Int. J. Plant Bas. Pharm. (2023) 3(2), 210-214

INTERNATIONAL JOURNAL OF PLANT BASED PHARMACEUTICALS

https://ijpbp.com

SHORT REPORT

OPEN ACCESS

Effect of *Nopalea cochenillifera* (L.) Salm-Dyck cladodes flour on glucose levels in streptozotocin-induced diabetic CD-1 mice

Héctor Enrique Fabela-Illescas[a], Juan Pablo Hernández-Uribe[a], Helen Belefant-Miller[b], Mónica Alonso de Jesús[c], Gabriel Betanzos-Cabrera[d*]

[a] *Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Av. Universidad Km 1, Rancho Universitario, C.P. 43600, Tulancingo de Bravo, Hidalgo, México*

[b] *Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA*

[c] *Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia Av. Universidad 3000, 04510, Ciudad de México, México*

[d] *Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias de la Salud, Área Académica de Nutrición, Ex-Hacienda de la Concepción, Tilcuautla, Pachuca de Soto 42080, Mexico*

ARTICLE INFO

Article History:

Received: 10 July 2023
Revised: 30 September 2023
Accepted: 08 October 2023
Available online: 14 October 2023

Edited by: B. Tepe

Keywords:

Cactus
Nopalea cochenillifera (L.) Salm-Dyck
Diabetes
Bioactive compounds
Diabetic mice

ABSTRACT

Nopalea cochenillifera (L.) Salm-Dyck is a cactus species native to Mexico, now widely distributed in the West Indies and tropical America. It is a species not usually consumed as food, but due to its composition of bioactive compounds, it could be used to treat diabetes. The present study aimed to evaluate the effect of *N. cochenillifera* cladode flour on glucose levels in streptozotocin-induced diabetic CD-1 mice. The flour obtained from cladodes absorbed glucose proportionally to the glucose concentration, and it was observed that the administration of flour (10.5 mg/kg body) decreased glucose levels in diabetic mice compared to the control group. This preclinical study demonstrates that *N. cochenillifera* flour could be used to improve glucose homeostasis in type 2 diabetes.

Reviewed by:
Aku Enam Motto: University of Lomé, BP 1515, Togo
Mehmet Akyüz: Kilis 7 Aralik University, Kilis, Türkiye
Diah Kartika Pratami: Universitas Indonesia, West Java, Indonesia
Mehmet Ali Temiz: Karamanoğlu Mehmetbey University, Karaman, Türkiye

* Corresponding author(s):
E-mail address:
gbetanzo@uaeh.edu.mx (G. Betanzos-Cabrera)
e-ISSN: 2791-7509
doi: https://doi.org/10.29228/ijpbp.34

1. Introduction

Nopalea cochenillifera (L.) Salm Dyck is a variety of cactus native to Mexico that is not commonly consumed by humans and is found in America, especially in dry regions of Mexico and Central America. It is a wild species primarily cultivated in gardens and used in landscaping, such as hedges (Lim, 2012). The current use of *N. cochenillifera* is mainly as fodder (Cardoso et al., 2019). Its cladodes are excellent sources of fiber, vitamins (vitamins C and K), minerals (calcium and magnesium), and hydrolyzable polyphenols (Fabela-Illescas et al., 2022; Lim, 2012). In addition, it is low in calories and fat, making it a healthy option to incorporate into the diet. *N. cochenillifera* has also been characterized by determining pH, color parameters, total soluble solids, water absorption capacity, oil absorption capacity, and swelling capacity of flour made from *N. cochenillifera* cladodes (Fabela-Illescas et al., 2022).

Please cite this article as: Fabela-Illescas, H. E., Hernández-Uribe, J. P., Belefant-Miller, H., de Jesús, M. A., & Betanzos-Cabrera, G. (2023). Effect of *Nopalea cochenillifera* (L.) Salm-Dyck cladodes flour on glucose levels in streptozotocin-induced diabetic CD-1 mice. *International Journal of Plant Based Pharmaceuticals*, *3*(2), 210-214, https://doi.org/10.29228/ijpbp.34.

Cacti are susceptible to microbial attack due to their high water content and low pH, which limits their marketability. Mature *N. cochenillifera* cladodes (flattened green stems) are often not incorporated into the human diet, representing a waste of a potential source of nutrition. Food technology can be used to maintain the quality of cladodes and maximize their shelf life for human consumption. Drying and milling processes would promote the consumption of *N. cochenillifera* by making it accessible for people to take advantage of its health benefits (Contreras-Padilla et al., 2011).

Diabetes is a global disease that impacts economically, socially, and on the quality of life of those who suffer from it (Bommer et al., 2018). Type 2 diabetes is characterized by hyperglycemia, insulin resistance, and loss of pancreatic β-cell function. In its initial stage, there are no symptoms. When detected late and not correctly treated, it causes serious health complications such as heart attack, blindness, kidney failure, amputation of the lower extremities, and premature death (Chatterjee et al., 2017). Therefore, it is essential to modify unhealthy lifestyles, such as sedentary lifestyles and poor diets, by over-consuming energy-dense industrialized foods. This lifestyle is typical in the Mexican population (Hernández-Ávila et al., 2013). Currently, there is an approach towards using medicinal plants to control and prevent chronic diseases such as diabetes (Ahad et al., 2020). *N. cochenillifera* has been used in traditional medicine to treat inflammatory diseases, dyslipidemia, high blood pressure, kidney problems, and diabetes (Tavares et al., 2023). All these benefits are attributed to phenolic compounds and their dietary fiber content (Magaña-Cerino et al., 2022; Tavares et al., 2023). In a pilot clinical trial before this work, it was reported that the administration of a drink based on fresh cactus of the *N. cochenillifera* variety showed a tendency to lower glucose and glycosylated hemoglobin levels in patients with type 2 diabetes in a rural Mexican population (Fabela-Illescas et al., 2015). Another study reported that a single dose of fresh, blended cladodes of *N. cochenillifera* decreased postprandial glycemia in rats after a starch load (Magaña-Cerino et al., 2022). This work focused on testing the hypoglycaemic activity of cladode flour in streptozotocin-induced diabetic CD-1 mice.

2. Materials and methods

2.1. *N. cochenillifera* cladodes flour

Mature (70 days after emergence) *N. cochenillifera* cladodes, with average morphometric variables of 25 cm long, 6 cm wide, and 1 cm thick (**Figure 1**), were manually harvested in the Tulancingo Valley, Hidalgo, in the spring of 2020. A voucher specimen (U10) was deposited by Manuel González Ledesma at the HGOM Herbarium of the Department of Botany, Universidad Autónoma del Estado de Hidalgo, Mexico. They were cut and dehydrated in a dryer at 45 °C for 48 h, as per Fabela-Illescas et al. (2022), and further pulverized in a pulverizing mill (UDY Cyclone Sample Mill, Fort Collins, CO, USA) for 15 min, then passed through a 100 mesh sieve.

2.2. Glucose adsorption capacity

The method for the determination of glucose adsorption capacity was performed as described by Peerajit et al. (2012); three glucose solutions (50, 100, and 200 mmol/g) were mixed each with 1 g of cladodes flour, incubated at 37 °C for 6 h and centrifuged at 4.000 x g for 20 min. The glucose retained in the supernatant (mmol/g of fiber) was calculated using a D-Glucose test kit (Megazymw, Bray, Ireland). Glucose adsorbed was calculated using the following formula:

$$Glucose\ adsorbed\ (mmol/g) = \left(\frac{C_i - C_s}{[W_s]}\right)\ x\ V_i$$

C_i = Glucose concentration of original solution (mmol/l)
C_s = Glucose concentration when the adsorption reached equilibrium (mmol/l)
W_s = Weight of cladodes flour (g)
V_i = Volume of glucose solution (ml)

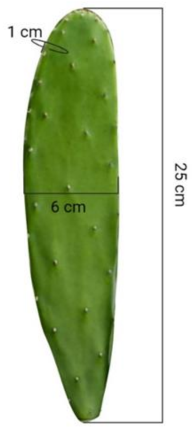

Figure 1. Morphometric variables of *N. cochenillifera* cladodes used in the study

2.3. Animal of study

Before experiments, the animals were housed in mouse cages at room temperature (27 ± 2 °C) with 12-h light/dark cycles. The care and use of the animals were approved by the Animal Care Committee of the Instituto de Ciencias de la Salud, UAEH, with the number CIECUAL/006/2020, as in other experiments (Estrada-Luna et al., 2018). Sixteen 8-9 week old male CD-1 mice weighing 35-40 g were used; we divided into four groups (n = 4): control (group 1), supplemented with 3.5, 7.0, and 10.5 mg/kg body of cladode flour, forming groups 2, 3 and 4, respectively.

2.3.1. Diets

The feed for the groups was a standard rat diet (Rodent Chow5008®). For the study groups, egg and lard were added to the same diet to increase caloric intake by approximately 30%, which contained 42% carbohydrate, 12% protein, and 42% saturated fat in the final feed, a high-fat diet similar to those reported by Romo-Araiza et al. (2018).

2.3.2. Induction of diabetes and treatments

Before the administration of flour, a group was diabetized (glucose values above 200 mg/dl) with a single dose of streptozotocin (Sigma, St. Louis, MO) at 180 mg/kg body, administered intraperitoneally (Betanzos-Cabrera et al., 2011).

After the animals were diabetized, three concentrations of cladode flour (3.5, 7.0, and 10.5 mg/kg body) were administered intragastrically to the mice in each group (n = 4). The control group was administered water and 10.5 mg/kg body flour intragastrically. Blood samples were obtained by venipuncture from the tail of each mouse at 0, 30, 60, 90, and 120 min after administration of flour. Glucose levels in the blood were measured using a glucose monitoring device (Accu-Chek Instant, Roche Indianapolis, IN, USA). Glucose levels were monitored for a more extended period (28 days) using the highest concentration of flour (10.5 mg/kg body).

2.4. Statistical analysis

Data were expressed as mean ± standard deviation. A one-way ANOVA with a Tukey test was used to analyze the results of all experimental groups. Differences were considered significant at $p < 0.05$, using GraphPad Prism version 8.4 (GraphPad Software, San Diego, USA).

3. Results and discussion

N. cochenillifera contains significant amounts of crude fiber, carbohydrates, and phenolic compounds (da Silva et al., 2015; Fabela-Illescas et al., 2022). Cladodes' concentration and fiber composition depends on their maturity stage, as does the content of phenolic compounds (Moussa-Ayoub et al., 2014; Rodríguez-García et al., 2007). The maturity stage of cladodes has shown a positive correlation with insoluble fiber content and a negative correlation with soluble fiber (Rodríguez-García et al., 2007). Peerajit et al. (2012) reported that dietary fiber absorbs glucose in a dose-dependent manner, which agrees with our results, as we found that flour absorbs glucose directly proportional to glucose concentration (mmol/g) (Figure 2).

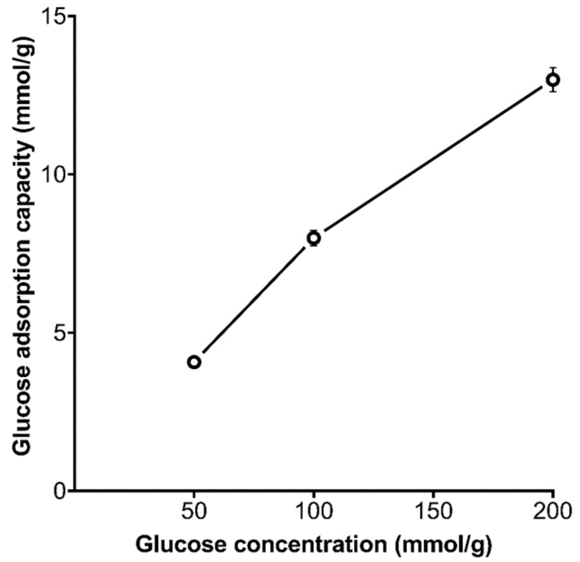

Figure 2. Glucose adsorption capacity of *N. cochenillifera* cladode flour

Figure 3 shows a decrease in glucose levels at the three administered flour concentrations. The 10.5 mg/kg concentration showed the best trend in decreasing glucose levels over time, with a significance level of $p < 0.05$. Villaseñor and Lamadrid (2006) reported a non-significant decrease in glucose levels upon administration of 5 mg of *N. cochenillifera*. Our results also show a reduction in glucose levels in mice supplemented with 10.5 mg/kg flour compared to diabetic and non-diabetic control mice (Figure 4). Furthermore, Figure 4 shows that 10.5 mg/kg flour in the control group did not affect the standard homeostatic control of glucose levels.

Glucose levels were also monitored for 28 days (Figure 5) in group 4 (supplemented with 10.5 mg/kg body). The mouse with the highest plasma glucose levels at the beginning of the experiment decreased the most at the end compared to the other animals in the same group. These results are related to those presented in Figure 2, where the glucose uptake capacity of flour was directly proportional to the glucose concentration, suggesting that the greater the uncontrolled glucose levels, the greater the hypoglycaemic effect of *N. cochenillifera*.

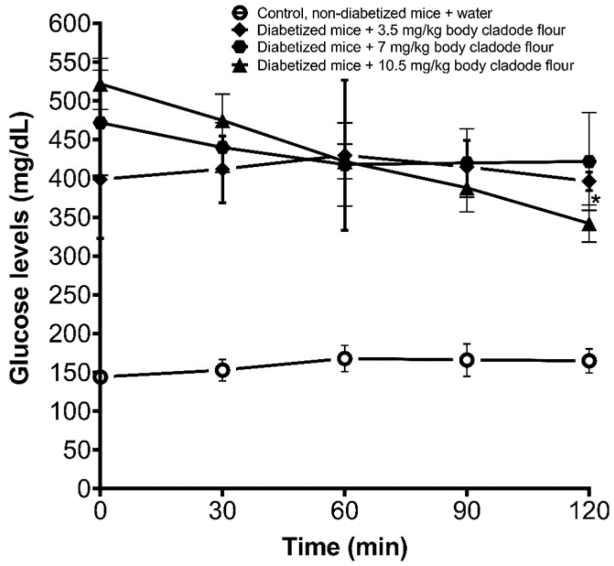

Figure 3. Effect of increasing concentrations of cladode flour on blood glucose levels in diabetic mice

Glucose values are the mean of four replicates ± standard deviation. Diabetic mice and control non-diabetic mice were orally administered with water or with 3.5, 7.0, or 10.5 mg/kg body cladode flour.

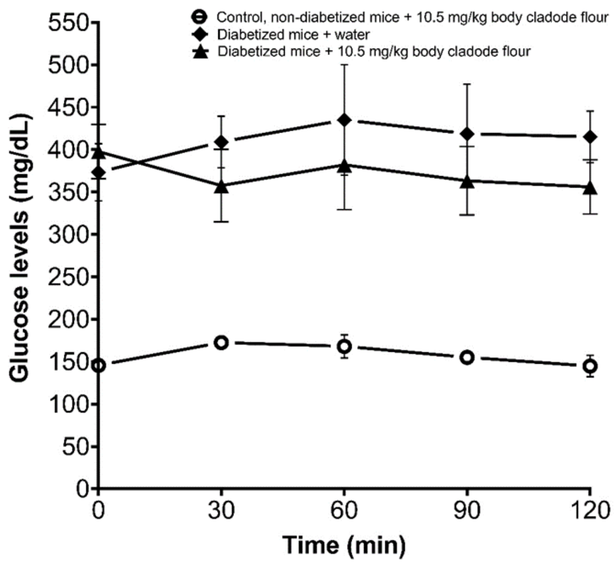

Figure 4. Glucose levels in control and diabetic mice after administration of water control or 10.5 mg/kg body cladode flour

Glucose levels are the mean of four repetitions ± standard deviation.

Supplemented groups 2 and 3 (3.5 and 7.0 mg/kg body, respectively) exhibited glucotoxicity and died before the end of the 28-day follow-up.

Although the decrease in glucose levels after flour supplementation could be related to fiber, the decrease could also be attributed to the effect of phenolic compounds (Leem et al., 2016). At least 31 compounds have been identified by HPLC in the cactus (Tavares et al., 2023). Previously, the following phenolic compounds were identified and quantified in cladode flour of *N. cochenillifera*, which could be related to its anti-diabetic effects: gallic acid (2708.49 µg/g), ferulic acid (796.73 µg/g), chlorogenic acid (179.70 µg/g), *p*-coumaric acid (80.58 µg/g), syringic acid (11.12 µg/g) and neochlorogenic acid (9.52 µg/g) (Fabela-Illescas et al., 2022). Chlorogenic acid has hypoglycaemic properties, delaying intestinal glucose absorption and inhibiting hepatic gluconeogenesis (Ong et al., 2012). Polyphenol conjugates, and dietary fiber are promising natural ingredients for improving nutrition. Non-extractable polyphenols bound to dietary fibers are implicated in preventing or progressing various chronic diseases. These polyphenolic fibers can be used to improve the nutritional content of food products, be used by consumers at high risk for human health problems (e.g., diabetes), or enable personalized nutrition (Fernandes et al., 2023). Andrade-Cetto and Wiedenfeld (2011) reported that pectins (complex polysaccharides present in plant cell walls) act as nutrient sequestrants in the digestive tract, preventing glucose absorption.

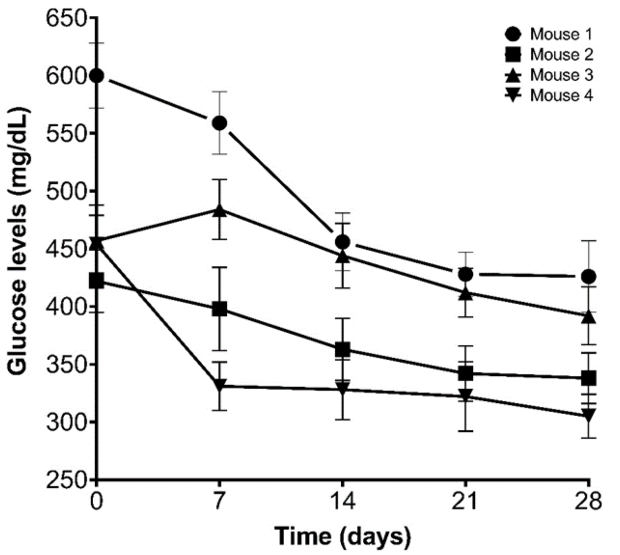

Figure 5. Glucose levels in four diabetic mice administered 10.5 mg/kg body cladode flour

Magaña-Cerino et al. (2022) evaluated the in vitro α-glucosidase activity of methanolic extract of *N. cochenillifera*. They found that the enzyme activity is not related to the glycaemic response, and perhaps the anti-diabetic effect may instead be due to compounds that cause changes in the gut microbiota (Sánchez-Tapia et al., 2017). Sánchez-Tapia et al. (2017) reported that incorporating nopal in the diet caused a decrease in serum glucose in obese rats, modified the intestinal microbiota, and increased occludin. It correlated with decreased metabolic endotoxemia, insulinotropic glucose peptide, glucose intolerance, lipogenesis, and metabolic inflexibility. The anti-diabetic mechanism of *N. cochenillifera* flour is unclear, and further testing is needed to identify the chemical source of the anti-diabetic effect.

4. Conclusions

Overall, the present work demonstrates the lowering of blood glucose levels by *N. cochenillifera* cladode flour in an in vivo model with induced diabetes. The flour could be a functional food and be a therapeutic option in disease treatment. However, further clinical trials are necessary on *N. cochenillifera* cladode to corroborate the anti-diabetic activity of the plant while considering the presence of comorbidities, pharmacological interaction, and dietary patterns.

Acknowledgments

We thank CONACyT for supporting Héctor Enrique Fabella-Illescas as a scholarship recipient.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

A veterinarian supervised the handling of the animals following the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

None.

CRediT authorship contribution statement

Héctor Enrique Fabela-Illescas: Data curation; Investigation; Visualization
Pablo Hernández-Uribe: Investigation; Resources
Helen Belefant-Miller: Formal analysis; Writing-review & editing
Mónica Alonso de Jesús: Methodology
Gabriel Betanzos-Cabrera: Formal analysis; Writing-original draft

ORCID Numbers of the Authors

H. E. Fabela-Illescas: 0000-0003-4421-4409
P. Hernández-Uribe: 0000-0002-2759-1192
H. Belefant-Miller: 0000-0002-9847-8824
M. A. de Jesús: 0009-0009-5368-7487
G. Betanzos-Cabrera: 0000-0003-2027-6904

Supplementary File

None.

Publisher's Note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ahad, A., Raish, M., Bin Jardan, Y. A., Alam, M. A., Al-Mohizea, A. M., & Al-Jenoobi, F. I. (2020). Effect of *Hibiscus sabdariffa* and *Zingiber officinale* on the antihypertensive activity and pharmacokinetic of losartan in hypertensive rats. *Xenobiotica, 50*(7), 847-857.

Andrade-Cetto, A., & Wiedenfeld, H. (2011). Anti-hyperglycemic effect of *Opuntia streptacantha* Lem. *Journal of Ethnopharmacology, 133*(2), 940-943.

Betanzos-Cabrera, G., Guerrero-Solano, J., Martínez-Pérez, M., Calderón-Ramos, Z., Belefant-Miller, H., & Cancino-Diaz, J. C. (2011). Pomegranate juice increases levels of paraoxonase1 (PON1) expression and enzymatic activity in streptozotocin-induced diabetic mice fed with a high-fat diet. *Food Research International, 44*(5), 1381-1385.

Bommer, C., Sagalova, V., Heesemann, E., Manne-Goehler, J., Atun, R., Bärnighausen, T., Davies, J., & Vollmer, S. (2018). Global economic burden of diabetes in adults: projections from 2015 to 2030. *Diabetes Care, 41*(5), 963-970.

Cardoso, D. B., de Carvalho, F. F. R., de Medeiros, G. R., Guim, A., Cabral, A. M. D., Véras, R. M. L., dos Santos, K. C., Dantas, L. C. N., & de Oliveira Nascimento, A. G. (2019). Levels of inclusion of spineless cactus (*Nopalea cochenillifera* Salm Dyck) in the diet of lambs. *Animal Feed Science and Technology, 247*, 23-31.

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *The Lancet, 389*(10085), 2239-2251.

Contreras-Padilla, M., Perez-Torrero, E., Hernández-Urbiola, M. I., Hernández-Quevedo, G., del Real, A., Rivera-Muñoz, E. M., & Rodríguez-García, M. E. (2011). Evaluation of oxalates and calcium in nopal pads (*Opuntia ficus-indica* var. *redonda*) at different maturity stages. *Journal of Food Composition and Analysis, 24*(1), 38-43.

da Silva, A. P. G., de Souza, C. C. E., Ribeiro, J. E. S., dos Santos, M. C. G., de Souza Pontes, A. L., & Madruga, M. S. (2015). Características físicas, químicas e bromatológicas de palma gigante (*Opuntia ficus-indica*) e miúda (*Nopalea cochenillifera*) oriundas do estado da Paraíba. *Revista Brasileira de Tecnologia Agroindustrial, 9*(2), 1810-1820.

Estrada-Luna, D., Martínez-Hinojosa, E., Cancino-Diaz, J., Belefant-Miller, H., López-Rodríguez, G., & Betanzos-Cabrera, G. (2018). Daily supplementation with fresh pomegranate juice increases paraoxonase 1 expression and activity in mice fed a high-fat diet. *European Journal of Nutrition, 57*, 383-389.

Fabela-Illescas, H. E., Ávila-Domínguez, R., Hernández-Pacheco, A., Ariza Ortega, J. A., & Betanzos-Cabrera, G. (2015). Efecto de una bebida a base de nopal (*Nopalea cochenillifera* (l) Salm-dyck) en pacientes de una población rural de Hidalgo, México: ensayo clínico piloto. *Nutrición Hospitalaria, 32*(6), 2710-2714.

Fabela-Illescas, H. E., Castro-Mendoza, M. P., Montalvo-González, E., Anaya-Esparza, L. M., Vargas-Torres, A., Betanzos-Cabrera, G., & Hernandez-Uribe, J. P. (2022). Identificación de compuestos bioactivos y características fisicoquímicas de la harina de cladodios de *Nopalea cochenillifera* (L.) Salm-Dyck. *Biotecnia, 24*(1), 46-54.

Fernandes, A., Mateus, N., & de Freitas, V. (2023). Polyphenol-Dietary Fiber Conjugates from Fruits and Vegetables: Nature and Biological Fate in a Food and Nutrition Perspective. *Foods, 12*(5), 1052.

Hernández-Ávila, M., Gutiérrez, J. P., & Reynoso-Noverón, N. (2013). Diabetes mellitus en México: El estado de la epidemia. *Salud Pública de México, 55*, 129-136.

Leem, K. H., Kim, M. G., Hahm, Y. T., & Kim, H. K. (2016). Hypoglycemic effect of *Opuntia ficus-indica* var. *saboten* is due to enhanced peripheral glucose uptake through activation of AMPK/p38 MAPK pathway. *Nutrients, 8*(12), 800.

Lim, T. K. (2012). *Edible medicinal and non-medicinal plants*: Springer.

Magaña-Cerino, J. M., Guzmán, T. J., Soto-Luna, I. C., Betanzos-Cabrera, G., & Gurrola-Díaz, C. M. (2022). Cladodes from *Nopalea cochenillifera* (L.) Salm-Dyck (Cactaceae) attenuate postprandial glycaemia without markedly influencing α-glucosidase activity. *Natural Product Research, 36*(4), 1105-1108.

Moussa-Ayoub, T. E., Abd El-Hady, E. S. A., Omran, H. T., El-Samahy, S. K., Kroh, L. W., & Rohn, S. (2014). Influence of cultivar and origin on the flavonol profile of fruits and cladodes from cactus *Opuntia ficus-indica*. *Food Research International, 64*, 864-872.

Ong, K. W., Hsu, A., & Tan, B. K. H. (2012). Chlorogenic acid stimulates glucose transport in skeletal muscle via AMPK activation: a contributor to the beneficial effects of coffee on diabetes. *PloS One, 7*(3), e32718.

Peerajit, P., Chiewchan, N., & Devahastin, S. (2012). Effects of pretreatment methods on health-related functional properties of high dietary fibre powder from lime residues. *Food Chemistry, 132*(4), 1891-1898.

Rodríguez-García, M., de Lira, C., Hernández-Becerra, E., Cornejo-Villegas, M., Palacios-Fonseca, A., Rojas-Molina, I., Reynoso, R., Quintero, L., Del-Real, A., *et al.* (2007). Physicochemical characterization of nopal pads (*Opuntia ficus indica*) and dry vacuum nopal powders as a function of the maturation. *Plant Foods for Human Nutrition, 62*, 107-112.

Romo-Araiza, A., Ríos-Hoyo, A., Ibarra, A., Hernández-Ortega, M., Gutiérrez-Salmeán, G., & Meneses-Mayo, M. (2018). Effect of a formulation with nopal (*Opuntia ficus-indica*), amaranth (*Amaranthus cruentus*) and mushroom (*Pleurotus ostreatus*) in a murine model of diet-induced cardiometabolic disruptions. *Insights in Nutrition and Metabolism, 2*, 5-13.

Sánchez-Tapia, M., Aguilar-López, M., Pérez-Cruz, C., Pichardo-Ontiveros, E., Wang, M., Donovan, S. M., Tovar, A. R., & Torres, N. (2017). Nopal (*Opuntia ficus indica*) protects from metabolic endotoxemia by modifying gut microbiota in obese rats fed high fat/sucrose diet. *Scientific Reports, 7*(1), 4716.

Tavares, E. D. A., Guerra, G. C. B., da Costa Melo, N. M., Dantas-Medeiros, R., da Silva, E. C. S., Andrade, A. W. L., de Souza Araújo, D. F., da Silva, V. C., Zanatta, A. C., *et al.* (2023). Toxicity and Anti-Inflammatory Activity of Phenolic-Rich Extract from *Nopalea cochenillifera* (Cactaceae): A Preclinical Study on the Prevention of Inflammatory Bowel Diseases. *Plants, 12*(3), 594.

Villaseñor, I. M., & Lamadrid, M. R. A. (2006). Comparative anti-hyperglycemic potentials of medicinal plants. *Journal of Ethnopharmacology, 104*(1-2), 129-131.