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Research Article

In vitro antioxidant and *in vivo* hypoglycemic activity of biophenols and polyunsaturated fatty acids from *Vitis vinifera* L. muscat and quebranta seeds from the Valley of Ica-Peru

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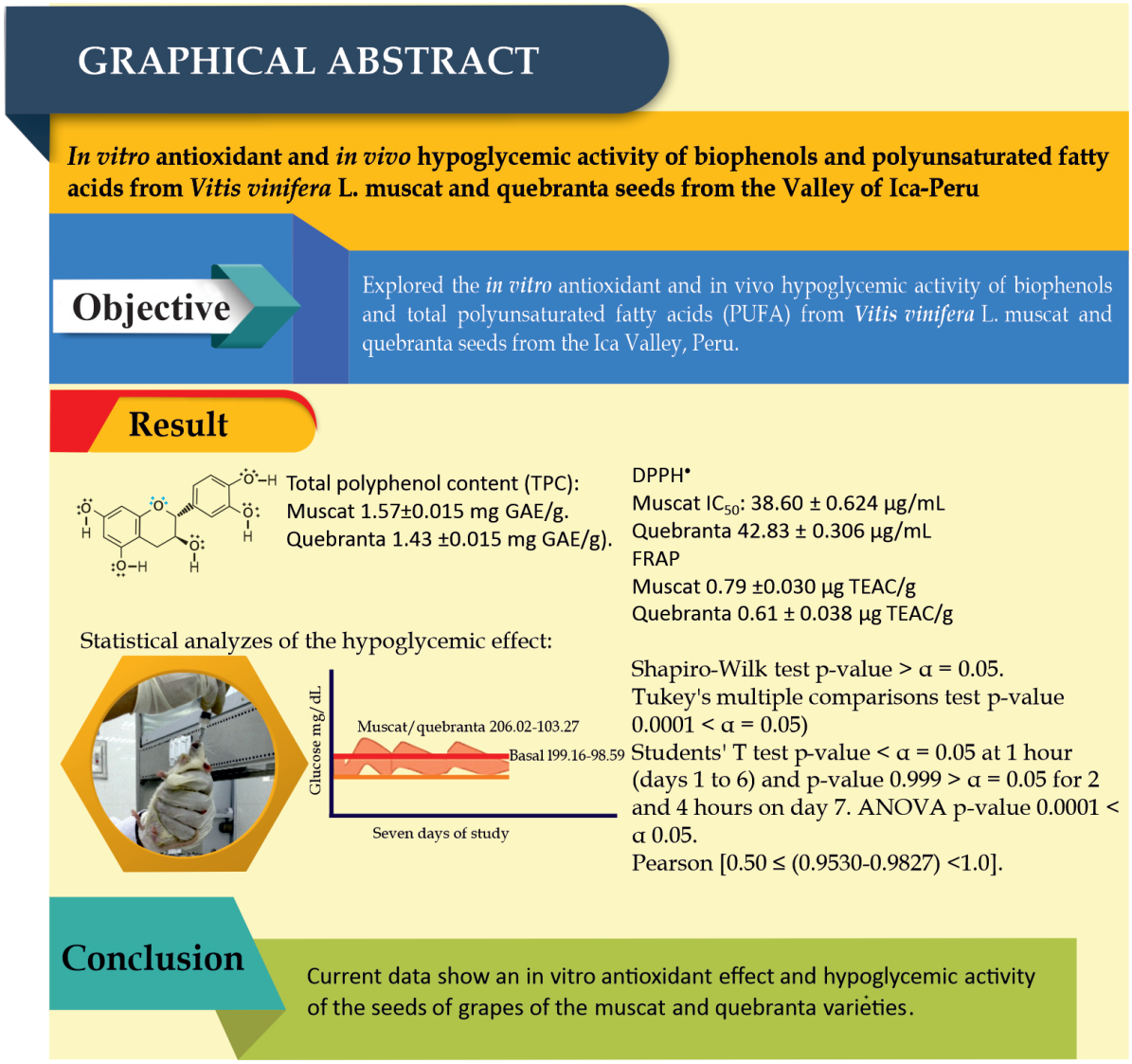
Abstract

Currently, there is a greater interest in using natural products in various chronic diseases such as type 2 diabetes. However, these investigations have not considered the components of grape seeds. In this context, the current study explored the *in vitro* antioxidant and *in vivo* hypoglycemic activity of biophenols and total polyunsaturated fatty acids (PUFA) from *Vitis vinifera* L. muscat and quebranta seeds from the Ica Valley, Peru. The total polyphenol content (TPC) of muscat (1.57±0.015 mg GAE/g) and quebranta (1.43±0.015 mg GAE/g) seeds was estimated by the Folin-Ciocalteu method. *In vitro* antioxidant activity was determined by DPPH$^{\bullet}$ free radical assay for muscat and quebranta (IC_{50}: 38.60±0.624 µg/mL and 42.83±0.306 µg/mL, respectively) and by FRAP 0.79±0.030 µg TEAC/g for muscat and 0.61±0.038 µg TEAC/g for quebranta. After inducing experimental hyperglycemia with alloxane in *Rattus norvegicus* strain Holtzman, treatment was carried out for 7 days and glucose levels were measured at 1, 2 and 4 hours. At a dose of 500 mg/kg, orally, of biophenols/PUFA from muscat and quebranta seeds, a hypoglycemic effect was observed; whose results were verified with the Shapiro-Wilk test (p-value > α = 0.05), Tukey's multiple comparisons test (p-value 0.0001 < α = 0.05), Student's T with p-value < α = 0.05 at 1 hour for days 1 to 6, and p-value 0.999 > α = 0.05 for 2 and 4 hours on day 7, indicates a small probability of difference; in ANOVA results the mean difference is significant (p-value 0.0001 < α 0.05). The Pearson analysis found a strong correlation [0.50 ≤ (0.9530–0.9827) < 1.0] between glibenclamide/biophenols-PUFA glucose levels. Current data show an *in vitro* antioxidant effect and hypoglycemic activity of the seeds of grapes of the muscat and quebranta varieties.

Graphical abstract



Keywords

Biophenols, polyunsaturated fatty acids, hypoglycemia, seeds of *Vitis vinifera* L., muscat grape, quebranta grape

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to defective insulin secretion and action; Over time, hyperglycemia leads to retinopathy, neuropathy, nephropathy, and heart disease (Castro-Juárez et al. 2017). According to the American Diabetes Association (ADA) diabetes can be classified into 4 general categories, which are type 1 diabetes (destruction of β cells), type 2 diabetes (insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance) specific types of diabetes (due to genetic functional defect of pancreatic beta cells, genetic dysfunction of insulin, genetic syndromes associated with diabetes, pancreatic diabetes, endocrinopathies, due to infections, due to drugs or chemical substances inducing beta cell disorders and immune problems) and gestational diabetes mellitus (American Diabetes Association 2023). These metabolic disorders are considered a global Public Health problem, which is increasing year by year, so that in 2019 there were more than 463 million people living with diabetes and with a projection of 578 million by 2030; in South and Central America, it was 32 million (2019) and 40 million is projected for 2030. In 2019, it was estimated that there would be 1,385,000 people (20–79 years) with type 2 diabetes in Peru, with a

higher prevalence in urban than rural areas, with a higher prevalence in women, and a very significant number of undiagnosed cases (Garmendia-Lorena et al. 2022).

Since the synthesis of the first insulin-stimulating dimethyl-biguanide drug in 1922 by Werner and Bell, biological and synthetic drugs have been sought to control blood glucose levels, but to date glycemic control is transitory, given the complex progression of diabetes, added to this, no drug is exempt from adverse reactions (Bailey et al. 1989); for this reason, natural products with bioactive compounds that have hypoglycemic effects are being sought in *in vitro* and *in vivo* models (Sok Yen et al. 2021).

In the Ica Valley, 8 varieties of *Vitis vinifera* L. (vine) are cultivated for the production of wine and Pisco, of which the quebranta variety (non-aromatic type, wedge-shaped leaf, short elliptical red berry) and muscat (aromatic type, pentagonal leaf, spherical and red berry) are widely consumed as table grapes (Surco-Laos et al. 2023). The seeds are obtained after pressing the berries (grapes) and are discarded without commercial value (Kapcsándi et al. 2021; Surco-Laos et al. 2023), but they are rich in biophenolic compounds, mono and polyunsaturated fatty acids (Garavaglia et al. 2016; Kaseke et al. 2020). In various studies it has been reported that the seeds of *Vitis vinifera* L. contain polyunsaturated fatty acids, anthocyanidin (cyanidin), flavan-3-ol catechins (catechin and epicatechin), flavonols (3-O-β-D-xylopyranoside, quercetin, rutin), and other biophenols, with antioxidant, anticancer, and antidiabetic properties (Alvarado et al. 2021a; Sok Yen et al. 2021). To evaluate the hypoglycemic activity of natural products, albino rats (*Rattus norvegicus*) of the Holtzman strain are used as an *in vivo* model; and to induce experimental diabetes, alloxane [2,4,5,6(1H,3H)-Pyridinetetrone] is used, whose mechanism of action is to activate superoxide dismutase 2 (SOD2) forming hydrogen peroxide (H_2O_2), increase in superoxides ($^{\cdot}O_2$), and activation of the Fenton reaction pathway, originating superhydroxyls ($^{\cdot}OH$) that are responsible for necrosis of pancreatic β cells (Dunn and Mcletchie 1943; Abdul-Hamid and Moustafa 2013).

The hypoglycemic molecular mechanism of action of the biophenolic compounds of grape pomace (grape seeds and grape skins) are diverse, among them, epigallocatechin gallate and epicatechin-2 gallate inhibit small intestinal Sodium-Glucose Transporter-1 (SGLT-1) (Ni et al. 2020; Hegedüs et al. 2022), quercetin inhibits glucose transporter-2 (GLUT-2) of the basolateral membrane of the enterocyte (Rodrigues et al. 2021; Hegedüs et al. 2022), tannins and anthocyanins inhibit the α-amylase enzymes responsible for the breakdown of glycogen (which increases blood glucose levels) and alpha-glucosidase in the intestinal wall responsible for glucose absorption (Hegedüs et al. 2022; Olvera-Sandoval et al. 2022). Fig. 1 graphically represents the mechanism of molecular action of biophenolic compounds from grape seeds, to visually understand this mechanism.

Fig. 1A shows the process of glucose absorption by means of Sodium-Glucose Transporter-1 (SGLT-1), which is found in the brush border and is then carried into the blood by glucose transporter 2 (GLUT2) of the basolateral membrane of the enterocyte. In Fig. 1B it is proposed that epicatechin inhibits SGLT-1 and quercetin inhibits GLUT2. Diagram made by the authors.

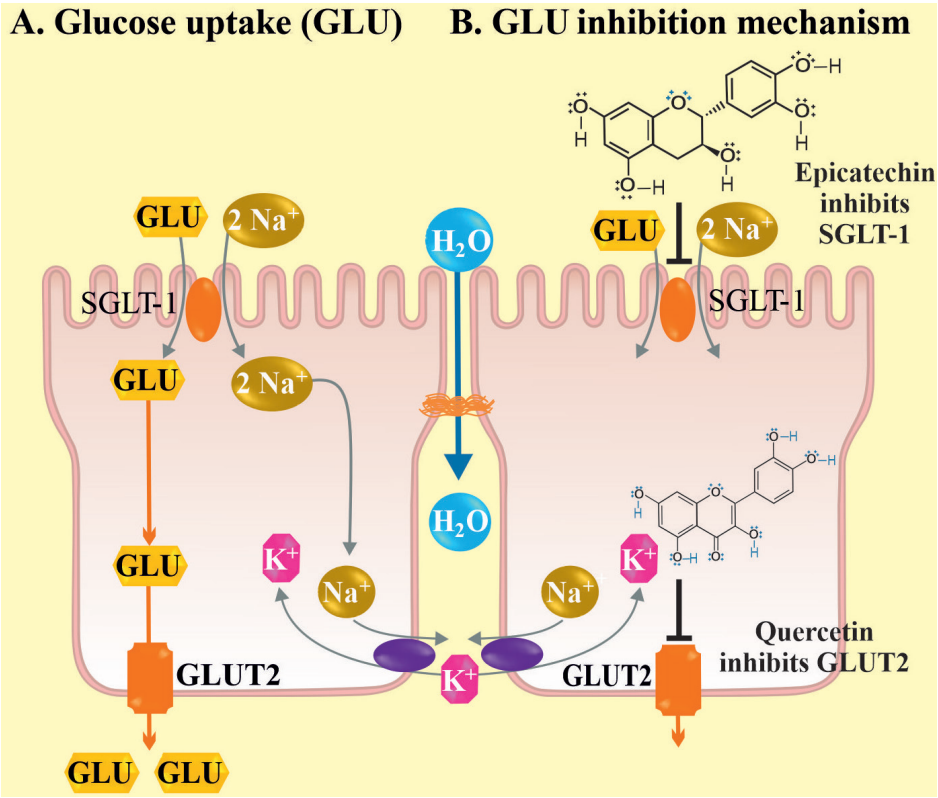


Figure 1. Mechanism of molecular action of the biophenolic compounds of the seeds of *Vitis vinifera* L.

After carrying out a review in the PubMed-NCBI database on the scientific literature on the effects of extracts and polyunsaturated fatty acids of *Vitis vinifera* L. in Peru, it has been shown that the studies of these seeds are still limited or scarce, so it is justified to investigate the *in vitro* antioxidant activity of the bioactive chemical components of the seeds and to carry out preclinical studies to demonstrate the potential hypoglycemic effect that will serve as scientific evidence to initiate clinical pharmacology studies. Therefore, the objective was to study the *in vitro* antioxidant and *in vivo* hypoglycemic activity of biophenols and polyunsaturated fatty acids from *Vitis vinifera* L. muscat and quebranta seeds from the Valley of Ica-Peru.

Materials and methods

Type and period of study

Preclinical study of experimental and explanatory pharmacology. The study was conducted between January to May 2023.

Reagents and standard

All chemicals and solvents used were reagent grade: distilled water, HPLC grade water, ethanol and methanol (Beaker Brand, USA); sodium acetate, acetic acid, hydrochloric acid, sodium carbonate, dimethylsulfoxide (DMSO), ferric trichloride and Folin ciocalteu reagent from Merck (Germany); gallic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPTZ [2,4,6-Tris(2- pyridyl)-s-triazine] brand Sigma-Aldrich (USA); 9% saline (RS No EN 02537, Medifarma), glibenclamide (5 mg Glidiabet, RS No. N-12347, Albis, Ferrer International SA), alloxane monohydrate (Sigma, Saint Louis, MO, USA), sodium pentobarbital (Halatal, Montana), test strips, and digital glucose meter (Accu Chek Active, Roche) (Ramos-Escudero et al. 2012; Chávez et al. 2021).

Population, sample, and sampling

The population consists of 85 artisanal Pisco-producing wineries that grow 8 varieties of *V. vinifera* L. (vine). The sample was a Pisco producing winery of the muscat and quebranta variety. The sampling was non-probabilistic and for convenience, when collecting the seeds of berries (grapes) of the muscat variety and quebranta *V. vinifera* L. from a winery that produces artisanal Pisco located on road of Kings in the San Juan Bautista district (14°00'41"S, 75°44'06"W at 426 masl), Ica province, Ica region, Peru (Surco-Laos et al. 2023).

Extraction process of fatty acids from grape seeds

The grape seeds were washed and gently cleaned with filter paper, then dried at 37 °C (Mermen Stove, Germany) for 8 days; then they were ground until obtaining fine particles (IVYMEN JP SELECTA manual mill, YCW-010E, Spain). The particles obtained were incorporated into a flask and distilled water at 90 °C was added (sample: solvent ratio 1:3), and it was placed in an ultrasonic bath (5.7 L MH series Model M3800H-E /Branson Ultrasonics, USA) for 30 min, then left to rest for 12 hours at a temperature of 20±5 °C. The obtained liquid extract was filtered and later, the solvent was evaporated in an oven at 37±2 °C to obtain a dry extract. The total dry extract (biophenols and polyunsaturated fatty acids) was stored in an amber bottle at 4° until analysis and experiment (Pérez-Navarro et al. 2019a; Kaseke et al. 2020; Surco-Laos et al. 2023).

Total polyphenol content using the Folin-Ciocalteu method

Before carrying out the *in vitro* studies, dilutions of the total extract of grape seeds were made in dimethylsulfoxide (DMSO) (Merck Brand); At the same time, a calibration curve of the gallic acid standard (Sigma-Aldrich) in 70% ($^{v}/_{v}$) alcohol in the concentration range of 1–5 mg/L was performed. 0.1 mL of total fatty acid sample was reacted with 0.25 mL of Folin-Ciocalteu reagent (Merck) in reagent: HPLC water ratio (1:2). This solution was homogenized for 5 min; subsequently, 1.25 mL of 20% sodium carbonate (Na_2CO_3) and 0.4 mL of water (HPLC grade) were added, and stirred to homogenize. At a temperature of 20±5 °C and under darkness, it was allowed to react for 90 min. The absorbances of the samples and the blank were read in triplicate at a wavelength of 760 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The total polyphenolic content was expressed in mg of gallic acid (GAE)/mL of total grape seed extract (Bouyahya et al. 2018; Fruehwirth et al. 2020; Surco-Laos et al. 2023).

Determination of antioxidant activity *in vitro*

DPPH radical scavenging assay

To 0.1 mL of the various dilutions of the total extract, 2.9 mL of DPPH solution was added, stirred, and allowed to react for 60 min protected from light. Subsequently, the reaction sample and the blank (methanol) were measured in triplicate at a wavelength of 517 nm (Spectrophotometer Peak Instrumental, model C-7100 USA); the percentage inhibition (% Inh) was determined by the following formula: %Inh. = [(blank abs - sample abs) / (blank abs)] x 100. From a curve of % inhibition vs µL of total extract, the IC_{50} was determined. Previously, the DPPH reagent solution was prepared, for which 3.1 mg of the DPPH reagent (Sigma) was weighed and incorporated into a vial, adding 80% methanol (Analytical Grade, Beaker) eqf 100 mL (Ramos-Escudero et al. 2012; García-Ceccarelli et al. 2022; Surco-Laos et al. 2022a, 2022b).

Ferric Reducing Antioxidant Power (FRAP)

To 0.10 mL of total extract samples, 1.5 mL of FRAP solution is added, it is homogenized for 30 seconds, then it is

left to react for 6 min at a temperature of 20±5 °C; after that time, said samples and the FRAP solution were measured in triplicate at a wavelength of 593 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). Trolox was used as reference compound at a concentration of 0.0312–1.0 mM. The final absorbance was obtained by subtracting the absorbance value from the initial FRAP solution. A quantification curve of mM of trolox/mL of total extract was made. The FRAP solution was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM diluted TPTZ (2,4,6-tripyridyl-s-triazine) solution and 40 mM HCl and ferric trichloride solution ($FeCl_3$. $6H_2O$) 20 mM (Ramos-Escudero et al. 2012; García-Ceccarelli et al. 2022).

Sample and population of experimental animals

The population was a vivarium dedicated to the reproduction of smaller experimental animals. The sample consisted of 40 male albino rats (*Rattus norvegicus*) of the Holtzman strain with an average weight of 200±100 g. Sampling was for convenience and intentional (Chávez et al. 2021; Alvarado et al. 2022).

Methodology for handling experimental animals

The 40 minor experimental animals (*Rattus norvegicus*) will be conditioned in five stainless steel and ventilated cages with dimensions of 57 × 48 × 48 cm. These animals were acclimatized for seven days in groups of eight albino rats per cage to standardized laboratory conditions (12-h light/dark cycle, temperature 22±2 °C). Balanced food for albino rats and drinking water were provided *ad libitum*. After seven days the experiment was started (Castañeda et al. 2008; Chávez et al. 2021; Alvarado et al. 2022).

Experiment design

After seven days of acclimatization, the albino rats were weighed and five groups were formed (G-1, G-2, G-3, G-4 and G-5) each with 8 experimental animals.

Physiological saline was administered to G1, and 5% monohydrated alloxane was administered to the other groups at a dose of 130 mg/kg, intraperitoneal (IP), after 48 hours, the 12-hour fasting glucose level was evaluated; those animals that experienced hyperglycemia (chemical experimental diabetes) with glucose values greater than 200 mg/dL were included in the study, in this case 30 albino rats. Based on said experimental hyperglycemia, four groups were formed (G-2, G3, G-4 and G-5) each with six experimental animals; and a negative or blank control group (G-1) made up of six animals. Blood samples were taken at 8:00 am after 1 hour of each administration of the total extract (biphenols and polyunsaturated fatty acids) from the seeds of muscat and quebranta grapes, saline solution and glibenclamide. The blood samples were obtained by cutting the tail, which compromised one of the marginal veins of each experimental animal, the first microdrop was discarded and the second microdrop was received directly on the reactive strip, whose glucose values were quantified directly with a digital glucometer (Accu Chek Active, Roche). The values obtained were expressed in mg/dL (Castañeda et al. 2008). The study design is summarized in Table 1.

Ethics for handling experimental animals

The Research Ethics Committee approved this study through certificate CEI-UNICA No. 005/05-2023. The study was carried out in strict compliance with national and international standards on ethics and handling of laboratory animals. After the experiment, all the animals were euthanized with an overdose of sodium pentobarbital, in-

Table 1. Design of the experiment of the hypoglycemic activity *in vivo* of the total extract of seeds of two varieties of *Vitis vinifera* L. from the Valley of Ica-Peru.

Group	Placebo/drug/total extract	Animal treatment
G-1 Blank group or negative control	Saline solution	Saline solution (2 mL/kg of body weight) was administered through an orogastric tube, once a day.
		The glycemia level was evaluated: 1, 2 and 4 hours, during 7 days of the experiment.
G-2- G5	Alloxane monohydrate 5%	Alloxane monohydrate 5% was administered at a dose of 130 mg/kg, by IP route, to groups G2, G3, G4 and G5.
		The administration was carried out 48 hours before starting the experimental study, and in a single dose.
		The glycemia level was evaluated after 48 hours.
G-2 Positive control group	Alloxane monohydrate 5%	The G-2 positive control was maintained for 7 days without treatment.
		The glycemia level was evaluated: 1, 2 and 4 hours, during 7 days of the experiment.
G-3 Comparator group	Glibenclamide as comparator drug	Glibenclamide was administered at a dose of 10 mg/kg of weight, orally through an orogastric tube.
		The dose was administered each day between 8:00–10:00 am.
		The glycemia level was evaluated: 1, 2 and 4 hours, during 7 days of the experiment.
G-4 Experimental group	Biophenols/PUFA extract from muscat grape seeds	Total muscat grape extract was administered at a dose of 500 mg/kg of weight, orally through an orogastric tube.
		The glycemia level was evaluated: 1, 2 and 4 hours, during 7 days of the experiment.
G-5 Experimental group	Biophenols/PUFA extract from quebranta grape seeds	Total quebranta grape extract was administered at a dose of 500 mg/kg of weight, orally through an orogastric tube.
		The glycemia level was evaluated: 1, 2 and 4 hours, during 7 days of the experiment.

traperitoneally (100 mg/Kg). The sacrificed animals were discarded according to NTSN°144-MINSA/2018/DIGESA, Technical Health Standard "Comprehensive Management and Management of Solid Waste in Health Establishments, Medical Support Services and Research Centers" (Castañeda et al. 2008; Huaccho et al. 2012; Carrasco et al. 2013; Alvarado et al. 2022).

Statistics

The results were transcribed into an Excel spreadsheet, from where they were exported for statistical analysis. The Shapiro-Wilk test, Tukey's multiple comparisons test, Student's T parametric test, ANOVA, linear regression analysis and Pearson's correlation coefficient were applied. The GraphPad Prism 9 Statistical Software was used Version 9.1.2 (Huaccho et al. 2012; Carrasco et al. 2013; Chávez et al. 2021).

Results

In this study, the total polyphenol content (TPC) was estimated using the Folin-Ciocalteu method of the aqueous extract of moscat and quebranta seeds (1.57±0.015 and 1.43±0.015 mg GAE/g, respectively) and the antioxidant activity *in vitro* using the DPPH$^{\bullet}$ free radical assay that accepts an electron from the biophenols, in this case from the biophenolic compounds and total polyunsaturated fatty acids from moscat and quebranta seeds (IC_{50}: 38.60±0.624 µg/mL and 42.83±0.306 µg/mL, respectively); and by the FRAP assay, which is based on the transfer of an electron from the biophenols to the ferric ion (Fe^{3+}) of the TPTZ-Fe^{3+} complex to reduce it to the ferrous ion Fe^{2+}, antioxidant activity was found with values of 0.79±0.030 µgTEAC/g for muscat and 0.61±0.038 µgTEAC/g for quebranta. The analysis of variance (ANOVA) for the three trials shows that the mean difference is statistically significant (p-value < α = 0.05) (Table 2). A low IC_{50} value indicates high antioxidant power of biophenolic compounds found in plant products (Surco-Laos et al. 2022a).

Table 3 and Fig. 2 show the hypoglycemic effect of biophenols and total polyunsaturated fatty acids (PUFA) from the seeds of *Vitis vinifera* L. muscat and quebranta observed *in vivo* model of albino rats (*Rattus norvegicus*) of the strain Holtzman. The total extract of biophenols/PUFA was administered at a dose of 500 mg/kg of animal weight. Glibenclamide was used as the comparator drug, which is a second generation sulfonylurea used in type 2 diabetes (Alvarado et al. 2021b), and according to the Biopharmaceutical Classification System, it is a class 2 drug with low aqueous solubility and high membrane permeability (Alvarado et al. 2020, 2021b, 2021c); and it is widely used as a standard in models of experimental hyperglycemia induced by alloxane (Castañeda et al. 2008).

Fig. 2 graphically shows the basal glucose levels (control with solution saline), glibenclamide, biophenols/PUFA of muscat and quebranta seeds, during the 7 days of treatment and evaluated at the pre-established times of 1, 2 and 4 hours.

Fig. 3 shows the linear regression analysis of glucose levels in each treatment vs. seven days.

A coefficient of determination (R^2) was observed at the hour of 0.9851, 0.9926 and 0.9928 (Fig. 3A); at 2 hours R^2 was 0.9786, 0.9822 and 0.9843 (Fig. 3B), and at 4 hours R^2 of 0.9717, 0.9746 and 0.9810 was observed (Fig. 3C) for glibenclamide, biophenols/total PUFA of muscat and quebranta seeds, respectively, all values close to 1.0, therefore it is a very reliable model that could be used in the future. In all cases p-value 0.0001 < α 0.05.

Fig. 4 shows the association result using the Pearson correlation coefficient.

These results indicate that there is a strong correlation [0.50 ≤ (0.9530–0.9827) < 1.0] between the glucose levels of glibenclamide/biophenols-PUFA of muscat and quebranta seeds. Being a positive association at the level of significance of 5% (p-value = 0.0001, 0.0002 < α = 0.05) and a coefficient of determination (R^2 = 0.9530–0.9827) that indicate that the linear relationship of both variables is between 97.46% to 99.26%.

Discussion

Our results are similar to those reported by Surco-Laos et al. who found TPC of 1.54±0.04 mg GAE/g and 1.39±0.04 mg GAE/g in seed of the muscat and quebranta variety, respectively; and the antioxidant activity by the DPPH method was 38.30±0.80 µg/mL and 42.70±0.40 µg/mL, and by the FRAP method it was 0.77±0.02 µgTEAC/g and 0.58±0.03 µgTEAC/g, respectively for seed varieties muscat and quebranta (Sur-

Table 2. Content of total polyphenols and *in vitro* antioxidant activity of polyunsaturated fatty acids of two varieties of seeds of the fruit *Vitis vinifera* (grape) muscat and quebranta.

Assay	Seed varieties				*Reference compounds	p-value
	Muscat		Quebranta			
	Mean ± SD	95%CI	Mean ± SD	95%CI		
TPC (mg GAE/g)	1.57 ± 0.015	0.017	1.43 ± 0.015	0.017	Gallic acid 33.5–700 µg/mL	3.588×10^{-4}
DPPH(IC_{50}) µg/mL	38.60 ± 0.624	0.707	42.83 ± 0.306	0.346	Trolox 0.032–0.5 mM	4.577×10^{-4}
FRAP (µgTEAC/g)	0.79 ± 0.030	0.034	0.61 ± 0.038	0.043	Trolox 0.032–0.5 mM	2.277×10^{-3}

TPC: total polyphenol content (mg GAE/g); TEAC: mg equivalent to 1 mM Trolox/g.

Table 3. Hypoglycemic effect of biophenols and total polyunsaturated fatty acids (PUFA) from the seeds of *Vitis vinifera* L. muscat and quebranta.

Hour	Basal		Alloxane		Glibenclamide		Muscat		Quebranta	
	Experiment day one									
	Mean ± SD (mg/dL)	CV%	Mean ± SD	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD	CV%	Mean ± SD (mg/dL)	CV%
1	99.16±0.014	0.014	261.80±4.01	1.53	221.33±0.89	0.40	209.22±2.98	1.42	206.02±1.83	0.89
2	99.15±0.005	0.006	260.78±3.58	1.37	216.88±1.47	0.68	204.92±0.40	0.19	203.13±1.68	0.83
4	99.13±0.020	0.020	260.14±3.73	1.43	210.87±0.81	0.38	203.48±1.52	0.75	198.60±1.17	0.59
	Experiment day two									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%
1	99.13±0.012	0.012	256.97±2.64	1.03	206.47±2.17	1.05	186.08±0.33	0.18	184.78±0.60	0.33
2	99.12±0.014	0.014	256.08±2.86	1.12	202.65±2.26	1.12	178.17±4.84	2.72	178.62±3.73	2.09
4	99.12±0.014	0.014	255.61±3.04	1.19	199.50±1.17	0.59	174.28±3.40	1.95	176.68±3.26	1.84
	Experiment day tree									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
1	99.02±0.230	0.233	258.28±5.54	2.15	192.42±0.70	0.36	170.07±0.23	0.13	169.13±0.77	0.46
2	98.61±0.230	0.130	254.50±4.08	1.60	189.08±1.97	1.04	165.80±2.81	1.69	166.83±1.04	0.62
4	98.60±0.470	0.470	252.62±3.59	1.42	187.03±2.24	1.20	161.60±2.63	1.63	164.90±1.37	0.83
	Experiment day four									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%
1	98.61±0.470	0.477	250.02±4.24	1.70	171.95±0.83	0.48	155.02±0.32	0.21	154.45±0.47	0.30
2	98.60±0.478	0.380	247.85±3.83	1.54	170.33±1.68	0.99	149.47±3.20	2.14	151.87±1.03	0.68
4	98.56±0.447	0.453	246.52±3.53	1.43	167.15±3.02	1.81	145.38±5.01	3.45	149.82±1.58	1.05
	Experiment day five									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%
1	98.71±0.156	0.158	245.38±3.81	1.55	151.53±0.80	0.53	130.15±0.52	0.40	129.48±0.53	0.41
2	98.67±0.169	0.171	244.08±2.98	1.22	140.80±7.42	5.27	124.38±4.35	3.49	125.12±2.22	1.77
4	98.66±0.166	0.168	242.77±2.90	1.19	136.50±7.68	5.62	120.80±4.49	3.72	123.33±2.49	2.02
	Experiment day six									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%
1	98.64±0.157	0.159	239.38±1.83	0.76	120.77±0.33	0.27	115.02±0.17	0.15	114.68±0.33	0.29
2	98.62±0.149	0.151	237.42±1.76	0.74	115.22±3.39	2.94	111.15±0.82	0.73	109.52±3.58	3.26
4	98.59±0.133	0.134	234.92±2.13	0.91	111.30±2.05	1.84	108.97±1.76	1.61	103.68±4.11	3.96
	Experiment day seven									
Hour	**Basal**		**Alloxane**		**Glibenclamide**		**Muscat**		**Quebranta**	
	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%	Mean ± SD (mg/dL)	CV%
1	98.59±0.119	0.121	231.42±0.97	0.42	102.15±2.34	2.29	102.50±1.65	1.61	103.27±0.34	0.33
2	98.53±0.207	0.210	229.43±0.64	0.28	99.80±0.29	0.29	99.90±0.28	0.28	100.12±0.48	0.48
4	98.15±0.210	0.214	226.87±1.37	0.60	98.96±0.74	0.75	99.25±0.86	0.87	98.93±0.76	0.76

SD: standard deviation, CV%: coefficient of variation, Muscat: biophenols and polyunsaturated fatty acids (PUFA), Quebranta: biophenols and polyunsaturated fatty acids (PUFA).

co-Laos et al. 2023). In another study Ferreira and Santos, estimated that the total content of polyphenols in the seeds and pomace of grapes from Portugal are 17.4±0.4% and 18.4±0.4%, respectively; and its antioxidant capacity measured in IC_{50} by the DPPH method is 55.9±0.7 and 48.9±0.5 µg/mL, of extract of seeds and pomace (µg of extract/mL DPPH), respectively (Ferreira and Santos 2022).

The Shapiro-Wilk test was applied to a sample of less than 50 (n = 30 animals), the results of which indicate that the hypoglycemic effect occurs normally at different times and days (p-value > α = 0.05). Tukey's multiple comparisons test was used to analyze the hypoglycemic effect by groups (glibenclamide vs biophenols/PUFA muscat; glibenclamide vs biophenols/PUFA quebranta; biophenols/PUFA muscat vs biophenols/PUFA quebranta; basal vs alloxane; basal vs glibenclamide; basal vs biophenols/ PUFA muscat; basal vs biophenols/PUFA; alloxane vs biophenols/PUFA muscat; alloxane vs biophenols/PUFA quebranta; alloxane vs glibenclamide), observing that there is no error rate in all the groups studied, whose p-values are equal to 0.0001 with a simultaneous confidence level of 95% (p-value < α = 0.05). Based on the previous statistical tests, the parametric Student's T test was applied to indicate that there is no probability of a difference in the hypoglycemic effect of biophenols/PUFA of the muscat and quebranta varieties during days 1 to 6 and at the first hour of treatment (p-value < α = 0.05); however, a p value = 0.999 was observed for both varieties of biophenols/PUFA, at 2 hours on days 6 and 7; at 4 hours,

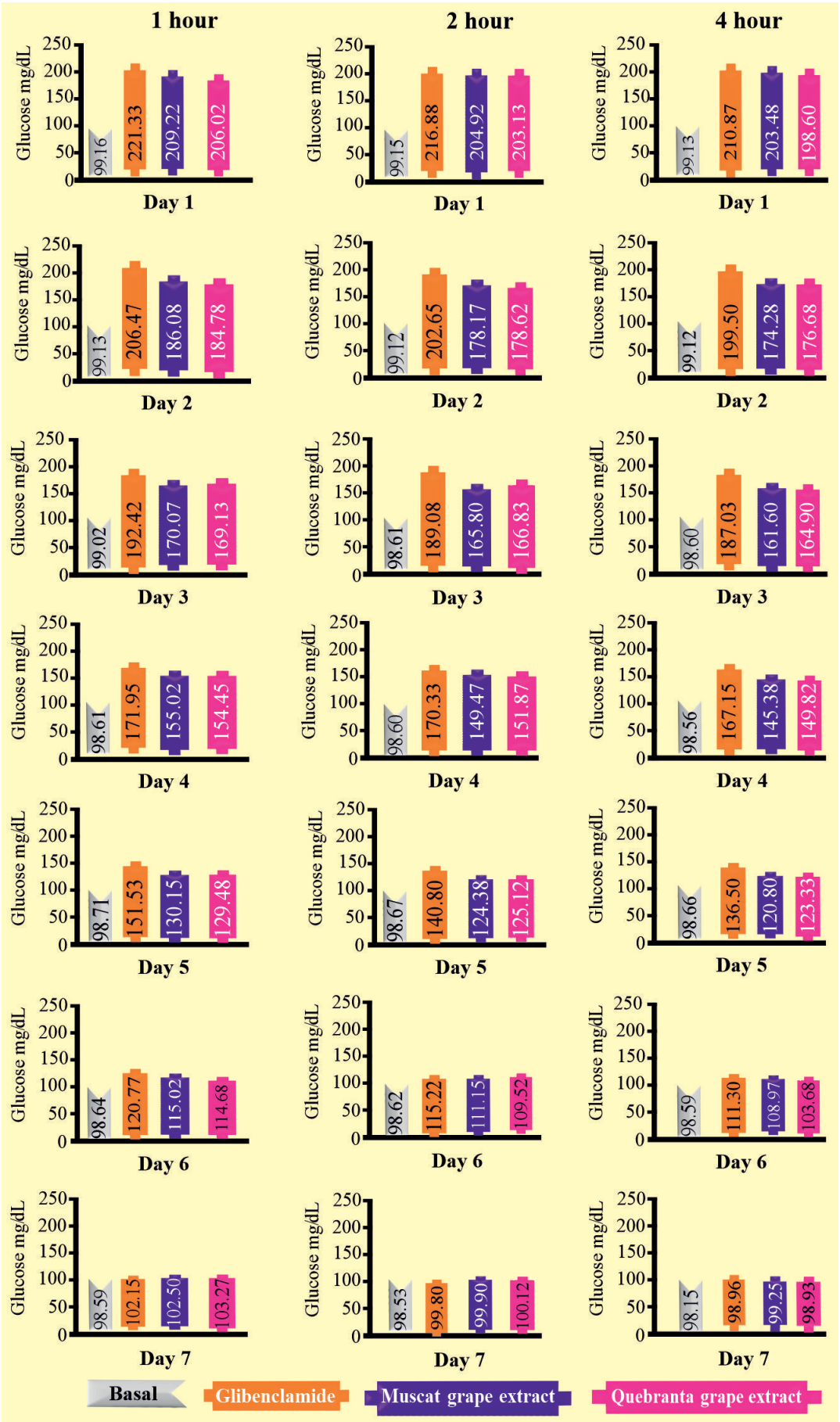


Figure 2. Hypoglycemic effect of biophenols and total polyunsaturated fatty acids (PUFA) from the seeds of *Vitis vinifera* L. Muscat and Quebranta.

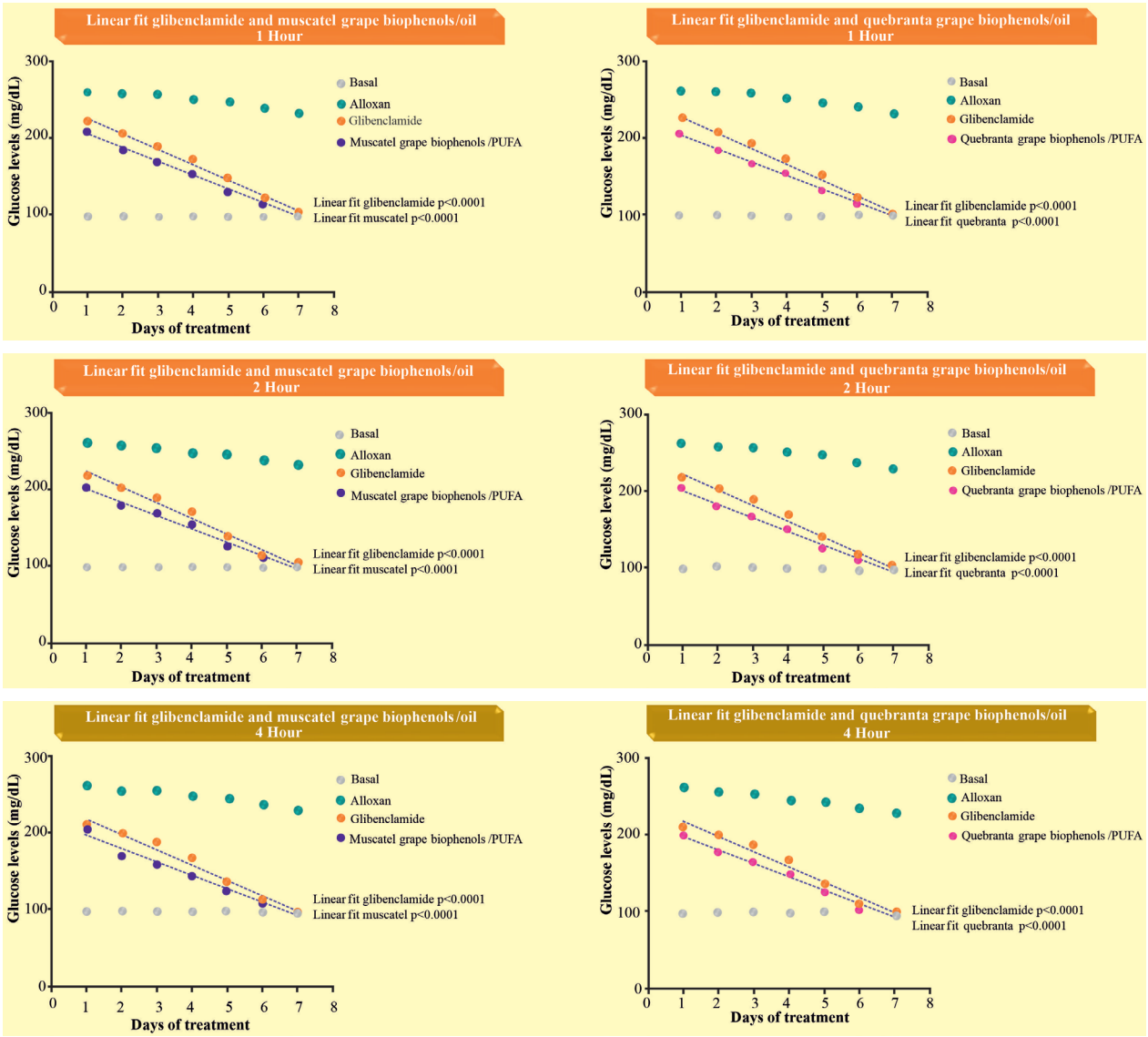


Figure 3. Representation of the linear regression analysis of glucose levels according to experiment/day of treatment.

p values = 0.4259 (day 6) and 0.999 (day 7) were observed for the muscatel variety; and a p-value = 0.999 on day 7 for the quebranta variety, these p-values indicate a small probability of difference (p-value > α = 0.05). At ANOVA, the difference in means is significant (p-value 0.0001 < α 0.05).

The biophenolic and PUFAS components of *Vitis vinifera* L. seeds could be responsible for the hypoglycemic effect of this study. In a recent study conducted by Surco-Laos et al. oleic acid was reported (muscat 19.70%; quebranta 18.35%), linoleic acid (muscat 70.07%; quebranta 71.87%), γ-linolenic acid (muscat 0.165%; quebranta 0.19%) and other fatty acids in a lower percentage (Surco-Laos et al. 2023). In other investigations various unsaturated fatty acids (60%), glycerophospholipids and PUFA such as linoleic acid has been reported in seeds of *V. vinifera* L.; while uvaol and oleanolic acid were quantified in the skins (Pérez-Navarro et al. 2019b). In another study Pérez-Navarro et al. found in seeds of *V. vinifera* L. from Castilla-La Mancha, a high content of glycosylated monomers, epicatechin, monomeric flavanol diglucosides and glycosylated flavanols (Pérez-Navarro et al. 2019a). Meanwhile, Gómez-Mejía et al. characterized oligomers of catechin (36.0±0.3 mg/g) and ellagic acid (3.14±0.02 mg/g) in seeds of *V. vinifera* L. var. Albariño and mulberry (*Morus nigra* L.) (Gómez-Mejía et al. 2021). While Di Stefano et al. gallic acid, caffeic acid, p-coumaric acid, epicatechin, myricetin, quercetin, resveratrol, flavonoids and anthocyanins were found in Sicily grape seeds (Di Stefano et al. 2022). Subsequently, Pérez-Navarro et al. demonstrated that "Karaerik" table grape seeds have a high content of total polyphenols and high antioxidant activity, which is attributed to proanthocyanidins (condensed tannins), derivatives of hydroxycinnamic acid, stilbenes, caffeic, coumaric and ferulic acids found in in the seeds. Quercetin and myricetin predominate in grape skins (Pérez-Navarro et al. 2022). These biophenolic compounds have the ability to neutralize superhydroxyls ($^{\cdot}OH$), superoxides ($^{\cdot}O_2$) and other free radicals that are involved in pancreatic β-cell necrosis (Zhang et al. 2019; Bartra et al. 2021). Chis et al. demonstrated antihyperglycemic effect and antioxidant capacity of biophenolic compounds from grape seeds, in diabetic Wistar rats induced by streptozotocin. Grape seed extract (dose 100 mg/kg/day) decreased the levels of lipid peroxides and car-

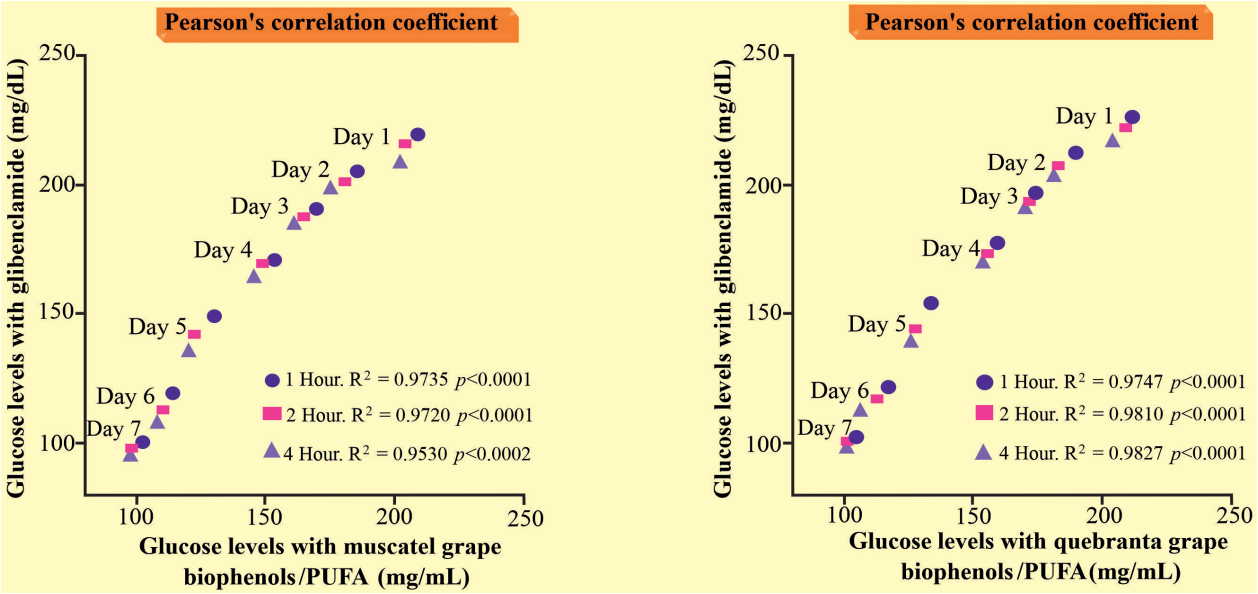


Figure 4. Pearson's scatterplot and correlation coefficient of glibenclamide glucose levels vs biophenols/PUFA from Muscat and Quebranta grape seeds.

bonylated proteins, improved antioxidant activity in plasma, and manifested a hepatoprotective effect (Chis et al. 2009). Likewise, Adam et al. reported that the aqueous extract of *V. Vinifera* L. seed eliminates free radicals *in vitro* and has an antidiabetic effect in albino rats, when glucose levels, HbA1c, lipid profile and serum insulin levels were observed almost normal with minor signs of pancreatic destruction (Adam et al. 2016). In another study Kong et al. demonstrated *in vitro* hypoglycemic activity (inhibition of α-glucosidase and α-amylase) of water-soluble grape seed oil, attributing said activity to catechins (44.12±0.21 mg/mL) and epicatechins (111.23±1.29 mg/g) (Kong et al. 2018). In a clinical trial conducted by Cao et al. biophenols from grape seeds, red wine, cocoa, coffee and olive oil have been observed to show antidiabetic effects in patients with type 2 diabetes, furthermore it is suggested that the mechanism of action is to increase glucose metabolism, improve vascular function, decrease HbA1c levels and reduce insulin resistance (Cao et al. 2019).

The limitation of the research is in the study population, since there are 85 artisan wineries that produce Pisco and we have only considered one winery, another limitation is the size of the sample studied, in this study two varieties of *V. vinifera* L. have been evaluated (muscat and quebranta) and the other six samples grown in the Ica valley were not considered; the other limitations that can lead to bias or confusion are: to evaluate the total content of biophenols and polyunsaturated fatty acids, and not having quantified or elucidated the chemical structure of the biophenolic compounds, which is why it is being considered by our research group, to be evaluated in future studies. Notwithstanding the foregoing, we consider that these preclinical studies on hypoglycemic effects in vivo form part of the scientific evidence, avoiding pseudoscience, and to promote other preclinical studies (molecular, cellular, isolated organs and in other small animals) with isolated biophenols and known chemical structure, based on these results to initiate clinical studies that are phase 0, I, II and III.

Conclusions

Our result suggests that the seeds of the muscat and quebranta grape varieties present antioxidant activity *in vitro* and a hypoglycemic effect in animal models *in vivo* that generate background and scientific evidence on their biological effects that merit further study to develop functional foods to prevent diabetes type 2 and subsequently encourage its use in the treatment and complications of the aforementioned chronic disease.

The seeds (by-products) of *Vitis vinifera* L. fruits could additionally be used as antioxidants in the nutrition and cosmetic industry.

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