https://doi.org/10.31925/farmacia.2026.4.11 **ORIGINAL ARTICLE**

EVALUATION OF SOME BIOACTIVE COMPOUNDS AND THE ANTIHYPERGLYCEMIC AND ANTIDIABETIC EFFECTS OF FREEZE-DRIED PEELS OF *MELICOCCUS BIJUGATUS* JACQ. (HUAYA) PEELS

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Abstract

This study aimed to investigate bioactive compounds, as well as the antihyperglycemic and antidiabetic effects of freeze-dried *Melicoccus bijugatus* Jacq. (huaya) peels. In the freeze-dried peels, the concentrations of total phenols (209.87 ± 3.40 mg Gallic Acid Equivalents/100 g freeze-dried) and the antoxidant capacity (282.85 ± 4.62 mg Ascobic Acid/100 g freeze-dried) were high and moderate in flavonoids (87.67 ± 0.97 Quercetin/100 g freeze-dried) compared to the reported in pulp fruit. The concentrations of organic acids *per* 100 g of freeze-dried were high: tartaric acid (21216.69 ± 1258.98 mg), malic acid (12336.9054 ± 555.05 mg), citric acid (10062.7239 ± 395.36 mg), and ascorbic acid (1941.56931 ± 68.20 mg). In male Wistar rats, the freeze-dried (25 mg/kg) reduced hyperglycemia by 10.54% in an oral glucose tolerance test, and the insulin signaling might be involved. The combination of metformin plus freeze-dried at low doses produced effects similar to monotherapy. In rats with type 2 diabetes (T2D), administration of freeze-dried (14 days) reversed hyperglycemia, glucose intolerance, and insulin resistance. Huaya peels represent a potential source of bioactive compounds for the treatment of T2D.

Rezumat

Studiul a avut ca scop investigarea compușilor bioactivi, precum și evaluarea efectelor antihiperglicemice și antidiabetice ale cojilor liofilizate de *Melicoccus bijugatus* Jacq. (huaya). În cojile liofilizate s-au evidențiat concentrații ridicate de compuși fenolici totali (209,87 ± 3,40 mg echivalenți acid galic/100 g produs liofilizat) și o capacitate antioxidantă crescută (282,85 ± 4,62 mg echivalenți acid ascorbic/100 g produs liofilizat), în timp ce conținutul de flavonoide totale a fost moderat (87,67 ± 0,97 mg echivalenți quercetină/100 g produs liofilizat), comparativ cu valorile raportate pentru pulpa fructului. Concentrațiile acizilor organici raportate la 100 g produs liofilizat au fost ridicate: acid tartric (21.216,69 ± 1.258,98 mg), acid malic (12.336,91 ± 555,05 mg), acid citric (10.062,72 ± 395,36 mg) și acid ascorbic (1.941,57 ± 68,20 mg). La șobolanii Wistar masculi, administrarea produsului liofilizat în doză de 25 mg/kg a redus hiperglicemia cu 10,54% în cadrul testului oral de toleranță la glucoză, sugerând o posibilă implicare a căii de semnalizare a insulinei. Asocierea metforminului cu produsul liofilizat, administrate în doze reduse, a determinat efecte comparabile cu cele ale monoterapiei. La șobolanii cu diabet zaharat de tip 2, administrarea produsului liofilizat timp de 14 zile a ameliorat hiperglicemia, intoleranța la glucoză și rezistența la insulină.

Keywords: *Melicoccus bijugatus* Jacq., freeze-dried huaya peels, antihyperglycemic effects, antidiabetic effects

Introduction

Type 2 diabetes (T2D) accounts for more than 90% of the 589 million global cases of diabetes reported in 2024 (1). Although pharmacological treatments and lifestyle modifications are available for its management, plant- and fruit-based natural alternatives have recently gained popularity; however, only a limited number have sufficient scientific evidence supporting their efficacy (2, 3). Epidemiological studies have shown that fruit consumption helps prevent chronic diseases such as T2D because of its antioxidant and anti-inflammatory properties, which are associated with the bioactive compounds present in fruits (4, 5). Although the peels of tropical fruits are usually discarded, they contain valuable bioactive compounds, primarily phenolics, anthraquinones, and triterpenoids, which are often concentrated in this tissue (6). *Melicoccus bijugatus* Jacq. (huaya) is a tropical fruit widely consumed in Mexico and has been associated with various pharmacological properties. The seeds have been reported to contain higher levels of

phenolic compounds and to exhibit greater antioxidant and antimicrobial activities than the pulp (7). Laxative effects have been attributed to the pulp, probably due to the presence of ferulic acid derivatives (8). The phenolic compounds p-coumaric acid and caffeic acid detected in pulp extracts may explain the traditional use of huaya fruit in the management of hypertension (9, 10).

Among the phenolic acids identified in *M. bijugatus* fruits, coumaric acid is one of the most abundant (11). This compound possesses antioxidant with antiplatelet activities and may have potential applications in the primary prevention of vascular diseases (9). Furthermore, the traditional use of huaya fruit for the treatment of asthma and other respiratory disorders could be related to the presence of resveratrol, which has been identified in the pulp by mass spectrometry (12). Resveratrol inhibits the NFκB transcription factor, a key regulator of inflammatory processes involved in the pathogenesis of asthma (13). Pulp extracts exhibited antimicrobial activity, whereas pulp and embryo extracts have demonstrated antifungal activity (14).

Although the peels and seeds are generally discarded as agro-industrial waste, Chel-Guerrero *et al.* 2018 (7) demonstrated that methanolic extracts of huaya peels can modulate acetylcholinesterase (AchE) activity. Therefore, huaya peels represent a promising source of bioactive compounds with potential applications as functional ingredients for the prevention and management of various diseases (15).

Accordingly, the aim of the present study was to evaluate the antihyperglycemic effects of freeze-dried huaya peels and to elucidate their possible mechanism of action in male Wistar rats. Subsequently, their antidiabetic effects were assessed in a T2D model induced in neonatal male Wistar rats. Based on the bioactive compounds present in huaya peels, it was hypothesized that their acute administration to healthy Wistar rats would produce antihyperglycemic effects following a glucose challenge and exert antidiabetic effects in the T2D model. To the best of our knowledge, this is the first study to provide comprehensive evidence regarding the antihyperglycemic mechanism of action of freeze-dried huaya peels and to propose their potential use as a therapeutic alternative for the management of hyperglycemia and T2D.

Materials and Methods

Huaya fruits were purchased from local markets in Mérida, Yucatán, Mexico. All procedures involving experimental animals were conducted in accordance with Mexican federal regulations (Ministry of Agriculture, SAGARPA, NOM-062-ZOO-1999, Mexico).

The experimental protocol was approved by the Institutional Ethics Committee of Universidad Autónoma Metropolitana-Iztapalapa (UAM-Iztapalapa) (approval number: 14605022) and was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals. Rats assigned to the different experimental groups were randomized using a simple randomization procedure.

Preparation of freeze-dried peels

Fruits with the same degree of ripeness, free of visible physical damage, of similar size, and exhibiting homogeneous peel and pulp coloration were selected. The peels were manually separated from the fruits and freeze-dried using a Labconco Model 6 freeze dryer (Labconco, MO, USA) at 0.04 mbar and 5°C for 48 h. Subsequently, the dried peels were ground into a fine powder and stored at -20°C until use. The yield of freeze-dried huaya peels was 25.12 ± 0.26%.

Preparation of methanolic extracts

Bioactive compounds were extracted by macerating 1.5 g of freeze-dried huaya peel powder in 30 mL of methanol. The mixture was maintained in a shaking incubator at 160 rpm for 24 h at 25°C. The resulting methanolic extract (ME) was filtered and stored at -20°C until analysis. The extraction yield of the methanolic extract was 9.91 ± 1.75%.

Quantification of total phenolic compounds

The total phenolic content of the ME was determined using the Folin-Ciocalteu method described by Singleton and Rossi (16). Briefly, 0.25 mL of huaya ME was mixed with 0.75 mL of distilled water. The diluted extract was transferred to a test tube, and 1.0 mL of 10% (v/v) Folin-Ciocâlteu reagent was added. After incubation for 1 min, 0.8 mL of 7.5% (w/v) Na_2CO_3 solution was added. The mixture was incubated in the dark for 1 h, and absorbance was measured at 765 nm using a JENWAY 6705 UV/Vis spectrophotometer. A gallic acid calibration curve was used for quantification. All measurements were performed in triplicate, and the results were expressed as milligrams of gallic acid equivalents *per* 100 g of freeze-dried peel (mg GAE/100 g FD).

Quantification of flavonoids

The flavonoid content of the ME was determined according to the method described by Chang *et al.*, (17). Briefly, 0.5 mL of ME was placed in a test tube and mixed with 1.5 mL of methanol, 0.1 mL of 10% (w/v) $AlCl_3$, 0.1 mL of 1M CH_3CO_2K, and 2.8 mL of distilled water. The mixture was vortexed, incubated in the dark for 30 min, and the absorbance was measured at 415 nm using a Thermo BIOMATE 3 UV/VIS spectrophotometer. A quercetin standard curve was used for quantification. All measurements were performed in triplicate, and the results were expressed as milligrams of quercetin equivalents *per* 100 g of freeze-dried peel (mg QE/100 g FD).

Antioxidant capacity

The antioxidant capacity of the ME was determined using the ABTS [2, 2'-azino bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay

described by Rivero-Pérez *et al.*, (18). Briefly, 75 µL of huaya ME was diluted in 925 µL of distilled water. Subsequently, 0.1 mL of the diluted extract was transferred to a test tube, and 1.0 mL of ABTS solution was added. The mixture was incubated in the dark for 7 min, and absorbance was measured at 734 nm using a JENWAY 6705 UV/VIS spectrophotometer.

Antioxidant capacity was quantified using an ascorbic acid standard curve. All measurements were performed in triplicate, and the results were expressed as milligrams of ascorbic acid equivalents *per* 100 g of freeze-dried peel (mg AAE/100 g FD). Data are presented as mean ± standard error of the mean (SEM).

Quantification of organic acids

The contents of citric, malic, ascorbic and tartaric acids in freeze-dried huaya peels were determined by high-performance liquid chromatography (HPLC). Briefly, 2 mg of freeze-dried huaya peel powder were resuspended in 2 mL of HPLC-grade water and subsequently filtered through 0.45 µm nylon membrane filters. Samples and standards were automatically injected into the HPLC system equipped with an XTerra MS C18 column, (5 µm, 4.6 x 250 mm). A phosphate buffer (50 mM, pH 2.8) was used as the mobile phase under isocratic conditions at a flow rate of 0.7 mL/min. Quantification was performed using calibration curves prepared with citric, malic, ascorbic, and tartaric acid standards. All analyses were conducted in triplicate, and the results were expressed as milligrams of compound *per* 100 g of freeze-dried peel.

Oral glucose tolerance test (OGTT)

Male Wistar rats weighing 200 - 250 g were used for the OGTT. Before the test, animals were fasted for 14 h. All experimental groups received an oral D-glucose load (2 g/kg) by gavage, and selected groups received the corresponding treatments before glucose administration. Capillary blood glucose levels were measured from tail vein blood samples before treatment and glucose administration (time 0) and subsequently at 30, 60, 90 and 120 min using a glucometer (Accu-chek® Roche Diagnostics).

The animals were randomly assigned to five experimental groups: 1. Control (D-glucose 2 g/kg), 2. Metformin (300 mg/kg), 3. Freeze-dried huaya peels (25 mg/kg), 4. Freeze-dried huaya peels (100 mg/kg) and 5. Freeze-dried huaya peels (200 mg/kg). Freeze-dried huaya peels were reconstituted in purified drinking water before administration. Dose extrapolation between species was performed using the allometric scaling equation: human dose x animal dose x (body weight $_{human}$/body weight $_{animal}$)$^{0.7}$ (19). None of the doses evaluated exceeded the reported LD50 value of 2,000 mg/kg described for several plant species of the *Sapindaceae* family (20). The metformin dose was selected according to previously published metformin tolerance test (MTT) protocols (21).

In addition to evaluating the antihyperglycemic effects of monotherapy, the efficacy of a low-dose combination of metformin and freeze-dried huaya peels was also assessed. The experimental groups were as follows: 1. Control (D-glucose 2 g/kg), 2. Metformin (300 mg/kg), 3. Freeze-dried huaya peels (25 mg/kg) and 4. Metformin (50 mg/kg) + Freeze-dried huaya peels (10 mg/kg). Glucose values are expressed as mean (n = 5 - 6) ± standard error of the mean (SEM). The area under the curve (AUC) of the OGTT glucose profiles was calculated using the trapezoidal method.

Evaluation of the possible mechanism underlying the antihyperglycemic effect of freeze-dried huaya peels

To investigate the possible mechanism responsible for the antihyperglycemic effect of freeze-dried huaya peels, a pharmacological inhibitor of the insulin signaling pathway was employed. Wortmannin (Sigma-Aldrich, USA), a specific phosphatidylinositol 3-kinase (PI3-K) inhibitor, was dissolved in dimethyl sulfoxide (DMSO) and administered at a dose of 1 mg/kg. Male Wistar rats (200 - 250 g) were fasted during 14 h before the OGTT. Animals were weighed, and baseline glucose levels (time 0) were measured. Subsequently, all groups received a D-glucose load (2 g/kg) while groups 2, 3, 4 and 5 also received the corresponding treatments. The experimental groups were as follows: 1. Control (D-glucose 2 g/kg), 2. Metformin (300 mg/kg), 3. Freeze-dried huaya peels (25 mg/kg), 4. Wortmannin (1 mg/kg) + D-glucose (2 mg/kg), 5. + Wortmannin (1 mg/kg) + Freeze-dried huaya peels (25 mg/kg).

Wortmannin was administered intraperitoneally (i.p.) 30 min before treatment administration. Blood samples were collected from the tail vein at 30, 60, 90 and 120 min after glucose loading, and capillary glucose concentrations were determined using a glucometer (Accu-chek® Roche Diagnostics). Data are expressed as mean (n = 5 - 6) ± SEM, and AUC values were calculated using the trapezoidal method.

Type 2 diabetes model in neonatal male Wistar rats

Male neonatal Wistar rats (3 - 4 days old) were used to establish the T2D model (22). Animals were randomly assigned to two experimental groups. Group 1: Control group (CT): rats received vehicle (0.1 M citrate buffer, pH 4.5) intraperitoneally (i.p.) and Group 2: Diabetic group (DB): rats received a single intraperitoneal injection of streptozotocin (STZ; 70 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5). Both CT and DB rats were evaluated at 8 weeks of age.

Evaluation of the antidiabetic effect of freeze-dried huaya peels

Eight weeks after vehicle or STZ administration, CT and DB rats received one of the following treatments by oral gavage for 14 consecutive days: 1.- Vehicle (water); 2.- Metformin (300 mg/kg) and 3.- Freeze-dried huaya peels (100 mg/kg). Blood glucose concentrations were monitored throughout the

treatments period. At the end of the treatment, an OGTT and an intraperitoneal insulin tolerance test (IPITT) were performed. The OGTT was conducted as previously described.

For the IPIIT, rats were fasted for 6 h at the end of the treatment period. Baseline glucose concentrations (time 0) were measured from tail vein samples. Subsequently, insulin (2 IU/kg) was administered intraperitoneally, and capillary glucose concentrations were measured at 30, 60, 90 and 120 min. Glucose response curves were generated for each experimental group, and the AUC values were calculated using the trapezoidal method.

Statistical analysis

All statistical analyses were performed using Sigma Plot version 14.0 (San José, CA, USA). Data are as mean ± SEM. OGTT and IPITT data were analyzed using two-way analysis of variance ANOVA followed by Duncan's *post hoc* test. Organic acid concentrations and AUC values were analyzed one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Antioxidant compounds present in freeze-dried huaya peels

The concentrations of selected antioxidant compounds present in freeze-dried huaya peels were determined using spectrophotometric methods. Table I summarizes the contents of total phenolic compounds, total flavonoids, and antioxidant capacity measured using the ABTS assay. In addition, HPLC analysis was used to quantify the concentrations of organic acids present in the freeze-dried peels. High concentrations of organic acids were detected, with the following abundance pattern: tartaric acid > malic acid > citric acid > ascorbic acid (Table II).

In recent years, there has been growing global interest in foods that provide health-promoting benefits. However, relatively few studies have specifically investigated tropical fruits as sources of secondary metabolites with potential therapeutic applications (4, 7). Interactions between tropical plants and their natural predators are often complex and may involve the production of unique bioactive compounds with significant pharmacological potential (23). Phenolic compounds present in fruits have attracted considerable attention because of their antioxidant properties. In the present study, the total phenolic content of freeze-dried huaya peels (209.87 ± 3.40 mg GAE/100 g FD, Table I) was comparable to that previously reported for huaya fruit (295.35 ± 17.54 mg GA/ 100 g fresh weight) (24). Similarly, the flavonoid content observed in our study (87.67 ± 0.97 mg QE/100 g FD, Table I) was moderate compared with the concentration reported by Moo-Huchin *et al*., (24) for the fruit (275.76 ± 8.08 mg QE/100 g fresh weight). The antioxidant capacity of plant-derived foods results from the cumulative and often synergistic action of a wide range of antioxidant compounds, which may contribute to the prevention of diseases associated with oxidative stress (25, 26). Therefore, the antioxidant activity of the freeze-dried huaya peels was evaluated using the ABTS assay.

The antioxidant capacity observed in freeze-dried huaya peels (282.85 ± 4.62 mg AAE/100 g FD, Table I) was comparable to that reported by Moo-Huchin *et al*., (24) for huaya fruit (194.27 ± 6.17 mg Vitamin C equivalents/100 g fresh weight). Quantitatively, polyphenols represent the major dietary antioxidants, followed by vitamins and carotenoids (27). Consequently, the antioxidant capacity observed in the present study is likely attributable, at least in part, to the phenolic compounds and flavonoids present in the freeze-dried peels.

Table I

Content of antioxidant compounds in methanolic extracts of freeze-dried huaya peels

	Antioxidant capacity (ABTS) (mg AAE/100 g FD)	**Total phenolic compounds (mg GAE/100 g FD)**	**Total flavonoids (mg QE/100 g FD)**
Methanolic extract of freeze-dried huaya peels	282.85 ± 4.62	209.87 ± 3.40	87.67 ± 0.97

Values are expressed as mean ± standard error of the mean (SEM) (n = 11)

HPLC analysis revealed high concentrations of organic acids in freeze-dried huaya peels, following the order: tartaric acid > malic acid > > citric acid > > ascorbic acid (Table II). Organic acids may contribute to the biological activity of huaya peels including potential modulation of the insulin signaling pathway. Previous *in vitro* studies have shown that citric acid and L-malic acid protect cardiomyocytes against hypoxia/reoxygenation-induced injury by reducing necrosis and apoptosis, possibly through activation of the PI-3K/Akt survival pathway (28). Furthermore, several studies have reported important biological activities associated with malic, citric, tartaric, and succinic acids (28-30). In agreement with these reports, our results demonstrated that freeze-dried huaya peels contain substantial concentrations of these organic acids (Table II).

Table II

Content of organic acids in freeze-dried huaya peels

	Tartaric Acid (mg/100 g FD)	Malic Acid (mg/100 g FD)	Citric Acid (mg/100 g FD)	Ascorbic Acid (mg/100 g FD)
Freeze-dried huaya peels	21,216.69 ± 1,258.98^{A}	12,336.9054 ± 555.05^{B}	10,062.7239 ± 395.36^{C}	1,941.56931 ± 68.20^{D}

Values are expressed as mean ± standard error of the mean (SEM) (n = 4). ^{A} = P < 0.05 Tartaric Acid *vs*. Malic, Citric and Ascorbic Acids; ^{B} = P < 0.05 Malic Acid *vs*. Tartaric, Citric and Ascorbic Acids; ^{C} = P < 0.05 Citric Acid *vs*. Tartaric, Malic and Ascorbic Acids; ^{D} = P < 0.05 Ascorbic Acid *vs*. Tartaric, Malic and Citric Acids; One-way ANOVA followed by Student-Newman-Keuls *post hoc* test

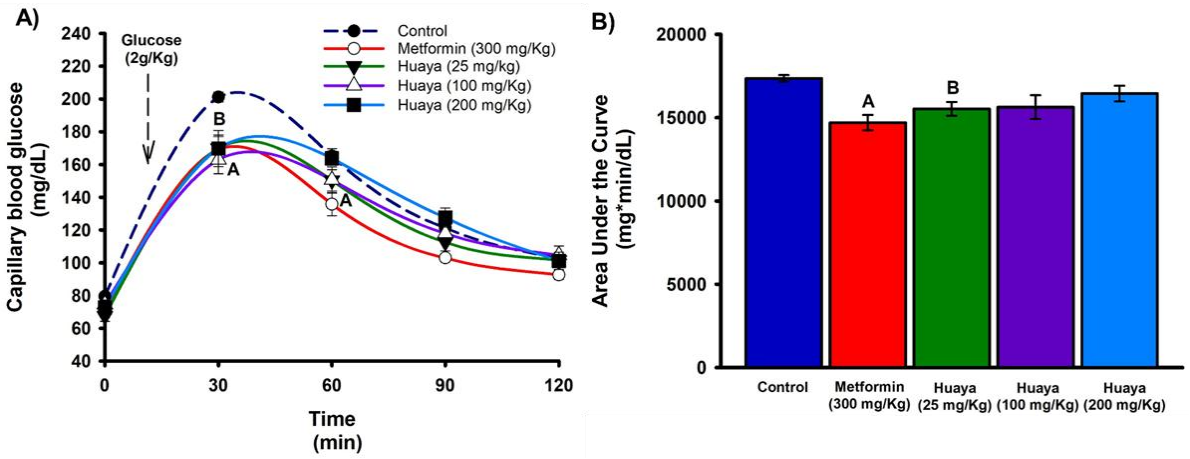

Figure 1.

Oral glucose tolerance test (OGTT) following administration of freeze-dried huaya peels. A) Antihyperglycemic effects were observed at 30 min during the OGTT with all tested doses of freeze-dried huaya peels (25, 100 and 200 mg/kg) compared with the control group. A = P < 0.05 Metformin (300 mg/Kg) *vs*. Control, B = P < 0.05 huaya peels (25, 100 and 200 mg/kg) *vs*. Control. Data were analyzed using two-way ANOVA followed by Duncan's post hoc test and are presented as mean ± SEM (n = 5 - 6 rats *per* group). B) The 25 mg/kg dose of freeze-dried huaya peels resulted in a significantly lower area under the curve (AUC) than that of the control group. A = P < 0.05 Metformin *vs*. Control, B = P < 0.05 Huaya peels (25 mg/kg) *vs*. Control. Data were analyzed using one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test and are presented as mean ± SEM (n = 5 - 6 rats *per* group).

Antihyperglycemic effect of freeze-dried huaya peels

In the OGTT performed in healthy rats, freeze-dried huaya peels produced significant antihyperglycemic effects at 30 min post-glucose administration at all tested doses (25, 100, and 200 mg/kg) compared with the control group. This reduction in blood glucose observed at 30 min was comparable to that achieved with metformin treatment (Figure 1A). Among the doses evaluated, 25 mg/kg of freeze-dried huaya peels resulted in a significantly lower area under the curve (AUC) than that of the control group (Figure 1B), suggesting that this dose exert a sustained antihyperglycemic effect. Interestingly, the freeze-dried huaya peels exhibited a non-linear, biphasic dose-response profile, with the lowest tested dose (25 mg/kg) showing the most consistent and prominent antihyperglycemic effect compared to higher doses. This lack of proportional improvement at higher doses is a characteristic hallmark of crude botanical extracts and complex phytochemical matrices (31). From a molecular perspective, this phenomenon can be explained by the saturation and subsequent desensitization of key intracellular components (receptors). Furthermore, unlike isolated pharmaceutical drugs, whole extracts contain an array of secondary metabolites that interact simultaneously with multiple biological targets. At lower doses (25 mg/kg), the concentrations of primary antidiabetic agents (such as specific organic acids or polyphenols) are sufficient to exert therapeutic effects, while counter-effective or mildly antagonistic compounds remain below their active thresholds. As the dose increases, these secondary compounds may reach concentrations high enough to compete for transporters, inhibit key metabolic enzymes, or induce minor counter-regulatory hyperglycemic responses, resulting in a plateau or reduction of the net therapeutic outcome (32).

Numerous pharmacological activities have been attributed to polyphenolic compounds, including the regulation of glucose homeostasis (33) and anti-inflammatory and analgesic effects (34).

In the present study, freeze-dried huaya peels exhibited high concentrations of total phenolic compounds. In this context, quercetin, a polyphenol isolated from *Cassia fistula*, has been reported to reduce plasma glucose concentrations when administered chronically to diabetic rats. This effect was associated with

the restoration of hepatic glucokinase, glucose-6-phosphatase (G6Pase), glycogen synthase, and glycogen phosphorylase activities to near-normal levels, as well as with increased GLUT4 mRNA and protein expression in skeletal muscle (35).

In addition, quercetin-3-rutinoside (rutin) has been identified in crude extracts of huaya seed coats. Several mechanisms have been proposed to explain the antihyperglycemic effects of rutin, including: (i) reduced intestinal glucose absorption through inhibition of α-glucosidase and α-amylase activities involved in carbohydrate digestion; (ii) suppression of hepatic gluconeogenesis through downregulation of the key gluconeogenic enzymes G6Pase and phosphoenolpyruvate carboxykinase (PEPCK); (iii) enhancement of insulin-mediated glucose uptake in peripheral tissues; (iv) stimulation of insulin secretion by pancreatic β-cells; and (v) protection of the islets of Langerhans against degeneration (36).

It is important to emphasize that our study did not specifically evaluate the presence of quercetin and rutin; rather, we performed an assessment of total phenolic compounds. It is essential to characterize the specific bioactive compounds in the freeze-dried *Melicoccus bijugatus* (huaya) peels using HPLC-MS/MS.

Possible mechanism underlying the antihyperglycemic effect of freeze-dried huaya peels

To investigate the mechanism underlying the antihyperglycemic effect of freeze-dried huaya peels during the OGTT, phosphatidylinositol 3-kinase (PI3-K), a key component of the insulin signalling pathway, was pharmacologically inhibited using wortmannin. Administration of freeze-dried huaya peels produced significant antihyperglycemic effects at 30 and 60 min during the OGTT. However, pretreatment with wortmannin abolished the antihyperglycemic effect observed at 30 min, suggesting that activation of the insulin signalling pathway may contribute to this response; however, it is essential to evaluate the expression of proteins involved in the insulin signaling pathway (IRS-1, PI3K, Akt, p-Akt, and GLUT4) in target tissues (muscle and adipose tissue) to confirm its activation. Interestingly, wortmannin did not completely reverse the effect observed at 60 min, suggesting the involvement of additional antihyperglycemic mechanisms (Figure 2A). Analysis of the AUC further demonstrated that the group receiving wortmannin (1 mg/kg) plus freeze-dried huaya peels (25 mg/kg) exhibited a significantly greater AUC than the huaya-treated group, indicating attenuation of the antihyperglycemic effect (Figure 2B).

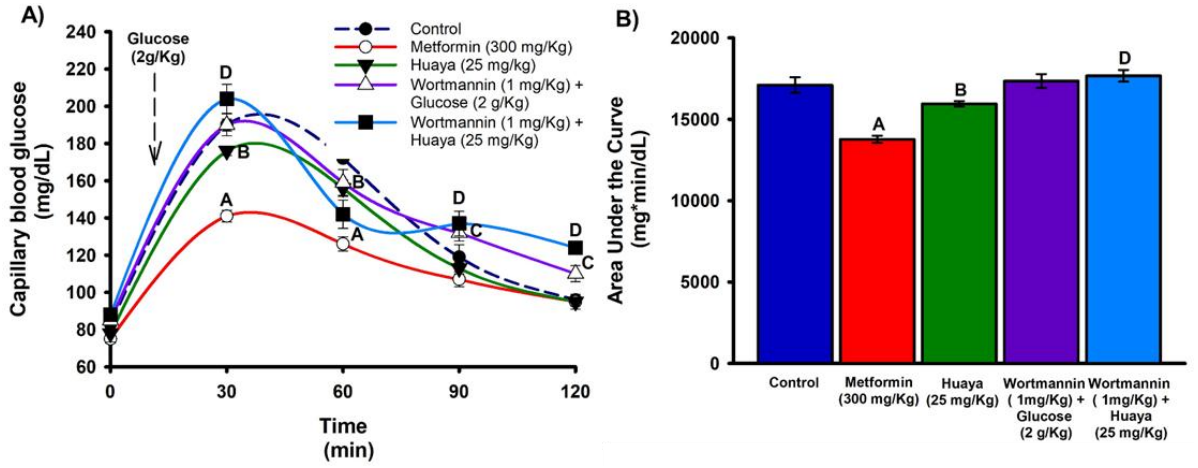

Figure 2.

Mechanism of action of the antihyperglycemic effect of freeze-dried huaya peels. A) The antihyperglycemic effects induced by the freeze-dried huaya peels were observed at 30 and 60 min during the OGTT compared with the control group. Pretreatment with wortmannin reversed the effect observed at 30 min but did not abolish the effect detected at 60 minutes. A = P < 0.05 Metformin *vs*. Control, B = P < 0.05 Huaya peels *vs*. Control, C = P < 0.05 Wortmannin (1 mg/kg) + D-glucose *vs*. Control, D = P < 0.05 Wortmannin (1 mg/kg) + Huaya peels (25 mg/kg) *vs*. Huaya peels (25 mg/kg). Data were analyzed using two-way ANOVA followed by Duncan's post hoc test (n = 5 - 6 rats *per* group). B) The AUC of Wortmannin (1 mg/kg) + Huaya peels (25 mg/kg) group was significantly higher than that of the Huaya peels (25 mg/kg) group, indicating attenuation of the antihyperglycemic effect. A = P < 0.05 Metformin *vs*. Control, B = P < 0.05 Huaya peels *vs*. Control, D = P < 0.05 Wortmannin (1 mg/kg) + Huaya peels (25 mg/kg) *vs*. Huaya peels (25 mg/kg). Data were analyzed using one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test and are presented as mean ± SEM (n = 5 - 6 rats *per* group).

Glucose homeostasis is maintained through a complex regulatory network involving pancreatic islet cells, the liver, and peripheral tissues, including skeletal muscle, adipose tissue and the brain. Insulin, secreted by β-cells in response to nutrient intake, suppresses hepatic glucose production while promoting

glucose uptake by skeletal muscle and adipose tissue (37). The canonical insulin signalling cascade responsible for maintaining glucose homeostasis involves activation of the serine/threonine kinase Akt, which requires insulin-receptor activation and subsequent stimulation of phosphatidylinositol 3-kinase (PI3-K) (38). The PI3-K/Akt signalling path-way plays a central role in insulin-mediated regulation of glucose transport in adipose tissue and skeletal muscle (39), as well as in the suppression of hepatic gluconeogenesis (40). In the present study, wortmannin – a fungal metabolite recognized as a potent, selective, and irreversible PI3-K inhibitor – (41), was used to evaluate the role of this specific component in the pathway. The reversal of the anti-hyperglycemic effect of freeze-dried huaya peels at 30 min following of the OGTT administration (Figure 2A) supports the involvement of the PI3-K. However, it is essential to evaluate the involvement of other components of the insulin pathway using molecular biology to define its role in the observed response.

Notably, the antihyperglycemic effect reappeared at 60 min despite PI3-K/Akt inhibition, suggesting that additional mechanisms may contribute to the glucose-lowering activity of freeze-dried huaya peels. In this regard, Manaharan *et al*., (42) reported that extracts from tropical plants exert antihyperglycemic effects through inhibition of the carbohydrate-hydrolyzing enzymes α-glucosidase and α-amylase. Therefore, inhibition of digestive enzymes may also contribute to the effects observed in the present study; however, additional experiments are required to confirm this hypothesis.

Maintenance of the antihyperglycemic effect through the combination of metformin and freeze-dried huaya peels at low doses

The combination of metformin and freeze-dried huaya peels at low doses was evaluated to determine whether antihyperglycemic efficacy could be maintained while reducing the doses of both treatments. The combination consisted of metformin (50 mg/kg) and freeze-dried huaya peels (10 mg/kg).

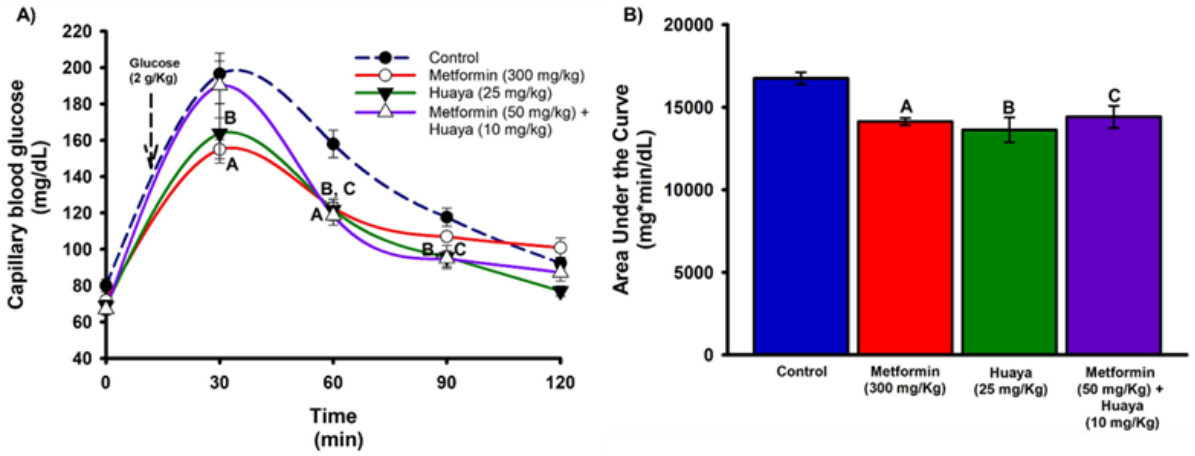

Figure 3.

Maintenance of the antihyperglycemic effect by the combination of metformin and freeze-dried huaya peels. A) The combination of metformin and freeze-dried huaya peels at low doses (50 and 10 mg/kg, respectively) produced significant antihyperglycemic effects at 60 and 90 min during the OGTT in comparison with metformin monotherapy. Data were analyzed using two-way ANOVA followed by Duncan's post hoc test (n = 5 - 6 rats *per* group). B) The combination of metformin and freeze-dried huaya peels resulted in a significantly lower AUC than that of the control group. A = P < 0.05 Metformin (300 mg/kg) *vs*. Control, B = P < 0.05 Huaya peels (25 mg/kg) *vs.* Control, C = P < 0.05 Metformin (50 mg/kg) and Huaya peels (10 mg/kg) *vs.* Control. OGTT data were analysed using two-way ANOVA followed by Duncan's post hoc test, whereas AUC values were analyzed using one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test. Data are presented as mean ± SEM (n = 5 - 6 rats *per* group).

Previous experiments demonstrated that the 25 mg/kg dose of freeze-dried huaya peels produced the most consistent antihyperglycemic response; therefore, this dose was included as a reference treatment in the present experiment. Consistent with previous findings, administration of freeze-dried huaya peels (25 mg/kg) significantly reduced capillary blood glucose concentrations at 30 and 60 min during the OGTT compared with the control group. Interestingly, the combination of metformin and freeze-dried huaya peels at low doses (50 and 10 mg/kg, respectively) maintained significant antihyperglycemic activity, with reductions in blood glucose concentrations observed at 60 and 90 min during the OGTT. The magnitude of this effect was comparable to that observed with metformin monotherapy (Figure 3A). Similarly, the combination treatment produced a significantly a lower AUC than that of the control group (Figure 3B).

Thus, dose reduction did not compromise antihyperglycemic efficacy, suggesting a potential additive or complementary interaction between metformin and the bioactive compounds present in huaya peels. Notably, maintenance of the antihyperglycemic effect was particularly evident at 60 and 90 min during the OGTT (Figure 3A). However, additional studies are required to elucidate the mechanisms responsible for the observed effects.

Currently, several pharmacological agents with distinct mechanisms of action are available for the management of T2D. Among these, metformin remains one of the most widely prescribed antihyperglycemic drugs because it suppresses hepatic gluconeogenesis, enhances glucose uptake in skeletal muscle, and improves insulin sensitivity (43). The antihyperglycemic efficacy of metformin generally increases with dose escalation, from 500 mg/day up to the upper limit of the recommended therapeutic range (≥ 2000 mg/day) (44). However, high doses may increase the risk of adverse effects, including gastrointestinal disturbances such as nausea, vomiting, abdominal discomfort, diarrhea, and, in rare cases, lactic acidosis (45). In the present study, maintenance of antihyperglycemic efficacy was observed when low doses of metformin and freeze-dried huaya peels were administered in combination (50 and 10 mg/kg, respectively). These findings suggest that this this therapeutic strategy may represent a promising complementary approach for glycemic control. Nevertheless, further preclinical and clinical studies are required to confirm its efficacy and safety.

Antidiabetic effect of the freeze-dried huaya peels

In the present study, the antidiabetic effect of the freeze-dried huaya peels was evaluated using a T2D model induced by the neonatal administration of streptozotocin (STZ). Eight weeks after STZ administration, diabetic (DB) rats exhibited hyperglycemia compared to the control group (CT) (Fig. 4). Subsequently, both CT and DB rats received oral treatment for 14 consecutive days with one of the following: (i) Vehicle (drinking water), (ii) metformin (300 mg/kg) or (iii) freeze-dried huaya peels (100 mg/kg). At the end of the treatment period, vehicle-treated DB rats remained hyperglycemic, whereas administration of metformin or freeze-dried huaya peels significantly reduced blood glucose concentrations, reversing the hyperglycemic state observed in untreated diabetic animals (Figure 4).

The first study to investigate the pharmacological properties of freeze-dried huaya peels was conducted by Chel-Guerrero *et al*., (7) who reported that extracts obtained from this tissue where capable of modulating acetylcholinesterase (AChE) activity. To the best of our knowledge, the present study is the first to evaluate both the antihyperglycemic and antidiabetic effects of freeze-dried huaya peels. Although limited information is available regarding the health-promoting properties of huaya fruit, several ethnomedicinal uses of its pulp and seeds have been documented. For example, the improvement in gastrointestinal disorders has been associated with the use of huaya seeds (11) due to the presence of epicatechin, catechin and procyanidin B2 (46). These compounds have been shown to inhibit chloride transport mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) in human colonic epithelial cells (47), which may contribute to their beneficial gastrointestinal effects. Caffeic acid has also been identified in huaya pulp (46).

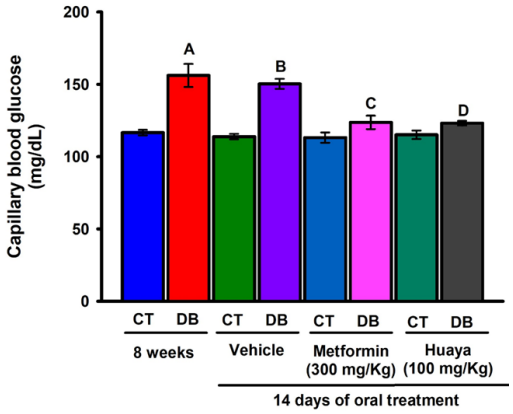

Figure 4.

Capillary blood glucose concentrations of control rats (CT) and type 2 diabetes (DB) rats after 14 days of treatment. Eight-week-old DB rats exhibited hyperglycemia compared with CT rats. Oral administration of metformin (300 mg/kg) or freeze-dried huaya peels (100 mg/kg) for 14 days reduced capillary blood glucose concentrations compared with those of untreated diabetic rats at 8 weeks of age. Neither treatment altered capillary blood glucose concentrations in CT rats. A = $P < 0.05$ DB (8 weeks) *vs.* CT (8 weeks); B = $P < 0.05$ DB and vehicle *vs.* CT-vehicle; C = $P < 0.05$ DB and metformin *vs.* DB (8 weeks); D = $P < 0.05$ DB and Huaya peels *vs.* DB (8 weeks). One-way ANOVA followed by the Student-Newman-Keuls *post hoc* test. Data are presented as mean ± SEM (n = 7 rats *per* group)

The traditional use of huaya fruit for the management of hypertension may be partially explained by the ability of caffeic acid to antagonize angiotensin II-induced vascular smooth muscle cell proliferation in stroke-prone spontaneously hypertensive rats (48). Similarly, the use of huaya pulp for the treatment of diarrhea may be associated with the presence of astringent polyphenolics and antimicrobial hydroxycinnamic acids such as coumaric acid, which may target the underlying causes of this condition (11). Furthermore, the traditional use of huaya pulp for respiratory disorders and asthma may be related to the presence of caffeic acid, which acts as a selective inhibitor of leukotriene biosynthesis. Because leukotrienes

play important roles in immunoregulation, inflammation, asthma, and allergic responses, inhibition of their synthesis may contribute to the therapeutic effects historically attributed to huaya fruit (49).

At the end of the treatment period, DB rats receiving vehicle exhibited glucose intolerance, as determined by the OGTT (Figure 5A), and insulin resistance, as assessed by the IIPT (Figure 5C). Treatment with metformin (300 mg/kg) or freeze-dried huaya peels (100 mg/kg) reversed these characteristics of T2D, as evidenced by lower AUC values in both the OGTT and the IIPT compared with those observed in vehicle-treated DB rats (Figure 5B and Figure 5D).

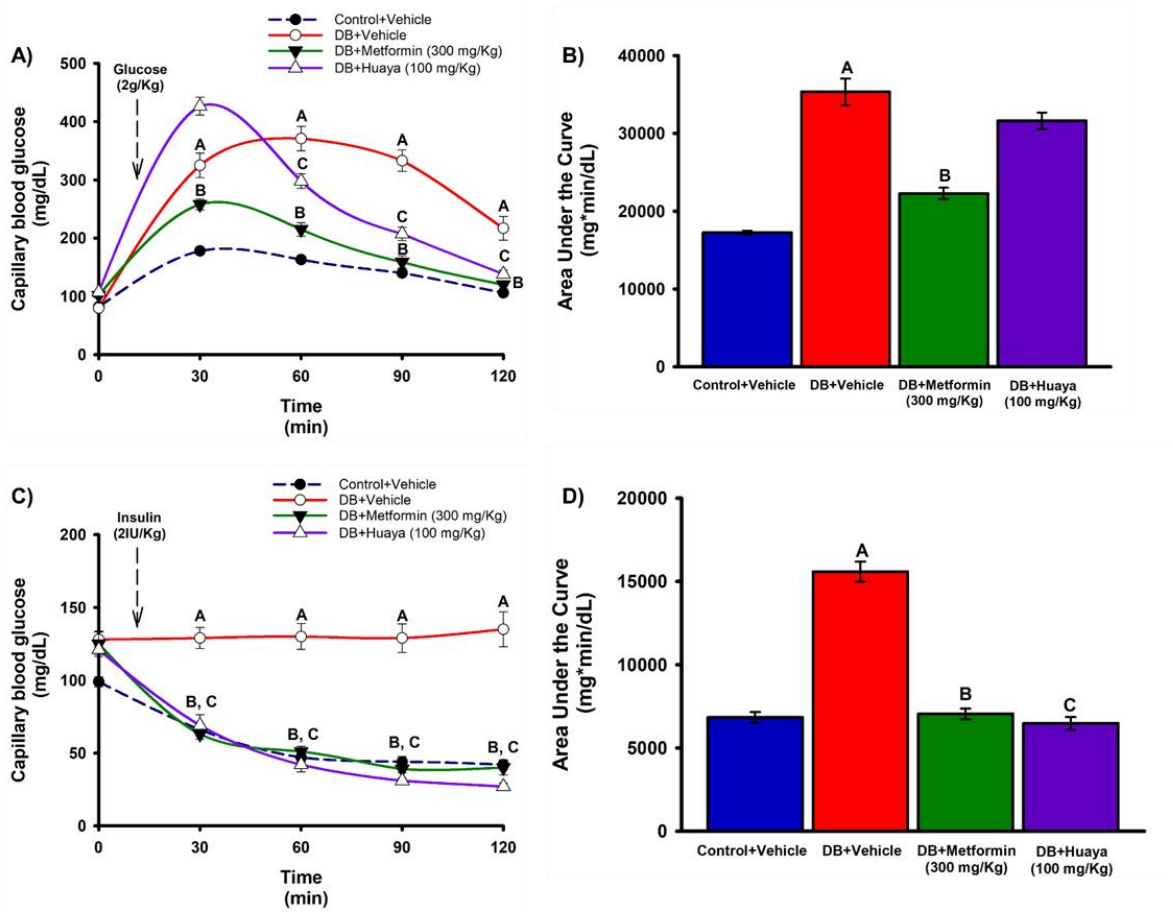

Figure 5.

Oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (IPITT) after 14 days of treatment. A) Vehicle-treated diabetic rats exhibited glucose intolerance compared with the control group. Subchronic administration of freeze-dried huaya peels reversed glucose intolerance. Treatment with metformin also reversed glucose intolerance in diabetic rats. This effect was additionally reflected in the AUC values B) compared with those of the DB and Vehicle group. C) DB and Vehicle rats exhibited insulin resistance, whereas treatment with freeze-dried huaya peels reversed this condition, producing a response similar to that observed with metformin treatment. D) AUC values were lower in rats treated with metformin or freeze-dried huaya peels than in the DB and Vehicle group. OGTT data were analysed using two-way ANOVA followed by Duncan's *post hoc* test, whereas AUC values were analysed using one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test for AUC. Data are presented as mean ± SEM (n = 5 - 6 rats *per* group).

In the present study, the antidiabetic effect of freeze-dried huaya peels was evaluated using a T2D model induced by neonatal administration of STZ, a model widely used to reproduce several characteristics of this disease (50, 51). Eight weeks after STZ administration, DB rats exhibited hyperglycemia compared with CT rats (Figure 4). Furthermore, it has been reported that, at this age, DB rats develop glucose intolerance and insulin resistance (52). In the present study, freeze-dried huaya peels contained high concentrations of organic acids (tartaric > malic > citric > ascorbic acid, Table II). Organic acids have been reported to exert dose-dependent inhibitory effects on α-amylase and α-glucosidase activities. The Inhibition of these digestive enzymes reduces the amount of absorbable monosaccharides, particularly glucose, thereby lowering blood glucose concentrations. The inhibitory effects of these organic acids

follow the descending order: citric acid > tartaric acid > malic acid > succinic acid > lactic acid > acetic acid (53). While a HPLC-MS/MS analysis is essential to fully characterize the bioactive compounds in freeze-dried huaya peels, our current HPLC assessment identified organic acids that, according to the literature, may contribute to reversing hyperglycemia in T2D rats.

Different tropical fruits, including the lychee (*Litchi chinensis*), longan (*Dimocarpus. longan*), and huaya, contain metabolites capable of regulating gluconeogenesis and β-oxidation (54). Among these metabolites are hypoglycin A (2-methylenecyclopropanealanine) and methylenecyclopropylglycine (MCPG). The metabolite of hypoglycin A, methylenecyclopropylacetyl coenzyme A ester (MCPA-CoA) induces hypoglycemia and depletes hepatic glycogen stores (55). Regarding MCPG, its principal metabolites target enzymes involved in hepatic β-oxidation, including 3-oxoacyl-CoA dehydrogenase and acetoacetyl-CoA thiolase. This results in reduced gluconeogenesis through decreased availability of NADH, which is required for glucose synthesis, and acetyl-CoA, an allosteric activator of pyruvate carboxylase involved in glucose production from pyruvate (54). Evaluating the presence of bioactive compounds -specifically hypoglycin A and MCPG- in the freeze-dried huaya peels using HPLC-MS/MS could offer valuable insights into their possible involvement in the observed hypoglycemic effects.

In the present study, treatment with metformin (300 mg/kg) or freeze-dried huaya peels (100 mg/kg) reversed key characteristics of T2D, including glucose intolerance and insulin resistance. These effects may be associated, at least in part, with antioxidant activity, since fruit polyphenols play a pivotal role in protecting the body against oxidative stress and chronic diseases such as T2D, cardiovascular disease, and cancer (56). In the present study, freeze-dried huaya peels exhibited high antioxidant capacity (282.85 ± 4.62 mg AAE/100 g FD, Table I) which may be associated with their high total phenolic content (209.87 ± 3.40 mg GAE/100 g FD, Table I). In addition to their antioxidant properties, polyphenols have been reported to interact with glucose transporters, thereby contributing to anti-hyperglycemic effects (57).

Interestingly, the present study is the first to evaluate the antidiabetic effect of freeze-dried huaya peels in a T2D model. Therefore, elucidating the mechanisms responsible for the observed hypoglycemic effects, as well as those involved in the reversal of glucose intolerance and insulin resistance, is of particular interest. Natural products and herbal extracts have been reported to improve insulin resistance through modulation of specific molecular targets, including peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor that functions as a transcription factor and plays a key role in lipid metabolism and glucose homeostasis (58). In addition, organic acids present in fruits, particularly citric acid and L-malic acid, have been reported to significantly increase Akt upregulated the expression of phosphorylation (28). This signaling pathway plays a central role in insulin-stimulated glucose uptake in skeletal muscle and adipose tissue while suppressing hepatic glucose production (59). Because natural products have been reported to activate PPAR-γ and organic acids to upregulate components of the insulin pathway, freeze-dried huaya peels could exert its antidiabetic effects by modulating these nuclear receptors and intracellular signaling cascade. Future studies in our laboratory will focus on elucidating the specific bioactive compounds present in the freeze-dried peels and evaluating the molecular mechanisms involved in the observed antidiabetic effects.

The limitations of this study include the absence of LC-MS/MS characterization of freeze-dried huaya peels, the lack of evaluation of proteins involved in insulin signaling, and the absence of an isobolographic analysis to determine the specific pharmacological interaction associated with the therapeutic combination. These aspects represent important research perspectives that are currently being addressed in our laboratory. Finally, while the freeze-dried huaya peels (*Melicoccus bijugatus*) demonstrated a significant antihyperglycemic and antidiabetic effect, it is important to acknowledge that a comprehensive toxicological profile was not established in this study. Parameters such as daily food intake, and specific biomarkers of renal or hepatic toxicity were not monitored. Preclinical screenings of agro-industrial by-products require strict safety clearances, as secondary metabolites can sometimes exert unintended adverse effects alongside their therapeutic benefits.

Conclusions

Administration of freeze-dried huaya peels to rats subjected to an OGTT produced antihyperglycemic effects. Furthermore, this effect was blocked by a PI3-K inhibitor, suggesting the involvement of one of the components of the insulin signaling pathway. The combination of metformin and freeze-dried huaya peels at low doses maintained antihyperglycemic efficacy, indicating that this therapeutic combination may represent a potential antihyperglycemic alternative. Subchronic administration of freeze-dried huaya peels to rats with T2D reversed hyperglycemia, glucose intolerance, and insulin resistance.

This study is the first to report both the antihyperglycemic mechanism of action and the antidiabetic effects of freeze-dried huaya peels. Furthermore, the results demonstrate that freeze-dried peels of *Melicoccus bijugatus* contain significant levels of bioactive compounds and exhibit promising antidiabetic activity. These findings support further investigation

of huaya peels as potential functional food ingredients or phytopharmaceutical agents.

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Availability of data and materials

All data generated or analysed during this study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

All experimental procedures were approved by the Institutional Ethics Committee of Universidad Autónoma Metropolitana-Iztapalapa (UAM-Iztapalapa) under approval number 14605022/2021) and were conducted in accordance with the Guidelines for Care and Use of Experimental Animals.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Duncan BB, Magliano DJ, Boyko EJ. IDF diabetes atlas 11th edition 2025: global prevalence and projections for 2050. Nephrol Dial Transplant. 2025;41(1):7-9.
2. Kalyani RR, Neumiller JJ, Maruthur NM, Wexler DJ. Diagnosis and treatment of type 2 diabetes in adults: a review. JAMA. 2025;334(11):984-1002.
3. Hekmat-Ardakani A, Morshed-Behbahani B, Rahimi-Ardabili H, Ayati MH, Namazi N. The effects of dietary supplements and natural products targeting glucose levels: an overview. Crit Rev Food Sci Nutr. 2023;63(23):6138-6167.
4. Sarkar T, Salauddin M, Roy A, Sharma N, Sharma A, Yadav S, et al. Minor tropical fruits as a potential source of bioactive and functional foods. Crit Rev Food Sci Nutr. 2023;63(23):6491-6535.
5. Georgescu A, Dascalu AM, Stana D, Alexandrescu C, Bobirca A, Cristea BM, et al. The role of inflammation in diabetic retinopathy in patients with type II diabetes; potential therapeutic perspectives. J Mind Med Sci. 2024;11(1):17-23.
6. Orozco-Sifuentes MM, Salas-Tovar JA, Soriano-Melgar LAA, Campos-Múzquiz LG, Nery-Flores SD, Palomo-Ligas L, et al. Agro-industrial by-products: alternative solution for functional food ingredients. In: Sanghvi G, Bishoyi AK, Joshi SJ, El Enshasy HA, editors. Sustainable food fortification: biobased approaches and strategies. Singapore: Springer Nature Singapore; 2025. p. 333-366.
7. Chel-Guerrero LD, Sauri-Duch E, Fragoso-Serrano MC, Pérez-Flores LJ, Gómez-Olivares JL, Salinas-Arreortua N, et al. Phytochemical profile, toxicity, and pharmacological potential of peels from four species of tropical fruits. J Med Food. 2018;21(7):734-743.
8. Badary OA, Awad AS, Sherief MA, Hamada FMA. In vitro and in vivo effects of ferulic acid on gastrointestinal motility: inhibition of cisplatin-induced delay in gastric emptying in rats. World J Gastroenterol. 2006;12(33):5363-5367.
9. Luceri C, Giannini L, Lodovici M, Antonucci E, Abbate R, Masini E, et al. p-Coumaric acid, a common dietary phenol, inhibits platelet activity in vitro and in vivo. Br J Nutr. 2007;97(3):458-463.
10. Logan K, Palacios J, Lopez S, Gray W, Nwokocha CR. Integrating experimental and computational approaches to cardioprotection: vascular reactivity, molecular docking, and ADMET modeling of Melicoccus bijugatus (Guinep). Int J Mol Sci. 2025;26(20):10228.
11. Bystrom LM. The potential health effects of Melicoccus bijugatus Jacq. fruits: phytochemical, chemotaxonomic and ethnobotanical investigations. Fitoterapia. 2012;83(2):266-271.
12. Bystrom LM, Lewis BA, Brown DL, Rodriguez E, Obendorf RL. Characterization of phenolics by LC-UV/Vis, LC-MS/MS and sugars by GC in Melicoccus bijugatus Jacq. 'Montgomery' fruits. Food Chem. 2008;111(4):1017-1024.
13. Wątroba M, Szukiewicz D. Anti-inflammatory properties of resveratrol. Int J Mol Sci. 2025;26(23):11710.
14. Bystrom LM, Lewis BA, Brown DL, Rodriguez E, Obendorf RL. Phenolics, sugars, antimicrobial and free-radical-scavenging activities of Melicoccus bijugatus Jacq. fruits from the Dominican Republic and Florida. Plant Foods Hum Nutr. 2009;64(2):160-166.
15. Nirmal NP, Khanashyam AC, Mundanat AS, Shah K, Babu KS, Thorakkattu P, et al. Valorization of fruit waste for bioactive compounds and their applications in the food industry. Foods. 2023;12(3):556.
16. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-158.
17. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-182.
18. Rivero-Pérez MD, Muñiz P, González-Sanjosé ML. Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity, and biomarkers of oxidative stress methodologies. J Agric Food Chem. 2007;55(14):5476-5483.
19. Janhavi P, Divyashree S, Sanjailal KP, Muthukumar SP. DoseCal: a virtual calculator for dosage conversion between human and different animal species. Arch Physiol Biochem. 2022;128(2):426-430.

20. da Silva MS, Tamashiro LK, Jorge BC, da Silva P, Heredia-Vieira SC, de Almeida GL, et al. Toxicological safety evaluation in acute and 28-day studies of aqueous extract from Serjania marginata Casar. (Sapindaceae) leaves in rats. J Ethnopharmacol. 2019;231:197-204.
21. Benedé-Ubieto R, Estévez-Vázquez O, Ramadori P, Cubero FJ, Nevzorova YA. Guidelines and considerations for metabolic tolerance tests in mice. Diabetes Metab Syndr Obes. 2020;13:439-450.
22. Oidor-Chan VH, Hong E, Pérez-Severiano F, Montes S, Torres-Narvaéz JC, Del Valle-Mondragón L, et al. Fenofibrate plus metformin produces cardioprotection in a type 2 diabetes and acute myocardial infarction model. PPAR Res. 2016;2016:8237264.
23. Vijayalakshmi V, Gokulakrishnan J, Prabhu V, Rahul S, Gopinath M. Systematic review of the therapeutic potential of medicinal herbs: insights into their biological applications and futuristic innovations. Proc Indian Natl Sci Acad. 2025.
24. Moo-Huchin VM, Estrada-Mota I, Estrada-León R, Cuevas-Glory L, Ortiz-Vázquez E, Vargas VM, et al. Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. Food Chem. 2014;152:508-515.
25. Nedea MI, Bădiceanu CD, Gheorghe-Barbu I, Marinaș IC, Pericleanu R, Dragomir RI, et al. Antifungal and antioxidant activity of thiourea derivatives against nosocomial Candida auris strains isolated in Romania. Molecules. 2025;30(8):1675.
26. Pérez-Jiménez J, Arranz S, Tabernero M, Díaz-Rubio ME, Serrano J, Goñi I, et al. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. Food Res Int. 2008;41(3):274-285.
27. Godos J, Guglielmetti M, Ferraris C, Frias-Toral E, Azpíroz DI, Lipari V, et al. Mediterranean diet and quality of life in adults: a systematic review. Nutrients. 2025;17(3):577.
28. Tang X, Liu J, Dong W, Li P, Li L, Lin C, et al. The cardioprotective effects of citric acid and L-malic acid on myocardial ischemia/reperfusion injury. Evid Based Complement Alternat Med. 2013;2013:820695.
29. Zhang QC, Zhao Y, Bian HM. Antiplatelet activity of a novel formula composed of malic acid, succinic acid and citric acid from Cornus officinalis fruit. Phytother Res. 2013;27(12):1894-1896.
30. Dharmappa KK, Kumar RV, Nataraju A, Mohamed R, Shivaprasad HV, Vishwanath BS. Anti-inflammatory activity of oleanolic acid by inhibition of secretory phospholipase A2. Planta Med. 2009;75(3):211-215.
31. Calabrese EJ, Agathokleous E. Hormesis: transforming disciplines that rely on the dose response. IUBMB Life. 2022;74(1):8-23.
32. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat Prod Rep. 2019;36(6):869-888.
33. Barik SK, Sengupta S, Arya R, Kumar S, Kim JJ, Chaurasia R. Dietary polyphenols as potential therapeutic agents in type 2 diabetes management: advances and opportunities. Adv Nutr. 2025;16(1):100346.
34. Tleubayeva MI, Datkhayev UM, Malm A, Józefczyk A, Wojtanowski K, Seitaliyeva AM, et al. Purslane (Portulaca oleracea L.) growing in south Kazakhstan – phytochemical composition of ultrasonic hydroethanolic extract and its pharmacological activity. Farmacia. 2025;73(3):665-675.
35. Daisy P, Balasubramanian K, Rajalakshmi M, Eliza J, Selvaraj J. Insulin mimetic impact of catechin isolated from Cassia fistula on the glucose oxidation and molecular mechanisms of glucose uptake on streptozotocin-induced diabetic Wistar rats. Phytomedicine. 2010;17(1):28-36.
36. Gaur YK, Kumar S, Gupta JK, Singh K, Saha S, Mukherjee S. Impact of the rutin on diabetic complications. Curr Funct Foods. 2024;2(1):e030823219378.
37. Accili D, Deng Z, Liu Q. Insulin resistance in type 2 diabetes mellitus. Nat Rev Endocrinol. 2025;21(7):413-426.
38. Li M, Chi X, Wang Y, Setrerrahmane S, Xie W, Xu H. Trends in insulin resistance: insights into mechanisms and therapeutic strategy. Signal Transduct Target Ther. 2022;7(1):216.
39. Li X, Hu S, Cai Y, Liu X, Luo J, Wu T. Revving the engine: PKB/AKT as a key regulator of cellular glucose metabolism. Front Physiol. 2024;14:1320964.
40. Nogueira-Ferreira R, Oliveira PF, Ferreira R. Liver metabolism: the pathways underlying glucose utilization and production. In: Ferreira R, Oliveira PF, Nogueira-Ferreira R, editors. Glycolysis. Elsevier; 2024. p. 141-156.
41. Arcaro A, Wymann MP. Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem J. 1993;296(2):297-301.
42. Manaharan T, Palanisamy UD, Ming CH. Tropical plant extracts as potential antihyperglycemic agents. Molecules. 2012;17(5):5915-5923.
43. Bailey CJ. Metformin: therapeutic profile in the treatment of type 2 diabetes. Diabetes Obes Metab. 2024;26 Suppl 3:3-19.
44. Scarpello JH. Review: optimal dosing strategies for maximising the clinical response to metformin in type 2 diabetes. Br J Diabetes Vasc Dis. 2001;1(1):28-36.
45. Yao L, Wang L, Zhang R, Soukas AA, Wu L. The direct targets of metformin in diabetes and beyond. Trends Endocrinol Metab. 2025;36(4):364-372.
46. Schuier M, Sies H, Illek B, Fischer H. Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. J Nutr. 2005;135(10):2320-2325.
47. Li PG, Xu JW, Ikeda K, Kobayakawa A, Kayano Y, Mitani T, et al. Caffeic acid inhibits vascular smooth muscle cell proliferation induced by angiotensin II in stroke-prone spontaneously hypertensive rats. Hypertens Res. 2005;28(4):369-377.
48. Yasuko K, Tomohiro N, Sei-Itsu M, Ai-Na L, Yasuo F, Takashi T. Caffeic acid is a selective inhibitor for leukotriene biosynthesis. Biochim Biophys Acta. 1984;792(1):92-97.

49. Barragán-Iglesias P, Oidor-Chan VH, Loeza-Alcocer E, Pineda-Farias JB, Velazquez-Lagunas I, Salinas-Abarca AB, et al. Evaluation of the neonatal streptozotocin model of diabetes in rats: evidence for a model of neuropathic pain. Pharmacol Rep. 2018;70(2):294-303.
50. Baig MA, Panchal SS. Streptozotocin-induced diabetes mellitus in neonatal rats: an insight into its applications to induce diabetic complications. Curr Diabetes Rev. 2019;16(1):26-39.
51. Takada J, Machado M, Peres SB, Brito LC, Borges-Silva CN, Costa CEM, et al. Neonatal streptozotocin-induced diabetes mellitus: a model of insulin resistance associated with loss of adipose mass. Metabolism. 2007;56(7):977-984.
52. Noh YH, Lee D, Lee YW, Pyo YH. In vitro inhibitory effects of organic acids identified in commercial vinegars on α-amylase and α-glucosidase. Prev Nutr Food Sci. 2020;25(3):319-324.
53. Spencer PS, Palmer VS. Food plant chemicals linked with neurological and neurodegenerative disease. In: Environmental factors in neurodegenerative diseases. Vol. 1. 1st ed., Elsevier Inc.; 2017. p. 247-278.
54. Panda B, Momin A, Devabattula G, Doneti R, Khandwaye A, Godugu C. A review on mechanistic aspects of litchi fruit induced acute encephalopathy. Toxicon. 2024;248:108052.
55. Uti DE, Atangwho IJ, Alum EU, Egba SI, Ugwu OP-C, Ikechukwu GC. Natural antidiabetic agents: current evidence and development pathways from medicinal plants to clinical use. Nat Prod Commun. 2025;20(3):1934578X251323393.
56. Rocha S, Santos I, Luísa CM, Fernandes E, Freitas M. The potential effect of polyphenols in emerging pharmacological liver targets for glucose regulation and insulin resistance: a review. Food Funct. 2025;16(13):5231-5277.
57. Shin EJ, Hur HJ, Sung MJ, Park JH, Yang HJ, Kim MS, et al. Ethanol extract of the Prunus mume fruits stimulates glucose uptake by regulating PPAR-γ in C2C12 myotubes and ameliorates glucose intolerance and fat accumulation in mice fed a high-fat diet. Food Chem. 2013;141(4):4115-4121.
58. Taheri R, Mokhtari Y, Yousefi AM, Bashash D. The PI3K/Akt signaling axis and type 2 diabetes mellitus (T2DM): from mechanistic insights into possible therapeutic targets. Cell Biol Int. 2024;48(8):1049-1068.