



## *Influence of *Ligaria Cuneifolia* (“Argentine Mistletoe”) Infusion on the Lipid Profile and Cellular Factors of Erythrocyte Deformability in Hypercholesterolemic Patients*

*Running Title: Ligaria cuneifolia Infusion and Plasma Cholesterol*

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### **Abstract**

*Ligaria cuneifolia* (Lc) is traditionally used to improve blood fluidity and reduce plasma cholesterol. **Objectives:** to analyze the effect of Lc infusion on plasma cholesterol [C] and erythrocyte deformability at high shear rate, estimated by the rigidity index [RI] in hypercholesterolemic patients.

**Methods:** Seventeen patients ( $50 \pm 14$  years) from the Cardiology Service, Hospital Provincial Centenario, received lyophilized Lc extract in tea bags three times per week for 30 days. Baseline [B] and after treatment with lyophilized Lc extract [TLc] blood samples were collected. Measurements: Plasma total C [TC], HDL-C and LDL-C, colorimetrically. In blood, erythrocyte membrane cholesterol [MC] extracting lipids from lysed erythrocyte membranes. RI was determined by a filtration method.

**Results:** Media  $\pm$ SD: TC, B:  $240.8 \pm 25.5$  vs TLc:  $231.2 \pm 31.8^*$ ; HDL-C: B:  $60.6 \pm 14.1$  vs TLc:  $61.3 \pm 16.1$  ns; LDL-C, B:  $183,2 \pm 27,2$  vs TLc:  $169,9 \pm 30,9^{**}$ ; Blood: IR B:  $13.7 \pm 5.6$  vs TLc:  $11.6 \pm 5.1$  \*; MC B:  $0.96 \pm 0.11$  TLc  $0.67 \pm 0.11$  \*\*. [\*\*\* $p < 0.01$  and \* $p < 0.05$  vs. B ; ns: non significant vs B].

**Conclusions:** in the patients studied, treatment with Lc generated a significant decrease in TC and LDL-C, without inducing changes in HDL-C. At the cellular level, decreases in MC and RI were observed, suggesting improved erythrocyte deformability at the microcirculatory level. Furthermore, considering that elevated LDL-C levels are associated with the development of atherosclerosis, these findings support the potential usefulness of Lc in the prevention of cardiovascular disease.

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## Introduction

*Ligaria cuneifolia* [Lc], commonly known as “liga,” “liguilla,” or “muérdago criollo,” is a hemiparasitic plant widely distributed throughout central and northern Argentina. It belongs to the Loranthaceae family and, like other species within this group, infusions prepared from its leaves and stems have been traditionally used in folk medicine due to their presumed therapeutic properties [1].

Traditionally, *Lc* infusions have been used to lower blood pressure and plasma cholesterol [C] levels, and to improve blood fluidity, similarly to European mistletoe, to which these effects were originally attributed [2]. Although *Lc* has been used as a natural substitute for European mistletoe due to their similar appearance, anatomical analyses have shown that they belong to different botanical families and that their macro- and micromolecular components differ significantly. This distinct chemical composition has led to the identification of mixed or adulterated preparations, in which European mistletoe is replaced by the Argentine species [3, 4].

Since 1998, our research team has conducted a project using *Ligaria cuneifolia* extracts, involving collaboration with the Department of Pharmacobotany at the Faculty of Pharmacy and Biochemistry [UBA], the Department of Biophysics at the Faculty of Medical Sciences [UNR], and the Institute of Experimental Physiology [IFISE-UNR].

Through both in vitro and in vivo experiments using Wistar rats, we have generated evidence supporting a scientific reinterpretation of *Lc*'s traditional use and opening new experimental perspectives.

Experimental studies demonstrated that intraperitoneal administration of crude *Lc* extract in Wistar rats (doses of 1.5, 2.5, 3.5, 5.5, and 8.3 mg/100g body weight)

produced an approximately 40% reduction in plasma cholesterol levels.

This reduction was associated with increased biliary excretion of cholesterol and bile salt end products of hepatic cholesterol metabolism—resulting in elevated overall bile flow [5]. However, a concurrent increase in blood viscosity and erythrocyte rigidity index (an indirect estimate of erythrocyte deformability) was also observed. Clinically, this finding contradicts traditional beliefs claiming that *Lc* consumption improves blood fluidity (i.e., by reducing viscosity).

Intravenous administration of crude *Lc* extract [at doses of 2.5 and 5.5 mg/100g body weight] did not significantly modify biliary parameters or plasma cholesterol levels. However, similarly to the intraperitoneal route, an increase in erythrocyte rigidity index and blood viscosity was observed, suggesting a direct effect of *Lc* on red blood cell internal viscosity [6].

In previously studied patients with hypercholesterolemia, treatment with an aqueous extract prepared from leaves and stems of *Ligaria cuneifolia* significantly reduced LDL-C levels without inducing changes in total cholesterol [TC], HDL-C, or blood fluidity, as assessed by standardized relative blood viscosity [RBV] [7].

Rheology is the science that studies the deformability and flow properties of fluids, whereas hemorheology specifically studies these properties in blood. From a hemorheological standpoint, red blood cells [RBCs] are the most important cellular components of blood because they are the most abundant. Under normal physiological conditions, RBCs can undergo reversible deformation during blood flow, a property determined by cell geometry and cytoplasmic viscosity.

This capability allows RBCs to pass through capillaries smaller than their diameter, thereby enabling efficient

gas exchange between blood and tissues [8]. At high shear rate, blood behaves as a fluid with lower viscosity than any other suspension of solid particles. This demonstrates that blood fluidity depends largely on erythrocyte deformability [9], which is determined by the following factors:

1. Excess membrane surface area relative to that of a sphere of equal volume, resulting in a high surface-area-to-volume ratio.
2. Intracellular fluid viscosity, mainly determined by the type and concentration of hemoglobin.
3. The viscoelastic properties of the cell membrane, which consists of three layers: a glycoprotein-rich glycocalyx on the outer surface, a lipid bilayer capable of resisting bending, and an underlying protein network constituting the cytoskeleton which is responsible for deformability, flexibility, and recovery of the discoid shape. The linear extensibility of this protein mesh determines the limits of erythrocyte deformability [10].

Blood viscosity varies according to shear rate: therefore, at high shear rates, blood viscosity depends primarily on erythrocyte deformability. [11]. In view of the role of excess serum cholesterol as a risk factor of atherosclerosis and its potential link to altered arterial cell and membrane function. It is relevant to determine whether membrane cholesterol is modified by *Lc* infusion treatment in patients with hypercholesterolemia [12]. Consequently, further investigation of cellular factors affecting blood viscosity is required, particularly erythrocyte membrane cholesterol and rigidity index, both of which are related to erythrocytes deformability.

The objective of this paper is to analyze the effect of oral administration of aqueous infusions prepared from extract of the leaves and stems of *Ligaria cuneifolia* [*Lc*] on plasma total cholesterol levels and blood fluidity at high flow rates as well as to characterize associated the cellular factors including membrane cholesterol and erythrocyte rigidity in patients with hypercholesterolemia.

## Materials and Methods

### Plant Material

Plant material was collected in the province of Córdoba from specimens parasitizing chañar trees and then dried at room temperature. Ground leaves and herbaceous stems of *Ligaria cuneifolia* were weighed in amounts

sufficient to prepare a 5% infusion, in accordance with the 8th edition of the Argentine Pharmacopeia. The infusion was freeze-dried, packaged into individual tea bags (2.5 g each), and sent to the Chair of Biophysics, Faculty of Medical Sciences, National University of Rosario [UNR].

Both the collection of specimens and their processing, packaging, and transportation were carried out by the team from the Department of Pharmacobotany, Faculty of Pharmacy and Biochemistry, University of Buenos Aires [UBA].

### Patients

Seventeen volunteer patients were enrolled in the study (mean age  $50 \pm 14$  years; 13 women and 4 men). All attended the outpatient clinic of the Cardiology Service at Hospital Provincial “Centenario” for routine follow-up. Medical history was obtained through an interview that included prior hypercholesterolemia, established atherosclerotic disease, diabetes, and cardiovascular risk factors other than dyslipidemia, to stratify individual cardiovascular risk according to ATP III guidelines and the Framingham risk tables. To support this assessment, we designed a questionnaire based on the WHO STEPwise approach to chronic disease surveillance.

Inclusion criteria included elevated cholesterol levels according to ATP III guidelines and Framingham tables (considering sex, age, hypertension, smoking status), without current indication for statin therapy. Exclusion criteria included patients with established cardiovascular disease, diabetes, strict indication for statins, chronic renal or hepatic disease, cancer, infectious diseases, or any medication with hemorheological effects.

All patients received clear and updated information regarding *Lc* and the objectives of the study. After providing written informed consent, participants completed the questionnaire to obtain demographic and clinical risk factor data.

The study protocol was approved by the corresponding Ethics Committee of the Faculty of Medical Sciences and [Resolution N° 3047/2023] and by the authorities of the Faculty of Medical Sciences and the National University of Rosario under the Resolution N° 5323/2023. All participants provided written informed consent prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Each participant was scheduled for an appointment at the Central Laboratory of the Provincial Hospital “Centenario.” Venous blood samples were collected after 9-12 hours of fasting to obtain baseline cholesterol levels and hemorheological parameters.

Additional laboratory tests included hematimetric indices, glycemia, renal, hepatic, and thyroid function to identify potential confounding factors. Following this initial evaluation, each patient was offered a regimen of *Lc* extract to be administered three times per week for a month. The extract preparation was delivered in pre-dosed tea bags with instructions for infusion preparation using hot drinking water.

### Administration of the Extract

The lyophilized *Lc* extract was provided in 2.5 g tea bags to be diluted in 100 ml of hot drinking water. The prescribed intake was three times per week, after meals, over a 30-day period.

### Laboratory Determinations

Biochemical analyses were performed at the Central Laboratory of the Hospital Centenario, whereas hemorheological assessments were carried out in the Blood Biology Laboratory of the Department of Biophysics, Faculty of Medical Sciences, UNR.

### The following biochemical and hemorheological tests were performed:

Lipid profile: Total Cholesterol [TC], High-Density Lipoprotein Cholesterol [HDL-C], Low-Density Lipoprotein Cholesterol [LDL-C] and triglycerides [TG] were measured using enzymatic esterase-oxidase methods [mg/dL] with commercial detection kits.

Hematologic tests: Red Blood Cell [RBC] count, White Blood Cell [WBC] count, platelet count, leukocyte differential, Hematocrit [Hct], and Hemoglobin [Hb] levels were determined. Hematocrit by micromethod. Red blood cell count using a Neubauer chamber. Mean corpuscular volume calculated from hematocrit and red blood cell count. Mean corpuscular hemoglobin [MCHb] calculated from hematocrit and hemoglobin concentration [Hb] by spectrophotometric method (cyanmethemoglobin method) [13].

Morphological Index [MI], was determined by direct microscopy of the whole blood aliquot, assigning an index according to the Bessis classification (Discocyte:

0, Stomatocyte I: -1, Stomatocyte II: -2, Stomatocyte III: -3, Spherostomatocyte: -4) [14]. The number of cells observed was 150 per aliquot. Then MI was calculated with the formula:  $\Sigma$  [shape index x number of cells] / total number of cells.

Hemorheological parameters: Blood viscosity [BV] and plasma viscosity [PV] measured with a Wells-Brookfield LVT cone-plate rotational viscometer [Brookfield, Stoughton, MA, USA] at a shear rate of  $230 \text{ s}^{-1}$  and a temperature of  $37 \text{ }^{\circ}\text{C}$ .

Relative blood viscosity [RBV] was standardized to a hematocrit of 45% and calculated using the equation of Matrai et al:  $\text{RBV} = (\text{BV}/\text{PV})^{45/\text{Hct}}$ . [15] Rigidity index [RI] was determined by a filtration method, with 5m nucleopore membranes [Nucleopore Corp., USA] in an automated device developed in our laboratory [16].

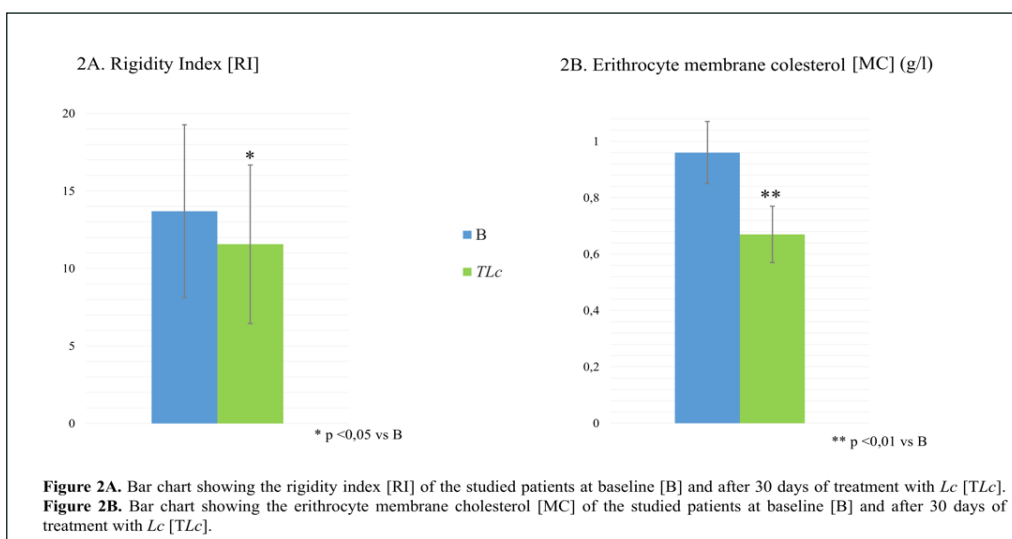
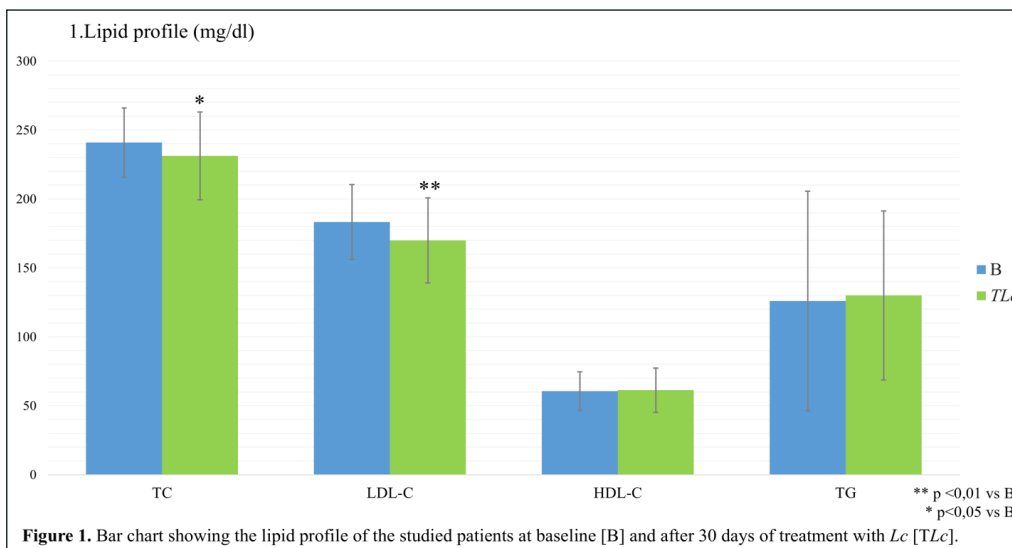
To determine erythrocyte membrane cholesterol [MC], lipids were extracted from the membrane of lysed erythrocyte membranes using 2-propanol and chloroform. After centrifugation, the supernatant was dried in a laboratory oven at  $37 \text{ }^{\circ}\text{C}$ . Then dried residue was resuspended in ethanol for colorimetric determination of cholesterol content [17].

All tests were conducted before and after the 30-day treatment period with *Lc* to assess statistically significant changes. Statistical analysis was performed using the paired Student’s t-test.

### Results

As shown in Figure 1, treatment with aqueous infusions of *Ligaria cuneifolia* for 30 days in hypercholesterolemic patients resulted in a significant reduction in LDL-C and total cholesterol levels [TC] without changes in HDL-C or triglyceride [TG] levels. As shown in Figure 2A, treatment with aqueous infusions of *Ligaria cuneifolia* in hypercholesterolemic patients resulted in a significant reduction in RI between baseline and post-treatment samples. Figure 2B shows a significant reduction in MC between baseline and post-treatment samples.

As shown in Table 1, no significant differences were observed in the relative blood viscosity standardized to a hematocrit of 45% [RBV] between baseline and post-treatment samples. Table 2 shows the cellular factors affecting RBC deformability. No significant differences in MCV, MCHC, or MI were observed.



**Table 1:** Blood and plasma viscosity of the studied patients at baseline [B] and after 30 days of treatment with *Ligaria cuneifolia* [TLc].

	BV [cP]	PV [cP]	Hct [%]	RBV
B	4,35 ± 0,54	1,59 ± 0,19	40,82 ± 3,07	3,07 ± 0,43
TLc	4,22 ± 0,36 [ns]	1,71 ± 0,34 [ns]	38,76 ± 3,11 **	2,86 ± 0,50 [ns]

Abbreviations: B: Baseline. TLc: after treatment with *Ligaria cuneifolia*. BV: Blood viscosity. VP: Plasma viscosity. Hct: Hematocrit. RBV: Standardized relative blood viscosity. \*\* significant vs B p<0,05. ns: non-significant vs B

**Table 2:** Cellular factors affecting red blood cells deformability of the studied patients at baseline [B] and after 30 days of treatment with *Ligaria cuneifolia* [TLc].

	MCV [fl]	MCHC [g/dl]	MI
B	87,87 ± 4,14	34,09 ± 0,96	-0,016 ± 0,011
TLc	88,15 ± 4,34 [ns]	34,12 ± 0,88 [ns]	-0,019 ± 0,019[ns]

Abbreviations: B: Baseline. TLc: after treatment with *Ligaria cuneifolia*. MCV: mean corpuscular volume. MCHC: Mean corpuscular hemoglobin concentration. MI: morphological index. ns: non-significant vs B.

## Discussion

Erythrocyte deformability is a key determinant of microcirculation and oxygen transport efficiency. Alterations in erythrocyte deformability have previously been described in various clinical contexts, including metabolic disorders [18].

Among the cellular factors analyzed that could modify blood viscosity at high shear rates, a decrease in the rigidity index was found, showing significant differences between baseline and post-treatment samples. This finding indicates that the patients treated with *Lc* infusion exhibited increased erythrocyte deformability, as estimated by the reduction in the rigidity index, without changes in mean corpuscular volume or erythrocyte shape. Consequently, the erythrocyte surface-area-to-volume ratio remained unchanged. Likewise, no modifications in intracellular viscosity were observed, as estimated by mean corpuscular hemoglobin concentration.

Interestingly, no significant differences in plasma viscosity were found between baseline and post-treatment samples measured at high shear rates, since triglyceride levels remained unchanged in these patients [19]. Similarly, relative blood viscosity corrected to a hematocrit of 45% was not significantly altered.

Unlike erythrocyte deformability, no significant differences were observed in total blood viscosity, suggesting that hemorheological changes initially occur at the cellular level before affecting the overall properties of blood flow. This observation is consistent with previous reports indicating that viscosity depends not only on the intrinsic properties of erythrocytes but also on plasma factors. Since these latter parameters remained unchanged in our sample, no marked variations in overall viscosity would be expected. We observed a decrease in LDL-C in patients treated with *Lc* infusion, together with a reduction in erythrocyte membrane cholesterol. This finding may be explained by the central role of lipoproteins in cholesterol transport to and from tissues. Recent studies have also demonstrated that red blood cells (RBCs), which contain large amounts of free cholesterol within their membranes, play an important role in reverse cholesterol transport. However, the exact role of [RBCs] in systemic cholesterol metabolism remains poorly understood. RBCs incubated with autologous

plasma or isolated lipoproteins showed a significant transfer of cholesterol from RBCs to HDL, whereas cholesterol from LDL moved in the opposite direction [20]. A previous study demonstrated that changes in erythrocyte plasma membranes are induced by elevated plasma cholesterol concentrations. Increased plasma cholesterol levels produce significant alterations in membrane lipid and protein structure, potentially impairing membrane function. Hypercholesterolemia constitutes a major cardiovascular risk factor because excess cholesterol alters normal blood rheology through its interaction with erythrocytes [21].

In our study, hypercholesterolemic patients showed increased erythrocyte membrane cholesterol and rigidity index values. Following treatment with *Lc* infusion, reductions in both MC and RI were observed.

## Conclusions

In the patients studied, treatment with extracts prepared from the leaves and stems of *Ligaria cuneifolia* produced a significant reduction in LDL-C and total cholesterol [TC] levels without inducing changes in HDL-C or blood fluidity, as assessed by standardized relative blood viscosity.

At the cellular level, decreases in MC and RI were observed, suggesting improved erythrocyte deformability at the microcirculatory level. Furthermore, considering that elevated LDL cholesterol levels are associated with the development of atherosclerosis, these findings support the potential usefulness of *Lc* in the prevention of cardiovascular disease.

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## Competing Interests

The authors declare that they have no competing interests. All authors read and approved of the final manuscript.

## Abbreviations

<i>Lc</i>	<i>Ligaria cuneifolia</i>
C	cholesterol
LDL-C	LDL cholesterol
HDL-C	HDL cholesterol
TG	triglycerides
BV	blood viscosity
PV	plasma viscosity
RBV	standardized relative blood viscosity
RI	rigidity index
MI	morphological Index
MC	erythrocyte membrane cholesterol
B	baseline determinations.
<i>Tlc</i>	After 30-day treatment with <i>Lc</i> determinations.

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