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Oral treatment with Davilla Elliptica A. St,-Hil. leaves improves liver steatosis and lipid metabolism on a diet-induced obese mice model

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ABSTRACT

Background: Davilla elliptica A. St.-Hil, also known as "lixeirinha or sambaibinha" is a shrub belonging to the Dilleniaceae family that occurs naturally in the Brazilian savannah (Cerrado). Research studies have shown evidence of its gastroprotective effect, as well as its benefits as an anti-nociceptive, anti-inflammatory, and antioxidant. However, there are no studies testing the potential effects of D. elliptica on treating metabolic parameters and obesity.

Purpose: The aim of the present study was to investigate D. elliptica effects on hepatic steatosis induced by a high-fat/high-sugar diet.

Methods: Animal experimentation was performed using male Swiss mice divided into four groups: ST (standard control), HLHS (obese control), HLHS+EAF (ethyl-acetate fraction), and HLHS+PL (leaf powder). The groups were treated for four weeks with 0.26 mg/kg/body weight.

Results: The main findings of the present study showed that D. elliptica reduced hepatic lipid deposition, body weight, triglycerides, and total cholesterol levels. Gene expression analysis showed that GPX4 and PPARγ mRNA were significantly suppressed in HLHS + EAF mice livers.

Conclusion: The present study contributes to elucidating the D. elliptica metabolic role in decreasing GPX4 and PPARγ expression in the HLHS group.

Introduction

Obesity is defined by excessive fat storage and frequently correlated with an imbalance between energy intake and energy expenditure (Faria et al., 2012). The excessive adipose cells produce triglyceride deregulation, which is commonly associated with lipodystrophy-inducing fat-liver deposition (Bray et al., 2017; Kachur et al., 2017). The prevalence of non-alcoholic fatty liver diseases (NAFLD) /steatosis is directly allied with obesity existing in 50% to 90% of overweight individuals, varying between obesity degrees: present in 65% of those with grade I-II obesity (BMI = 30–39.9 kg / m^2) and in 85% of those with grade III obesity (BMI = 40–59 kg / m^2) (Divella et al., 2019; Piche et al., 2020).

NAFLD development is linked with imbalances in the fatty acid uptake and de novo synthesis augmenting lipid oxidation and secretion. The excessive circulating triglycerides are deposited in hepatocyte

Abbreviations: AU, Arbitrary Units; BMI, body mass index; DNL, De Novo lipogenesis; EAF, Ethyl-acetate fraction; FFAs, Free fatty acids; GPX4, Glutathione Peroxidase 4; GPX1, Glutathione Peroxidase 1; GAPDH, Glyceraldehyde 3- phosphate dehydrogenase; GPXs, Glutathione peroxidases; HLHS, High- lard/ High-sugar; HE, Hematoxylin and eosin; HFD, high-fat diet; NAFLD, non-alcoholic fatty liver steatosis; PL, Leaf powder; PPARy, Peroxisome proliferator-activated receptor y; qRT-PCR, quantitative real -time reverse transcriptase PCR; ROS, reactive oxygen species; ST, Standard control; WHO, World Health Organization.

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cytoplasm (Birkenfeld and Shulman, 2014; Musso et al., 2009; Vasconcellos et al., 2016). Liver steatosis occurs in conditions of increased lipogenesis, mitochondrial ß-oxidation, or the decreased ability of the liver to export lipids with high oxidative stress levels, which can induce steatohepatitis (Lee et al., 2010; Polyzos et al., 2019).

Recent studies have shown that endemic species of the Brazilian savanna (Cerrado) may exert positive effects on obesity treatment (Guimaraesa et al., 2021; Ribeiro et al., 2021). Davilla elliptica (Dilleniaceae) A. St.-Hil, also popularly known as "lixeirinha", "sambaibinha", "muricizinho", "cipó-caboclo" or "cipó-de-carijó" (Rodrigues et al., 2002; Rodrigues and Carvalho, 2001; Soares et al., 2005), is a native species of the Brazilian savanna occurring in the Pandeiros river basin and presenting shrub habits and branched sub-bushes (Jácome et al., 2010). This plant presents in its chemical composition a wide diversity of secondary metabolic substances such as flavonoids, saponins, steroids, tannins, coumarins, and triterpenoids. These bioactive compounds have anti-inflammatory, antioxidant, antitumoral, anti-nociceptive, anti-microbial, anti-mutagenic, and gastroprotective activities (Carlos et al., 2005; Sousa et al., 2020a). However, there is a lack of studies showing D. elliptica molecular mechanisms and metabolic effects. Therefore, the aim of the present study is to investigate the D. elliptica effects on treating hepatic steatosis and metabolic parameters on high-fat-fed mice.

Materials and methods

Plant material and extract preparation

Davilla elliptica St.-Hil leaves were harvested during winter from July to August of 2018 in the municipality of Bonito de Minas – Minas Gerais state, Brazil (15°13′31.37 "S and 44°55′1.52″ W). Harvesting was previously authorized by license number 66,693–1 (SISBIO/ IBAMA) and registered under Protocol No. 00000.024121/0120-18 at SisGen (National System for Management of Genetic Heritage and Traditional Knowledge Associated). Herbarium specimens of D. elliptica may be found in the Herbarium of the State University of Montes Claros, under registry No. 6773. The scientific name and botanical description were validated by the following website: www.theplantlist.org.

After harvesting, D. elliptica leaves were washed thoroughly in water, dried at 38 °C (±2) in a drying stove with air circulation (New Ethics), pulverized in a Willey-type mill, and kept under refrigeration (5 °C). The leaves were prepared according to Rotta and collaborators (Rotta et al., 2008). The plant material that was pulverized was conditioned in the proportion of 10 mL of absolute ethanol for each gram of plant powder. The mixture was stored for one week, and then it was filtered and placed in a drying stove at 35 ± 2 °C. After drying the solvent, the samples were stored in the refrigerator at 10 °C. For the fractionation of the flavonoid extract, the samples obtained in the previous procedure were resuspended in a mixture of ethanol, composed as follows: water (7:3), in the ratio of 3 g of extract to 250 mL of 70% ethanol. At the first wash of the mixture, 200 mL of hexane PA were added three times. A volume of 200 mL ethyl acetate was added three times to the first wash residue. Partitions containing the compounds of interest ethyl-acetate fraction (EAF) were brought to a greenhouse and kept under air circulation at 38 °C until the solvents dried (Andreo and Jorge, 2006). The percent yield was calculated using the following formula:% yield = weight of the extract obtained/weight of powdered sample multiplied by 100 (Bhat et al., 2016).

Qualitative phytochemical characterization

Qualitative tests were performed using dry leaves to detect the presence of tannins, flavonoids, saponins, alkaloids, and terpenes. Tannins were evaluated by a reaction with 2% ferric chloride and a reaction with neutral lead acetate, flavonoids by the Shinoda reagent, saponins by the persistent foam test, and alkaloids by a reaction with reagents by Mayer, Bouchadart, Bertrand, and Dragendorf and a reaction with 2% ferric chloride. Finally, a test with sodium hydroxide was used for the detection of phenolic compounds (Royo et al., 2015)

Animals

The experiment was conducted with 24 Swiss (Mus musculus) male mice (six weeks old) obtained from the State Federal University of Minas Gerais. The mice were randomly divided into four groups ($n = 6$ per treatment) after an adjustment period of seven days. The animals were housed in cages exposed to 12 h light-dark cycles (lights from 12 a.m. to 12 p.m.) at 25.0 ± 2.0 °C. The Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros, Brazil (process No. 164/2018) approved this study. The experiments followed the ARRIVE guidelines.

Diets and experimental design

The sample size consisted of the number of animals needed to achieve statistical significance, that is, $p<0.05$ between treatment groups. This number was based on data described in the literature, and considering population variability (for example, standard deviation) around 20% and alpha (type I error) and beta (type II error) values of 5% and 80% respectively. Therefore, the sample used was the "n" sample to reach statistical significance of $p<0.05$ with a coefficient of variation (CV) of 20% $n = 6$ animals per group (Damy et al., 2010; Scheibe, 2008).

As there is individual variation even in inbred strains of mice, it is important to collect baseline data on the mice before experimenting. We excluded the male mice with body weights below 15 g or over 25 g at six weeks of age. Mice that looked ill or had pelage diseases were also excluded from the study (Wang and Liao, 2012).

The obesity induction was carried out for three months. Diets consisted of a standard (ST) diet (Purina - Labina ®) composed of 50.3% carbohydrates, 41.9% protein, and 7.8% lipids, representing a total of 2.18 kcal per 1 g of diet and a high-lard/high-sugar diet (HLHS) diet composed of 36.59% carbohydrates, 12.88% protein, and 50.53% lipids, presenting a total of 5.1 kcal per 1 g of diet (Guimaraes et al., 2020). All components of the high-fat diet were purchased from Rhoster LTDA (São Paulo, Brazil). The experimental design was carried out as follows: ST diet + vehicle; HLHS diet + vehicle; HLHS + EAF 0, 26 mg kg/ body weight (Azevedo Ade et al., 2015); and HLHS diet + powder of D. elliptica leaves (PL) 0, 26 mg kg/body weight. The animals were treated daily over four weeks with food and water offered ad libitum. Food intake was measured twice a week throughout the treatment to ensure food efficiency (food intake/body weight). The energy intake was calculated through analysis of grams ingested multiplied by the Kcal/g value of the respective diets – 5.1 Kcal/g for the high-lard/high-sugar diet (HLHS) and 2.18 Kcal/g for the standard diet (ST). Adiposity was calculated as the sum of white adipose tissues: mesenteric, epididymal, retroperitoneal, and subcutaneous. At the end of the experiment, the animals were fasted overnight (12 h) and euthanized by decapitation. Samples were collected, weighed, and stored immediately in liquid nitrogen at - 80 °C for further analysis.

Histological analysis

Liver samples were fixed in formaldehyde solution (10%), embedded in paraffin, and sectioned at 5 μm. Slides were stained with hematoxylin and eosin (HE), and images were photographed using an Olympus FSX100® microscope (Tokyo, Japan) under the 20 × optical magnification. For each slide, images from the three most affected fields (x20 objective lenses) were considered. Biopsies analyses were classified according to Hübscher (2006) depending on fat accumulation (Hubscher, 2006).

Hepatic lipid content determination

Lipids were extracted from 200 mg of liver tissues using a chloroform-methanol extraction protocol (Bligh and Dyer, 1959) with slight modifications. Briefly, the liver tissue (200 mg) was homogenized with 200 µl chloroform and 400 µl of methanol and vortexed. Chloroform (400 µl) and ultrapure water (400 µl) were added and vortexed again. The homogenate was centrifuged at 1000 rpm for 5 min. The lower phase (chloroform) was collected and used for lipid measurements. Triglycerides and total cholesterol levels were quantified according to the manufacturer's instructions (Labtest Diagnóstica, Brazil) and analyzed on a spectrophotometer (490 nm, Biotek Instruments, USA).

Gene expression analysis

Total RNA was isolated from liver tissues using TRIzol reagent (Invitrogen Corp. ®, San Diego, California, USA) and treated with DNAse (Promega ®). Reverse transcription was carried out with M-MLV (Promega ®) using random hexamer primers. The expression level of the target genes Glutathione Peroxidase 4 (GPX4), Glutathione Peroxidase 1 (GPX1), Peroxisome proliferator-activated receptor y (PPARy), was determined by quantitative real -time reverse transcriptase PCR (qRT-PCR) using SYBR Green reagent® (Applied Biosystems®) on 384-well QuantStudio 6 flex equipment (Applied Biosystems®). Gene expression was normalized to the endogenous glyceraldehyde 3- phosphate dehydrogenase (GAPDH) and the relative expression was estimated using the $2^{\Delta\Delta}$CT method (Livak and Schmittgen, 2001) (Table 1).

Statistical analyzes

All data were analyzed by Graph Pad Prism software (version 5.0®, San Diego, USA) and subjected to specific tests with 95% reliability ($p<0.05$). Data were expressed as the mean ± SEM. The statistical significance of differences in mean values between the groups of mice was assessed by two-way ANOVA, with Tukey's post-test for multiple comparisons.

Results

Phytochemical screening of D. elliptica leaves revealed an abundance of hydrolyzable tannins, as well as alkaloid and flavonoid compounds

The extract yield was measured and presented as a percent value in hydroalcoholic extract. The yield was 28.94%. A low amount of extract yield was obtained for ethyl acetate fraction (2.243%). In the present investigation, the phytochemical analysis of D. elliptica leaves revealed the presence of important phytochemicals, including flavonoids, alkaloids, phenolics, saponins, and tannins (Table 2).

Energy intake, food intake and body weight

Energy intake was similar between treatments subjected to the same type of diet, with statistical differences between the ST control group and the obese group HLHS ($p<0.001$). The HLHS + PL group showed a reduction in energy consumption (Kcal) compared to the HLHS control ($p < 0.01$). The statistical analysis reported a decrease in Kcal consumption between the treatment groups HLHS + EAF and HLHS + PL ($p<0.01$), suggesting that the presence of polyphenols in the ethyl acetate fraction was responsible for this effect. In addition, food intake was comparable only between the control ST and the HLHS groups ($p<0.001$), suggesting that the energy intake was not a major cause of decreased adiposity, as reported in the treatment group HLHS + EAF. No statistical differences were observed in the liver weights of the animals (Table 3).

The adiposity was higher in the HLHS compared to ST group ($p<0.001$), as expected. In addition, a decrease in adiposity was found in obese mice treated with an EAF fraction of D. elliptica compared to the HLHS control group ($p<0.01$). Statistical differences were also observed between treatment groups HLHS + EAF and HLHS + PL ($p<0.01$) (Fig. 1A). Body weight significantly decreased in the HLHS + EAF and HLHS + PL groups when compared to the HLHS control group ($p<0.001$). There were also differences between treatment groups HLHS + EAF and HLHS + PL ($p<0.001$) (Fig. 1B).

Hepatic tissue morphologic analysis

The hepatic fat deposition was measured by histopathological analysis using hematoxylin and eosin (H&E). Accordingly, HLHS treatment for three months induced hepatocyte diameter increases with large lipid droplets diffusely present, phenomena notably attenuated by administrations of fraction and leaf of D. elliptica. Statistical differences were observed between groups based on hepatocyte diameter, with a significant decrease in steatotic area between obese groups treated with fraction (201.7 ± 78.06 µm) and leaf powder (335.3 ± 154.0 µm) compared to the obese control group (1629 ± 230.4 µm) as well as a

Table 2
. Phytochemical screening of leaves of Davilla elliptica.

Class	Test	Observation	Leaf
Tannins	Ferric chloride	Blue color	+++
	Copper acetate	Precipitate	+++
	Neutral lead acetate	Precipitate	++
Flavonoids	Iron chloride	Blue color	++
	Sodium hydroxide	Yellow color	+
Saponins	Persistent foam	Persistent foam	++
Alkaloids	Mayer	Precipitate	++
	Bouchadart	Precipitate	+
	Bertrand	Precipitate	+++
	Dragendorff	Precipitate	++
Phenolic compounds	Iron chloride	Green color	+
	Sodium hydroxide	Brownish color	++

Table 3
. Energy intake, food intake, body weight, and selected organs mass.

Groups	ST	HLHS	HLHS +EAF	HLHS +PL
Energy Intake	0.3090 ± 0.008	0.381 ± 0.015	0.3750 ± 0.012	0.320 ± 0.009
Food Intake	0.143 ± 0.004	0.070 ± 0.003	0.075 ± 0.003	0.065 ± 0.002
Liver	0.043 ± 0.002	0.040 ± 0.003	0.041 ± 0.001	0.037 ± 0.002

Data are mean ± SEM (standard error). Number of mice: N (6) of groups. Abbreviations: ST, standard; HLHS, High- lard/ High-sugar; HLHS +EAF, fraction of ethyl acetate; HLHS +PL, leaf pulverized.

Table 1
. Primers sequences used for Real -time PCR analysis.

Gene	NCBI code	Forward	Reverse
GAPDH	NM_008084	AAGAAGGTGGTGAAGCAGGCATC	CGAAGGTGGAAGAGTGGGAGTTG
GPX1	NM_008160	TGCAATCAGTTCGGACACCAGGAG	AGCCTTCTCACCATTCACTTCGC
GPX4	NM_008162	TGTGTGCATCCCGCGATGATTG	CCTTGGCTGAGAATTCGTGCATGG
PPARy	NM_011146	AGGAAAGACAACGGACAAATCACC	ATTCGGATGGCCACCTCTTTGC

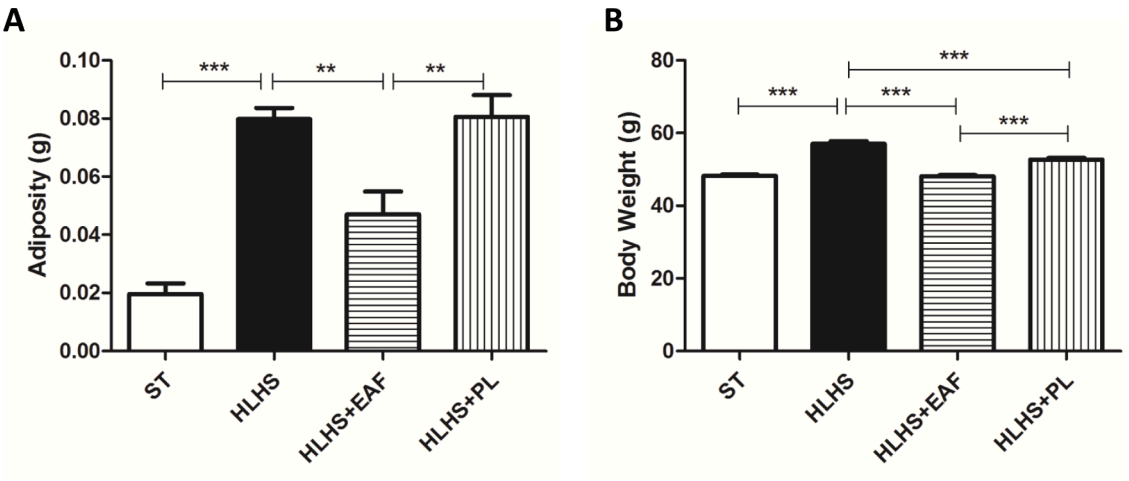

Fig. 1. Adiposity (A) and Body weight (B). Effects of oral administration of D. elliptica in mice fed standard or high-fat diet. Values shown are mean ± SEM ($n = 06$). Significant differences, using one-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).

reduction in lipid droplets in the livers of ST animals compared to HLHS ($p<0.001$) (Fig. 2A-B).

Tissue dosage of triglycerides and cholesterol

High-fat diets cause the accumulation of lipids in the hepatocyte cytosol. This change was confirmed by a hepatic tissue dosage of triglycerides and cholesterol. In mice fed with HLHS, EAF fraction and PL of D. elliptica treatments reduced the content of triglycerides and cholesterol compared to the control group. A significant reduction was observed of hepatic triglyceride levels in the HLHS + EAF (270. 90 ± 24.35 mg/g) ($p < 0.001$) and HLHS + PL (334.80 ± 32.05 mg/g) ($p < 0.01$) treatment groups compared to the HLHS group (489.00 ± 16.13 mg/g). There was a significant decrease in cholesterol showing a statistical association with HLHS + EAF (220.30 ± 29.74 mg/g) ($p < 0.05$) compared to HLHS (297. 30 ± 12.39 mg/g) and with HLHS + PL (335.80 ± 6.583 mg/g) compared to HLHS + EAF (220.30 ± 29.74 mg/g) ($p < 0.01$) (Fig. 3A-B).

Effects of D. elliptica on expressions of PPARγ and GPX4 signaling pathway-related genes in mRNA expression

Previous research demonstrated that the PPARγ signaling pathway is related to lipid metabolism. An analysis of mRNA expression showed significantly lower levels of expression of PPARγ/ GAPDH in group HLHS verses HLHS + EAF (0.4875± 0.1358 AU) ($p < 0.01$) and in group HLHS verses HLHS + PL (0.8186± 0.2063 AU) ($p < 0.01$). In addition, we observed that GPx4 / GAPDH decreased in groups HLHS verses HLHS + EAF (0.6518 ± 0.0757 AU) ($p < 0.05$) and HLHS verses HLHS + PL (0.4943± 0.0767 AU) ($p < 0.01$) while no statistical difference was observed for the expression of GPx1 between the groups (Fig. 4A-C).

Discussion

The present study demonstrates for the first time the anti-obesogenic effects of D. elliptica and its ability to improve metabolically associated disorders. The main results showed a reduced adiposity followed by improved dyslipidemia (diminished cholesterol and triglycerides) and reduced hepatic lipid deposition (hepatic steatosis) after leaf powder or extract administration.

Previously published studies described the D. elliptica chromatographic profile under certified chromatograph standards technics or even using magnetic resonance. The presence of compounds such as epicatechin, gallic acid, kaempferol, quercetin derivatives, myricetin derivatives, and routine were corroborated (Kushima et al., 2009; Rodrigues et al., 2008). Many other less concentrated bioactive substances are also present in the crude extract, producing synergisms that may provide several benefits through combination. The Cerrado (Brazilian savanna) plants have being described as potentially effective for treating obesity; recently, Freitas et al. demonstrated that Acosmium

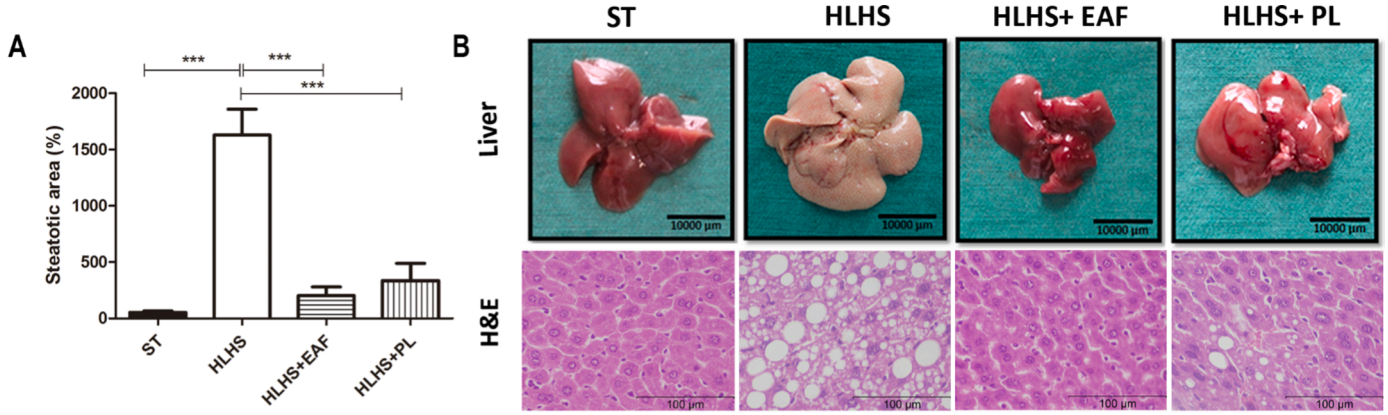

Fig. 2. Hematoxylin and eosin (HE). Staining in mice-fed standard diet (ST), High- lard/ High-sugar (HLHS), HLHS plus ethyl acetate fraction (HLHS + EA), HLHS plus pulverized leaves (HLHS + PL). Scale bar, 100 µm. Values shown are mean ± SEM ($n = 06$). Significant differences, using one-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).

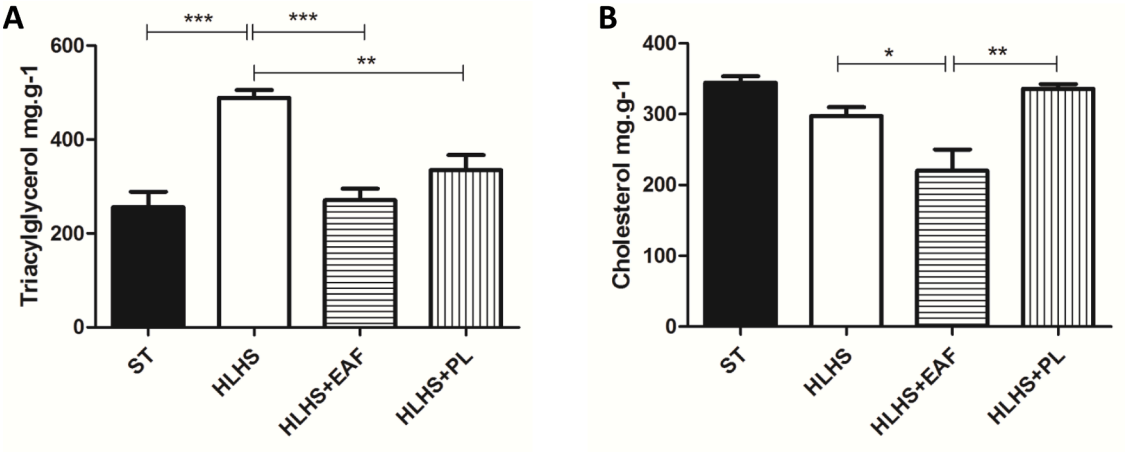

Fig. 3. Hepatic Lipid Content Determination. Effects of oral administration of D. elliptica in triglycerides and total cholesterol in mice fed standard or high-fat diet. Values shown are mean ± SEM ($n = 06$). Significant differences, using one-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).

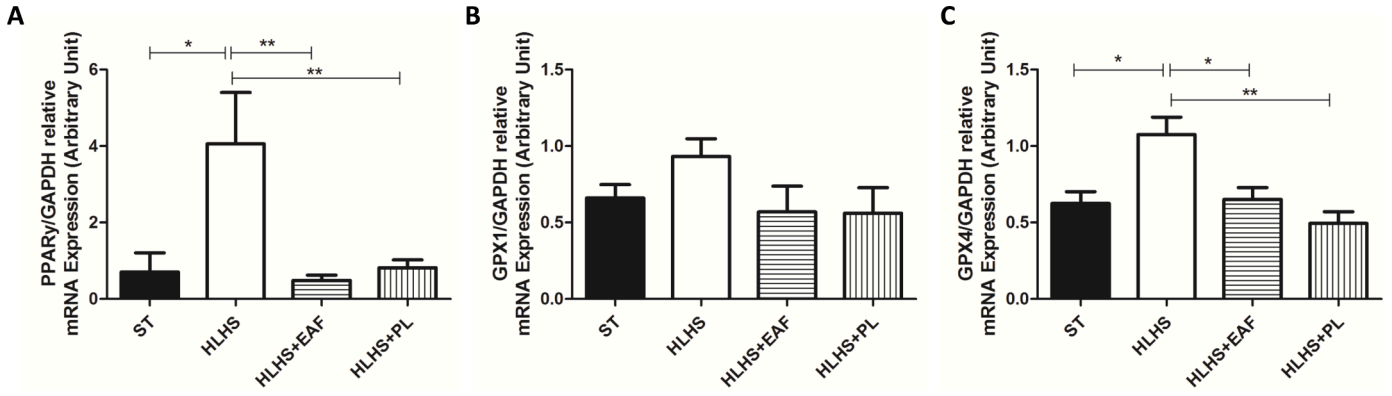

Fig. 4. Gene expression. 4-week D. elliptica effects on PPARγ, GPX1 and GPX4 expression in the liver tissue of high fat fed mice. (A) PPARγ. (B) GPX1. (C) GPX4.

dasycarpum bark improved lipid profile, weight loss, and adiposity in animals with diet-induced obesity (Freitas et al., 2021).

The main compounds found in D. elliptica were flavonoids, myricetin, and quercetin as isolated from the leaves (Bisoet al., 2010; Rinaldo et al., 2006). These compounds were previously described as able to reduce the body weight of obese animals without altering food intake (Chang et al., 2012). The myricetin-3-O-β-D-galactopyranoside presence significantly reduced adiposity in mice fed a high-fat diet without differences reported in food consumption (Kim et al., 2017). Another well-described compound isolated from D. elliptica is gallic acid, which is able to reduce body weight and decrease adiposity in animals fed high-fat diets (Paraisoet al., 2019; Sousa et al., 2020b).

Xia et al. confirmed that myricetin significantly reduces high-fat diet-induced liver lipid accumulation by normalizing circulating triglycerides and cholesterol. Taken together, these results suggest that myricetin plays a positive role in hepatic steatosis (Xia et al., 2016). In the same context, animals treated with quercetin have shown decreases in plasma triglycerides and cholesterol levels with reduced body weight along with decreased liver lipid accumulation (Marcolin et al., 2013; Tan et al., 2021). The present study found similar results for both leaf powder and extract.

Corroborating these findings, Bonacorsi et al. detected an antioxidant effect (above 80%) of the methanol extract of D. elliptica (Bonacorsi et al., 2013). Interestingly, corroborating our results, the gene expression of glutathione peroxidases (GPXs) was decreased in the mouse livers after D. elliptica fraction treatment. GPXs enzymes are responsible for neutralizing simple and complex lipid hydroperoxides, and GPX4 is described as neutralizing cholesterol hydroperoxides (Maiorino et al., 1991). Studies have shown that GPX4 variants result in decreased content and catalytic activity associated with obesity (Ruperez et al., 2014), cardiovascular disease (Crosley et al., 2013; Polonikov et al., 2012), and inflammation (Du et al., 2012). In the same context, PPARγ is considered a transcription factor of lipid metabolism (Sekulic-Jablanovic et al., 2017). In addition to regulating metabolism in hepatocytes, PPARγ is an inducer of free fatty acid (FFA) synthesis (Skat-Rordam et al., 2019). We also observed a reduced hepatic expression of PPARγ after plant fractions administration.

According to our knowledge, this is the first study investigating the effects of leaf powder and polyphenol fraction of the hydroalcoholic extract of D. elliptica in obese mice. Nevertheless, our study has some limitations. First, we do not have detailed scientific composition data for the plant. Despite previous studies already having made descriptions, it is always relevant to corroborate anterior findings. Second, the number of animals used in this research was reduced, although it was still enough to show statistical differences between groups. According to the principle of the "Three Rs" of Russel and Burch (Russell and Burch, 1959), the number of animals used in each experiment should be reduced to a minimum consistent with the achievement of the study's scientific objectives in order to avoid unnecessary pain and suffering to the animals. Third, the analysis of the chromatographic profile of the species under study was not performed by HPLC or UCPL. However, a previous study described the chromatographic profile of the Davilla elliptica species (Kushima et al., 2009; Rodrigues et al., 2008). We understand that there may exist some variation in the amounts of

compounds due to region and seasonality, however, the presence of the main compounds (such as myricetin and quercetin) will be maintained.

Conclusion

In conclusion, the present results show that obese mice orally treated with D. elliptica leaf powder and polyphenol hydroalcoholic fraction show improved adiposity, reduced circulating lipids, and hepatic steatosis. These effects were associated with altered PPARγ and GPX4 gene expressions. These results point to a potential use of D. elliptica in the treatment of obesity and associated comorbidities; however, more studies are necessary to elucidate the main pathways and mechanisms by which this plant modulates the metabolism.

Author's contributions

JNS and SHSS conceived the study. JNS, BMM and VM wrote the manuscript. JNS, LPO and VHDG performed graphic designer and figures. VM and SHSS provided a critical revision of the paper. All authors read and approved the final version of the paper.

Declaration of Competing Interest

All authors do not have conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100130.

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