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Posted Date: 26 September 2025

doi: 10.20944/preprints202509.2109.v1

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Article

Effect of Guava Seeds on the Biochemical Parameters and Composition of HDL Subclasses in Ovariectomized Rats

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Abstract

Estrogen deficiency is associated with endothelial dysfunction, vascular inflammation, increased lipoprotein oxidation, accumulation of lipid-rich material, and platelet activation. The absence of estrogen causes physiological, metabolic, and biochemical changes that increase the risk of cardiometabolic disease development caused by a deregulation in metabolic processes such as lipid metabolism and plasma lipoproteins levels. High-density lipoprotein (HDL) has cardioprotective properties related to the quality and the quantity of its components that can be modified by some nutritional factors. Guava (*Psidium guajava* L.), a widely cultivated fruit in Mexico, is notable for its high polyunsaturated fatty acid and dietary fiber content in its seeds, but its effect on health is understudied. The study aimed to evaluate the effect of guava seed supplementation on body weight, blood pressure, lipid profile, HDL composition, and paraoxonase-1 (PON1) activity in an ovariectomized rat model (OVX). Four groups with 6 adults female Wistar rats each were classified as SHAM group: rats with simulated ovariectomy; OVX group: rats with ovariectomy; OVX+DGS group: rats supplemented with 6 g of defatted guava seeds; OVX+GS group: rats supplemented with 6 g of guava seeds. Biochemical parameters, size, and lipid concentration of HDL subclasses, apolipoproteins, and PON1 activity were determined. A decrease in body weight gain, systolic blood pressure, mean arterial pressure, and triglycerides in plasma was observed at the end of the experiment in the supplemented groups. The supplementation of 6 g of guava seeds for 30 days decreased biochemical parameters in ovariectomized rats; these results could be attributed to the seed composition, suggesting a protective effect against the risk of developing diseases in menopausal states.

Keywords: HDL subclasses; lipid profile; ovariectomized; menopause; guava seeds

1. Introduction

The substantial reduction and deficiency in circulating levels of estrogen affects almost all organ systems, including the cardiovascular, immune, and digestive systems. In this context, menopause is characterized by the depletion of the oocytes stored in the ovaries and the significant decrease in estrogen levels. Menopausal women experience significant physiological, metabolic and, biochemical changes such as an increase in total cholesterol, triglycerides and low-density lipoprotein-cholesterol (LDL-c), and a decrease in high-density lipoprotein-cholesterol (HDL-c) as well as the shift in the distribution of lipids composition [1] in this sense, estrogens and other ovarian hormones possess cardioprotective properties and it plays a critical regulatory role in glucose metabolism, lipid homeostasis, and inflammatory pathways [2,3], including insulin sensitivity, blood pressure, and prothrombotic processes [4].

The decrease in estrogen secretion leads to a reduced capacity of HDL to efflux cholesterol, which is mainly related to HDL core and surface lipid remodeling (free cholesterol, triglycerides, phospholipids, sphingolipids), which has been described in women during menopause, suggesting indirect effects of estrogen signaling pathways on the lipid profile [5,6]. The lipidome and proteome have also been described to be altered with estrogen deficiency; this could potentially compromise the other cardioprotective properties of HDL. Therefore, this suggests that HDL may become dysfunctional when circulating estrogen levels are reduced, such as in a state of menopause, affecting their quality more than the quantity of these lipoproteins [7,8]. On the other hand, some nutritional factors and bioactive compounds have been shown to influence the metabolism of estrogen, HDL structure and composition, and cardiovascular health [9,10], so lowering cardiovascular risk factors during and after menopause through diet intervention is possible.

Guava (*Psidium guajava* L.) is a fruit from the Myrtaceae family and is widely cultivated in Mexico, ranking third worldwide for production [11]. During the processing of this fruit, pulp is obtained as the main product and seeds and peels as by-products [12]. Due to the presence of bioactive compounds, the pulp and the by-products have the potential to be used in healthy processed foods [13]. Guava is notable for its high vitamin C content, as well as its seeds for their polyunsaturated fatty acid and dietary fiber content [14]. Guava seeds and oil have been characterized; however, their effect on health remains understudied. Significant improvements in lipid profile were also reported: total cholesterol, LDL-c, and triglycerides decreased, while HDL-c increased in diabetic mice supplemented with guava seed extract [15]. Also, guava seed oil has been shown to have antioxidant activity [16], anti-LDL peroxidation, and gram-negative bacteria inhibitory activity [17,18].

Therefore, in the present study, we aimed to evaluate the effect of guava seed supplementation on body weight, blood pressure, lipid profile, HDL composition, and PON1 activity in an ovariectomized rat model.

2. Materials and Methods

2.1. Animals

Twenty-four adults female Wistar rats weighing 200–250 g and aged 3 months were maintained under temperature-controlled conditions with light-dark cycles (12 h) and received commercial feed (LabDiet 5008) and water *ad libitum*. Four groups were formed, with $n=6$ experimental animals in each group, classified as follows: SHAM group: negative control, rats with simulated ovariectomy. OVX group: positive control, rats with ovariectomy. OVX+DGS group: rats supplemented with 6 g of defatted guava seeds. OVX+GS group: rats supplemented with 6 g of guava seeds. The treatment groups (OVX+DGS and OVX+GS) followed the same procedure as the OVX group and received a supplement of guava seeds prepared as described above and mixed with commercial LabDiet 5008 rat food in a 70-30% ratio (14 g of commercial food + 6 g of guava seeds) in pellet form for 30 days. The experimental protocols were carried out according to the guidelines for the protection of animals

used for scientific purposes (2010/63/EU) and national guidelines (NOM-O62-ZOO-1999) [19] and were approved by the Animal Ethics Committee of the Universidad Autonoma del Estado de Hidalgo(CICUAL/-V-I/02/2025).

2.2. Ovariectomy

To remove the ovaries, a dorsal ovariectomy was performed in which the rats were anesthetized for surgery by a combination of xylazine hydrochloride (2.5 mg/kg of body weight) and Zelazol® (10 mg/kg of body weight). To remove the ovaries, an incision was made on the left side of the vertebral column of the animal, the uterine horns were identified, fixed to the ovary at one end and to the uterus at the other. Ligatures were established on both sides of the ovary, sectioning and removing them. Once the ovaries were removed, the incision was sutured. The SHAM group underwent the same procedure, but the ovaries were not removed [20,21]. After surgery, meloxicam (0.3 mg/kg of body weight) was administered for 5 days for pain management.

2.3. Guava Seeds

For the supplement, ripe, undamaged guavas were obtained from a local market in the city of Pachuca, Hidalgo, which are harvested in the state of Michoacan. The fruits were washed and disinfected to remove the seeds. The guava seeds were washed with purified water to eliminate any pulp that could remain attached. Once clean, the guava seeds were air-dried at room temperature for 24 hours and ground in a commercial coffee grinder; a portion was used in the group OVX+GS and stored until use in darkness at room temperature.

2.4. Seed Delipidation

The rest of the ground seeds were used for lipid extraction with dichloromethane at a ratio of 1:10 (w/v), and the mixture was stirred at 100 rpm/35 °C for 8 hours. Following this, the mixture was centrifuged at 5000 rpm for 30 minutes to separate the solvent and then dried in an oven at 60 °C for 40 minutes to remove the solvent entirely, and stored for the group OVX+DGS.

2.5. Blood Pressure

Blood pressure levels were measured in the tail of rats after heating by a non-invasive method and recorded in a CODA TM system from Kent Scientific Corporation (Torrington, Connecticut), with at least six blood pressure recordings for each animal, and the data collected using CODA TM software for further processing.

2.6. Lipid Profile

Total cholesterol, triglycerides, and phospholipids levels in plasma, as well as HDL-c, HDL triglycerides (HDL-Tg), and HDL phospholipids (HDL-PPL) levels, were determined using commercial colorimetric enzymatic methods (Randox®, UK; Wako, Ltd, Osaka, Japan). Absorbance was read at a wavelength of 505 nm for cholesterol and triglycerides and 600 nm for phospholipids in a spectrophotometer (BECKMAN COULTER DU® UV/Vis Spectrophotometer, Germany). For the lipid composition of HDL, we used the phase containing the HDL fraction of plasma after ultracentrifugation, following the methodology described below.

2.7. HDL Size and Lipid Concentration of HDL Subclasses

HDL particles were separated via sequential ultracentrifugation in a (Beckman Optima TLX table) [22–25]. Total apo B-containing lipoproteins were obtained by density <1.063 mg/dL, whereas total HDL 1.063 <density <1.21 g/mL. The HDL were dialyzed against 0.09 M Tris/0.08 M boric acid/3 mM EDTA (TBE) buffer, pH 8.4. Then, HDL were separated further by their hydrodynamic diameters in non-denaturing 3–30% gradient polyacrylamide gel electrophoresis (PAGE). Gels were stained for total cholesterol, phospholipids, and triglycerides using enzymatic mixtures previously described

[26–28]. The electrophoresis gels were then scanned (GS-670 BioRad densitometer) and stained again for proteins with Coomassie blue R-250, before being scanned once more. The relative proportions of each HDL subclass were estimated via optical densitometry analysis, using as reference globular proteins (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase 10.4 nm; lactate dehydrogenase, 8.2 nm; and albumin, 7.1 nm; high-molecular-weight calibration kit, Amersham Pharmacia Biotech, Buckinghamshire, U.K.) [24,28]. The relative proportion of every HDL subclass is expressed as the percentage of the total HDL area under the curve, integrated from 7.9 to 12.36 nm. For the classification of the HDL subclasses, we considered the following size intervals: HDL3c, 7.94e8.45 nm; HDL3b, 8.45e8.98 nm; HDL3a, 8.98e9.94 nm; HDL2a, 9.94e10.58 nm; and HDL2b, 10.58e13.59 nm [28]. Enzymatic staining of total cholesterol, triglycerides, and phospholipids of HDL subclasses was performed according to the methodology previously described [27].

2.8. Determination of Apolipoproteins

Apolipoprotein (apo) composition was determined on an SDS-denaturing 4–21% PAGE gradient. Fifteen µg of previously isolated HDL protein was used. The bands of each apo were stained with Coomassie blue and destained with a solution of methanol, acetic acid, and water (30/58/12, v/v/v). The Aepos were identified by comparison with molecular mass standards and subsequently analyzed by optical densitometry. The results are expressed as the percentage of each apo relative to total HDL protein. apo CI and CII are expressed as a percentage of apo C [29].

2.9. Paraoxonase-1 Activity

PON1 was determined using the method described by Gan et al. (1991). The trial mixture included 1 mM phenylacetate as substrate and 0.9 mM CaCl₂ in 20 mM Tris HCl, pH 8, and 10 µL rat plasma heparin (diluted 1:10). Enzymatic hydrolysis of the substrate was measured spectrophotometrically at 270 nm (UVVIS Beckman Coulter, Brea, CA, USA). The absorbance at 270 nm for the reaction was 1310 M⁻¹cm⁻¹. Enzyme activity was expressed as the number of micromoles of phenylacetate hydrolyzed per minute per milliliter of plasma.

2.10. Data Analysis

Normal distribution of all data was verified using the Shapiro-Wilk test. Since these data were normally distributed, they are presented as mean ± standard deviation (SD). Differences between groups were determined using analysis of variance (ANOVA) and post hoc analysis (Tukey test). A p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 24 statistical software (SPSS Inc. IBM, Chicago, IL, USA).

3. Results

3.1. Biochemical Parameters

Table 1 shows a final body weight decrease of 50% in the OVX+GS compared to the OVX group. The OVX+DGS group showed a significant lower blood level than the OVX group. Concerning the results of the lipid profile, the OVX+DGS group had 50% less triglycerides and phospholipids than the OVX group in plasma, as well as 30% less than the OVX+GS group. In addition, there was a 70% increase in non-HDL-c in OVX+DGS (Table 1). Surprisingly, c-HDL (60%) was lower in OVX+DGS than in the other two groups (Table 2).

Table 1. Biochemical parameters post-supplementation in rats.

Parameter	SHAM	OVX	OVX+DGS	OVX+GS
Body weight gain (g)	28.00±9.57	63.00±25.16 ^c	57.20±23.73 ^d	32.83±3.82
Systolic blood pressure (mmHg)	145.20±10.60	166.94±9.35 ^b	138.89±17.78	149.38±10.80
Media blood pressure (mmHg)	121.30±6.50	144.31±10.35 ^{a,b}	102.42±21.22	122.70±12.01
Cholesterol (mg/dL)	37.01±8.42	40.88±5.59	33.77±2.84	32.42±6.18
Triglycerides (mg/dL)	49.42±14.62	55.63±10.04 ^{b,c}	28.72±5.25 ^d	37.83±6.95
Phospholipids (mg/dL)	115.46±15.67	134.29±19.88 ^{b,c}	80.37±12.88 ^d	99.81±8.06
Non-HDL-c (mg/dL)	9.15±6.90	7.56±3.92	15.64±1.17	4.86±4.09

± Standard deviation. *n*=6. SHAM: SHAM-operated; OVX: ovariectomized; OVX+DGS: ovariectomized+defatted guava seeds OVX+GS: ovariectomized+guava seeds. Tukey test. ^a*p*<0.05 vs SHAM; ^b*p*<0.05 vs OVX+DGS; ^c*p*<0.05 vs OVX+GS, ^dOVX+DGS vs SHAM.

Table 2. HDL lipid profile post-supplementation in rats.

HDL lipid profile	SHAM	OVX	OVX+DGS	OVX+GS
HDL-c (mg/dL)	27.86±4.59	33.32±2.42	18.13±2.31 ^{a,b,c}	27.55±6.90
HDL-Tg (mg/dL)	11.65±3.18	10.42±1.88	8.48±1.35	7.32±2.57
HDL-PPL (mg/dL)	58.80±13.86	64.71±4.38	52.06±5.31	61.66±12.68
HDL-c/HDL-PPL ratio	0.44±0.08	0.52±0.05	0.35±0.04 ^b	0.52±0.06
HDL-Tg/HDL-PPL ratio	0.18±0.09	0.16±0.04	0.17±0.03	0.14±0.04

± Standard deviation. *n*=6. SHAM: SHAM-operated; OVX: ovariectomized; OVX+DGS: ovariectomized+defatted guava seeds OVX+GS: ovariectomized+guava seeds. Tukey test. ^a*p*<0.05 vs SHAM; ^b*p*<0.05 vs OVX; ^c*p*<0.05 vs OVX+GS.

3.2. Size and Lipid Composition of HDL Subclasses

After 30 days of supplementation, significant changes were observed in the protein composition of subclass 2b in the OVX+DGS group compared to the SHAM group; a similar increase was noted in HDL3c subclass compared to the OVX+GS group (Table 3). Moreover, an increase in cholesterol was found in the DGS group in the small subclasses 3b and 3c, compared to the OVX group, and in 3c in comparison with the SHAM group.

Table 3. Size and lipid composition of HDL subclasses.

HDL subclasses	SHAM	OVX	OVX+DGS	OVX+GS
Protein (%)				
HDL 2b	40.60±3.32	46.56±2.08	48.12±1.64 ^a	43.19±5.06
HDL 2a	13.06±2.46	12.03±1.20	13.26±2.12	12.23±2.28
HDL 3a	16.68±2.00	14.39±1.06	14.84±1.21	15.10±2.11
HDL 3b	8.25±0.92	8.23±0.73	7.48±1.21	8.29±1.14

HDL 3c	19.00±3.10	18.51±1.74	16.31±1.07	21.19±3.75 ^c
Cholesterol (%)				
HDL 2b	40.53±1.36	43.46±2.33	36.82±1.60	39.74±2.93
HDL 2a	12.09±0.53	11.77±0.44	11.04±1.38	11.79±0.82
HDL 3a	17.05±1.42	15.65±2.10	19.71±4.05	17.19±1.63
HDL 3b	9.24±0.80	8.42±1.19	11.38±1.91 ^b	9.77±1.29
HDL 3c	21.09±3.20	20.69±2.26	27.18±1.30 ^{a,b}	23.83±2.52
Triglycerides (%)				
HDL 2b	34.90±3.37	36.94±2.34	39.53±3.08	36.50±2.75
HDL 2a	11.13±1.13	11.15±0.65	11.75±0.53	10.58±1.28
HDL 3a	17.63±2.57	17.29±1.58	16.53±0.44	17.05±1.42
HDL 3b	9.49±0.41	9.28±0.95	9.00±0.54	9.63±1.19
HDL 3c	26.84±1.90	25.33±2.93	23.37±2.62	26.25±3.05
Phospholipids (%)				
HDL 2b	41.34±5.21	40.54±3.64	39.70±0.94	41.42±6.00
HDL 2a	10.87±0.76	10.39±0.78	11.97±1.06	10.01±0.88 ^c
HDL 3a	16.57±2.18	15.64±1.03	17.21±0.39	15.47±1.34
HDL 3b	8.99±1.01	9.27±0.80	8.92±0.74	9.00±0.78
HDL 3c	22.24±2.29	24.79±1.64	22.20±0.93	24.11±3.49

± Standard deviation. *n*=6. SHAM: SHAM-operated; OVX: ovariectomized; OVX+DGS: ovariectomized+defatted guava seeds OVX+GS: ovariectomized+guava seeds. Tukey test. ^a*p*<0.05 vs SHAM; ^b*p*<0.05 vs OVX; ^c*p*<0.05 vs OVX+GS.

3.3. Apolipoproteins

The apolipoprotein composition in the experimental groups is listed in Table 4, showing a significant decrease in apo E content and a significant increase in Apo C concentration in the OVX+DGS group compared to the other test groups.

Table 4. Apolipoprotein percentage of the total HDL protein.

Apolipoproteins	SHAM	OVX	OVX+DGS	OVX+GS
Apo AIV	19.79±1.53	18.22±3.64	22.76±3.13	21.01±3.12
Apo E	19.92±3.26	18.95±1.85	13.70±3.38 ^{a,b,c}	21.68±3.33
Apo AI	44.17±3.20	45.60±5.38	41.79±7.65	39.93±8.10
Apo AII	8.71±1.12	8.99±1.58	8.34±3.28	9.18±1.30
Apo C	5.89±0.90	6.75±0.88	13.41±5.03 ^{a,b,c}	8.20±0.93

± Standard deviation. *n*=6. SHAM: SHAM-operated; OVX: ovariectomized; OVX+GS: ovariectomized+defatted guava seeds OVX+GS: ovariectomized+guava seeds. Tukey test. ^a*p*<0.05 vs SHAM; ^b*p*<0.05 vs OVX; ^c*p*<0.05 vs OVX+GS.

3.4. PON1 Activity

Finally, we determined the antioxidant activity of PON1. Interestingly, we found a significant decrease in the OVX+DGS group compared to the other test groups. Likewise, there was a significant decrease in the OVX+GS group compared to the SHAM group (Figure 1).

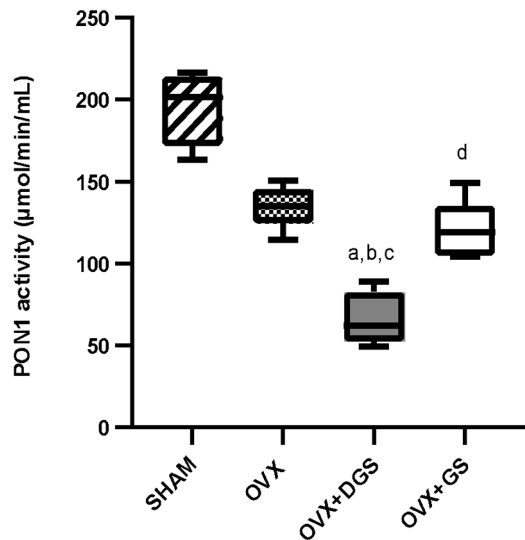


Figure 1. Paraoxonase-1 activity in rat plasma. SHAM (*n*=6): SHAM-operated; OVX (*n*=6): ovariectomized; OVX+DGS (*n*=6): ovariectomized+defatted guava seeds OVX+GS (*n*=6): ovariectomized+guava seeds. Tukey test. ^a*p*<0.05 vs SHAM; ^b*p*<0.05 vs OVX; ^c*p*<0.05 vs OVX+GS, ^dOVX+GS vs SHAM.

4. Discussion

In the present study, we determined the effect of guava seed consumption in an ovariectomized animal model by simulating the biological processes due to a reduction in estrogen levels. In this model, estrogen depletion is related to metabolic effects and significant changes in body composition, including visceral fat accumulation, lipid metabolism disorders, and increased blood pressure, factors that significantly influence the high risk of developing cardiovascular diseases (CVD) [31].

An increase in body mass was observed in ovariectomized rats; nevertheless, a lower increase in body weight was observed in supplemented rats. We suggest that polyunsaturated fatty acids could be responsible for the reduction in weight gain, given that the group supplemented with defatted seeds did not show significant differences compared to the ovariectomized group without supplementation.

Any of the components of GS, including insoluble dietary fiber, may have an impact on improving blood pressure, but the mechanisms are not totally clear. Nevertheless, it has been suggested that these mechanisms could include a reduction in inflammation levels [32,33] and an improvement in endothelial function [34]. Additionally, cellulose has been shown to inhibit starch digestion by binding to α -amylase [35], reducing glucose absorption, enhancing insulin sensitivity, and, as a result, lowering the risk of hypertension [36].

Diseases characterized by increased inflammatory processes, such as cardiovascular diseases, are associated with changes in the lipidome of HDL, particularly a decrease in HDL phospholipid content and an increase in HDL triglycerides [37]. Plasma lipid depletion in rats from the OVX+DGS and OVX+GS groups suggests a reduction in hepatic cholesterol and triglyceride synthesis [38], as well as an increase in intestinal transit by insoluble fibers, which can create a physical barrier and, as a result, a decrease in lipid absorption [39].

A larger number of small HDL particles and their cholesterol efflux capacity could be related to the lowest HDL-c levels in the OVX+DGS group. There is controversy regarding whether the larger particles (HDL2) or the smaller ones (HDL3) are more atheroprotective. It has been suggested that the large HDL subclass is more prone to oxidative modification with respect to the small subclasses [40], whereas HDL3 plays a central role in the reverse cholesterol transport (RCT) by removing cholesterol from the periphery and maturing into HDL2 particles through progressive lipidation by the action of lecithin: cholesterol: acyltransferase (LCAT) [41].

Apolipoproteins are the most abundant group of proteins in HDL. Apo E is considered an atheroprotective protein because it removes more saturated than unsaturated lipids, and atherogenic plaques are rich in saturated lipids and cholesterol [42]. Furthermore, HDL particles containing apo E promote the efflux of cholesterol from extrahepatic cells [43] through ABCA1- and ABCG1-dependent processes, and this process is antagonized by the presence of apo CIII, which can negatively affect the antiatherogenic properties of HDL [44,45].

It has been shown in some animal models that PON1 can prevent the harmful effects of oxidative stress on serum and that serum levels of PON1 correlate with levels of HDL and apolipoprotein AI, although this correlation is not strong. We might suggest that increased PON1 activity is a consequence of higher HDL levels. In our study, the increase in PON1 activity in SHAM rats can be attributed to estrogens, given that estradiol enhances PON1 activity [46]. In addition, a pro-oxidative environment could lead to an increase in the binding of free radicals to PON1, resulting in a less active enzyme in the circulation [47]. It has been reported that dietary factors have a significant effect on PON1 activity, specifically polyunsaturated fatty acids [48], which could be associated with higher activity in the OVX+GS group compared to the OVX+DGS group. Furthermore, according to the observed results of apo and PON activity, the OVX+DGS group could be a population at risk for the development of cardiovascular disease.

Differences shown between OVX+DGS and OVX+GS groups are related to the lipid composition of the seed; when supplementing with a defatted seed, the protective properties previously described were lost indicating that such properties are associated with the polyunsaturated fatty acids [49] and probably other lipidic molecules contained in the whole seed, such as phytosterols [50], that has been reported its use on health, owing to its cholesterol-lowering and anti-inflammatory effects [51,52]. Studies on biomodels fed a high-fat diet have proven the efficacy of supplementation with defatted seeds such as safflower [53] (50-100 mg/kg of weight/day) and poppy [54] (33% fiber and 27% protein) on plasma and hepatic lipid levels, reducing triglyceride and cholesterol levels. In addition, it has been described that defatted grape seed affects the 3T-L1 pre-adipocyte cells, showing a significant decrease in lipid accumulation, possibly due to a regulation of the mRNA expression of leptin and lipoprotein lipase (LPL), both of which have been demonstrated to regulate lipid metabolism [55].

5. Conclusions

The supplementation of guava seeds during 30 days limited the gain of body weight, blood pressure, triglycerides, and non-HDL-c in ovariectomized rats. These results could be attributed to lipidic components and insoluble dietary fiber in the seed, suggesting a discrete protective effect against the risk of developing diseases during a physiological cessation of estrogen secretion.

Author Contributions: Conceptualization: E.C.-T., D.E.-L.; investigation, E.C.-T., D.E.-L., L.M.R.-M. and A.C.-O.; writing—original draft preparation, E.C.-T., D.E.-L., L.M.R.-M.; writing—review and editing, E.C.-T., D.E.-L., A.C.-O., Ó.P.-M. and E.F.-M.; funding acquisition, D.E.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The experimental protocols were approved by the Animal Ethics Committee of the Autonomous University of the State of Hidalgo (CICUAL/-V-I/02/2025).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Special gratitude to the university's animal facility employees for their support in the animal handling and surgery processes.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HDL	High-density lipoprotein
PON1	Paraoxonase-1
LDL	Low-density lipoprotein
SHAM	SHAM-operated rats
OVX	Ovariectomized rats
DGS	Defatted guava seeds
GS	Guava seeds
HDL-c	HDL cholesterol
HDL-Tg	HDL triglycerides
HDL-PPL	HDL phospholipids
Apo	Apolipoprotein
SD	Standard deviation
CVD	Cardiovascular diseases
RCT	Reverse cholesterol transport
LCAT	Lecithin: cholesterol: acyltransferase
E2	Estradiol
LPL	Lipoprotein lipase

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