

Leaf extract of *Coffea arabica* L. reduces lipid peroxidation and has anti-platelet effect in a rat dyslipidemia model

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This study aimed to evaluate the antioxidant potential of the *Coffea arabica* Lineu (L.) leaf extract and its effects on platelet aggregation of dyslipidemic rats. The extract was obtained by the percolation of *C. arabica* L. leaves in hydroethanolic solution 70% (v/v). The mass spectrometry FIA-ESI-MS² suggested the presence of chlorogenic acid, rutin acid, and quinic acid. The DPPH[•] radicals scavenging capacity was demonstrated (IC₅₀ = 0.06 mg/mL). The extract was administered to rats by gavage (300 mg/kg/day) for 56 days. Dyslipidemia was induced by administering Triton WR-1339 (300 mg/kg body weight) on the 54th day. On day 56, blood was collected by puncturing the abdominal aorta artery and the aortic artery was removed. Lipid profile, markers of renal and hepatic injury, lipid peroxidation, and platelet aggregation tests were carried out. The ingestion of extract reduced the lipid peroxidation (aorta and plasma) and platelet aggregation in dyslipidemic rats. The extract did not affect markers of renal and hepatic function as analyzed in this study, suggesting neither impaired liver nor kidney function in these animals. Therefore, our results demonstrate that the extract of leaves of *C. arabica* L. show antioxidant potential *in vitro* and *in vivo* as well as anti-platelet aggregation in dyslipidemic animals.

Keywords: Coffee. Antioxidant. Dyslipidemia. Lipid peroxidation. Platelet aggregation.

INTRODUCTION

Different studies present well-established correlations between atherosclerosis, the major risk factor for coronary artery disease, and the concentration of serum lipids and retention of cholesterol in the arterial intima (Kathiresan, Srivastava, 2012; Moriya, 2019). The increase of oxidative

metabolic activity due to hyperlipidemia associated with low concentrations of endogenous antioxidants can lead to oxidative stress (Abdou, Wahby, 2016). The activation of inflammatory cells and platelets mediated by hyperlipidemia as well as the interactions between vascular elements, lipoproteins, and oxidative stress play an important role in the atherosclerosis process establishing links between inflammation, thrombosis, and atherogenesis (Colas *et al.*, 2011; Escárcega *et al.*, 2018).

Studies have shown that the ingestion of products with high level of polyphenols may reduce the risk of coronary artery disease development, which has been

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identified as the leading cause of morbidity and mortality in industrialized societies (Graziano *et al.*, 2010; Sanchis-Gomar *et al.*, 2016; WHO, 2017; CDC, 2020).

Coffee (*Coffea arabica* L.) is one of the most valuable commodities. Although coffee beverage is consumed worldwide by all social classes as dietary habit, tea of coffee leaves has been used by the population of several countries. Thus, coffee leaves have traditionally been used as a stimulant, tonic, abortifacient, and to treat flu, respiratory and circulatory diseases, rheumatic disorders, anemia, edema, diarrhea, intestinal pain, HIV/AIDS control, migraine pain, and to relief fever (Rezende, Cocco, 2002; Souza, Mendonça, Silva, 2013; Chen, Ma, Kitts, 2018).

Among the several bioactive substances present in coffee leaves, phenolic compounds, such as phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic, and sinapic acids) and flavonoids (anthocyanins, quercetin glucoside, quercetin, isoquercitrine, rutin, and kaempferol) as well as caffeine, trigonelline, kahweol, cafestol, amino acids, sucrose, tannins, mangiferin catechin, epicatechin, alpha-tocopherol, carotenoids, and ascorbate have been reported in the literature (Lepelley *et al.*, 2007; Magalhães *et al.*, 2008; Chen, Ma, Kitts, 2018). However, scientific data about the biological activity as well as adverse effects of compounds extracted from coffee leaves are still scarce.

Therefore, this study aimed to evaluate the antioxidant potential of *Coffea arabica* L. leaf extract and its effects on platelet aggregation using *in vivo* model of dyslipidemic rats. This dyslipidemia model promotes the inhibition of the enzyme lipoprotein lipase and, consequently, promotes the accumulation of triglycerides and VLDL in the blood plasma. Furthermore, the enzymatic action of HMG-CoA reductase in the liver increases, resulting in a high cholesterol content. Both effects are the result of the administration of a non-ionic detergent called Triton WR-1339 (Zarzecki *et al.*, 2014).

MATERIAL AND METHODS

Chemicals and reagents

Ethanol, sodium chloride, sodium citrate, and sodium carbonate were obtained from Merck

Millipore (Germany). Folin & Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), triton WR-1339 (Tyloxapol), adenosine diphosphate, 1,1,3,3-tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), gallic acid, and 3,5-Di-tert-4-butylhydroxytoluene (BHT) were purchased from Sigma Aldrich (USA). Reagent kits for total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine determinations were purchased from Labtest Diagnóstica S.A. (Brazil).

Obtaining *Coffea Arabica* L. leaf samples

Coffea arabica L. leaves were collected from plants cultivated in sandy soil containing organic matter, in March 2013, in Alfenas-MG with the following geographic coordinates of latitude 21°25'48" S and longitude 46°02'23" W. The voucher specimen of this sample was identified by Dr. Geraldo Alves da Silva, preserved and deposited in the herbarium of the Federal University of Alfenas under registration No. 2,440.

Preparation of *Coffea arabica* L. leaves extract

The fresh leaves of *Coffea Arabica* L. were oven-dried at $44.8 \pm 0.3^\circ\text{C}$ for 168 hours. The dried leaves were grounded and then extracted by percolation using a hydroethanol solution of 70% (w/v). The extract obtained was evaporated to dryness *in vacuo* ($45.3 \pm 4.1^\circ\text{C}$) and lyophilized at -53°C under a pressure 429 μHg for 120 hours (Prista, Alves, Morgado, 2002).

Phytochemical screening

Total phenolic content (TP) was determined using the Folin-Ciocalteu assay (Singleton, Orthofer, Lamuela-Raventos, 1999). TP levels of the sample were standardized regarding gallic acid and expressed as "mg gallic acid equivalents/100 g extract (GAE)." Each determination was performed in triplicate in independent days.

The lyophilized extract was solubilized in water at a concentration of 1 mg/mL, filtered with a 0.45 μm filter and analyzed by Flow-injection analysis-electrospray

ionization (FIA-ESI-MS2). The mobile phase was double deionized with water obtained from Milli-Q Plus, Millipore Merck (0.055 S/cm, 27°C).

The spectra from the FIA-ESI-MS/MS procedure was obtained on the Thermo Scientific LTQ XL linear ion trap analyzer equipped with an ESI interface, in negative mode. A capillary tube made of stainless-steel at 280 °C was used, with a spray voltage of 5.00 kV, capillary voltage of -90 V, tube lens of -100 V and a 5 µL/min flow. Full scan analysis was recorded in the mass range between 100 and 2000 Da. Multiple-stage fragmentations (ESI-MS) were performed using the collision-induced dissociation (CID) method applying helium for ion activation. The first event was a full-scan mass spectrum to acquire data about ions in that mass range. The second scan event was an MS/MS experiment performed using a data-dependent scan on the [M-H] – molecules from the compounds of interest at a collision energy of 30% and an activation time of 30 ms. The product ions were then submitted to further fragmentation under the same conditions until no more fragments were observed. The identification of the different compounds in the chromatographic profile of the hydroethanolic extract was accomplished by the mechanisms of fragmentation and comparing their mass spectral data with the literature (Mendonça, 2008; Michelin, 2008; Santos *et al.*, 2014; Araújo *et al.*, 2019; Bastos *et al.*, 2019).

DPPH radical scavenging assay

DPPH[•] radical scavenging activity measurement was performed according to the methodology described by Dudonné *et al.* (2009). Data were compared with a standard curve made by butylated hydroxytoluene (BHT) ethanolic solution at the same concentration of the *Coffea arabica* L. extract (1%). Each determination was performed in triplicate, in independent days.

Animal experimental protocol

The study was conducted in accordance with the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation, and this study was approved by the Research Ethics

Committee on Animals at the Federal University of Alfenas (UNIFAL-MG), protocol No. 504/2013. In total, 38 adult male Wistar rats (*Rattus norvegicus*) weighing 350 ± 25g, obtained from the UNIFAL-MG vivarium, were used in this study. The animals were housed in a temperature-controlled room and maintained on a 12 hours light/dark cycle with food and water available *ad libitum*.

After one week of acclimatization, the animals were divided into the following four groups (n=8): animals treated with water and 0.9% saline (Control group); animals treated with water and Triton WR-1339 (Dyslipidemic group); animals treated with the extract of *Coffea arabica* L. leaves and 0.9% NaCl (Extract group) and animals treated with the extract of *Coffea arabica* L. leaves and Triton WR-1339 (Dyslipidemic-Extract group).

The dose selection of ethanolic extract of *Coffea arabica* L. leaves was based on preliminary studies. Thus, ethanolic extract of *Coffea arabica* L. leaves (300 mg/Kg/day) was administered to animals by gavage during 56 days of treatment. The control group received the same dosage of water.

On the 57th day after treatment, the dyslipidemia was induced intraperitoneally by the administration of Triton WR-1339 (Tyloxapol, Sigma Aldrich) dissolved in 0.9% NaCl to fasting animals, in a single dose of 300 mg/kg of body weight, according to Silvério *et al.* (2013). Control animals received 0.9% (p/v) NaCl intraperitoneally.

Six rats were treated with clopidogrel suspended in 5% (w/v) solution of gum arabic (1mL/kg) 6 hours before blood collection to obtain platelet-rich plasma (PRP) which was used as abnormal controls (Sugidachi *et al.*, 2007) in the evaluative assays of the platelet aggregation.

Blood sample collection

After the Triton WR-1339 injection, the animals were fasted for 12 hours and anaesthetized with sodium pentobarbital (40 mg/Kg, i.p.). Blood samples were collected by puncturing the abdominal aorta and distributed into siliconized tubes without additives, tubes with 1.8 mg/mL tripotassic ethylene diaminetetraacetic acid (K3EDTA), and tubes with sodium citrate. Blood

aliquots were distributed in siliconized glass tubes (without additive) and tubes with K3EDTA were centrifuged at 1500g for 10 minutes and stored at -80 °C to obtain serum and plasma. The serum samples were used to determine biochemical parameters. Protease inhibitors (50 µg/mL phenyl-methyl-sulphonyl fluoride, 100 µM benzamidine and 50 µM trypsin) were added to plasma obtained from blood collected with K3EDTA and the samples were stored at -80°C for evaluation of lipid peroxidation. Sodium citrate tubes were used to obtain *platelet rich plasma* samples and to assess platelet aggregation.

Animals were euthanized by cervical dislocation, and the aorta was then removed and homogenized in 0.1 M phosphate buffered saline (PBS) containing EDTA and it was also centrifuged at 3,000 g at 4°C for the evaluation of lipid peroxidation (Silvério *et al.*, 2013). The aorta was removed and stored at -80°C in 0.1M phosphate buffered saline (PBS) containing protease inhibitors (50 mg/mL phenyl-methyl-sulphonyl fluoride, 100 µM benzamidine and 50 µM trypsin) and homogenized at 4°C.

Biochemical parameters

The serum lipid profile was assessed by enzymatic assays (Trinder) for total cholesterol, HDL cholesterol, and triacylglycerides (Burtis, Ashwood, 2008). Non-HDL cholesterol was estimated based on a lipid profile (non-HDL-C = total cholesterol - HDL cholesterol). This parameter measures the cholesterol content of all atherogenic lipoproteins, including LDL and VLDL cholesterol (Virani, 2011).

The renal function was assessed by determining the urea and creatinine concentrations in serum with the enzymatic method and the modified Jaffé method, respectively (Burtis, Ashwood, 2008).

The hepatic injury was assessed by determining aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) activities in serum as outlined in both the Reitman and Frankel method and the modified Roy method (Burtis, Ashwood, 2008).

All analyses were determined in automatic analyzer LabMax Plenno, using *commercial kits* from Labtest.

Platelet aggregation

Platelet aggregation was assessed in aliquots of platelet-rich plasma (PRP) as outlined in Baldissera-Jr. *et al.* (2010), in a dual channel aggregometer (Qualitem) standardized at 37 °C. Briefly, the washed platelet solution and 1mM calcium chloride were previously incubated at 37°C. Then, platelet aggregation was induced using 5µM adenosine diphosphate (ADP) as an agonist. The result of the test was expressed as a percentage of aggregation by the quantity of light transmitted through the test solution after the addition of 5µM ADP.

Lipid peroxidation

Lipid peroxidation was determined in plasma and homogenized aorta by quantification of malonaldehyde (MDA) using fluorescence spectrophotometer Cary Eclipse Varian®, according to PUNCHARD and KELLY (1996). The MDA concentration was estimated based on a calibration curve using tetraethoxypropane (Sigma Aldrich-USA). The results were expressed as nmol MDA/mg protein. The protein content of the aorta homogenate was measured using the Bradford method (Bradford, 1976), using BSA as the standard and spectrophotometry Genesys. The plasma protein was measured by Biuret method (Burtis, Ashwood, 2008), using automatic analyzer LabMax Plenno and *commercial kits* from Labtest.

Statistical Analysis

The results were expressed as mean ± standard deviation. The results were also submitted to Shapiro-Wilk test, two-way analysis of variance and they were compared using the Tukey's or Scott-Knott test at 5% significance. Analyses were performed using the statistical program SISVAR (System for Analysis of Variance).

RESULTS AND DISCUSSION

Standardization and phytochemical screening of the *Coffea arabica* L. leaf extract

It was observed that plant material particles were distributed predominantly in sieves with a mesh aperture of 850 micrometers. The average particle size of the leaves powder was 688.145 ± 1.480 mm. Cutting the sheets of *Coffea arabica* L. leaf was classified as semi-thin sectioning (Simões *et al.*, 2003).

For the preliminary evaluation of the chemical composition of the *Coffea arabica* L. leaf extract, the total phenolic content and mass spectrometry were determined. The extract phenolic levels was 6.8 ± 0.1 g GAE per 100 g extract. This result (68 g/Kg of extract) was higher than the values 10.30, 24.79, and 40.80 g/Kg, obtained for methanolic extracts of *Coffea arabica* leaves, from three regions of Brazil (de Almeida *et al.*, 2019). In this research, the mass spectroscopy was applied to identify the probable structures of the compounds in the extract. Thus, the negative ion mode was applied. The results presented peaks that indicate the presence of compounds as quinic acid, chlorogenic acid, and rutin in the extract analyzed (Figures 1-4). The precursor ion at m/z 191

corresponds to deprotonated quinic acid molecule (Figure 1). The MS² spectrum showed product ions at m/z 127 (Figure 2) is compatible with the loss of four hydroxyls, according to Michelin (2008). The data provided by the mass spectrum (Figure 2) and the presence of precursor ion of m/z 191 (Figure 1) are suggestive of the presence of quinic acid molecule in the extract composition. The second-order fragmentation of the precursor ion m/z 353 (Figure 3) showed product ion of m/z 191 [$M - 162 - H$] suggesting the presence of a unit of caffeic acid (162 Da) esterified by a unit of quinic acid (191 Da), which is compatible with the structure of chlorogenic acid presented by Michelin (2008).

According to Santos *et al.* (2014), the mass spectrum in negative mode, the precursor ion of m/z 609 (Figure 1), may correspond to the rutin. The MS² spectrum showed product ions at m/z 301 (Figure 4), which may be due to loss of hexose sugars (162 Da) and deoxyhexose (146 Da), generating quercetin (301 Da) (Santos *et al.*, 2014).

The presence of these compounds corroborates with the literature and indicates to the presence of natural antioxidants in the leaves of *Coffea arabica* L. (de Almeida *et al.*, 2019; Silva *et al.*, 2020). Chlorogenic acid levels are higher in *Coffea arabica* leaf extracts than in coffee beans (Silva *et al.*, 2020).

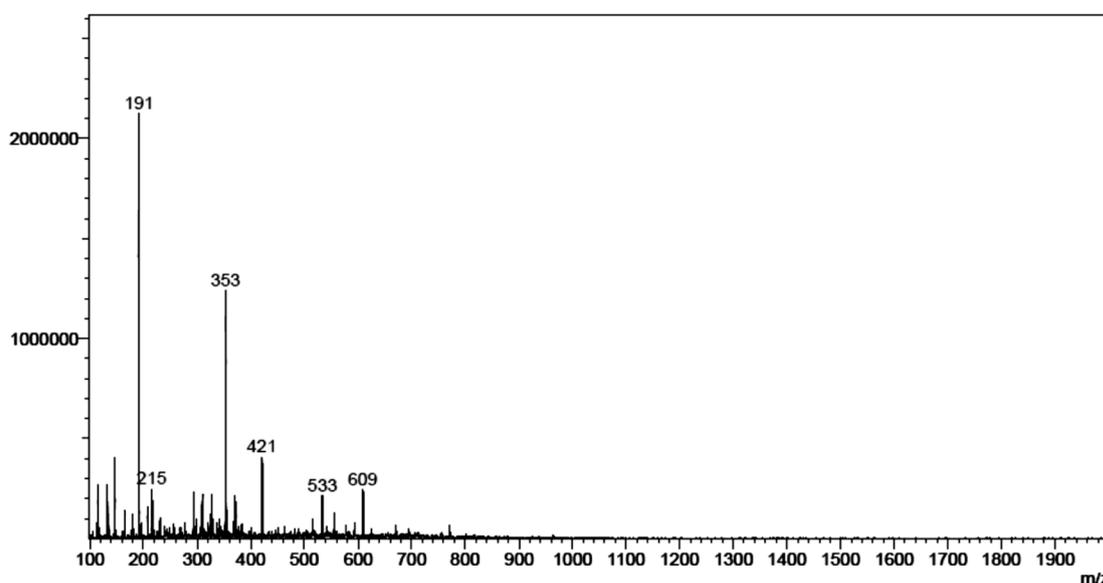


FIGURE 1 - First order mass spectrum in full-scan mode of the *C. arabica* L. dry extract analyzed in negative mode by FIA-ESI-MS². Range of ions m/z 100 to 1900 Da.

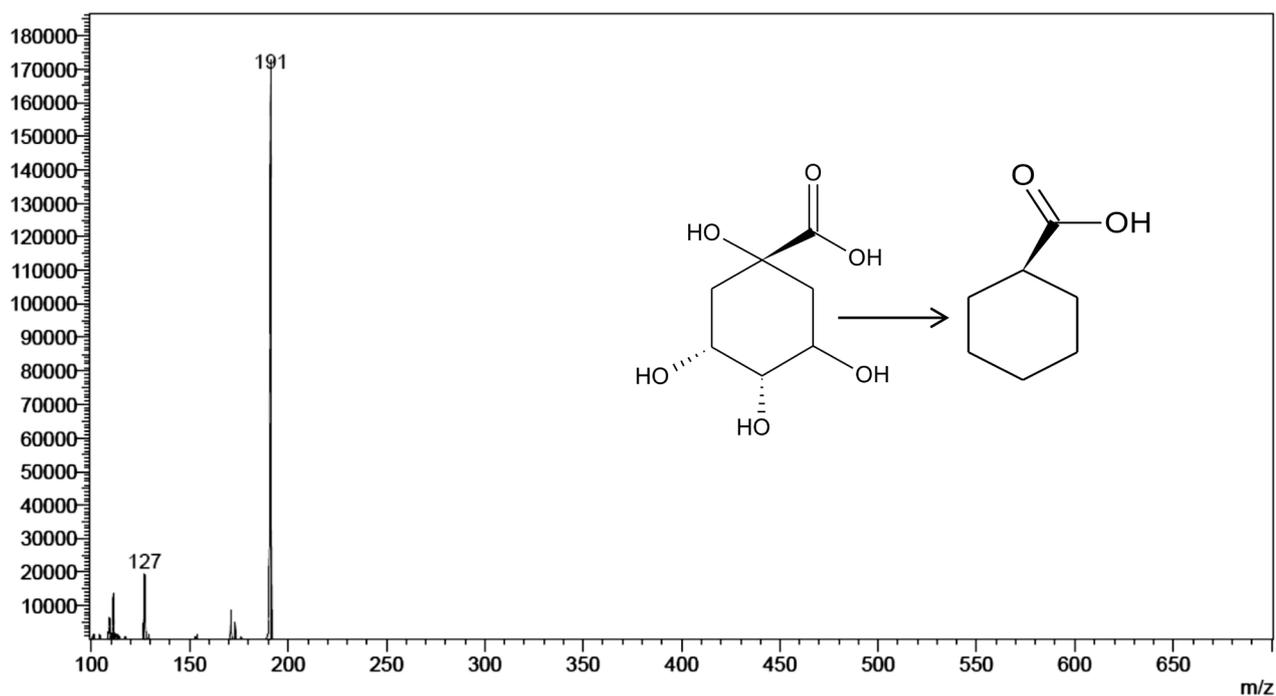


FIGURE 2 - The MS² spectrum of the precursor ion m/z 191 using 20eV of collision energy.

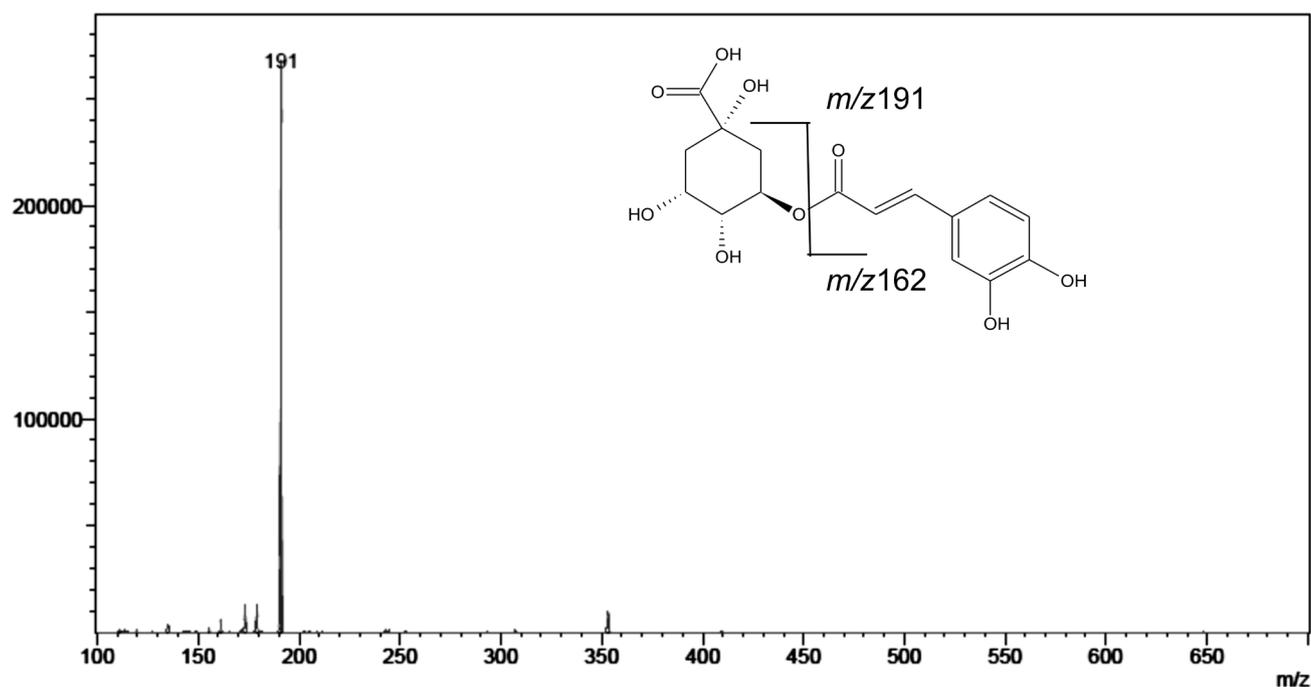


FIGURE 3 - The MS² spectrum of the precursor ion m/z 353 using 20eV of collision energy.

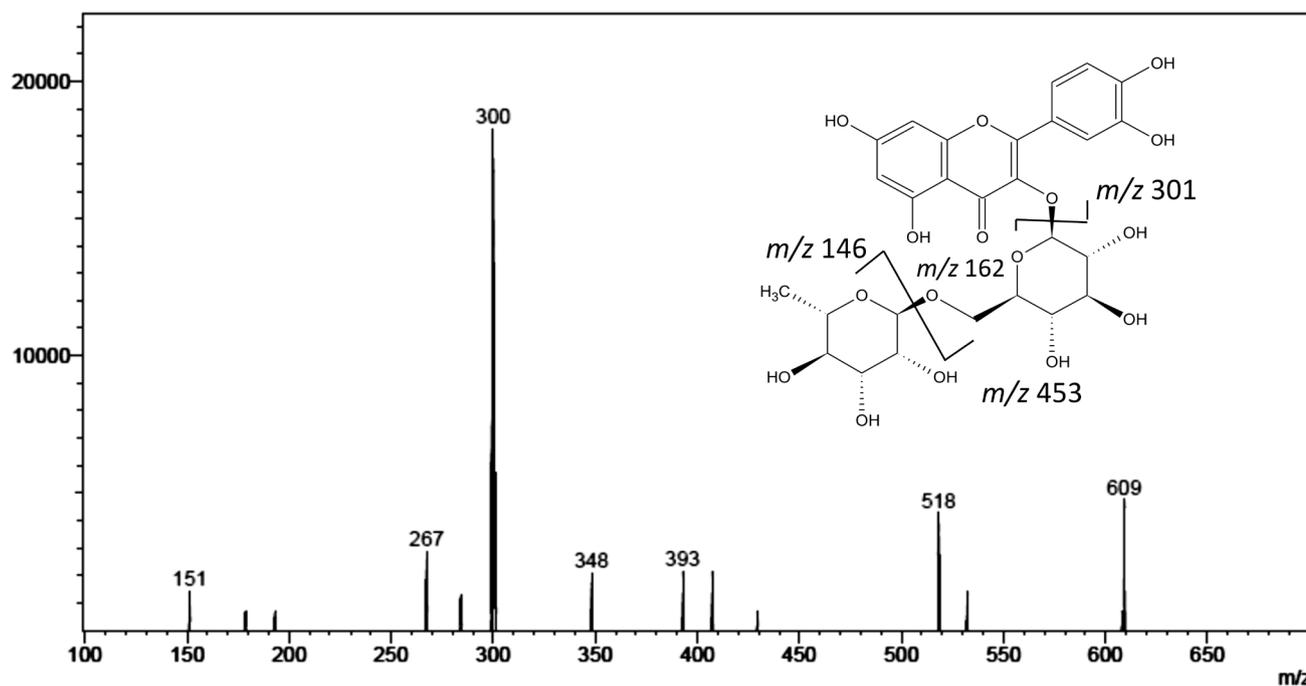


FIGURE 4 - The MS² spectrum of the precursor ion *m/z* 609 using 20eV of collision energy

Evaluation of DPPH radical scavenging activity of the *Coffea arabica* L. leaf extract

The antioxidant capacity was evaluated using the DPPH[•] radical scavenging assay. This assay is one of the most widely used methods to assess antioxidant capacity of plant samples (Marcheafave *et al.*, 2019). The results of the leaves extract scavenging capacity was expressed as concentration-dependent and ranged between 14.72 and 90.14%. The EC₅₀ value was 60 µg/mL (concentration Log = 1.7781) for DPPH[•]-radicals, whereas the maximum activity of 90.140 ± 0.108% occurred with a concentration of 160 µg/mL

(concentration Log = 2.2041). These results were similar to those observed with the BHT standard (Figure 5), demonstrating that the *Coffea arabica* L. leaf extract was able to donate hydrogen to DPPH[•] and it had *in vitro* antioxidant potential.

Among compounds with the ability to donate hydrogen atoms or reductive capacity, phenolic compounds have been subject of great interest in the scientific literature because these compounds are effective free radical scavengers. Thus, the antioxidant activity observed can be attributed to the presence of phenolic compounds as chlorogenic acid, rutin, and quinic acid in the leaves extract (Chiang *et al.*, 2011).

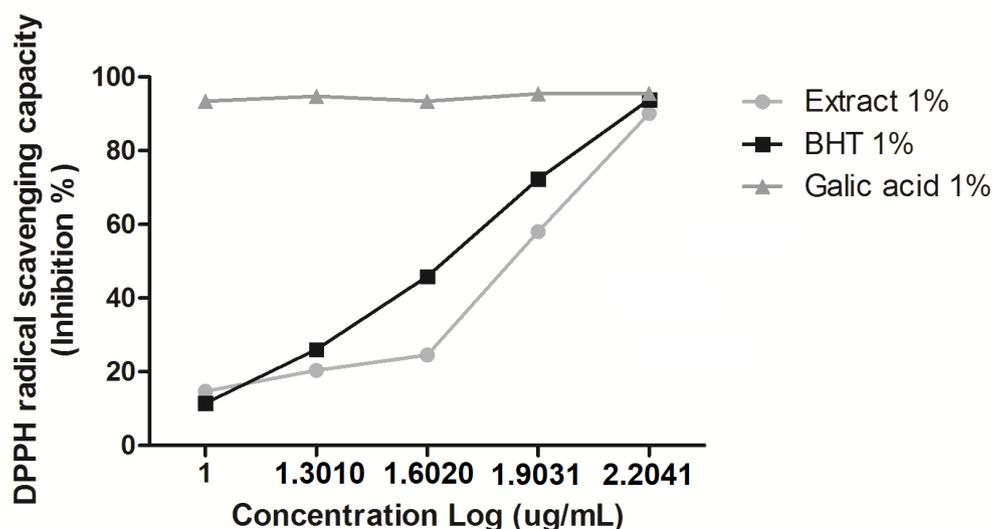


FIGURE 5 - Evaluation of the *in vitro* antioxidant activity of the *Coffea Arabica* L. leaf ethanolic extract by the DPPH radical scavenging assay. Data were expressed as the mean of triplicate \pm standard deviation (SD), performed in independent days. Different letters in same column indicate significance at $p < 0.05$. DPPH: 2,2-diphenyl-1-picrylhydrazyl, BHT: 3,5-Di-tert-4-butylhydroxytoluene.

Evaluation of antioxidant effect of the *Coffea arabica* L. leaf extract in rats

Medicinal plants have been an alternative strategy to prevent and treat several diseases, including atherosclerosis (Rodrigo, Miranda, Vergara, 2011; Ji *et al.*, 2019). Bioactive compounds have demonstrated efficacy, safety, and they have been well tolerated as a lipid-lowering alternative, as well as resulting in fewer side effects, such as fatigue (Ji *et al.*, 2019; Karri *et al.*, 2019).

Active substances such as alkaloids, saponins, polyphenols, and flavonoids can modulate metabolic pathways and monitor the concentration of plasma lipids by different mechanisms of action (Ji *et al.*, 2019). Its exploration can result in the effective and safe development of anti-obesity drugs (Yun, 2010; Balaji *et al.*, 2016).

Coffea arabica is cited as a natural anti-obesity agent. Constituents such as flavonoids and polyphenols are able to control body weight (Karri *et al.*, 2019). Phytochemical screening of *Coffea arabica* L. leaf extract confirmed the presence of chlorogenic acid and rutin. Chlorogenic acid presents antioxidant, anti-inflammatory, anti-oxidation, anti-glycation, and

potential regulation of glucolipid metabolism and it is able to exert important role in the lipid regulation and on related disorders, as cardiovascular diseases (Tajik *et al.*, 2017; Cao *et al.*, 2020). It can inhibit obesity and reduce blood lipids (Liu *et al.*, 2018). Rutin is one of the most studied flavonoids and its effects on lipid peroxidation inhibition has been reported (Pisoschi, Pop, 2015; Gues *et al.*, 2017).

Thus, this study evaluated the effects of the ingestion of *C. arabica* L. leaf extract on lipid peroxidation in dislipidemic animals. In preliminary studies, other doses (50 mg/Kg/day, 150 mg/kg/day and 300 mg/kg/day) of this extract were assessed (data not shown). However, the dose of 300 mg/kg/day was the lowest concentration of extract that showed efficacy to inhibit lipid peroxidation, according to our experiments.

It was observed that the animals treated with Triton WR-1339 presented high level of serum total cholesterol, non-HDL cholesterol, and triglycerides. The consumption of the extract (300 mg/kg/day) did not change the serum lipid profiles (Table I). However, the concentration of MDA in the plasma and in the aorta of dyslipidemic animals decreased when they were treated with *Coffea arabica* L. leaf extract in the concentration 300 mg/kg/day (Table II).

TABLE I - Effect of *Coffea arabica* L. leaf ethanolic extract on serum lipid profile in non-dyslipidemic and dyslipidemic animals

Experimental group	Total Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	Non-HDL Cholesterol (mg/dL)	Triglycerides (mg/dL)
Control	86.7±5.3 ^a	33.6±3.1 ^a	53.1±5.2 ^a	95.6±26.4 ^a
Extract	86.8±7.2 ^a	35.6±5.1 ^a	51.3±6.4 ^a	96.7±33.8 ^a
Dyslipidemic	296.2±11.4 ^b	33.6±2.1 ^a	262.5±10.9 ^b	2316.1±212.9 ^b
Dyslipidemic-Extract	302.1±13.3 ^b	31.5±1.7 ^a	270.6±13.9 ^b	2361.1±260.3 ^b

The values represent the average ± standard deviation of eight measurements per treatment. Averages followed by the same letter in the same column do not differ statistically. Different letters (a, b) in same column indicate statistical significance by Scott-Knott Test ($p < 0.05$). HDL: high-density lipoprotein.

TABLE II - Effect of *Coffea arabica* L. leaf extract on the lipid peroxidation and platelet aggregation in non-dyslipidemic and dyslipidemic animals

Experimental group	Lipid Peroxidation (μmol MDA/g protein)		Platelet aggregation (%)
	Aorta	Plasma	
Control	2.24 ± 0.08 ^a	4.16 ± 0.26 ^a	58.80 ± 8.00 ^a
Extract	2.15 ± 0.27 ^a	4.32 ± 0.32 ^a	55.20 ± 7.50 ^a
Dyslipidemic	9.36 ± 0.33 ^b	7.99 ± 0.17 ^b	68.70 ± 4.20 ^b
Dyslipidemic-Extract	4.85 ± 0.34 ^c	6.62 ± 0.26 ^c	59.50 ± 8.60 ^a

The values represent the average ± standard deviation of eight experiments per treatment. Averages followed by the same letter in the same column do not differ statistically. Different letters (a, b, c) in same column indicate statistical significance by Scott-Knott Test, $p < 0.05$. MDA: malonaldehyde.

These data are relevant since the accumulation of lipoproteins, mainly low-density lipoprotein (LDL), in the vascular wall has been considered as a key process in early atherogenesis, which may undergo oxidative processes that are mediated by ROS and RNS. This ultimately leads to an increased formation of lipid peroxidation products, such as MDA (Badimón, Vilahur, Padró, 2009). These results corroborate literature data, which have shown a reduction in lipid peroxidation in different tissues after ingestion of phenolic compounds, which include chlorogenic acid and rutin. The antioxidant activity of these compounds may be responsible for the reduction of ROS levels and oxidative stress, reflecting in the reduction of lipid peroxidation (Jurgonski *et al.*, 2012; Vinothkumar *et al.*, 2014; Tajik *et al.*, 2017).

Rutin may also have contributed with the results, considering it is able to activate antioxidant enzymes and to suppress lipid peroxidation, reducing levels of lipid damage in animals treated with leaf extract (Manzoni *et al.*, 2020; Sharma *et al.*, 2020).

Evaluation of anti-platelet effect of the *Coffea arabica* L. leaf extract in rats

Hyperlipidemia can activate platelet aggregation and trigger vascular occlusion and atherothrombotic diseases (El-Seweidy *et al.*, 2019). Considering that oxidized LDL particles can bind platelets and induce their activation, leading to morphological changes and increased aggregation (Badimón, Vilahur, Padró, 2009),

the effect of extract intake on platelet aggregation was also evaluated. Although the effect of *Coffea arabica* L. extract on inhibition of platelet aggregation in dyslipidemic and non-dyslipidemic rats (59.50 ± 8.60 and 55.20 ± 7.50 , respectively) was less than that observed in animals treated with clopidogrel ($42.5\% \pm 2.74$, $p < 0.05$), a significant inhibition of platelet aggregation was evidenced in dyslipidemic animals treated with the extract when compared to the group of dyslipidemic animals that did not receive *Coffea arabica* L. leaf extract (Table II).

These results corroborate the data from literature and they may be related to the presence of chlorogenic acid in *Coffea arabica* L. leaf extract, which can suppress the expression of P-selectin in blood platelets, inhibiting the activity of cyclooxygenase and it can also act as an antioxidant (Olas, Bryś, 2019). Most flavonoids are able to reduce platelet aggregation by arachidonic acid and rutin, which was identified in the leaf extract, may have contributed to the observed results (Faggio *et al.*, 2017).

Therefore, the results obtained were able to protect platelets and a large number of rats against the dangerous effects of dyslipidemia, suggesting that the antiplatelet effect of the extract is related to its antioxidant activity. The antioxidant potential was confirmed by the DPPH radical assay and decreased lipid peroxidation in the plasma and aorta of dyslipidemic rats treated with the extract.

However, the possible involvement of other mechanisms on the antiplatelet effect of the extract

should not be discarded since it has been shown that chlorogenic acid inhibited platelet activation by A2A receptor/adenylate cyclase/cAMP/PKA activation, and consequently, suppression of activation of the GPIIb/IIIa receptor and platelet secretion (Fuentes *et al.*, 2014).

Evaluation of effect of the *Coffea arabica* L. leaf extract on hepatic and renal functions in rats

Despite several health benefits attributed to the presence of phenolic compounds in food, beverages, and vegetables extracts, the adverse reactions caused by whichever product may be studied before used in humans. Thus, considering that many ingestible phenolic compounds can be metabolized or conjugated to sulfate in the liver and excreted in urine (Thilakarathna, Rupasinghe, 2013), and that nephrotoxicity and hepatotoxicity are some of the undesirable effects of the extract studied, the effect of the extract *Coffea arabica* L. on renal function and hepatic injury was evaluated.

Table III shows the results of the serum activity assays for liver enzymes that are indicative of lysis, as well as for the levels of urea and creatinine in the serum of animals belonging to different groups. It was observed that the consumption of leaf extracts of *C. arabica* L. at a dose of 300 mg/kg body weight for 56 days did not significantly alter these markers, suggesting no impairment of liver and kidney function in these animals.

TABLE III - Effect of *Coffea arabica* L. leaf extract on the renal function and hepatic injury in non-dyslipidemic and dyslipidemic animals

Experimental group	Renal function		Hepatic injury		
	Urea (mg/dL)	Creatinine (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	42.80±4.72 ^a	0.41±0.06 ^a	120.90±5.41 ^a	38.50±3.46 ^a	31.90±1.10 ^a
Extract	42.00±3.47 ^a	0.40±0.04 ^a	125.20±4.27 ^a	39.20±3.74 ^a	28.50±3.43 ^a
Dyslipidemic	44.70±5.22 ^a	0.43±0.05 ^a	125.90±4.43 ^a	38.50±4.59 ^a	44.30±3.35 ^b
Dyslipidemic-Extract	44.50±3.54 ^a	0.44±0.05 ^a	134.70±25.40 ^a	37.50±7.32 ^a	47.60±5.01 ^b

The values represent the average ± standard deviation of eight experiments per treatment. Averages followed by the same letter in the same column do not differ statistically. Different letters (a, b) in same column indicate statistical significance by Scott-Knott Test, $p < 0.05$. AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

The hepatoprotective effects observed can be attributed to the presence of chlorogenic acid due to its antioxidant and anti-inflammatory actions. Its antioxidant property relieves oxidative reactions and protects the liver from oxidation, acting on the expression of antioxidant enzymes and preventing lipid peroxidation (Tofalo *et al.*, 2016; Hosseinabadi *et al.*, 2020).

There are few studies on the biological effects of coffee leaves and, consequently, on renal function. However, a study with green coffee extract presented improvement in kidney function and relief of inflammation and oxidative stress markers (AlAmri *et al.*, 2020) and coffee consumption provides lower levels of uric acid, reduces the incidence of renal failure and helps prevent kidney disease (Catalano *et al.*, 2015). The beneficial effect of coffee on kidney function may be related to the presence of chlorogenic acid, its metabolites and Mg²⁺ that act positively on the kidney through its antioxidant and anti-inflammatory properties (Nakajima, Kanno, 2013). The results found for the coffee leaf extract are consistent with the works of the previously reported literature.

CONCLUSIONS

The *Coffea arabica* L. leaf extract presented antioxidant activity both *in vitro* and *in vivo*, as well as protective effect against platelet aggregation in dyslipidemic rats. These results justify its medicinal value and suggest that the leaf extract may have a beneficial effect in preventing atherosclerosis or other thrombotic events. Future stages of the study include the fractionation of the extract to identify its bioactive fraction.

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