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Anti-hyperglycemic Effects of Agave Fructans in Murine Model and *in vitro* Assay

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Abstract

Diabetes mellitus is a complex disease that involves various disorders related to the metabolism of macronutrients. It is characterized by chronic hyperglycemia that is a consequence of defects in the action and secretion of insulin. The treatment of diabetes leads to the use of drugs with notable side effects and alternatives have been sought through the use of various plant components. Agave fructans have shown in animal studies effects on the reduction of blood glucose. The objective of this study was to evaluate the anti-hyperglycemic activity of *Agave tequilana* fructans using *in vitro* and murine models to test optimal concentration and dose at several times to assure the biological effect of these compounds. The glucose release delay test was performed using dialysis membranes to identify the kinetics of glucose release. For the in *vivo* test, the study of postprandial hypoglycemic activity was performed. The results showed that Agave fructans decrease glucose absorption probably due to the delay in the release of glucose to the organism. Because of the above, Agave fructans could be used as food ingredients with the ability to control glucose levels in diabetic people.

Keywords

Agave tequilana, Fructans, Hypoglycemic Activity

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1. Introduction

According to International Diabetes Federation in 2016 there were more than 415 million people with Diabetes mellitus (DM) and it can be estimated that in the coming 25 years this tendency will result in an increase that will peak more than 642 million [1].

DM is a complex disease that involves various disorders related to metabolism of macronutrients (carbohydrates, lipids and proteins). It is characterized by high levels of blood glucose (chronic hyperglycemia) that are a consequence of defects in the action and secretion of insulin. Most prevalent forms of diabetes are type I or insulin dependent, and type II or noninsulin dependent [2].

The hyperglycemia generates reactive oxygen species that cause lipid peroxidation, membrane damage, and also play an important role in secondary diabetes-related complications such as cataracts, neuropathy and nephropathy.

The antioxidants protect cells from peroxidation and therefore help in the treatment of diabetes. Plants that contain antioxidants such as polyphenols, which are classified into several groups among which are the tannins and flavonoids, have shown an effect on the elimination of free radicals, anti-inflammatory action and antidiabetic potential [3].

The treatment of DM depends on the administration of insulin or oral antidiabetic agents that have potential side

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effects [4], however they present a number of undesired side effects associated with their uses, so it has been suggested that the use of safer, specific and effective plant products for treatments of DM (particularly type II DM) be promoted [5].

Traditional herbal remedies used for DM have identified more than 1,200 species of plants with hypoglycemic activity and more than 200 chemical components of different plants are reported as blood glucose reducers with various mechanisms proposed [6].

Fructans are fructose polymers that form the reserve of carbohydrates of several plants, including *Agavaceae* family that belongs to this genus [7]. Moreover, fructans has important functions in the physiology of plants, mainly in the resistance to adverse conditions like cold and heat.

Recently, they have shown beneficial effects on health due to beta (2-1) and (2-6) bonds found in fructans which are not fermentable by any enzyme in the human body, besides having flavonoids present with potential antioxidant activity [8].

Urías and cols. in 2014 showed that some fructans isolated of *Agave dasylirion* in obese animals reduced their food intake in addition to losing weight and lowering glucose, cholesterol levels and increasing levels of lectins involved in decreasing appetite [9].

In another study some fructans of *Agave angustifolia* showed usefulness in controlling some parameters like body weight, blood metabolites, etc. in obese and diabetic rats [10].

It has been observed that fructans serve as prebiotics in addition to showing hypoglycemic activity [11, 12]. However, in the case of the fructans from *Agave tequilana*, no studies have been carried out regarding its postprandial hypoglycemic activity and its possible mechanism of action. Therefore, the present study sought to evaluate the anti-hyperglycemic activity of *Agave tequilana* fructans in models *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Materials

A) Reagents

α-cellulose, glucose, maltose and acarbose were purchased from Sigma-Aldrich, while the kit for glucose determination was RANDOX brand. The glucometer used was brand Accu-chek.

B) Agave Fructans

Agave fructans were prepared from plants of *Agave tequilana Weber* collected from the state of Jalisco. Soluble carbohydrates obtained from agave heads juice were spray dried until a relative humidity of 4.1%. Characterization was realized according to its carbohydrate profile by HPLC. Fructans with degree of polymerization >10 were the main component with 69% [13].

C) Animals

For the present study, 15 male Wistar rats were used, which were purchased from animal facilities of the University of Guadalajara.

2.2. Determination of Glucose Dialysis Retardation

In this test the rate of glucose release at intestinal level was simulated. The experiment was performed according to the proposed by Abiramí, 2014; Bhutkar, 2013 with modifications. The assay was performed in triplicate, a 100 mM / L glucose solution was prepared for fill membrane dialysis [14, 15].

Cellular dialysis membranes with molecular cut-off of 12,000 Daltons were used for the assay. The membranes were filled with 25 mL of a 100 mM glucose solution combined with the samples: (0.5 grams of fructans), positive control (0.5 grams of cellulose) and a negative control without sample.

Once the samples were prepared, they were dialyzed in 80 mL of distilled water and incubated at 37°C in flasks. After that 1 mL of dialysate was taken from each vial to determine glucose by a kit based on the glucose oxidase reaction, at the following times 30, 60, 120 minutes [16].

2.3. Postprandial Hypoglycemic Activity Test in Rats

The postprandial hypoglycemic test was proposed by Jaiswal et al. (2009) [17]. In this study we used non-diabetics male Wistar rats each one with 180 g and 9 weeks old. They were subjected to 12 hours of light and 12 hours of darkness at controlled temperature and humidity. Prior to experimentation they were subjected to 8 hours of fasting. Animals were randomly grouped into 3 groups of 5 animals for each one (positive control, negative control and treatment).

Initial blood glucose was taken from the animals by a slight tail cut, the measurement was performed with glucometer, and then an oral dose of maltose 3 g/kg was given to all rats in 1 mL of distilled water.

After administration of maltose, 200 mg of fructans dissolved in 1 mL of distiller water were administered to the first animal group, positive control was administered using acarbose at 50 mg / kg for the second group and as negative control were administered with distiller water.

After 30 minutes of treatment a second glucose measure was

performed on all animals.

3. Results

3.1. Determination of Glucose Dialysis Retardation

In the Figure 1 the release of glucose is presented over the course of two hours. The fructans sample begins with a glucose release of 4.5 mM through the dialysis tube. This value is slightly higher when compared to the cellulose positive control with 4.0 mM. However as time passes, the opposite occurs, and the fructans sample retards the release of glucose presenting values of 1.5 and 0.25 mM for the 60 and 120 minutes, respectively, compared with 4.5 and 2.4 mM of positive control.

3.2. Postprandial Hypoglycemic Activity Test in Rats

The Fasting blood glucose levels was 98.93 mg/dL; this values increased with the maltose administration. As can be seen in Figure 2, the group of animals that received only maltose solution (negative control) showed an average increase in glucose values after 30 minutes of 46 mg/dL. Glucose measurement was performed at 30 minutes because it is the time when the highest blood glucose level is reached after administering maltose 3 gr/kg [18], while the animals receiving the maltose and fructans treatment had an increase of 28.4 mg/dL in blood glucose. This value was significantly different. However, this value did not reach the level shown by acarbose drug which had an average increase of blood glucose of 11.8 mg/dL.

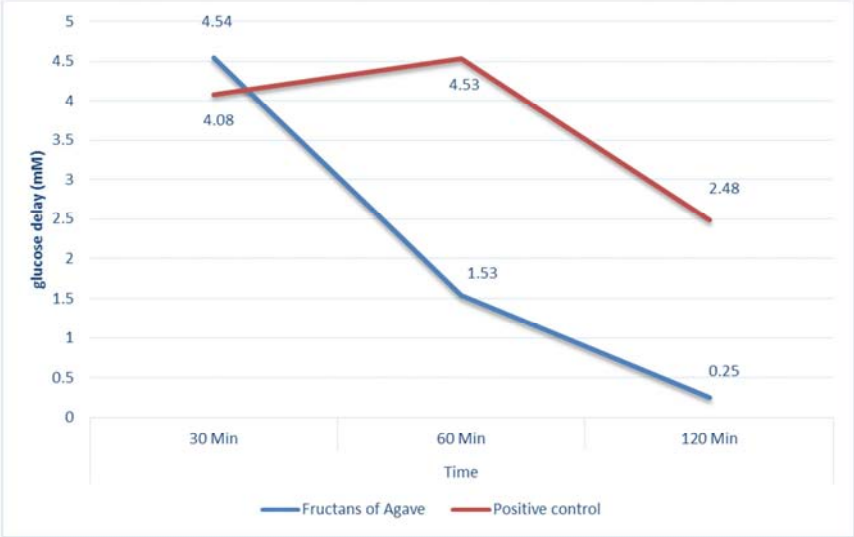

Figure 1. Delay in glucose release from samples: acarbose (positive control) fructans treatment at different times.

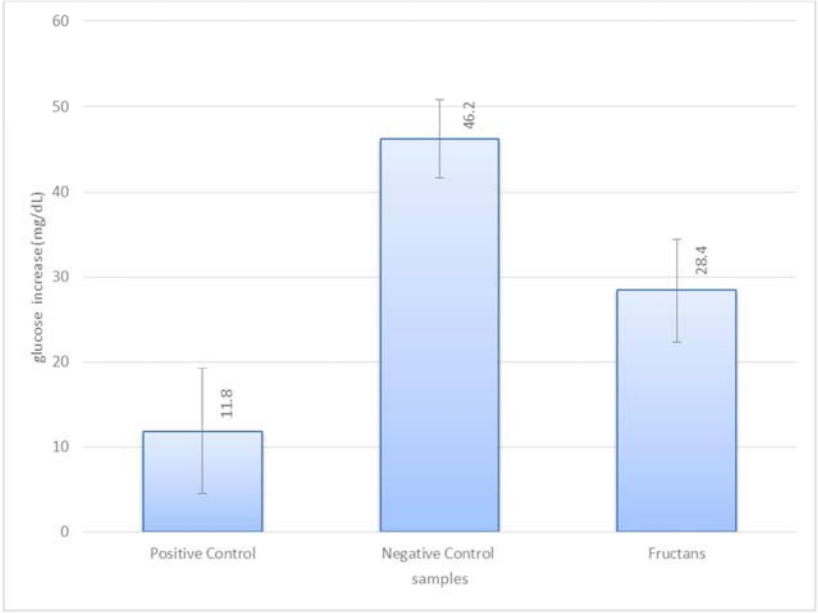

Figure 2. Glucose increase in different animal groups. Negative control (Distilled water), positive (acarbose) and treatment with fructans.

4. Discussion

4.1. Determination of Glucose Dialysis Retardation

The glucose dialysis retardation test attempts to represent the rate at which glucose is absorbed by the gastrointestinal tract [13].

Agave fructans exhibited their greatest effect in delaying glucose release at 30 minutes with 4.5 mM compared with 4.0 mM of positive control. The glucose dialysis retardation shown by Agave fructans may be related to their viscosity and the soluble fiber content that can form nets that would entrap the glucose molecules, which results in a gradual release of carbohydrates. This represents a beneficial physiological effect in diabetic people since it can avoid glucose peaks levels when consuming carbohydrates which significantly affect the health [17].

Similar results in delayed glucose dialysis have been reported for plant extracts [15] and dietary fibers [18].

4.2. Postprandial Hypoglycemic Activity Test in Rats

The determination of postprandial hypoglycemic activity aims to evaluate the effect of treatments on blood glucose levels after carbohydrate intake. Agave Fructans showed a tendency to prevent drastic increases in glucose levels in rats with temporary hyperglycemia induced by maltose. The increase were significantly lower in comparison to the negative control, 28.4 mg/dL and 46.2 mg/dL, respectively; however, they were not as effective as the acarbose drug with 11.8 mg/dL of glucose increase, because there was no significant difference.

Some plant products such as red ginseng [18] and fungal extracts [19] have also shown beneficial effects on glucose control using the postprandial hyperglycemic rodent model and plant extracts such as *Moringa oleifera* demostrated hypoglycemic activity in the diabetic rat model [20].

Agave fructans have shown beneficial effects on weight loss, lipid profile and hyperglycemia in experiments performed on obese mice with a high-fat diet. However, no studies have been reported regarding the postprandial anti-hyperglycemic effect.

Postprandial hypoglycemic activity is a characteristic of drugs such as acarbose that prevent glucose uptake by the body, due in part to the inhibition of enzymes that convert disaccharides to monosaccharides in the gastrointestinal tract. These types of therapies are effective in patients newly diagnosed with type 2 diabetes because it helps control glucose levels [21].

Based on the results obtained in this study, it is not possible to suggest a hypoglycemic effect, since in order to be considered, the rats used should have been diabetic, however in this case, the rodents used were normo-glycemic, so ours results can be considered an anti-hyperglycemic effect.

5. Conclusion

The present study strongly suggests that Agave fructans possess anti-hyperglycemic effect through the decrease of glucose and their mechanism of action may be due to the fructans could effectively adsorb glucose, delay the glucose diffusion and then might promote the glucose adsorption in the gastrointestinal tract preventing their immediate release to the organism.

These results indicate that Agave fructans could be utilized to develop functional food products to lower postprandial serum glucose and potential antidiabetic agents.

However, further research is needed in preclinical models of diabetic animals that support its possible food or pharmacological application.

References

[1] International Diabetes Federation. IDF (2016) Diabetes Atlas 7th ed http://www.diabetesatlas.org.

[2] Achi, N. K., Ohaeri, O. C., Ijeh, I. I., & Eleazu, C. (2016) Modulation of the lipid profile and insulin levels of streptozotocin induced diabetic rats by ethanol extract of *Cnidoscolus aconitifolius* leaves and some fractions: Effect on the oral glucose tolerance of normoglycemic rats, Biomedicine and Pharmacotheraphy, vol. 86, pp. 562-569.

[3] Patel, D. K., Kumar, R., Laloo, D., & Hemalatha, S. (2012) Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity, Asian Pacific Journal of Tropical Biomedicine, pp. 411-420.

[4] Ejike, C., Awazie, S., Nwangozi, P., & Godwin, C. (2013) Synergistic postprandial blood glucose modulatory properties of *Vernonia amygdalina* (Del.), *Gongronema latifolium* (Benth.) and *Occimum gratissimum* (Linn.) aqueous decoctions, Journal of Ethnopharmacology, vol. 149, pp. 111-116.

[5] Sharma, N., Sharma, V. K., & Seo, S. Y. (2005) Screening of some medicinal plants for anti-lipase activity, Journal of Ethnopharmacology," vol. 97, pp. 453–456.

[6] Sairam S., &Urooj A. (2013) *Artocarpus altilis* mode of anti-hyperglycemic activity: elucidation by suitable in vitro and *ex vivo* techniques, International Journal of Pharmaceutical Sciences and Research, Vol. 4(8), pp. 3013-3019.

[7] Montañez, J., Venegas, J., & Vivar, M. (2011) Extracción, caracterización y cuantificación de los fructanos contenidos en la cabeza y en las hojas del *Agave tequila*na weber azul, Bioagro, Vol. 23(3), pp. 199-206.

[8] Vargas Vázquez, C. (2009) Obtención de insumos de interés industrial a partir de las fructanos del Agave mezcalero potosino *Agave salmiana*, CIIDIR-Michoacán, pp. 4-14.

[9] Urías, J., Cani, P., & Delmée. (2008) Physiological effects of dietary fructanos extracted from *Agave tequilana* Gto. and *Dasylirion* spp, British Journal of Nutrition, Vol. 99, pp. 254-261.

[10] Rendón, J., Juarez, B., & Pinos, J. (2012) Effects of differents Sources of Fructans on body weight, blood metabolites and fecal bacteria in normal and obese non-diabetic and diabetic rats, Plant Foods Human Nutrition, Vol. 67, pp. 64-70.

[11] Rendón Huerta, J. A., Juárez Flores, B. I., Pinos Rodríguez, J. M., Aguirre Rivera, J. R., & Delgado Portales, R. E. (2011). Effects of different kind of fructans on in vitro growth of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium lactis*, African journal of microbiology research, Vol. 5(n 18), pp. 2706-2710.

[12] Santos Zea, L., Leal Díaz, A. M., Cortés Ceballos, E., & Gutiérrez Uribe, J. A. (2012) *Agave (Agave spp.)* and its Traditional Products as a Source of Bioactive Compounds, Current Bioactive Compounds, Vol. 2012 (n 8), pp. 218-231.

[13] Márquez-Aguirre, A. L., Camacho-Ruiz, R. M., Arriaga-Alba, M., Padilla-Camberos, E., Kirchmayr, M. R., Blasco, J. L., & González-Avila, M. (2013) Effects of Agave tequilana fructans with different degree of polymerization profiles on the body weight, blood lipids and count of fecal Lactobacilli/Bifidobacteria in obese mice, Food & function, Vol. 4(n 8), pp. 1237-1244.

[14] Abirami, A., Nagarani, G., & Siddhuraju, P. (2014) Measurement of functional properties and health promoting aspects-glucose retardation index of peel, pulp and peel fiber from *Citrus hystrix* and *Citrus maxima*, Bioactive Carbohydrates and dietary fibre," Science Direct, Vol. 4, pp. 16-26.

[15] Bhutkar, M., Bhise, S. (2013) *In vitro* hypoglycemic effects of *Albizzia lebbeck* and *Mucuna pruriens*, Asian Pacific Journal of Tropical Biomedicine, Vol. 3 (n 11), pp. 866-870.

[16] Ou S, Kwok KC, Li Y, Fu L. (2001) *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose, J Agric Food Chem, Vol. 49, pp.1026–1029.

[17] Céspedes, M. A. L., & Bustos, F. M. (2010) The effect of extruded orange pulp on enzymatic hydrolysis of starch and glucose retardation index," Food and bioprocess technology, Vol. 3(n 5), pp. 684-692.

[18] Trinh, H. T., Han, S. J., Kim, S. W., Lee, Y. C., & Kim, D. H. (2007) Bifidus fermentation increases hypolipidemic and hypoglycemic effects of red ginseng," Journal of microbiology and biotechnology, Vol. 17(n 7), pp. 1127-1133.

[19] Choi, H. J., Kim, N. J., & Kim, D. H. (2000) Inhibitory effects of crude drugs on α-glucosidase, Archives of pharmaceutical research, Vol. 23(n 3), 261-266.

[20] Jaiswal, D., Rai, P., & Kumar, A. (2009) Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats, Journal of Ethnopharmacology, Vol. 129, pp. 392-396.

[21] Breuer, H. W. (2003) Review of acarbose therapeutic strategies in the long-term treatment and in the prevention of type 2 diabetes, International journal of clinical pharmacology and therapeutics, Vol. 41(n 10), pp. 421-440.