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Pharmacological evaluation of *Prosopis ruscifolia* extract on lipid profile in hyperglycemic and hyperlipidemic mice

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ABSTRACT

Diabetes is a serious chronic pathology, with long-term effects including damage to blood vessels or diabetic dyslipidemia. Diabetic dyslipidemia is characterized by increasing concentrations of low-density triglycerides and lipoproteins and a decrease in high-density lipoproteins HDL-cholesterol (HDL-c). This study aimed to evaluate the effect of *Prosopis ruscifolia* on lipid profile in albino Swiss mice with hyperglycemia and hyperlipidemia. Hyperglycemia was induced by alloxan and the animals were orally treated with Pr (50, 100, and 200 mg/kg) for 45 days. Hyperlipidemia was induced with tyloxapol and the animals were treated with Pr (50, 100, and 200 mg/kg). In hyperglycemic animals treated with 100 mg/kg, there was a decrease in the concentration of cholesterol, a decrease in the concentration of triglycerides, and an increase in HDL-c at the end of treatment compared to untreated hyperglycemic animals. In mice with hyperlipidemia treated with 50 and 100 mg/kg of Pr, serum cholesterol and triglyceride concentration were reduced. HDL-c increased in animals treated with Pr 50, 100, and 200 mg/kg compared to untreated animals. It was observed that the administration of *P. ruscifolia* in hyperglycemic and hyperlipidemic animals had a favorable effect on the lipid profile.

1. INTRODUCTION

Diabetes is a serious chronic disease and a global health problem that affects more than 400 million people. Each year, over 1.6 million deaths occur as a result of diabetes or associated pathologies, such as cardiovascular diseases, deterioration of kidney function, dyslipidemia, and others [1]. Diabetes is a metabolic disorder that affects different parts of the body, with short- and long-term consequences, such as ketoacidosis due to hyperglycemia or diabetic coma. Chronic exposure to hyperglycemia causes an imbalance in cellular homeostasis which then leads to damaged blood vessels, among other effects [2,3]. All this implies a great expense in public health systems [1,4].

Cardiovascular disorders in diabetic patients involve atherosclerosis, which causes broken atherogenic plaques to pass into the circulation to obstruct a vessel and induce myocardial infarction or stroke [5]. This constitutes the main cause of morbidity and mortality in people with diabetes, and one of the predominant risk factors is dyslipidemia. These patients generally present a pattern characterized by high concentrations of triglycerides and low-density lipoproteins (LDL) LDL-cholesterol (LDL-c) and a reduction in high-density lipoproteins (HDL) HDL-cholesterol (HDL-c) called atherogenic dyslipidemia [2,5].

Type 2 diabetes mellitus (T2D) is mostly associated with being overweight or obese. Patients who suffer from it, in addition to presenting altered glucose metabolism, also suffer from alterations in lipid metabolism. Obesity, characterized by excessive accumulation of abdominal and visceral fat, is associated with insulin resistance and hyperinsulinemia. Due to this insulin resistance, there is a greater release of free fatty acids from adipocytes, which in the liver induce an increase in the synthesis of triglycerides and stimulate the production of apolipoprotein B. An increase in the production of triglyceride-rich very low-density lipoprotein (VLDL) particles is also observed, which increases the concentration of triglycerides in the blood [5,6]. This increase in VLDL causes an increase in the hepatic protein cholesterol ester transfer protein, which transfers cholesteryl esters from HDL to

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apolipoprotein B containing particles VLDL and LDL, reducing HDL concentration. There is transfer of triglyceride in the opposite direction such that cholesteryl ester-depleted HDL and LDL become triglyceride-rich. Levels of small dense LDL are thus increased [7,8].

Treatment of diabetes includes changes in lifestyle, diet, increased physical activity, and decreased carbohydrate intake. Additionally, patients with T2D are treated with an oral hypoglycemic drug. Historically, herbal medicines have also been utilized in the treatment and prevention of diseases such as diabetes [9]. Several medicinal plants have been shown to be effective in different stages of diabetes.

Previously, this research group determined the hypoglycemic effect of *Prosopis ruscifolia* in Wistar rats and in Swiss albino mice, in addition to a favorable effect on the cardiovascular risk index and atherogenic risk index in hyperglycemic mice [10,11]. This medicinal plant was also demonstrated to protect liver and kidney from the damage induced by acetaminophen and gentamicin, respectively [12]. Other species of *Prosopis*, such as *Prosopis farcta*, demonstrated a favorable effect on the decrease of glycemia and blood lipid concentrations in models of diabetic animals. *Prosopis cineraria* also proved to have favorable effects on glycemia and lipidemia [13–15]. This work reports the effect of the *P. ruscifolia* extract on the lipid profile in a mouse model with experimental hyperglycemia by alloxan in an observation period of 45 days and in hyperlipidemic mice by tyloxapol.

2. MATERIAL AND METHODS

2.1. Plant Material and Extract

Aerial parts of *P. ruscifolia* Griseb (Fabaceae), named locally viñal, were collected in Teniente Irala Fernández, Chaco, Paraguay, identified by experts, and deposited in the FCQ herbarium (Degen 4581 herbarium specimen). First, the extract of the leaves was obtained by refluxing with ethanol, then preserved in a desiccator, and resuspended in water for use [10].

2.2. Drugs and Reagents

Ethanol was purchased locally and distilled before use. The lipid profiling kits used were from the HUMAN brand. Alloxan and Triton WR-1339 (tyloxapol) were from Sigma-Aldrich Co. (St. Louis, MO). Blood glucose test strips were from HUMAN.

2.3. Experimental Animals

Swiss albino mice, male and female, 10–12 weeks old, weighing 25–35 g were obtained from the Bioterium of the Department of Pharmacology of the Facultad de Ciencias Químicas, UNA. They were kept in polypropylene boxes in the Bioterium, with a light/dark cycle of 12/12 hours, in a controlled environment with a temperature of 20°C–24°C and relative humidity of the environment of 60 ± 5%. The animals received food ration and water *ad libitum*.

2.4. Induction of Hyperglycemia by Alloxan

Male animals were selected and randomly assigned into eight groups (n = 6). Four groups were induced to hyperglycemia by alloxan monohydrate (150 mg/kg, i.p.), after having been deprived of food for 18 hours. After induction, they received free food and a 10% glucose solution overnight. One week after induction, the fasting glucose concentration was determined. Blood from the tail vein was obtained for fasting blood glucose measurement with a glucometer. Mice with a glycemia greater than 180 mg/dl were considered hyperglycemic and treated for 45 days with water (Hv), 50 (HPr 50), 100 (HPr 100), or 200 mg/kg (HPr 200) of the *P. ruscifolia* extract. Additionally, four groups of normoglycemic animals were treated for 45 days with water (Nv), 50 (NPr 50), 100 (NPr 100), or 200 mg/kg (NHPr 200) of the *P. ruscifolia* extract. On day 46, after 6 hours of fasting, the animals were anesthetized with an i.p. injection of sodium pentobarbital (40 mg/kg) to obtain blood through cardiac puncture. Thus, the blood of each animal was obtained, and the obtained blood was incubated to promote the formation of the clot and the separation of the serum by centrifugation of the samples. Next, this serum was fractionated to measure the lipid profile, according to instructions for HUMAN reagents. The glycemia of the animals was monitored every 15 days [16,17]. Very low-density lipoprotein cholesterol (VLDL-c) and LDL-c were calculated using Friedewald's equation. VLDL-c = serum triglyceride/5; LDL = TC − VLDL-c − HDL-c. Results were expressed in mg/dl. Atherogenic index (AI) was calculated as LDL-c/HDL-c and coronary risk index (CRI) was calculated as TC/HDL-c.

2.5. Induction of Hyperlipidemia in Male and Female Mice

To induce elevation of cholesterol and triglycerides in male and female Swiss albino mice, i.p. injection was used. Tyloxapol (Triton WR-1339) was injected in doses of 350–400 mg/kg, according to sex. Animals were randomly assigned to one of eight work groups ($n = 6$). Four groups of female hyperlipidemic animals received water (Hv), 50 (HPr 50), 100 (HPr 100), or 200 mg/kg (HPr 200) of the *P. ruscifolia* extract, 48, 24, and 1 hours before injection of tyloxapol, and 24 hours after induction, the animals were anesthetized with an i.p. injection of sodium pentobarbital (40 mg/kg) to obtain blood through cardiac puncture [16]. Similarly, four groups of female animals with normal lipemia received water (Nv), 50 (NPr 50), 100 (NPr 100), or 200 mg/kg (NHPr 200) of the extract of *P. ruscifolia*, 48, 24, and 1 hours before tyloxapol injection, and 24 hours after induction, blood was obtained by the same procedure already mentioned. The same procedure was performed with four groups of hyperlipidemic male animals with tyloxapol and four normolipemic animals. The extraction of samples from the mice was carried out after 6 hours of fasting in all cases.

2.6. Ethical Issues

The animals were considered as biological reagents; therefore, the work was carried out in accordance with the standards established by the Ethics Committee of the European Commission. The handling of the biological reagents was carried out by standardized procedures in accordance with the Principles of Laboratory Animal Care. The minimum number of animals required for each trial was used, and the shortest observation duration was required to obtain consistent data. Each animal was employed once [18]. The protocol was submitted for consideration by the Research Ethics Committee of the Facultad de Ciencias Químicas, UNA, and approved (CEI 470/19).

2.7. Statistical Analysis of the Results

The GraphPad Prisma 7.0 program was used and the data from the various groups were expressed as means ± standard deviation. The statistical analysis used was one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test; a level of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

First, the effect of *P. ruscifolia* extract on the glycemia of normo- and hyperglycemic mice was verified, after chronic treatment for 45 days in eight groups of male mice ($n = 6$): Nv (normoglycemic treated with vehicle, water); NPr 50, NPr 100, and NPr 200 mg/kg (normoglycemic treated with the extract, 50, 100, and 200 mg/kg, resp.); Hv (hyperglycemic, vehicle-treated water); HPr 50, HPr 100, and HPr 200 mg/kg (hyperglycemic treated with the extract, 50, 100, and 200 mg/kg, resp.). A statistically significant difference was established between the initial glycemia of the animals in group Hv and those in group Nv, which was maintained during the 45 days of treatment. In addition, it was found that the glycemia of the animals in the Nv group remained unchanged and the glycemia of the hyperglycemic animals, Hv, remained high throughout the treatment (Table 1).

3.1. Effect of *P. ruscifolia* on Normal and Hyperglycemic Animals the Lipid Profile

In all the groups of animals with normal glycemia, it was observed that there was no significant difference in any parameter of the lipid profile between those that received vehicle or the extract. The hyperglycemic animals that received water presented a significant difference in the cholesterol level with respect to the normoglycemic (Nv: 103.1 mg/dl ± 8.4; Hv: 130.5 mg/dl ± 20.03, $p < 0.001$), and in the groups of hyperglycemic animals treated with 100 mg/kg (HPr 100: 108.7 mg/dl ± 5.78, $p < 0.1$), the cholesterol level was significantly different with respect to the control group Hv. In addition, a nonsignificant reduction was observed in the group HPr 50. So, a certain hypocholesterolemic effect of the extract in hyperglycemic animals was demonstrated (Fig. 1A).

In the group of Hv animals (113.5 mg/dl ± 39.8, $p < 0.01$), the triglyceride level was significantly higher compared to the groups of Nv animals (70.5 mg/dl ± 11.5). The animals in the HPr 50 and HPr 100 groups showed a significant decrease compared to the Hv group (HPr 50: 55.00 mg/dl ± 4.15, $p < 0.0001$; HPr 100: 46.58 mg/dl ± 3.80, $p < 0.0001$) after treatment with the extract of *P. ruscifolia*. This indicated that the extract of *P. ruscifolia* has a lipid-lowering effect in hyperglycemic animals due to alloxan (Fig. 1B).

Table 1: Effect after 45 days oral treatment of normo- and hyperglycemic mice with *P. ruscifolia* leaves extract.

Group	Initial glycemia	Final glycemia
Nv	153.0 ± 17.30	105.2 ± 13.51
NPr 50	167.2 ± 17.72	126.0 ± 17.74
NPr 100	157.7 ± 21.89	142.8 ± 12.56
NPr 200	179.5 +/− 20.47	128.5 ± 22.34
Hv	432.0 ± 22.14	278.3 ± 48.87***
HPr 50	448.5 ± 32.27	341.0 ± 61.93****
HPr 100	479.3 ± 8.43	395.3 ± 31.87***
HPr 200	578.0 +/− 15.89	No data

Data are expressed as mean ± SD, after one-way ANOVA, Tukey's posttest.
***$p < 0.001$; ****$p < 0.0001$ en relación con Nv.

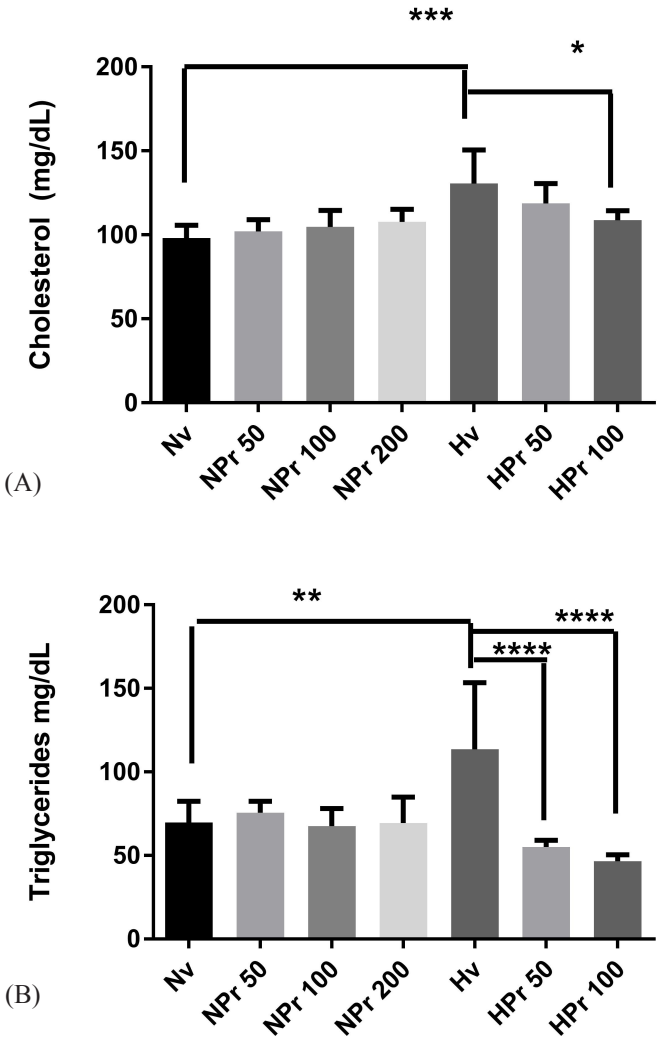

Figure 1: Cholesterol (A) and triglyceride (B) levels after 45 days of oral treatment with *P. ruscifolia* extract in normal and hyperglycemic mice. Data are expressed as mean ± SD, after one-way ANOVA, Tukey's posttest; *$p < 0.1$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Regarding HDL-c, the group of hyperglycemic animals treated with the vehicle, Hv (31.00 mg/dl ± 2.68), presented a difference in the Nv group (30.3 mg/dl ± 2.4). Interestingly, in the HPr 50 and HPr 100 groups, a significant elevation was observed (84.1 mg/dl ± 10.7 and 32.9 mg/dl ± 5.4, resp.), so it is presumed with these doses a cardioprotective effect (Fig. 2A).

In the hyperglycemic animals that received the vehicle, the LDL-c level was 72.5 mg/dl ± 10, significantly different from the Nv (61.9 mg/dl ± 8.13). Those hyperglycemic animals treated with

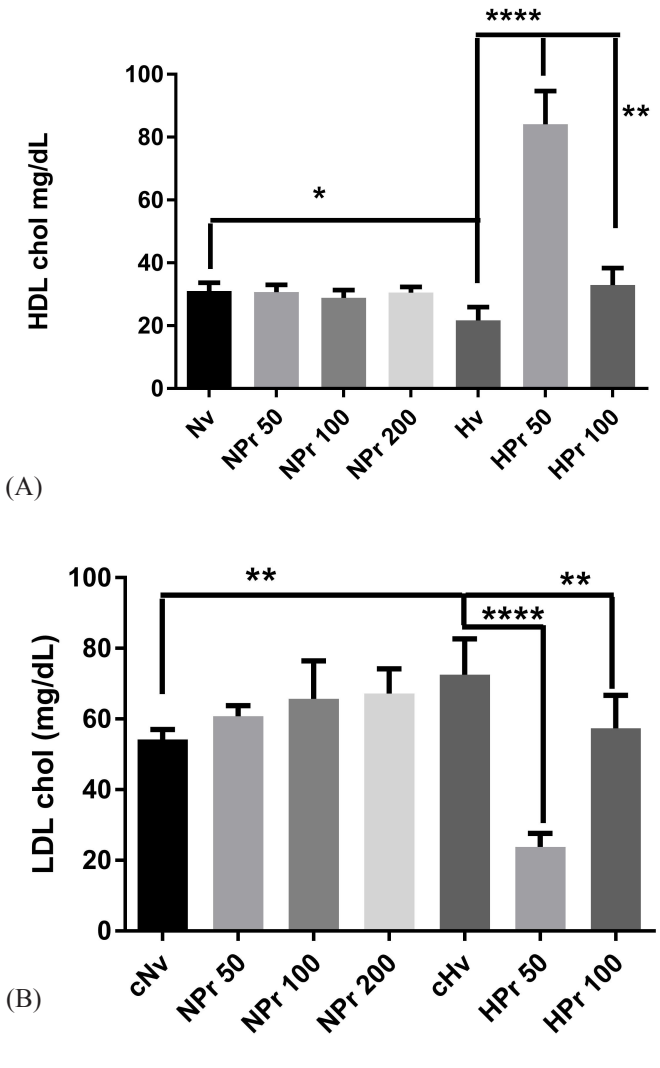

Figure 2: HDL (A) and LDL (B) cholesterol level after 45 days of oral treatment with *P. ruscifolia* extract, in normal and hyperglycemic mice. Data are expressed as mean ± SD, after one-way ANOVA, Tukey's posttest; **p* < 0.1; ***p* < 0.01; *****p* < 0.0001.

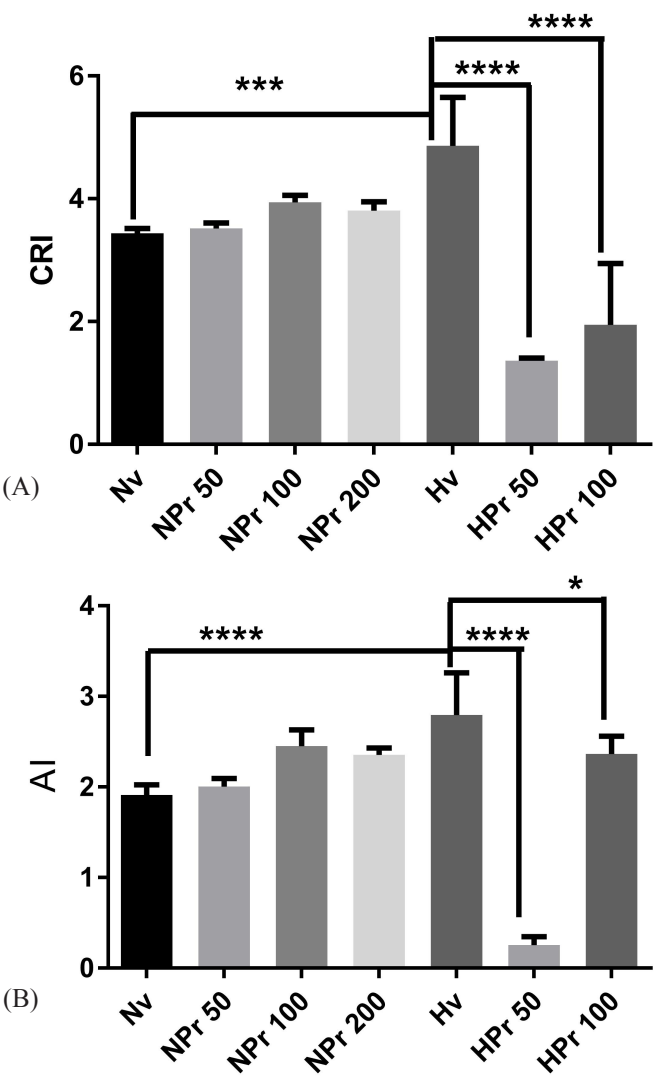

Figure 3: CRI (A) and AI (B) after 45 days of oral treatment with *P. ruscifolia* extract, in normal and hyperglycemic mice. Data are expressed as mean ± SD, after one-way ANOVA, Tukey's posttest; **p* < 0.1; ****p* < 0.001; *****p* < 0.0001.

the extract of *P. ruscifolia* HPr 50 and HPr 100 (23.8 mg/dl ± 3.8 and 57.3 mg/dl ± 9.3, resp.) presented a significant decrease in the concentration of cholesterol – LDL (Fig. 2B). In addition, a significant difference was observed in the level of VLDL-c between the groups HPr 100 (8.95 mg/dl ± 0.78 $p < 0.01$) and Hv (13.67 mg/dl ± 2.01). Additionally, the CRI and AI were calculated, and the results are shown in Figures 3A and 3B (CRI: HPr 50 1.36 ± 0.043; HPr 100 1.95 ± 0.99; both $p < 0.0001$. AI: HPr 50 0.26 ± 0.92, $p < 0.0001$ and HPr 100 2.37 ± 0.195, $p < 0.1$). Both showed a significant reduction, indicating a cardioprotective effect in the animals that were treated with the extract.

3.2. Effect of *P. ruscifolia* on the Lipid Profile of *Male* Mice with Hyperlipidemia Induced by Tyloxapol

For the evaluation of the effect of the extract of *P. ruscifolia* on the lipid profile of mice, 8 groups of male mice ($n = 6$) were used, and four groups were induced hyperlipidemia with tyloxapol. Among the animals that were not induced, Nv, NPr 50, NPr 100, and NPr 200, no difference was observed between the lipid profile parameters measured. On the other hand, the animals that were induced to hyperlipidemia with tyloxapol (Hv) presented an elevation in the concentration of cholesterol and triglycerides, compared to the noninduced ones (Table 2). The animals treated with the extract of *P. ruscifolia* had significantly reduced cholesterol level (Hv 50) and triglyceride level. However, between the values, there was no statistically significant difference (Hv 50 and Hv 100), compared to the group without treatment. Between the group of hyperlipidemic mice, Hv, and the normolipemic mice, Nv, no statistically significant difference was found in HDL-c. Nevertheless, the treated animals of the HPr 50 and HPr 200 groups showed a significant increase in this parameter (Table 2).

Table 2: Effect of *P. ruscifolia* leaves extract on lipid profile, in tyloxapol-induced hyperlipidemia in male and female mice.

Group	Cholesterol mg/dl	Triglycerides mg/dl	HDL–Chol mg/dl
		Male	
Nv	97.5 ± 9.80	77.67 ± 10.65	24.83 ± 4.31
NPr 50	105.8 ± 24.24	97.50 ± 13.40	25.83 ± 5.57
NPr 100	108.5 ± 9.65	93.00 ± 18.81	24.83 ± 6.21
NPr 200	114.5 ± 21.59	81.33 ± 12.86	23.33 ± 3.67
Hv	256.5 ± 116.9****	1192 ± 938.5*	28.00 ± 7.62
HPr 50	146.3 ± 24.86 *	788.7 ± 921.8	40.67 ± 8.94*
HPr 100	180.0 ± 79.09	1423 ± 581.2	31.00 ± 4.29
HPr 200	230.5 ± 67.05	883.3 ± 463.0	39.83 ± 3.13*
		Female	
Nv	81.00 ± 9.01	115.8 ± 51.44	21.00 ± 1.27
NPr 50	84.50 ± 8.87	102.5 ± 38.98	21.50 ± 3.21
NPr 100	85.17 ± 11.60	98.33 ± 40.52	22.00 ± 6.72
NPr 200	82.50 ± 6.09	77.17 ± 30.75	20.33 ± 1.86
Hv	251.7 ± 26.89****	1651 ± 464.7****	8.50 ± 3.08***
HPr 50	153.2 ± 42.47**	402.7 ± 330.5***	13.33 ± 3.93
HPr 100	151.8 ± 52.57**	976.7 ± 622.1	20.67 ± 5.76***
HPr 200	234.0 ± 99.96	987.7 ± 940.9	17.67 ± 6.12*

Hv was compared with Nv; HPr 50, HPr 100, and HP 200 was compared with Hv.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

3.3. Effect of *P. ruscifolia* on the Lipid Profile of Female Mice with Hyperlipidemia Induced by Tyloxapol

As in the male mice, the female mice that were not induced to hyperlipidemia with tyloxapol, Nv, NPr 50, NPr 100, and NPr 200, did not show changes in any parameters of the lipid profile measured. In this group of female mice, there was a significant elevation of cholesterol and triglyceride levels and a significant decrease in HDL-c (Table 2). After the acute treatment with the extract of *P. ruscifolia* (HPr 50 and HPr 100), the groups of female mice presented a lower concentration of cholesterol in serum, compared to the Hv group. In the same way, the serum triglyceride concentration was significantly reduced when they were treated with 50 mg/kg, and with the other doses, although a marked difference is observed, it was not significant. Finally, all the hyperlipidemic groups treated with the extract increased the concentration of HDL-c, and a statistically significant difference was demonstrated between the HPr 100 and HPr 200 groups, compared to the untreated group, Hv (Table 2).

Animal models for the study of diabetes and hyperlipidemia have been widely used [19, 20]. Alloxan produces a diabetogenic effect through two mechanisms, the inhibition of insulin, and the formation of reactive oxygen species, which lead to selectively necrosis of pancreatic beta cells [21]. The nonionic detergent Triton WR-1339, tyloxapol, produces hyperlipidemia because the inhibition of lipoprotein lipase activity [22,23] produces an elevation of plasma VLDL with a rapid decrease in HDL without altering the albumin concentration. In addition, it reduces the activity of liver acyl-Co A cholesterol acyltransferase and two liver enzymes more associated with lipogenesis with dehydrogenase activity, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. It has also been observed that the lipemia of these animals was elevated, with the presence of lipid vacuoles in the liver tissue, loss of elastic tissue fibers in the aorta, and marked DNA fragmentation in the brain, which indicates that the effect of tyloxapol goes further than just raising lipids concentrations [23,24].

In our study, a marked increase in glycemia was observed in the groups of animals induced with alloxan [21] compared to the noninduced groups and these levels remained high after treatment with *P. ruscifolia* when compared to the control group Nv (Table 1). In previous works, we have shown that the extract of *P. ruscifolia* leaves has a hypoglycemic effect at 28 days of treatment [10,11]. Other researchers demonstrated the hypoglycemic effect of the bark of *P. cineraria* after a treatment period of 45 days [14] and of the leaves after treatment for 12 weeks [15]. It should be noted that previous studies in our research group were carried out with animals with more moderate hyperglycemia and in one of these studies, Wistar rats were used [10,11]. In this work, hyperglycemia was very severe, above 500 mg/dl, and could not be reversed by treatment with the tested extract.

Regarding the effect of chronic treatment with *P. ruscifolia* extract on the lipid profile of normal and hyperglycemic animals, it was observed that animals induced with alloxan had a higher concentration of cholesterol, triglycerides, LDL-c, and a lower concentration of HDL-c in serum than the animals of the control group, Nv. Furthermore, the animals treated with the extract (HPr100) reduced the concentration of cholesterol and VLDL-c in serum compared to the untreated induced animals, Hv. Regarding triglycerides and LDL-c, it was observed that both doses, 50 and 100 mg/kg, presented a significant decrease in the concentration of these parameters and both doses showed a favorable increase

in the concentration of HDL-c. In a previous study, in which the animals received the treatment with 100 mg/kg of the extract of *P. ruscifolia* leaves for 28 days, we found that the concentration of both cholesterol and LDL-c decreased, and HDL-c increased, but no effect on triglycerides was observed after 28 days in alloxan-induced hyperglycemic mice [11].

Our results after treatment for 45 days coincide with the findings of other researchers who conducted studies with the steam bark of *P. cineraria* in Swiss albino mice for the same observation period of 45 days [14]. In another study carried out with the leaves of *P. cineraria* in Wistar rats, after 12 weeks of treatment, this same result was obtained [15]. Taking all these results into account, we can affirm that *P. ruscifolia* has lipid-lowering effects in mice with alloxan-induced hyperglycemia, comparable to those found by other groups of researchers. Additionally, the results of this study demonstrated a decrease in coronary and atherogenic risk in the groups treated with the extract, thus showing a cardioprotective effect [6,25].

Additionally, tyloxapol produces hyperlipidemia mainly by inhibiting the activity of lipoprotein lipase [22,23]. As expected, the acute injection of tyloxapol in both male and female mice produced a significant increase in lipid profile parameters [26,27]. In both male and female mice, a decrease in cholesterol and triglycerides was observed, as demonstrated by other authors [28], and in both groups, an increase in HDL-c concentration was observed after treatment with the extract. These results agree with what was observed by our research group in a 28-day model [11].

The results obtained with this study demonstrate that *P. ruscifolia* could be used as an adjunctive treatment to treat dyslipidemia associated with diabetes and dyslipidemia. The mechanism of action by which the components of this plant produce their effect remains to be elucidated. *Prosopis ruscifolia* has been reported to possess different metabolites such as flavonoids, saponins, and alkaloids. From this group of compounds, quercetin stands out as a flavonoid that has been shown to have favorable effects in diabetic and hyperlipidemia animal models. Quercetin was identified in the extract of *P. ruscifolia* and in other species of the same genus [11,29].

In both diabetic patients and patients with hyperlipidemia, the tissues are exposed to oxidative stress due to the imbalance in the metabolism of both carbohydrates and lipids [30]. Our obtained results, regarding the lipid profile of the animals treated with *P. ruscifolia*, may be explained by a powerful antioxidant effect of the components present in the plant [31] or the hepatoprotective effect of the extract [12] which promotes correct lipid metabolism and both effects could be related to the quercetin content in the extract [11].

Finally, when analyzing males and females in parallel in the hyperlipidemia induction model by tyloxapol, our results indicated a difference between both sexes regarding their respective intrinsic variability. This result is significant because it demonstrates the importance of working with both male and female animals as they display distinct hormonal imbalances [32].

4. CONCLUSION

In this study, it was demonstrated that animals with alloxan-induced hyperglycemia constitute a valid model for studying the effect of viñal on glycemia and lipid profile for a period of 45 days, since hyperglycemia was maintained throughout that period. In addition, treatment for 45 days with the ethanolic extract of *P. ruscifolia* improves the lipid profile parameters in animal models with alloxan-induced hyperglycemia and has a cardioprotective effect, since it improves the values of the coronary risk indices and AI. Mice with tyloxapol-induced hyperlipidemia improved lipid profile values, which was verified by a decrease in cholesterol, an increase in HDL-c in males, a decrease in cholesterol and triglyceride, and an increase in HDL-c in females. Finally, a better effect of the extract on the lipid profile in female mice and less variability between measurements was demonstrated.

5. AUTHORS' CONTRIBUTIONS

All authors have made a substantial contribution to this work, read the final manuscript, and approved the submission.

6. ACKNOWLEDGMENTS

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

Ethics Committee of the Facultad de Ciencias Químicas, UNA, and approved (CEI 470/19).

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