

Chemical Profile and Study of the Antidiabetic Effect of *Annona squamosa* L. peel

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Research Article

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Abstract

Type 2 Diabetes Mellitus (T2DM) is considered a public health problem worldwide. Current treatment involves the use of oral antidiabetics, whereas natural products could be a therapeutic alternative because of their bioactive compounds. The objective of this research was to investigate the chemical profile and antidiabetic effect of the peel of *Annona squamosa* L. (sugar apple) and evaluate a possible mechanism of action. We analyzed the content of secondary metabolites finding the following in 100 g of freeze-dried peel: 5000.13 ± 30.44 GAEq mg, 82.04 ± 1.12 QEq mg, 3023.07 ± 75.83 AA mg, 13919.07 ± 492.11 CA mg, 16099.56 ± 492.11 L-MA mg, 21346.73 ± 1134.01 D-MA mg, 5261.92 ± 34.59 TA mg, 2920.15 ± 290.13 Glu mg, and 5271.59 ± 562.07 Fru mg. The antihyperglycemic effect of the freeze-dried peel of sugar apple was demonstrated using an Oral Glucose Tolerance Test (OGTT) in healthy Wistar rats, discovering an effect at doses of 100 and 200 mg/kg, which was reversed by administering Wortmannin at 1 mg/kg. Regarding the antidiabetic effect in a T2DM model after 14-days of treatment, we found a decrease in non-fasting glucose, and capillary glucose levels during an OGTT, as well as an improvement in insulin response. These results allow us to conclude that sugar apple peel has a beneficial effect on the regulation of glucose levels in both healthy and diabetic rats, seemingly to be related to the insulin signaling pathway; therefore, this could be an alternative therapy for glucose control in prediabetes and T2DM

Statement Of Novelty

This work presents the phytochemical study and the glucose-modulating effect of freeze-dried peel of *Annona squamosa* (sugar apple) in healthy and diabetic rats. Although the sugar apple peel extracts have been studied previously, our work focuses on using only the freeze-dried peel, finding that acute doses of the freeze-dried peel significantly decrease postprandial glucose in healthy rats, exhibiting a similar behavior in subchronic doses in diabetic rats. In addition, a possible mechanism of action was evaluated using Wortmannin, indicating that the postprandial glucose decrease could be related to the insulin signaling pathway. These findings indicate that the freeze-dried peel of sugar apple could work as a therapeutic alternative for the treatment of type 2 diabetes.

1. Introduction

According to the 2021 estimate of the International Diabetes Federation (IDF), around 536.6 million adults aged 20–79 have diabetes, projecting that by 2045 this figure would increase to 738.2 million [1]. Type 2 Diabetes Mellitus (T2DM) is the most common type of diabetes, accounting for 90% of diabetes cases globally. T2DM is the ninth leading cause of mortality worldwide, with more than one million deaths reported in 2017, and the seventh leading cause in terms of human suffering (Disability-Adjusted Life Years, DALYs) [2]. The basis of T2DM treatment includes the promotion of a healthy diet along with regular physical activity, accompanied by the use of medications that help control blood glucose concentration [3]. In recent years, fruit and vegetable products have been identified as one of the main sources of discovery of antidiabetic agents. Their acceptance and use as alternative therapies have

increased rapidly since side effects and costs are lower compared to conventional therapies [4]. In this context, the peel of the tropical fruit commonly known as sugar apple or saramuyo (*Annona squamosa* L.) is a valuable source of bioactive compounds [5–7] which have shown that they can be an alternative for the search of new substances with pharmacological activity such as antineoplastics, antivirals and anti-inflammatories [6, 7]. The ability of sugar apple peel to modulate blood glucose levels has been studied previously [8, 9], however, we consider it important to evaluate it since studies in *Stenocereus stellatus* indicate that the presence of total phenols correlates with antioxidant and antihyperglycemic capacities [10]. By the way, the same correlation is observed for *Litchi chinensis* Sonn. [11], so it is possible to infer that, by containing this type of metabolites, the *Annona squamosa* L. (sugar apple) peel may have a biological effect. Thus, the objective of this work was to obtain the phytochemical profile of the *Annona squamosa* L. peel and to appraise its antihyperglycemic and antidiabetic effects.

2. Material And Methods

2.1 Procurement of raw material

The *Annona squamosa* L. (sugar apple) fruits at commercial maturity were provided by the Technological Institute of Mérida, Yucatán, México.

2.2 Obtainment of Lyophilized Peel and Methanolic Extract

The lyophilized peel (freeze-dried peel) was obtained by vacuum dehydration in a Labconco Model 6 lyophilizer (Labconco, MO, USA). The powder obtained was stored at -20°C. The Methanolic Extract (ME) was obtained by macerating 1.5 g of lyophilized peel powder with 30 mL of methanol under agitation at 160 rpm for 24 h at 25°C. The Dry Methanolic Extract (DME) was obtained by filtering and evaporating the ME in a rotary evaporator (BUCHI, model R-3, Flawil, Switzerland) at 40°C and 130 mBar.

2.3 Chemical analysis

2.3.1 Qualitative analysis

Determination of triterpenoids [12], reducing sugars, alkaloids, anthraquinones, tannins, saponins, and coumarins [13, 14] was carried out following previously reported methodologies. The results were reported on a hedonic scale.

2.3.2 Total Phenol Content (TPC) and Total Flavonoid Content (TFC)

25 µL of sugar apple ME was diluted in 975 µL of distilled water to measure the TPC, according to the method described by Singleton & Rossi [15]. Absorbance was quantified at 765 nm (JENWAY 6705 UV/VIS) and results were expressed as mg Gallic Acid Equivalents in 100 g of Lyophilized Peel (mg GAEq/100 g LP).

As for the Total Flavonoid Content, 500 μL of the ME was used, following the methodology described by Chang *et al.* [16] Absorbance was detected at 415 nm (Thermo BIOMATE 3 UV/VIS), expressing the results as mg Quercetin Equivalents in 100 g of Lyophilized Peel (mg QEq/100 g LP).

2.3.3 Organic acids and sugars

Organic acids (citric, malic, ascorbic, and tartaric acids) in the lyophilized peel, as well as sugars (glucose, fructose and sucrose) in the DME, were quantified by a High-Performance Liquid Chromatography (HPLC), following the methodology proposed by Aarland *et al.* [14]. The results were expressed as mg of compound in 100 g of lyophilized peel.

2.3.4 Antioxidant Capacity (AC)

10 μL of sugar apple ME diluted in 990 μL of distilled water was used to measure the AC employing the radical acid ABTS+ (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic) method described by Yan *et al.* [17]. Absorbance was determined at 734 nm (JENWAY 6705 UV/VIS) and results were expressed as millimole Trolox Equivalent in 100 g of Lyophilized Peel (mmol TEq/100 g LP).

2.4 Pharmacological analysis

2.4.1 Experimental animals

The Wistar rats were acquired from the biotherium of the Universidad Autónoma Metropolitana-Iztapalapa in Mexico City (Mexico). They remained housed in acrylic boxes with light/dark cycles of 12/12 h, at a temperature of $22 \pm 1^\circ\text{C}$ and humidity of $50 \pm 10\%$, with water and food *ad libitum*. All experimental procedures were performed under Mexican regulations of NOM-062-ZOO-199 [18].

2.4.2 Oral Glucose Tolerance Test (OGTT)

The antihyperglycemic effect of the lyophilizate was established by a 2-h Oral Glucose Tolerance Test (OGTT) in healthy 8-week-old Wistar rats (200–250 g). After a 14-hour fast, body weight was measured, in addition to basal glucose (T0) utilizing a glucometer (Accu-chek® Roche). Animals were randomly distributed into 5 groups, with 6 animals each. Group 1: Negative control, CT (D-glucose anhydrous 2g/kg); group 2: Positive control, Metformin, MF (300 mg/kg); groups 3, 4 and 5, Sugar Apple (SA) lyophilizates in three doses (25, 100 and 200 mg/kg). Metformin was prepared in a saline solution and the lyophilized peel powder was dissolved in purified water to be administered orally every 30 and 15 min, respectively, prior to glucose loading (2 g/kg). Capillary glucose, in mg/dL, was obtained at min 30, 60, 90, and 120 through a puncture into the caudal tail vein of the rat. With the results of glycemia during the OGTT, curves of glucose concentrations at the different times were obtained, and the Area Under the Curve (AUC) of each of these was calculated using the trapezoid method [19].

2.4.3 Inhibition of the insulin signaling pathway

Wortmannin (WT) (Sigma-Aldrich, USA), a specific inhibitor of phosphatidylinositol-3 kinase (PI3K), a protein involved in insulin-mediated glucose uptake, was used to elucidate a possible mechanism of

action of the antihyperglycemic effect of the sugar apple peel lyophilizate. For this purpose, an OGTT was performed in 8-week-old Wistar rats (200–250 g). After a 14-h fast, the animals were randomly distributed into 5 groups, with 5–6 animals each. Group 1: control, CT, (D-glucose anhydrous 2g/kg); group 2: metformin (300 mg/kg); group 3: sugar apple, SA, (100 mg/kg); group 4: wortman, WT, (1 mg/kg); group 5: WT (1 mg/kg) + SA (100 mg/kg). WT was prepared with Dimethyl Sulfoxide (DMSO) and administered intraperitoneally 30 min before glucose loading (2 g/kg). Capillary glucose was measured at min 30, 60, 90, and 120.

2.4.4 Neonatal STZ-induced rat model of T2DM

The T2DM model described by Oidor-Chan *et al.* [20] was applied in the research. Briefly, male neonatal Wistar rats were randomly distributed into 2 groups. Group 1: healthy rats (HR), which received a single dose of vehicle, 0.1 M citrate buffer, pH 4.5 intraperitoneally (i.p.); and group 2: diabetic rats (DM), which received a single dose of Streptozotocin (STZ, 70 mg/kg, i.p.) (Sigma-Aldrich, USA). Capillary glucose and non-fasting body weight were evaluated weekly for 8 weeks. An OGTT and an Intraperitoneal Insulin Tolerance Test (IITT) were performed at weeks 4 and 8 after STZ administration, following the methodology described by Salvador-Adriano *et al.* [21] with minor modifications. In the case of the OGTT, the animals received a D-glucose anhydrous load (2 g/kg) orally, prior to a 14-h fast; meanwhile, for the IITT, the rats received a 6-h fast and a load (2 IU/kg, i.p.) of human isophane insulin (AMSA Laboratories). In each of the tests, capillary glucose was measured at min 30, 60, 90, and 120. The results were expressed as the mean $\pm$ EE in mg/dL.

2.4.5 Analysis of the antidiabetic effect

At week 8, healthy rats and diabetic rats (DM) that complied with the T2DM model were divided into 4 groups of 5–7 rats and received orally, with the aid of a rat metal cannula, one of the following treatments during 14 days: 1) vehicle (purified water); 2) metformin, MF (300 mg/kg); 3) sugar apple, SA (100 mg/kg), and 4) SA (200 mg/kg). Each treatment was administered every morning. At the end of each treatment, capillary glucose and non-fasting body weight were evaluated; in addition, OGTT and IITT were performed.

2.5. Statistical Analysis

Non-fasting capillary glucose, body weight, OGTT, IITT, and AUC results were expressed as mean $\pm$ EE and analyzed by One-Way Analysis of Variance (ANOVA) followed by a Tukey *post hoc* test with a significance level of $p < 0.05$. For the experimental results of the OGTT in the presence of WT (section 2.4.3) a data transformation was carried out by calculating the square root of each value to obtain a normal distribution; they were then analyzed by applying an ANOVA followed by a Tukey *post hoc* test with a significance level of $p < 0.05$. Experimental results of the OGTT and IITT at weeks 4 and 8, as well as the non-fasting capillary glucose and body weight during 8 weeks after STZ administration were analyzed by applying a *student t-test* with a significance level of $p < 0.05$. The Statistical Package for the Social Sciences (SPSS Version 24) was used in all cases. The software SigmaPlot 12 was used to create the artwork.

3. Results And Discussion

3.1 Chemical Analysis

3.1.1 Qualitative Analysis

Phytochemical analysis of the sugar apple peel revealed the presence of anthraquinones, tannins, triterpenoids, reducing sugars, and saponins (Table 1). These compounds have also been found in other fruits of the *Annonaceae* family such as *A. muricata* [22], and *A. cherimoya* M. [23]. These types of chemical groups are associated with a wide range of biological activities such as antioxidant, antimicrobial, cytotoxic, and anti-inflammatory, among others [6, 7, 24].

Table 1
Qualitative-quantitative analysis and antioxidant capacity of sugar apple

Chemical compounds	<i>Biological material</i>
	Sugar apple
Qualitative	
^A Alkaloids	-
^A Anthraquinones	+++
^B Reducing sugars	++
^A Coumarins	-
^B Saponins	+
^B Tannins	+++
^B Triterpenoids	++
Quantitative	
^A Total Polyphenols ^a	5000.13 ± 30.44
^A Total Flavonoids ^b	82.04 ± 1.2
^B Sucrose ^c	-
^B Glucose ^c	2920.15 ± 290.13
^B Fructose ^c	5271.59 ± 562.07
^C Ascorbic acid ^d	3023.07 ± 75.83
^C Citric ^d	13919.07 ± 492.11
^C L-Malic ^d	16099.56 ± 203.76
^C D-Malic ^d	21346.73 ± 1134.01
^C Tartaric ^d	5261.92 ± 34.59
Antioxidant Capacity	
^A ABTS ^e	354.27 ± 3.9

Chemical compounds	Biological material
	Sugar apple
Qualitative parameters: (-) Not detected; (+) Sparingly present; (++) Moderately present; (+++) Abundantly present. ^A Methanolic Extract, ^B Dry Methanolic Extract, ^C Lyophilized Peel. ^a TPC in mg of Gallic Acid Equivalents in 100 g of Lyophilized Peel (mg GAEq/100 g LP). ^b Total Flavonoids in mg of Quercetin Equivalents in 100 g of Lyophilized Peel (mg QEq/100 g LP), ^c Content of sucrose, glucose and fructose in mg of compound in 100 g of Lyophilized Peel (mg/100 g LP), ^d Content of ascorbic, citric, malic and tartaric acids in mg of compound in 100 g of Lyophilized Peel (mg/100 g LP), ^e Antioxidant Capacity in millimole Trolox Equivalent in 100 g of Lyophilized Peel (mmol TEq/100 mg LP), mean $\pm$ EE (n = 3–9).	

3.1.2. Quantitative Analysis

Total Phenol Content (TPC) and Total Flavonoid (TF). Phenolic compounds correspond to a diverse group of secondary metabolites that are widely distributed in different organs or structures of plants, such as fruits. These compounds include an extensive diversity of structures, from the simplest ones, such as phenolic acids, to more complex structures called polyphenols, like flavonoids [25]. Phenol content was obtained through the Folin-Ciocalteu method, while flavonoids were determined via a colorimetric method using aluminum chloride (Table 1). The concentration of total phenols in the Methanolic Extract (ME) of sugar apple peel was 5000.13 ± 30.44 mg Gallic Acid Equivalents/100 g of Lyophilized Peel (LP) and the concentration of total flavonoids was 82.04 ± 1.2 mg Quercetin Equivalents/100 g of LP. The content differs in quantity from that previously reported by Can-Cauich *et al.* [5] but it is consistent in the presence of a higher content of TPC compared to TF. They additionally report the content of 11 phenolic compounds, namely hydroxycinnamic acids, hydroxybenzoic acids, flavonols, isoflavones, and flavanols [5].

Antioxidant Capacity. Phenolic compounds are related to antioxidant properties since they act as free radical collectors and metal ion chelators [26]. The antioxidant capacity was obtained using the ABTS + radical method. This test allows measuring the relative capacity of antioxidants to trap the ABTS + radical in an aqueous phase and comparing it with a standard antioxidant such as Trolox or ascorbic acid [27]. Sugar apple peel presented 55.23 ± 0.43 mmol Trolox Equivalents/100 mg of LP. Can-Cauich *et al.* [5] reported a correlation between Antioxidant Capacity and TPC of 0.94. Combining the data from Can-Cauich *et al.* [5] and Chel-Guerrero *et al.* [7] with our measurement, a correlation between Antioxidant Capacity and TPC of 0.86 is obtained (SI, Fig. 1).

Sugars. The ability to synthesize soluble sugars is one of the main features of plant physiology. Sugars fulfill a large number of essential functions for plants, being a general source of metabolic energy that contributes to their growth and development, defense, flowering, and apical dominance [28]. Sugars play a key role in the organoleptic quality of the fruits and, consequently, in consumer acceptance. Usually, the main sugar found is sucrose, which can be hydrolyzed to produce fructose and glucose [29]. Sugar content in the sugar apple peel (Table 1) was calculated via High-Performance Liquid Chromatography (HPLC), observing that the predominant monosaccharide was fructose (5271.59 ± 562.07 mg/100 mg

LP), followed by glucose (2920.15 ± 290.13 mg/100 mg LP), whilst sucrose presence was not detected. The investigation conducted by Chel-Guerrero *et al.* [7] concurs with the absence of sucrose in sugar apple peel. We can also note the discoveries of Pal & Kumar [30] revealing that sugar apple fruit contains 15% of total sugar content during the commercial maturity stage, while Fang *et al.* [31] report that during fruit ripening there is an accumulation of sugars, mainly glucose and fructose, which contributes to the sweetness of the fruit. Furthermore, genes involved with starch degradation are up-regulated while genes related to starch synthesis are highly repressed during late development and ripening stages.

Organic Acids. The content of organic acids along with sugars has an effect on sweetness and acidity, which are key characteristics of the flavor of the fruit. It should be noted that the most abundant organic acids in fruits are citric acid and malic acid, and that the content of these metabolites depends on several aspects, such as temperature, soil quality, stage of development, etc. [32]. In this research, the content of organic acids (Table 1) was measured by means of an HPLC, with the following retention times for the acids found: 3.8 min for Tartaric acid, 4.6 min for L-Malic acid, 5.2 min for Ascorbic acid, 6.4 min for Citric acid, and 7.6 min for D-Malic acid. According to our findings, D-Malic and L-Malic, as well as Citric acid, are predominant (21346.73 ± 1134.01 , 16099.56 ± 203.76 , and 13919.07 ± 492.11 mg/100 g LP, respectively), which is consistent with the breakthroughs reported by Lee *et al.* [33]. During fruit ripening, the increase in sugar content is due to the starch degradation process and increased sugar accumulation, while organic acids generally decrease. Malate and citrate are the predominant organic acids in mature climacteric and non-climacteric fruits. In climacteric fruits, such as sugar apple, malate/citrate serve as a substrate during cellular respiration, so their content tends to decrease [34]; however, sugar apple, like other *anonaceous* fruits, has an opposite response, since citric acid increases significantly at the consumption maturity stage, as proved by Pal & Kumar [30].

3.2 Pharmacological Analysis

3.2.1 Antihyperglycemic Analysis

A Glucose Tolerance Test is used to determine the ability of a person/animal to metabolize a glucose load. This type of test allows knowing if the body is able to metabolize a standardized amount of glucose by measuring capillary glucose concentrations within a certain period of time. As a result of this test, an antihyperglycemic effect was found in two doses of sugar apple peel lyophilizate. In the OGTT curves of the 100 and 200 mg/kg doses, they present a statistically significant difference respecting to the CT (glucose 2 g/kg) at min 30 and 60; nevertheless, the 200 mg/kg dose curve is still below that of the control at min 90 (Fig. 1a). When looking at the results as the Area Under the Curve (AUC) of glucose (Fig. 1b), the doses of 100 and 200 mg/kg of sugar apple are statistically different from the CT (glucose 2 g/kg) and there is no significant difference with the positive control, MF (300 mg/kg). In the literature, there is evidence of the antihyperglycemic effect of sugar apple leaves: Shirwaikar *et al.* [35] assessed the effect of two doses (250 and 500 mg/kg) of the alcoholic extract of sugar apple leaves during an OGTT in healthy rats, concluding that the dose of 500 mg/kg has a better antihyperglycemic effect given that glucose levels decrease to 86.1 ± 1.22 and 66.33 ± 2.33 mg/dL at minute 60 and 120, respectively,

comparing to the control. The mechanism of the antihyperglycemic effect of *Annona* family species is still unknown; however, said effect has been attributed to the high polyphenol content, specifically glycoside flavonols such as rutin [36].

3.2.2 PI3K Inhibition

For the present study, it was proposed to elucidate a possible mechanism of action of the antihyperglycemic effect produced by sugar apple peel lyophilizate using Wortmannin (WT), which is a specific inhibitor of the phosphatidylinositol-3 kinase (PI3K) involved in the insulin-mediated glucose uptake in skeletal muscle and adipose tissue. Through an OGTT, the effect of WT, together with the 100 mg/kg sugar apple dose, was analyzed. As described in the previous section, the antihyperglycemic effect of sugar apple is present from minute 30; however, such effect is reversed with the administration of WT (1 mg/kg) (Fig. 2a). These results are confirmed by the AUC (Fig. 2b), where the AUC of the 100 mg/kg sugar apple dose is significantly lower than that administered in combination with WT, indicating that the lyophilized sugar apple peel affects postprandial glucose-lowering through the insulin signaling pathway. *In vitro* studies carried by Davis *et al.* [37] have shown that the hexane extract of sugar apple leaves produces an effect on IR- β /IR-1 phosphorylation, which proves to be a key step in the insulin signaling cascade. They also demonstrate that it increases PI3 kinase expression, as well as GLUT-4 glucose transporter mRNA expression. The authors propose that the bioactive compounds of the hexane extract of sugar apple leaves may be acting as insulin sensitizers or insulin receptor activators, which increases glucose transport through the PI3K pathway.

3.2.3 Analysis of the antidiabetic effect

After noticing the antihyperglycemic effect in the sugar apple peel, we set out to evaluate its antidiabetic effect in a neonatal STZ-induced rat model of T2DM. The T2DM model (SI, Figs. 2a-f) concurs with that reported by Barragán-Iglesias *et al.* [38]. The 14-day administration of 200 mg/kg of sugar apple peel lyophilizate was found to have an effect on the glucose levels of diabetic rats compared to vehicle-diabetic rats, by significantly decreasing non-fasting capillary glucose (Fig. 3a) and having a similar effect to that observed in the metformin 300 mg/kg treatment. Regarding body weight, no differences were observed in the weight gain of diabetic rats between lyophilized doses (Fig. 3b).

During IITT (Fig. 4a), diabetic rats treated with the 200 mg/kg dose improved their response to exogenous insulin administration by significantly decreasing capillary glucose from min 30, compared to vehicle-diabetic rats. Glucose values from that moment on were similar to those of diabetic rats treated with metformin and to those of healthy rats that received vehicle. These results can be confirmed by observing it as the Area Under the Curve (Fig. 4b). Previous studies had shown that the aqueous extract of sugar apple leaves (300 mg/Kg) decreases capillary glucose in diabetic rats with a simultaneous increase in plasma insulin [39]. They attribute that the hypoglycemic effect of sugar apple could be due to increased insulin secretion in β -pancreatic cells or enhanced glucose transport from the blood to peripheral tissues [39]. Similarly, Sahu *et al.* [8] compared the antidiabetic effect of the aqueous extract of sugar apple leaves and peel in a high-fat diet-induced diabetic mice model with multiple low doses of STZ, finding,

with regard to the peel extract, that the effect of aqueous extract of leaves in diabetic mice significantly improved plasma glucose, triglycerides, cholesterol, glucose tolerance, and insulin levels when compared to control diabetic mice. Moreover, the peel showed higher antioxidant activity compared to the other treated groups.

Concerning the OGTT (Fig. 4c), both doses of sugar apple were able to reverse the glucose intolerance observed in vehicle-diabetic rats since they significantly decreased capillary glucose from min 60. These data are confirmed when observing that the AUC of both sugar apple doses significantly decreased compared to the group of diabetic rats that were administered the vehicle alone (Fig. 4d). In the literature, Gupta *et al.* [40] had previously reported the hypoglycemic effect of a 15-day treatment of ethanolic extracts of sugar apple leaves (350 mg/kg). This extract lowers fasting glucose levels and also reduces glucose intolerance after 1 h when applying an OGTT in STZ-induced diabetic rats (50 mg/kg). The authors found out that the active ingredients of the extract take about 1 h to enter the circulation and thus carry out the hypoglycemic effect in the target tissues.

Petroleum ether, ethyl acetate, alcohol [9], and aqueous [8] extracts of sugar apple peel had already been used to estimate the effect on capillary glucose. In this research, however, we decided to evaluate the lyophilized peel, noting that an acute dose (100 mg/kg or 200 mg/kg) of the lyophilizate significantly decreases the capillary glucose levels in normoglycemic rats, exhibiting the same behavior in a subchronic dose (200 mg/kg) in diabetic rats. Although the mechanism of action is still unknown, our results seem to indicate that this effect could be related to the insulin signaling pathway.

4. Conclusions

In the present study, the phytochemical content of the sugar apple (*Annona squamosa* L.) peel lyophilizate was evaluated, observing a higher Total Phenol Content with respect to Total Flavonoid Content. In terms of primary metabolites, the predominant sugar was fructose while D-malic and L-malic acids, as well as citric acid, were the most abundant organic acids.

Oral Glucose Tolerance Tests revealed that the lyophilized peel (freeze-dried peel) of sugar apple decreases postprandial glucose in healthy rats. This effect is reversed upon administration of Wortmannin, a specific phosphatidylinositol-3 kinase inhibitor, indicating that the antihyperglycemic effect could be related to the insulin signaling pathway. Additionally, the antidiabetic effect was determined in a T2DM model in rats, finding that a 14-day treatment at a dose of 200 mg/kg in diabetic rats decreases non-fasting capillary glucose, and also reverses glucose intolerance and insulin resistance. These results indicate that sugar apple peel lyophilizate could be a therapeutic alternative for the treatment of T2DM.

Declarations

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Conflicts of interest

The authors have declared no conflict of interest.

References

1. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B.B., Stein, C., Basit, A., Chan, J.C., Mbanya, J.C., Pavkov, M.E., Ramachandran, A., Wild, S.H., James, S., Herman, W.H., Zhang, P., Bommer, C., Kuo, S., Magliano, D.: IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res. Clin. Pract.* (2022). <https://doi.org/10.1016/j.diabres.2021.109119>
2. Khan, M.A.B., Hashim, M.J., King, J.K., Govender, R.D., Mustafa, H., Kaabi, A.: Epidemiology of type 2 diabetes—global burden of disease and forecasted trends. *J. Epidemiol. Glob Health.* (2020). <https://doi.org/10.2991/jegh.k.191028.001>
3. Aschner, P.: New IDF clinical practice recommendations for managing type 2 diabetes in primary care. *Diabetes Res. Clin. Pract.* (2017). <https://doi.org/10.1016/j.diabres.2017.09.002>
4. Alam, F., Islam, M.A., Kamal, M.A., Gan, S.H.: Updates on managing type 2 diabetes mellitus with natural products: towards antidiabetic drug development. *Curr. Med. Chem.* (2018). <https://doi.org/10.2174/0929867323666160813222436>
5. Can-Cauich, C.A., Sauri-Duch, E., Betancur-Ancona, D., Chel-Guerrero, L., González-Aguilar, G.A., Cuevas-Glory, L.F., Perez-Pacheco, E., Moo-Huchin, V.M.: Tropical fruit peel powders as functional

- ingredients: Evaluation of their bioactive compounds and antioxidant activity. J. Funct. Foods. (2017). <https://doi.org/10.1016/j.jff.2017.08.028>
6. Chel-Guerrero, L.D., Gomez-Cansino, R., Guzman-Gutierrez, S.L., Campos-Lara, M.G., Saury-Duch, E., Díaz de León-Sánchez, F., Reyes-Chilpa, R., Mendoza-Espinoza, J.A.: *In vitro* antiviral activity and phytochemical screen in the extracts of peels from four species of tropical fruits collected in Merida Yucatan, Mexico. Phyton-Int. J. Exp. Bot. (2018). <https://doi.org/10.32604/phyton.2018.87.068>
 7. Chel-Guerrero, L.D., Sauri-Duch, E., Fragoso-Serrano, M.C., Pérez-Flores, L.J., Gómez-Olivares, J.L., Salinas-Arreortua, N., Sierra-Palacios, E.D.C., Mendoza-Espinoza, J.A.: Phytochemical profile, toxicity, and pharmacological potential of peels from four species of tropical fruits. J. Med. Food (2018). (2018). <https://doi.org/10.1089/jmf.2017.0124>
 8. Sahu, M., Sahoo, N., Alagarsamy, V., Rath, B.: Comparative evaluation of antidiabetic and antioxidant activities of aqueous fruit peel and leaf extracts of *Annona squamosa* on high fat diet and multiple low dose streptozotocin mouse model of diabetes. Austin J. Pharmacol. Ther. **4**(1), 1081 (2016)
 9. Sharma, A.K., Chand, T., Khardiya, M., Yadav, K.C., Mangal, R., Sharma, A.K.: Antidiabetic and antihyperlipidemic activity of *Annona squamosa* fruit peel in streptozotocin induced diabetic rats. Int. Toxicol. Pharmacol. Res. **5**(1), 15–21 (2013)
 10. Cervantes-Arista, C., Roman-Guerrero, A., Oidor-Chan, V.H., Díaz de León-Sánchez, F., Álvarez-Ramírez, E.L., Pelayo-Zaldívar, C., Sierra-Palacios, E.D.C., Mendoza-Espinoza, J.A.: Chemical characterization, antioxidant capacity, and anti-hyperglycemic effect of *Stenocereus stellatus* fruits from the arid Mixteca Baja region of Mexico. Food Chem. (2020). <https://doi.org/10.1016/j.foodchem.2020.127076>
 11. Contreras-Castro, A.I., Oidor-Chan, V.H., Bustamante-Camilo, P., Pelayo-Zaldívar, C., de Díaz, F., Mendoza-Espinoza, J.A.: Chemical characterization and evaluation of the antihyperglycemic effect of lychee (*Litchi chinensis* Sonn.) cv. J. Med. Food (2022). <https://doi.org/10.1089/jmf.2021.0098>
 12. Roopalatha, U.C., Nair, V.M.G.: Phytochemical analysis of successive reextracts of the leaves of *Moringa oleifera* Lam. Int. J. Pharm. Pharm. Sci. **5**(3), 629–634 (2013)
 13. Aarland, R.C., Peralta-Gomez, S., Sanchez-Morales, Parra-Bustamante, F., Villa-Hernandez, J.M., de Díaz, F., Perez-Flores, L.J., Rivera-Cabrera, F., Mendoza-Espinoza, J.A.: A pharmacological and phytochemical study of medicinal plants used in Mexican folk medicine. Indian J. Tradit Knowl. **14**(4), 550–557 (2015)
 14. Aarland, R.C., Bañuelos-Hernández, A.E., Fragoso-Serrano, M., Sierra-Palacios, E.D.C., Díaz de León-Sánchez, F., Pérez-Flores, L.J., Rivera-Cabrera, F., Mendoza-Espinoza, J.A.: Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of *Echinacea purpurea* and *Echinacea angustifolia* extracts. Pharm. Biol. (2017). <https://doi.org/10.1080/13880209.2016.1265989>
 15. Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. **16**(3), 144–158 (1965)

16. Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C.: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* (2002).
<https://doi.org/10.38212/2224-6614.2748>
17. Yan, F., Yu, X., Jing, Y.: Optimized preparation, characterization, and antioxidant activity of chitooligosaccharide–glycine Maillard reaction. *J. Food Sci. Technol.* (2018).
<https://doi.org/10.1007/s13197-017-2982-0>
18. Mexican Official Standard: : *Technical Specifications for the Production, Care and Use of Laboratory Animals* (NOM-062-ZOO-1999). SENASICA. (2001). <http://publico.senasica.gob.mx/?doc=743>
Accessed 6 June 2022
19. Tai, M.M.: A mathematical-model for the determination of total area under glucose-tolerance and other metabolic curves. *Diabetes Care.* (1994). <https://doi.org/10.2337/diacare.17.2.152>
20. Oidor-Chan, V.H., Hong, E., Pérez-Severiano, F., Montes, S., Torres-Narváez, J.C., Del Valle-Mondragón, L., Pastelín-Hernández, G., Sánchez-Mendoza, A.: Fenofibrate plus metformin produces cardioprotection in a type 2 diabetes and acute myocardial infarction model. *Ppar Res.* (2016).
<https://doi.org/10.1155/2016/8237264>
21. Salvador-Adriano, A., Vargas-Chávez, S., Hernández-Vázquez, A.J., Ortega-Cuellar, D., Tovar, A.R., Velázquez-Arellano, A.: Insulin sensitivity is inversely related to cellular energy status, as revealed by biotin deprivation. *Am. J. Physiol. Endocrinol. Metab.* (2014).
<https://doi.org/10.1152/ajpendo.00442.2013>
22. Rubio-Melgarejo, A., Balois-Morales, R., Palomino-Hermosillo, Y.A., López-Guzmán, G.G., Ramírez-Ramírez, J.C., Cervantes-García, E., Bautista-Rosales, P.U.: Phytochemical and antioxidant dynamics of the soursop fruit (*Annona muricata* L.) in response to *Colletotrichum* spp. *J. Food Qual.* (2020).
<https://doi.org/10.1155/2020/3180634>
23. Benarba, B., Mendas, O.I.N., Righi, S.: Phytochemical analysis, antioxidant and anti-*Candida albicans* activities of Mill. fruit pulp. *Nor. Afr. J. Food Nutr. Res.* (2018).
<https://doi.org/10.51745/najfnr.2.4.121-130>
24. Amudha, P., Varadharaj, V.: Phytochemical and pharmacological potential of *annona* species: a review. *Asian J. Pharm. Clin. Res.* (2017). <https://doi.org/10.22159/ajpcr.2017.v10i7.18073>
25. Haminiuk, C.W.I., Maciel, G.M., Plata-Oviedo, M.S.V., Peralta, R.M.: Phenolic compounds in fruits - an overview. *Int. J. Food Sci. Technol.* (2012). <https://doi.org/10.1111/j.1365-2621.2012.03067.x>
26. Perron, N.R., Brumaghim, J.L.: A Review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell. Biochem. Biophys.* (2009). <https://doi.org/10.1007/s12013-009-9043-x>
27. Opitz, S.E., Smrke, S., Goodman, B.A., Yeretjian, C.: Processing and Impact on Antioxidants. In: Preedy, V. (ed.) *Beverages*, pp. 253–264. Elsevier (2014)
28. Van den Ende, W.: Sugars take a central position in plant growth, development and, stress responses. A focus on apical dominance. *Front. Plant. Sci.* (2014). <https://doi.org/10.3389/fpls.2014.00313>
29. Duran-Soria, S., Pott, D.M., Osorio, S., Vallarino, J.G.: Sugar signaling during fruit ripening. *Front. Plant. Sci.* (2020). <https://doi.org/10.3389/fpls.2020.564917>

30. Pal, D., Sampath Kumar, P.: Changes in the physico-chemical and biochemical compositions of custard apple (*Annona squamosa* L.) fruits during growth, development and ripening. J. Hortic. Sci. (1995). <https://doi.org/10.1080/14620316.1995.11515328>
31. Fang, R., Huang, W., Yao, J., Long, X., Zhang, J., Zhou, S., Deng, B., Tang, W., An, Z.: Characterization of full-length transcriptome and mechanisms of sugar accumulation in *Annona squamosa* fruit. Biocell (2020). <https://doi.org/10.32604/biocell.2020.012933>
32. Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J.G., Walker, R.P.: The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents - a review. Rev. Chapingo Ser. Hortic. (2015). (2015). <https://doi.org/10.5154/r.chsh.2015.01.004>
33. Lee, P.R., Tan, R.M., Yu, B., Curran, P., Liu, S.Q.: Sugars, organic acids, and phenolic acids of exotic seasonable tropical fruits. Nutr. Food Sci. (2013). <https://doi.org/10.1108/00346651311327927>
34. Batista-Silva, W., Nascimento, V.L., Medeiros, D.B., Nunes-Nesi, A., Ribeiro, D.M., Zsögön, A., Araújo, W.L.: Modifications in organic acid profiles during fruit development and ripening: correlation or causation? Front. Plant. Sci. (2018). <https://doi.org/10.3389/fpls.2018.01689>
35. Shirwaikar, A., Rajendran, K., Kumar, C.D., Bodla, R. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. J. Ethnopharmacol. (2004). (2004). <https://doi.org/10.1016/j.jep.2003.12.017>
36. Calzada, F., Solares-Pascasio, J.I., Ordoñez-Razo, R.M., Velazquez, C., Barbosa, E., García-Hernández, N., Mendez-Luna, D., Correa-Basurto, J.: Antihyperglycemic activity of the leaves from *Annona cherimola* miller and rutin on alloxan-induced diabetic rats. Pharmacogn Res. (2017). <https://doi.org/10.4103/0974-8490.199781>
37. Davis, J.A., Sharma, S., Mittra, S., Sujatha, S., Kanaujia, A., Shukla, G., Katiyar, C., Lakshmi, B.S., Bansal, V.S., Bhatnagar, P.K.: Antihyperglycemic effect of *Annona squamosa* hexane extract in type 2 diabetes animal model: PTP1B inhibition, a possible mechanism of action? Indian J. Pharmacol. (2012). <https://doi.org/10.4103/0253-7613.96304>
38. Barragán-Iglesias, P., Oidor-Chan, V.H., Loeza-Alcocer, E., Pineda-Farias, J.B., Velazquez-Lagunas, I., Salinas-Abarca, A.B., Hong, E., Sánchez-Mendoza, A., Delgado-Lezama, R., Price, T.J., Granados-Soto, V.: Evaluation of the neonatal streptozotocin model of diabetes in rats: Evidence for a model of neuropathic pain. Pharmacol. Rep. (2018). <https://doi.org/10.1016/j.pharep.2017.09.002>
39. Kaleem, M., Medha, P., Ahmed, Q.U., Asif, M., Bano, B.: Beneficial effects of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singap. Med J. **49**(10), 800–804 (2008)
40. Gupta, R.K., Kesari, A.N., Murthy, P.S., Chandra, R., Tandon, V., Watal, G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals, J. Ethnopharmacol. (2005). (2005). <https://doi.org/10.1016/j.jep.2005.01.048>

Figures

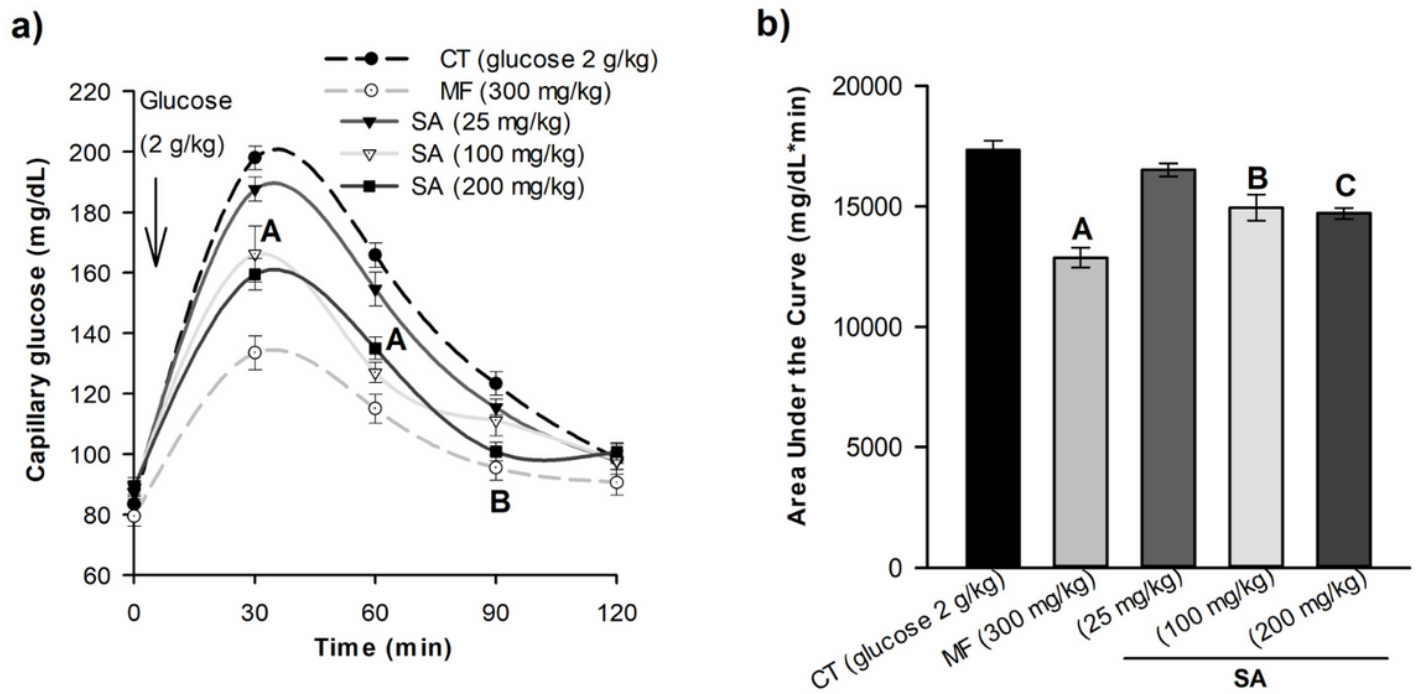


Figure 1

Antihyperglycemic effect. Oral Glucose Tolerance Test (OGTT). **a)** OGTT curve, **A=** Indicates significant difference SA (100 mg/kg) and SA (200 mg/kg) vs CT (glucose 2 g/kg) min 30 and 60, **B=** SA (200 mg/kg) vs CT (glucose 2 g/kg) min 90. **b)** **Area Under the Curve of the OGTT**, **A=** MF (300 mg/kg), **B=** SA (100 mg/kg), and **C=** SA (200 mg/kg) vs CT (glucose 2 g/kg). One-way ANOVA followed by a Tukey *post hoc* test, respectively. Mean $\pm$ SD (n= 5-6, $p<0.05$).

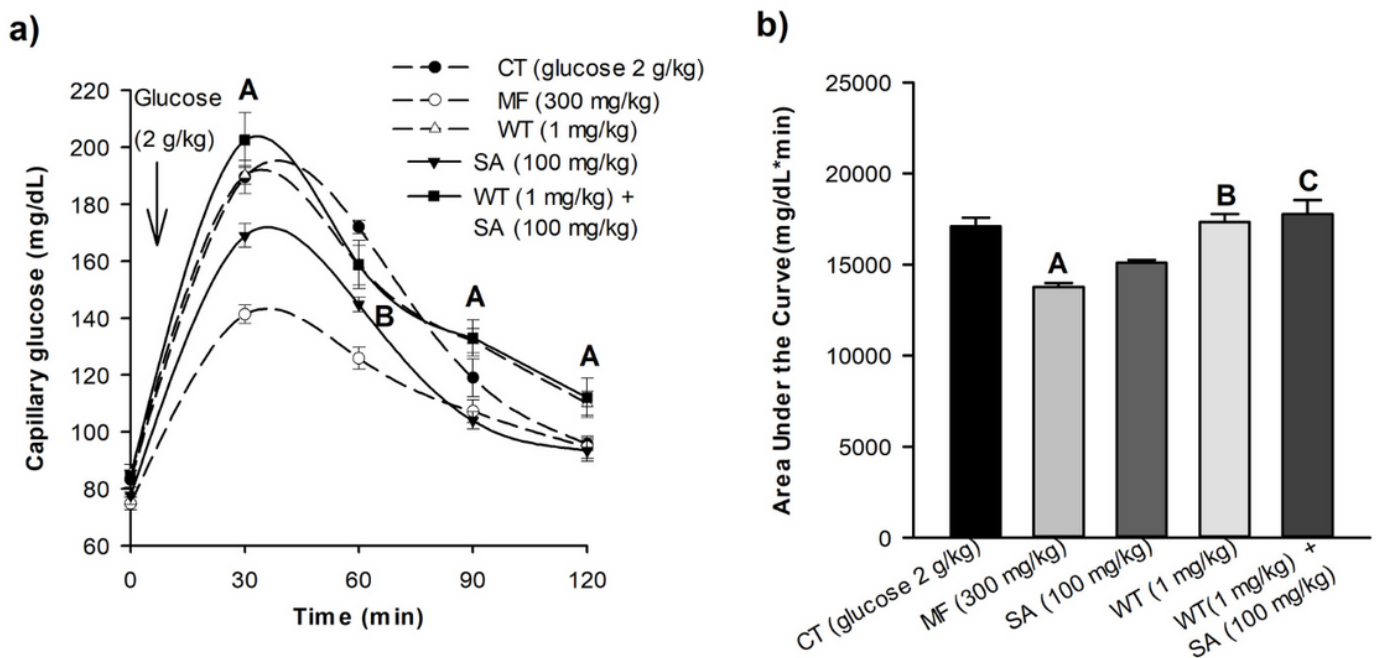


Figure 2

PI3-K inhibition. a) Effect of Wortmannin (WT) before administration of sugar apple lyophilizate during an OGTT. A= Indicates significant difference WT (1 mg/kg) + SA (100 mg/kg) vs SA (100 mg/kg), min 30, 90 and 120. **B=** SA (100 mg/kg) vs. CT (glucose 2 g/kg). **b) AUC of the OGTT. A=** Indicates significant difference MF (300 mg/kg) vs CT (glucose 2 g/kg), **B=** SA (100 mg/kg) vs CT (glucose 2 g/kg), **C=** WT (1 mg/kg) + SA (100 mg/kg) vs SA (100 mg/kg). One-way ANOVA followed by a Tukey *post hoc* test. Mean $\pm$ EE (n= 4, p<0.05).

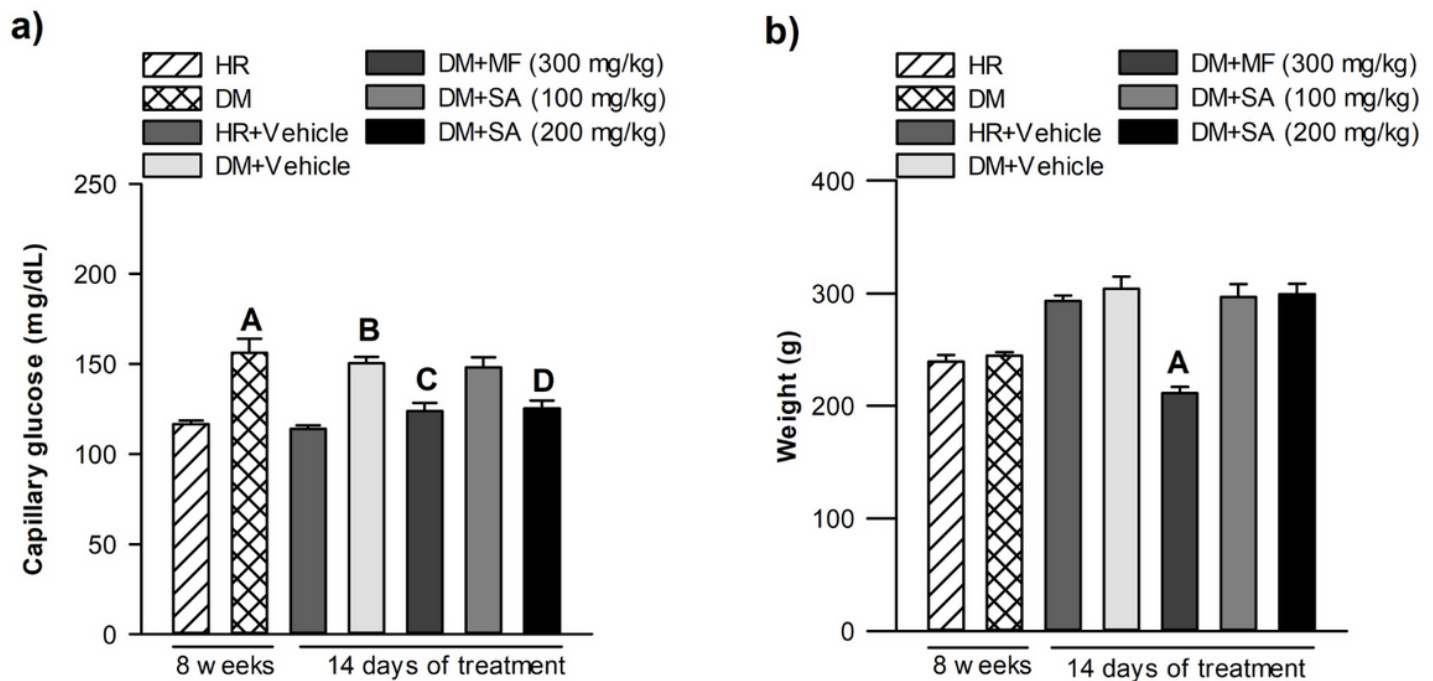


Figure 3

Hypoglycemic effect and body weight after 14 days of treatment. a) Non-fasting capillary glucose. A= Indicates statistically significant differences, diabetic rats (DM) vs healthy rats (HR), **B=** DM+Vehicle vs HR+Vehicle, **C=** DM+MF vs DM+Vehicle, **D=** DM+ SA (200 mg/kg) vs DM+Vehicle. **b) Body weight. A=** Indicates statistically significant differences, DM+MF (300 mg/kg) vs DM+ Vehicle. One-way ANOVA followed by a Tukey *post hoc* test. Mean $\pm$ SD (n= 5-6, p<0.05).

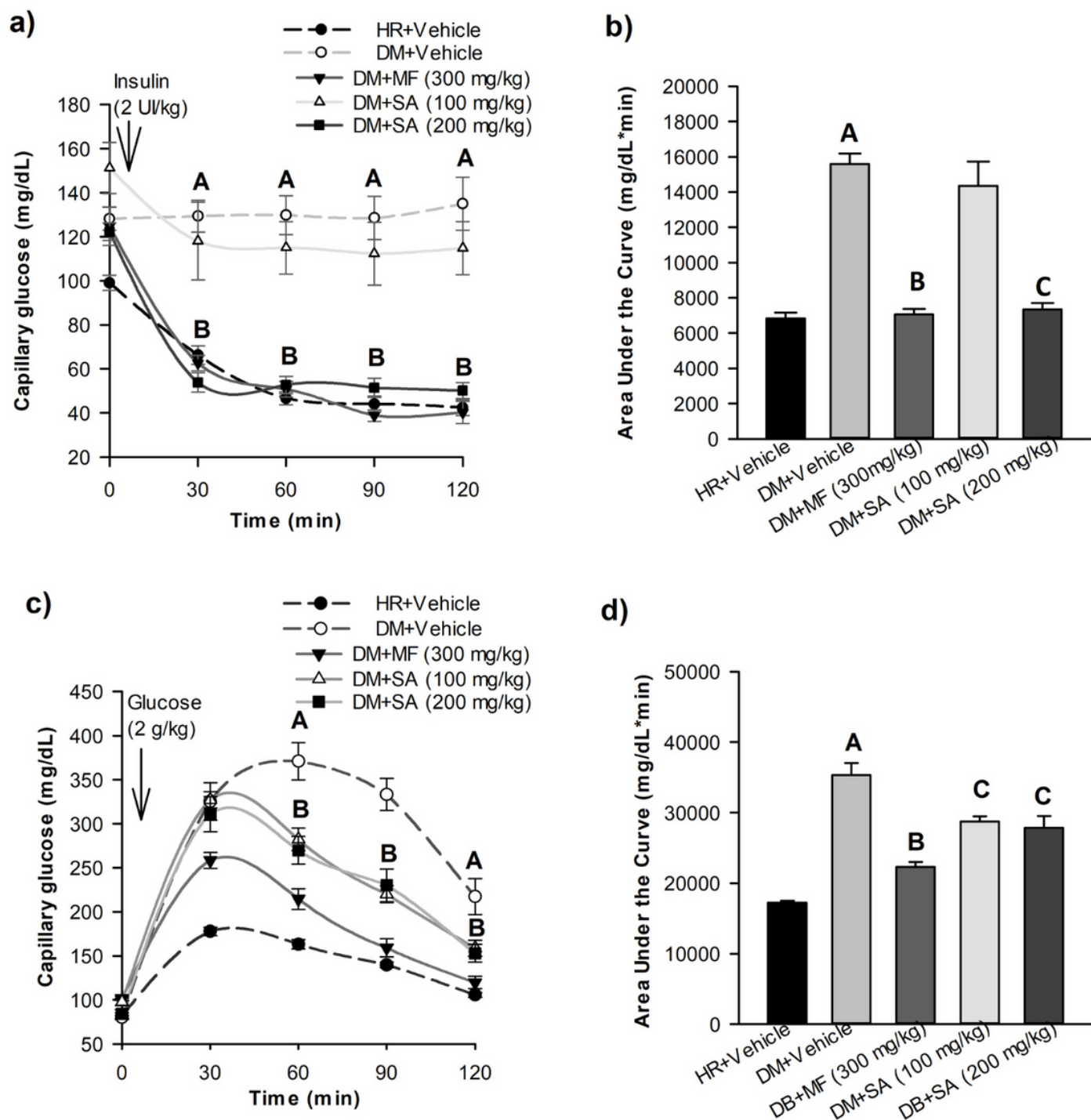


Figure 4

Antidiabetic effect after 14 days of treatment. a) IITT curve. A= Indicates statistically significant differences, DM+Vehicle vs HR+Vehicle, B= DM+MF (300 mg/kg) and DM+ SA (200 mg/kg) vs DM+Vehicle. **b) Area Under the Curve of the IITT.** A= Indicates significant differences DM+Vehicle vs HR+Vehicle, B= DM+MF (300 mg/kg) vs DM+Vehicle and C= DM+ SA (200 mg/kg) vs DM+Vehicle. **c) OGTT curve.** A= Indicates significant differences DM+Vehicle vs HR+Vehicle, B= DM+ SA (100 and 200 mg/kg) vs DM+Vehicle. **d) Area Under the Curve of the OGTT .** A= Indicates significant differences

DM+Vehicle vs HR+Vehicle, **B**= DM+MF (300 mg/kg) vs DM+Vehicle and **C**= DM+ SA (200 mg/kg) vs DM+Vehicle. One-way ANOVA followed by a Tukey *post hoc* test. Mean $\pm$ SD (n= 5-6, p<0.05).

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