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Dyslipidemic effect of aqueous and ethanolic extracts of raw and roasted xoconostle (*Opuntia joconostle*) peel

[Efecto dislipidémico de extractos acuosos y etanólicos de cáscara cruda y asada de xoconostle (*Opuntia joconostle*)]

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Abstract: The aim of the present study was to assess the impacts of roasting and the type of extraction solvent (ethanol or water) on the hypolipidemic activity of xoconostle fruit peel extracts in a tyloxapol-induced model of hyperlipidemia. Water and ethanol extracts from raw and roasted *Opuntia joconostle* peels were obtained to quantify the phytochemicals contained within and assess their hypolipidemic activity in rats (n=5) against tyloxapol-induced dyslipidemia (400 mg/kg). The raw ethanol and water extracts, as well as the roasted water extract (200 mg/kg), showed hypolipidemic activity in the tyloxapol-treated group ($p < 0.05$). In contrast, the roasted sample extracted with ethanol did not show this effect. The concentrations of phenolic compounds (39.80 mg GAE/g) and flavonoids (16.42 ± 0.14 mg QE/g) were higher in the ethanolic extracts than in the aqueous extracts. Conversely, the concentration of betalains (115.51 ± 1.66 mg/100 g) was higher in the water extracts than in the ethanol extracts. It was concluded that the roasting process modified the concentration of some phytochemicals and their antioxidant capacity *in vitro*, producing a hypolipidemic effect in tyloxapol-induced hyperlipidemic rats.

Keywords: *Opuntia joconostle*; Tyloxapol; Hypocholesterolemic; Hypotriglyceridemic; Roasting peel

Resumen: El objetivo del presente estudio fue evaluar el impacto del tostado y del tipo de disolvente de extracción (etanol o agua) sobre la actividad hipolipidémica de los extractos de cáscara de frutos de xoconostle en un modelo de hiperlipidemia inducido por el tyloxapol. Se obtuvieron extractos acuosos y etanólicos de cáscara cruda y asada de *Opuntia joconostle* para cuantificar los fitoquímicos que contienen y evaluar su actividad hipolipidémica en ratas (n=5) contra la dislipidemia inducida por el tyloxapol (400 mg/kg). Los extractos acuosos y etanólicos crudos, así como el extracto acuoso tostado (200 mg/kg), mostraron actividad hipolipidémica en el grupo tratado con tyloxapol ($p < 0,05$). En cambio, la muestra asada y extraída con etanol no mostró este efecto. Las concentraciones de compuestos fenólicos (39,80 mg GAE/g) y flavonoides ($16,42 \pm 0,14$ mg QE/g) fueron mayores en los extractos etanólicos que en los acuosos. Por el contrario, la concentración de betalainas ($115,51 \pm 1,66$ mg/100 g) fue mayor en los extractos acuosos que en los etanólicos. Se concluyó que el proceso de asado modificó la concentración de algunos fitoquímicos y su capacidad antioxidante *in vitro*, produciendo un efecto hipolipidémico en ratas hiperlipidémicas inducidas por el tyloxapol.

Palabras clave: *Opuntia joconostle*; Tyloxapol; Hipocolesterolémico; Hipotrigliceridémico; Exfoliación

INTRODUCTION

Dyslipidemias occur when there is an imbalance in the serum lipid concentration and is characterized by hypercholesterolemia and hypertriglyceridemia (Real & Ascaso, 2021), which heightens the risk of cardiovascular diseases. In addition, elevated concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (c-LDL) contribute to atherogenesis (Douglas & Channon, 2014; Jamkhande *et al.*, 2014; Cabral & Klein, 2017) and increase the risk of a heart attack (Yusuf *et al.*, 2004). In Mexico, the prevalence of dyslipidemia in the population grew from 13% to 19.5% over 20 years (Shamah *et al.*, 2020).

Treatment strategies for dyslipidemia comprise pharmacological (FDA, 2015) and nonpharmacological methods, namely, medicinal plants (Bye & Linares, 2016; Alonso *et al.*, 2017; Aumeeruddy & Mahomoodally, 2022) that contain bioactive compounds or phytochemicals shown to reduce serum lipid levels, e.g., phenolic compounds, flavonoids, and betalains (Ramchoun *et al.*, 2012; Ma *et al.*, 2015; Tacherfiout *et al.*, 2018; Salazar-Gomez *et al.*, 2019; Yahaghi *et al.*, 2020). *Xoconostle* fruit (*O. joconostle*) is a good source of the previously mentioned bioactive compounds (Osorio-Esquivel *et al.* 2011; Morales *et al.*, 2012; Morales *et al.*, 2014). These fruits are used in Mexican culture to treat diabetes and dyslipidemias (Pimienta-Barrios *et al.*, 2008; Osorio-Esquivel *et al.*, 2011; Osorio-Esquivel *et al.*, 2012). Some studies have attributed their therapeutic effect to their content of phytochemicals (Paiz *et al.*, 2010; Osorio-Esquivel *et al.* 2011; Osorio-Esquivel *et al.*, 2012; Morales *et al.*, 2014; Medina-Perez *et al.*, 2019), which are extracted using solvents such as water, ethanol, and methanol (Tlili *et al.*, 2013; Boussahel *et al.*, 2015). When consumed, *xoconostle* fruits are usually cooked to increase palatability (van Boekel *et al.*, 2010; Sarkar *et al.*, 2011). However, heat may affect the concentrations of phytochemicals (Tiwari & Cummins, 2013; Cortéz-García *et al.*, 2015; De Santiago *et al.*, 2018) and their biological activity in the organism (van Boekel *et al.*, 2010; Sarkar *et al.*, 2011).

Animal models of tyloxapol-induced hyperlipidemia are used to evaluate phytochemicals with hypolipidemic activity and study cholesterol and triglyceride metabolism (Adeneye *et al.*, 2010; Irudayaraj *et al.*, 2010; Jo *et al.*, 2014; Rasouli *et al.*, 2016; Baldissera *et al.*, 2017; Salazar-Gomez *et al.*,

2019). Furthermore, tyloxapol augments the TC plasma concentration by promoting the hepatic synthesis of cholesterol through accelerated HMG-CoA reductase activity (Iqbal *et al.*, 2015) and modification of lipoprotein lipases, promoting an increase in the concentrations of triglycerides (TGs) in the blood (Irudayaraj *et al.*, 2013). The aim of the present study was to assess the impacts of roasting and the type of extraction solvent (ethanol and water) on the hypolipidemic activity of *xoconostle* fruit peel extracts in a tyloxapol-induced hyperlipidemia model.

MATERIALS AND METHODS

Plant material

Xoconostle fruits were collected in November 2021 from a pesticide-free crop located in San Martín de las Pirámides, Mexico State, Mexico (coordinates 19.712944, -98.836879).

Preparation of the extracts

The *xoconostle* fruit peels were removed with a stainless steel knife, cut into small pieces, and divided into two samples: one raw and the other roasted at 170-180°C for eight minutes (De Santiago *et al.*, 2018). Subsequently, 250 g of peel from both portions was macerated for 72 hours with 750 mL of ethanol (A and C) or water (B and D) to prepare the extracts. Later, the samples were filtered through filter paper and concentrated with a rotary evaporator (Rotavap), and the solid extract of each sample was obtained and refrigerated at 4°C until use.

Phytochemical screening

Solutions were prepared with 0.5 g of each extract dissolved in 30 mL of ethanol and utilized to perform a preliminary phytochemical analysis to determine the alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, and anthraquinones present according to previously published methods (Harborne, 1973; Raman, 2006; Rondón *et al.*, 2018).

Bioactive compounds

A solution was prepared at a ratio of 1:100 of solid extract (A, B, C, and D) to solvent (ethanol or water) to quantify the total contents of phenolic compounds (CFs) and flavonoids (FTs).

Total phenolic content

The Folin-Ciocalteu method was applied to assess the

total phenolic content as follows. First, 100 μL of each extract was mixed with 500 μL of Folin-Ciocalteu reagent and the mixture was left to stand for seven minutes. Later, 4 mL of 7% sodium bicarbonate was added to the samples, which remained at rest for two hours at room temperature. Finally, the absorbance was measured at 760 nm using a BioSpec-mini spectrophotometer (Shimadzu Corp., Japan). The total phenolic contents in the extracts are expressed as mg of gallic acid equivalents (GAE) per gram of sample dry weight (mg/g) and determined by using a gallic acid calibration curve.

Total flavonoid content

The total flavonoid content was determined using a solution of aluminum trichloride (AlCl_3) in 2% methanol. One milliliter of each of the xoconostle fruit peel extracts was mixed with 1 mL of AlCl_3 solution and left to stand in the dark for 10 minutes. The absorbance was measured in a BioSpec-mini spectrophotometer (Shimadzu Corp., Japan) at 415 nm. The total flavonoid content results are expressed in milligrams of quercetin equivalents per gram of fresh weight (mg EQ/g) of xoconostle peel and determined by using a quercetin calibration curve (Medina-Perez *et al.*, 2019).

Total betalain content

The total betalain (BT) content was analyzed with solutions consisting of 1 g of each extract (A, B, C and D) dissolved in 1 mL of water of ethanol and then diluted 1:100 according to the method described by Betancourt *et al.* (2017), with absorbance measurements at 538 nm and 483 nm in a BioSpec-mini spectrophotometer (Shimadzu Corp., Japan). The following equation was used to calculate concentration from absorbance: $B \text{ (mg/g)} = (\text{AxFDxPMxV})/(\epsilon\text{xPxL})$, where B corresponds to betacyanin (BC) or betaxanthin (BX), A is the absorbance at 538 nm for BC and 483 nm for BX, FD is the dilution factor at the time of reading in the spectrophotometer, PM is the molecular weight (betanin = 550 g/mol and indicaxanthin = 308 g/mol), V is the volume of the extract, ϵ is the molar extinction coefficient (betanin = 60 000 L/mol·cm, and indicaxanthin = 48 000 L/mol·cm) and L is the path length of the cuvette (1 cm) (Stintzing & Carle, 2004).

Compound analysis by high-performance liquid chromatography (HPLC)

To identify the phenolic compounds by HPLC, an Agilent Technologies 1260 Infinity HPLC with a Diode Array Detector (DAD) was used. The methodology consisted of using a 250 \times 4.0 mm Lichrospher RP18 column with a 5 μm particle size. The mobile phases were A (water with 1% acetic acid) and B (acetonitrile). Gradient elution was performed as follows (time and ratio of A:B): 0-30 minutes, 99:1; 30-33 minutes, 45:55; and 33-36 minutes, 99:1. The column temperature was maintained at 30°C, and the absorbance was measured at a wavelength of 350 nm. To identify the phenolic compounds present in the extract samples using this method, standards of phenolic compounds (chlorogenic acid, rutin, apigenin and quercetin) were injected prior to the analysis of the xoconostle extracts.

Antioxidant activity assays

DPPH assay

Samples were prepared by mixing 0.2 mL of each extract with 1.8 mL of 0.1 mM DPPH diluted with 80% methanol; the extraction solvents were used as blank controls. All samples were prepared in triplicate and left to stand at room temperature in the dark for 30 minutes. The absorbance of each sample was measured in a BioSpec-mini spectrophotometer (Shimadzu Corp., Japan) at 517 nm. Finally, the percent DPPH inhibition was calculated with Equation 1 (Noreen *et al.*, 2017):

$$(1) \quad \% \text{ DPPH inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

where:

- A control= absorbance of the control and
- A sample= absorbance of the reaction sample.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP reagent was prepared using a 10:1:1 ratio (v/v/v/v) of 300 mM sodium acetate at pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine diluted in 40 mM hydrochloric acid, and 20 mM ferric chloride. This solution was kept in the dark for 30 minutes.

Antioxidant activity was determined by mixing 0.1 mL of each extract with 2.9 mL of FRAP reagent and then placing each sample at 37°C for 30

minutes. The absorbance of each reaction sample was measured using a spectrophotometer at 593 nm; ethanol and water were employed as controls. All samples were prepared in triplicate (Noreen *et al.*, 2017). The final absorbance of each sample was compared with the calibration curve of the Trolox standard (0-200 g/mL). Data are expressed as μg Trolox eq/g of extract (Peñarrieta *et al.*, 2008).

In vivo study with experimental animals

Acute toxicity test

To evaluate the acute toxicity, male and female ICR mice with an average weight of 30.00 ± 4.00 g were used. The animals were maintained in a vivarium at 23-25°C on a 12/12 hour light-dark cycle and given ad libitum access to food and drinking water. The acute toxicity test was conducted according to OECD Guideline 423, which consisted of intragastric administration of the extracts at a single dose of 2000 mg/kg body weight to three mice of each sex. After administration, the animals were monitored every hour for seven hours and then once a day for 14 days. To detect changes in body weight and the appearance of toxic effects (skin, hair, eyes, membranes, or mucous membranes; tremors, convulsions, salivation, diarrhea, lethargy, drowsiness, coma; and affectations in circulatory, respiratory, or autonomic systems). After 14 days, all mice were sacrificed by cervical dislocation. The kidneys, liver, spleen, and heart were extracted for macroscopic evaluation.

Tyloxapol-induced hyperlipidemia model (Triton WR-1339)

The hypolipidemic effect of *xoconostle* peel extracts was evaluated in 30 female Wistar rats weighing 200 ± 50.00 g maintained at 23-25°C on a 12/12-hour light-dark cycle and receiving food and drinking water ad libitum. After one week of adaptation, they were randomly allocated to six groups (n=5). Group I was the negative control (saline solution as the vehicle), Group II to Group VI were induced hyperlipidemia with a single intraperitoneal dose of tyloxapol at 400 mg/kg (Mendieta *et al.*, 2014); Group III to Group VI were administered 200 mg/kg doses of each of the extracts via intragastric gavage 1 hour before and 22 hours after tyloxapol injection. Blood samples were taken by retro-orbital puncture 24 hours after tyloxapol administration, followed by sacrifice by cervical dislocation and collection of the liver for further analysis.

Determination of the serum lipid profile

The serum lipid profiles in the various groups of rats were analyzed. First, serum samples were obtained from blood samples centrifuged at 13000 rpm for 15 minutes; then, the concentrations of total cholesterol (TC), HDL cholesterol (c-HDL), and triglycerides (TGs) were measured using the reagents from certain kits and a Vitalab Selectra 2 analyzer (Wiener Lab., Amsterdam, The Netherlands) (Argüelles *et al.*, 2010). The levels of c-VLDL and c-LDL were calculated according to Equations 2 and 3 shown below (Friedewald *et al.*, 1972):

$$(2) \text{ c-LDL} = \text{TC} - (\text{c-VLDL} + \text{c-HDL})$$

$$(3) \text{ c-VLDL} = \text{TG}/2.21$$

Finally, the atherogenic index of plasma was determined with Equation 4 (Kanthé *et al.*, 2012):

$$(4) \text{ AIP} = \text{LDL}/\text{HDL}$$

Hepatic lipid peroxidation

Portions of 0.5 g of liver tissue from all specimens in each experimental group were added to 3 mL of PBS (phosphate-buffered saline) and homogenized with a LabGen 125 homogenizer (Cole Palmer, USA). The homogenate was centrifuged at 13000 rpm for 15 minutes at 18°C. Then, 500 μL of supernatant was mixed with 500 μL of 20% trichloroacetic acid (TCA), and the samples were incubated for 30 minutes on ice and centrifuged at 13000 rpm for 5 minutes at 20°C. Afterward, 500 μL of the supernatant was combined with 500 μL of 0.7% thiobarbituric acid (TBA), and the mixtures were incubated at 95°C for 15 minutes and cooled on ice (Beuret *et al.*, 2005). The absorbance of each sample was measured at 535 nm, and the data are expressed as malondialdehyde (MDA) concentration (mol MDA/g tissue) calculated with an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (Buege & Aust, 1978).

All *in vivo* studies were approved by a bioethics committee with approval number ENCB/CEI/036/2022.

Statistical analysis

The *in vitro* assays were performed in triplicate. All results are reported as the means \pm standard errors. Statistical analyses were performed with GraphPad Prism version 9.1.1 (GraphPad Software, Inc., USA).

Two-way ANOVA with Tukey's post hoc test was applied to analyze the concentrations of bioactive compounds and the antioxidant capacity determined by FRAP assay. Additionally, the nonparametric Kruskal-Wallis test with Dunn's post hoc test was used to assess the antioxidant activity data obtained from the DPPH assay. Ultimately, a one-way ANOVA with Dunnett's post hoc test was performed to examine the murine model results; a p value ≤ 0.05 was considered to indicate statistical significance.

RESULTS

Xoconostle fruit peel extraction yields

The xoconostle fruit peel weighed 13.95 ± 0.70 g,

representing $16.31 \pm 0.55\%$ of the total weight of the fruit. The percent yield from each of the extracts was D (2.99%) > B (2.53%) > A (2.38%) > C (2.32%), with no significant differences ($p > 0.05$).

Phytochemical screening

The presence of phenols and flavonoids was qualitatively observed in all extracts. Alkaloids, quinones, anthraquinones, steroids, and triterpenoids were present only in extracts B, A and C (Table No. 1).

Table No. 1

Phytochemical screening of ethanolic and aqueous extracts of raw and roasted *Opuntia joconostle* peel

Phytochemical	Tests	A	B	C	D
Alkaloids	Mayer	-	-	-	-
	Hager	+	+	+	-
Flavonoids	Shinoda	-	-	-	-
	Mg	+	-	+	-
	NaOH	+	+	+	+
Saponins	Frothing	-	-	-	-
Quinones y antroquinones	NH ₄ OH	+	+	+	-
	H ₂ SO ₄	-	-	+	-
Steroids / Triterpenoids	Salkowski	+	+	+	-
	Liberman	+	-	+	-
Phenols	FeCl ₃	+	+	+	+

A: Ethanolic fresh extract, B: Aqueous fresh extract, C: Ethanolic roast extract, D: Aqueous roast extract, (+) = presence, (-) = absence

Effect of roasting on the bioactive components of the extracts from the xoconostle fruit peel

There were greater concentrations of CFs and FTs in the ethanol extracts ($p < 0.05$). Conversely, the concentration of betalains (BTs) was lower in these extracts ($p < 0.05$) (Table No. 2). Roasting decreased the concentrations of CFs and FTs in the ethanol extracts by 1.78% and 5.02%, respectively, although

this result was not significant ($p > 0.05$). In water extracts, the concentration of CFs was reduced by 10.38% and the concentration of TFs increased by 50.02%. Moreover, roasting increased the extraction of BTs (43.16%) and BXs (25.42%) ($p < 0.05$); however, roasting reduced the levels of BCs (13.9%) and BXs (11.09%) ($p < 0.05$) in the water extracts (Table No. 3).

Table No. 2

Bioactive compounds in ethanolic and aqueous extracts from raw and roasted *Opuntia joconostle* peel

Extract	Phenols (mg GAE/g)	Flavonoids (mg QE/g)	Betalains (mg/100 g)	
			Betacianins	Betaxantins
A	39.80 ± 0.78 ^a	16.42 ± 0.14 ^a	30.41 ± 0.37 ^a	32.75 ± 0.12 ^a
B	33.96 ± 0.16 ^b	4.27 ± 0.06 ^c	115.51 ± 1.66 ^c	83.85 ± 0.72 ^c
C	39.08 ± 0.57 ^a	15.61 ± 0.88 ^a	43.52 ± 0.34 ^b	41.07 ± 0.46 ^b
D	30.43 ± 0.55 ^c	6.40 ± 0.09 ^b	99.40 ± 0.53 ^d	74.51 ± 3.29 ^d

Data are represented as mean ± SEM; n=3, Two-way ANOVA, Tukey <0.05; values with different letters show a statistically significant difference between groups ($p < 0.05$)

A: ethanolic raw, B: aqueous raw, C: ethanolic roast, D: aqueous roast

Table No. 3

Effect of roasting on the concentration of bioactive compounds in ethanolic and aqueous extracts of roasted *Opuntia joconostle* peel

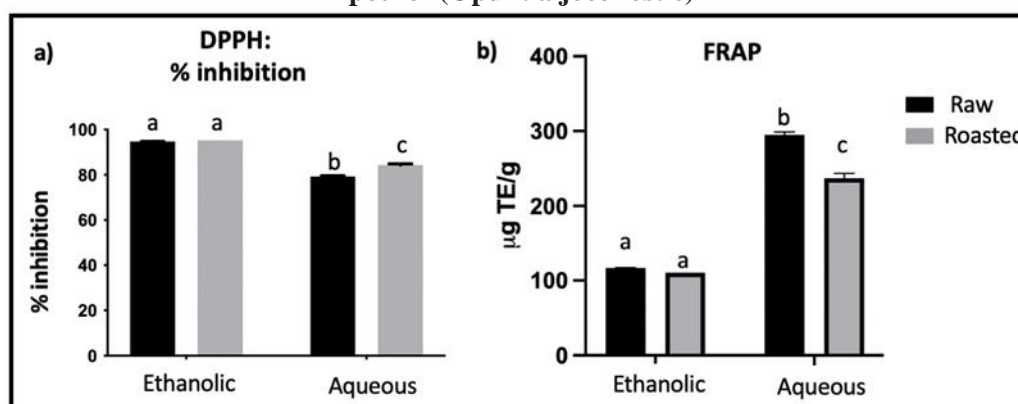
	Ethanolic extract	Aqueous extract
Phenols (mg GAE/g)	↓ 1.78 ± 1.23 %	↓ 10.38 ± 1.19 %
Flavonoids (mg QE/g),	↓ 5.02 ± 4.59 %	↑ 50.02 ± 2.66 %
Betacianins mg/100 g	↑ 43.16 ± 1.07 %	↓ 13.91 ± 0.88 %
Betaxantins mg/100 g	↑ 25.42 ± 1.74 %	↓ 11.09 ± 4.32 %

Data are represented as mean ± SEM; n=3

Table No. 3 and Figure No. 1 display the findings from the *in vitro* DPPH and FRAP antioxidant assays, which indicate that in the ethanolic extracts, both the percent inhibition and µg Trolox eq/g extract were higher than those in the water extracts (Figure No. 1). Extracts A and C presented inhibition percentages of 94.77 ± 0.03%

and 95.32 ± 0.00% and values of 116.94 ± 0.39 and 110.59 ± 0.03 µg Trolox eq/g, respectively, while extracts B and D had inhibition percentages of only 79.31 ± 0.34% and 84.34 ± 0.56% and values of 294.83 ± 4.00 and 237.05 ± 6.44 µg Trolox eq/g, respectively.

Figure No. 1

Antioxidant capacity evaluated with DPPH and FRAP in ethanolic and aqueous extracts of raw and roasted peel of (*Opuntia joconostle*)

Each bar represents the mean ± standard error, n=3, for % inhibition Kruskal Wallis was applied with Dunn's post hoc test; FRAP was analyzed with two-way ANOVA and Tukey, $p < 0.05$, means with different letters indicate statistically significant difference between groups

HPLC analysis of the phenolic compounds in the ethanolic and aqueous extracts of fresh and roasted xoconostle (*Opuntia joconostle*) peels.

Figure No. 2 shows the HPLC chromatograms corresponding to the ethanolic and aqueous extracts of raw and roasted *Opuntia joconostle* peels. The conditions used for the reversed-phase HPLC analysis of the xoconostle peel extracts allowed us to observe, in a general way, the presence of different peaks that coincided with the retention time and

wavelength of the previously analyzed phenolic compounds. Therefore, we can highlight the presence of compounds such as chlorogenic acid, rutin, quercetin and apigenin, as well as the unidentified predominant peak at a retention time of 15 minutes, whose height also varied according to the solvent and whether the sample was roasted but did not coincide with any of the retention times presented by the different phenolic compounds previously analyzed.

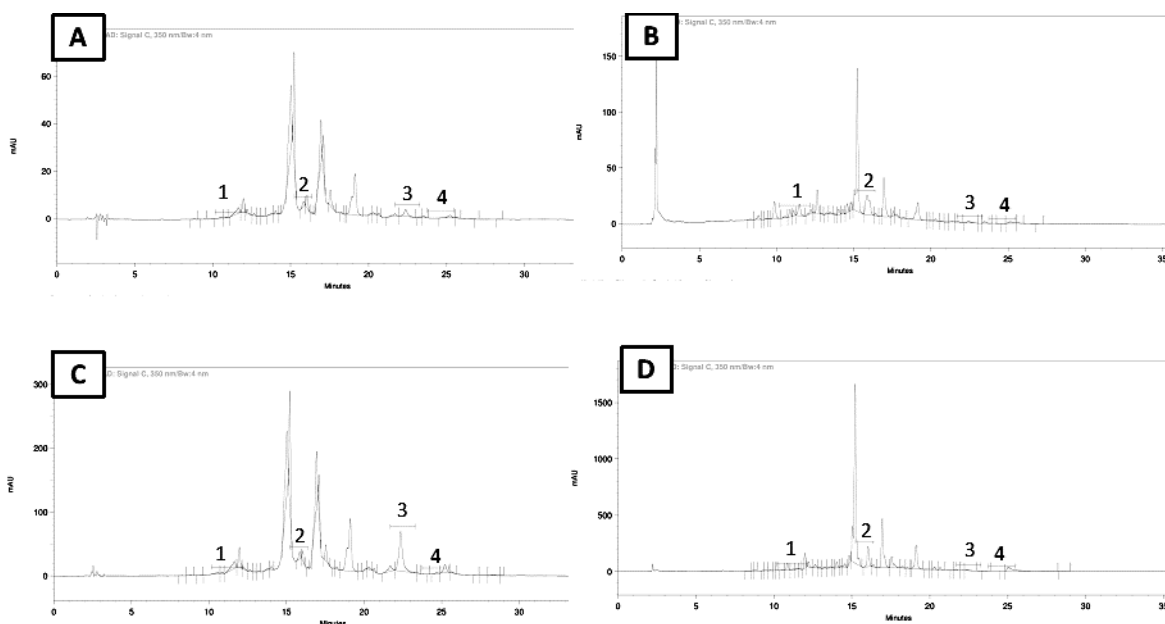


Figure No. 2

HPLC chromatograms of phenols in ethanolic and aqueous extracts of raw and roasted xoconostle (*Opuntia joconostle*) peel at wavelengths 350. 1. Chlorogenic acid; 2. Rutin, 3. Quercetin and 4. Apigenin.

Acute toxicity test

During the 14 days of the test, no abnormal behavior or lethality was observed in the mice administered a single dose of 2000 mg/kg xoconostle fruit peel extract. In addition, no significant pathological changes in the color or texture of the vital organs were detected by macroscopic examination. These data determined that the safe dose of the xoconostle fruit peel extracts might be >2000 mg/kg, classifying the extracts as category 5 based on OECD Guideline 423. Moreover, the therapeutic dose was taken as 200 mg/kg (1/10 of the lethal dose) to evaluate the hypolipidemic activity.

Effect of the xoconostle fruit peel extracts on blood lipid levels

After tyloxapol injection, the plasma concentrations of TGs and TC increased significantly in Group II compared to the negative control (Group I), indicating the effective induction of hyperlipidemia (Table No. 4A and Table No. 4B). Conversely, in groups treated with 200 mg/kg xoconostle fruit peel extracts (Group III to Group VI), the concentrations of TGs and TC decreased compared to those in Group II, mainly after the administration of A (82.71% and 90.99%, respectively), B (92.90% and 94.27%, respectively), and D (85.68% and 90.09%,

respectively). In contrast, no significant difference was observed in the reduction of TGs (5.69%) or TC (14.16%) between the C extract-administered groups and Group II ($p>0.05$).

The administration of tyloxapol did not affect the concentration of c-HDL but increased the concentrations of c-LDL and c-VLDL. Moreover, treatment with the *xoconostle* fruit peel extracts did not modify c-HDL levels. Extracts FE, FA, and AA reduced c-LDL concentrations by 90.19%, 99.09%, and 97.15%, respectively, and decreased c-VLDL concentrations by 82.73%, 92.91%, and 85.70%, respectively. Extract C had a nonsignificant effect on the concentration of these lipoproteins, augmenting c-LDL by 14.47% and diminishing c-VLDL by 5.80%.

Therefore, extracts A, B and D at a dose of 200 mg/kg significantly reduced the concentrations of TGs, TC, and c-LDL after 24 hours of treatment. Additionally, extracts A, B and D lowered the atherogenic index of plasma ($p\leq 0.05$) in rats with induced hyperlipidemia, promoting cardioprotection by reducing the probability of developing atherosclerosis. Hepatic lipid peroxidation analysis showed no significant differences between the groups, indicating that tyloxapol-induced dyslipidemia and treatment with extracts A, B, C and D did not cause excessive production of reactive oxygen species to promote an impaired antioxidant capacity in the tissues (Table No. 4A and Table No. 4B).

Table No. 4A
Triglycerides and total cholesterol in serum collected 24 hours after administration of tyloxapol and treated with the ethanolic and aqueous extracts of raw and roasted *Opuntia joconostle* peel

Treatment	Triglycerides (T) mmol/L		Total cholesterol (CT) mmol/L		HDL mmol/L	
Group I (Vehicle)	0.54 ± 0.06 ^a		1.46 ± 0.02 ^a		0.94 ± 0.01 ^a	
Group II (400 mg/kg tyloxapol)	22.39 ± 1.18 ^b		28.66 ± 3.83 ^b		0.86 ± 0.10 ^a	
Group III (400 mg/kg tyloxapol + 200 mg/kg A extract)	3.87 ± 1.18 ^a	↓ 82.71%	2.58 ± 0.54 ^a	↓ 90.99 %	0.76 ± 0.05 ^a	↓ 10.74 %
Group IV (400 mg/kg tyloxapol + 200 mg/kg B extract)	1.59 ± 0.37 ^a	↓ 92.90%	1.64 ± 0.16 ^a	↓ 94.27 %	0.74 ± 0.06 ^a	↓ 13.08 %
Group V (400 mg/kg tyloxapol + 200 mg/kg C extract)	21.12 ± 2.89 ^b	↓ 5.69%	32.72 ± 7.33 ^b	↑ 14.16 %	0.92 ± 0.14 ^a	↑ 7.94 %
Group VI (400 mg/kg tyloxapol + 200 mg/kg D extract)	3.20 ± 1.30 ^a	↓ 85.68%	2.84 ± 0.99 ^a	↓ 90.09 %	0.78 ± 0.10 ^a	↓ 9.34 %

Data expressed as mean ± SEM; n=5, one-way ANOVA and Dunnett's test for group II (dyslipidemic); different letters represent a statistically significant difference ($p<0.05$) with respect to group II (dyslipidemic); ↓: decrease, ↑: increase

Table No. 4B
Triglycerides and total cholesterol in serum collected 24 hours after administration of tyloxapol and treated with the ethanolic and aqueous extracts of raw and roasted *Opuntia joconostle* peel.

Treatment	LDL mmol/L		VLDL mmol/L		Atherogenic index (IA)	Lipoperoxidación hepática mol MDA/g tejido
Group I (Vehicle)	0.27 ± 0.08 ^a		0.24 ± 0.03 ^a		0.27 ± 0.07 ^a	302.98 ± 9.86 ^a
Group II (400 mg/kg tyloxapol)	18.92 ± 1.94 ^b		10.14 ± 0.53 ^b		15.54 ± 2.56 ^b	297.20 ± 26.83 ^a
Group III (400 mg/kg tyloxapol + 200 mg/kg A extract)	1.86 ± 1.67 ^a	↓ 90.19 %	0.92 ± 0.77 ^a	323.94 ± 14.92 ^a	0.92 ± 0.77 ^a	323.94 ± 14.92 ^a
Group IV (400 mg/kg tyloxapol + 200 mg/kg B extract)	0.17 ± 0.11 ^a	↓ 99.09 %	0.21 ± 0.13 ^a	308.46 ± 36.30 ^a	0.21 ± 0.13 ^a	308.46 ± 36.30 ^a
Group V (400 mg/kg tyloxapol + 200 mg/kg C extract)	21.66 ± 5.81 ^b	↑ 14.47 %	13.36 ± 3.36 ^b	344.68 ± 15.49 ^a	13.36 ± 3.36 ^b	344.68 ± 15.49 ^a
Group VI (400 mg/kg tyloxapol + 200 mg/kg D extract)	0.54 ± 0.49 ^a	↓ 97.15 %	0.87 ± 0.79 ^a	293.75 ± 28.67 ^a	0.87 ± 0.79 ^a	293.75 ± 28.67 ^a

Data expressed as mean ± SEM; n=5, one-way ANOVA and Dunnett's test for group II (dyslipidemic); different letters represent a statistically significant difference ($p < 0.05$) with respect to group II (dyslipidemic); ↓: decrease, ↑: increase

DISCUSSION

Phytochemical analysis showed the presence of phenols and flavonoids in the *xoconostle* fruit peel extracts A, B, C and D. Using ethanol as the solvent allowed the extraction of more CFs and FTs, while BT extraction was optimized when utilizing water. Roasting did not affect the concentrations of CFs and FTs in the ethanol extracts but diminished the concentration of CFs and augmented the concentration of FTs in water extracts. In addition, roasting contributed to a higher extraction of BCs and BXs compared to the raw *xoconostle* fruit peel. However, roasting reduced the levels of BC and BX in the water extracts. The variation in the areas under the curve of the peaks observed in the HPLC chromatograms shows that some thermal treatments, such as roasting, release more phytochemicals from the food matrix, making them more accessible after thermal processing. Thermal processing favors the breakdown of components (such as glycosidic bonds in the polysaccharides of dietary fiber) present in the

cell walls of vegetables, inducing the release of bioactive compounds from the food matrix and increasing the amounts of free compounds (Ramírez-Moreno *et al.*, 2013; De Santiago *et al.*, 2018). This could explain the variation in some peaks according to the solvent used (water or ethanol) and the thermal treatment to which the *xoconostle* peel was subjected. The antioxidant activity was higher in the ethanol extracts and remained unaffected after roasting.

The results of the oral acute toxicity test indicated that the DL₅₀ values of extracts A, B, C and D were >2000 mg/kg, which would label them as category 5 substances. In the hyperlipidemia model, injection of 400 mg/kg Triton WR-1339 (tyloxapol) promoted increases in TGs and TC in plasma serum compared to Group I. Rats with tyloxapol-induced hyperlipidemia treated with 200 mg/kg *xoconostle* fruit peel extracts A, B and D displayed a reduction in plasma TGs, TC and atherogenic index. In contrast, extract C did not have a significant impact on these variables compared to Group II. According

to the hepatic lipid peroxidation results, neither the tyloxapol-induced dyslipidemia model animals nor those treated with xoconostle fruit peel extracts A, B, C and D showed altered liver tissue.

Regarding the CF and FT concentrations in extracts A, B, C and D, the results from other studies differ from the present findings. For example, previous authors reported 2.23 ± 0.01 mg GAE/g p.f. and 0.84 ± 0.14 mg QE/g p.f. for CF and FT, respectively, after testing a methanol extract of xoconostle peel (*O. oligacantha* CF Först) (Medina-Perez et al., 2019). Furthermore, they determined concentrations of 2.07 ± 0.01 mg GAE/g p.f. CFs and 23.03 ± 0.029 mg betanins/100 g p.f. in a methanol extract (80%) of xoconostle (*Opuntia joconostle*) peel (Osorio-Esquivel et al., 2011). Correspondingly, after roasting, the xoconostle (*Opuntia joconostle*) mesocarp displayed an almost 20% reduction in CFs ($p \leq 0.05$), while the TF content remained without significant changes (Cortez et al., 2015). The hypolipidemic effects of extracts A, B and D were superior to the administration of 200 mg/kg of *R. alaternus* methanolic extract in a tyloxapol-induced hyperlipidemia model, which reduced the levels of TGs by 70% and TC by 60% (Tacherfiout et al., 2018). The administration of 100 mg/kg *T. angustifolia* lowered the concentration of TGs by 44% (Salazar-Gomez et al., 2019). On the other hand, the results of this study were similar to those reported for *T. atlanticus* aqueous extracts reducing TGs by 96.4% and TC by 82.8% ($p < 0.01$) (Ramchoun et al., 2012). As in this study, tyloxapol administration did not affect c-HDL concentration, nor did treatment with an aqueous extract of *Ocimum basilicum* (Amrani et al., 2006).

Compared to other plant extracts, the contents and composition of bioactive compounds (CFs and FTs) in extracts A, B, C and D might differ depending on the solvent used (Tlili et al., 2013; Boussahel et al., 2015). Nonpolar solvents more efficiently extract nonpolar compounds (lipids, aliphatic compounds, and carotenoids) and polar solvents more efficiently extract polar compounds (iridoids, flavonoids, and phenolic acids) (Ponomarev, 1976; Suchinina et al., 2011). Therefore, changing the polarity of the extraction solvent can influence the spectrum of extracted compounds and affect their biological activity (Suchinina et al., 2011). Accordingly, methanol and ethanol more effectively extracting CFs (Siddhuraju

& Becker, 2003). On the other hand, water is better for extracting betalains (BTs and BXs) (Herbach et al., 2006), which explains the higher content of BTs in the water extracts compared to the ethanol extracts (Siddhuraju & Becker, 2003).

Variations in bioactive compound quantity can be associated with the fruit genotype and growth environment, such as climatic conditions, growing location, and maturity stage (Nuutila et al., 2003; Vallejo et al., 2003; Naczk & Shahidi, 2006; Padilla et al., 2007; Singh et al., 2007; Sariburun et al., 2010; Hernández-Fuentes et al., 2015). The functions of flavonoids in plants include pigmentation, protection against ultraviolet light and microorganisms, and the regulation of enzymatic activity (Treutter, 2005). A study showed that the fruit peels of a xoconostle variety (*O. oligacantha* C.F. Först) contain higher levels of TFs (0.84 ± 0.14 mg QE/g) and CFs (2.23 ± 0.01 mg GAE/g) than other structures (pulp, seeds, and whole fruit) (Medina-Perez et al., 2019). These outcomes differ from the results obtained for extracts A, B, C and D, which might be related to the bioactive compounds' chemical structure, oxidation state, localization within fruit tissue, interactions with other compounds, and thermal processing (van Boekel et al., 2010).

The increase in the concentration of bioactive compounds in extracts from roasted xoconostle fruit peels may be a result of the greater release of phytochemicals from the matrix, since heating favors the breakdown of components in the cell walls of plants (such as glycosidic bonds in polysaccharides) (Ramirez-Moreno et al., 2013; De Santiago et al., 2018). Nevertheless, heat treatment also facilitates the degradation of these compounds, reducing their content in fruit structures (Zadernowski et al., 1999; Yen & Hung, 2000) depending on the temperature applied. For instance, Durmaz & Alpaslan (2007), roasted apricot (*Prunus armeniaca* L.) kernels at 150°C for 5, 10, 15, and 20 minutes and found that after 10 minutes, this treatment favored the loss of bioactive compounds. In addition, high temperature processes induce chemical reactions and accelerate the rate at which they occur (van Boekel et al., 2010). For example, roasting promotes nonenzymatic browning reactions, known as the Maillard reaction (Durmaz & Alpaslan, 2007), which occurs between the carbonyl group of sugars and the amino group of amino acids during food processing and storage. A large number of Maillard reaction products (PRMs)

affect the characteristics of foods, including their aroma, color, flavor, and texture (Fayle & Gerrard, 2002). PRMs have a phenolic-type structure; therefore, their content can be determined by the Folin-Ciocalteu method (Scalbert & Wilhamson, 2000), which explains the preservation of CF and FT concentrations in the *O. joconostle* peel ethanol extracts even after roasting. The fructose, glucose, and sucrose in the *xoconostle* fruit peels (Morales et al., 2014) enabled the Maillard reaction.

As stated in the DPPH and FRAP assay results section, both the percent DPPH inhibition and the μg Trolox eq/g extract values were higher for the ethanol extracts than the water extracts, demonstrating that the latter have lower free radical scavenging activity and ferric-reducing ability. Roasting did not affect the antioxidant capacity of the ethanol extracts but modified it in the water extracts. Research indicates that bioactive compounds such as polyphenols (Crozier et al., 2009), including flavonoids (Buratti et al., 2007) and betalains (Cai et al., 2003), provide plants with antioxidant properties by reducing free radical-mediated oxidative stress, resulting in positive effects on health. Consequently, a relationship can be established between the presence and concentration of bioactive compounds, the type of extraction solvent (ethanol or water), antioxidant capacity, and heat treatment in *xoconostle* peel extracts A, B, C and D. As mentioned above, roasting induces the Maillard reaction, from which reducing compounds are formed (Eichner, 1981; Hwang et al., 2001). Such compounds can donate H⁺ atoms that reduce the DPPH radical (Morales & Pérez, 2001), which explains the nonsignificant difference between extracts A and C ($p>0.05$).

Stabilizing flavonoids by adding a hydroxyl group or an alkyl or methoxy electron donor group to the ortho position enhances their antiradical capacity (Rice et al., 1995). Analogously, a study conducted in plants of the *Amaranthaceae* family demonstrated that the antioxidant potential of betalains is related to their structural characteristics, i.e., the free radical scavenging capacity of betaxanthins increased with the number of hydroxy and imino residues, whereas the antioxidant capacity of betacyanins decreased with glycosylation and improved with acylation (Cai et al., 2003). Another study reported that the antiradical activity of betalains was not exclusive to the presence of hydroxyl groups (Gandía et al., 2009). In betaxanthins, the presence of charges

derived from secondary amines reduced their antioxidant capacity (Gandía et al., 2010). Therefore, all of the bioactive compounds extracted from or conserved and produced by *Opuntia joconostle* peels are responsible for its antioxidant activity as evaluated by the DPPH and FRAP methodologies.

The hypolipidemic activity of *Opuntia joconostle* peel extracts was exhibited through the modified TG and TC plasma concentrations in tyloxapol-induced hyperlipidemic rats, indicating that extracts A, C and D could stimulate triglyceride hydrolysis by activating the lipoprotein lipase that was formerly inactive in response to tyloxapol administration and deactivation of HMG-CoA reductase. The effects of these extracts might be due to their flavonoid content since they reduced serum lipids in animal models of hyperlipidemia (drug-induced, diet-induced, and genetically induced) through several mechanisms, including downregulating the expression of intestinal Apo-B48 (Ma et al., 2015), inhibiting hepatic HMG-CoA reductase activity (Lee et al., 1999; Kuang et al., 2018; Khamis et al., 2017), reducing hepatic lipogenesis by suppressing the expression of SREBP-1 (sterol regulatory element binding protein), which is involved in activating enzyme genes that regulate fatty acid synthesis (Bao et al., 2016; Kuang et al., 2018), and promoting hepatic fatty acid oxidation (Chang et al., 2011; Mulvihill et al., 2011). Furthermore, studies have shown that betanin modifies lipid homeostasis in mice with hepatosteatosis (Yahaghi et al., 2020) by enhancing the expression of SREBP-1c (Shimano et al., 1999) and diminishing the expression of peroxisome proliferator-activated receptor (PPAR- α) (Kersten, 2014). Other authors reported that betaine acts as a methyl donor in one-carbon metabolism, including DNA methylation in the promoter region of the LPL gene at the cytosine bases of CpG dinucleotides, which correlates with transcriptional status and gene expression intensity (Xing et al., 2011).

Atherosclerosis is a pathological condition underlying several major adverse events, including stroke and coronary artery disease. Both are characterized by elevated plasma levels of TC, oxidative stress, and lipid accumulation (Douglas & Channon, 2014; Chavez-Sanchez et al., 2014). Increased c-LDL and decreased c-HDL levels play vital roles in the incidence of atherosclerosis (Duarte et al., 2009). The atherogenic index of plasma is an

indirect measure of c-LDL particle size and is also associated with the plasma concentrations of TGs and c-HDL (Arbogast & Dreher, 1987; Khot *et al.*, 2003).

Oxidative stress links hypercholesterolemia to atherogenesis through the formation of reactive oxygen species (ROS) in the vascular wall, mainly via c-LDL oxidation (Shi *et al.*, 2000). Some phytochemicals from plants can modulate oxidative stress conditions (Morales *et al.*, 2012), including hypercholesterolemic atherosclerosis. The evaluated extracts from xoconostle fruit peel influenced the atherogenic index of plasma with the exception of extract C, which could be due to the effects roasting and ethanol extraction had on the concentration of bioactive compounds, where minimal variation was observed in contrast to the A extract. The synergistic interaction between compounds from plant extracts or their multifactorial effects modify their biological activities (Wagner & Ulrich-Merzenich, 2009). Therefore, the hypocholesterolemic activity of extracts A, B, and D might be due to the enhancement of c-LDL catabolism through c-LDL hepatic receptors (Khanna *et al.*, 1992) and the stimulation of lipolytic activity of plasma lipoprotein lipase (LPL) (Shattat *et al.*, 2010).

Hypertriglyceridemia and hypercholesterolemia are associated with functional and structural changes in liver tissue, including increased levels of ROS (Yang *et al.*, 2008; Devaki *et al.*, 2013; Klisic *et al.*, 2018). Studies have shown that after intraperitoneal administration of tyloxapol in rodents, both enzymatic and nonenzymatic antioxidant capacities are affected (Oh *et al.*, 2006; da Rocha *et al.*, 2009). The endogenous antioxidant system comprises enzymes such as superoxidase dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), which can transform ROS into nontoxic substances, thus building the first line of defense against oxidative stress (Burton & Jauniaux, 2011). On the other hand, oxidative stress increases hepatic lipid peroxidation, which is generated by an

imbalance in the homeostasis of prooxidant and antioxidant mechanisms (Kumari & Menon, 1987). Consequently, lipid peroxidation induced by dyslipidemia can alter the physical properties of cell membranes (Engelmann *et al.*, 1992). However, in the present study, no hepatic damage was observed after 24 hours of tyloxapol treatment, probably due to the action of the endogenous antioxidant system coupled with the antioxidant capacity of the bioactive compounds, such as CFs, FTs, and BTs (Velioglu *et al.*, 1998; Sesso *et al.*, 2003; Vaya *et al.*, 2003; Girija & Lakshman, 2011), in the extracts, which prevented oxidative damage as indicated by lipid peroxidation.

CONCLUSION

Extracts A, B, C and D from *Opuntia joconostle* peel possess antioxidant and hypolipidemic activity.

ETHICAL ISSUES

All *in vivo* studies were approved by a bioethics committee with approval number ENCB/CEI/036/2022. Furthermore, all experiments were conducted in compliance with the Mexican Official Standard (NOM—062-Z00-1999) technical specifications for the production, care, and use of laboratory animals, and the protocol and use of the mice were approved by our institutional committee on animal care and use.

Oral acute toxicity assessments were carried out according to guideline No. 423 provided by the Organization of Economic Cooperation and Development (OECD) with slight modifications. This procedure was used to minimize the number of animals required for testing.

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