

Functional and Hypoglycemic Properties of Nopal Cladodes (*O. ficus-indica*) at Different Maturity Stages Using in Vitro and in Vivo Tests

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ABSTRACT: Nopal (*Opuntia ficus-indica*) cladodes are recommended for their therapeutic properties; their maturity stage may affect their biological properties. Cladodes of three maturity stages, from the same crop and location, were dehydrated and evaluated for some of their physicochemical and nutritional characteristics and antidiabetic properties. The flours of small and medium cladodes (SCF and MCF, respectively) had higher contents of dietary fiber, water absorption, swelling, and viscosity compared to those of the large cladode flour (LCF). Streptozotocin-induced diabetic rats, treated with MCF and SCF (doses of 50 mg/kg body weight), showed reduction of postprandial blood glucose on 46.0 and 23.6%, respectively ($p < 0.05$), in relation to the control; and LCF had no significant effect. In vitro, glucose diffusion tests showed similar ranking by the two former samples, whereas the latter was close to the control. Cladode maturity stages showed different fiber content and produced suspensions with differences in viscosity, which may affect in vitro and in vivo glucose responses.

KEYWORDS: *O. ficus-indica*, maturity stages, functional properties, diabetes

INTRODUCTION

The use of nopal (*Opuntia* spp.) has been recorded in Mexico since pre-Hispanic times given its important role in the agricultural economy of the Aztec Empire and is thus considered as one of the oldest cultivated plants in Mexico.¹ Cacti grow in semiarid areas and produce stems known as cladodes, which are the edible part of the plant. Its production and consumption is not limited to Mexico; this crop is gaining popularity in various other countries because of its effects on human health and the palatability of its fruits.^{2,3}

In Mexico, cladodes from all maturity stages are traditionally used to treat diabetes.⁴ In vivo studies with some of its polysaccharides and different extracts from *Opuntia* spp. have shown considerable antioxidant, hypolipidemic, and antidiabetic effects tested on streptozotocin-induced diabetic mice.^{5,6} Additionally, dehydrated extracts tested on diabetic patients have shown attenuation of postprandial hyperglycemia; and broiled stems administered under fasting conditions to diabetic subjects were able to decrease serum glucose.^{7,8}

Frati et al.⁹ suggested that the hypoglycemic mechanism of nopal is related to its fiber content, and Alarcon-Aguilar et al.¹⁰ tested in alloxan-diabetic mice polysaccharides isolated from *Opuntia* sp. and showed discrete antihyperglycemic effects attributable to a fiber, although only *O. streptacantha* polysaccharide caused an evident hypoglycemic effect attributable to unknown substances. Additionally, it has been reported that polysaccharides extracted from *O. monacantha* cladodes could improve the insulin sensitivity in peripheral insulin target tissues.⁵ However, the mechanism by which nopal exerts these effects remains to be elucidated.¹¹

Interestingly, both the concentration and composition of fibers from cladodes are dependent on their maturity stage. The maturity stage of cladodes has shown a positive correlation with the content of insoluble fiber and a negative one with soluble fiber.¹² Additionally, other maturity-related nutrimental changes of the cladode have been reported, such as a decrease in protein and starch and increase of simple carbohydrates as the cladode matures.^{13,14} The nutrimental variations may have important effects on their functional and antidiabetic properties. The aim of this study was to evaluate the possible link between physicochemical and functional properties of cladode flours from different maturity stages and their glucose lowering effects using in vitro and in vivo studies.

MATERIALS AND METHODS

Chemicals. Microcrystalline cellulose and streptozotocin were purchased from Sigma-Aldrich (St. Louis, MO); the diet Nu3 containing 22% proteins, 5.5% fat, 5% dietary fiber, 7% ash, and 60.5% carbohydrates was supplied by Lab Research Global Solution (Mexico City, Mexico); the glucometers and strips were purchased from Roche (Mannheim, Germany); and the glucose GOD-PAP kit was purchased from Randox (Diamond Road, Crumlin, Co. Antrim, United Kingdom).

Plant Material. Fresh cactus cladodes of *O. ficus-indica* var. Milpa Alta were collected in the month of September, 2010 in the locality of El Refugio, Guanajuato, Mexico and identified by C. Mondragon Jacobo from the National Research Institute of Forest, Agriculture and

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Livestock (INIFAP). The cladodes were classified according to their age (days) and size (weight and length) as small (12 days, 40 g, 14 cm), medium (20 days, 74 g, 20 cm), and large (30 days, 293 g, 32 cm); the cladodes were in a range of maturity for optimal human consumption.

Cladodes were washed with distilled water and disinfected using commercial 10% (w/v) sodium hypochlorite solution, and the spines were manually removed. They were cut into squares (about 2×2 cm²) and desiccated in a tray drier at 40 °C for 24 h. The dried material was ground using a mill with mesh size <80 (0.18 mm), and different samples were obtained: small cladode flour (SCF), medium cladode flour (MCF), and large cladode flour (LCF).

Proximate Analysis. Moisture, ash, protein, and total, insoluble and soluble dietary fibers were analyzed using AOAC methods.¹⁵ The caloric content was measured using a combustion calorimeter IKA C2000 (IKA works, Inc., Wilmington NC, USA).

Functional Properties. Bulk density was measured using the procedure reported by Narayana and Narasinha.¹⁶ Water absorption capacity (WAC) was determined according to the method described by Sathe and Salunkhe¹⁷ and swelling (SW) according to Robertson et al.¹⁸

Viscosity. All viscosity measurements were made using cladode flour (SCF, MCF, and LCF) suspensions of 0.005 g/mL, and cellulose was tested at the same concentration of samples using distilled water at 37 °C, using a rotational Brookfield viscometer model LVDV-E (Brookfield Engineering Laboratories INC., Middleboro MA, USA) with spindle number 1 at 100 rpm shear rate. Results were expressed as mPa·s.

Maximum Velocity of Glucose Diffusion (V_{max}). Glucose diffusion was determined based on the method reported by Ou et al.¹⁹ Briefly, the technique involved the use of a sealed dialysis tube filled with a solution of glucose with and without the sample, which was submerged in distilled water and shaken at 37 °C. The amount of glucose in the external solution was quantified with the GOD-PAP kit (glucose oxidase-peroxidase-4-aminophenazone-phenol) from Randox at different times. V_{max} was calculated with values of glucose in the dialysate, using the parabola equation, where Y is the glucose content (μmol); x is time (min); and a, b, and c are coefficients. The equation to calculate the diffusion rate (Y') at any time is $Y' = 2ax + b$. When x is close to 0, $Y' = V_{max} = b$.

Animals. Male Wistar rats (220–250 g) of 8 weeks of age were purchased from Harlan S. A. (Mexico City, Mexico) and fed with a commercial diet. They were maintained at 24 ± 1 °C under a 12/12 h light dark cycle. Experiments on animals were performed following the guidelines for the use of experimental animals of the National Research Council, approved by the Bioethics Committee of the Universidad Autonoma de Queretaro, Mexico.

Diabetes Induction. Diabetes was induced by a single intra-peritoneal injection of freshly prepared streptozotocin (STZ), 45 mg/kg body weight (b.w.) dissolved in citrate buffer, 0.01 M at pH 4.5, after an overnight food deprivation. Five days later, fasting blood glucose levels were measured with a glucometer. Animals with basal glucose levels higher than 180 mg/dL or between 80 and 110 mg/dL and with levels higher than 200 mg/dL after 2 h of an oral glucose tolerance test were classified as diabetic animals.²⁰

Oral Glucose Tolerance Tests (OGTT). Two independent experiments were performed as follows: fasting healthy and STZ-induced diabetic rats were orally administered with cladode flours (SCF, MCF, and LCF) at a dose of 50 mg/kg b.w. and after 10 min were given a glucose solution (2 g/kg b.w.). The cladode flours and glucose were administered by intragastric intubation. Blood glucose concentrations, from the tail vein, were measured with a glucometer at 0, 30, 60, and 120 min. AUC (area under the curve) was calculated using the trapezoidal rule.²¹

Fasting Glucose (FG). Fasting STZ-induced diabetic rats were orally administered with cladode flours (SCF, MCF, and LCF) at a dose of 50 mg/kg b.w.; these flours were also administered by intragastric intubation; blood glucose concentrations were measured with a glucometer from the tail vein at 0, 60, 120, and 180 min.²²

Statistical Analysis. Data are represented as the mean ± standard error (SE). Statistical significance of differences ($p < 0.05$) between the mean values for the treatment groups was analyzed by Tukey's and Dunnett's multiple range test and a one-way analysis of variance (ANOVA) using the statistical software package JMP 8.0.2 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Proximate Analysis. Table 1 shows the proximate analysis results of the cladodes flours at three different maturity stages.

Table 1. Proximate Composition and Fiber and Caloric Contents of Cladode Flours (*O. ficus-indica* var. Milpa Alta)^a

parameters	SCF	MCF	LCF
moisture ^b	6.8 ± 0.2 a	6.4 ± 0.1 a	6.3 ± 0.1 a
ash ^b	15.5 ± 0.2 a	14.9 ± 0.3 a	14.1 ± 0.2 a
fat ^b	3.0 ± 0.0 a	2.5 ± 0.0 b	2.7 ± 0.0 ab
protein ^b	18.5 ± 0.3 a	14.4 ± 0.3 b	15.3 ± 0.2 b
total dietary fiber ^b	38.3 ± 0.2 a	35.2 ± 0.2 b	33.7 ± 0.2 c
insoluble fiber ^b	29.8 ± 0.0 a	25.9 ± 0.6 c	27.6 ± 0.6 b
soluble fiber ^b	8.5 ± 0.2 b	9.3 ± 0.2 a	6.1 ± 0.2 c
caloric content ^c	31.3 ± 0.0 a	29.8 ± 0.0 b	28.1 ± 0.0 c

^aValues are the means ± SE ($n = 3$). Different letters (a–c) indicate significant statistical difference between cladode flours ($p < 0.05$) by Tukey's test. SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour. ^bg/100 g dry weight. ^cCalories/100 g dry weight.

Moisture and ash contents showed some slight changes among all flours, and the fat content was slightly higher in SCF. Hernández-Urbiola et al.²³ reported a lower fat content (1.5 to 2.2 g/100 g d.w.) and higher ash content (17.6–21.6 g/100 g d.w.) for cladode powder from *O. ficus-indica* from higher maturity stages. For all parameters, except for soluble fiber, the SCF sample showed similar or higher values in comparison with those of MCF and LCF. The higher percentage of protein in young cladodes may be related to their higher metabolic activity,²⁴ and the lower content in mature cladodes may be due to the transport of nitrogen from mature to young tissues.²⁵ While soluble and insoluble fiber contents were not related to their maturity stages, total dietary fiber decreased as the size of the cladode increased (Table 1). Rodriguez-García et al.¹² reported higher contents of soluble fiber in cladodes of 60 g as compared to 200 g of pad weight. This behavior may be due to changes of the parenchyma/chlorenchyma ratio;¹³ when the ratio is increased, the mucilage content of the cladodes is also increased, and then the soluble fiber follows the same trend. The differences in composition could be due to the use of different varieties of *Opuntia ficus-indica*, as well as to other factors such as, water availability, soil composition, agronomic production conditions, and the age of cladodes used in the studies.^{12,26}

It should be mentioned that the sum of main components given in Table 1 ranges from 70.1 to 82.1%; the difference to 100% is mainly due to other carbohydrates,²⁷ which were not measured. The caloric content seemed to have an inverse relationship to the maturity stage (SCF > MCF > LCF) (Table 1); based on the proximate composition, carbohydrates may be the main compounds responsible for the caloric changes.

Functional Properties. As observed in Table 2, the values obtained for the bulk density of cladode flours decreased as the maturation of the cladodes increased.

Table 2. Physical Characteristics and Functional Properties of Cladode Flours (*O. ficus-indica* var. Milpa Alta)^a

sample	bulk density ^b	water absorption capacity (WAC) ^c	swelling (SW) ^d	viscosity ^e
cellulose	5.7 ± 0.0 d	2.9 ± 0.0 c	5.1 ± 0.0 d	2.1 ± 0.2 d
SCF	7.1 ± 0.0 a	8.0 ± 0.3 a	25.0 ± 0.0 a	22.8 ± 0.2 a
MCF	6.3 ± 0.0 b	8.0 ± 0.4 a	20.1 ± 0.0 b	21.4 ± 0.1 b
LCF	6.0 ± 0.0 c	6.2 ± 0.1 b	10.2 ± 0.0 c	14.4 ± 0.0 c

^aValues are the means ± SE ($n = 3$). Different letters (a–d) indicate significant statistical difference between cladode flours and cellulose ($p < 0.05$) by Tukey's test. SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour. ^bg/dL. ^cg of water/g of dry weight. ^dmL/g of dry weight. ^emPa·s.

The high proportion of total dietary fiber (Table 1) in the cladode samples is expected to affect the value of the bulk density,²⁸ both the insoluble and the soluble fiber fractions change during the maturation process.¹² However, it is not possible to assign the responsibility for the decrease in bulk density to either the insoluble fiber (mainly cellulose and insoluble hemicelluloses) or the soluble fraction (mainly mucilage and pectins).^{29,30} Further specific studies are required to elucidate the effect of the different fiber fractions on bulk density.

The WAC values of SCF and MCF were the same but that for LCF was significantly lower (Table 2). Both the swelling property and the viscosity of the samples decreased with the age of cladodes, showing a trend similar to that for the bulk density (Table 2). The differences observed between flours for WAC and swelling may be attributed to their dietary fiber contents, such as pectins,³¹ since the carbohydrates interact with free polar groups through hydrophilic bonds or get retained within the matrix.^{32,33} The values for the viscosity of SCF and MCF were similar to those obtained for glucomannan, a soluble fiber, at the same concentration (21.1 mPa·s).³⁴ This result is particularly interesting since glucomannan is known as an antidiabetic soluble fiber.³⁴ Finally, it should be noted that all parameters shown in Table 2 were higher than those of the cellulose control and showed similar values to those reported by Huang et al.³⁵ and Palanuvej et al.³⁴ for the swelling and viscosity properties. The functional properties may produce beneficial physiologic effects, given that higher extract viscosity may increase the viscosity of intestinal contents. Swelling is directly related to the hydration properties of the soluble dietary fiber, which produces a gel-like matrix, which can trap water and nutrients, especially those soluble in water, such as glucose. Additionally, these properties cause low diffusion and dilution of enzymes, substrates, and nutrients to the absorptive surface in the gut, leading to decreased nutrients absorption, including glucose.³⁶

Effect of Cladode Flour on Glucose Diffusion in Vitro.

The decrease of intestinal glucose absorption could be related to some viscous components from plants that exert an antihyperglycemic effect.³⁷ The MCF and SCF showed similar lower glucose values in the dialysate at most experimental times evaluated compared to that of all other samples (Figure 1A); the former sample exhibited a V_{max} reduction of 35.2% and the latter of 37.9% (Figure 1B). Figure 1B shows similar V_{max} values for MCF and SCF of 6.6 and 6.3 $\mu\text{mol}/\text{min}$, respectively, and LCF exhibited values (9.3 $\mu\text{mol}/\text{min}$) closer to those of the control and cellulose.

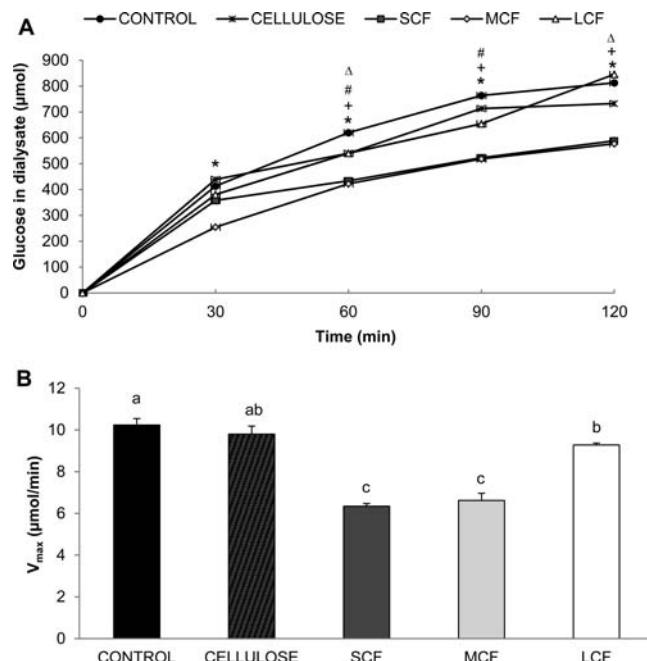


Figure 1. Effect of cladode flour of *O. ficus-indica* var. Milpa Alta on the diffusion of glucose in a dialysis system. (A) Glucose in the dialysate. (B) Maximum velocity of glucose diffusion (V_{max}). Data are the means ± SE ($n = 3$). $+p < 0.05$ for SCF, $*p < 0.05$ for MCF, $#p < 0.05$ for LCF, and $\Delta p < 0.05$ for cellulose compared with the control assessed by Dunnett's test. SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour.

It has been suggested that there is a direct relationship between the viscosity of soluble plant polysaccharides and their ability to inhibit glucose absorption, which is also dependent on the concentration and molecular mass of the soluble fibers.^{37,38} In our experiments, a high soluble fiber content and viscosity were found for SCF and MCF but not for the LCF (Tables 1 and 2). These results suggest that the viscosity of cladode flours affect glucose absorption and may explain the *in vivo* antihyperglycemic effect of the samples under study (Figures 2 and 3).

Antihyperglycemic Effect of Cladode Flours on the Oral Glucose Tolerant Test Evaluated in Healthy and Diabetic Rats. The antihyperglycemic effect of cladode flours at three maturity stages was tested using an oral glucose tolerance test (Figure 2A and B). Basal glucose levels were similar for all groups; 30 min after the glucose load, blood glucose concentration increased over 2-fold for the healthy control (HC) group; thereafter, glucose levels decreased. All cladode samples significantly decreased ($p < 0.05$) the hyperglycemic peak (Figure 2A).

The AUC test represents in this case the behavior of glucose absorption after a glucose load in relationship to time (Figure 2B); the results showed the same trend of those presented in Figure 2A. The AUC values were significantly reduced ($p < 0.05$): for the SCF, 28.4% ($p < 0.05$); while for MCF and LCF treatments, reductions were numerically but not significantly lower, 19.7% and 16.2%, respectively in relationship to the HC ($p < 0.05$) (Figure 2B).

The antihyperglycemic effect of cladode flours was also evaluated in diabetic rats. Figure 3A shows that basal glucose levels were similar for all experimental groups; at 30 min, the diabetic control (DC) group presented an almost 7-fold

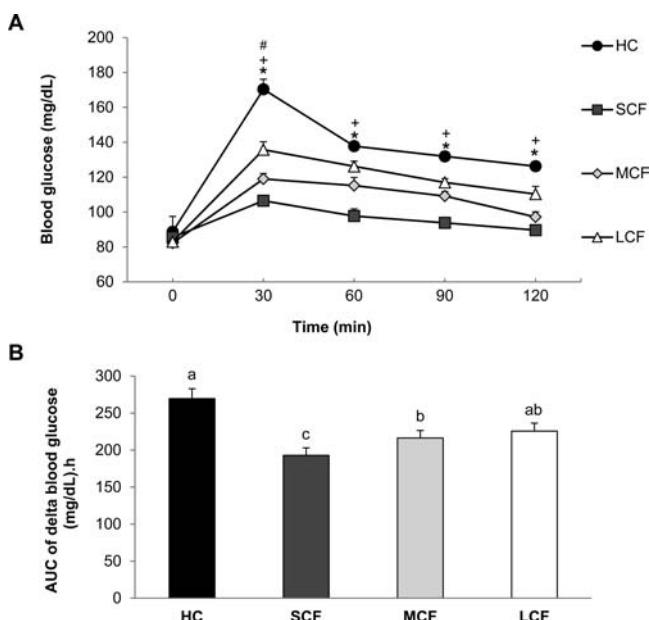


Figure 2. Oral glucose tolerance test. (A) Blood glucose and (B) area under the curve (AUC) of healthy rats administered with cladode flour 50 mg/kg b.w. Data are the means \pm SE ($n = 5$). $+p < 0.05$ for SCF, $*p < 0.05$ for MCF, and $^{\#}p < 0.05$ for LCF compared with the control assessed by Dunnett's test. (a–c) Different letters indicate significant statistical differences between cladodes flours ($p < 0.05$) assessed by Tukey's test. HC, healthy control; SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour; AUC, area under the curve; b.w., body weight.

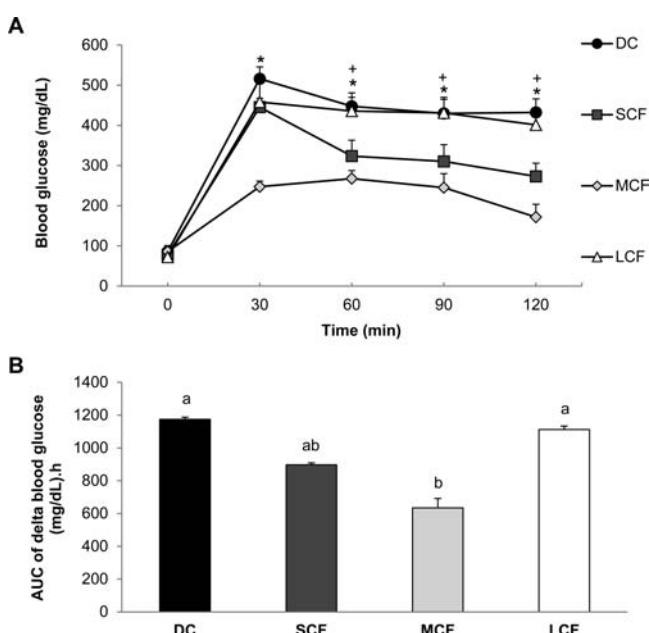


Figure 3. Oral glucose tolerance test. (A) Blood glucose and (B) area under the curve (AUC) of diabetic rats administered with cladode flour 50 mg/kg b.w. Data are the means \pm SE ($n = 5$). $+p < 0.05$ for SCF and $*p < 0.05$ for MCF compared with the control assessed by Dunnett's test. (a–b) Different letters indicate significant statistical differences between cladodes flours ($p < 0.05$) assessed by Tukey's test. DC, diabetic control; SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour; AUC, area under the curve; b.w., body weight.

increase of blood glucose concentration, and it was unable to return to or approach the basal levels (120 min). The MCF sample significantly attenuated ($p < 0.05$) the postprandial glucose peak (30 min) by 52% compared to that of the diabetic control group and significantly ($p < 0.05$) attenuated glucose levels especially at 120 min. All AUC values for diabetic rats (Figure 3B) were about 4-fold higher than those for healthy rats, which may be due to a higher glucose intolerance as a consequence of pancreas damage for the STZ-induction. MCF presented the highest decreases on AUC (46%) ($p < 0.05$), twice as much of the reduction of the healthy rats (Figure 2B); this difference could be due to an additional mechanism of dietary fiber entrapment that synergistically might take place in the diabetic rats, related to nonidentified compounds. More studies are needed to explain these results. Meanwhile, the SCF treatment did not significantly attenuate ($p < 0.05$) the hyperglycemic peak but significantly decreased ($p < 0.05$) the glucose levels at 60, 90, and 120 min. Rats treated with SCF did not reach basal glucose levels, but significant ($p < 0.05$) differences were exhibited with the diabetic control, and they showed a lower AUC (23.6%) (Figure 3A and B). As in the healthy rats, again the role of LCF was weaker in diabetic rats. The trend of Figure 3A and B was not observed for healthy rats (Figure 2A and B), which may be due to differences in their glucose metabolism related to the physiological state of the animals.

The reduction of the AUC values with SCF and MCF may be associated with a glucose entrapment mechanism produced by the viscosity and swelling properties of the soluble fiber inside the intestine, as shown by the flour in solution (Table 2). Similar results have been reported by other authors, who suggest that the antihyperglycemic effect of nopal may be due to its fiber content, mainly pectin, which may decrease carbohydrate absorption because of the gel forming properties of pectins.^{39,40}

Another mechanism related to decreased blood glucose levels is through an improvement of insulin sensitivity by suppressing the production of hepatic glucose.²² It has been reported that nondigestible fermentable dietary fibers increase the release of intestinal glucagon-like peptide 1 (GLP-1) receptor, leading to the stimulation of insulin secretion⁴¹ and reduction of hepatic glucose production by the improvement of insulin-stimulated phosphorylation of IRS-2 and Akt in the liver of treated mice, thus normalizing the excessive hepatic glucose production.⁴²

Hypoglycemic Effect of Cladode Flour on Diabetic Rats. In a diabetic condition, blood glucose is elevated, and this hyperglycemic state is worsened by a glucose contribution from the liver and a lower glucose incorporation on insulin dependent cells such as muscle and fat.⁴³ Basal glucose levels were similar for all experimental groups; blood glucose levels corresponding to the diabetic control group were constantly high throughout the experiment. The highest significant decrease ($p < 0.05$) was observed for the MCF treatment (40%), followed by SCF (30% decrease) ($p < 0.05$); as for LCF, no significant difference with the diabetic control was observed (Figure 4).

Hahm et al.⁴⁴ reported that STZ-induced diabetic rats treated with freeze-dried cladode powder from *O. humifusa* (150–500 mg/kg b.w.) significantly decreased the fasting glucose levels, mainly attributed to a higher insulin production, and that an aqueous extract prepared from the cladodes increased the plasma insulin in healthy rats.⁴⁵ Also, Morán-Ramos et al.⁴⁶ suggested that dehydrated cladodes from *O. ficus-indica* var.

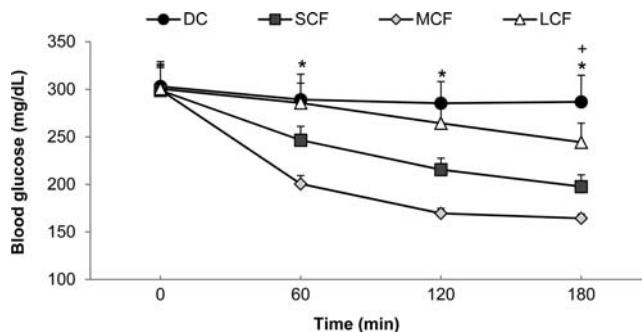


Figure 4. Fasting glucose of STZ-diabetic rats administered with cladode flour 50 mg/kg b.w. Data are the means \pm SE ($n = 7$). $+p < 0.05$ for SCF and $*p < 0.05$ for MCF compared with the control assessed by Dunnett's test. DC, diabetic control; SCF, small cladode flour; MCF, medium cladode flour; LCF, large cladode flour; b.w., body weight.

Milpa Alta improved hepatic insulin sensitivity, consequently reducing hepatic gluconeogenesis in obese Zucker (*fa/fa*) rats since gluconeogenesis is modulated by the insulin signaling pathway. It has been reported that a diet supplemented with viscous and no fermentable soluble fibers improved altered hepatic gene expression profiles associated with fatty liver, insulin resistance, and intestinal permeability.⁴⁷

In conclusion, cladode flours obtained from different maturity stages showed different antihyperglycemic and hypoglycemic effects; the small and medium cladode samples exhibited the greatest reduction of glucose, most likely due to entrapment mechanisms and delay of intestinal absorption. Apparently, viscosity is a prominent factor directly affecting the antidiabetic potential. Thus, further studies using different levels of viscosity are needed.

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Notes

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