Potential Hypoglycemic Effect of Pomegranate Juice and Its Mechanism of Action: A Systematic Review

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ABSTRACT Pomegranate juice (PJ) has gained popularity attributed to its phenolic compounds and their medicinal properties. Its potential hypoglycemic effect has been related to enzymatic inhibition, insulin release, and the protection of pancreatic tissue. These effects depend on several aspects, such as the content and composition of phenols in pomegranate and characteristics of the organism that consumes the juice. The objective of this study was to systematically review scientific evidence investigating the hypoglycemic effect of PJ; the factors that affect bioactive compounds; and the mechanisms of action attributed to this effect. Human and rodent *in vivo* and *in vitro* studies were retrieved from PubMed, Scopus, and ScienceDirect databases. After reviewing the articles, it was identified that the methodologies and results among the scientific evidence were quite heterogeneous. Despite these limitations, many of the *in vivo* and *in vitro* studies found important hypoglycemic effects from PJ, as well as an increase in the function of β -cell, insulin secretion, a significantly lower activity of α -amylase enzyme, an inhibition of the enzyme α -glucosidase and dipeptidyl peptidase-4 (DPP-4), and the protection against DNA damage. Determining the potential health benefits of polyphenols contained in the pomegranate is limited for multiple factors that could affect the efficacy of PJ. Overall, the results of this review suggest the need for further experimentation, using controlled variable factors and testing the effect of PJ under similar experimental conditions.

KEYWORDS: • antihyperglycemic effect • antioxidants • phenolic compounds

INTRODUCTION

POMEGRANATE (*PUNICA GRANATUM* LINN) fruit has been used as medicinal source since ancient times in the Middle East.¹ It has recently gained more attention due to the growing evidence on its therapeutic effects and the increasing costs of medical treatment for metabolic disorders. Finding effective bioactive compounds and phytochemicals derived from fruits and plants that provide health benefits at a reduced cost has become important.²

Hyperglycemia associated with diabetes mellitus increases complications and premature death due to constant oxidative stress, so it is the principal dysfunction that has to be treated. Many parts of the pomegranate, like the peel, seeds, flowers, and juice,^{3,4} have shown hypoglycemic effects attributed to their phenolic compounds by the suggested effects on the inhibition of enzymes

related to carbohydrate metabolism,^{5,6} stimulation of insulin release by β -cells,⁷ and protection of pancreatic tissue.^{8,9}

The juice seems to be the most popular derivative because of its ease to extract. The main phenolic compounds in pomegranate juice (PJ) are as follows: punicalagin, ellagic acid, flavonoids, and anthocyanins,⁶ which provide the juice the highest antioxidant capacity when compared *in vitro*, with other antioxidant beverages,¹⁰ placing it among the 100 foods with the highest polyphenol content.¹¹ This content was higher when the preparation included seeds,¹² and 20 times more antioxidant activity has been demonstrated in the juice when it was prepared from the whole fruit versus using just the arils.¹³ The content and composition of the phenols in the pomegranate and its derivatives are largely influenced by the type of crop and the variety of the fruit.^{14–16} Among other aspects, the intestinal metabolites derived from the intake of pomegranate polyphenols, such as urolithins, proved to modulate the metabolism of carbohydrates. However, these metabolites depend on the individual colonic microbiota.¹⁷

Various factors of the fruit production, processing, and transformation, as well as physiological aspects, have a

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major influence on the phenolic compounds, related to the hypoglycemic properties. This review aims to gather scientific evidence about the hypoglycemic effect of PJ, the factors that affect bioactive compounds, and the mechanisms of action attributed to this effect.

METHODS

Studies retrieved from three bibliographic sources were included in this systematic review using PRISMA-P guidelines¹⁸ to perform the search.

Search strategy

The selected sources used for this review were as follows: PubMed, Scopus and ScienceDirect. The bibliographic search, in both English and Spanish, was carried out from August 2018 to January 2019. The following keywords were sought in both languages: “pomegranate juice” AND “diabetes”; “pomegranate juice” AND “hypoglycemic”; “pomegranate juice” AND “glucose,” and the terms were used alone or in combination. Articles published in the last 15 years were selected.

Eligibility criteria

This review includes research conducted on humans and rodents, and *in vitro* techniques that have evaluated effects on glucose levels or have reported results related to the hypoglycemic capacity of PJ.

The inclusion criteria of this systematic review were as follows: studies written in English or Spanish assessing outcomes that could attribute an effect on glucose by the PJ in healthy patients or with any disease condition, hyperglycemic rodent models, and *in vitro* techniques related to enzyme inhibition assays and cell cultures.

The exclusion criteria included articles with outcomes different from those of interest or with incomplete results; articles written in other languages; and articles that used the PJ in combination with another derivative.

Reviews were considered only for the construction of background and discussion.

Data collection

The articles were systematically reviewed and analyzed in search of results that could attribute an effect in the reduction of glucose levels and/or in some physiological process that would lead to the reduction of these levels by PJ.

The most relevant information was organized for its presentation in tables, which in turn are divided into clinical studies in humans shown in Table 1, *in vivo* rodent models in Table 2, and *in vitro* methods in Table 3.

The realization of a meta-analysis was not considered due to the significant heterogeneity between study designs and interventions.

RESULTS

Search for articles and data collection

Through the search of the keywords in bibliographical sources and additional records identified through other sources, this review recovered 2639 abstracts, of which 2616 were excluded due to duplication and evaluation of different effects and different topics and reviews. One article was excluded due to the lack of information about the results,¹⁹ and another because of the inconsistency between the results.²⁰ Finally, 23 articles that addressed the eligibility criteria were selected: 12 were clinical studies in humans, 8 *in vivo* rodent models, and 3 *in vitro* methods. Figure 1 depicts the flowchart of the selection process for the articles included in this systematic review.

Characteristics of the studies

Most of PJ studies in humans were randomized studies, placebo and double and triple blind^{17,21–28}; as well as quasi-experimental studies.^{29,30} One did not report the trial design in the article.³¹ All of the studies had different hypotheses, which meant a significant heterogeneity among the population included. The studies contained between 10 and 86 participants in the 15- to 79-year-old range and were inclusive of both genders.

In the *in vivo* studies, the hypotheses were related to the PJ on metabolic alterations, comparing their effects using different doses³² and different pomegranate varieties³³; evaluating their interaction with hypoglycemic drugs^{34,35}; comparing its effect against pomegranate seeds⁸; and when subjects were exposed *ad libitum*.⁹ All of these studies included rodent models with drug-induced hyperglycemia.^{8,9,32–37} Between the studies, among 18–48 rodents, with groups of 5–8 animals of the Wistar strain,^{9,33–35} Sprague Dawley,^{8,36} *Rattus norvegicus*,³² and *Rattus rattus*; mostly males^{8,9,32,33,36}; in two cases both genders^{34,35}; and in one case only females,³⁷ were exposed to PJ.

In the *in vitro* methods, one study in cell cultures that evaluated the effect of PJ on β -cells⁷ and two studies about the inhibition of enzymes related to carbohydrate metabolism were recovered.^{5,6}

HUMAN CLINICAL STUDIES

Characteristics of participants

The studies have evaluated the effect of PJ on parameters related to metabolic alterations in healthy,^{17,24,28} obese,²³ and diabetic people,^{21,26,27,29,30} with metabolic syndrome²⁵ and arterial pathologies,^{27,31} and in women with polycystic ovarian syndrome.²²

In most studies, patients were excluded if they were taking any hypoglycemic drug, due to possible interaction with PJ compounds. There were some studies that included patients on medication, but the type of drug was not reported.^{26,27,30} None of these studies showed a positive effect with PJ.

The study design did not control the ingestion of polyphenols; but in some cases, they considered the normal diet and the consumption of polyphenols by other sources. In these studies, the researchers provided a list of restricted foods and monitored the ingestion with diary meal records.^{19,24,25,28,30}

Characteristics of pomegranate and interventions

Enough evidence has reported the influence of the type of crop and variety on the amount and composition of phenolic compounds.^{14–16} However, only three studies reported using Shiraz,²⁵ Mollar,¹⁷ and Wonderful²⁷ pomegranate varieties.

The method of juice extraction affects considerably the phenolic content.^{12,14} The studies that reported it used manual extractions^{25,29,31} and commercial juice extractor²¹; in both cases, including arils (seeds and pulp). There was one study that included husk, membranes, and seeds.¹⁷ Among the studies that quantified phenolic compounds, a fairly wide ranges of values were observed and impossible to compare with each other due to the omission on the variety and extraction method (Table 1).

The amount of PJ used among human studies has been quite heterogeneous, with different ranges of mL/day. Exposure time indicates the same trend, with one-time exposures, up to 12-week interventions. In one study, participants consumed PJ for at least 1 year, while at least half of participants continued for up to 3 years.³¹ For control groups, placebo drinks contained the same calories and color and similar taste and flavor to PJ, to equalize the organoleptic characteristics of the juice, which could have been perceived by participants. In other cases, the same participants were used as their own controls^{29,30}; in others, controls were participants without any exposure²⁴ and even with different health conditions than experimental group.²¹

Actions related to PJ

Hypoglycemic action observed after the ingestion of PJ has been independent of gender. However, older people showed less response to PJ.²¹

The glucose effect on the groups before and after consumption of PJ, in some cases, no changes were observed,^{24,28} while in some cases, glucose was increased.^{22,23,25–27} In the majority of people where glucose reduction was observed, the levels were significantly reduced 3 h after the ingestion of PJ and after 4 and 6 weeks of exposure.^{21,29,30} In participants who consumed juice between 1 and 3 years, glucose levels were reduced, but not significantly.³¹

Insulin levels behaved similar to glucose; in some cases, consumption of PJ increased insulin^{23,25}; in others, it reduced it^{21,28}; and in one case, no changes were observed.²²

For the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, there was equality of evidence referring to a decrease in the insulin resistance index^{21,28} and an increase in it,^{22,25} before and after the PJ ingestion.

As a part of the determination of the mechanism of action, the percentage function of β -cell (HOMA-% β) increased significantly 3 h after the ingestion of PJ.²¹

IN VIVO RODENT STUDIES

Characteristics of subjects

Of the studies included in Table 2, all of the subjects reported in this review were hyperglycemic by drug induction with 45 mg/kg/bw (body weight)³³ or 60 mg/kg/bw^{9,32} of streptozotocin alone or in combination with 120 mg/kg/bw of nicotinamide^{8,36} or 120–150 mg/kg/bw of alloxan.^{34,35,37}

Characteristics of pomegranate and interventions

Only one study reported using “Wonderful” variety pomegranates⁹; in other studies, authors reported using “sweet red” pomegranates,^{8,36} “dark red and sour,” and “dark red and sweet.”³³ All studies reported the juice extraction method. The studies used manual extraction^{34,35} and commercial extractors,^{8,32,33,36,37} in both cases, including arils (seeds and pulp). One study used manual extraction, including husk, membranes, and seeds³²; and in one case, the extraction was carried out with industrial machinery, including arils.⁹

Among the studies that quantified polyphenols, the higher amount of total phenolic compounds, quantified in 4976.46 ± 258.68 mg GAE (gallic acid equivalent)/L, was in the “Wonderful” variety,⁹ followed by “dark red and sour” whose content was 1344.2 ± 40.8 mg GAE/g, the dark red and sweet with 1196.4 ± 53.1 mg GAE/g,³³ and in less quantity was 91.34 ± 0.03 mg GAE/g of an unknown variety.³⁵

In most cases, the methodology used a gavage to supply the PJ in different doses based on mL per kilogram of body weight,^{33,37} per 200 g of body weight,³² or per day.^{8,34–36} Only one evidence considered free access to the juice, with the intention of evaluating the consumption and acceptance, due to its organoleptic characteristics. In addition, only this antecedent considered the exposure of control subjects to a solution with the same sugar content as the PJ.⁹ The rest of the studies supplied saline or distilled water to the control group. The exposure to PJ was between 21^{8,9,36} and 28 days.^{32–35,37}

Actions related to PJ

Basal glucose levels were only reported in two studies, which made it difficult to compare the real effect of PJ in the exposed subjects. In cases that were possible to compare the initial glucose level of the rats with the level at the end of the exposure, it was observed that in both, when using three different doses of PJ³² and when exposing *ad libitum* juice consumption,⁹ glucose levels were significantly reduced. When the effects of PJ were compared with the control hyperglycemic groups without intervention, a reduction of glucose levels were observed in all cases.^{8,34,35} A significant reduction in glucose levels was observed in three studies,^{33,36,37} in one of which two varieties of PJ were used and their effects were compared with the control.³³

TABLE 1. CHARACTERISTICS OF THE HUMAN CLINICAL STUDIES AND THE EFFECT OF POMEGRANATE JUICE

<i>Trial design</i>	<i>Study hypothesis</i>	<i>Controlled ingestion of dietary polyphenols</i>	<i>Population (No./gender/age)</i>	<i>Health condition</i>	<i>Use of hypoglycemic drugs</i>	<i>Variety of Punica granatum</i>	<i>Extraction of juice</i>	<i>Quantification of phenolic compounds</i>
nr	Effect of PJ consumption on carotid lesions, oxidative stress, and blood pressure in patients with CAS	No	19/14 m, 5 f/65–75 years	Asymptomatic CAS	No	nr	Manual (nr the included parts)	Reported as 1561 mg/L of punicalagins, 417 mg/L of hydrolyzable tannins, 384 mg/L of anthocyanins, and 121 mg/L of ellagic acid derivatives
Randomized double-blind, placebo controlled	Effect of PJ consumption on myocardial perfusion in patients with CHD	No	45/40 m, 5 f/69 ± 10 years	All with CHD, 11 with DM2	Yes (only 9 participants)	Wonderful	POM wonderful commercial juice	No
Randomized double-blind, placebo controlled	Effect of PJ in insulin secretion and sensitivity	No	20/nr/25–55 years	Obese	No	nr	nr	No
Quasi-experimental	Effect of PJ in oxidative stress mediated by PON1	No	50/nr/45 ± 8 years	DM2	nr	nr	Manual extraction, including arils (seeds+pulp)	No
Randomized placebo-controlled crossover	Influence of PJ on cardiometabolic risk factors	Yes	28/12 m, 16 f/50.4 ± 6.1 years	Healthy	No	nr	Pomegranate Pure® commercial juice	Reported as 1685 mg/L polyphenols
Randomized clinical	Reduction of insulin-resistant state and FPG	No	135/DM2: 85 (40 m, 45 f), healthy: 50/37–60 years	DM2	No	nr	Commercial juice extractor, including arils (seeds+pulp)	No
Randomized double-blind, placebo controlled	Effect of PJ on plasma advanced glycated end products	No	44/23 m, 21 f/56 ± 6.8 years	DM2	Yes (nr specifically)	nr (commercial juice)	nr (commercial juice)	No
Quasi-experimental	Effect of CPJ on inflammatory factors	No	31/15 m, 16 f/46 ± 8.3 years	DM2	Yes (nr specifically)	nr (commercial juice)	nr (commercial juice)	Reported as total phenols 6.3 mg/100 g
Randomized counterbalanced, within-subjects experimental	Effect of PJ consumption on complete blood counts, glucose, lipids, and C-reactive protein	No	10/5 m, 5 f/31.8 ± 6.6 years	Healthy	No	nr	Commercial juice (CBH-VITOM S.A., Athens, Greece)	Reported as total phenols 405 mg/L of GAE, flavonoids 12.67 mg/L of equivalent quercetin
Randomized double-blind, crossover controlled	Effect of PJ in metabolic syndrome patients	No	30/14 m, 16 f/51.57 ± 10.04 years	Metabolic syndrome	nr	Shiraz pomegranate	Manual extraction, including arils (seeds+pulp)	Reported as total phenols 69 mg/L, anthocyanins 100.46 mg/L, and total flavonoids 283.02 mg/L
Randomized double-blind, crossover controlled, unblinded	Glycemic response to a high glycemic index food and PJ	No	16/nr/31 ± 5 years	Healthy	No	Mollar	Manual extraction, including husk, membranes, and seeds	Reported as Punicalin 357.3 ± 1.1 mg/L, punicalagin 61.9 ± 0.6 mg/L, and ellagic acid 24 ± 0.3 mg/L
Randomized triple-blind, parallel controlled	Effect of PJ on metabolic alterations related to PCOS	No	86/f/15–48 years	PCOS	No	nr (commercial juice)	nr (commercial juice)	No

^aStatistically significant.^bCrude data not shown.

±, mean value; ↓, reduction; ↔, no significant changes; bw, body weight; CAS, carotid artery stenosis; CHD, coronary heart disease; CPJ, concentrated pomegranate juice; Ctr, control; DM2, type 2 diabetes mellitus; f, feminine; FPG, fasting plasma glucose; GAE, gallic acid equivalent; HDL, high-density lipoproteins; HOMA-IR, homeostatic model assessment for insulin resistance; m, masculine; nr, not reported; PCOS, polycystic ovarian syndrome; PJ, pomegranate juice; PON1, human serum paraoxonase 1; SB, symbiotic beverage; SPJ, symbiotic pomegranate juice.

<i>Intervention</i>	<i>Time of exposure</i>	<i>Baseline glucose (FPG)</i>	<i>Glucose value after intervention ($\pm$)</i>	<i>Glucose change in PJ group</i>	<i>HOMA-IR index before and after PJ</i>	<i>Baseline insulin concentration</i>	<i>Insulin value after intervention ($\pm$)</i>	<i>References</i>
PJ ($n=10$): 50 mL/day of PJ versus Ctr ($n=9$): placebo nr characteristics	1 Year (10 participants); 3 years (5 participants)	PJ: 1 year ($n=10$): 123 ± 9 mg/dL; PJ: 3 years ($n=5$): 120 ± 7 mg/dL Ctr: nr	PJ: 1 year ($n=10$): 120 ± 11 mg/dL; PJ- 3 years ($n=5$): 106 ± 5 mg/dL Ctr: nr	PJ: 1 years: $\downarrow$; PJ: 3 years: $\downarrow$	No	No	No	Aviram <i>et al.</i> ³¹
PJ ($n=26$): 240 mL/day of PJ versus Ctr ($n=19$): placebo with isocaloric and similar color and flavor solution	3 Months	PJ: 113 ± 30 mg/dL; Ctr: 117 ± 52 mg/dL	PJ: 116 ± 31 mg/dL; Ctr: 121 ± 63 mg/dL	PJ: $\uparrow$	No	No	No	Sumner <i>et al.</i> ²⁷
PJ ($n=10$): 120 mL/day of PJ versus Ctr ($n=10$): placebo (nr characteristics) both in fasted state	1 Month	PJ: 4.7 ± 0.3 mmol/L; Ctr: 4.7 ± 0.5 mmol/L	PJ: 4.8 ± 0.4 mmol/L; Ctr: 4.7 ± 0.4 mmol/L	PJ: $\uparrow$	nr	PJ: 105 ± 45 pmol/L; Ctr: 99 ± 61 pmol/L	PJ: 127 ± 39 pmol/L; Ctr: 88 ± 40 pmol/L	González-Ortiz <i>et al.</i> ²³
PJ ($n=50$): 200 mL/day of PJ versus Ctr: same group as control	6 Weeks	PJ: 195 ± 31 mg/dL	PJ: 160 ± 37 mg/dL	PJ: $\downarrow^a$	No	No	No	Parsaeyan <i>et al.</i> ²⁹
PJ ($n=28$): 500 mL/day of PJ versus Ctr ($n=28$): 500 mL placebo isocaloric solution	4 Weeks	PJ: 4.89 ± 0.37 mmol/L; Ctr: 4.65 ± 0.48 mmol/L	PJ: 4.81 ± 0.53 mmol/L; Ctr: 4.74 ± 0.27 mmol/L	PJ: $\leftrightarrow$	$\downarrow$ (2.21 ± 1.43 vs. 1.82 ± 1.1)	PJ: 9.3 ± 5.81 mL U/I; Ctr: 10.65 ± 9.12 mL U/I	PJ: 7.53 ± 4.12 mL U/I; Ctr: 10.54 ± 7.72 mL U/I	Tsang <i>et al.</i> ²⁸
PJ ($n=85$): 1.5 mL/kg bw versus Ctr ($n=50$): healthy individuals+PJ	3 h	PJ ^b : 9 mmol/L; Ctr ^b : 5.2 mmol/L	PJ: 8.5 ± 0.2 mmol/L; Ctr: 9.44 ± 0.24 mmol/L	PJ: $\downarrow^a$	$\downarrow$ (4.3 vs. 3.9) ^b	PJ: 11 ± 0.9 μ IU/mL	PJ: 9.84 ± 0.59 μ IU/mL	Banihani <i>et al.</i> ²¹
PJ ($n=22$): 250 mL/day versus Ctr ($n=22$): with isocaloric and similar color solution	12 Weeks	PJ: 160 ± 47 mg/dL; Ctr: 148 ± 42 mg/dL	PJ: 162 ± 35 mg/dL; Ctr: 158 ± 49 mg/dL	PJ: $\uparrow$	No	No	No	Sohrab <i>et al.</i> ²⁶
CPJ ($n=31$): 50 g/day of CPJ versus Ctr: same group as control	4 Weeks	CPJ: 143.61 ± 7.22 mg/dL	CPJ: 140.77 ± 6.31 mg/dL	CPJ: $\downarrow$	No	No	No	Shishehbor <i>et al.</i> ³⁰
PJ ($n=10$): 500 mL/day (250 mL morning/250 mL afternoon) versus Ctr ($n=10$): nothing	14 Days	PJ: 5.2 ± 0.1 mmol/L; Ctr: 5.3 ± 0.1 mmol/L	PJ: 5.1 ± 0.1 mmol/L; Ctr: 5.2 ± 0.1 mmol/L	PJ: $\leftrightarrow$	No	No	No	Manthou <i>et al.</i> ²⁴
PJ ($n=30$): 500 mL/day of PJ versus Ctr ($n=30$): placebo with isocaloric and similar color and taste solution	1 Week	PJ: 144.57 ± 11.89 mg/dL; Ctr: 144.73 ± 12.27 mg/dL	PJ: 147.76 ± 12.78 mg/dL; Ctr: 142.56 ± 12.03 mg/dL	PJ: $\uparrow$	$\uparrow$ (3.76 ± 0.39 vs. 4.65 ± 0.51)	PJ: 11.94 ± 1.30 μ g/dL; Ctr: 12.4 ± 1.46 μ g/dL	PJ: 14.2 ± 1.63 μ g/dL; Ctr: 12.7 ± 6.9 μ g/dL	Moazzen and Alizadeh ²⁵
PJ ($n=16$): 200 mL of PJ+bread versus Ctr ($n=16$): with isocaloric solution+bread	Two times	4.7 ± 0.5 mmol/L of the total persons ($n=16$)	PJ: 6.8 ± 1.0 mmol/L; Ctr: 7.7 ± 0.9 mmol/L ($\pm$ value of two exposures)	$\downarrow$ Compared with Ctrl	No	No	No	Kerimi <i>et al.</i> ¹⁷
PJ ($n=22$): 2 L of PJ, per week; SPJ ($n=22$): 2 L of PJ+lactobacillus, per week SB ($n=21$): 2 L of SB with lactobacillus and pomegranate flavoring, per week versus Ctr ($n=21$): 2 L of placebo beverage with water and pomegranate flavoring, per week	8 Weeks	PJ: 112 ± 12 mg/dL; SPJ: 112 ± 9 mg/dL; SB: 112 ± 8 mg/dL; Ctr: 114 ± 8 mg/dL	PJ: 113 ± 10 mg/dL; SPJ: 110 ± 6 mg/dL; SB: 111 ± 6 mg/dL; Ctr: 114 ± 7 mg/dL	PJ: $\uparrow$	$\uparrow$ (6.16 ± 1.17 vs. 6.20 ± 1.23)	PJ: 22 ± 3 μ IU/mL; SPJ: 22 ± 3 μ IU/mL; SB: 22 ± 4 μ IU/mL; Ctr: 24 ± 3 μ IU/mL	PJ: 22 ± 3 μ IU/mL; SPJ: 21 ± 3 μ IU/mL; SB: 20 ± 3 μ IU/mL; Ctr: 25 ± 3 μ IU/mL	Esmacilinezhad <i>et al.</i> ²²

TABLE 2. CHARACTERISTICS OF THE *IN VIVO* RODENT STUDIES AND THE EFFECT OF POMEGRANATE JUICE

Study hypothesis	Subjects (No./strain/sex)	Rodent model	Variety of Punica granatum	Extraction of juice	Quantification of phenolic compounds	Intervention and route	Time of exposure
Protective effect of PJ and peel extract against alloxan-induced diabetic	32 ($n=8$ p/g)/ <i>Rattus Rattus</i> /female	Alloxan 120 mg/kg/bw	nr	Commercial juice extractor, including arils (seeds+pulp)	No	PJ: 5 mL/kg/bw, orally versus Ctr: none exposure	4 Weeks
Effect of PJ on antioxidant status, amelioration of oxidative stress, and histology of liver	48 ($n=8$ p/g)/Sprague Dawley/male	STZ 60 mg/kg/bw and NAD 120 mg/kg/bw	nr (Sweet red from Spain)	Commercial juice extractor including arils (seeds+pulp)	No	PJ: 1 mL/day, orally versus Ctr: 1 mL/day of distilled water orally	21 Days
Hypoglycemic and antioxidant effect of PJ in three different doses	20 ($n=5$ p/g)/ <i>Rattus Norvegicus</i> /male	STZ 60 mg/kg/bw	nr	Commercial juice extractor, including arils (seeds+pulp)	No	PJ-dose 1: 1 mL/200 g/bw orally; PJ-dose 2: 2 mL/200 g/bw orally; PJ-dose 3: 4 mL/200 g/bw orally	4 Weeks
Protective effect of different PJ on kidney	28 ($n=7$ p/g)/Wistar/male	STZ 45 mg/kg/bw	Reported as: (1) RDAc; (2) RDS	Commercial juice extractor, including arils (seeds+pulp)	Total phenols—PJ (RDAc): 1344.2 ± 40.8 mg GAE/g; PJ (RDS): 1196.4 ± 53.1 mg GAE/g; Total anthocyanin—PJ (RDAc): 320.8 ± 2.6 mg RE/g; PJ (RDS): 211.3 ± 1.7 mg RE/g; Total flavonoid—PJ (RDAc): 351.8 ± 3.5 mg C3G/g; PJ (RDS): 366.2 ± 13.8 mg C3G/g	PJ (RDAc): 4 mL/kg/bw orally, PJ (RDS): 4 mL/kg/bw, orally versus Ctr: saline solution	4 Weeks
Effect of herb-drug (nateglinide) interaction against DM2	40 ($n=8$ p/g)/Wistar/either sex	Alloxan 150 mg/kg/bw IP	nr	Manual extraction, including arils (seeds+pulp)	Total phenols— 91.34 ± 0.03 mg GAE/g; flavonoids— 2.93 ± 0.02 mg QE/g	PJ: 3 mL/day, orally versus Ctr: saline solution	4 Weeks
Effect of herb-drug (tolbutamide) interaction against DM2	40 ($n=8$ p/g)/Wistar/either sex	Alloxan 150 mg/kg/bw IP	nr	Manual extraction, including arils (seeds+pulp)	No	PJ: 3 mL/day, orally versus Ctr: saline solution	4 Weeks
Effect of fresh PJ versus pomegranate seed powder on metabolic indices and pancreatic tissue	40 ($n=8$ p/g)/Sprague Dawley/male	STZ 60 mg/kg/bw and NAD 120 mg/kg/bw	nr (Sweet red from Spain)	Commercial juice extractor, including arils (seeds+pulp)	Ellagic acid (not quantified)	PJ: 1 mL/day orally versus Ctr: 1 mL/day water	21 Days
Effect of free access to PJ on metabolic indices and pancreatic tissue	18 ($n=6$ p/g)/Wistar/male	STZ 60 mg/kg/bw	Wonderful	Extraction with industrial machinery, including arils (seeds+pulp)	Total phenols— 4976.46 ± 258.68 mg GAE/L	PJ: free access to PJ in the drinking bottle versus Ctr: free access to isocaloric solution	21 Days

The rest of the groups not exposed to PJ were not included in the review.

^aStatistically significant.

^bCrude data not shown.

↓, reduction; C3G, cyanidin-3-glucoside; IP, intraperitoneal; NAD, nicotinamide; p/g, per group; QE, quercetin equivalent; RDAc, dark red and sour; RDS, dark red and sweet; RE, rutin equivalents; STZ, streptozotocin.

The basal levels of insulin were not reported by any of the studies; therefore, the HOMA-IR index was not determined either. Those that reported final insulin levels, expressed higher insulin levels than the control group.^{8,34–37}

One of the *in vivo* studies showed a significantly lower activity of α -amylase enzyme in the group exposed to juice, as well as a slight inhibition of DNA damage generated by the injection of alloxan, and decreased DNA fragmentation in the liver tissue.³⁷ In addition, treatment with PJ for 21 days increased the number and size of islets of Langerhans,⁸ and for the same time of exposure, free access to PJ showed slight damage to pancreatic architecture and more islets of Langerhans, in comparison with control groups.⁹

IN VITRO METHODS

The hypoglycemic response of PJ is attributed to its bioactive compounds. It has been suggested that polyphenols may affect glucose levels by improving the sensitivity of insulin receptors with increased activity of the peroxisome proliferator-activated gamma receptor (PPAR- γ), reduction of glucose absorption by inhibiting digestive enzymes, modulating the expression of glucose transporter GLUT4, increasing glucose uptake by peripheral tissues, inhibition of gluconeogenesis, and increasing insulin secretion, insulin levels, and the number of β cells.^{3,38–40}

Baseline glucose (FPG)	Glucose value after intervention ($\pm$)	Glucose change in PJ group	HOMA-IR index after PJ	Baseline insulin concentration ($\pm$)	Insulin concentration after intervention ($\pm$)	References
nr	PJ: 164.38 $\pm$ 6.65 mg/dL; Ctr: 227.8 $\pm$ 7.72 mg/dL	$\downarrow$ ^a PJ compared with Ctrl	No	No	PJ: 3.54 $\pm$ 0.26 mIU/mL; Ctr: 2.10 $\pm$ 0.19 mIU/mL	Osman <i>et al.</i> ³⁷
Nr	PJ ^b : 150 mg/dL; Ctr ^b : 230 mg/dL	$\downarrow$ ^a PJ compared with Ctrl	No	nr	PJ ^b : 210 μ U/mL; Ctr ^b : 110 μ U/mL	Aboonabi <i>et al.</i> ³⁶
PJ-dose 1: 479.62 $\pm$ 164.7 mg/dL; PJ-dose 2: 415.75 $\pm$ 78.92 mg/dL; PJ-dose 3: 397.89 $\pm$ 214.8 mg/dL	PJ-dose 1: 130.65 $\pm$ 100.59 mg/dL; PJ-dose 2: 82.36 $\pm$ 39.04 mg/dL; PJ-dose 3: 144.14 $\pm$ 174.97 mg/dL	$\downarrow$ ^a In all doses of PJ with respect to the baseline	No	No	No	Prasetyastuti <i>et al.</i> ³²
nr	PJ (RDAC): 23.92 $\pm$ 2.38 mg/dL; PJ (RDS): 21.68 $\pm$ 1.95 mg/dL; Ctr: 30.52 $\pm$ 2.07 mg/dL	$\downarrow$ ^a Both types of PJ compared with Ctrl	No	No	No	Reynoso-Camacho <i>et al.</i> ³³
nr	PJ: 143.57 $\pm$ 0.57 mmol/L; Ctr: 189.22 $\pm$ 0.15 mmol/L	$\downarrow$ Compared with Ctrl	No	nr	PJ: 14.73 $\pm$ 0.81 μ u/mL; Ctr: 8.14 $\pm$ 0.15 μ u/mL	Chakraborty <i>et al.</i> ³⁵
nr	PJ: 143.57 $\pm$ 0.57 mmol/L; Ctr: 189.22 $\pm$ 0.15 mmol/L	$\downarrow$ Compared with Ctrl	No	nr	PJ: 15.39 $\pm$ 0.73 μ u/mL; Ctr: 9.21 $\pm$ 0.24 μ u/mL	Chakraborty <i>et al.</i> ³⁴
nr	PJ: 300.55 mg/dL; Ctr: 321.09 mg/dL	$\downarrow$ Compared with Ctrl	No	nr	PJ: 27.42 μ g/L; Ctr: 26.20 μ g/L	Taheri <i>et al.</i> ⁸
PJ: 417 $\pm$ 51 mg/dL; Ctr: 447 $\pm$ 37 mg/dL	PJ: 356 $\pm$ 24 mg/dL; Ctr: 535 $\pm$ 54 mg/dL	$\downarrow$ ^a PJ with respect to the baseline	No	No	No	Virgen-Carrillo <i>et al.</i> ⁹

As shown in Table 3, in the case of PJ, it is suggested that a mechanism involved in the hypoglycemic action of the juice is related to the absorption of carbohydrates mediated by the inhibition of intestinal enzymes and the interaction with glucose transporters. Studies with PJ have compared the juice effect with the effect of the drug acarbose by its action in enzymatic inhibition.¹⁷ The literature in this area is very heterogeneous and scarce. In an *in vitro* assay, a commercial PJ inhibited α -glucosidase enzyme in a similar manner to acarbose, used as a reference for the inhibition of this enzyme.⁶ Nevertheless, another assay reported noninhibition by a nonreported variety of PJ against α -glucosidase and α -amylase, while the same authors reported

that other pomegranate derivatives as seed and flower extracts showed a potent effect in the inhibition of the enzymes.⁵ In addition, it has been proven that when the main polyphenols from PJ are isolated, there is a higher inhibition effect.⁶

The ability of PJ to inhibit the enzyme dipeptidyl peptidase-4 (DPP-4) in a similar manner to sitagliptin⁶ was demonstrated. This enzyme is related to the metabolism of glucose by degrading the incretin hormones GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide), which stimulate insulin secretion. Therefore, the inhibition of DPP-4 increases the half-life of incretins, prolonging the duration of their effects.⁴¹

TABLE 3. CHARACTERISTICS OF THE *IN VITRO* METHODS AND THE EFFECT OF POMEGRANATE JUICE

Study hypothesis	Variety of Punica granatum	Extraction of juice	Quantification of phenolic compounds	Enzyme inhibition assays/cell culture	Outcome IC_{50}	References
Effect of PJ on oxidative status, insulin release and, insulin cell content in β -cells	Wonderful	POM wonderful commercial juice	No	β TC3 cells treated with 50 μ M of PJ	Reduction in oxidative stress; significantly increased on insulin release; no effect on insulin content	Koren-Gluzer <i>et al.</i> ⁷
Effect of different pomegranate parts, including PJ, on porcine pancreatic α -amylase and rat intestinal α -GLU activities	nr	Manual extraction, including arils (seeds+pulp)	No	α -amylase; α -GLU	Reported NI	Kam <i>et al.</i> ⁵
Interaction of PJ polyphenols with enzymes related to obesity and DM2	nr	Rabenhorst® commercial juice	No	Lipase; α -GLU; DPP-4	Lipase: 2.50 mg/mL; α -GLU: 1.01 mg/mL; DPP-4: 0.96 mg/mL	Les <i>et al.</i> ⁶

α -GLU, α -glucosidase; β TC3, β -tumor cell line; DPP-4, dipeptidyl peptidase-4; NI, no inhibition.

FIG. 1. Flowchart of the selection process for the articles included in the systematic review.

Adapted from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med* 2009;6:e1000097.

In cell cultures of a β -tumor cell line, it has been demonstrated that POM Wonderful commercial juice and punicalagin, a polyphenol present in pomegranate, significantly increased insulin release from cells, but showed no effect on insulin content.⁷

DISCUSSION

The functional effects of pomegranate and its derivatives have been attributed to the phenolic compounds^{5,6,8,9}; it is known that their presence, types of compounds, and the quantity are elements influenced by diverse factors, such as the agriculture and the processing type to obtain the by-products of the fruit. In addition, when the pomegranate has been used experimentally, it has been demonstrated that polyphenols are affected by biological factors of the organism, such as the microbiota.¹⁷ Through this review, we searched for scientific evidence that has addressed the effect of PJ, trying to identify in the publications all of those factors that could determine or modify the hypoglycemic response of phenolic compounds, as well as the suggested mechanism of action for this effect.

We identified that the evidence about the effects of PJ on glucose levels was quite heterogeneous. Among clinical trials, we found diversity of health conditions and a wide range of ages between the participants, while in the *in vivo* studies, all rodents were induced to hyperglycemia. Characteristics of the juice, as the variety of the fruit and the method of extraction, are determinants of the amount of phenolic compounds.^{12–16} In most human studies, this information was scarcely reported, while in rodent studies, the extraction method was widely reported, and only in a few cases, the variety of the fruit was reported. Reporting both factors is important to provide context to the observed polyphenol content, as the biological action hypothesized in the studies will be attributed to these compounds. In the cited studies, the variety of pomegranate and the amount of phenolic compounds were reported; it was impossible to determine which variety had more of these compounds, since the measurements between the studies were different.

Regarding the actions related to juice, despite the diversity of doses used, effects on serum glucose levels were not overwhelming to conclude a hypoglycemic effect, although some studies have reported significant changes in glucose levels.^{9,21,29,32,33,36,37} This does not mean that phenolic compounds do not have an effect. *In vitro* studies, although scarce, mostly attributed positive biological effects on glycemic related processes. It was reported that Wonderful PJ and punicalagin, a polyphenol widely present in pomegranate, significantly increased insulin release from cells.⁷ This agrees with the humans findings, in which an increase in β -cell function was demonstrated in diabetic patients who ingested PJ.²¹ The ability of the juice compounds to inhibit enzymes related to the absorption of carbohydrates has been tested.⁶ A histopathological examination showed protection to pancreatic tissue of rodents exposed to PJ.^{8,9}

Studies with significant reductions in glucose levels were predominantly in rodent models. Of these findings, we can

mention that experimental control with rodents is better, because their food intake is limited to a balanced diet and free of other sources of compounds.

This level of experimental control is not possible in humans due to the differences in diet, health condition, and microbiota.^{42,43} It is important to consider those factors, as it was recently demonstrated that metabolites derived from the intake of phenols contained in the pomegranate can affect the carbohydrate metabolism.¹⁷ However, these metabolites depend on the gut microbiota of each subject. This could be an explanation for the heterogeneity between the results reported in human studies.¹⁷ In addition, the bioavailability of phenols, meaning the digested proportion, absorbed and metabolized, differs between each type. Its biological activity will not depend on its food concentration, but the chemical structure will determine the degree of absorption and the nature of its metabolites.⁴⁴ The intake of Wonderful PJ by humans was verified *in vivo*. The bioavailability of punicalagin, which was quickly converted to ellagic acid after ingestion, remaining at its maximum concentration for 1 h and continuing in this form in the systemic circulation for up to 5 h, to be subsequently converted to urolithins with the support of the gut microbiota, reaching its maximum concentration at 6 h, persisting up to 48 h in the circulation to be eliminated later in the urine.⁴⁵ It is suggested that the possibility of the functional effect of prolonged supplementation with PJ may be due to the final metabolites, urolithins, as these remain circulating in the body for a longer period of time, although it was observed that punicalagin was mainly responsible for the intestinal inhibition of the α -amylase enzyme.¹⁷

Glucose levels before exposure is another factor that could affect the hypoglycemic response of PJ. In human interventions, it has been suggested that the hypoglycemic response to PJ depends on the initial levels of FPG (fasting plasma glucose), as one of the studies observed a greater hypoglycemic response in patients with lower initial FPG.²¹ In the case of clinical trials, all reported basal and final glucose levels.^{17,21–31} However, these data were only reported in two of the animal studies,^{9,32} apparently without considering that diverse factors such as animal species, strain, age, and gender, as well as the dose and route of administration, influence the chemically pancreatic damage.⁴⁶ This in turn could interfere with the effect of the phenolic compounds in the following manner: if the basal level of glucose in rodents is very high, it means that pancreatic damage is too. Thus, the increase in secretion of insulin by the β cells,^{7,21} as the principal hypoglycemic mechanism of action suggested in PJ, could not be evident with severely damaged pancreas. Therefore, it is important to express basal glucose levels.

Regarding the safety in the intake of PJ, no side effects were reported with the acute and subchronic use of pomegranate in rats⁴⁷; and no adverse effects have been reported for human juice consumption,^{17,23} even when it has been consumed for up to 3 years.³¹ It is necessary to investigate the consumption behavior and its acceptance, due to the organoleptic characteristics of acidity and astringency that

characterize it,⁴⁸ which could also affect its intake over time and the dose needed to evidence its hypoglycemic effects. Studies in humans did not report these aspects, while in animal models, one study exposed rodents freely to PJ consumption to evaluate the acceptance through behavioral consumption.⁹

It is relevant to point out the growing research in the study of herb-drug interaction as another option for therapy of chronic diseases such as diabetes mellitus, as this interaction may be able to affect the pharmacokinetics and pharmacodynamics of both. Recently, it has been suggested that PJ can interact with hypoglycemic drugs such as tolbutamide and nateglinide to increase their effect, through inhibition of cytochrome P450 (CYP) 2C9 by PJ and the subsequent potentiation of these drugs, which are principally metabolized in the liver by these proteins.^{34,35} However, it is unknown if this effect is dependent on factors such as the variety of the pomegranate and the phenolic compounds.

Finally, although it has been demonstrated that the potential mechanisms of hypoglycemic action by PJ *in vivo*: the increase in the function of β -cell,²¹ a significantly lower activity of α -amylase enzyme, protection against DNA damage,³⁷ protection of pancreatic tissue^{8,9}; and *in vitro*: an increase in insulin secretion,⁷ inhibition of the enzyme α -glucosidase and DPP-4,⁶ in similar way to common hypoglycemic drugs; we consider that determining the health potentials of polyphenols contained in the pomegranate, in particular the hypoglycemic action of the juice, and its prompt pharmaceutical incorporation, is limited for multiple reasons: the type of crop, the harvest, the variety of the fruit, the parts of the fruit used to extract the juice and the method of extraction; the individual characteristics of the organism; the dose administered and the bioavailability of these compounds are some of the factors mentioned in this review, so it is not surprising the difficulty to establish, with the previous *in vivo* and *in vitro* studies, the functionality of PJ. Overall, the results of this review suggest the necessity for further experimentation, using controlled variables and factors, and testing the effect of PJ under similar experimental conditions.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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