl acetic acid.

results are in agreement with previous findings that use of the same plant extract (Andrade-Cetto and Wiedenfeld, 2011).

In the free-enzyme assay, the only reduction in activity was produced by AE5, but this result could be attributed to the pH and not to the extract itself. It is well established that the acidification of the assay medium has an impact on the enzyme activity. Intestinal disaccharidases function at an optimal pH between 5.5 and 6.5, and their activity decreases rapidly in environments outside of this range (Dahlqvist, 1964). These results were confirmed with the everted small intestine assay, in which we demonstrated that the TE of the plant does not affect alpha-glucosidase activity.

The *Opuntia* preparation used in the everted small intestine assay was a total extract, including the mucilage and pectins (similar to the traditional used preparation). The activity results demonstrate that the extract does not affect the hydrolysis of disaccharides, neither by inhibition of the corresponding enzymes or by mechanical means. There are two hypotheses referring to the mechanical effect; one mentions that the components of the extract form a barrier between the hydrolysis-absorption enzyme complex and the substrate, while the other theory points toward the sequestration of the substrate (Fрати-Munari et al., 1983, 1989; Ibanez-Camacho and Roman-Ramos, 1979).

With our results, it is clear that the sequestration of the substrate does not occur because the hydrolysis of the disaccharide was not altered by the TE. In addition, when a mechanical barrier exists, it does not affect the hydrolysis step in disaccharide catabolism.

The anti-hyperglycemic effect of *O. streptacantha* has been well established, the results presented here contribute to the understanding that the observed effect is not due to action on alpha-glucosidases or related to the intestinal hydrolysis of disaccharides. Further theories, such as the stimulation in the insulin secretion observed in *Opuntia ficus-indica* (Butterweck et al., 2011), should be applied to studies on *O. Streptacanta*. The present study confirms that the traditional used, total extract of the plant, is an anti-hyperglycemic agent.

#### Conflict of interest statement

The authors have no conflicts of interest to disclose.

#### Acknowledgements

This work was partially supported by the DGAPA, PAPIIT project IN228510.

#### References

- Andrade-Cetto, A., Wiedenfeld, H., 2011. Anti-hyperglycemic effect of *Opuntia streptacantha* Lem. Journal of Ethnopharmacology 78, 145–149.
- Argueta, V. (Ed.), 1994. Atlas of the traditional Mexican medicinal plants, vol. II. National Indigenous Institute, Mexico, pp. 1051–1052.
- Butterweck, V., Semlin, L., Feistel, B., Pischel, I., Bauer, K., Verspohl, E., 2011. Comparative evaluation of two different *Opuntia ficus-indica* extracts for blood sugar lowering effects in rats. Phytotherapy Research 25, 370–375.
- Dahlqvist, A., 1964. Method for assay of intestinal disaccharidases. Analytical Biochemistry 7, 18–25.
- Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26, S5.
- Fрати-Munari, C., Fernández-Harp, A., De la Riva, H., Ariza-Andraca, R., Del Carmen Torres, M., 1983. Effects of nopal (*Opuntia* sp.) on serum lipids, glycemia and body weight. Archivos Investigación Médica 14, 117–125.
- Fрати-Munari, C., Altamirano-Bustamante, E., Rodríguez-Barcenas, N., 1989. Hypoglycemic action of *Opuntia streptacantha* Lemaire: study using raw extracts. Archivos Investigación Médica 20, 321–325.
- Godard, M., Ewing, B., Pischel, I., Ziegler, A., Benedek, B., Feistel, B., 2010. Acute blood glucose lowering effects and long-term safety of OpunDia™ supplementation in pre-diabetic males and females. Journal of Ethnopharmacology 130, 631–634.
- Ibanez-Camacho, R., Roman-Ramos, R., 1979. Hypoglycemic effect of *Opuntia cactus*. Archivos Investigación Médica 10, 223–230.
- Institutional Animal Care and Use Committee, 1999. Guideline of Selected Techniques for Rat and Mouse Blood Collection. Guideline 9 IACUC USA, p6.
- Lee, E., Weiss, S., Lam, M., Torres, R., Diamond, J., 1998. A method for assaying intestinal brush-border sucrase in an intact intestinal preparation. Proceedings of the Natural Academy of Sciences 95, 2111–2116.
- Matsui, T., Oki, T., Osajima, Y., 1999. Inhibitory effect of alpha-glucosidase inhibitors varies according to its origin. Journal of Agriculture Food Chemistry 47, 550–553.
- Najm, W., Desiree, L., 2010. Herbs used for diabetes, obesity and metabolic syndrome. Primare Care: Clinics in Office Practice 37, 237–254.
- Nishioka, T., Kawabata, J., Aoyama, Y., 1998. Baicalein, an alpha-glucosidase inhibitor from *Scutellaria baicalensis*. Journal of Natural Products 61, 1413–1415.
- Nolte, M., 2009. Pancreatic hormones and antidiabetic drugs. In: Bertram, G., Katzung, S., Masters, B., Trevor, A. (Eds.), Basic and Clinical Pharmacology. The McGraw-Hill Companies, p. 1232.
- Office of Animal Care and Use, of the National Institute of Health, USA, 1996. <http://oacu.od.nih.gov/regs> (accessed 15.08.11).
- Shane-Mc Whorter, L., 2001. Biological complementary therapies: a focus on botanical products in diabetes. Diabetes Spectrum 14, 199–208.
- Shane-Mc Whorter, L., 2005. Botanical dietary supplements and the treatment of diabetes: what is the evidence? Current Diabetes Reports 5, 391–398.
- Shapiro, K., Gong, W., 2002. Natural products used for diabetes. Journal of the American Pharmaceutical Association 42, 217–226.
- Yeh, G.Y., Eisenberg, D.M., Kaptchuk, T.J., Phillips, R.S., 2003. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care 26, 1277–1281.
- Zareba, T., Serradell, N., Castañer, R., Davies, S.L., Prous, J., Mealy, N., 2005. Phytotherapies for diabetes. Drugs of the Future 30, 1253–1282.