

Biological effect of olive leaves (*Olea europaea l.*) extracts on rats subjected to hyperlipidic diet

Efeito biológico do extrato de folhas de oliveira (*Olea europaea l.*) em ratos submetidos a dieta hiperlipídica

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Abstract

Objective: In order to explore the nutritional and bioactive characteristics of the olive leaf, the study aimed to evaluate the biological potential of olive leaves by analyzing the effect of leaf extract on the glycemic and lipid profile of Wistar rats subjected to a hyperlipidic diet. **Methodology:** The experiment lasted 70 days; animals were divided and randomly distributed into 4 groups of 6 rats each, being N: normocaloric diet + water; H: hyperlipidic diet + water; NE: normocaloric diet + extract; and HE: hyperlipidic diet + extract. At the end of the experiment the effect of the aqueous extract of olive leaves on glycemia, lipid profile and lipid peroxidation were determined in rats. **Results:** The consumption of olive leaf extract by rats promoted changes in lipid profile and beneficial effect on the thiobarbituric acid reactive substances (TBARS) levels. **Conclusions:** Due to their chemical composition, rich in nutrients and compounds with bioactive properties, in addition to its considerable antioxidant and biological activities, olive leaves can be an option as a food supplement with biological properties.

Keywords: Olive leaves. Agroindustrial waste. Oxidative stress. Wistar rats.

Resumo

Objetivo: A fim de explorar as características nutricionais e bioativas da folha de oliveira, este estudo teve como objetivo avaliar o potencial biológico das folhas de oliveira, analisando o efeito de seu extrato no perfil glicêmico e lipídico de ratos da linhagem Wistar submetidos a dieta hiperlipídica. *Metodologia:* O experimento teve duração de 70 dias; os animais foram divididos e distribuídos aleatoriamente em quatro grupos de seis ratos cada, sendo N: dieta normocalórica + água; H: dieta hiperlipídica + água; NE: dieta normocalórica + extrato; e HE: dieta hiperlipídica + extrato. Ao final do experimento, determinou-se o efeito do extrato aquoso das folhas de oliveira na glicemia, perfil lipídico e na peroxidação lipídica dos ratos. *Resultados:* O consumo do extrato de folhas de oliveira pelos ratos promoveu alterações no perfil lipídico e efeito benéfico em relação aos níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS). *Conclusões:* Devido a sua composição química, rica em nutrientes e compostos com propriedades bioativas, além de suas consideráveis atividades antioxidante e biológica, as folhas de oliveira podem ser uma opção como suplemento alimentar com atividade biológica.

Palavras-chave: Oliveira. Resíduo agroindustrial. Estresse oxidativo. Ratos Wistar.

Introduction

The olive tree (*Olea europaea* L.), one of the oldest fruit plants grown by man, produces a large amount of leaves that are discarded during the production cycle.¹ The leaves contain considerable amounts of phenolic compounds, among which is oleuropein, to which are attributed antioxidant, antimicrobial, hypoglycemic and anti-inflammatory activities, among others. Thus, considering these possible beneficial properties of olive leaves and the need to foster their use, it is vital to study their actual biological value.²

The world population has expressed increasing concerns about foods,³ since the kind and quality of foods that are consumed have been considered one of the factors in the prevention of some noncommunicable diseases (NCDs) such as cancer, diabetes, high blood pressure, heart diseases, and obesity.⁴

Considering that cardiovascular diseases have been one of the main causes of death and disability and that one of the major risk factors are dyslipidemias,⁵ this reality has urged the food industry to invest in healthy foods and foods with functional properties. Thus, it is vitally

important to evaluate the hypocholesterolemic action of olive leaves considering that studies have shown that they improve lipid profile in blood.⁶

Cholesterol plays various essential functions in the body, being a component of cell membranes and a precursor of bile salts, hormones and vitamin D.⁷ It is synthesized in the liver and excreted in the circulation as a component of lipoproteins, also derived from diet. Because it is not considered an essential nutrient, cholesterol intake is not necessary because the body can synthesize it in sufficient amount.⁸ The recommended daily intake of dietary cholesterol is less than 300 mg and 200 g for people with history of cardiovascular diseases.⁹ Total cholesterol levels and the LDL fraction may increase with an excessive intake of calories, saturated fats and dietary cholesterol. In contrast, cholesterol levels can be lowered with weight loss, a dietary replacement of saturated fatty acids with polyunsaturated fatty acids, soluble dietary fiber,¹⁰ and the intake of some cholesterol-reducing foods such as fruits and vegetables.¹¹

In turn, *diabetes mellitus* is a chronic metabolic disease caused by absolute or relative insulin deficiency. It is characterized by hyperglycemia, and in the long term it causes complications in the eyes, kidneys, nerves, pancreas, the most common being endocrine disorders.¹² The mechanism of the disease complications is still unclear, but much attention has been drawn to the role of oxidative stress.¹³ It is known that many of the adverse effects of oxidative stress decline after supplementation with dietary antioxidants, such as vitamins and other non-nutrient antioxidants such as phenolic compounds.¹⁴

Literature has documented that high concentrations of free radicals in the cell cause uncontrolled serial reactions and peroxidation of lipids, resulting in various pathological conditions including atherogenesis and cancer.¹⁵ There are reports stating that hypercholesterolemic atherosclerosis is associated with an increased level of the product of lipid peroxidation, i.e., thiobarbituric acid-reactive substances (TBARS). Therefore, a decrease in lipid peroxidation may reduce atherosclerosis caused by hypercholesterolemia.¹⁶ Oxygen-reactive species react with lipids, causing lipoperoxidation and subsequent formation of malondialdehyde (MDA). The accumulation of these byproducts is considered a marker of the oxidation process in the body, and reduced lipid peroxidation represents a mechanism of protection against injuries caused by oxidative stress.¹⁷

To investigate the relationship between cholesterol metabolic disfunctions and atherogenesis, several animal models have been used. These models are very important to understand the origin of atherosclerosis and to discover new therapeutic agents. The rat is the most appropriate animal species to provide information on lipogenesis because its pathway is well represented in adipose tissue and liver.¹⁸ Some studies have shown the efficacy of hyperlipidic diets on the genesis of obesity and its comorbidities, especially in Wistar rats. This diet-induced obesity model has also been used to investigate endothelial disorders since in most of the studies where this animal was subjected to this kind of treatment, it exhibited metabolic changes, such as increased cholesterol levels.¹⁹

Given the above, aiming to explore the nutritional and bioactive characteristics of olive leaves, this study had the purpose of evaluating the biological potential of aqueous extract of this plant on the glycemic and lipid profile of Wistar rats fed a hyperlipidic diet.

Methodology

Sampling

Olive leaves of cultivar Koroneiki were obtained from Estância Guarda Velha, a farm located in Pinheiro Machado-RS (31°29'59,4" S and 53°30'32,7" W). Nearly 2 kg of leaves were harvested at random from various olive plants. The leaves were then ground and kept under refrigeration at - 80°C for further conduction of analyses and the biological experiment.

Experiment

A completely randomized bifactorial design with six replications was used in the experiment. The treatment factors consisted of the kind of feed (normocaloric and hyperlipidic diet) and the liquid (water and olive extract).

The biological assay was carried out with 24 Wistar/UFPel strain rats (male, adults), provided by the *Biotério Central da Universidade Federal de Pelotas* (Central Vivarium of the Federal University of Pelotas). Three individuals per unit were housed in polypropylene housing boxes (41 x 34 x 16 cm) equipped with 300-ml polypropylene drinkers and anti-acid rubber stopper and stainless-steel nozzle coupled to the boxes.

The animals were maintained in the *Laboratório de Nutrição Experimental da Faculdade de Nutrição* (Laboratory of Experimental Nutrition, Faculty of Nutrition) of the Federal University of Pelotas, under a temperature of 23±1°C, 50-60% relative air humidity and automatic alternating light-dark cycles in 12-hour periods. The position of the boxes was changed every week in order to provide better distribution of light and noises for the animals and, consequently, reduce environmental stressors.

The experiment lasted 70 days, including four days for adaptation to the experimental environment. The study was approved by the Ethics Committee on Animal Research (CEEA)²⁰ of the Federal University of Pelotas with number 23110.004842/2016-64. All necessary arrangements for the animals' well-being were provided, following recommendations of the *Colégio Brasileiro de Experimentação Animal (COBEA)*²¹ (Brazilian College of Animal Experimentation) described in the manual for the care and use of laboratory animals.

To conduct the biological assay, the rats were divided and distributed randomly into four groups of six individuals each, with diet and water provided *ad libitum*. The experiment had two control groups (N and H) and two treated groups (NE and HE), in which the animals were fed normocaloric diet – commercial feed (Nuvilab®) and commercial feed mixed with 20% pork fat. To prepare the plant extracts, an infusion of olive leaves was made at a concentration of 0.008 g.mL⁻¹, which was offered to the animals, as shown in Table 1. In the first four weeks of the experiment, 12 animals received feed (N) and 12 animals received feed with pork fat (H). In the four subsequent weeks, six animals continued receiving feed (N) and other six individuals received feed and the extract (NE); six rats continued with feed and pork fat (H), and other six with feed, pork fat and extract (HE). At the end of the experiment, after fasting for six hours, the animals were euthanized using intraperitoneal injection of sodium pentobarbital at a concentration of 100 mg.Kg. Subsequently, blood was collected from the animals, which had their liver, heart and kidneys removed.

Table 1. Experimental diets prepared for experiments with Wistar rats.

Groups	Diets
NE	Commercial feed and extract
N	Commercial feed and water
HE	Commercial feed + 20% of pork fat and extract
H	Commercial feed + 20% of pork fat and water

Biochemical analyses

Blood glucose was determined by Advantage Roche® glucose meter using one drop of blood collected after euthanasia, expressed in mg.dL⁻¹.

For analysis of the lipid profile, the collected blood was centrifuged at 3500 rpm for 10 minutes to obtain the blood plasma, which was transferred to Eppendorf tubes and frozen at -20°C until analyses of the lipid fractions. Total cholesterol (TC) was quantified by the enzymatic system of Labtest Diagnóstica® cholesterol liquiform cat. 76 -2/100. The HDL was determined by precipitation of low-density and very low-density lipoproteins (LDL and VLDL), using the enzymatic system of Labtest Diagnóstica® cholesterol liquiform cat. 13. The VLDL was calculated according to Equation 1. The triacylglycerols (TAG) were determined by the enzymatic system of Labtest Diagnóstica® (GPO-ANA cat. 59-4/50), and subsequently read in spectrophotometer at 510 nm.^{22,23}

$$VLDL = \frac{\text{Triacylglycerols}}{5} \quad \text{Eq. 1}$$

Malonaldehyde formation (MDA), which is an index of lipid peroxidation, was determined for the liver, heart and kidneys of the animals by quantifying the thiobarbituric acid-reactive substances (TBARS), as described by Esterbauer & Cheeseman.²⁴ The samples were prepared by pooling the organs of each group, which were ground separately. Then, 1 g of the pool of organs was added with 10 ml of 0.9% NaCl and homogenized in a potter-type homogenizer, and then centrifuged at 10,000 rpm for five minutes.

It was used 200 μ L of sample, added with 500 μ L of ultrapure water, 200 μ L of 8.1% sodium lauryl sulfate (SLS), and 500 μ L of acetic acid buffer (pH 3.4) and 0.6% thiobarbituric acid (pH 4.0). The mixture was placed in a thermostatic bath for one hour at 100°C and then centrifuged at 10,000 rpm for five minutes. For each sample a blank was made, without addition of TBA. Reading of the sample absorbance was done in a Femto Cirrus 80 MB spectrophotometer, using methanol for the blank reading. The results were expressed in mmol of malonaldehyde (MDA) per gram of sample.

Statistical analysis

The results were expressed as a function of the mean and standard deviation, and statistically assessed. Using analysis of variance (ANOVA), the significant differences between means ($p < 0.05$) were established by the statistical Tukey's test. For the analyses, the Microsoft Excel 2013 program and Statistica software, version 11.0 were used.

Results and Discussion

The intake of feed, water and olive leaves extract by the rats was calculated by the sum of the daily evaluations between the supplied diet and the consumed diet during the experiment. Table 2 shows the dietary consumptions during the experiment. It can be seen that there was no significant difference between the feed intake between the groups NE and N and between the groups N, HE and H, indicating that the olive leaf extract did not affect the rats' feed intake for the different groups. However, by observing the use of the olive leaf extract and hyperlipidic diets alone, a positive effect was found when it was eaten together with the normocaloric diet.

In addition, among the animals fed hyperlipidic diets, the higher lipid content in the diet might have an effect on satiety, reducing the liquids intake.²⁵ With respect to liquids intake, the groups of rats showed significant differences ($p < 0.05$) between each other, with a higher intake of olive leaf extract by the group NE and, therefore, more intake of antioxidants and/or bioactive compounds.

Table 2. Total intake of feed, water and olive leaves extract during the experiments conducted with Wistar rats. Pelotas-RS, 2018.

Groups	Feed intake (Kg)	Water or extract intake (L)
NE	4.81±0.34b-	4.17±0.03a
N	5.15±0.10ab	3.74±0.06b
HE	5.24±0.06 ^a	2.86±0.14c
H	5.30±0.06 ^a	2.78±0.05c

- Means (\pm standard deviation) followed by the same letter in column do not differ from each other by the Tukey' test ($p < 0.05$).

The animals' weight gain was calculated by the difference between the weights measured at the beginning and end of the experiment (Fig. 1). The group NE exhibited the lowest weight gain, differing only from the hyperlipidic group; the extract showed a positive effect when combined with the normocaloric diet. Jemai et al.²⁶ evaluated Wistar rats fed cholesterol-rich diets and treated with hydroxytyrosol to assess their effect on cholesterol reduction and did not find a statistical difference in total weight gain compared to the control group, indicating that hydroxytyrosol did not have an action in weight gain with the normocaloric diet and hyperlipidic diet. This result differs from the findings in the present study, in which there was a significant difference ($p < 0.05$) in the consumption of olive leaves extract associated with normocaloric diet when compared to the hyperlipidic diet.

Figure 1

The total weight gain was due to the total calorie intake by the animals, which, according to data of consumption, was lower for the group of normocaloric diet, which consumed the olive leaves extract; therefore, data are justified. Furthermore, weight gain can also result from a fat-rich diet, which is definitely one of the factors that induce weight gain and may be associated with the appearance of morbidities such as dyslipidemias, besides influencing lipid peroxidation, since there are positive correlations between lipid peroxidation markers and cholesterol and triacylglycerols levels in the body.^{4,9,27}

Table 3 shows the values for liver, kidneys and heart of the experimental animals. It can be seen that the highest absolute weight values for liver and kidneys were found in animals that fed hyperlipidic diets with the extract (15.03 g for group HE) and normocaloric diet with water (2.87 g for group N). With respect to heart, the highest weight occurred in group NE, i.e., 1.34 g, and the lowest weight was found in groups HE and H (1.18 g in both groups). The weight of the organs did not differ significantly among the groups.

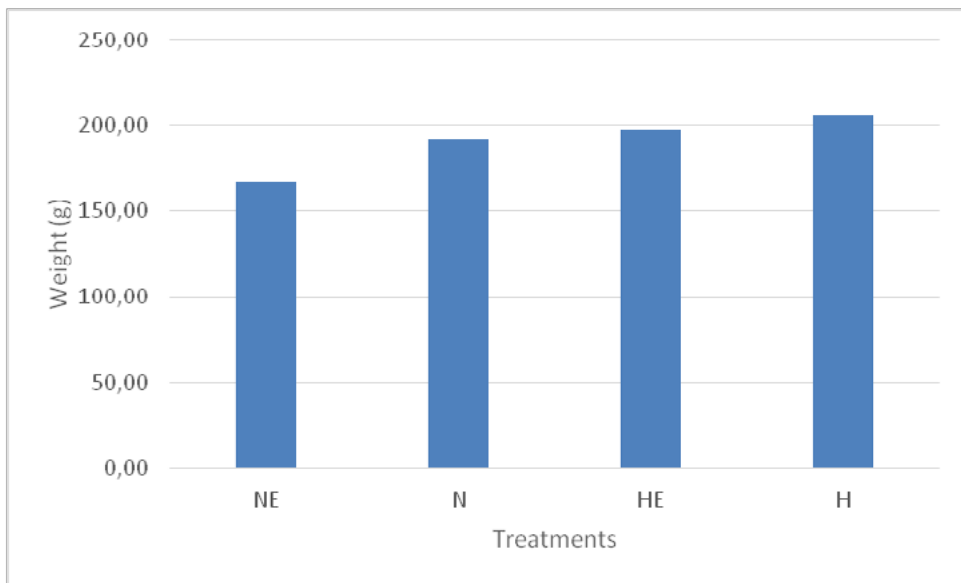


Figure 1. Weight gain by Wistar rats during the experiment. Pelotas-RS, 2018.

Source: Data collected by authors.

Poudyal et al.,²⁸ when they assessed rats that fed a diet supplemented with dry extract of olive leaves, found a reduction in the liver weight, which is probably due to the reduced deposition of collagen and fat in this organ. The same effect was found by Jemai et al.,²⁶ when they assessed the effect of water-diluted hydroxythirosol, a polyphenol present in olive leaves, in rats fed a cholesterol-rich diet, which exhibited decreased weight of liver, kidneys and heart. The authors concluded that the phenolic compounds might reduce lipids accumulation in the organs. Fki et al.²⁹ observed the same effect on the organs, with reduced weight, when rats were fed a diet supplemented with extract of olive leaf from cultivar Chemlali in Tunísia, evaporated and mixed with sampled water.

Table 4 shows the glucose contents in the rats supplied with experimental diets. It can be seen that for fasting glucose, there was no significant difference ($p < 0.05$) in the comparison between the groups that consumed olive leaves extract with the control groups.

Natural antioxidants have become popular for treatment of diabetes and its complications, as a strategy to alleviate oxidative damage. Among these natural antioxidants, olive plant has been considered one of the species with high antioxidant activity due to the presence of compounds such as oleuropein, hydroxythirosol and thyrosol.³⁰ However, it was not possible to observe this

effect on the present study, which can be justified by the fact that the extract was used integrally, which could be very diluted, differently from the studies found, which isolated and used specific compounds of olive leaf extracts.

Table 3. Weight of organs of Wistar rats in experiments with and without use of olive leaves extract. Pelotas, RS, 2018.

Groups	Liver (g)	Kidneys (g)	Heart (g)
NE	14.45±1.05 ^{NS}	2.76±0.21 ^{NS}	1.34±0.11 ^{NS}
N	14.90±0.71	2.87±0.30	1.33±0.13
HE	15.03±1.09	2.71±0.45	1.18±0.10
H	13.96±1.15	2.48±0.13	1.18±0.09

^{NS} Not significant by the Tukey's test of analysis of variance ($p < 0.05$)

Table 4. Blood glucose in Wistar rats in experiments with and without intake of olive leaves extract. Pelotas-RS, 2018.

Groups	Glucose (mg.dL ⁻¹)
NE	138±22.63 ^{NS}
N	137±9.27
HE	137±4.18
H	142±6.71

NS Not significant by the Tukey's test of analysis of variance ($p < 0.05$).

Al-Azzawie & Alhamdani³¹ tested blood glucose in diabetic rabbits treated with oleuropein and found that the glucose levels in these animals declined significantly after the beginning of the treatment, compared to the control. The same tendency was observed in a study conducted by Jemai et al.,³² when they evaluated glucose levels in diabetic rats treated with oleuropein and hydroxythirosol. After treatment, the sugar levels were significantly restored to values that were not different from normal, as in the control group.

In the present study, the cholesterol-rich diet induced hypercholesterolemia, which appeared in an increased level of total cholesterol (TC), low density lipoproteins (LDL), triglycerides (TAG) and very low-density lipoproteins (VLDL), as shown in Table 5. In a study conducted by Jemai et

al.,²⁶ after inducing hypercholesterolemia in rats, these authors observed a rise in TC, TAG and LDL levels. When hydroxythirosol was administered, a significant reduction of the serum levels of CT, TAG and LDL and an increase of the serum level of HDL were observed. Similar results were found in a study conducted by Fki et al.,³³ in which a cholesterol-rich diet induced a rise in TC and LDL levels, and by administering aqueous olive extracts, the serum levels of TC and LDL declined significantly, also occurring an increase of HDL serum level. But in the present study, we did not observe the same effect, which indicates that in the concentration of olive leaf extract used, the isolated compounds that provide a protective effect on the tested lipids were not effective, considering that there was no significant difference in the lipid profile of the rats that consumed the olive leaf extract, compared to the control.

Table 5. Lipid profile of Winstar rats in experiments with and without intake of olive leaves extract. Pelotas-RS, 2018.

Groups	TC (mg.dL-1)	HDL (mg.dL-1)	TAG (mg.dL-1)	VLDL (mg.dL-1)
NE	141.36±9.25NS	64.34±7.04ab1/	103.54±6.30b	20.71±1.26b
N	130.97±9.15	33.16±3.16b	107.40±9.41b	21.48±1.88b
HE	126.10±7.98	79.04±8.33a	358.96±8.88a	81.47±2.98a
H	145.94±7.23	62.43±1.80ab	338.54±6.25a	96.88±1.07a

Although most of the data found in literature indicate a possible tendency of decline in the levels of the groups treated with olive leaves, or some of its chemical constituents, compared to the control group, such reduction was not statistically demonstrated in the present study. This result can be explained by the extract concentration used, which was the same as that found in commercial teas, and other studies might have used higher concentrations or even a compound isolated from olive leaves, such as the polyphenols oleuropein and hydroxythirosol. So, it is suggested that more studies be conducted to investigate extracts with higher concentration, or from components extracted/isolated from olive leaf and their respective concentrations, in order to clearly demonstrate the action of these compounds in the body.

Figure 2 shows the index of lipid peroxidation found in the organs removed from the animals. There was no significant difference between the groups NE, HE and H with respect to the index of lipid peroxidation in the heart. In the liver, there was no significant difference in the groups NE, HE and H, and in the groups HE and H. In the kidneys, there was no difference ($p < 0.05$)

between the groups NE, N and H, and the groups HE and H. The results of this study indicate a beneficial effect of olive leaves in the TBARS levels, demonstrating the antioxidant potential of olive leaf extracts in *in vivo* assays.

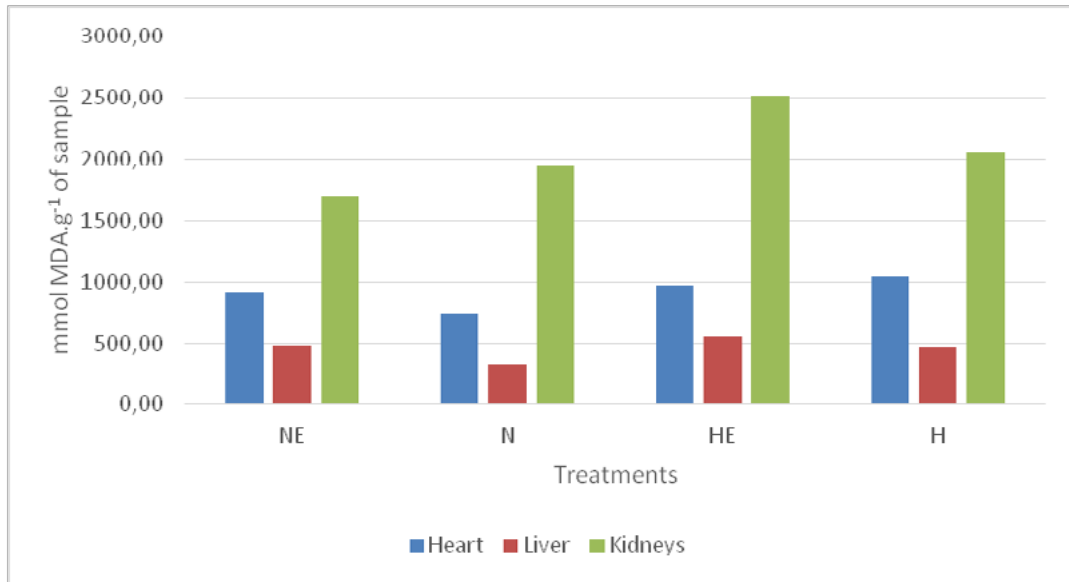


Figura 2. Índice de peroxidação lipídica do coração, fígado e rins de ratos Wistar, Pelotas-RS, 2018.

Fonte: Dados coletados pelos autores.

In the present study, it was observed that the hyperlipidic diet did not produce oxidative damages in the rats' organs, which is demonstrated by the increased level of lipid peroxidation. This effect was reported by other authors, who associated an increased intake of cholesterol with lipid peroxidation processes and increased levels of MDA, suggesting that hypercholesterolemia is directly associated with the formation of free radicals, and that these compounds might cause cell injury and, consequently, endothelial dysfunction, giving rise to atherosclerosis. Therefore, a reduction of cholesterolemia has an effect in reducing oxidative stress.³⁴⁻³⁶

It should be emphasized that *in vivo* antioxidant defense depends on endogenous mechanisms and their synergistic action with exogenous antioxidants. Furthermore, TBARS values in animals may vary, and factors such as the age of the biological models may interfere with the mechanisms of adaptive responses to the diet's antioxidant compounds.³

In a study conducted by Jemai et al.,³² diabetes-induced rats exhibited hepatic damages and the TBARS levels increased significantly in the livers of the diabetic rats compared to the control group. When oleuropein and hydroxythirosol were administered in two different doses, the TBARS concentration declined significantly. Fki et al.³³ obtained a significant increase in the MDA levels in the liver, heart and kidneys of the animals fed a cholesterol-rich diet, compared to the diet of the control group. In the group treated with olives extracts, this increase was significantly reduced. The data obtained suggested that the phenolic compounds present in olives are capable of diminishing or mitigating the oxidative effects.

In other study, the TBARS contents in the liver, heart and kidney decreased significantly when hydroxythirosol was administered in hypercholesterolemic rats, when compared with the control group. These results suggested that the hypolipidemic effect of hydroxythirosol may be due to its properties of diminishing the serum levels of total cholesterol, triglycerides and low-density lipoproteins as well as its antioxidant activities, which inhibit the lipid peroxidation process.²⁶

The analysis of the results of this study indicates that even when there are no differences between weight gains and consumption between the groups, the intake of olive leaves resulted in an improved lipid profile and lipoperoxidation in the organs, demonstrating the biological activity of olive leaves. It was also clear that the type of diet consumed is a factor of great influence on the development of pathologies, such as dyslipidemias.

It is still unclear whether the hypolipidemic effect of olive leaves is due to a single specific component or interactions between their constituents, which indicates the need for more studies to elucidate this point.

Conclusions

The intake of aqueous olive leaf extract resulted in an improved lipid profile and lipoperoxidation of Wistar rats' organs, demonstrating their potential in the biological activity. More studies are suggested to elucidate the minimum extract concentrations and whether the hypolipidemic effect of olive leaves is due to a single component or interactions between their components.

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Contributors

Antunes BF participated in all stages, from the conception of the study to the revision of the final version of the manuscript; de Leon CAC participated in the conduction of the experiment; Lorini A participated in the conduction of the experiment; Nogueira CC participated in the experiment; Helbig E and Zambiasi RC participated in all stages of the study, from conception to the final revision of the manuscript.

Conflict of interests: The authors declare that there is no conflict of interests

References

1. Coutinho EF. A cultura da Oliveira. Pelotas: Embrapa Clima Temperado; 2007.
2. Roig A, Cayuela ML, Sánchez-Monedero MA. An overview on olive mill wastes and their valorisation methods. *Waste Management*. 2006; 26(9):960-969.
3. Lima FEL, Menezes TN, Tavares MP, Szarfarc SC, Fisberg RM. Ácidos graxos e doenças cardiovasculares: uma revisão. *Rev Nutr*. 2000; 13(2):73-80.
4. Cintra DE, Costa AV, Peluzio MC, Matta SL, Silva MT, Costa NM. Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout or chicken skin. *Nutrition*. 2006; 22(2):197-205.
5. Riediger ND, Othman R, Fitz E, Pierce GN, Suh M, Moghadasian MH. Low n-6: n-3 fatty acid ratio, with fish- or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. *Eur J Nutr*. 2008; 47(3):153-160.
6. Andrikopoulos N, Antonopoulou S, Kaliora A. Oleuropein inhibits LDL oxidation induce by cooking oil frying by-products and platelet aggregation induced by platelet-activating factor. *Lebensmittel-Wissenschaft und Technologie*. 2002; 35(6):479-484.
7. Champe PC, Harvey RA, Ferrier DR. *Bioquímica ilustrada*. 4 ed. Porto Alegre: Artmed; 2009.
8. Araújo JMA. *Química de alimentos: teoria e prática*. 3 ed. Viçosa: Universidade Federal de Viçosa; 2004.
9. Santos RD, Gagliardi ACM, Xavier HT, Magnoni CD, Cassani R, Lottenberg AM. Sociedade Brasileira de Cardiologia. I Diretriz sobre o consumo de Gorduras e Saúde Cardiovascular. *Arquivos Brasileiros de Cardiologia*. 2013; 100(1 Supl. 3):1-40.
10. Anderson EL, Van Voris LP, Bartram J, Hoffman HE, Belshe RB. Pharmacokinetics of a single dose of rimantadine in young adults and children. *Antimicrobial Agents and Chemotherapy*. 1987; 31(7):1140-1142.
11. Shutler SM, Low AG, Walker AF. Influence of baked beans on plasma lipids in pigs fed on a hypercholesterolaemic diet. *Proceedings of the Nutrition Society*. 1988; 47:97A.
12. Ceriello A. Oxidative stress and glycemic regulation. *Metabolism*. 2000; 49(2):9-27.

13. Kamtchouing P, Kahpui MS, Dzeufiet DPD. Antidiabetic activity of methanol/Methylene chloride stem bark extracts of *Terminalia superba* and *Canarium schweinfurthii* on Streptozotocin-induced diabetic rats. *J Ethnopharmacol.* 2006; 104(3):306-309.
14. Devalaraja S, Jain S, Yadav H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. *Food Research International Essex.* 2011; 44(7):1856-1865.
15. Halliwell B. Biochemistry of oxidative stress. *Biochemical Society Transactions.* 2007; 35(5):1147-1150.
16. Prasad K. Flaxseed and cardiovascular health. *J Cardiovasc Pharmacol.* 2009; 54(5):369-377.
17. Halliwell B. Free radicals and antioxidants - quo vadis?. *Trends in Pharmacological Science.* 2011; 32(3):125-129.
18. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ. Development of hypertension in a rat model of diet-induced obesity. *Hypertension.* 2000; 35(4):1009-1015.
19. Petry CJ, Ozanne SE, Wang CL, Hales CN. Effects of early protein restriction and adult obesity on rat pancreatic hormone content and glucose tolerance. *Hormone and Metabolic Research.* 2000; 32(6):23-39.
20. Universidade Federal de Pelotas. Comissão de Ética em Experimentação Animal. Regimento da comissão de ética em experimentação animal da Universidade Federal de Pelotas [Internet]. [acesso em: 10 out. 2016]. Disponível em: <http://wp.ufpel.edu.br/ceea/regimento/>
21. Colégio Brasileiro de Experimentação Animal. Princípios éticos na experimentação animal [Internet]. 1991. [acesso em: 10 out. 2016]. Disponível em: <http://www.feis.unesp.br/Home/comissaodeeticaeusoanimal/principios-eticos-na-experimentacao-animal.pdf>
22. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc writing Committee on the reformulation of the AIN-76A Rodent diet. *J Nutr.* 1993; 123(11):1939-1951.
23. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clinical Chemistry.* 1972; 18(6):499-502.
24. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malondialdehyde and 4-hydroxynonenal. *Methods Enzymol.* 1990; 186:407-421.
25. Kristensen M, Jensen MG, Aarestrup J, Petersen KEN, Sondergaard L, Mikkelsen MS, et al. Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type. *Nutr Metab.* 2012; 9(8). Disponível em: <https://nutritionandmetabolism.biomedcentral.com/articles/10.1186/1743-7075-9-8>
26. Jemai H, Fki I, Bouaziz M, Bouallagui Z, El Feki A, Isoda H, Sayadi S. Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. *J Agric Food Chem.* 2008; 56(8):2630-2636.
27. Muniz LC, Schneider BC, Silva ICM, Matijasevich A, Santos IS. Accumulated behavioral risk factors for cardiovascular diseases in Southern Brazil. *Rev Saúde Pública.* 2012; 46(3):534-542.

28. Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats¹⁻³. *J Nutr.* 2010; 40(5):946-953.
29. Fki I, Sahnoun Z, Sayadi S. Hypocholesterolemic effects of phenolic extracts and purified hydroxytyrosol recovered from olive mill wastewater in rats fed a cholesterol-rich diet. *J Agric Food Chem.* 2007; 55(3):624-631.
30. Visioli F, Galli C. Biological properties of olive oil phytochemicals. *Crit Rev Food Sc Nutr.* 2002; 42(3):209-221.
31. Al-Azzawie HF, Alhamdani MSS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sciences.* 2006; 78(12):1371-1377.
32. Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. *J Agric Food Chem.* 2009; 57(19):8798-8804.
33. Fki I, Bouaziz M, Sahnoun Z, Sayadi S. Hypocholesterolemic effects of phenolic-rich extracts of Chemlali olive cultivar in rats fed a cholesterol-rich diet. *Bioorg Med Chem.* 2005; 13(18):5362-5370.
34. Levin BE, Keesey RE. Defense of differing body weight set points in diet induced obese and resistant rats. *Am J Physiol.* 1998; 274(2 pt 2):412-419.
35. Pellizzon MA, Billheimer JT, Bloendon LT, Szapary PO, Rader DJ. Flaxseed reduces plasma cholesterol levels in hypercholesterolemic mouse models. *J Am Coll Nutr.* 2007; 26(1):66-75.
36. Bressan J, Hermsdorff HHM, Zulet MA, Martínez JA. Impacto hormonal e inflamatório de diferentes composições dietéticas: ênfase em padrões alimentares e fatores dietéticos específicos. *Arq Bras Endocrinol Metab.* 2009; 53(5):572-581.

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