Baru almond flour (*dipteryx alata vog.*) improves hepatic glutathione system in a high-fat diet murine model

A farinha de amêndoa de baru (dipteryx alata vog.) melhora o sistema hepático da glutationa em um modelo murino de dieta rica em gordura

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Abstract

Nonalcoholic fatty liver disease (NAFLD) has an effect on a range of individuals worldwide and it is characterized by steatosis, oxidative stress, lipotoxicity and inflammation. Dietary antioxidants are important for the efficient defense against oxidative damage. We hypothesized that baru almond (Dipteryx Alata Vog.) can be promising therapeutic agent to the NAFLD treatment. The objective of the present study was to evaluate the effect of baru almond flour (BAF) on the lipid profile and antioxidant defenses in rats fed a high-fat diet. Forty Fisher rats were divided into the control group (C); baru control (CB); high-fat (H); and high-fat baru (HB) for 12 weeks. The high-fat diet reduced HDL-cholesterol, increased serum levels of non-HDL fraction, total cholesterol. Induced hepatic steatosis, liver damage and oxidative stress, as demonstrated by the presence of elevated aminotransferases and oxidized glutathione levels. BAF demonstrated a high content of phenolic compounds and antioxidant activity. Treatment with BAF attenuated serum cholesterol levels and improved the hepatic glutathione system, increasing glutathione content. These results show that BAF is a promising source of natural food antioxidants and has beneficial effects on the redox status in vivo. Further studies to elucidate its functional properties and underlying mechanisms are, therefore, required.

Keywords: Dipteryx Alata Vog.. High-fat diet. Antioxidant. Liver. Glutathione.

Resumo

A doença hepática gordurosa não alcoólica (DHGNA) afeta indivíduos em todo o mundo e é caracterizada por esteatose, estresse oxidativo, lipotoxicidade e inflamação. Nesse contexto, os antioxidantes dietéticos são importantes para a defesa eficiente contra os danos oxidativos. Nossa hipótese é que a amêndoa de baru (Dipteryx Alata Vog.) pode ser um agente terapêutico promissor para o tratamento da DHGNA. Assim, o objetivo do presente estudo foi avaliar o efeito da farinha de amêndoa de baru (BAF) no perfil lipídico e nas defesas antioxidantes em ratos alimentados com dieta hiperlipídica. Quarenta ratos Fisher foram divididos no grupo controle (C) (dieta AIN-93M); controle de baru (CB) (AIN-93M com 2% de BAF); alto teor de gordura (H) (AIN-93M com 2% de colesterol e 25% de óleo de soja); e baru com alto teor de gordura (HB) (dieta hiperlipídica com 2% de BAF) por 12 semanas. A dieta hiperlipídica reduziu o HDL-colesterol, aumentou os níveis séricos da fração não-HDL, colesterol total. Além disso, induziu esteatose hepática, dano hepático e estresse oxidativo, como demonstrado pela presença de ALT elevadas e níveis de GSSG. A amêndoa de baru demonstrou alto teor de compostos fenólicos e atividade antioxidante. O tratamento com BAF atenuou os níveis séricos de colesterol. Além disso, BAF melhorou o sistema de glutationa hepática, aumentando o conteúdo de glutationa. Esses resultados mostram que o BAF é uma fonte promissora de antioxidantes alimentares naturais e tem efeitos benéficos no estado redox in vivo. Mais estudos para elucidar suas propriedades funcionais e mecanismos subjacentes são, portanto, necessários.

Palavras-chave: *Dipteryx Alata Vog. Dieta hiperlipídica. Antioxidante. Fígado. Glutationa.*

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INTRODUÇÃO

Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation that can involve a variety of presentations, from a simple accumulation of lipids in hepatocytes without liver damage, such as inflammation, necrosis, ballooning and fibrosis, to severe liver disease and eventually cirrhosis and/or hepatocellular carcinoma[1]. It is indicated in the literature that the degree of fat deposition in the liver predicts the risk of steatohepatitis, as well as the risk of progression to cirrhosis[2].

It is also known that metabolic disorders, including insulin resistance and altered lipid profile, could contribute to the pathogenesis of NAFLD[3,4], and its progression is influenced by environmental and genetic factors in a "multiple parallel-hit model", in which oxidative stress functions, primarily, as the starting point of hepatic and extrahepatic injury.

Regarding the above, these processes occur in the presence of a significant amount of fat in the liver and systemic and hepatic insulin resistance that, when combined, can create multiple alterations, leading to an instability between the protection system of the liver against lipotoxicity and the production of reactive oxygen species in gut and adipose tissue. Which in turn causes endoplasmic stress, oxidative stress, and hepatocyte apoptosis[1,5].

Oxidative stress in NAFLD patients[6,7] is considered the third injury that occasionally leads to hepatocyte death. The pathogenesis of NAFLD appears to be an endless loop of steatosis, lipotoxicity and inflammation that give rise to complicated alterations in the histopathological and biochemical characteristics of the liver[8].

Nuts and edible seeds contain elevated quantities of lipids, proteins, phenolic compounds, carotenoids, tocopherols, dietary fiber and minerals and show a propitious essential amino acid profile. They are important sources of protein and bioactive compounds for vegetable-based diets, as the consumption of these foods is related to a lower chronic disease risk and is also associated with a reduction in oxidative stress and inflammation[9–11] due to their nutritional constituents[12–14].

In this context, the baru tree (*Dipteryx alata Vog.*) is a native plant of the Brazilian Savanna that produces an almond nutritionally composed of high amounts of monounsaturated fatty acids, especially oleic acid, polyunsaturated fatty acids, and protein; low amounts of saturated fatty acids and carbohydrate; dietary fiber; and

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high levels of calcium, iron, phytates, tannins, vitamin E, and zinc[15–18]. Oliveira-Alves et al.[19] showed that the main phenolic compounds identified in baru nuts were gallic acid and its derivatives, such as gallic acid esters and gallotannins. Among all, gallic acid and methyl gallate seemed to be the main compounds responsible for the high antioxidant activity.

Because of its bioactive compounds and based on *in vitro* and *in vivo* previous studies that show positive effects on lipid profiles[11,20,21], hepatoprotective effects[22], antioxidant and anti-inflammatory properties[17,19,22–25], enough attention has been paid to baru almonds with respect to their health benefits. However, as it is possible to verify, studies on their bioactive potential are still scarce. We hypothesized that baru almond (*Dipteryx Alata* Vog.) can be promising therapeutic agent to the NAFLD treatment. Therefore, we investigated the effect of baru almond flour (BAF*)* on the lipid profile and antioxidant defenses in a high-fat diet-induced NAFLD murine model.

METHODS AND MATERIALS

Baru nuts

Baru seed, roasted and skinned, was commercially purchased from Urucuia Grande Sertão (Arinos, Minas Gerais). The seeds were ground to obtain the flour.

Baru evaluation

The contents of moisture, ash, total lipids, proteins, fibers, and carbohydrates were analyzed at the Bromatology Laboratory of the Food Department of the School of Nutrition, Federal University of Ouro Preto.

The humidity was determined by drying in an oven at 105 °C until constant weight. The fixed mineral residue, according to the method recommended by Soest[26], was obtained by calcining the sample in a muffle between 550 and 600 °C for 5 hours. In the Soxhlet extractor, the total lipids were extracted with petroleum ether, according to the Analytical Standards of the Adolfo Lutz Institute[27]. The protein content was determined according to the Kjeldahl method[26]. To quantify dietary fibers, the neutral detergent fiber determination method was used. All analyses were performed in triplicate. Finally, the carbohydrate content was calculated by the percentage difference of the sum of the contents of proteins, total lipids, moisture, mineral residue, and fibers.

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The content of total polyphenols was determined using the Folin-Ciocalteu method, described by Georgé et al.[28]. This method consists of reducing the Folin-Ciocalteu reagent, a mixture of phosphotungstic acids ($H_3PW_{12}O_{40}$), and phosphomolybdic ($H_3PMo_{12}O_{40}$), oxidizing the phenolic compounds and leading to the production of tugstico (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxide. The blue color is absorbed at a wavelength of 760 nm. All analyses were performed in triplicate. The absorbance values versus the gallic acid concentration were used for linear regression analysis, and the line equation was obtained. The sample absorbance values were used to calculate the polyphenol concentration. The content was expressed in mg of gallic acid equivalents (GAE) per 100 g of flour.

The antioxidant capacity of BAF was determined by the 2,2-diphenyl-1-picryl-hydrazil (DPPH) method[29]. This method is based on the electron transfer of an antioxidant compound to the DPPH radical in methanol solution, read at 515 nm. The absorbance values versus the Trolox concentration were used to construct a graph, and the absorbance values of the samples were replaced in the line equation, constituting the calculation of the antioxidant capacity (Trolox equivalent antioxidant capacity - TEAC), expressed in µM of equivalent of Trolox/g.

Animals

Forty female Fischer rats were used, with approximately 40 days of life and an average weight of 100 g, provided by the Experimental Nutrition Laboratory, School of Nutrition, Federal University of Ouro Preto (UFOP). The animals were placed in individual cages, and kept in an environment with controlled light, temperature, and humidity. They also received water and food ad libitum. The Ethics Committee on the Use of Animals (CEUA) at UFOP approved all procedures, protocol number 2013/66.

Diets and experimental design

The animals were randomly divided into 4 groups of 10 animals each: a control group (C), received a standard diet AIN-93 M, a control group baru (CB), received a standard diet + 2% BAF, a high-fat group (HF), fed a modified standard diet plus 25% soy oil and 2% cholesterol and a high-fat group baru (HFB), receiving a high-fat diet + 2% BAF. The composition of the diets is described in **Table 1.**

All animals were weighed weekly during the 12-week experimental period, and food intake and excretion were monitored between the fifth and sixth weeks of the

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experiment. In the end, the animals were subjected to 12 hours of fasting and then anesthetized with isoflurane and euthanized by exsanguination. Blood was collected in polypropylene tubes in the animal's axillary space through an incision in the vessels adjacent to the brachial plexus. The tubes were centrifuged to obtain the serum and stored at -80 °C, as well as the liver for further analysis. The feces were collected during the fifth and sixth weeks of the experiment, dried in an oven at 60ºC, and stored for analysis of the fat content.

Table 1 - Composition of the experimental diets

Nutrients (g)	Diets			
	AIN-93M	AIN-93M Baru	High-fat	High-fat+ Baru
Casein	140	140	140	140
Mineral Mixture[1]	35	35	35	35
Vitamin Mixture[2]	10	10	10	10
Cellulose	50	50	50	50
Sucrose	100	100	100	100
Choline	2.5	2.5	2.5	2.5
Corn Starch	622.5	602.5	392.5	372.5
Soybean oil	40	40	250	250
Cholesterol	0	0	20	20
Baru Nut Flour[3]	0	20	0	20
Total	1000	1000	1000	1000
Energetic Content (kcal/kg)	3810	3817	4960	4967

[1]Salt mixture (grams per kilogram of mixture): NaCl, 74; KI, 0.01; tripotassium citrate, 28; CaCO3, 357; MnCO3, 0.63; iron citrate, 6.06; MgO, 24; K2SO4, 46.6; KH2PO4, 250; ZnCO3, 1.65; CuCO3, 0.3; Na2SeO4, 0.01; (NH4)6MoO24·4H2O, 0.00795. The salts were purchased from Reagen, Rio de Janeiro, Brazil

[2]Vitamin mixture (international unit or gram per kilogram of mixture): Niacin, 3; cholecalciferol (400,000 IU/g), 0.25; calcium pantothenate, 1.6; riboflavin, 0.6; thiamin HCl, 0.6; pyridoxine HCl, 0.7; folic acid, 0.20; biotin, 0.02; vitamin B12, 2.5; vitamin K, 0.075; vitamin A (500,000 IU/g), 0.8; α-tocopherol (500 IU/g), 15; sucrose, quantity sufficient to 1 kg. The vitamins were purchased from Merck, Darmstadt, Germany.

[3]Chemical composition of Baru Nut Flour (100 g): moisture, 4.4 g; proteins, 18.8 g; ash, 2.18 g; fat, 26.7 g; dietary fiber, 22.2 g; and carbohydrates, 25.5 g.

Serum Analyses

Biochemical measurements of the lipid profile, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed based on a colorimetric assay using commercial kits from Labtest Diagnóstica SA. The concentration of non-HDL cholesterol was calculated from the difference between total cholesterol and HDL cholesterol and expressed in mmol / L.

Liver Analyses

The concentration of total glutathione was determined using a Sigma CS0260 kit (St. Louis, MO., USA). To determine the concentration of oxidized glutathione, the liver

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sample was prepared with 5% sulfosalicylic acid, 2 μL of vinylpyridine was added, and the pH was adjusted with triethanolamine (TEA) to a range between 6 and 7. The samples were incubated, and GSSG concentration was determined using Sigma Kit CS0260 (St. Louis, MO., USA). The reduced glutathione concentration in the samples was determined by subtracting the total glutathione concentration from the oxidized glutathione concentration.

The measurement of catalase activity was based on Aebi[30], observed by the decomposition of hydrogen peroxide at 240 nm in a spectrophotometer for 3 minutes. The activity of the superoxide dismutase (SOD) enzyme was determined in liver homogenate using a Cayman Kit 706002 (Ann Arbor, MI., USA), which uses tetrazolium salt to detect superoxide radicals formed by xanthine oxidase and hypoxanthine. The determination of the lipid content of the liver was based on the method of Folch, Lees e Sloane[31], in which the fat is extracted with a chloroform/methanol solution (2:1) and presented as a percentage.

The histological analysis was performed in the left lobe of the liver, which was sectioned and fixed in buffered formaldehyde. The fabrics were processed in an increasing series of alcohols and embedded in paraffin. Paraffin sections of approximately four micrometers in thickness were obtained in a semi-automatic microtome and mounted on glass sheets previously cleaned and degreased. The cuts were dewaxed in xylene, stained with hematoxylin and eosin (H&E) using the standard technique and then examined under a microscope equipped with a digital camera.

Real-time quantitative RT-PCR

Total RNA was isolated from the liver sample using the RNAgents Total RNA Isolation System (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. Complementary deoxyribonucleic acid (cDNA) was synthesized from 2 μg of total RNA, and the High Capacity cDNA Reverse Transcription kit from Applied Biosystems (Foster City, CA, USA) was used according to the manufacturer's instructions. The following primer sequences were used: **GPx:** 5'-CAGTTCGGACATCAGGAGAAT-3' (forward); 5'-AGAGCGGGTGAGCCTTCT-3' (reverse); **γ-GCS**: 5'-ATCTGGATGATGCCAACGAGTC-3' (forward); 5'-CCTCCATTGGTCGGAACTCTACT-3' (reverse). **GAPDH** 5'-GGATGCAGGGATGATGTTCT-3' (forward); 5'-AAGGGCTCATGACCACAGTC-3' (reverse). For the analysis of the expression of the genes under study, the

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quantitative polymerase chain reaction technique after reverse transcription (qRT-PCR) was used, using the Power SYBR® Green PCR Master Mix reagent (Applied Biosystems) in an ABI 7500 thermocycler (Applied Biosystems). All analyses were performed in triplicate. The data obtained were analyzed using the method of relative quantification of gene expression (comparative Cq or ΔΔCq). The expression of the target genes was determined as a function of the expression of the endogenous control gene GAPDH, and the control group was used as a basis for the results of the comparative expression.

Statistical analyses

All data were previously submitted to the Kolmogorov-Smirnov normality test. Those who followed a parametric distribution were evaluated by analysis of bivariate variance (two-way ANOVA), and the values were expressed as the mean ± standard deviation. For data that did not follow a normal distribution, the Kruskal-Wallis nonparametric test was used followed by the Dunn posttest, and the results were expressed as median and minimum and maximum values. The differences were considered significant for $p < 0.05$. GraphPad Prism® Software version 6.00 for Windows (San Diego, California, USA) was used.

RESULTS

The results of the composition as well as the total energy value (VET) of the flour are shown in **Table 2**.

Table 2 - Proximate composition of baru almond flour.

Components (g.100^{-1})*						
Humidity	**Ashes**	**Fibers**	**Proteins**	**Ethereal extract**	**Carbohydrates**	**VET (kcal)**
4,47 ± 0,14	2,18 ± 0,02	22,26 ± 0,99	18,81 ± 0,54	26,7 ± 0,005	25,56	417,79

* Mean ± standard deviation of samples in triplicate, except carbohydrates, which were estimated by difference.

The BAF polyphenol content is 200.88 ± 5.84 mg GAE/100g and its antioxidant capacity is represented by 5.60 ± 0.82 µMTEAC/g.

At the end of the experimental period, it was observed that the animals that received a high-fat diet had an increase in the final body mass as well as the mass gain when compared to the groups that received a control diet. When analyzing dietary intake by animals, those who received a high-fat diet had a lower intake in terms of the amount of food; however, they ingested more calories than those fed with control

diets. The high-fat and BAF increased fecal excretion. The high-fat diet increased liver mass and lipid deposition **(Table 3)**.

Table 3 - Biological variables in rats fed control (C) or high-fat (H) diets with (B) or without baru almond flour treatment.

	Experimental group				ANOVA p-value		
	C	**CB**	**H**	**HB**	**Baru**	**Diet**	**Interaction**
Final body mass (g)	188.33 ± 12.27	202.24 ± 19.53	223.39 ± 16.15	228.83 ± 11.12	0.0508	<0.0001	0.3822
Mass gain (g)	84.51 ± 17.07	99.36 ± 23.36	121.1 ± 21.16	125.7 ± 17.60	0.1328	<0.0001	0.4210
Food intake (g/wk)	67.74 ± 5.49	71.45 ± 7.59	57.53 ± 4.42	59.75 ± 3.73	0.0974	<0.0001	0.6714
Caloric intake (kcal/wk)	258.08 ± 20.92	273.69 ± 29.10	285.34 ± 21.93	297.58 ± 18.61	0.0634	0.0012	0.8182
Feces (g/wk)	5.69 ± 0.52	6.38 ± 0.71	7.25 ± 0.53	7.54 ± 0.52	0.0114	<0.0001	0.2765
Liver mass (g)	4.92 ± 0.29	5.01 ± 0.68	13.38 ± 1.81	14.14 ± 1.54	0.2987	<0.0001	0.4200
Liver Fat	6.02 ± 0.93	7.07 ± 1.26	40.19 ± 4.38	42.04 ± 3.66	0,1288	<0.0001	0,6719

Values are means ± SD. Data were tested by two-way ANOVA and analysis of variance.

The triacylglycerol concentration was higher in the CB group than in the other groups, and lower levels were observed in the HB group. The consumption of a high-fat diet promoted a significant increase in the serum concentration of total cholesterol concerning the groups with the control diet, and in the animals treated with the flour, a partial reduction of this steroid was observed. The levels of HDL cholesterol were affected by the diet, in which the groups fed with a high-fat diet had lower levels than the groups fed the control diet. In relation to the non-HDL-C fractions, high-fat diet groups showed higher levels **(Table 4)**.

The analysis of liver damage marker enzymes are shown in **Table 4**. The high-fat diet elevated AST and ALT activities.

Table 4 - Serum analysis of rats fed control (C) or c (H) diets with (B) or without baru almond flour treatment.

	Experimental group				ANOVA p-value		
	C	**CB**	**H**	**HB**	**Baru**	**Diet**	**Interaction**
Serum	mmol/L						
Triacylglycerols	0.48 ± 0.14[b]	0.67 ± 0.11[a]	0.35 ± 0.09[b,c]	0.25 ± 0.06[c]	0.2057	< 0.0001	0.0002
Cholesterol*	2.23 ± 0.40[b]	2.15 ± 0.49[b]	3.69 ± 1.46[a]	2.55 ± 0.65[a,b]	–	–	–
HDL-C	1.60 ± 0.32	1.38 ± 0.27	0.78 ± 0.32	0.82 ± 0.18	0.3417	< 0.0001	0.1664

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Continuação da tabela 4

	Experimental group				ANOVA p-value		
	C	CB	H	HB	Baru	Diet	Interaction
Serum	mmol/L						
Non HDL-C*	0.64 ± 0.22[b]	0.76 ± 0.28[b]	2.91 ± 1.65[a]	1.73 ± 0.53[a]	–	–	–
ALT (U/mL) *	21.90 ± 6.47[b]	22.55 ± 3.40[b]	65.14 ± 26.58[a]	55.35 ± 10.21[a]	–	–	–
AST (U/mL)	71.15 ± 11.63	61.91 ± 15.60	161.98 ± 40.83	153.78 ± 25.74	0.3142	< 0.0001	0.9518

Values are means ± SD. Data were tested by two-way ANOVA and analysis of variance. When interactions were significant (p < 0.05), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, significantly different values are marked with different superscript letters when a significant interaction was observed (p < 0.05).

* Data subjected to the nonparametric Kruskal-Wallis test; different values are marked with different superscript letters (p < 0.05).

The **figure 1** represents the photomicrographs of the histological sections of the liver. The presence of micro and macrovesicular steatosis was observed in high–fat groups, and there was no significant difference in the treatment with BAF. No other degenerative processes were detected.

Figure 1 - Representative hematoxylin and eosin-stained histological sections of livers from rats fed a control or high-fat diet treated or not with baru.
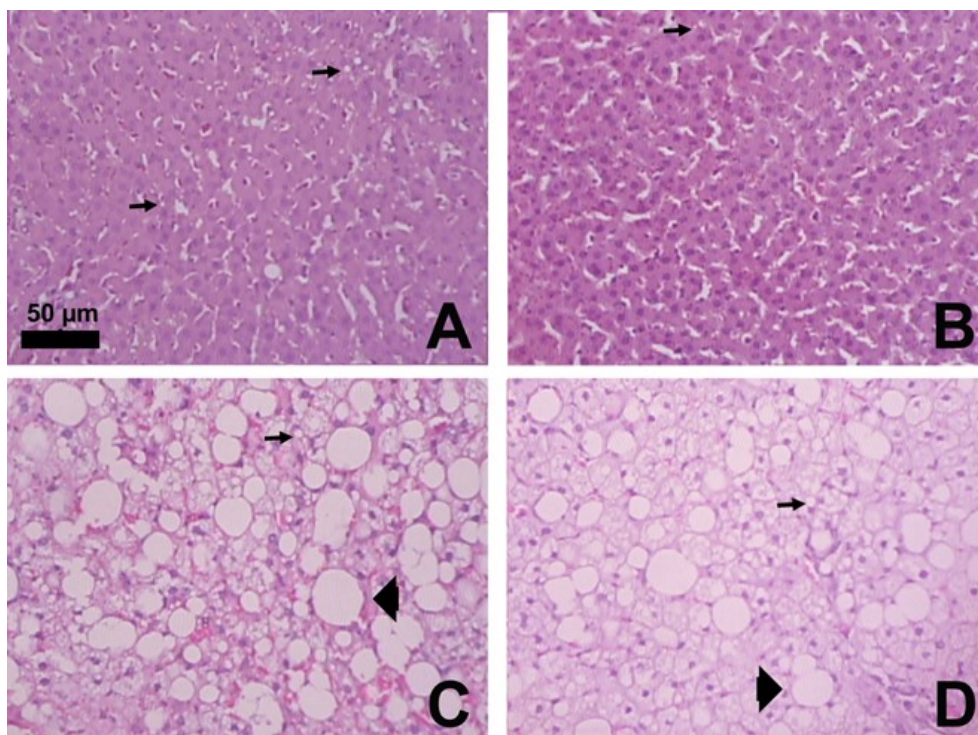
In the C group (A) and CB group (B), normal histological aspects were observed, with the presence of microvesicular steatosis (thin arrow). Note the loss of normal liver architecture in the H group (C), with numerous hepatocyte-shaped signet rings, featuring macrovesicular steatosis (arrowhead), and the presence of steatotic microvessels (thin arrow). HB group (D) presents a similar aspect, but less intense. Scale bar = 50 µm.

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In the liver, the high-fat diet had lower SOD activity than the control groups. In addition, BAF reduced catalase activity (**Figure 2 A-B)**. BAF affected the concentration of total glutathione and reduced glutathione, and in both cases, the treated groups had higher levels than the untreated groups. The consumption of a high-fat diet influenced the hepatic concentration of oxidized glutathione, since the groups that received this diet showed higher levels when compared to the groups fed with the control diets. The GSH/GSSG ratio, used in biological systems to estimate the redox state, was lower in the H group compared to the control group **(Figure 2 C-F)**.

Figure 2 - SOD (A), catalase (B), total glutathione (C), oxidized glutathione (D), reduced glutathione (E) and reduced/oxidized glutathione ratio (F) in the liver.
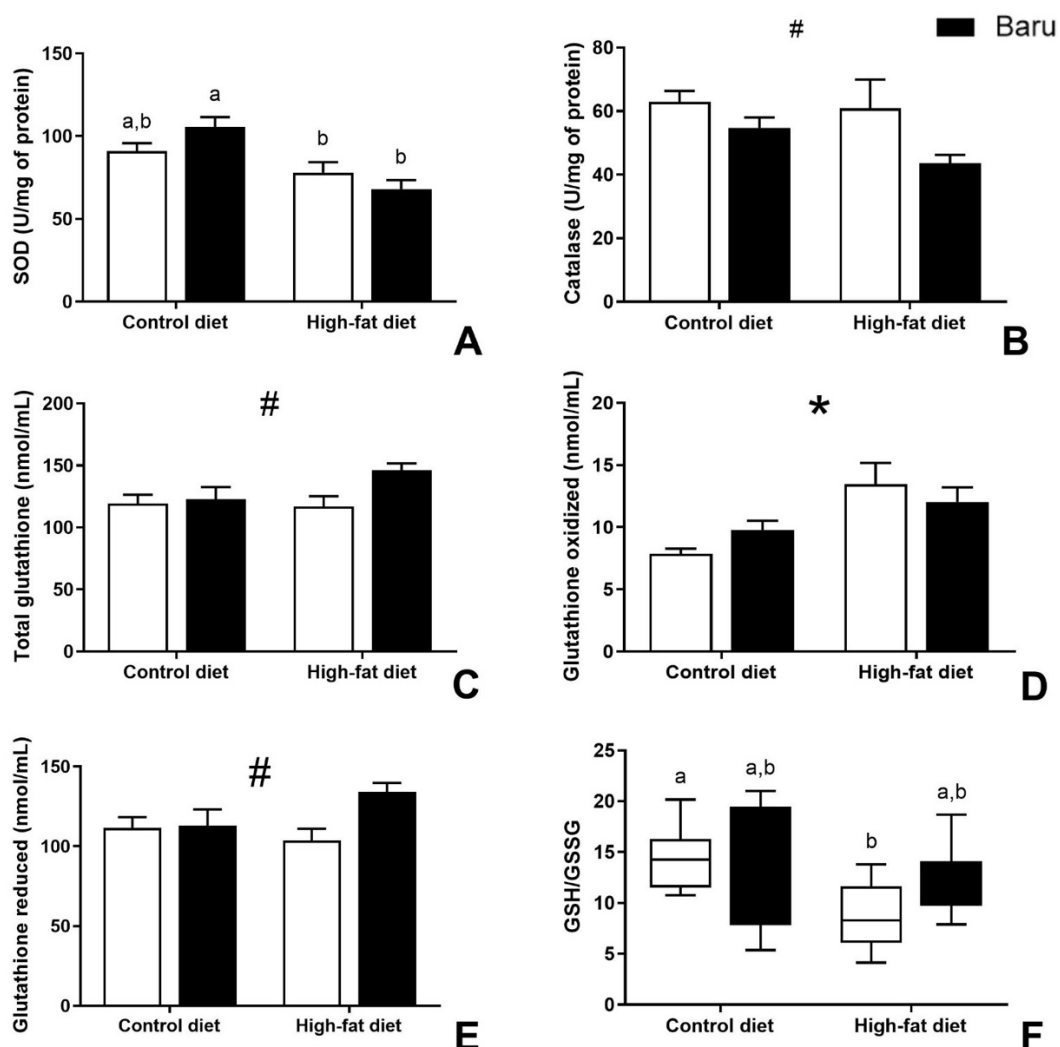
Data (A-F) are expressed as the mean ± SD, and data (F) are expressed as medians and percentiles. C control, CB baru control, H high-fat and HB baru high-fat. SOD, superoxide dismutase; Different letters indicate p <0.05. * Diet effect p <0.05; # Treatment effect p <0.05.

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The analysis of gene expression in the liver showed that the high-fat diet decreased the relative expression of GPx mRNA and γ-GCS (**Figure 3**).

Figure 3 - Gene expression levels in the liver of gamma-glutamylcysteine synthetase (A) and glutathione peroxidase (B) in rats fed a control or high-fat diet treated or not with baru.
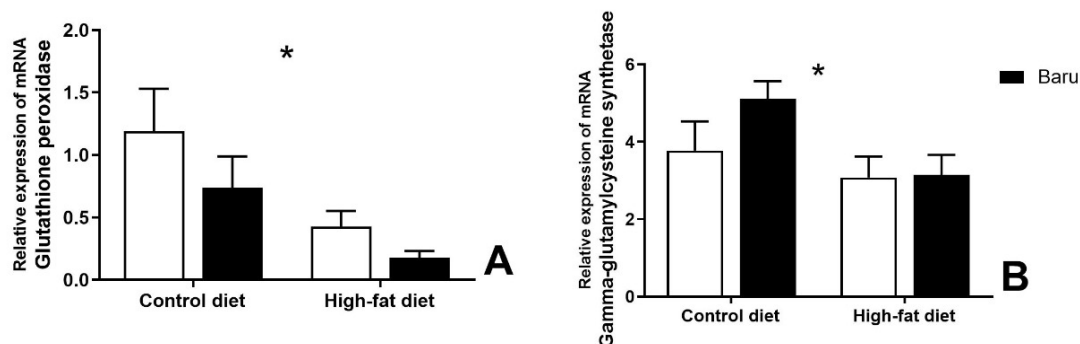
Data are expressed as the mean ± SD. C control, CB baru control, H high-fat and HB baru high-fat. * Diet effect p <0.05.

DISCUSSION

Our results demonstrated that BAF is a source of natural antioxidants defenses and has beneficial effects on the redox status *in vivo,* since it improved the hepatic glutathione system. Thus, we accepted the hypothesis that BAF is a promising therapeutic agent to the NAFLD treatment.

It is already known in the literature that true nuts and edible seeds present biologically active properties and baru almond is a part of these groups of seeds that presents a high nutritional value. These stand out for their fatty acid profile, especially oleic, linoleic, linolenic, gadoleic, erucic, phytic acids, considerable phytosterol content, high levels of vitamin E and selenium, and, in some cases, dietary fiber, especially insoluble fibers. The high intake of these foods is related to the decreased risk of cardiovascular diseases and some types of cancer, such as prostate, esophagus, stomach, colon, and rectum[10,19,32].

In the evaluation studies of the composition of baru almond can be found the evaluation of different parts: skin, pulp, toast and raw [33]. The composition of roasted almonds showed a similar profile in studies with higher lipid content, followed by carbohydrates and proteins or fibers[18,19,33]. The baru nut is a food with a good amino acid profile, quality and value of the proteins offered [34]. Its source of lipids contains, in greater proportion, monounsaturated fatty acids, specifically oleic acid (47.20g/100g)[19].

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The almond flour, roasted almond and baru husk show relevant amounts of total phenolics and antioxidant capacity[16,18,33]. The main phenolic compounds identified in the roasted almond were gallic acid and its derivatives, such as gallic acid esters and galotanins[19]. The analyzed profile of different baru almonds showed that their lipids, proteins and amino acids are representative of edible seeds and similar to those of nuts. They also contain appreciable amounts of calcium and high levels of iron, zinc and dietary fiber[15].

It has been demonstrated a positive correlation between the content of polyphenols and the antioxidant capacity of fruits and vegetables[35]. In addition to the content of phenolic compounds, baru almonds have a considerable antioxidant capacity (25.60 µM TEA/g), however[23] demonstrated lower values of antioxidant capacity (0.8 µM TEA/g) when compared to our results. In contrast, Lemos et al.[16] found 149.1 µM TEAC/g and showed that the seed process of roasting and peeling interfered with the polyphenol content and antioxidant capacity. Furthermore, roasting did not significantly reduce the phenolic content of the almond skin but reduced the DPPH radical scavenging capacity by 50%.

These *in vitro* results show the importance of investigating the baru effects *in vivo* models of metabolic impairment, such as those that can be induced by the diet. Therefore, we evaluated the baru antioxidant potential in an experimental model that promotes oxidative stress, through a high-fat diet in rats.

As expected, the high-fat diet promoted deleterious effects in lipid profile and in the liver, also showed in the Lopes et al.[36] study. In addition, it is important to note that there was no significant difference between the HB group and the control groups, pointing to a positive effect of the seed in reducing total cholesterol. The high-fat diet also promoted a reduction in HDL-C levels and a consequent increase in non-HDL cholesterol levels, which is expected when there is consumption of a high-fat diet.

We showed that rats fed a high-fat diet developed hepatic steatosis, evidenced by the presence of micro and macrovesicular lipid droplets. The liver injury caused by the high-fat diet in this study could be also demonstrated by the increased activity of the enzymes ALT and AST. Our results corroborate other studies, indicating these changes as characteristics of a high-fat model and its effectiveness to induce a NAFLD[37,38].

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The SOD, CAT, and GPx are enzymes that constitute an antioxidant system reacting against oxidative stress damage in the NAFLD. Regarding the activity of SOD, the high-fat diets demonstrated lower activity when compared to control groups, which reflected the imbalance of antioxidant response in our diet model. Unexpected BAF did not act on SOD activity, and also it reduced CAT activity. Several studies indicate that CAT activity is reduced in NAFLD patients[39-41], although, in opposite to the above, there are a variety of studies that demonstrate an increased CAT activity as the fatty liver is aggravated[42-45].

In this context, we consider that in spite of our results did not demonstrated BAF reduction of liver lipid accumulation, the decrease on liver CAT activity could indicate an antioxidant protection, since excessive fatty acids provided by consumption of a high-fat diet increase fatty acid oxidation and thus catalase activity[46]. A recent study with obese women fed a normocaloric diet and regular consumption of 20g of baru almonds showed an improvement in antioxidant status due to the increased GPx activity, but it did not show differences between groups in the CAT and SOD activities[25].

In respect to the enzymes involved in the glutathione cycle, we observed that the groups that received a high-fat diet had higher levels of oxidized glutathione. BAF increased total and reduced glutathione, and therefore improved the GSH/GSSG ratio. The concentration of GSH is a sensitive indicator of the cellular redox state, and in the literature, several studies have shown the reduction of GSH as a consequence of oxidative stress and its increase after ingestion of natural compounds[47-50]. The increase in GSH levels found in this study may be related to the bioactive compounds present in baru acting on the transcription of gamma-glutamylcysteine synthetase (γ-GCS), the enzyme that limits glutathione synthesis. It was only possible to partially observe this effect in the CB group since the relative expression of γ-GCS mRNA was found to be reduced in the groups fed a high-fat diet. The consumption of a high-fat diet also led to a decrease in the relative expression of GPx mRNA, indicating the induction of oxidative stress[50,51].

Ameliorating glutathione levels has been suggested as a way of health promotion and disease prevention, so that phytochemicals and foods are nutritional strategies to improve glutathione status[52]. Our results highlight the importance of baru as a source of bioactive compounds and its benefits on serum lipid profile and glutathione system balance. Also, we support other studies that demonstrate the

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biological potential of baru due to the worldwide increased interest in almond consumption.

CONCLUSION

The present study showed the *in vitro* and *in vivo* BAF antioxidant activity. The bioactive compounds present in baru almond can improve the lipid profile and antioxidant defenses. However, the importance of further research is emphasized to verify the mechanisms underlying these effects.

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Author Contributions

 L.L.T. R., M.M.F.C., J.M.M.L., N.N.L., J.F.C.G. and L.F.B. performed the study, analysed the data, and prepared the manuscript. W.G.L. contributed to histological analysis. M. E. S., M. L. P. and L.F.B. conceived and coordinated the project.

Author Declarations

The authors declare no competing interests.

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