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HYDROETHANOLIC EXTRACT OF *CEDRELA ODORATA* IMPROVES HYPERLIPIDEMIA AND REDUCES THE ACTIVITY OF ENZYMES IN THE LIVER OF ETHANOL-INDUCED HYPERLIPIDEMIC RATS

E. W. Allebrandt Neto [1], S. F. Santos [1], F. H. S. Silva Gomes [1], C. V. Santos [3], M. I. Watanabe [2], M. A. Giordani [3], N. H. Kawashita [1] and M. P. Pereira [*1]

Universidade Federal de Mato Grosso [1], Departamento de Química, Cuiabá, Mato Grosso, Brazil.
Universidade Federal de Mato Grosso [2], Departamento de Ciências da Saúde Básicas, Cuiabá, Mato Grosso, Brazil.
Universidade Federal de Mato Grosso [3], Instituto de Ciências da Saúde, Sinop, Mato Grosso, Brazil.

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Correspondence to Author:
M. P. Pereira

Ph.D,
Department of Chemistry,
Federal University of Mato Grosso,
Cuiabá, Brazil.

E-mail: mayara.pereira@ufmt.br

ABSTRACT: Hydroethanolic extract of the *Cedrela odorata* (HeECo) showed hypolipidemic potential in diabetic rats. Thus, we evaluate the hypolipidemic effect and the mechanism of the action of the HeECo in a rat model of ethanol-induced hyperlipidemia. Male Wistar rats are divided: normolipidemic (C); hyperlipidemic non-treated (Hl); hyperlipidemic treated with HeECo 500 $mg.kg^{-1}$ (HlCo); and hyperlipidemic treated with fenofibrate 200 $mg.kg^{-1}$ (HlFen). The relative weight, lipid content of some tissues, serum lipid profile, enzymatic activity and content of lipase lipoprotein (LPL) in retroperitoneal white adipose tissue (retWAT) were measured. Protein content of SREBP-1c and enzyme activities of glucose-6-phosphate dehydrogenase (G6PD), malic enzyme and ATP-citrate lyase (ACL) were measured in the liver. We observed an increase in the levels of triacylglycerol, total cholesterol and VLDL in the Hl group that the C group. The HlCo group showed reduced serum parameters, relative weight, lipid content and LPL activity in retWAT, that the Hl group. We observed lower activity in G6PD and ACL in the HlCo group that the Hl group. We suggest that HeECo has a hypolipidemic effect, possibly reducing de novo fatty acid synthesis in the liver.

INTRODUCTION: Dyslipidemia is a known disorder in lipid metabolism with an increase in cholesterol and triglycerides (TAG) in the plasma [1-2]. It is among the main risk factors for atherosclerosis, myocardial infarction and stroke. The reduction of blood lipids is an important strategy for preventing the occurrence and progression of these diseases.

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In some instances, changes in lifestyle, such as lower caloric intake, the introduction of physical activity and quitting smoking are not enough, or perhaps the patient cannot wait for results due to clinical priority. In those cases, pharmacological treatment is necessary, using drugs with lipid-lowering action from the statins and fibrates groups [3]. The use of allopathic medicine is not always accessible for all people in general.

The leading cause of irregular treatment, in addition to the side effects, is the high cost of continuous therapy [4]. With this in mind, the development of new, effective and affordable therapeutic alternatives for the low-income population has become increasingly important.

Since, these alternatives are already in popular medical use it is also important to prove their efficacy. In this context, medicinal plants have become great allies since they are potential sources of therapeutic compounds .

Cedrela odorata, from the *Meliaceae* family, also known as pink cedar, white cedar, and red cedar, is a large tree reaching up to 35 meters in height. It grows widely throughout Brazil and in the countries of South America, occurring in all plant environments, but mainly in the Atlantic Forest [5]. It is used for wound healing [6] and in the treatment of diarrhea, vomiting, fever and inflammation [7]. Giordani *et al* [8] conducted studies with hydroethanolic extract of the inner bark of *Cedrela odorata* (HeECo), based on an ethnopharmacological study [9]. The extract, at a dose of 500 $mg.kg^{-1}$, presented low toxicity and was able to reduce blood glucose levels in diabetic animals treated for 21 days with the extract and which received an overload of glucose. The same authors observed a 36.6% reduction in plasma TAG values [8]. The HeECo showed the presence of higher quantities of gallic acid, (-)- gallocatechin and (+)-catechin by high performance liquid chromatography analysis [8]. In summary, the objective of the present study was to confirm the potential hypolipidemic effect of the hydroethanolic extract of the inner stem bark of *Cedrela odorata* in hyperlipidemic rats induced by acute ethanol administration and to propose the mechanism of action.

MATERIAL AND METHODS:

Preparation of the Crude Inner Stem Bark Hydroethanolic Extract of *Cedrela odorata*: The inner stem bark of *Cedrela odorata L. (Meliaceae)* used in this study was harvested at Chácara Paraíso, line 6, Juína, Mato Grosso, Brazil, (at geographic coordinates S 15º37,139, W 056º05, 100; elevation: 348 meters) in April 2016. The plant was registered on Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen: www.sisgen.org.br). Voucher specimens were deposited at the Herbarium of Federal University of Mato Grosso (identification number 44341). The hydroethanolic extract of *Cedrela odorata* (HeECo) was prepared as described by Giordani *et al* [8]. The inner stem bark was cleaned, fragmented, and completely dried at 40 ºC and then ground to a powder in an electric mill. Subsequently, the powder was macerated (1:10 m/v) in 70% ethanol for seven days. After this period, the macerate was filtered and concentrated in a rotary evaporator (model 801, 60 Hz, Fisatom, Brazil) under reduced pressure and at a temperature of approximately 50 ºC to 60 ºC and lyophilized (Lyophilizer model LS3000, Terroni). The extract was kept protected from light and stored at 2 ºC to 8 ºC until use. The HeECo was dissolved in 2% dimethyl sulfoxide (DMSO) (vehicle) at the time of use.

Determination of Total Polyphenols Content: Total phenolics content was determined by Folin-Ciocalteu methods. For that, 200 uL the diluted extract solution ($300\mu g.mL^{-1}$) was added in 1000 uL of 20% Na_2CO_3 solution. Lastly, it was added 500 uL of 10% Folin-Ciocalteu solution. The mixture was agitated in the dark. After 30 min, the absorbance at 760 nm was evaluated in a spectrophotometer. A curve was constructed with gallic acid (10, 20, 40, 60, 80 e $100\mu g.mL^{-1}$) and used to determine a concentration of total phenolic which is expressed in mg of gallic acid per g of dry plant extract.

Induction of Hyperlipidemia in Rats and Treatment: This experimental protocol was previously approved by the Animal Research Ethics Committee of the Federal University of Mato Grosso (UFMT) under number 23108.113431/2015-27. Male Wistar rats (*Rattus norvergicus*) weighing between 240g and 250g were used from the Central Animal House of UFMT. The animals were divided into four experimental groups: 1) control group (C) – normolipidemic rats and receiving vehicle as treatment; 2) hyperlipidemic group (Hl) – hyperlipidemic rats that received vehicle as treatment; 3) hyperlipidemic group treated with HeECo (HlCo) – hyperlipidemic rats that received 500 $mg.kg^{-1}$ of HeECo as treatment; and 4) hyperlipidemic group treated with fenofibrate (HlFen) – hyperlipidemic rats that received 200 $mg.kg^{-1}$ of fenofibrate as treatment. The ethanol-induced hyperlipidemia protocol was performed as described by Silva *et al* [10] and is represented in **Fig. 1**. In summary, for five days after water fasting, the animals received a 26% ethanol solution (0.1 $mL.10\ g^{-1}$) by a gastroesophageal tube and a 10%

ethanol solution was offered ad libitum. Only on the 4th and 5th days did the animals receive their respective treatments (vehicle, HeECo or fenofibrate). On the 5th day after 2 hours of the treatments, the animals were euthanized to collect blood and tissues for analysis. The normolipidemic animals received water.

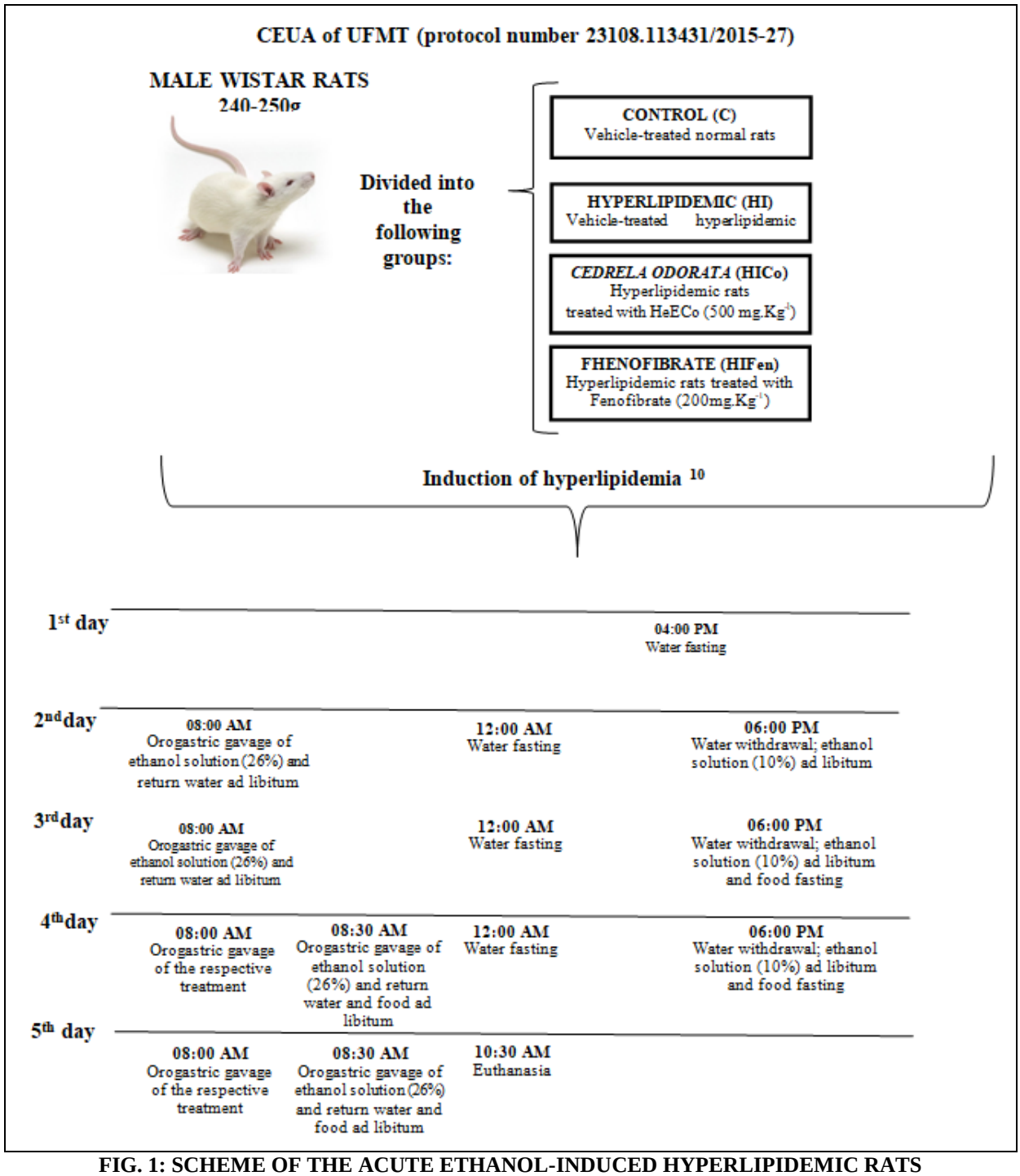

FIG. 1: SCHEME OF THE ACUTE ETHANOL-INDUCED HYPERLIPIDEMIC RATS

Determination of Total Lipid Content in the Liver and Adipose Tissues and Triglycerides in the Liver: Total lipids in the liver, retroperitoneal (retWAT), epididymal (epiWAT), perirenal (periWAT), inguinal (ingWAT) white adipose tissues, and brown adipose tissue (BAT), were determined by gravimetric methods after chloroform-methanol (2:1) extraction according to Folch *et al.* [11]. For the quantification of total cholesterol in the liver, after quantifying the total lipids, the liver lipids contained in the bottles were resuspended and homogenized using isopropanol.

The homogenate was used for total cholesterol measurement through Gold Analisa®, a colorimetric commercial kit, following the protocol provided by the supplier. The results are expressed in mg.g of tissue^{-1}.

Biochemical Parameters and Hepatic Total Cholesterol: At the end of the experimental period (5th day), the animals were euthanized by decapitation between 9am and 11am. Blood samples were collected into tubes containing an anticoagulant to determine the enzyme activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the plasma. Serum was collected to determine the concentration of triglycerides (TAG), total cholesterol (TC) and HDL cholesterol (c-HDL). The levels of LDL and VLDL cholesterol were determined by the Friedewald equation [12]. Biochemical analyses were performed by enzymatic colorimetric methods using commercial kits (Labtest® and Gold Analisa® Belo Horizonte, MG, Brazil) and expressed in mg.dL^{-1}. The coronary risk indices and the atherogenic index were calculated using the formula

(Atherogenic index = Total cholesterol / HDL)

Immunoblotting Analysis: Frozen liver and retWAT samples were homogenized in 50 mmol. L^{-1} Tris– HCl buffer (pH 7.4) containing 150 mmol. L^{-1} NaCl, 1 mmol. L^{-1} EDTA, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 10 mmol. L^{-1} sodium pyrophosphate, 100 mmol. L^{-1} sodium fluoride, 10 mmol. L^{-1} sodium orthovanadate and 1 mmol. L^{-1} phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged at 21 000 g at 4 °C for 40 minutes, and the supernatant was used for protein determination, with bovine serum albumin as the standard [13].

Four volumes of sample buffer (125 mmol.L^{-1} Tris–HCl, 20% glycerol, 4% SDS, 100 mmol.L^{-1} DTT, 0.02% bromophenol blue; pH 6.8) were added to the supernatant. Samples containing 100 µg of protein were subjected to SDS–PAGE analysis on 10% acrylamide gels and electroblotted into nitrocellulose membranes. Proteins were detected by overnight incubation at 4 °C with specific primary antibodies: anti-SREBP-1c (1:200) and anti-lipase lipoprotein (LPL) (1:200). Anti-tubulin (1:200) was used as the internal control. Primary antibodies were detected by peroxidase-conjugated secondary antibodies and visualized with enhanced chemiluminescence reagents (Super Signal West Pico Chemiluminescent Substrate). Band intensities were read and analyzed with Bio-Rad ImageLab 5.0 software.

Activity of ATP-Citrate-Lyase, Malic and Glucose-6-Phosphate Dehydrogenase Enzymes: The activities of the enzymes glucose-6-phosphate-dehydrogenase (G6PD), malic (ME) and ATP-citrate-lyase (ACL) were determined using the same homogenate from the liver. Approximately 100 mg aliquots of liver were homogenized in 1 mL of 10 mM Tris buffer, pH 7.4, containing 0.32 M sucrose, 2 mM EDTA and 5 mM 2-b-mercaptoethanol and centrifuged at 9545 g for 10 minutes at 10 ºC. The upper layer was discarded, and the supernatant was centrifuged again at 18222 g for two hours at 4 ºC to obtain the cytosolic fraction. The protein content of the homogenates was determined by the Bradford method [13] and the enzymatic activity was determined by the spectrophotometric method at a wavelength of 340 nm. The G6PD activity was evaluated based on the reduction of $NADP^+$ [14]. The ME activity was assessed by measuring the formation of NADPH from malate and $NADP^+$ [15]. For the ACL, the assay was performed which is based on the oxidation of NADH [16]. The enzymatic activities of G6PD and ME are expressed in nmol.NADP.mg protein. min^{-1} and for the ACL in nmol.NADH.mg protein^{-1}. min^{-1}.

Lipoprotein Lipase Enzyme Activity: The determination of the enzymatic activity of LPL in the retWAT was performed using 250 mM sucrose buffer-EDTA 1 mM-heparin 20 U.mL^{-1} at a pH of 7.4. The retWAT was homogenized in a buffer (100 mg.1 mL^{-1} buffer) and subsequently centrifuged at 19000 g for 15 minutes at 4 °C using the supernatant for analysis, according to Nilsson-Ehle & Shotz, [17]. The analysis was performed using a commercial colorimetric kit from the Gold Analisa® laboratory (Belo Horizonte, MG, Brazil). The results are expressed in mg of protein^{-1}.

Statistical Analysis: The results are expressed as the mean ± standard error of the mean. The normally distributed data were compared statistically using Student's t-test for groups C vs

HI and by one-way ANOVA, followed by Tukey's post-test comparing groups HI, HICo, and HIFen. The differences found were considered statistically significant when "p" < 0.05.

RESULTS: The hydroethanolic extract of *Cedrela odorata* (HeECo) demonstrates 22.88 mg of gallic acid equivalent per g of dry plant extract **Table 1.**

TABLE 1: TOTAL POLYPHENOLIC COMPOUNDS OF HeECo

Cedrela odorata	Polyphenols
	22.88 mg GAE.g^{-1}

mg GAE.g^{-1}: mg of gallic acid equivalent per g of dry plant extract.

There was no statistical difference in the initial and final body weights **Table 2**. The relative weight of the retWAT had an increase of about 48% in the Hl group, when compared with group C and a reduction of 28% and 34% in the HlCo and HlFen groups, respectively, compared with the Hl group.

The relative weight of the liver had an increase of 11% and 12% only in the HlFen group, when compared with the Hl and HlCo groups, respectively. No difference in the relative weight of the other evaluated tissues was observed **Table 2.**

TABLE 2: INITIAL, FINAL, AND RELATIVE WEIGHT TISSUE WEIGHT OF RATS IN THE C, HL, HlCo, HlFen GROUPS

Variables	Groups			
	C (6)	Hl (6)	HlCo (6)	HlFen (6)
Initial weight (g)	246.656 ± 2.959	248.433 ± 4.266	245.425 ± 4.662	246.465 ± 4.113
Final weight (g)	249.532 ± 3.086	246.745 ± 3.870	241.487 ± 5.746	239.918 ± 4.013
Inguinal WAT (g.100g^{-1})	0.467 ± 0.034	0.534 ± 0.030	0.533 ± 0.033	0.501 ± 0.035
Epididymal WAT (g.100g^{-1})	0.557 ± 0.035	0.614 ± 0.048	0.546 ± 0.036	0.497 ± 0.023
Retroperitoneal WAT (g.100g^{-1})	0.362 ± 0.013	0.535 ± 0.026^{*a}	0.383 ± 0.04^{b}	0.355 ± 0.039^{b}
Perirenal WAT (g.100g^{-1})	0.088 ± 0.011	0.111 ± 0.017	0.081 ± 0.009	0.0811 ± 0.009
Brown adipose tissue (g.100g^{-1})	0.055 ± 0.002	0.077 ± 0.011	0.086 ± 0.017	0.072 ± 0.009
Heart (g.100g^{-1})	0.380 ± 0.011	0.417 ± 0.013	0.403 ± 0.018	0.404 ± 0.011
Liver (g.100g^{-1})	3.215 ± 0.085	3.347 ± 0.080^{a}	3.330 ± 0.044^{a}	3.722 ± 0.063^{b}
Soleus muscle (g.100g^{-1})	0.104 ± 0.002	0.101 ± 0.003	0.102 ± 0.001	0.105 ± 0.002
EDL muscle (g.100g^{-1})	0.097 ± 0.002	0.092 ± 0.001	0.091 ± 0.001	0.098 ± 0.001

WAT: white adipose tissue; Values represent the mean ± standard error of the mean of the number of animals in parentheses. *Student's t-test C *vs* HI (p < 0.05). Different letters indicate statistical difference (Hl *vs* HlCo *vs* HlFen) one-way ANOVA, followed by the Tukey post-test.

The total lipid content was 23% higher in the ingWAT of the Hl group when compared with the C group **Table 3** and the HlFen group showed a reduction of 19% when compared with the Hl group. The treatment with HeECo did not reduce the content of lipids in this tissue. The lipid content increase in the retWAT was about 350% in the Hl group in relation to the C group. The treatment with HeECo was able to reduce this increase by 70%. In the periWAT the lipid content in the Hl group was 23% higher than in the C group **Table 3** and no effect was observed with treatment of the HeECo and fenofibrate (Fen), reference drugs in the hyperlipidemia treatment, on the lipid content of this tissue.

TABLE 3: TOTAL LIPID CONTENT IN THE WHITE ADIPOSE TISSUES, LIVER, AND HEPATIC CHOLESTEROL OF RATS IN THE C, Hl, HlCo, HlFen GROUPS

Variables	Groups			
	C (6)	Hl (6)	HlCo (6)	HlFen (6)
Inguinal WAT	0.376 ± 0.017	0.461 ± 0.015^{*a}	0.416 ± 0.016^{a,b}	0.373 ± 0.009^{b}
Epididymal WAT	0.601 ± 0.010	0.614 ± 0.018	0.571 ± 0.011	0.551 ± 0.028
Retroperitoneal WAT	0.146 ± 0.019	0.661 ± 0.014^{*a}	0.195 ± 0.046^{b}	0.639 ± 0.021^{a}
Perirenal WAT	0.474 ±0.026	0.555 ± 0.023*	0.519 ± 0.029	0.513 ± 0.037
Brown adipose tissue	0.353 ± 0.010	0.344 ± 0.028	0.312 ± 0.038	0.335 ± 0.024
Liver	0.033 ± 0.003	0.041 ± 0.002	0.043 ± 0.002	0.039 ± 0.003
Hepatic cholesterol	37.952 ± 3.155	34.465 ± 2.783	27.257 ± 2.757	33.749 ± 2.009

WAT: white adipose tissue; Values represent the mean ± standard error of the mean of the number of animals in parentheses. *Student's t-test C *vs* HI (p < 0.05). Different letters indicate statistical difference (Hl *vs* HlCo *vs* HlFen) one-way ANOVA, followed by the Tukey post-test.

Table 4 presents the data of the biochemical analysis of the blood. The TAG serum was 131% higher in the Hl group than in the C group. The HlCo and HlFen groups had about 43% lower TAG levels than in the Hl group. The TC did not alter in the Hl group when compared with the C group. However the treatment with HeECo reduced the TC level by 16%, compared with the Hl group and the Fen reduced the TC levels by 23%, compared with the HeECo treatment. cHDL and cLDL did not change in hyperlipidemic rats (Hl group) compared with the C group. However, the HeECo and Fen administration produced 27% and 11% reductions in cHDL, respectively, compared with the Hl group. The cLDL was only reduced in the HlFen group (by about 60%) when compared with the Hl and HlCo groups **Table 4**. The VLDL in Hl rats was almost 150% higher than in the C group and was reduced by about 45% in the HlCo and HlFen groups compared with the Hl group. The Hl group had an increase in the activity of the alanine aminotransferase (ALT) enzyme (53%) compared with the C group, and the treatment with HeECo promoted a reduction (HlCo group) when compared with the Hl group.

TABLE 4: SERUM BIOCHEMICAL PARAMETERS, ATHEROGENIC INDEX, AND LDL/HDL RATIO OF RATS IN THE C, Hl, HlCo, HlFen GROUPS

Variables	Groups			
	C (6)	Hl (6)	HlCo (6)	HlFen (6)
Triglycerides (mg.dL^{-1})	115.9 ± 8.2	267.9 ± 35.1^{*a}	150.2 ± 18.7^{b}	153.2 ± 10.3^{b}
Total cholesterol (mg.dL^{-1})	111.7 ± 8.7	125.8 ± 3.4^{a}	105.5 ± 3.5^{b}	81.0 ± 7.9^{c}
c-HDL (mg.dL^{-1})	54.0 ± 3.6	59.0 ± 0.3^{a}	43.2 ± 3.5^{b}	34.6 ± 2.4^{b}
c-LDL (mg.dL^{-1})	35.9 ± 5.4	45.8 ±7.5^{a}	42.1 ± 3.7^{a}	15.7 ± 3.4^{b}
VLDL (mg.dL^{-1})	23.2 ± 1.6	57.8 ± 7.3^{*a}	30.1 ± 3.7^{b}	34.9 ± 4.5^{b}
Atherogenic index	2.1 ± 0.1	2.4 ± 0.3	2.1± 0.2	2.5 ± 0.2
LDL/HDL ratio	0.6 ± 0.1	0.8 ± 0.3	0.7 ± 0.1	0.5 ± 0.1
Albumin (mg.dL^{-1})	2.8 ± 0.4	2.6 ± 0.3	2.5 ± 0.1	2.4 ± 0.1
Alanine aminotransferase (U/L)	23.6 ± 1.9	36.1 ± 1.0*^{a}	17.0 ± 3.4^{b}	32.4 ± 3.9^{a}
Aspartate aminotransferase (U/L)	66.7 ± 5.1	70.8 ± 4.5	72.0 ± 4.9	74.0 ± 2.7

Values represent the mean ± standard error of the mean of the number of animals in parentheses. *Student;s t-test C *vs* Hl ($p < 0.05$). Different letters indicate statistical difference (Hl *vs* HlCo *vs* HlFen) one-way ANOVA, followed by the Tukey post-test.

There was no significant difference in the LPL content (% of control) in the retWAT among the groups (C: 100.00 ± 18.66; Hl: 131.80 ± 23.37; HlCo: 75.40 ± 16.79; HlFen: 56.11 ± 9.88) **Fig. 2A**. The LPL activity (mg of protein^{-1}) in the retWAT did not show a difference in the Hl group (308.4 ± 2.4) when compared with the C group (318.3 ± 8.8). However, reductions of 55% and 52% were observed in the HlCo group (137.7 ± 7.7) compared with the Hl and HlFen groups (289.4 ± 6.1), respectively **Fig. 2B.**

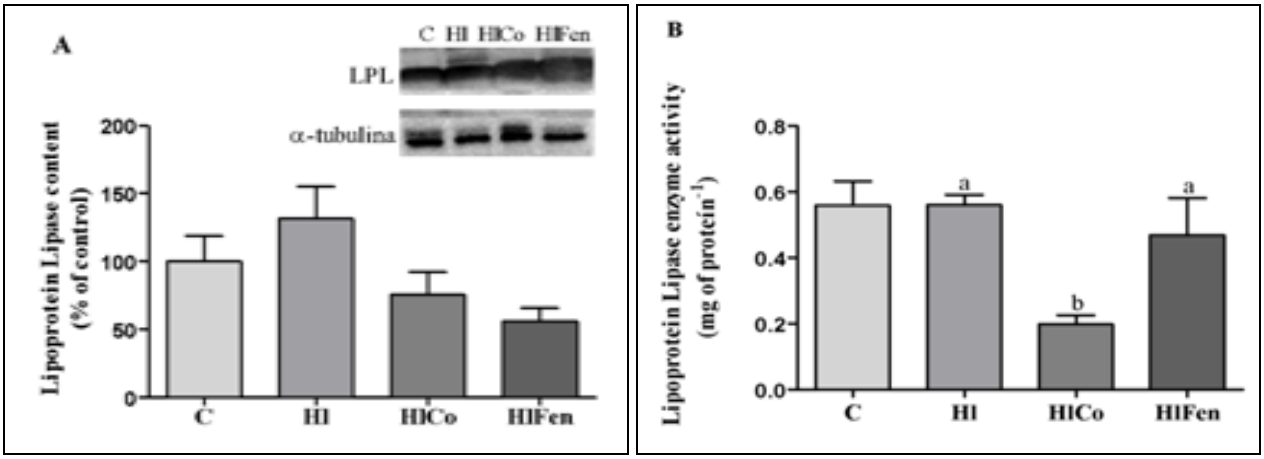

FIG. 2: PROTEIN CONTENT (A) AND ACTIVITY (B) OF LPL IN THE RETROPERITONEAL WHITE ADIPOSE TISSUE OF RATS IN THE C, Hl, HlCo, HlFen GROUPS. Values represent the mean ± standard error of the mean of the number of 5-6 animals. *Student's t-test C vs Hl ($p < 0,05$). Different letters indicate statistical difference (Hl vs HlCo vs HlFen) one-way ANOVA, followed by the Tukey post-test.

The content of SREBP-1c protein (% of control) was 82% higher in the Hl group (Hl: 182.55 ± 24.29) than in the C group (C: 100.00 ± 21.36). Treatments of the hyperlipidimic rats with HeECo (HlCo group) and Fen (HlFen group) did not promote a reduction (HlCo: 145.50 ± 40.42; HlFen: 110.55 ± 26.38) in the content of that protein **Fig. 3.**

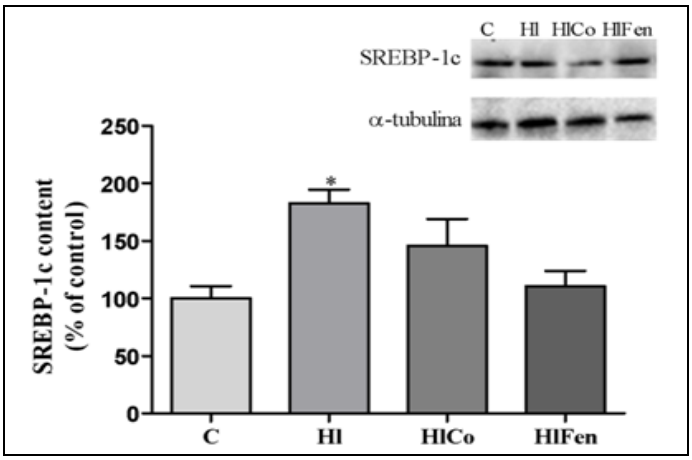

FIG. 3: PROTEIN CONTENT SREBP-1C IN THE LIVER OF RATS IN THE C, Hl, HlCo, HlFen GROUPS. Values represent the mean ± standard error of the mean of the number of 5-6 animals. *Student's t-test C vs Hl ($p < 0,05$). Different letters indicate statistical difference (Hl vs HlCo vs HlFen) one-way ANOVA, followed by the Tukey post-test.

The activity of the enzymes involved in the synthesis of fatty acids was measured in the liver. A 21% increase in the G6PD activity (nmol NADP.mg protein^{-1}. min^{-1}) was observed in the Hl group (Hl: 27.56 ± 1.59), compared with the C group (C: 22.71 ± 1.23) and a 29% reduction in the HlCo group (19.73 ± 1.59) compared with the Hl group. No difference was observed in the HlFen group (20.95 ± 2.16) when compared with the Hl and HlCo groups **Fig. 4A**. For the ME, activity (nmol NADP.mg protein^{-1}. min^{-1}) was not significantly different among groups (C: 2.48 ± 0.29; HI: 2.05 ± 0.24; HICo: 1.98 ± 0.21; HIFen: 2.01 ± 0.22) **Fig. 4B**. The ACL enzyme activity (nmolNADH.mg protein^{-1}. min^{-1}) was 70% higher in the HI group (3.70 ± 0.66) than in the C group (2.18 ± 0.37), and 25% lower in the HICo (2.77 ± 0.62) and HIFen (2.77 ± 0.23) groups than in the Hl group, without a significant difference between the HlCo and HlFen groups **Fig. 4C.**

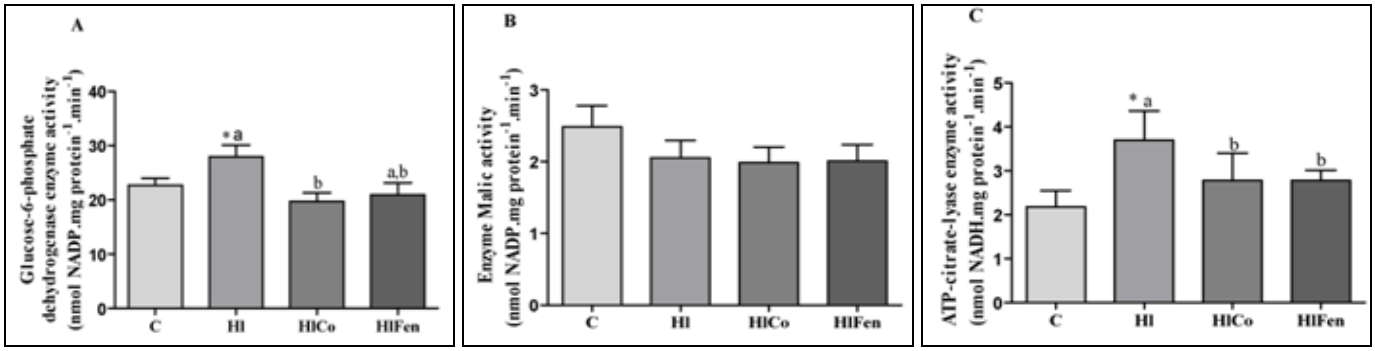

FIG. 4: GLUCOSE-6-PHOSPHATE DEHYDROGENASE (A), MALIC ENZYME(B), AND ATP-CITRATE-LYASE (C) ENZYME ACTIVITY IN THE LIVER OF RATS IN THE C, Hl, HlCo, HlFen GROUPS. Values represent the mean ± standard error of the mean of the number of 5-6 animals. *Student's t-test C vs HI ($p < 0,05$). Different letters indicate statistical difference (Hl vs HlCo vs HlFen) one-way ANOVA, followed by the Tukey post-test.

DISCUSSION: The model of rats used to evaluate the hypolipidemic effects of HeECo was obtained by acute ethanol administration. After the ingestion of ethanol, about 90% is metabolized in the liver through oxidative reactions [18-19] in the cytosol. The ethanol is transformed to acetaldehyde catalyzed by alcohol dehydrogenase (ADH). In humans, seven genes (ADH1 to ADH7) code different subunits of ADH (α, β1, β2, β3, γ1, γ2, π, χ, σ and μ) [20]. Next, acetaldehyde is oxidized to acetate by mitochondrial acetaldehyde dehydrogenase, and both enzymes have NAD as an oxi-reduction

coenzyme [20]. Acetate is converted to acetyl-CoA by the action of acetyl-CoA synthetase, which then follows different pathways, such as the Krebs cycle, ketogenesis, fatty acid (FA) and cholesterol synthesis. The metabolism of large amounts of ethanol increases the $NADH/NAD^+$ ratio, which causes a reduction in FA oxidation and increases the availability glycerol-3-phosphate through the stimulation of acyl transferases and phosphatidic acid phosphatases enzymes, culminating in an increase in the formation of TAG [21]. The increase in the TAG synthesis can increase its storage in the liver (hepatic steatosis) or its movement outward to blood, carried by lipoproteins called very low-density lipoproteins (VLDL). The Hl group showed an increase in serum levels of TAG and VLDL, compared with the animals that did not receive ethanol. The treatment with the HeECo was able to reduce those increases in hyperlipidemic animals. The reduction of TAG and VLDL caused by the HeECo confirms the hypolipidemic potential of the extract initially observed by Giodani *et al* [8].

There is a consensus that the concentration of TAG in the blood results from the difference between the rate of uptake by extrahepatic tissues and the rate of VLDL released by the liver [21]. The LPL enzyme found in the capillary wall is the enzyme that hydrolyzes TAG from VLDL (and other lipoproteins) to glycerol and FAs, allowing that the latter are taken up by adipocytes (or other cells). Thus, the extrahepatic uptake of TAG was verified by evaluating the activity and protein content of the LPL enzyme in the retWAT, since an increase in the content of lipids in this tissue was observed in the animals of the Hl group and there was a reduction in the content of lipids in hyperlipidemic animals treated with the HeECo (HlCo group). However, the reduction in serum TAG levels by the HeECo does not seem to be related to the extrahepatic uptake of TAG because the LPL activity in retWAT was lower, explaining, at least in part the lower weight and lipid content in this tissue, observed in animals of the HlCo group.

The HeECo did not alter the lipid content by the relative weight of the other extrahepatic tissues evaluated. According to Pak-Dek *et al* [22] the activity of LPL in the muscles and adipose tissue determines if the lipids are used or stored, influencing the amount of deposited fat. Studies have observed that the effect of some extracts on the accumulation of lipids in 3T3-L1 adipocytes is due, at least in part, to the decrease in the expression levels of LPL [23-24]. The studies conducted on the liver give us insights to help clarify one of the objectives of this work, since the findings exposed so far explain the reduction in the mass of the adipose tissue by the HeECo, but do not explain the reduction in TAG and VLDL observed in the blood. For this purpose the action of the HeECo on the de novo FA synthesis in the liver was investigated, because the FA and VLDL production is related also to additional ethanol ingested [25], and to a higher consumption of calories ($7.1\ kcal.g^{-1}$). The hypertriglyceridemia induced by the use of ethanol increases the synthesis, secretion, and retention of TAG-rich lipoproteins in the liver, which is responsible for the development of hepatic steatosis [26]. The transcription sterol regulatory element binding proteins (SREBPs) regulate the expression of enzymes of cholesterol, FA, and TAG synthesis pathways [27].

The isoform 1c (SREBP-1c) preferentially activates genes related to FA generation and TAG metabolism [28] such as FA synthase, acetyl-CoA carboxylase, and ATP-citrate lyase (ACL) [29]. Several studies report that an increase in the ethanol metabolism accelerates the transcription of SREBP-1c [30]. It describes that administration of ethanol accelerates hepatic lipogenesis via activating SREBP-1c and production of acetaldehyde is responsible for increasing the expression of SREBP-1c, via Egr-1 [31]. The administration of ethanol for four days caused an increase in the content of SREBP-1c in the liver (Hl group), as well as an increase in the activity of the enzyme ACL. The ACL is a cytosolic enzyme that cleaves citrate from mitochondria in acetyl-CoA, which can be a substrate for FA de novo or cholesterol synthesis [32]. An increase in G6PD activity in the HI group reinforces this hypothesis, producing reducer equivalents (NADPH) needed for FA synthesis [33].

However, the HeECo was not able to reduce the protein expression of SREBP-1c. Vijayakumar & Nachiappan [34] observed a reduction in the gene expression of SREBP-1c in the liver after 14 days of treatment with ethanolic extract of *Cassia auriculata* flowers in hyperlipidemic rats induced

by Triton WR-1339. In our model, the treatment with the extract was only for two days, perhaps a short time for a reduction of the level of SREBP-1c, since this parameter depends also on the rate of degradation. It seems that there is a difference between the means of the hyperlipidemic groups, however there was no statistical difference. By increasing the number of samples processed, a statistical difference could be obtained. However, we observed a lower activity of the enzymes ACL and G6PD in the liver of hyperlipidemic animals treated with the extract (HlCo group). The enzyme ACL is regulated by malonyl-CoA, palmitoyl-CoA, and the phosphorylation/dephosphorylation process and G6PD is regulated by the NADPH/NADP rate [35].

The inhibition of ACL in the liver by benpedoic acid causes lipid-lowering decreased concentrations of acetyl-CoA and malonyl-CoA in the tissue and the serum levels of TAG and free FA [36]. These findings lead us to suggest that the hydroethanolic extract of *Cedrela odorata* a may have an action on hepatic FA synthesis, contributing to the decrease of serum TAG, cholesterol and VLDL.

The animals in the Hl group showed an increase in serum alanine aminotransferase (ALT) levels. The ALT is a marker for liver inflammation, injury or hepatic steatosis [37]. However, we did not observe an increase in the lipid content in the liver of animals in the Hl group. Experiments conducted by Chen *et al* [38] established a model of alcoholic hepatic steatosis in male mice with high fat and ethanol liquid diet for 5 weeks. Therefore, hepatic steatosis seems to take longer to establish.

Several studies have reported the hypolipidemic effect of polyphenols [39-43]. The administration of a single dose (2 mg/kg body weight) of polyphenol suppressed the effect of Triton injection on plasma total cholesterol, triglycerides, and LDL-C in male albino mice. In addition, the supplementation of the high-fat diet with polyphenol fraction (2 mg/kg body weight/day) prevented the increase of total cholesterol, triglycerides and LDL-C, and increased HDL-C level when compared to mice feeding only the high-fat diet [40]. The epigallocatechin-3-gallate decreased lipid droplet and triglyceride contents in HCT116 and HT-29 cancer cells. Addition, too deregulated the expression of the key genes involved in FA de novo synthesis and lipid uptake [42]. Polyphenols, such as catechin, quercetin, resveratrol, preserve HepG2 cell morphology, mitochondrial dysfunction and prevent oleic acid-induced lipid accumulation by induced mitochondrial biogenesis and bioenergetics [43].

Giordani *et al* [8] performed high performance liquid chromatography on the hydroethanolic extract of *Cedrela odorata* and observed the presence of polyphenols such as gallic acid, (-)- gallocatechin and (+)- catechin. We observed that HeECo showed 2.22 mg GAE.g^{-1}. The polyphenolic compounds presented in HeECo may can responsible for the antioxidant activity (increased in superoxide dismutase and glutathione peroxidase enzymes) of the species, as observed by Giodani *et al* [8] and the hypolipidemic effect observed by us.

CONCLUSION: Our results lead us to conclude that the hydroethanolic extract of the inner stem bark of *Cedrela odorata* husk has a hypolipidemic action, since it reduced TAG and VLDL levels in rats with hyperlipidemia induced by acute ethanol use, possibly by reducing the de novo fatty acids synthesis in the liver, due to reduced activity of the ACL and G6PD enzymes.

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REFERENCES:

1. Trautwein EA and McKay S: The role of specific components of a Plant-based diet in management of dyslipidemia and the impact on cardiovascular risk. Nutrients 2020; 12(9): 2671-2692.
2. Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z and Spasojevic-Kalimanovska V: Obesity and dyslipidemia. Metabolism 2019; 92: 71-81.
3. Lu Y, Zhanh H, Lu J, Ding Q, Li X, Wang X, Sun D, Tan L and Mu L: Prevalence of dyslipidemia and availability of lipid-lowering medications among primary health care settings in china. JAMA Net Open 2021; 4(9): 2127573-2127588.
4. Mansur AP, Mattar APL, Tsubo CE, Simão DT, Yoshi FR and Daci K: Prescription and adherence to statins of patients with coronary artery disease and hypercholesterolemia. Ar Bras Cardiol 2001; 76: 115-123.
5. Carvalho PER: Espécies arbóreas brasileiras. Coleção espécies Arbóreas 2014; 4: 644.
6. Bieski IG, Rios SF, Oliveira RM, Espinosa MM, Macedo M, Albuquerque UP and Oliveira MDT: Ethnopharmacology of Medicinal Plants of the Pantanal Region (Mato Grosso, Brazil). Evidence Based Complementary and Alternative Medici 2012; 2012: 1-36.
7. Lee H, Park J, Kwon O, Lim Y, Kim JH, Kim S, Zamora N, Rosales K, Choi S, Oh S and Ahn K: Anti-inflammatory effects of ethanol extract from the leaves and shoots of *Cedrela odorata* L. in cytokine stimulated keratinocytes. Experimental and Therapeutic Medicine 2019; 1: 833-840.
8. Giordani MA, Collicchio TCM, Ascêncio SD, Martins DTO, Balogun SO and Bieski L GC: Hydroethanolic extract of the inner stem bark of *Cedrela odorata* has low toxicity and reduces hyperglycemia induced by an overload of sucrose and glucose. Journal of Ethnopharmacology 2015; 162: 352-361.
9. Bieski IGC, Leonti M, Arnason JT, Ferrier J, Rapinski M and Violante IMP: Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil. J Ethnopharmacol 2015; 173: 383-423.
10. Silva RM, Santos FA, Rao VSN, Maciel MA and Pinto AC: Blood glucose and triglyceride-lowering effect of trans-dehydrocrotonin, a diterpene from Croton cajucara Benth in rats. Diabetes, Obesity and Metabolism 2001; 3: 452–458.
11. Folch J, Lees M and Stanley GHS: A simple method for the isolation and purification of total lipids from animal tissues. Biol Chem 1957; 226: 497-509.
12. Friedewald WT, Levy RI and Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 1972; 18: 499-502.
13. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976; 72: 248-254.
14. Lee CY: Glucose-6-phosphate dehydrogenase from mouse. Methods Enzymol 1982; 89: 252-257.
15. Ochoa S: Malic enzyme. Methods Enzymol 1955; 1: 739-753.
16. Srere PA: The citrate cleavage enzyme. Distribution and purification. J Biol Chem 1959; 234: 2544-2547.
17. Nilsson-Ehle P and Schotz MC: A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J Lipid Res 1976; 17: 536-541.
18. Lieber C: Metabolism and metabolic effects of alcohol. Medical Clinics of North America 1984; 68: 3-31.
19. Kubiak-Tomaszewska G, Tomaszewski P, Pachecka J, Struga M, Olejarz W, Mielczarek-Puta M and Nowicka G: Molecular mechanisms of ethanol biotransformation: enzymes of oxidative and nonoxidative metabolic pathways in human. Xenobiotica 2020; 50(10): 1180-1201.
20. Daré BL, Lagente V and Gicquel T: Ethanol and its metabolites: update on toxicity, benefits, and focus on immunomodulatory effects. Drug Metabolism Reviews 2019; 51: 1-17.
21. You M and Gavin AE: Effect of ethanol on lipid metabolism. Journal of Hepatology 2019; 70: 237–248.
22. Pak-Dek MS, Abdul-Hamid A, Osman A, Soh CS. Inhibitory Effect of *Morinda Citrifolia* L. on Lipoprotein Lipase Activity. Journal of Food Science 2008; 78: 595-598.
23. Lee HJ, Le B, Lee DR, Choi BC and Yang SH: *Cissus quadrangularis* extract (CQR-300) inhibits lipid accumulation by dowregulating adipogenesis and lipogenesis in 3T3-L1 cells. Toxicol Rep 2018; 30(5): 608-614.
24. Kim HJ, Lee MJ, Jang JY and Lee SH: *Allium hookeri* Root extract inhibits adipogenesis by promoting lipolysis in high fat diet-induced obese mice. Nutrients 2019; 20:11(10): 2262-2275.
25. Wilson DA and Matschinsky FM: Ethanol metabolism: the good, the bad and the ugly. Med Hypotheses 2020; 19(140): 109638.
26. Yang L, Yang C, Thomes PG, Kharbanda KK, Casey CA, McNiven MA and Donohue T: Lipophagy and alcohol-induced fatty liver. Front Pharmacol 2019; 10: 495.
27. Kusnadi A, Park SH, Yuan R, Pannellini T, Giannopoulou E, Oliver D, Lu T, Park-Min KH and Ivashkiv LB: The cytokine TNF promotes transcription factor SREBP activity and binding to inflammatory genes to activate macrophages and limit tissue repair. Immunity 2019; 51(2): 241-257.
28. Ferré P, Phan F and Foufelle F: SREBP-1c lipogenesis in the liver: an update. Biochemical Journal 2021; 478(20): 3723-3739.
29. Qiu Z, Deng W, Hong Y, Zhao L, Li M, Guan Y, Su Y, Chen C, Shi Q, Yu J and Wang W: Biological behavior and lipid metabolism of colon cancer cells are regulated by a combination of Sterol Regulatory Element-Binding Protein 1 and ATP Citrate Lyase. Onco Targets Ther 2021; 14: 1531-1542.
30. Lee DH, Park MH, Hwang CJ, Kim Y, Hwang DY, Han SB and Honga JT: Parkin deficiency prevents chronic ethanol-induced hepatic lipid accumulation through b-catenin accumulation. Cell Communication and Signaling 2019; 17: 104-116.
31. Li P, Chen Y, Ke X, Zhang R, Zuo L, Wang M, Chen Z, Luo X and Wang J: Baicalin ameliorates alcohol-induced hepatic steatosis by suppressing SREBP1c elicited PNPLA3 competitive binding to ATGL. Archives of Biochemistry and Biophysic 2022; 22: 109236.
32. Batchuluun B, Pinkosky S and Steinberg GR: Lipogenesis inhibitors: therapeutic opportunities and challenges. Nature Reviews Drug Discovery 2022; 21: 283-305.
33. Reisz JA, Tzounakas VL, Nemkov T, Vougaridou AI, Papassideri IS, Kriebardis AG, D'Alessandro A and Antonelou MH: Metabolic linkage and correlations to storage capacity in erythrocytes from glucose 6-phosphate

dehydrogenase-deficient donors. Front Med (lausanne) 2018; 11(4): 248.
34. Vijayakumar R and Nachiappan V: *Cassia auriculata* flower extract attenuates hyperlipidemia in male Wistar rats by regulating the hepatic cholesterol metabolism. Biomed Pharmacother 2017; 95: 394-401.
35. Pinkosky SL, Groot PH, Lalwani ND and Steinberg GR: Targeting ATP-Citrate Lyase in Hyperlipidemia and Metabolic Disorders. Trends in Molecular Medicine 2017; 23: 1047-1063.
36. Brandts J and Ray K: Bempedoic acid, an inhibitor of ATP citrate lyase for the treatment of hypercholesterolemia: early indications and potential. Expert Opin Investig Drugs 2020; 29(8): 763-770.
37. Hu L, Shao X, Qiu C, Shao X, Wang X, Niu R and Wang Y: Hepatic steatosis is associated with abnormal hepatic enzymes, visceral adiposity, altered myocardial glucose uptake measured by 18 F-FDG PET/CT. BMC Endocr Disord 2020; 20(1): 75.
38. Chen M, Sun X, Wei W, Cucarella C, Martíns-Sanz P, Casado M, Pi L, Ren B and Cao Q: Hepatic COX-2 expression protects mice from an alcohol-high fat diet-induced metabolic disorder by involving protein acetylation related energy metabolism. Alcohol 2021; 92: 41-52.
39. Santhakumar AB, Battino M and Alvarez-Suarez JM: Dietary polyphenols: structures, bioavailability and protective effects against atherosclerosis. Food Chem Toxicol 2018; 113: 49–65.
40. Ramchoun M, Khouya T, Harnafi H, Alem C, Benlyas M, Simmet T, Ouguerram K and Amrani S: Effect of polyphenol, flavonoid, and saponin fractions from *Thymus atlanticus* on acute and chronic hyperlipidemia in mice. Future J of Pharmaceutical Sciences 2020; 6(69): 1-9.
41. Feldman F, Koudoufio M, Desjardins Y, Spahis S, Delvin E and Levy E: Efficacy of polyphenols in the management of dyslipidemia: a focus on clinical studies. Nutrients 2021; 19:13(2): 672.
42. Wang Y, Pan H, Chen D, Guo D and Wang X: Targeting cancer energy metabolism and lipid droplet formation as new treatment strategies for epigallocatechin-3-gallate (EGCG) in colorectal cancer cells. Journal of Functional Foods 2021; 83: 104570.
43. Rafiei H, Omidian K and Bandy B: Dietary polyphenols protect against oleic acid-induced steatosis in an *in-vitro* model of NAFLD by modulating lipid metabolism and improving mitochondrial function. Nutrients 2019; 11: 541-558.

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