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Original Article

Vaccinium corymbosum: Phenolic Compound Content and Effect of Fruit Extract on Blood Glucose in Healthy Mice

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ABSTRACT

Introduction: In the context of the increasing prevalence of metabolic diseases such as diabetes, the search for natural compounds with potential impact on glycemic regulation has become a crucial area of research. Among the numerous options available, *Vaccinium corymbosum* extract, commonly known as "blueberry", has emerged as a promising candidate due to its rich composition of phytochemicals with antioxidant, anti-inflammatory and hypoglycemic properties. The aim of this study was to determine the total phenolic content (TPC) and the activity of *Vaccinium corymbosum* ("blueberry") fruit extract on glycemia in healthy mice. **Methods**: The Folin-Ciocalteau method was applied in order to quantify the phenolic compounds and the BE was administered to 25 mice distributed in six groups: control, negative control, experimental-D1- D2-D3, which were administered the BE in doses of 40, 80 and 120 mg/kg b.w. respectively; and insulin group; which were subjected to the glucose tolerance test (GTT) taking blood samples after 30, 60, 120 and 180 minutes. **Results**: The total phenolic content (TPC) amount found in the berries was 3.79±0.06 GAE/dry weight (mg/g) and 18.96±0.28 GAE/solution (mg/L). Statistically significant differences were observed between the three doses of BE and the negative control during GTT as well as induced a significant reduction in area under the curve (AUC) compared to the negative control. **Conclusions**: the three doses of the BE decreased glucose levels being the dose of 40 mg/kg b.w. the one that produced a statistically significant decrease with respect to the doses of 80 and 120 mg/kg b.w. during GTT.

Keywords: Animal studies, Phenols, Blueberry, Hypoglycemic Effect, Insulin, Type 2 Diabetes.

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INTRODUCTION

Excessive and frequent fluctuations in postprandial glucose and insulin levels represent a risk factor for developing type 2 diabetes mellitus (DM2), associated with impaired glucose intolerance (IGT) and insulin intolerance (ITI), inflammation, dyslipidemia, *β-cell* dysfunction and endothelial dysfunction.[1] The maintenance of healthy blood sugar levels and controlled carbohydrate metabolism is a rapidly growing concern in most developed countries and increasingly also in developing countries, due to increased awareness of the risks of hyperglycemia resulting from unhealthy diets and sedentary lifestyles.[2] In addition to dietary self-limitation and physical activity efforts, consumption of plant secondary metabolites can contribute substantially to improved carbohydrate and lipid metabolism.[3–6]

Long-term epidemiological studies have pointed to dietary factors that affect the risk of developing diabetes and have shown interesting findings related to the regular high intake of different classes of flavonoids and the reduction of disease risk.[7] Higher consumption of anthocyanins was associated with a lower risk of DM2 in adult men and women, decreased risk of myocardial infarction[8] and an increased likelihood of good health and well-being in people who survive to older ages. *Vaccinium* berries are enriched in anthocyanins, a group of polyphenols recognized for their ability to provide and activate cellular antioxidant protection and inhibit the expression of proinflammatory genes, which would explain the efficacy of *Vaccinium* in ameliorating DM2.[9,10]

Blueberries represents a rich source of anthocyanins, compounds that in recent years have gained attention in research due to their numerous functions and applications.[11,12] Blueberries has the highest total anthocyanin content (~300-700 mg/100 g of fresh fruit) with respect to other berries such as elderberry, raspberry, strawberry, among others[13]. Fresh blueberries also provide ascorbic acid (3 mg/100 g), quercetin (3 mg/100g) and catechins (20 mg/100 g).[14,15]

There are a number of in vitro and in vivo studies suggesting that polyphenols such as anthocyanins influence carbohydrate digestion and absorption, resulting in improved postprandial blood glucose. Dietary polyphenols[16,17], catechins and theaflavins[18] showed potential inhibitory activity against α-amylase and α-glucosidase. Likewise, quercetin glycosides[19] as well as dietary polyphenols[20,21], and flavonoids[22] have been shown to inhibit glucose transport in vitro. In humans, several studies have examined the effect of polyphenols on postprandial glycemic response[23]. The overall evidence suggests that consumption of edible berries, particularly of

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the genus *Vaccinium*, which have high concentrations of anthocyanins could provide a complementary intervention to improve glycemia in subjects with DM2 or impaired glucose tolerance.[24–26]

Blueberries, due to their enriched content in the aforementioned phytochemicals, have aroused great interest as a viable dietary intervention for the prevention and treatment of diseases of metabolic origin as their known pharmacological effects include vascular regulation and antioxidant and anti-inflammatory actions, which are important in the treatment of DM2, diabetic retinopathy, as well as (pre)diabetes.[27–31] In this context, blueberry, with its bioactive profile, represents a promising approach to improve glycemic homeostasis and insulin sensitivity.[32–36] The aim of this study was to determine the total phenolic content (TPC) and the activity of *Vaccinium corymbosum* ("blueberry") fruit extract on glycemia in healthy mice. The results obtained will not only contribute to the understanding of the biological effects of this particular extract, but could also provide valuable information for the development of dietary and therapeutic strategies aimed at improving metabolic health.

MATERIALS AND METHODS

Plant materials

Blueberry fruits were obtained from the wholesale market "Las Capullanas", Piura. Fresh fruits were selected whole, ripe and in good condition and those that showed contamination and infection, dry or unripe were excluded. The plant species was identified by biologist José Campos de la Cruz (CBP N° 3796), registered in the registry of professionals who perform taxonomic identification certifications of specimens and flora products (RD N°0311-2013- MINAGRI-DGFFS-DGEFFS).

Determination of Total Phenolic content (TPC)

TPC were determined by the Folin Ciocalteau method, for this from a sample of 0.5 grams of dry extract dissolved in 10 mL volumetric flasks 200 µL of the plant extract was taken, 200 µL of Folin ciocalteu reagent at 10% v/v was added and mixed gently. Then 400 µL of 4% Na2CO3 was added, made up with distilled water. It was waited 90 minutes, and the absorbance was measured in a UV-Vis Genesys 150 ThermoFisher-Scientific® UV-Vis spectrophotometer, wave length: 765 nm, measurements were performed in triplicate.[37,38]

The calibration curve was performed with gallic acid as standard at concentrations of 10-50 µg/mL. The concentration of total phenols was expressed as gallic acid equivalent/g dry drug (mg GAE/g DS).[38]

Preparation of blueberry fruit extract (BE)

The fruits were taken to the Pharmacology laboratory of the Universidad Privada Antenor Orrego, Piura, where those in the best condition and with excellent organoleptic characteristics were selected and then washed with potable water by immersion and water jet to eliminate foreign substances or particles adhering to their surface. The whole fruits were manually crushed in a mortar until a homogeneous paste was obtained, which was placed in an amber-colored container and 500 milliliters of 70° alcohol was added for each 200 grams of sample, mixed by mechanical agitation for 10 minutes and left to macerate for 7 days in darkness at room temperature, with daily mechanical agitation for five minutes. After this time, the macerate was filtered with Whatman No. 1 filter paper in a Buchner funnel and subsequently the filtrate was subjected to a water bath at 40°C to eliminate the alcohol until a dry extract was obtained, which was stored in an amber-colored flask refrigerated at 10°C until its later administration. The dry extract was reconstituted with distilled water so that it could be administered to the experimental animals at doses of 40 mg/kg b.w., 80 mg/kg b.w., and 120 mg/kg b.w.[39–48]

Experimental animals

Twenty-five albino mice (*Mus musculus var. albinus*), between females and males, 3 to 5 months of age, weighing 25–35 g, were obtained from the National Institute of Health (Chorrillos, Lima, Perú). Mice were maintained in room under temperature- and humidity-controlled conditions at 22°C ± 2°C, a relative humidity between 45-65%, with a 12 h light/dark cycle was maintained, with lights on from the time of 07:00 to 19:00, with free access to water and food. All animals have been acclimatized into the Laboratory of Pharmacology, seven days prior experiments.[49–51]

Glucose tolerance test (GTT)

To evaluate the effect of BE on blood glucose level, the PTG was used, which is the most widely used method to evaluate glucose homeostasis in rodents and allows determining the effect of extracts, fractions or compounds that are administered to experimental animals subjected to a glucose overload.[52] Briefly, mice are routinely fasted prior to administration of a glucose load (2 g/kg), most commonly by oral gavage or by a single intraperitoneal (IP) injection. Basal (fasting) blood glucose measurements are taken prior to glucose administration and, thereafter, additional measurements are taken at regular intervals (usually 30, 60, 90, and 120 minutes).[53,54] The results of this test can be expressed as a time course of absolute blood glucose measurements and as the area under the curve (AUC).[52,55,56] This test is similar to the human oral glucose tolerance test (OGTT), which is widely used in medical practice for the diagnosis of glucose intolerance, insulin resistance and DM2.[57–59]

Fasting and administration of extracts

This study used mice subjected to an overnight fast of 16 hours and during this period no substance was administered, and no procedures were performed. Overnight fasting is most commonly used in published studies for GTT in both mice and rats, and has the advantage of producing low and stable basal blood glucose and insulin levels.[52,59,60]

Determination of blood glucose level

The tail of the rat was cleaned with a cotton swab with alcohol (70°), then the tail of the rat was rubbed from the proximal to the distal part. A puncture was made in the caudal vein of the rat tail, discarding the first drop and receiving the next one on the reagent strip, previously inserted in the digital glucometer (Accu-Chek softclix Roche Diagnostics), the values obtained were expressed in mg/dL.[52–55]

Distribution of animals and treatments

On the eighth day, the mice were weighed, with their respective labeling and were assigned the pertinent observations on a data collection form. Then, the specimens were divided into 5 groups of 5 specimens each: Control, Negative Control, Experimental-D1, Experimental-D2, Experimental-D3 and Standard Group (insulin group), whose basal glycemia was taken (T=0). The administration of all treatments, including the oral glucose overload, was performed orally through a nasogastric tube, with the volume equivalent to the pre- established doses, except for insulin, which was administered subcutaneously. After 40 minutes of the basal glycemia measurement, the corresponding treatments were administered to each group, and finally the GTT was performed, which consisted of the administration of an oral glucose overload (2g/kg b.w.), and the measurement of glycemia at 30, 60, 120 and 180 minutes. The distribution of the groups was as follows:

a) NEGATIVE CONTROL: GTT was performed, taking blood samples at 30, 60, 120 and 180 minutes.

b) EXPERIMENTAL-D1: BE was administered at a dose of 40 mg/kg b.w., and GTT was performed by taking blood samples after 30, 60, 120 and 180 minutes.

c) EXPERIMENTAL-D2: BE was administered at a dose of 80 mg/kg b.w., and GTT was performed by taking blood samples after 30, 60, 120 and 180 minutes.

d) EXPERIMENTAL-D3: BE was administered at a dose of 120 mg/kg b.w., and GTT was performed by taking blood samples after 30, 60, 120 and 180 minutes.

e) STANDARD GROUP (Insulin group): Insulin was administered at a dose of 4 IU/kg b.w. and GTT was performed by taking blood samples after 30, 60, 120 and 180 minutes.

Statistical analysis

Descriptive statistics were used to express both the data of glucose levels and the area under the curve (AUC), as well as the mean value ± standard deviation (SD), then the SPSS Program v.25.0 was used to previously determine if the data present a normal distribution, using the Shapiro Wilk test, the Tukey Post HOC test for the comparison before and after treatment in each group, and the ANOVA test for the comparison of the variation of the parameters between the groups with a significance level of 0.05. The AUC of GTT (AUC_{GTT}) was calculated using the trapezoidal method.[61–63]

Ethical Aspects

The principles of reduction in the number of species to be used, through small groups and guaranteeing the least possible suffering through the principle of refinement were considered, according to the Guide for the Handling and Care of Laboratory Animals by the National Institute of Health[49] and the principles of ethics in animal research[64–66]. For the euthanasia procedure, an overdose of sodium pentobarbital (100 mg/kg b.w.) was administered intraperitoneally (IP), so as not to cause suffering to the experimental animal.

RESULTS

The results are shown in Tables 1-5 and Figures 1-2.

DISCUSSION

Pre-diabetes, characterized by *impaired glucose tolerance* (IGT) and/or *impaired fasting glucose* (IFG)[67,68], represents a high-risk state for the development of DM2. Annually, about 5 to 10% of prediabetic patients progress to DM2, and estimates indicate that up to 70% of patients with prediabetes will eventually develop overt diabetes during their lifetime.[69,70] Lifestyle changes focused on improving diet quality can slow or even halt the progression of (pre)diabetes[71–73], as an inverse association between plant-based diets and the progression of DM2 has been demonstrated. [74–78] Blueberries, one of the most popular berries due to their taste and nutritional/phytochemical composition, exhibit a variety of health-related properties in various metabolic diseases, including DM2, and thus the present study evaluates the total phenolic content (TPC) of blueberry extract (BE) and the effect on blood glucose

Table 1: Total phenolic content of *Vaccinium corymbosum* fruits.

Sample	Concentration in GAE/solution (mg/L)	Concentration GAE/dry weight (mg/g)
V. corymbosum fruit	18.96±0.28	3.79±0.06

Note: GAE= Galic acid equivalent

Table 2: Evaluation of the effect of *Vaccinium corymbosum* extract (BE) on blood glucose levels (mg/dL) in *Mus musculus* var. *albinus*.

Group/Time (minutes)	0	30	60	120	180
Negative control	93.80 ± 11.62	108.80 ± 16.03	124.20 ±10.54	135.00±10.12	120.80 ± 3.11
Experimental-D1 (BE: 40 mg/Kg)	98.20 ± 6.76	114.00 ± 10.14	99.00 ± 7.10	68.80 ± 4.81	63.00 ± 8.24
Experimental-D2 (BE: 80 mg/Kg)	98.60 ± 12.44	137.20 ± 10.70	111.40 ± 7.09	84.60 ± 8.79	74.20 ± 11.36
Experimental-D3 (BE: 120 mg/Kg)	104.20 ± 13.71	137.80 ± 25.24	108.20 ±15.75	94.20 ± 5.84	68.80 ± 11.43
Standard Group (Insulin)	82.40 ± 19.41	44.80 ± 17.38	26.40 ± 2.70	23.60 ± 2.96	37.00 ± 11.95
Significance [a]	0.007	0.000	0.000	0.000	0.000

BE=blueberry extract (*Vaccinium corymbosum*); a = ANOVA test for p<0.050 (Statistically significant).

Table 3: Comparison of the effect of *Vaccinium corymbosum* extract (BE) on blood glucose levels (mg/dL) by experimental group in *Mus musculus var. albinus*.

Group/Time (minutes)	Blood glucose levels (mg/dL)				
	0	30	60	120	180
Negative VS Exp D1 (BE: 40mg/Kg b.w.)	93.80 ± 11.62 98.20 ± 6.76	108.80 ± 16.03 114.00 ± 10.14	124.20 ± 10.54 99.00 ± 7.10	135.00 ± 10.12 68.80 ± 4.81	120.80 ± 3.11 63.00 ± 8.24
Negative VS Exp D2 (BE: 80 mg/Kg b.w.)	93.8 ± 11.62 98.60 ± 12.44	108.80 ± 16.03 137.20 ± 10.70	124.20 ± 10.54 111.40 ± 7.09	135.00 ± 10.12 84.60 ± 8.79	120.80 ± 3.11 74.20 ± 11.36
Significance	0.990[c]	0.080[c]	0.260[c]	0.000[b]	0.000[b]
Negative VS Exp D3 (BE:120 mg/Kg b.w.)	93.80 ± 11.62 104.20 ± 13.71	108.80 ± 16.03 137.80 ± 25.24	124.20 ± 10.54 108.20 ± 15.75	135.00 ± 10.12 94.20 ± 5.84	120.80 ± 3.11 68.80 ± 11.43
Significance	0.786[c]	0.070[c]	0.094[c]	0.000[b]	0.000[b]
Negative VS Standard (Insulin)	93.80 ± 11.62 82.40 ± 19.41	108.80 ± 16.03 44.80 ± 17.38	124.20 ± 10.54 26.40 ± 2.70	135.00 ± 10.12 23.60 ± 2.96	120.80 ± 3.11 37.00 ± 11.95
Significance	0.717[c]	0.000[b]	0.000[b]	0.000[b]	0.000[b]

BE=blueberry extract (*Vaccinium* corymbosum); TUKEY test obtained in IBM SPSS v 25.00; b=Statistically significant (p-value < 0.050); c=Statistically not significant (p-value > 0.050).

Table 4: Comparison of *Vaccinium corymbosum* extract (BE) of the effect on blood glucose levels (mg/dL) doses in *Mus musculus var. albinus*.

Groups/Time (minutes)	Blood glucose levels (mg/dL)				
	0	30	60	120	180
Exp D1 (BE: 40 mg/Kg b.w.) VS Exp D2 (BE: 80 mg/Kg b.w.)	98.20 ± 6.76	114.00 ± 10.15	99.00 ± 7.11	68.80 ± 4.82	63.00 ± 8.25
	98.60 ± 12.44	137.20 ± 10.71	111.40 ± 7.09	84.60 ± 8.79	74.20 ± 11.37
Significance	1.000[c]	0.215[c]	0.291[c]	0.010[b]	0.403[c]
Exp D1 (BE:40 mg/Kg b.w.) VS Exp D3 (BE:120 mg/Kg b.w.)	98.20 ± 6.76	114.00 ± 10.15	99.00 ± 7.11	68.80 ± 4.82	63.00 ± 8.25
	104.20 ± 13.72	137.80 ± 25.24	108.20 ± 15.75	94.20 ± 5.85	68.80 ± 11.43
Significance	0.974[c]	0.194[c]	0.604[c]	0.000[b]	0.912[c]
Exp D2 (BE: 80 mg/Kg b.w.) VS Exp D3 (BE: 120 mg/Kg b.w.)	98.60 ± 12.44	137.20 ± 10.71	111.40 ± 7.09	84.60 ± 8.79	74.20 ± 11.37
	104.20 ± 13.72	137.80 ± 25.24	108.20 ± 15.75	94.20 ± 5.85	68.80 ± 11.43
Significance	0.981[c]	1.000[c]	0.993[c]	0.225[c]	0.933[c]

BE=blueberry (*Vaccinium corymbosum*) extract; TUKEY test obtained in IBM SPSS v 25.00; b=Statistically significant (p-value< 0.050); c=Statistically not significant (p-value > 0.050).

Table 5: Area under the curve (AUC) of plasma glucose levels (mg/dL) of the different treatments during the glucose tolerance test in *Mus musculus var. albinus*.

Group	AUC/min	
	Mean ± SD	Significance
Negative	122.13 ± 9.50	
Exp D1 (BE: 40 mg/Kg b.w.)	85.37 ± 5.17	
Exp D2 (BE: 80 mg/Kg b.w.)	99.50 ± 5.33	0.000[c]
Exp D3 (BE: 120 mg/Kg b.w.)	101.57 ± 20.97	
Standard (Insulin)	34.97 ± 5.47	

BE=blueberry (*Vaccinium corymbosum*) extract; c=ANOVA test for p<0.050 (statistically significant).

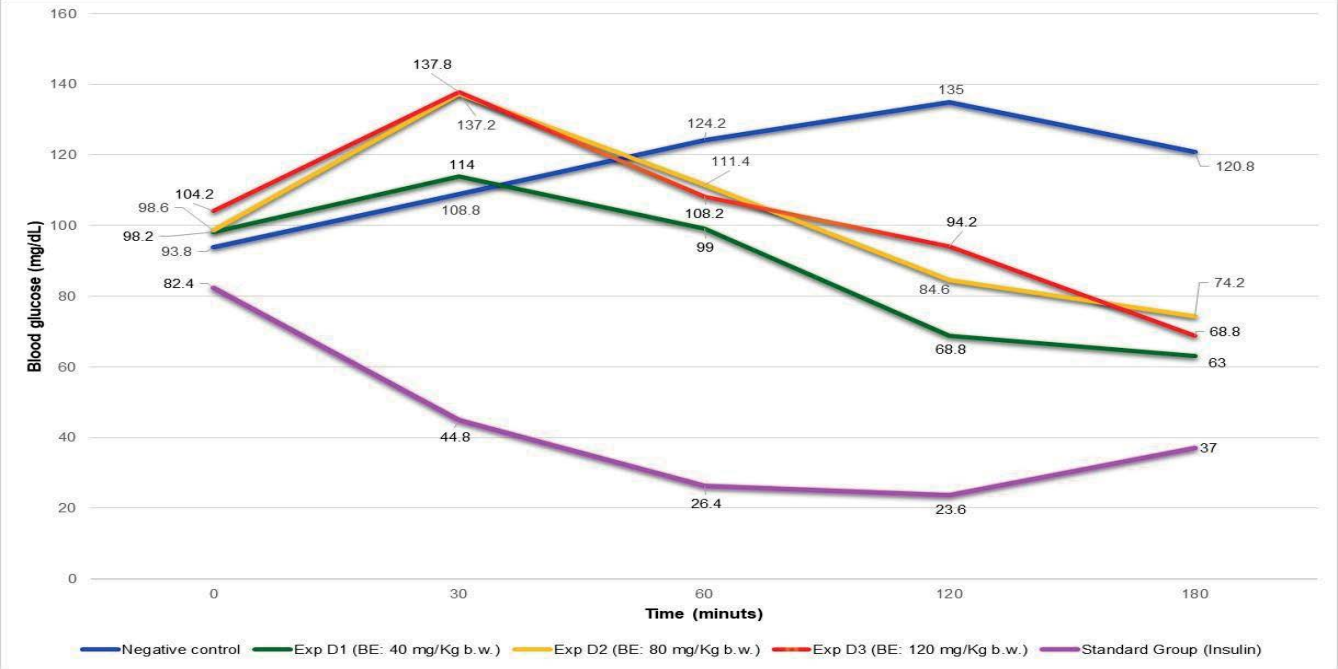

Figure 1: Blood glucose levels in the glucose tolerance test. BE: blueberry extract (*Vaccinium corymbosum*).

levels, with the aim of determining its ability to maintain glucose homeostasis within the normal physiological range.

GTT is the most widely used test in the literature to assess glucose homeostasis in rodents and can be influenced by several variables that should be considered when designing the study, including the duration of the fasting period, the dose of glucose and its route of administration. In addition, when comparing between studies, factors such as the age, strain, and sex of the animals used should be considered, as different strains of mice commonly used are known to have variations in glucose metabolism, further complicating direct comparisons between studies[54,56], and there is also an increase in insulin resistance with aging[79,80], furthermore, there are differences in glucose metabolism between male and female animals[81,82]. Therefore, it is essential that all studies of glucose homeostasis be conducted in animals of the same age, sex, and breed, and from the same provider when possible, and

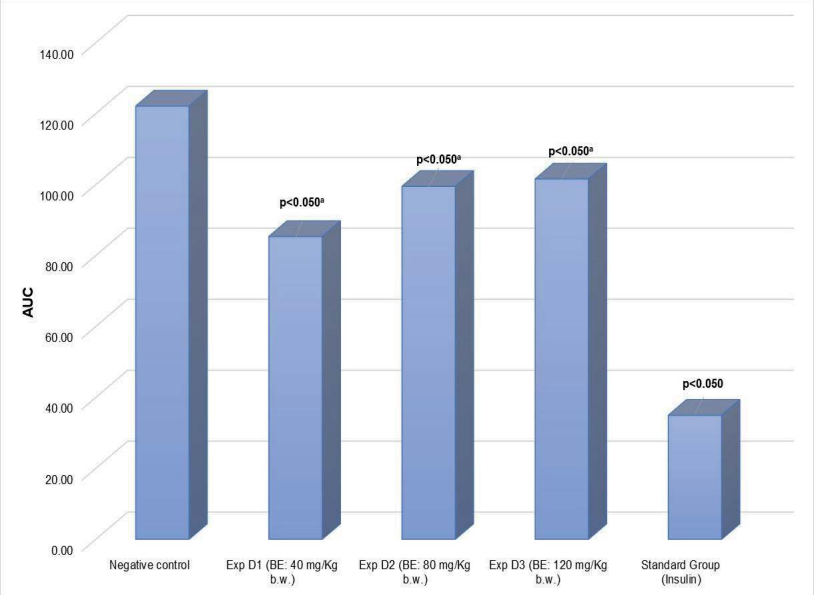

Figure 2: Area under the curve (AUC) of plasma glucose levels (mg/dL) of the different treatments during the glucose tolerance test in *Mus musculus var. albinus.* BE: blueberry extract (*Vaccinium corymbosum*). TUKEY test obtained in IBM SPSS v 25.00; a=Statistically significant (p-value<0.050). Negative Control vs Exp-D1, Exp-D2, Exp-D3, Standard Group (Insulin).

that these details be reported.[83,84] In this study the mice were of the same age, breed, and from the same supplier; however, they were not of the same sex, but the possible influence of this factor was managed by using probability sampling to select the members of each working group.

Overnight fasting prior to the initiation of GTT was used as it is most used in published studies for GTT in both mice and rats; and has the advantage of producing low and stable basal blood glucose and insulin levels.[57,59] However, several recent studies have expressed concern that overnight fasting in mice is not ideal because, as nocturnal animals, they consume most of their daily calories at night. This, combined with their relatively high metabolic rate, means that an overnight fast is a relatively long time for mice to be deprived of food, and may induce a state more similar to starvation than an overnight fast in humans.[54] Furthermore, prolonged fasting inhibits insulin-stimulated glucose uptake in humans, but the opposite occurs in mice, were overnight fasting increases insulin sensitivity.[85]

Table 2 shows that there is a significant difference between the treatment groups, so it can be affirmed that the EA influences blood glucose levels. Blueberry is a low-calorie food, enriched with dietary fibers and a variety of polyphenolic secondary metabolites (flavonols, anthocyanins, phenolic acids) that are responsible for its antioxidant and anti-inflammatory properties.[86–89] Although blueberries contain many bioactive compounds, anthocyanins are the essential compounds with antidiabetic properties. Several animal and human studies revealed a relationship with the antidiabetic properties of anthocyanins[90–93], these compounds have the ability to inhibit different enzymes involved in carbohydrate digestion, stimulate insulin secretion when applied to rodent pancreatic β-cell cultures, with cyanidins and delphinidins (the main anthocyanins in blueberry) showing the greatest effect among the different anthocyanins tested[94] as well as influencing gene expression and metabolic pathways, and may modulate diabetes and other associated disorders, such as hyperlipidemia, overweight, obesity, and cardiovascular disease.[95–99]

In addition, oleanolic acid, a triterpenoid found in the genus Vaccinium, has been reported to increase the release of acetylcholine from nerve terminals. Acetylcholine stimulates M3 muscarinic receptors on pancreatic cells and increases insulin release. Therefore, oleanolic acid is one of the active constituents likely responsible for the observed increases in serum insulin concentrations after supplementation with blueberry.[100,101]

In Table 3, where the effect of BE on blood glucose levels (mg/dL) during GTT is compared between the negative control (no treatment) and the different treatment groups (Exp-D1, Exp-D2, Exp-D3 and insulin), both the three groups that were administered BE and the insulin group experienced a decrease in blood glucose levels greater than that of the negative control, Thus, after 60 minutes, a dose of 40 mg/kg b.w. of BE significantly lowers glucose levels, and from 120 minutes to 180 minutes it was observed that the three doses of BE significantly decreased blood glucose levels with respect to the negative control. As for the comparison between the doses of the extract (Table 4), the dose of 40 mg/kg b.w. manages to decrease glucose more than the doses of 80 and 120 in a statistically significant way at 120 minutes. There is no significant difference between the 80 and 120 mg/kg p.c. doses at any time. As for the insulin group, after 30 minutes and up to 180 minutes, a greater and significant decrease in blood glucose levels was observed with respect to both the negative control and all the groups treated with BE.

Insulin, which is usually the main treatment for DM1 and for many people with DM2, was used as a Standard Group. Although its use in DM2 is unusual for newly diagnosed patients, there are several cases in which the use of insulin is considered, such as severe hyperglycemia, gestational diabetes, presence of significant weight loss, and ketonuria.[102,103] The goal of adding insulin to the treatment regimen is to mimic the physiologic insulin profile, covering both nocturnal and postprandial glucose levels. The American Diabetes Association (ADA) recommends that long-acting insulin be incorporated to cover the basal insulin requirement after failure of non-insulin agents.[103] These agents include oral hypoglycemic agents, among which one could consider those that share targets of action with the study extract, such as second-generation sulfonylureas, glibenclamide, and glimepiride, as well as α-glucosidase inhibitors that slow starch absorption such as acarbose and miglitol.[102,104,105] In studies where hypoglycemic and antihyperglycemic activities of plant extracts are evaluated, glibenclamide is generally

used as Standard Group[106] but also acarbose, so these options should be considered later studies.[107]

About the doses used, the study of Kiliçalp *et al*[108], where the differences in hematological and biochemical parameters were investigated in rats administered *Vaccinium myrtillus* extract in doses of 20 mg/kg b.w. administered once a day for 8 weeks, and no significant difference was found between the control and experimental groups, so it was decided to use BE doses of 40 mg, 80 and 120 mg/kg b.w. to observe its effect on glycemia. The reduction in glycemia found in the present study is similar to that found by Feshani *et* al[109], who demonstrated that the ethanolic extract of *Vaccinium arctostaphylos* fruits had a significant antihyperglycemic effect in diabetic rats in the first half hour after treatment with a dose of 200 mg/kg b.w. and 400 mg/kg b.w. with respect to the negative control group (untreated diabetic rats) during OGTT, likewise, Herrera-Balandrano *et* al[110] demonstrated that blueberry (*Vaccinium ashei*) anthocyanin extract at two doses (100 mg/kg and 400 mg/kg per day) administered for 5 weeks significantly reduced blood glucose levels in diabetic mice at 30, 60 and 120 minutes after glucose loading during OGTT.

The reduction in glycemia was observed in studies where BE supplementation was performed as part of the diet and for a minimum period of 4 weeks, as demonstrated by Eid *et al*[111], who used *Vaccinium vitis-idaea* extract at doses of 125, 250, 500 mg/kg in mice fed a diet high in saturated fat for a treatment period of 8 weeks and observed an antihyperglycemic activity, this effect being more pronounced as the dose of the plant extract increased from 125 to 250 mg/kg and, however, decreased thereafter (dose of 500 mg/kg b.w.). Baheg et al [112] also demonstrated that *Vaccinium uliginosum* L. extract in doses of 100, 200 and 300 administered orally for 4 weeks decreased blood glucose levels from 48 hours after administration until the end of the experiment (28 days) in a statistically significant manner with respect to the group of diabetic rats, with the dose of 200 mg/kg b.w. producing the greatest reduction in glycemia ($p<0.05$). This "bell-shaped" dose-response relationship is not unusual for pharmacological agents, particularly from medicinal plants[113]. As shown in Figure 1, blood glucose concentrations increased 30 min after glucose solution administration in all groups and subsequently decreased at 60 min with the 40 mg/kg b.w. dose and at 120 min and 180 min in all doses, both 40, 80, and 120 mg/kg b.w. significantly compared to the negative control group ($p <0.05$), during GTT, indicating that BE has a time-dependent effect on postprandial glucose level, which could be mediated by regulation of glucose uptake from the intestinal lumen, through inhibition of carbohydrate digestion or absorption[114]; or by facilitating glucose utilization by peripheral tissues[115] and by the increased sensitivity and release of insulin.[116]

Table 5 shows the plasma glucose levels (mg/dL) AUC for the different treatments during GTT. The results indicate a significant difference between all treatment groups, including the three doses of extracts and insulin. Also, in Figure 2 shows that all the extracts tested induced a significant reduction in AUC compared to the negative control, being the dose of 40 mg/kg b.w. the one that produced the lowest glycemia levels in the 180 minutes of duration, compared to higher doses of the extract; however, the standard group (insulin) produced the lowest AUC over all the treatments analyzed. The dose of the BE that showed the greatest reduction in blood glucose levels (40 mg/kg b.w.) differed from the results of previous studies where generally the dose of 200 mg/kg b.w. had the best antihyperglycemic effect, as demonstrated by Yanglin *et al*[107] who used the extract in doses of 100 and 200 mg/kg of p.c., obtaining a reduction in postprandial hyperglycemia, which was greater and statistically significantly higher as the dose of the extract was increased, even surpassing that produced by the control (acarbose). Oliveira et al[117] demonstrated that *Vaccinium virgatum* extract at a dose of 200 mg/kg/day orally prevented the increase in serum glucose levels significantly in rats receiving a sucrose-enriched diet. This variation could be explained by variations in the blueberry species used, the part of the plant species used (leaves, fruit, peel or whole species), the extraction methods used, the use of extract or anthocyanin-enriched fraction, as well as whether they used a single dose for OGTT or whether they used daily supplementation for a minimum period of 4 weeks.

CONCLUSION

Vaccinium corymbosum extract (BE) decreased glucose levels being the dose of 40 mg/kg b.w. the one that produced a statistically significant decrease with respect to the doses of 80 mg/kg b.w. and 120 mg/kg b.w. during the GTT, however, the decrease was not greater than that obtained with insulin. Also, the effect of the 40 mg/kg b.w. dose is revealed to be time-varying, since, compared to other doses, a more notable decrease in glucose levels was observed at 60 minutes after administration of the extract. This finding indicates that the efficacy of the treatment is time-dependent, suggesting a maximal impact on the reduction of glucose levels at that specific period. These results not only support the effect of BE on glycemia, but also underscore the importance of dose and time in the manifestation of its effects. These findings may have significant implications in the development of therapeutic approaches related to glucose regulation, opening doors for future research and potential clinical applications, however, further studies on the mechanisms involved in glucose homeostasis are urgently needed.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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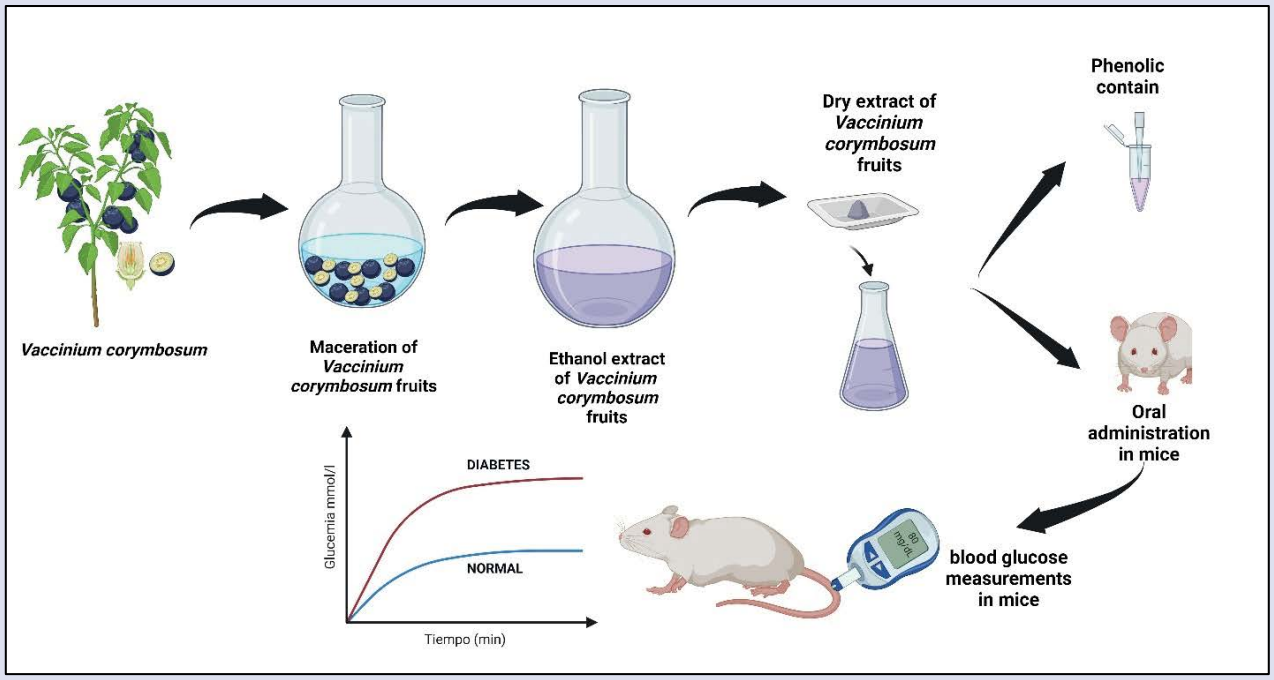
GRAPHICAL ABSTRACT



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