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RESEARCH ARTICLE

The Enriched Proanthocyanidin Extract of *Ligaria cuneifolia* Shows a Marked Hypocholesterolemic Effect in Rats Fed with Cholesterol-Enriched Diet

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Abstract: ***Background:*** *Ligaria cuneifolia* (*Lc*) (R. et P.) Tiegh. (Loranthaceae) (Argentine mistletoe) is usually used in local folk medicine.

Objective: We studied the effect of treatment with the *Lc* proanthocyanidin-enriched fraction (*PLc*) in rats fed with Cho-enriched diet on plasma lipids levels, the hemorheological parameters, and biliary secretion.

Method: Adult male Wistar rats were fed ad libitum with a Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) during 28 days. Then, were separated in six experimental groups (n=5 each one), which were injected ip every 24 h with: 1) saline solution (control group, C) and 2) P*Lc*, 3 mg/100 g body weight (treated group, C+P*Lc*), during 3, 7 and 10 days. Group C presented an increase in plasma levels of Cho and Triglycerides (TG), and also, accumulation of hepatic lipid droplets. Also, cell shape and their corresponding morphological index (MI) were altered too.

Results: The treatment with P*Lc* at 3, 7 and 10 days produces a diminution in the plasma Cho, LDL-Cho and serum TG levels, accompanied by a diminution of the lipid accumulation in the liver. The rates of bile acid output in bile can explain the diminution of plasma Cho, evidencing that some of the enzymes involved in the cholesterol conversion into bile acids could be up regulated by the treatment with P*Lc*, leading to the observed increase bile flow. P*Lc* treatment leads to a diminution of plasma levels of Cho and TG.

Conclusion: Essentially, the treatment with P*Lc*, despite the duration produces a modification in hemorheological parameters approaching the values of the experimental group with standard diet. Plasma levels of Cho, LDL-Cho and TG represent selected markers to evaluate the effect of enriched extract from *Ligaria cuneifolia*. Further work is necessary to better evaluate the mechanisms by which P*Lc* induces modifications in the lipids metabolism.

Keywords: *Ligaria cuneifolia*, proanthocyanidin, cholesterol-enriched diet, Hypocholesterolemic marked, biliary secretion, hemorheological properties, Hypotriglyceredemic.

1. INTRODUCTION

Ligaria cuneifolia (R. et P.) Tiegh. (Loranthaceae) (*Lc*), popularly known as "Argentine mistletoe", is a widely distributed hemiparasite plant from the Northern and Central regions of Argentina [1]. *Lc* has been used traditionally in the forms of crude extracts, infusions, to decrease blood pressure and to give more fluidity to the blood, lowering the excess of cholesterol (Cho) [2]. Earlier studies in our laboratories revealed that the treatment with the crude extract of *Lc* by intraperitoneal injection to adult male Wistar rats fed with standard diet produced an increase in blood viscosity as a consequence of an augmentation of erythrocyte rigidity, and also a diminution of plasma Cho levels. Moreover, we suggest that the diminution of plasma Cho could be caused by an increase in the biliary excretion rate of Cho and bile salts (products of the hepatic metabolism of Cho) which was obtained in our studies in the treated rats [3].

The micromolecular study of the flavonoid composition from *Lc* extracts, disclosed the presence of free-quercetin, glycosylated-quercetin, catechin and proanthocyanidins corresponding to cyanidin monomers [4]. Based on this

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knowledge of the composition of the extract of *Lc*, we also investigated the effect of some fractions extracted from *Lc*, rich in various flavonoids. Advancing in the analysis of different extracts of *Lc*, we studied in rats fed with standard diet, the effect of intraperitoneal treatment with the *Lc* glycosylated-quercetin-enriched fraction (Q*Lc*). In this study, we observed that the treatment with Q*Lc* produced similar hemorheological effects to those observed for the crude extract of *Lc*, such as the increased erythrocyte rigidity which caused the augmentation of blood viscosity, but contrary to the observed for the crude extract of *Lc*, the glycosylated-quercetin-enriched fraction did not lead to a diminution in the plasma Cho levels, thus suggesting that another compound from *Lc* would be responsible for the decrease in plasma Cho levels [5].

Progress in the analysis of the different extracts from *Lc*, we studied in rats fed with standard diet, the effect of intraperitoneal treatment with the *Lc* proanthocyanidin-enriched fraction (P*Lc*). In this regard, we observed that treatment with P*Lc* in rats fed with standard diet is involved in the diminution of the plasma level of Cho without impairment of hemorheological parameters [6].

In the present work, we studied the effect of treatment with *PLc* in rats fed with Cho-enriched diet on plasma lipids levels, and the hemorheological parameters.

2. MATERIALS AND METHODS

2.1. Plant Material

Samples of *Lc* growing on *Geoffroea decorticans* (Hook. & Arn.) Burkart (Fabaceae) were collected from Córdoba, a province in the center of Argentina. Classification of the specie was performed by means of the key according to Abbiatti. Voucher specimen is kept at the Pharmacobotanical Museum "Juan A. Dominguez", School of Pharmacy and Biochemistry, University of Buenos Aires (BAF 809).

2.2. Preparation of Plant Extracts

20 g of air-dried leaves of *Lc* were grounded in a rotary blade mill and extracted with 200 mL 80% methanol (v/v) for 48 h at room temperature (crude extract). The crude extract was filtered and the solution was dried by evaporation under reduced pressure at 40 °C and the residue was successively extracted with 50 mL of ethyl acetate (ethyl acetate fraction, EAF). The EAF, enriched in proanthocyanidin, was concentrated and dried by evaporation under reduced pressure, and the residue was stored in the absence of light at -20°C (https://portaltramites.inpi.gob.ar/Boletines/Index [7]).

2.3. Animals and Treatment

Adult male Wistar rats were housed two per cage and maintained under a 12 h light/dark cycle. Animals were fed *ad libitum* with a Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) to obtain an increase of plasma cholesterol. The diet was maintained for 28 days. Animal care and treatments were conducted in conformity with institutional guidelines in compliance with National and International laws and policies (Expedient 6109/012 E.C. Resolution 267/02).

The animals were divided into six experimental groups (n=5 each one), which were injected intraperitoneally every 24 h with: 1) saline solution (control group, C) and 2) P*Lc*, 3 mg/100 g body weight (treated group, T), during 3, 7 and 10 days. The P*Lc* dose used was calculated considering their relative concentrations in the plant, so that its effects may be comparable with the obtained by the total extract treatment. Other group of rats (n=5) were fed *ad libitum* with standard diet during 28 days.

2.3.1. Experimental Procedures

On the day of experiment, 24 h after the last administration, animals were weighed and anaesthetized with Ketamine/Xylazine (100mg/kg/3mg/kg, intraperitoneal). The bile duct was cannulated and bile was collected on ice into preweighed tubes, every 15 min for 60 min. Body temperature was maintained at 38.0±0.5ºC throughout the experiments by means of a heating lamp [8]. At the end of bile collection blood was obtained by heart puncture and the liver was promptly removed, washed and weighed.

2.3.2. Hemorheological Assays

The rheological measurements were performed according the guidelines of Committee for Standardization in Haematology [9]. All measurements were made at room temperature (22±1ºC) within 4 hours after blood collection. Heparinized blood samples (heparin 5 IU/mL) were collected to obtain plasma. Whole blood viscosity and plasma viscosity measurements were performed in a Wells-Brookfield LVT-CP viscometer at 230 s^{-1} shear rate. Relative blood viscosity was calculated at a standard hematocrit of 45%, thus avoiding the influence of both plasma viscosity and percentage of red blood cells on viscosity measurements [10]. Thus, the obtained parameter is mainly affected by erythrocyte deformability. The erythrocyte deformability was estimated using the method of filtration through Nuclepore™ membranes by means of an instrument built in our laboratories and it was calculated its inverse, the Rigidity Index (RI) [11]. Hematocrit was measured by the micromethod. Red blood cells were counted using an improved Neubauer chamber. Cell shape was assessed by direct microscopy of the whole blood sample (150 cells per aliquot), assuming an index according to Bessis classification. Morphological Index (MI) was calculated as follows: Σ (shape index × cell number / total cell number) [12]. To determine the osmotic fragility, red blood cells were incubated for 30 min in NaCl solution of concentrations ranging from 0 to 0.9 mM. Percentage of hemolysis was measured photocolorimetrically, considering the tube containing distilled water as 100% of hemolysis. Hemolysis was plotted vs. NaCl concentration. The parameters X_{50} (concentration of NaCl which produces 50% of hemolysis (mM)) and β (slope of the curve) were determined. β, a parameter of sample homogeneity, indicates the presence of different forms of erythrocytes [13, 14].

2.3.3. Plasma Cho Concentration and Triglycerides Concentrations

The heparinized blood samples were used to determine: 1- Plasma Cho concentration by the enzymatic esterase-oxidase method and also of LDL-Cho levels (low-density lipoprotein) and the HDL-Cho (high-density lipoprotein) [15]. 2- Plasma Triglycerides concentration (TG) by the enzymatic method using the kit of Wiener Lab.

2.3.4. Histological Studies

Liver pieces were fixed in 10% formaldehyde; dehydrated, 24 hours later they were embedded in paraffin, cut and stained with hematoxylin-eosin and trichromica Masson-Alcian blue. Light microscopic analysis was performed in Olympus BX40, U-MDOB model (magnification 400×).

We applied the score described by Kleiner *et al.* [16] that designed and validated a histological scoring system that allowed a semi-quantitative evaluation of steatosis accepted by the Nash Clinical Research Network Pathology Committee, detailed in the following table:

Steatosis Grade (% of parenchyma involved by steatosis)	Score
< 5%	0
5%-33%	1
>33%-66%	2
>66%	3

Microvesicular Steatosis	
No present	0
Present	1

Localization	
Zone 3 (centrilobular zone)	0
Zone 1 (periportal zone)	1
Azone	2
Panacinar	3

2.3.5. Determination of Bile Flow and Biliary Output of Bile Components

The bile flow (BF) was estimated by gravimetry, assuming a bile density of 1.0 g/mL; BF was expressed as µL/min.g of liver. Bile salts concentration in bile was determined by Talalay's method modified by Berthelot [17, 18]. The biliary output of bile salt was calculated as the product of BF times the bile salt concentration.

2.4. Statistical Analysis

All results are expressed as mean ± SD. Statistical evaluation was carried out by one-way analysis of variance (one-way ANOVA). The Student's t-test employed to determine statistical differences between the two experimental groups. P value less than 0.05 was considered statistically significant.

3. RESULTS

In Table **1**, we observed that the Cho-enriched diet (C group) produces a significant diminution of the MI with respect to STD group. The treatment with P*Lc* leads to an increase in MI reaching the value of the STD group at 10 days. The Cho-enriched diet did not exert any significant modifications of the others hemorreological parameters tested: total blood viscosity (TBV) and plasma viscosity (PV). Standardized relative BV (SBV) at 45% hematocrit [$(BV/PV)^{45/Hto}$]; rigidity index (RI); Osmotic fragility (X50 and β). Besides this the P*Lc*-treatment did not show any significant modifications.

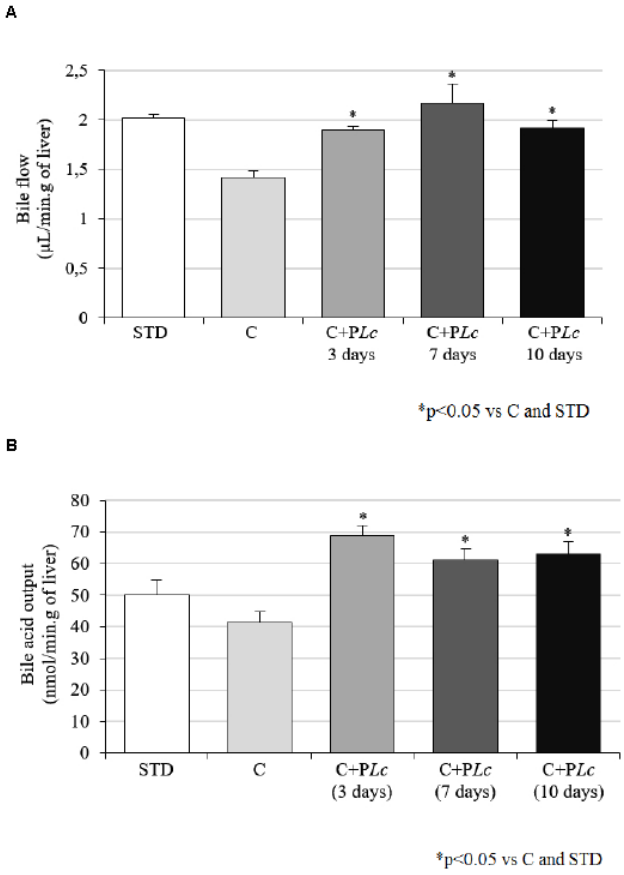

Fig. (1). Effect of enriched proanthocyanidin extract of *Ligaria cuneifolia* on bile flow and biliary parameters in rats fed with cholesterol-enriched diet. A. Bile flow expressed in µL/min. g of liver. B. Bile acid output expressed in nmol/min. g of liver. STD: rats fed with standard diet during 28 days. C: rats fed with Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) during 28 days and injected i.p. every 24 h with saline solution in the last 3 or 7 or 10 days (In all the cases there is not diferences to the diferentes times of treatment). C+P*Lc* (3 days) rats fed with Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 3 days. C+P*Lc* (7 days) rats fed with Cho-enriched diet during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 7 days. C+P*Lc* (10 days) rats fed with Cho-enriched diet during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 10 days. Values are expressed as mean ± SE of at least five animals pergroup. *Significant difference $p < 0.05$ vs. theC group.

In Table **2**, we observed that the Cho-enriched diet (C group) produces a significant increase in levels of plasma Cho and LDL-Cho, without change in HDL-Cho concentration. Besides, we observed an increase in the levels of TG in

plasma. The treatment with P*Lc* during 3, 7 and 10 days, leads to a significant decrease in plasma in plasma Chol, LDL-Cho and TG in comparison with the group fed with Cho-enriched diet (C group).

The plasma levels of hepatic enzymes ASAT and ALAT did not show any significant modifications between all the studies groups.

The histological analysis of the liver samples was shown in Fig. **1** and the distribution of recorded scores is shown in Table **3**. As can be observed, there is an increase in the degree of steatosis in the group fed a diet rich in cholesterol compared to the standard diet. Treatment with P*Lc* causes a decrease in the number and size of steatosis observed in Cho-enriched diet group. Moreover, the microvesicular steatosis disappears in P*Lc*-treated groups. The steatosis localization is in all the groups are in periportal zone (zone 1).

Fig. **1** shows the values for the bile flow (A), and also the biliary excretion of bile salts (B) for all the experimental groups studied. The Cho-enriched diet group was found to be associated with a decrease in the biliary output of bile salts (Fig. **1**, Panel B) and these changes were accompanied by a diminution in total bile flow (Fig. **1**, Panel A) in comparison with STD group. Treatment with P*Lc* during 3, 7 and 10 days, produced a significant increase in the biliary output of bile salts, and these changes were accompanied by an increment in total bile flow in comparison with Cho-enriched diet group.

4. DISCUSSION

In previous works, it was demonstrated in normocholesterolemic rats a decrease in plasma cholesterol associated with a decrease in blood fluidity, due to an increase in blood viscosity

Table 1. Hemorheological parameters.

	STD	C	C+P*Lc* 3days	C+P*Lc* 7days	C+P*Lc* 10days
MI	-1.30±0,56	-2.45±0.08$^{\&}$	-2.18±0.11(ns)$^{\&}$	-2.22±0.08(ns)$^{\&}$	-1.98±0.29*
RI	7.50±0.40	8.02±0.96	6.06±0.17**	5.68±0,46 **	6.30±0.92**
SBV	5.55±0.26	5.76±0.18	5.16±0.41(ns)	4.93±0.09*	5.01±0.19*
X_{50}	0.58±0.10	0.48±0.04	0.49 ± 0.02(ns)	0.47±0.02(ns)	0.44±0.02(ns)
β	10.33±1.96	9.88±0.84	9.48 ± 0.63(ns)	10.30±1.06(ns)	9.86±0.58(ns)

SBV: Standardized Relative Blood Viscosity (BV) at 45% Hematocrit [(BV/PV) $^{45/Hto}$] calculated with Total Blood Viscosity (TBV) and Plasma Viscosity (PV); RI: Rigidity Index; MI: Morphological Index; parameters of osmotic fragility (X_{50} and β). Experimental groups: Standard Diet (STD), Control (Cho-enriched diet, C), C+ P*Lc* 3days, C+P*Lc* 7days, C+P*Lc* 10days. N= 4-5 rats,* p<0.05 vs. C, ** p<0.01 vs. C, and ns p>0.05 vs. C. $^{\&}$p<0.05 vs. STD

Table 2. Plasma TG, Cho, LDL-Cho, and HDL-Cho concentrations. ASAT and ALAT enzymes activity.

	STD	C	C+P*Lc* 3days	C+P*Lc* 7days	C+P*Lc* 10days
Plasma TG	70.38±1.84	109.47±7.38$^{\&}$	59.80±2.53*	64.00±2.94*	64.60±3.61*
Plasma Cho	69.90±4.25	125.95±3.58$^{\&}$	79.00±3.30*	75.36±0.97*	68.50±1.86*
LDL-Cho	19.51±2.40	29.92±2.27$^{\&}$	16.13±1.33*	18.00±0.29*	18.16±0.59*
HDL-Cho	28.50±0.62	32.18±1.129	21.13±0.40*	28.02±1.29*	23.17±0.86*
ASAT	80.8 ±2.02	97.30±1.19$^{\&}$	77.50±1.09*	90.86±1.19^{ns}	88.33±1.57^{ns}
ALAT	33.15±0.84	31.37±1.45	31.6±0.76	33.33±1.02^{ns}	28.92±0.87^{ns}

ASAT: Aspartate Aminotransferase, UI/L; ALAT: Alanine Aminotransferase UI/L.
Plasma TG (Triglycerides), Cho (Cholesterol), LDL-Cho and HDL-Cho are expressed as mg/dl.
Experimental groups: Standard diet (STD), Control (Cho-enriched diet, C), C+ PLc 3days, C+PLc 7days, C+PLc 10days. N= 5 rats,* p<0.05 vs. C, and ns p>0.05 vs. C. & p<0.05 vs. STD.

Table 3. Validated histological feature scoring system which allowed a semiquantitative evaluation of steatosis.

	STD	C	C+P*Lc* 3days	C+P*Lc* 7days	C+P*Lc* 10days
Score	0.0±0.0	2.04±0.54&	1.0±0.0**&	1.75±0.80*&	0.5±0.2**&
Microvesicular Steatosis	0	1	0	0	0
Localization	-	1	1	1	1

Steatosis Grade (% of parenchyma involved by steatosis): **Score**: < 5% = 0, 5%-33% = 1; >33%-66% = 2.**Microvesicular Steatosis**: No present = 0; Present = 1. **Localization:** Zone 3= 0; Zone 1= 1; Azone = 2; Panacinar = 3. Experimental groups: Standard diet (STD), Control (Cho-enriched diet, C), C+ *PLc* 3days, C+P*Lc* 7days, C+*PLc* 10days. N= 4-5 rats. ,* p<0.05 vs. C, ** p<0.01 vs. C, and ns p>0.05 vs. C. & p<0.05 vs. STD

as well as a lower erythrocyte deformability, using the following *Lc* extracts: crude, methanolic fraction, catequine, and quercetine enriched fractions by via intraperitoneal [5, 19-21]. The administration of PLc was by via intraperitoneal and was not included in the diet so as to have safety of the administered dose, which was calculated considering their relative concentrations in the plant, so that its effects may be comparable with the obtained by the total extract treatment. In addition to being included in the diet we should use a dose at least an order of magnitude greater than would require a significant amount of plant material. Besides, we demonstrated that *Ligaria cuneifolia* proanthocyanidin enriched extract, which is a normally present flavonoide in *Lc*, administered intraperitoneally during three days to normocholesterolemic rats produced a significant diminution in plasma cholesterol, LDL-Cho and HDL-Cho without alterations in the blood viscosity or hepatic tissue, confirmed by the absence of hepatotoxicity through histological examination [22]. On the other side, it is known that the increase in the Cho content in the diet leads to an augmentation in plasma lipids and an accumulation of drop lipid in the liver [23]. In this connection, we observed in the group fed with Cho-enriched diet, an increase in the Cho and TG plasma levels, and also, accumulation of hepatic lipid droplets. The treatments with P*Lc* during 3, 7 and 10 days produce a similar diminution in plasma lipids, accompanied for a diminution of the lipid accumulation in the liver, being in this case more important after 10 days of P*Lc*-injection (Tables **2**, **3** and Fig. **1**).

It is known that the liver plays a central role in the regulation of cholesterol levels, in which the major pathway responsible for elimination of cholesterol from the body is degradation to bile acids and their biliary secretion [24, 25]. The conversion of cholesterol to bile acids takes place through a number of enzymatic steps [26]. In rats hypercholesterolemic, the P*Lc* -treatment during 3, 7 and 10 days, leads to a similar decrease in the total plasma Cho. The significant diminution of total Cho can be explained, at least partly, by an increase in the rate of bile acid synthesis, evidencing that some of the enzymes involved in the cholesterol conversion to bile acids, as cholesterol 7alpha-hydroxylase could be up regulated by the treatment with P*Lc*, and also, an augmentation of biliary secretion (BS). In this connection, the observed increase in biliary secretion of bile acids provides enlargement of osmotically active entities in the biliary canaliculus leading to increase in the FB observed (Fig. **2**).

On the other hand, cholesterol administration has been reported to influence hepatic lipid metabolism in rats [27]. Numerous studies have been done to seek the effect of dietary cholesterol on hepatic lipid homeostasis. In early 1990s, Thomas *et al.* [28, 29] have investigated the effect of cholesterol on the accumulation of liver lipids through the radioisotope 14C-fatty acid and proposed that the hepatic TG accumulation was developed by the enhancement of hepatic TG synthesis and the reduction of fatty acid beta-oxidation. Liu *et al.* [30] have suggested the roles of increased lipogenesis, decreased oxidation of fatty acids and decreased secretion of VLDL as causes for the accumulation of TG in the liver in the cholesterol-fed rats. Xu *et al.* [31] have also reported that the impaired hepatic lipid homeostasis because of lipid accumulation attributed to the increasing activity of the enzymes involved in fatty acid biosynthesis in the rats by the dietary cholesterol.

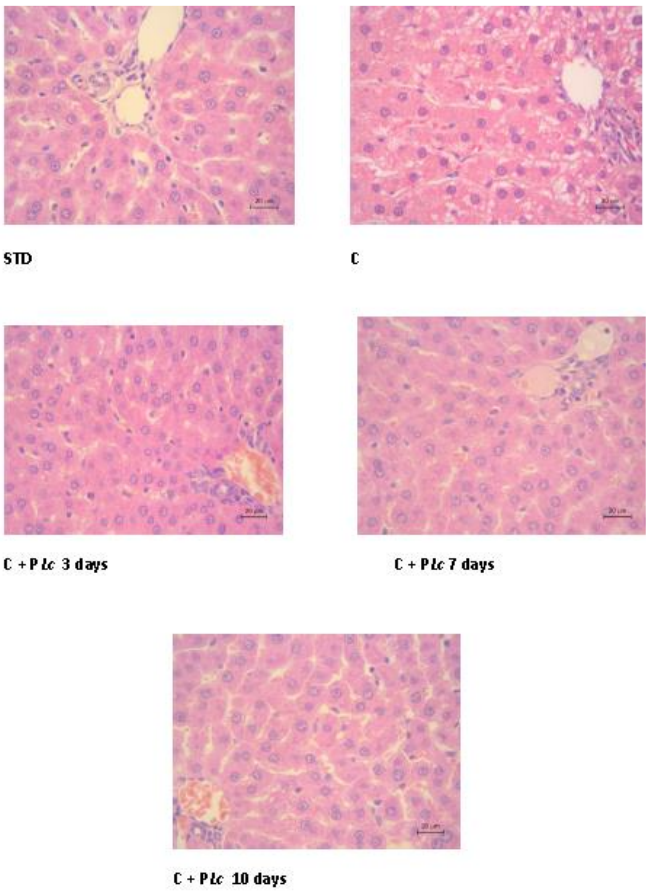

Fig. (2). Effect of enriched proanthocyanidin extract of *Ligaria cuneifolia* on the microvesicular steatosis in liver of rats fed with cholesterol-enriched diet. Photomicrograph of cells of liver of the all groups studied. STD: rats fed with standard diet during 28 days. C: rats fed with Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) during 28 days and injected i.p. every 24 h with saline solution in the last 3 or 7 or 10 days (In all the cases there is not diferences to the diferentes times of treatment). C+P*Lc* (3 days) rats fed with Cho-enriched diet (Cho (97% purity) 8 g/kg of diet and corn oil 280 g/kg of diet) during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 3 days. C+P*Lc* (7 days) rats fed with Cho-enriched diet during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 7 days. C+P*Lc* (10 days) rats fed with Cho-enriched diet during 28 days and injected i.p. every 24 h with 3 mg/100 g body weight of P*Lc* the last 10 days. (All photomicrographs: Hematoxylin and eosin; original magnification x 400).

Moreover, Fukada *et al.* [32] have shown that the suppression of fatty acid beta-oxidation may be responsible for the conversion of fatty acid to TG and cholesterol ester (CE) to afflux to LDL-Cho throughout the onset of biosynthesis and secretion of LDL-Cho, showing also that hypercholesterolemia rats induced by high cholesterol diet had higher serum TG level. Our finding shows that there is hepatic lipid accumulation (Fig. **2** and Table **3**), high serum TG level, and increase in plasma LDL-Cho, all of it caused by dietary cholesterol (Table **2**). P*Lc* treatment leads to a diminution of plasma LDL-Cho and serum TG level. We hypothesized that P*Lc* could inhibit the enzyme acyl-CoA: cholesterol acyl-

transferase (ACAT) acts as a rate-controlling enzyme of cholesterol esterification since the CE preferentially flew into LDL particle thus produce a decrease in lipoprotein. Besides, P*Lc* could act on of fatty acid beta-oxidation decreasing the conversion of fatty acid to TG.

Reportedly, diets supplemented with cholesterol induce changes in rat erythrocyte membrane [33] which leads to changes in hemorheological proprieties [34]. In concordance in the present study we observed that the Total Blood Viscosity (TBV) and Plasma Viscosity (PV). Standardized Relative BV (SBV) at 45% hematocrit [$(BV/PV)^{45/Hto}$]; Rigidity Index (RI) the inverse of Erythrocyte Deformability (ED); cell shape and the corresponding Morphological Index (MI) were altered in the Cho-enriched diet group in comparison with standard diet group. Essentially, the treatment with P*Lc* despite the duration produces a modification in hemorheological parameters approaching the values of the experimental group with standard diet (Table **1**).

CONCLUSION

We demonstrated that the treatment with proanthocyanidin enriched extract from *Ligaria cuneifolia* in rats fed with Cho-enriched diet produces a diminution in plasma lipids, accompanied by a diminution of fats accumulation in the liver. The significant diminution of total Cho can be explained by the onset of biosynthesis and secretion of bile salts. Besides, this treatment causes a diminution of plasma LDL-Cho and produces a modification in hemorheological parameters as well, approaching the values to the normal. Plasma levels of Cho, LDL-Cho and TG represent selected markers to evaluate the effect of enriched extract from *Ligaria cuneifolia*. Nevertheless, the detailed mechanism still needs further investigation.

CURRENT & FUTURE DEVELOPMENTS

Further work is necessary to better evaluate the mechanisms by which proanthocyanidin enriched extract from *Ligaria cuneifolia* produces or induces modifications in the lipids metabolism.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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