

Consumption of *Terminalia catappa* flour: modulation of lipid metabolism, reduction of cardiovascular risk, and hepatic protection in aged *Wistar rats*

BRUNO DANTAS

Federal University of Campina Grande

NATÁLIA OLIVEIRA

Federal University of Paraíba

ARIELLY OLIVEIRA

Federal University of Campina Grande

LARISSA MARIA DUTRA

Federal University of Paraíba

JOSEFA DAYSE SILVA

Federal University of Campina Grande

JULIANO CARLO FREITAS

Federal University of Campina Grande

RAPHAELA DANTAS

Federal University of Campina Grande

CARLOS EDUARDO OLIVEIRA

Federal University of Paraíba

ANA CRISTINA MARTINS

Centro Universitário Maurício de Nassau

VANESSA VIERA

Federal University of Campina Grande

DIEGO PEREIRA

`diego.elias@ufcg.professor.edu.br`

Federal University of Campina Grande

JULIANA KÉSSIA SOARES

Federal University of Paraíba

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Abstract

The objective of this study was to evaluate the impact of *Terminalia catappa* flour consumption on biochemical, morphometric, cardiovascular risk, and hepatic markers in aged Wistar rats. Three groups were formed (n = 10): the control group (CG) was treated with distilled water, and the P500 and P1000 groups were treated with 500 and 1000 mg/kg of *Terminalia catappa* flour, respectively. Animal body weight and food intake were monitored weekly. At the end of the study, feces samples were collected for cholesterol, triglycerides (TG), and fatty acid analysis. Additionally, murinometric and biochemical parameters were assessed. Hepatic tissue was harvested to evaluate cholesterol, TG, and malondialdehyde (MDA) levels. Food consumption and body weight showed no significant differences. In the P500 and P1000 groups, retroperitoneal fat weight was reduced, with P1000 also decreasing triglycerides (TG) and HDL levels. Both experimental groups lowered total cholesterol (TC), TG, and hepatic malondialdehyde (MDA) levels, with more pronounced effects in P1000, which also exhibited a higher proportion of unsaturated fatty acids. Feces cholesterol increased in P1000, while feces TG levels decreased in both treated groups. P1000 stood out for significantly reducing cardiovascular and coronary risk indices and achieving the greatest reduction in MDA levels in coronary tissue. These results suggest that *Terminalia catappa* improves plasma and hepatic lipid metabolism, reduces body fat, and attenuates lipid peroxidation. Given its effects on cardiovascular risk factors, consumption of this fruit may contribute to reduced cardiovascular and coronary risks.

1 INTRODUCTION

Life expectancy is projected to increase significantly over the coming decades, with estimates indicating that by 2050, approximately one-quarter of the global population will be 60 years or older ^{1,2}. This demographic shift raises a critical question: Will the increase in life expectancy translate into healthier and more fulfilling lives, or will a rise in age-related diseases accompany it?^{3,4} Achieving healthier aging requires a proactive approach, emphasizing early interventions and promoting lifelong care through lifestyle modifications and dietary habits ⁵.

A literature review study indicated that the high consumption of Westernized diets, characterized by high amounts of sugars and saturated fats, is strongly associated with the development of non-communicable chronic diseases (NCDs)⁶. Research using experimental models has shown that high-fat diets promote increased oxidative stress in multiple organs^{7,8}, which is one of the main underlying mechanisms in the pathogenesis of NCCDs⁹. Oxidative stress has, in turn, been widely recognized as a crucial factor in modulating inflammatory and degenerative processes, directly implicating the deterioration of cellular and tissue function^{10–12}.

On the other hand, the bioactive compounds present in plant matrices play a crucial role in mitigating oxidative stress, reducing inflammatory processes, and promoting cellular regeneration^{13–15}. Some of

these compounds exhibit antioxidant activity, directly neutralizing excess free radicals in the body and contributing to increased activity of the endogenous antioxidant system¹⁶.

Among the natural sources of antioxidants is the fruit of *Terminalia catappa*, which is native to tropical and subtropical regions and belongs to the Combretaceae family¹⁷. It contains a range of antioxidant compounds, such as gallic acid, ellagic acid, quercetin, and its derivatives, as well as tannins and anthocyanins, which give the fruit a vibrant pigmentation and significant therapeutic potential¹⁸.

In addition to antioxidants, *Terminalia catappa* also contains a lipid profile primarily composed of unsaturated fatty acids, known for their benefits related to fat metabolism, inflammation reduction, and improved cardiovascular health¹⁹. Research involving rodent models has highlighted the bioactive potential of *Terminalia catappa* by correlating its bioactive compounds with the reduction of oxidative stress and inflammation²⁰, regulation of glucose metabolism²¹, and prevention of chronic diseases²². However, none of this research has sought to evaluate the impact of *Terminalia catappa* consumption on the metabolism and health of aged rats.

Given the urgent need for interventions that improve the quality of life for the elderly, we hypothesize that the incorporation of *Terminalia catappa* flour into the diet may reduce hepatic oxidative stress and improve important biochemical parameters during this stage of life. Therefore, the objective of this study is to evaluate the impact of *Terminalia catappa* flour consumption on biochemical parameters, morphometric measurements, cardiovascular risk, and hepatic markers in aged Wistar rats.

2 MATERIAL AND METHODS

2.1 *Terminalia catappa*

The fruits of *Terminalia catappa* used in these experiments belongs to the family família Combretaceae and taken from the city of Cuité/PB, Brazil: (Latitude: -6.48173, Longitude: -36.1496; 6° 28' 54" South, 36° 8' 59" West. he species was deposited in the CES/UFCG Herbarium, with record 2893. To produce *Terminalia catappa* flour, the fruits were manually depulped before the drying process. The pulp was then spread onto stainless steel trays and dried in a forced-air circulation oven (Biopar, Model S480 AD, Porto Alegre, RS, Brazil) at $50 \pm 1^\circ\text{C}$ for 48 hours. After drying, the material was ground using a blender and subsequently sieved through a 0.5 mm mesh to ensure uniform particle size. The resulting flour was weighed, vacuum-sealed in sterile polypropylene bags, and stored at room temperature ($23 \pm 1^\circ\text{C}$) until further analysis.

2.2 Analysis physical-chemical

The physical-chemical analyses were performed on the *Terminalia catappa* flour. For this, water activity analysis was performed using the AQUALAB device (DECAGON, Model AQUALAB 4TE, USA), the pH was determined using a digital pH meter (GEHAKA, model PG1800, São Paulo - SP, Brazil), the ash content was quantified by incineration in a muffle furnace (JUNG, Model 0612, Blumenau - SP, Brazil) stabilized at

550°C, humidity was determined by oven drying (Medclave, Model n° 4, Brazil) being stabilized at 105°C, and acidity was determined by titration according to the *Association of Official Analytical Chemists* – AOAC²³. Lipids were determined by the Folch, Less and Stanley method, and the fatty acid profile was performed using the transesterification methodology of Hartman and Lago^{24,25}. Total insoluble and soluble fiber contents were determined using an enzymatic–gravimetric method.

2.3 Analysis of Antioxidant Compounds

2.3.1 Extraction

Terminalia catappa flour constituents were extracted with an 80% methanol solution and evaluated for ABTS• removal capacity, ferric-reducing activity (FRAP), flavonoids, and total phenolics. Crushed baru almond (1 g) was placed in a test tube and then 10 mL of solvent was added. The test tube was left at room temperature for 24 hours, and after filtration, the volume was completed to 10 mL with extraction solvent and stored at 18°C until analysis. All extractions were performed in triplicate.

2.3.2 Determination of the Total Phenolic Content

To measure the total phenolic compounds present in the sample, we used the methodology described by Liu et al. (2022) with minor adaptations²⁶. The absorbance of the extract was compared with a standard gallic acid curve to estimate the concentration of phenolic compounds in the sample. Results were expressed in milligrams equivalent to gallic acid/100g of sample (mg EAG/100g).

2.3.3 Determination of total flavonoids

The colorimetric assay developed by Zhishen et al. (1999) measured the total flavonoid content²⁷. To estimate the concentration of flavonoid contents in the sample, the extract's absorbance was compared with a catechin standard curve. The total flavonoid content was expressed in mg equivalent to catechin/100g of sample (mg EC/100g).

2.3.4 Antioxidant activity - FRAP methods

The FRAP method was performed according to Benzie and Strain, (1999), with modifications proposed by Liu et al. (2022)²⁸. The FRAP solution was used as a reference reagent and the absorbance was read in nm. Results were expressed as μmol trolox equivalents per gram sample ($\mu\text{mol TE/g}^{-1}$).

2.3.5 Antioxidant activity - ABTS methods

The ABTS method was performed by the methodology described by Surveswaran et al. (2007), with modifications. Results were expressed as μmol Trolox equivalents per gram of sample ($\mu\text{mol TE/g}^{-1}$)²⁹. Where A_0 is the absorbance of the control. The effective concentration presented 50% radical inhibition activity (IC_{50}), expressed in mg extract/mL, which was determined from the graph of the free radical scavenging activity (%) against the extract concentration (Table 1).

Table 1
Physicochemical characteristics and antioxidant potential of *Terminalia catappa* flour.

Parameters	CP
Humidity	14.12 ± 0.18
Lipids	1.38 ± 0.29
Ashes	5.92 ± 0.02
Acids	2.27 ± 0.02
aw	0.52 ± 0.00
pH	4.2 ± 0.08
Dietary fiber (g/100 g)	
Insoluble dietary fiber	31.45 ± 0.10
Soluble dietary fiber	2.32 ± 0.31
Total dietary fiber	33.77 ± 0.41
Antioxidant potencial	
Total Phenolics (mg GAE)	106.6 ± 0.01
Total Flavonoids (mg CE/100 g)	4.6 ± 0.03
FRAP (μmol TEAC/100 g)	2.07 ± 0.02
ABTS (μmol TEAC/100 g)	8.64 ± 0.00
Data are expressed as mean ± SD, n = 3; GAE: Gallic acid equivalent; CE: Catechin equivalent; TE: Trolox equivalent.	

PLEASE, INSERT Table 1 ABOUT HERE!

2.4 Animals and Experimental Groups

All of the experimental methods were previously approved by the Ethics Committee for Animal Use - CEUA of UFCG - Certification No. 53-2020, in compliance with the standards established by the National Council for the Control of Animal Experimentation (CONCEA, Brazil), under Law No. 11,794 /2008 (Arouca Law), and with the guidelines for *in vivo* experiments with animals of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0³⁰. The *Terminalia catappa* used for the production diet administered to animals was registered in SisGen; protocol No. A92B6F0 (see attachment). Thirty male Wistar strains, aged 18 months and weighing 350 ± 20 g from the Federal University of Pernambuco were used, and throughout the research remained at the Experimental Nutrition Laboratory of the Federal University of Campina Grande, Cuité campus (LANEX/ UFCG/ CUITÉ). The animals were housed in individual polypropylene cages (60 cm long, 50 cm wide, and 22 cm high), and kept under standard laboratory conditions (temperature 22 ± 1°C, humidity 55 ± 5%, light/dark cycle of 12/12 hours - artificial

light from 6:00 to 18:00). Three groups were formed: Control - supplemented with distilled water; P500 - supplemented with 500 mg/kg of *Terminalia catappa* flour of animal weight; and P1000 - supplemented with 1000 mg/kg of *Terminalia catappa* flour of animal weight. The gavage treatment was administered for 35 days, during which all animals were provided with standard feed (Presence Purina®, São Paulo, Brazil) and water *ad libitum*. The dosages administered to the animals were based on studies conducted by Behl, Valpadian, and Kotwani (2021), which explored the effects of *Terminalia catappa* fruit extract at doses of 20 mg/kg, 30 mg/kg, and 40 mg/kg on streptozotocin-induced diabetic retinopathy in rats³¹. Additionally, we considered a study by Naitik, Prakash, Kotrsha, and Rao (2012), which evaluated the impact of consuming 250 mg/kg and 500 mg/kg of *Terminalia catappa* for 20 days on its antitumor and lipid-lowering activities in transplanted fibrosarcoma in Wistar albino rats³².

2.5 Experimental Design

The elderly animals were supplemented via gavage for 35 days. Before being anesthetized, feces samples were collected for cholesterol and triglyceride analysis. Following anesthesia, the animals underwent a murinometric evaluation. Subsequently, blood was collected via cardiac puncture for biochemical profiling. The organs of interest were removed and weighed, along with the carcass. Mesenteric, epididymal, and retroperitoneal fats were also removed and weighed. The liver tissue, after being weighed, was sectioned: the right lobe was separated for cholesterol and triglyceride analyses, while the left lobe was designated for the analysis of lipid peroxidation products, specifically malondialdehyde (MDA), and fatty acid profiling. The experimental protocol is detailed in Fig. 1.

PLEASE, INSERT FIGURE 1 ABOUT HERE!

2.6 Food Consumption and Body Weight

Throughout the experiment, during the light cycle, the animal's body weight and feed consumption were measured weekly using a Balmak® digital electronic scale (Model: ELP-10, Santa Bárbara do Oeste/SP, Brazil) with a capacity ranging from 20 g to 10,000 g.

2.7 Murinometric Assessment

The animals were subjected to physical evaluation. Nasal-anal length, abdominal (AC) and thoracic (TC) circumferences were measured. The weights of the following organs were evaluated: liver, kidney, and heart, as well as the carcass. Body fat was also assessed by weighing the mesenteric, retroperitoneal, and epididymal fat, with the results expressed in grams.

2.8 Blood Collection and Biochemical Profile Analyses

The blood collected was subjected to a centrifugation process (Digital Bench Centrifuge – NT 810, Novatecnica brand, Piracicaba – São Paulo, Brazil) at 1308 G force for 15 min. The supernatant was used to measure glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). An enzymatic method, using the

Labtest commercial kit (Minas Gerais, Brazil), and the reading were carried out using a spectrophotometer (Kasuaki, model IL-226-NM-BI, Araucaria, Brazil).

2.9 Adiposity Index (ADI), Coronary Risk Index (CRI), and Cardiovascular Risk Index (CVRI)

The coronary risk index (CRI) and cardiovascular risk index (CVRI) were determined using the equations: $CRI = CTr/HDL$; $IRCV = TG/HDL$, respectively (CTr = Cholesterol; TG = triglycerides) (Friedewald et al., 1972). The adiposity index (AI) was calculated using the formula: $[\text{body fat weight (epididymal + visceral + retroperitoneal)}/\text{body weight}] \times 100$ (Nascimento et al., 2011)³³.

2.10 Hepatic and Feces Cholesterol and Triglycerides

The analysis of feces and hepatic lipids was performed following the method described by Folch, Less, and Stanley (1957)²⁴. After lipid extraction, cholesterol and triglyceride concentrations were determined. An enzymatic method, using the Labtest commercial kit (Minas Gerais, Brazil) and the reading were carried out using a spectrophotometer (Kasuaki, model IL-226-NM-BI, Araucaria, Brazil).

2.11 Determination of malondialdehyde in the liver and heart

At the end of the experiment, after 6 hours of fasting, the animals were anesthetized with Ketamine Hydrochloride and Xilasin (1 ml/kg body weight) and were sacrificed. Then, the liver and heart tissues were removed to determine the content of MDA. To assess lipid peroxidation, MDA production was measured in an assay described by Esterbauer and Cheeseman, (1990)³⁴. Tissue (5 samples per group) homogenates (T Tris-HCl 20 mm, 1:5 p/v) were centrifuged at 2500 g at 48°C for 15 min, then were added to a 750 ml solution (1-Methyl-2-phenylindole 10.3 mm in acetonitrile + 225 ml HCl 37%) and the mixture was placed in a water bath and heated to 4°C for 40 min. Next, it was centrifuged at 2500 g at 4°C for 5 min. Absorbance was measured at 586 nm (Genesys 10 s UV-VIS, Thermo Fisher Scientific, Loughborough, UK). The concentration of MDA was expressed as nmol of MDA per gram of liver and heart tissues.

2.12 Determination of the fatty acid profile in the liver

To determine the fatty acid profile of the liver, the lipid extract of the products was first obtained using the method of Folch et al. (1957)²⁴. From this extract, methyl esters were obtained by esterification following the methodology of Hartman and Lago, (1973)²⁵. The methyl esters were identified and quantified in a Ciola & Gregori Ltda gas chromatograph (model CG-Master), with a flame ionization detector. The chromatographic analysis used a polyethylene glycol column (Carbowax 20M), with fused silica, 30 m long, 0.53 mm in diameter, and 0.25 µm thick stationary phase film. The vaporizer and detector temperatures were 150 °C and 200 °C, respectively. The oven program was 80 °C for 30 min, with an increase of 10 °C/min up to 180 °C. The mobile phase was hydrogen, with a flow rate of 5

mL/min. A volume of 1 μ L was injected, with a split ratio of 1:25. The characterization of fatty acids was carried out by comparing the mass spectrum obtained with standards also injected into GC-MS.

3 STATISTICAL ANALYSIS

For data analysis, analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test when significant differences between groups were detected. A normality test was conducted prior to analysis. A significance level of 5% was considered for rejecting the null hypothesis.

4 RESULTS

4.1 Food Consumption and Body Weight

The analysis of weekly feed intake and weight over the 35-day treatment period, consisting of five weeks with *Terminalia catappa* flour, revealed no statistically significant differences between the groups ($p > 0.05$) (Fig. 2).

PLEASE INSERT FIGURE 2 ABOUT HERE!

4.2 Murinometric Assessment

Measurements of naso-anal length, thoracic circumference, and abdominal circumference showed no significant differences between the groups [$F(2, 30) = 1.339, p > 0.05$; $F(2, 30) = 0.696, p > 0.05$; $F(2, 24) = 1.246, p > 0.05$]. Similarly, no significant differences were observed in the weights of the organs and carcass of the animals [$F(2, 30) = 0.290, p > 0.05$; $F(2, 30) = 0.008, p > 0.05$; $F(2, 24) = 0.028, p > 0.05$; $F(2, 30) = 0.592, p > 0.05$]. Regarding the assessment of fat weights, no statistical differences were found between the groups for mesenteric or epididymal fats [$F(2, 26) = 1.047, p > 0.05$; $F(2, 26) = 0.601, p > 0.05$]. However, a reduction in retroperitoneal fat weight was observed in the experimental groups compared to the control group [$F(2, 14) = 25.20, p < 0.0001$] (Table 2).

Table 2
Murinometric assessment of experimental groups.

Parameters	CG	P500	P1000
Naso-anal length (cm)	24.41 ± 0.83 ^a	24.35 ± 0.57 ^a	23.91 ± 0.92 ^a
TC (cm)	14.86 ± 0.81 ^a	14.86 ± 0.64 ^a	14.23 ± 0.68 ^a
AC (cm)	16.09 ± 1.02 ^a	15.77 ± 0.82 ^a	15.45 ± 0.99 ^a
Organs and carcass weights (g)			
Liver weight	12.11 ± 1.60 ^a	12.58 ± 1.42 ^a	12.03 ± 2.33 ^a
Kidney weight	2.39 ± 0.18 ^a	2.38 ± 0.14 ^a	2.38 ± 0.28 ^a
Heart weight	1.23 ± 0.08 ^a	1.24 ± 0.11 ^a	1.24 ± 0.13 ^a
Carcass weight	241.00 ± 23.65 ^a	244.82 ± 18.93 ^a	233.20 ± 30.84 ^a
Visceral fats (g)			
Mesenteric fat	5.31 ± 1.52 ^a	5.22 ± 1.52 ^a	5.46 ± 1.15 ^a
Epididymal fat	4.40 ± 1.53 ^a	4.47 ± 1.17 ^a	4.65 ± 1.31 ^a
Retroperitoneal fat	6.06 ± 0.44 ^a	4.60 ± 0.47 ^b	4.10 ± 0.41 ^b
Data are expressed as mean ± standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). TC - Thoracic circumference and AC - Abdominal circumference. Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test (p < 0.05).			

PLEASE INSERT Table 2 ABOUT HERE!

4.3 Blood Collection and Biochemical Profile Analyses

The analysis of biochemical parameters indicated no significant differences among the groups for blood glucose, creatinine, or urea levels [F (2. 30) = 2.502, p > 0.05; F (2. 30) = 0.547, p > 0.05; F (2. 30) = 1.165, p > 0.05] (Fig. 3A, E, F). Notably, total cholesterol concentrations were significantly reduced in the experimental groups compared to the control group [F (2. 21) = 6.961, p < 0.01] (Fig. 3B). Furthermore, a significant reduction in triglyceride and HDL levels was observed exclusively in the P1000 experimental group when compared to both the control and P500 groups ([F (2. 21) = 6.961, p < 0.01] (Fig. 3C, D). Additionally, liver enzyme activities, specifically AST and ALT, demonstrated a significant decrease in the P1000 experimental group relative to the other groups [F (2. 21) = 6.961, p < 0.01] (Fig. 3G, H).

PLEASE INSERT FIGURE 3 ABOUT HERE!

4.4 Adiposity Index (ADI), Coronary Risk Index (CRI), Cardiovascular Risk Index (CVRI), and Malondialdehyde Levels in Coronary Tissue

The findings revealed that the P1000 group exhibited a significantly reduced coronary and cardiovascular risk compared to the P500 group [$F(2, 30) = 14.74, p < 0.0001$; $F(2, 25) = 19.33, p < 0.0001$] (Fig. 4A, B). In contrast, no significant differences were observed in the adiposity index among the groups [$F(2, 17) = 4.022, p < 0.0001$] (Fig. 4C). Regarding malondialdehyde levels in coronary tissue, a significant reduction was observed in the experimental groups compared to the control [$F(2, 15) = 165.8, p < 0.0001$] (Fig. 4D). Post hoc analysis further indicated that the P1000 group exhibited markedly greater reductions than the P500 group, underscoring the enhanced efficacy of the higher intervention dose.

PLEASE INSERT FIGURE 4 ABOUT HERE!

4.5 Hepatic Cholesterol, Triglycerides, and Malondialdehyde

Significant reductions in hepatic cholesterol, triglyceride and malondialdehyde concentrations were observed in the P500 and P1000 groups compared to the control group CG [$F(2, 15) = 72.01, p < 0.0001$; $F(2, 15) = 24.62, p < 0.0001$; $F(2, 15) = 4.517, p < 0.05$] (Fig. 5A, B, C).

PLEASE INSERT FIGURE 5 ABOUT HERE!

4.6 Feces Cholesterol and Triglycerides

The analysis of feces cholesterol revealed a significant increase in concentration in the P500 group compared to the control group, while the P1000 group exhibited a notable elevation in feces cholesterol relative to all groups [$F(2, 15) = 12.19, p < 0.01$]. Specifically, the P500 group demonstrated higher cholesterol levels compared to CG, and the P1000 group showed the highest concentration $p < 0.001$ (Fig. 6A). In contrast, the feces triglyceride levels decreased significantly in the P500 group when compared to CG $p < 0.01$, with the P1000 group presenting the lowest triglyceride values across all groups [$F(2, 15) = 11.82, p < 0.001$] (Fig. 6B).

PLEASE INSERT FIGURE 6 ABOUT HERE!

4.7 Liver Fatty Acid Composition

The analysis of liver fatty acid composition revealed significant differences among the experimental groups. For saturated fatty acids, palmitic acid (C16:0) exhibited higher concentrations in the control group (CG) compared to the P500 and P1000 groups, which displayed reduced levels. Stearic acid (C18:0) showed a progressive increase from the CG to the P1000 group. Myristic acid (C14:0) was found at the lowest concentrations in the P1000 group ($p < 0.05$).

In terms of monounsaturated fatty acids, oleic acid (C18:1 ω -9) demonstrated a significant decrease in both treated groups (P500 and P1000) relative to the control group.

Regarding polyunsaturated fatty acids, a decrease in linoleic acid (C18:2 ω -6) concentrations was observed in the treated groups compared to the control. Conversely, arachidonic acid (C20:4 ω -6) and docosahexaenoic acid (C22:6 ω -3) exhibited significant increases in the P500 and P1000 groups, with

the P1000 group showing the highest levels for both. Docosatetraenoic acid (C22:4 ω -6) was detected exclusively in the P500 and P1000 groups, with a significant elevation noted in the P1000 group ($p < 0.05$) (Table 3).

Table 3
Fatty acid composition in the liver.

Liver fatty acids		CG	P500	P1000
SATURATED				
Palmitic acid	C16:0	21.11 $\pm$ 0.2 ^a	20.04 $\pm$ 0.1 ^a	18.33 $\pm$ 0.3 ^b
Stearic acid	C18:0	16.72 $\pm$ 0.1 ^a	18.90 $\pm$ 0.1 ^b	20.59 $\pm$ 0.2 ^c
Myristic acid	C14:0	0.32 $\pm$ 0.2 ^a	0.27 $\pm$ 0.1 ^b	0.19 $\pm$ 0.1 ^c
Σ SFA		38.15	39.21	39.11
MONOUNSATURATED				
Oleic acid	C18:1 ω -9	12.22 $\pm$ 0.2 ^a	9.24 $\pm$ 0.3 ^b	9.10 $\pm$ 0.2 ^b
Σ MUFA		12.22	9.24	9.10
POLYUNSATURATED				
Linoleic acid	C18:2 ω -6	23.42 $\pm$ 0.1 ^a	21.04 $\pm$ 0.3 ^a	19.10 $\pm$ 0.1 ^b
Arachidonic acid	C20:4 ω -6	18.28 $\pm$ 0.1 ^a	21.98 $\pm$ 0.1 ^b	25.62 $\pm$ 0.1 ^c
Docosahexaenoic acid	C22:6 ω 3	1.16 $\pm$ 0.1 ^a	1.78 $\pm$ 0.1 ^a	2.40 $\pm$ 0.1 ^b
Docosatetraenoic acid	C22: 4 ω -6	-	0.30 $\pm$ 0.1 ^a	0.41 $\pm$ 0.1 ^b
Σ PUFA		42.86	44.8	47.53
Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test ($p < 0.05$).				

PLEASE INSERT Table 3 ABOUT HERE!

5 DISCUSSION

This study investigated, for the first time, the effects of consuming different concentrations of *Terminalia catappa* flour on the physical and biochemical parameters of aged Wistar rats. The results demonstrated that supplementation improved liver function by reducing oxidative stress, levels of transaminases, and the deposition of triglycerides and cholesterol in the liver. Additionally, a

hypolipidemic effect was observed, particularly when the highest dose of *Terminalia catappa* flour was administered (P1000).

According to the results of our study on the physicochemical analysis of *Terminalia catappa* flour, along with findings from another study conducted by our laboratory, the nutritional and bioactive potential of the flour was confirmed. The analysis highlighted significant levels of proteins, carbohydrates, and dietary fibers, particularly cellulose and lignin, which contribute to digestive health, increased satiety, and reduced fat absorption. Furthermore, the lipid profile analysis revealed a predominance of unsaturated fatty acids over saturated ones, with emphasis on oleic acid, which is associated with cardiovascular benefits, and linoleic acid, an essential fatty acid for the human diet due to its role in regulating lipid metabolism, inflammation, and LDL levels. Additionally, the flour also contains significant levels of total phenolic compounds and flavonoids. Among the primary phenolic compounds identified are gallic acid, ellagic acid, quercetin, and its derivatives, which are widely recognized for their antioxidant and anti-inflammatory properties¹⁸.

In light of this, we decided to evaluate the effects of consuming *Terminalia catappa* flour in vivo, considering doses of 500 and 1000 mg/kg of body weight. Despite the functional composition of the flour, the data from this study indicated that consumption at the tested doses did not result in significant changes in food intake or body weight among the animals in the experimental groups compared to the control group. These results contrast with previous studies that link the consumption of dietary fiber sources to reduced body weight gain in rodents^{35,36}. However, it is important to emphasize that aged rats may exhibit metabolic alterations related to aging, such as a lower basal metabolic rate and changes in gut microbiota, which can impact the response to dietary interventions^{37,38}.

Although we did not observe a significant difference in the body weight of the animals, our results demonstrated that supplementation had a selective impact on visceral fat deposits, with a significant reduction in retroperitoneal fat in both experimental groups (P500 and P1000) compared to the control group, while mesenteric and epididymal fat weights remained unchanged. This specific effect may be attributed to the presence of the unsaturated fatty acids oleic and linoleic acids found in the flour, which are known to regulate lipid metabolism by stimulating the mobilization of fats from adipose tissue and their mitochondrial oxidation through the modulation of Peroxisome Proliferator-Activated Receptors (PPARs)^{39–41}. Furthermore, dietary fibers, such as lignin and cellulose, also present in the flour, likely contributed to lower absorption and increased excretion of lipids^{42–44}.

Upon analyzing the concentrations of cholesterol and triglycerides in the feces of the animals, we indeed observed a higher feces excretion of cholesterol in both the P500 and P1000 groups. This effect can be explained by the ability of fibers and phenolic compounds to bind to bile acids in the intestinal lumen, thereby reducing their reabsorption and consequently increasing the feces elimination of cholesterol^{45,46}. Regarding the levels of feces triglycerides, we noted lower concentrations in the feces of the supplemented animals, particularly in the P1000 group. This reduction may indicate greater efficiency in the absorption and/or utilization of triglycerides in the intestinal tract or an influence of the

bioactive compounds on the digestion and absorption of fats. Consistent with our results, a study conducted by Sugiyama et al. (2007) suggests that phenolic compounds can interact with digestive enzymes, such as lipases, thereby reducing the release of triglycerides for feces excretion⁴⁷. These data suggest that *Terminalia catappa* flour plays a balancing role in lipid homeostasis, reducing the cholesterol available for storage while simultaneously optimizing triglyceride metabolism, which impacts the prevention of visceral fat accumulation and supports metabolic health.

Our results also reveal significant data regarding cardiovascular health markers, particularly in the P1000 group, which demonstrated a considerably improved lipid profile. Total cholesterol and triglyceride levels were significantly reduced compared to the control group, supporting recent evidence that indicates a decrease in these lipids is associated with a reduced atherosclerotic risk⁴⁸. Although high-density lipoprotein (HDL) levels showed a decline in the P1000 group, the reduction in coronary and cardiovascular risk indices underscores the importance of a multifactorial approach in assessing cardiovascular risk. This assessment should not only include lipid parameters but also inflammatory and metabolic factors⁴⁹.

Furthermore, it is important to note that when evaluating malondialdehyde levels in coronary tissue, we observed a significant reduction of this compound in the P1000 group compared to both the P500 and control groups. This finding highlights the potential role of antioxidant mechanisms in mitigating oxidative stress associated with atherosclerotic processes⁵⁰. Malondialdehyde, a marker of lipid peroxidation, is closely linked to oxidative damage in cell membranes, and its reduction suggests a relevant protective effect that complements the improvements observed in lipid parameters^{51–53}.

Moreover, the absence of significant changes in the adiposity index suggests that the observed benefits may be attributed to direct metabolic modulation rather than weight-related mechanisms. This finding is consistent with emerging research that highlights the role of bioactive compounds in lipid homeostasis and vascular health^{54–57}.

The analysis of fatty acid composition in the liver of the treated groups revealed an increase in the concentrations of polyunsaturated fatty acids (PUFAs), particularly arachidonic and docosahexaenoic acids, contrasting with a decrease in saturated and monounsaturated fatty acids, such as oleic acid. The elevation of PUFAs in the P1000 group suggests a protective role of these compounds against inflammatory processes and oxidative stress, contributing to lipid homeostasis. Recent studies highlight the relevance of PUFAs in modulating inflammatory processes and cellular protection, recognizing them as anti-inflammatory agents with potential to promote liver health^{58–60}. The combination of reduced saturated fatty acids, such as palmitic and myristic acids, along with the increase in PUFAs, may significantly impact the reduction of lipid accumulation in the liver and the maintenance of metabolic health⁶¹.

These findings reinforce the hypothesis that supplementation with *Terminalia catappa* flour not only optimizes lipid metabolism but also minimizes lipid accumulation, contributing to liver health. The

interrelationship among biochemical markers suggests a synergistic effect that warrants exploration in future research to gain a deeper understanding of the underlying mechanisms involved.

These results demonstrate that *Terminalia catappa* flour not only improves liver health by reducing lipid accumulation but also promotes a less oxidative cellular environment, with potential implications for the prevention of diet-related liver conditions, such as non-alcoholic fatty liver disease (NAFLD). Despite these promising results, future translational research involving elderly individuals is recommended. It is important to note that, according to Nair and Jacob (2016), the doses of flour used in our study with rodents (500 and 1000 mg/kg) are equivalent to approximately 7.14 and 14.29 mg/kg in elderly humans⁶².

6 CONCLUSION

This study highlights the potential benefits of *Terminalia catappa* flour on liver function and lipid metabolism modulation in aged Wistar rats. Although there were no significant changes in body weight or food intake, supplementation reduced retroperitoneal fat deposits and improved lipid profiles, particularly at the higher dose (P1000). The reductions in total cholesterol, triglycerides, and hepatic enzyme activities, along with the increased feces excretion of cholesterol and triglycerides, suggest a beneficial effect on metabolic health and protection against fat accumulation in the liver.

The fatty acid analysis showed an increase in polyunsaturated fatty acids and a decrease in saturated and monounsaturated fatty acids in the livers of the treated groups, corroborating the importance of bioactive compounds in modulating lipid homeostasis. Thus, *Terminalia catappa* flour not only reduces lipid accumulation but also promotes a less oxidative cellular environment, which may help prevent diet-related liver diseases, such as non-alcoholic fatty liver disease.

Declarations

AUTHORS CONTRIBUTION

B. S. Dantas and J. K. B. Soares: Designed the theme of the study, performed the experimental methods, analyzed the data, interpreted the results, and wrote the manuscript;

N. D. de Oliveira, A. C. S. Oliveira, and J. G. Melo: Performed the experimental methods;

J. C. R. de Freitas: Performed the sample injections in chromatography and conducted the reading and interpretation of the results;

V. B. Viera, C. E. V. de Oliveira, and A. C. S. Martins: Conducted the physicochemical analysis of the matrix used in the study;

D. E. Pereira, R. V. R. Dantas, J. D. L. Silva, and L. M. G. Dutra: Wrote the manuscript;

D. E. Pereira and **J. K. B. Soares**: Reviewed the manuscript.

ETHICAL APPROVAL

All of the experimental methods were previously approved by the Ethics Committee for Animal Use - CEUA of UFCG - Certification No. 53-2020, in compliance with the standards established by the National Council for the Control of Animal Experimentation (CONCEA, Brazil), under Law No. 11,794 /2008 (Arouca Law), and with the guidelines for *in vivo* experiments with animals of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0³⁰.

CONSENT FOR PUBLICATION

The authors declare their agreement with all the information included in this manuscript.

COMPETING INTERESTS

The authors have declared that no competing interests exist in the attached article, "Consumption of *Terminalia catappa* flour: modulation of lipid metabolism, reduction of cardiovascular risk, and hepatic protection in aged Wistar rats," by DANTAS BS et al.

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References

1. Dogra S, Dunstan DW, Sugiyama T, Stathi A, Gardiner PA, Owen N. Active Aging and Public Health: Evidence, Implications, and Opportunities. *Annu Rev Public Health*. 2022;43(Volume 43, 2022):439-459. doi:10.1146/ANNUREV-PUBLHEALTH-052620-091107/CITE/REFWORKS
2. Wise J. Global life expectancy to increase by almost five years by 2050, study predicts. *BMJ*. 2024;385:q1126. doi:10.1136/BMJ.Q1126
3. Menassa M, Stronks K, Khatmi F, et al. Concepts and definitions of healthy ageing: a systematic review and synthesis of theoretical models. *eClinicalMedicine*. 2023;56:101821. doi:10.1016/J.ECLINM.2022.101821
4. Tenchov R, Sasso JM, Wang X, Zhou QA. Aging Hallmarks and Progression and Age-Related Diseases: A Landscape View of Research Advancement. *ACS Chem Neurosci*. 2024;15(1):1-30. doi:10.1021/ACSCHEMNEURO.3C00531
5. Murphy T, Dias GP, Thuret S. Effects of diet on brain plasticity in animal and human studies: Mind the gap. *Neural Plast*. 2014;2014. doi:10.1155/2014/563160
6. Adolph TE, Tilg H. Western diets and chronic diseases. *Nat Med*. 2024;30(8):2133-2147. doi:10.1038/S41591-024-03165-6

7. Tursun S, Şahin Y, Alçiğir ME, Çınar M, Karahan İ. Cafeteria diet can cause systemic inflammation and oxidative damage in the various tissues. *Med J Nutrition Metab.* 2024;17(1):81-91. doi:10.3233/MNM-230068
8. Yazıcı E, Şahin E, Alvuroğlu E, Yuluğ E, Menteşe A. Bergamottin reduces liver damage by suppressing inflammation, endoplasmic reticulum and oxidative stress in cafeteria diet-fed mice. *Food Biosci.* 2023;52:102371. doi:10.1016/J.FBIO.2023.102371
9. Minich DM, Brown BI. A review of dietary (Phyto)nutrients for glutathione support. *Nutrients.* 2019;11(9). doi:10.3390/nu11092073
10. Jomova K, Raptova R, Alomar SY, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch Toxicol.* 2023;97(10):2499-2574. doi:10.1007/S00204-023-03562-9
11. Hajam YA, Rani R, Ganie SY, et al. Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells.* 2022;11(3). doi:10.3390/CELLS11030552
12. Behl T, Makkar R, Sehgal A, et al. Current Trends in Neurodegeneration: Cross Talks between Oxidative Stress, Cell Death, and Inflammation. *Int J Mol Sci.* 2021;22(14). doi:10.3390/IJMS22147432
13. Varesi A, Chirumbolo S, Campagnoli LIM, et al. The Role of Antioxidants in the Interplay between Oxidative Stress and Senescence. *Antioxidants 2022, Vol 11, Page 1224.* 2022;11(7):1224. doi:10.3390/ANTIOX11071224
14. Mucha P, Skoczyńska A, Małecka M, Hikisz P, Budzisz E. Overview of the Antioxidant and Anti-Inflammatory Activities of Selected Plant Compounds and Their Metal Ions Complexes. *Mol 2021, Vol 26, Page 4886.* 2021;26(16):4886. doi:10.3390/MOLECULES26164886
15. Zhou G, Xu R, Groth T, et al. The Combination of Bioactive Herbal Compounds with Biomaterials for Regenerative Medicine. *Tissue Eng Part B Rev.* 2024;30(6). doi:10.1089/TEN.TEB.2024.0002
16. Halliwell B. Understanding mechanisms of antioxidant action in health and disease. *Nat Rev Mol Cell Biol.* 2024;25(1):13-33. doi:10.1038/S41580-023-00645-4
17. Ramanan S S, Arunachalam A, Singh R, Verdiya A. Tropical almond (*Terminalia catappa*): A holistic review. *Heliyon.* 2025;11(1):e41115. doi:10.1016/J.HELİYON.2024.E41115
18. de Oliveira ND, Martins ACS, Cirino JA, et al. Exploring the potential of the tropical almond (*Terminalia catappa* L.): Analysis of bioactive compounds, morphology and metabolites. *Ind Crops Prod.* 2024;221:119378. doi:10.1016/J.INDCROP.2024.119378
19. Tabansi D, Dahiru D, Patrick AT, Jahng WJ. Anti-Atherosclerosis and Anti-Hyperlipidemia Functions of *Terminalia catappa* Fruit. *ACS Omega.* 2023;8(39):35571-35579. doi:10.1021/ACSOMEGA.3C00685
20. Iheagwam FN, Batiha GES, Ogunlana OO, Chinedu SN. *Terminalia catappa* Extract Palliates Redox Imbalance and Inflammation in Diabetic Rats by Upregulating Nrf-2 Gene. *Int J Inflamm.* 2021;2021. doi:10.1155/2021/9778486

21. Iheagwam FN, Iheagwam OT, Onuoha MK, Ogunlana OO, Chinedu SN. Terminalia catappa aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats. *Sci Rep.* 2022;12(1). doi:10.1038/S41598-022-15114-9
22. Divya N, Rengarajan RL, Radhakrishnan R, et al. Phytotherapeutic efficacy of the medicinal plant Terminalia catappa L. *Saudi J Biol Sci.* 2019;26(5):985-988. doi:10.1016/J.SJBS.2018.12.010
23. Official Methods of Analysis, 22nd Edition (2023) - AOAC INTERNATIONAL.
<https://www.aoac.org/official-methods-of-analysis/>. Accessed January 13, 2025.
24. FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957;226(1):497-509.
25. Hartman L, Lago RC. Rapid preparation of fatty acid methyl esters from lipids. *Lab Pract.* 1973;22(6):475-476.
26. Liu G, Nie Y, Huang C, et al. Ferulic acid produces neuroprotection against radiation-induced neuroinflammation by affecting NLRP3 inflammasome activation. *Int J Radiat Biol.* 2022. doi:10.1080/09553002.2022.2055798
27. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999;64(4):555-559. doi:10.1016/S0308-8146(98)00102-2
28. Li D, Rui Y xin, Guo S duo, Luan F, Liu R, Zeng N. Ferulic acid: A review of its pharmacology, pharmacokinetics and derivatives. *Life Sci.* 2021;284. doi:10.1016/J.LFS.2021.119921
29. Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chem.* 2007;102(3):938-953. doi:10.1016/j.foodchem.2006.06.033
30. du Sert NP, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* 2020;18(7). doi:10.1371/JOURNAL.PBIO.3000410
31. Behl T, Velpandian T, Kotwani A. Terminalia catappa Fruit Extract Reverses Streptozotocin-Induced Diabetic Retinopathy in Rats. *Endocr Metab Immune Disord Drug Targets.* 2021;21(6):1053-1065. doi:10.2174/1871530320666201029142113
32. Naitik P, Prakash T, Kotresha D, Rao NR. Effect of Terminalia catappa on lipid profile in transplanted fibrosarcoma in rats. *Indian J Pharmacol.* 2012;44(3):390-392. doi:10.4103/0253-7613.96345
33. Nascimento AF, Luvizotto RAM, Leopoldo AS, et al. Long-term high-fat diet-induced obesity decreases the cardiac leptin receptor without apparent lipotoxicity. *Life Sci.* 2011;88(23-24):1031-1038. doi:10.1016/J.LFS.2011.03.015
34. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* 1990;186(C):407-421. doi:10.1016/0076-6879(90)86134-H
35. Ji Y, Ma N, Zhang J, et al. Dietary intake of mixture coarse cereals prevents obesity by altering the gut microbiota in high-fat diet fed mice. *Food Chem Toxicol.* 2021;147.

doi:10.1016/J.FCT.2020.111901

36. Wang B, Yu H, He Y, et al. Effect of soybean insoluble dietary fiber on prevention of obesity in high-fat diet fed mice via regulation of the gut microbiota. *Food Funct.* 2021;12(17):7923-7937. doi:10.1039/D1FO00078K
37. Pontzer H, Yamada Y, Sagayama H, et al. Daily energy expenditure through the human life course. *Science.* 2021;373(6556). doi:10.1126/SCIENCE.ABE5017
38. Walrath T, Dyamenahalli KU, Hulsebus HJ, et al. Age-related changes in intestinal immunity and the microbiome. *J Leukoc Biol.* 2021;109(6):1045-1061. doi:10.1002/JLB.3RI0620-405RR
39. Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clin Sci (Lond).* 2009;116(1):1-16. doi:10.1042/CS20070456
40. Shin S. Regulation of Adipose Tissue Biology by Long-Chain Fatty Acids: Metabolic Effects and Molecular Mechanisms. *J Obes Metab Syndr.* 2022;31(2):147-160. doi:10.7570/JOMES22014
41. Varga T, Czimmerer Z, Nagy L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta.* 2011;1812(8):1007-1022. doi:10.1016/J.BBADIS.2011.02.014
42. Mueller MA, Cleary MP, Kritchevsky D. Influence of dietary fiber on lipid metabolism in meal-fed rats. *J Nutr.* 1983;113(11):2229-2238. doi:10.1093/JN/113.11.2229
43. Jesch ED, Carr TP. Food Ingredients That Inhibit Cholesterol Absorption. *Prev Nutr food Sci.* 2017;22(2):67-80. doi:10.3746/PNF.2017.22.2.67
44. Nie Y, Luo F. Dietary Fiber: An Opportunity for a Global Control of Hyperlipidemia. *Oxid Med Cell Longev.* 2021;2021. doi:10.1155/2021/5542342
45. Liu Y, Huang K, Zhang Y, Cao H, Guan X. Dietary polyphenols maintain homeostasis via regulating bile acid metabolism: a review of possible mechanisms. *Food Funct.* 2023;14(21):9486-9505. doi:10.1039/D3FO02471G
46. Joyce SA, Kamil A, Fleige L, Gahan CGM. The Cholesterol-Lowering Effect of Oats and Oat Beta Glucan: Modes of Action and Potential Role of Bile Acids and the Microbiome. *Front Nutr.* 2019;6. doi:10.3389/FNUT.2019.00171
47. Sugiyama H, Akazome Y, Shoji T, et al. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *J Agric Food Chem.* 2007;55(11):4604-4609. doi:10.1021/JF070569K
48. Europeia De Cardiologia S. Comissão para as Recomendações Práticas. doi:10.1093/eurheartj/ehz455
49. Tian X, Chen S, Zuo Y, et al. Association of lipid, inflammatory, and metabolic biomarkers with age at onset for incident cardiovascular disease. *BMC Med.* 2022;20(1):1-12. doi:10.1186/S12916-022-02592-X/FIGURES/4

50. Młynarska E, Hajdys J, Czarnik W, et al. The Role of Antioxidants in the Therapy of Cardiovascular Diseases-A Literature Review. *Nutrients*. 2024;16(16). doi:10.3390/NU16162587
51. Mas-Bargues C, Escrivá C, Dromant M, Borrás C, Viña J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. *Arch Biochem Biophys*. 2021;709. doi:10.1016/J.ABB.2021.108941
52. Lankin VZ, Tikhaze AK, Melkumyants AM. Malondialdehyde as an Important Key Factor of Molecular Mechanisms of Vascular Wall Damage under Heart Diseases Development. *Int J Mol Sci* 2023, Vol 24, Page 128. 2022;24(1):128. doi:10.3390/IJMS24010128
53. Seenak P, Kumphune S, Malakul W, Chotima R, Nernpermpisooth N. Pineapple consumption reduced cardiac oxidative stress and inflammation in high cholesterol diet-fed rats. *Nutr Metab (Lond)*. 2021;18(1). doi:10.1186/S12986-021-00566-Z
54. Gibert-Ramos A, Martín-González MZ, Crescenti A, Josepa Salvadó M. A Mix of Natural Bioactive Compounds Reduces Fat Accumulation and Modulates Gene Expression in the Adipose Tissue of Obese Rats Fed a Cafeteria Diet. *Nutrients*. 2020;12(11):1-17. doi:10.3390/NU12113251
55. de Resende PE, Kaiser S, Pittol V, et al. Influence of crude extract and bioactive fractions of *Ilex paraguariensis* A. St. Hil. (yerba mate) on the Wistar rat lipid metabolism. *J Funct Foods*. 2015;15:440-451. doi:10.1016/J.JFF.2015.03.040
56. Barragán-Zarate GS, Alexander-Aguilera A, Lagunez-Rivera L, Solano R, Soto-Rodríguez I. Bioactive compounds from *Prosthechea karwinskii* decrease obesity, insulin resistance, pro-inflammatory status, and cardiovascular risk in Wistar rats with metabolic syndrome. *J Ethnopharmacol*. 2021;279. doi:10.1016/J.JEP.2021.114376
57. da Costa KCM, Oliveira L de S, Silva JC, et al. Enhancing Vascular Health and Lowering Blood Pressure in Spontaneously Hypertensive Rats through Syrah Grape (*Vitis vinifera*) Pomace: The Role of Phenolic Compounds. *Nutrients*. 2024;16(14):2312. doi:10.3390/NU16142312/S1
58. El-Mowafy AM, Abdel-Aziz AA, Abdel-Dayem MA. Novel protection by Omega-3-FAs (DHA or EPA) against carbamazepine's liver-injury: differential suppression of oxidative-stress and inflammatory markers, and the influence on carbamazepine-clearance. *Clin Nutr Open Sci*. 2022;42:14-26. doi:10.1016/J.NUTOS.2022.01.006
59. Saleh DO, Mahmoud SS, Hassan A, Sanad EF. Doxorubicin-induced hepatic toxicity in rats: Mechanistic protective role of Omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK-3 β axis modulation. *Saudi J Biol Sci*. 2022;29(7):103308. doi:10.1016/J.SJBS.2022.103308
60. Zhang Y, Zhao Q, Zhao R, Lu Y, Jiang S, Tang Y. Efficacy of DHA-enriched phosphatidylserine and its underlying mechanism in alleviating polystyrene nanoplastics-induced hepatotoxicity in mice. *Int Immunopharmacol*. 2024;142:113154. doi:10.1016/J.INTIMP.2024.113154
61. Šmíd V, Dvořák K, Stehnová K, et al. The Ameliorating Effects of n-3 Polyunsaturated Fatty Acids on Liver Steatosis Induced by a High-Fat Methionine Choline-Deficient Diet in Mice. *Int J Mol Sci*. 2023;24(24). doi:10.3390/IJMS242417226

Figures

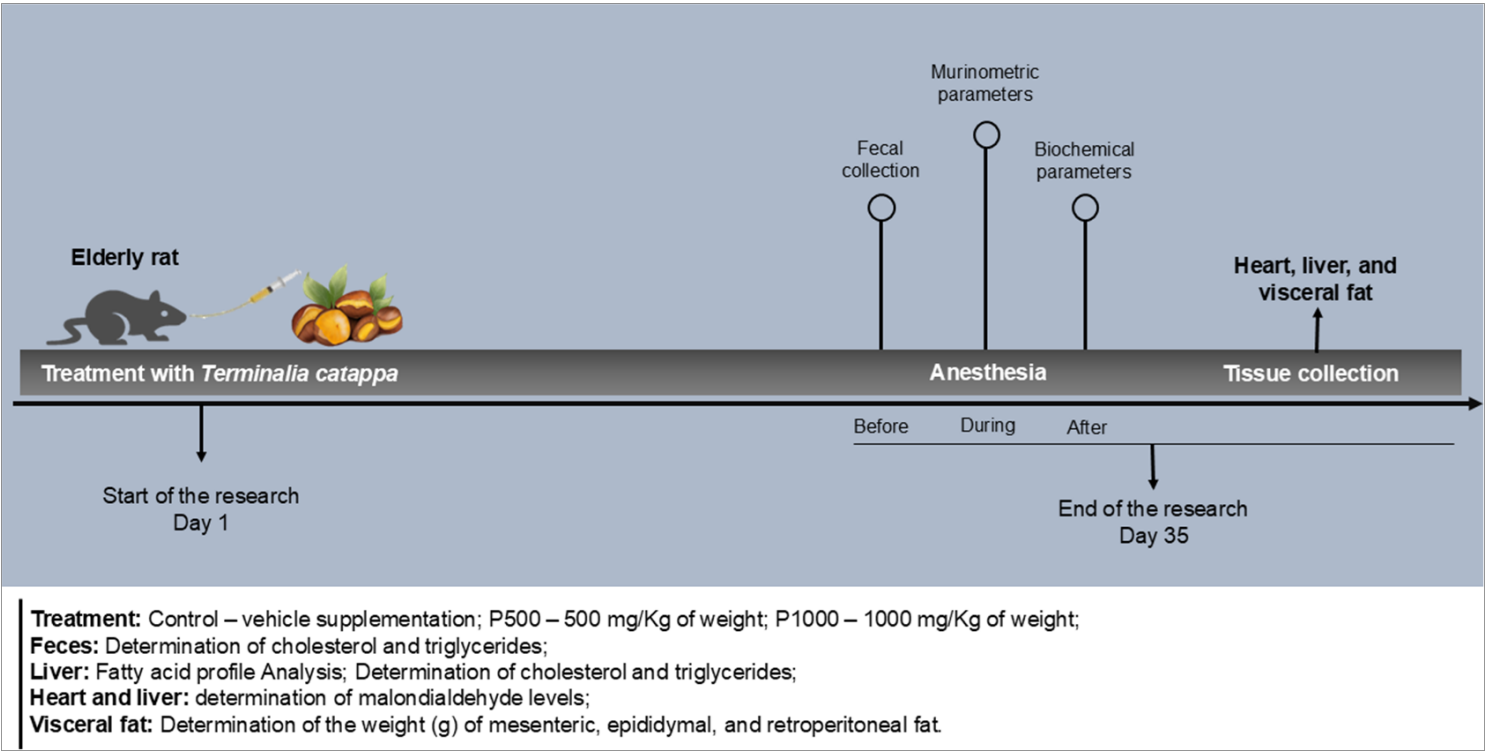


Figure 1

Experimental Protocol. Timeline of the experimental procedure (days) involving Wistar rats treated during the aging phase with distilled water (CONTROL), 500 mg/kg of *Terminalia catappa* flour (P500), and 1000 mg/kg of *Terminalia catappa* flour (P1000).

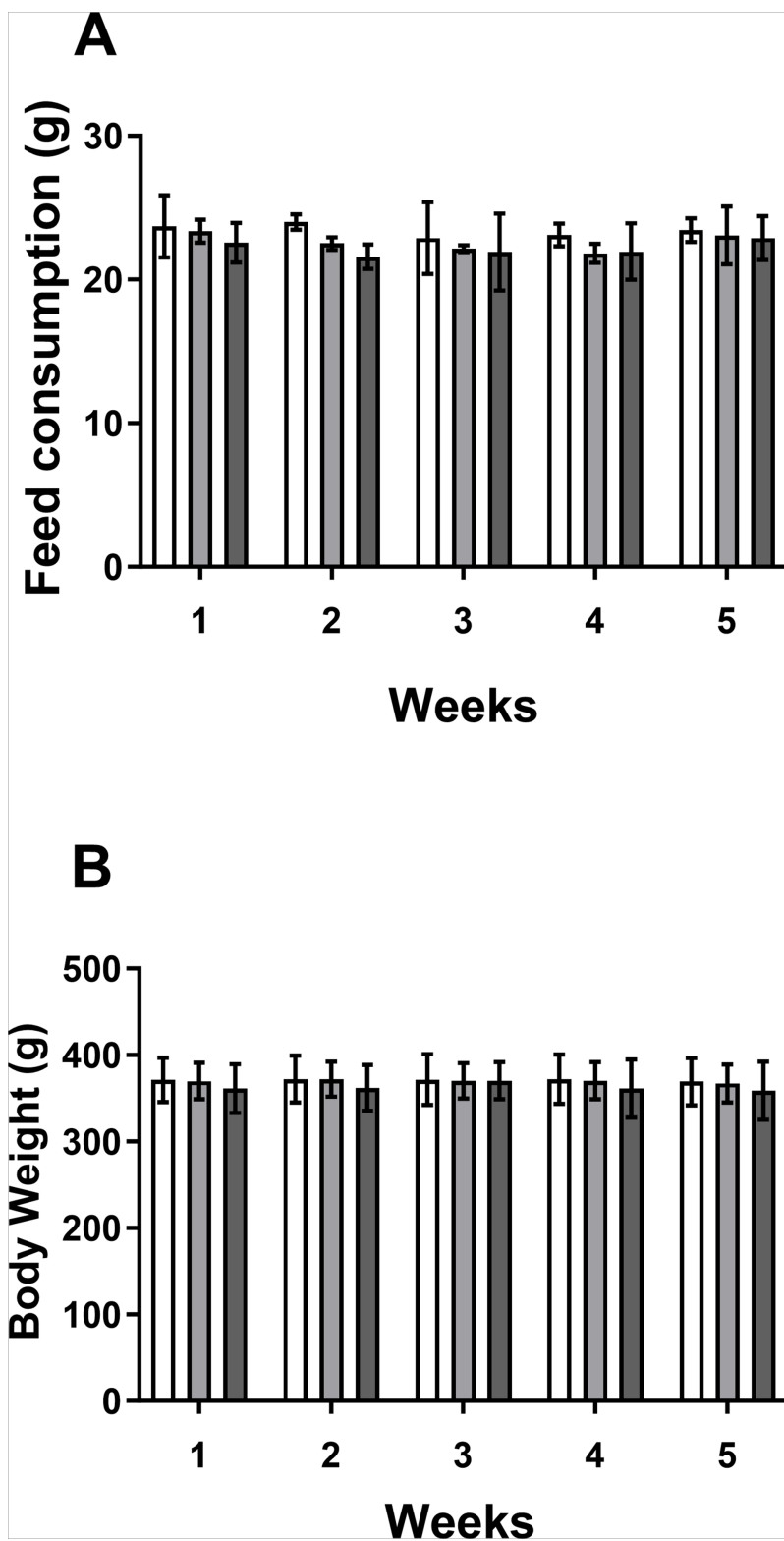


Figure 2

Food consumption and body weight of animals treated with 500 and 1000 mg/kg of *Terminalia catappa* flour during the aging phase.

Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

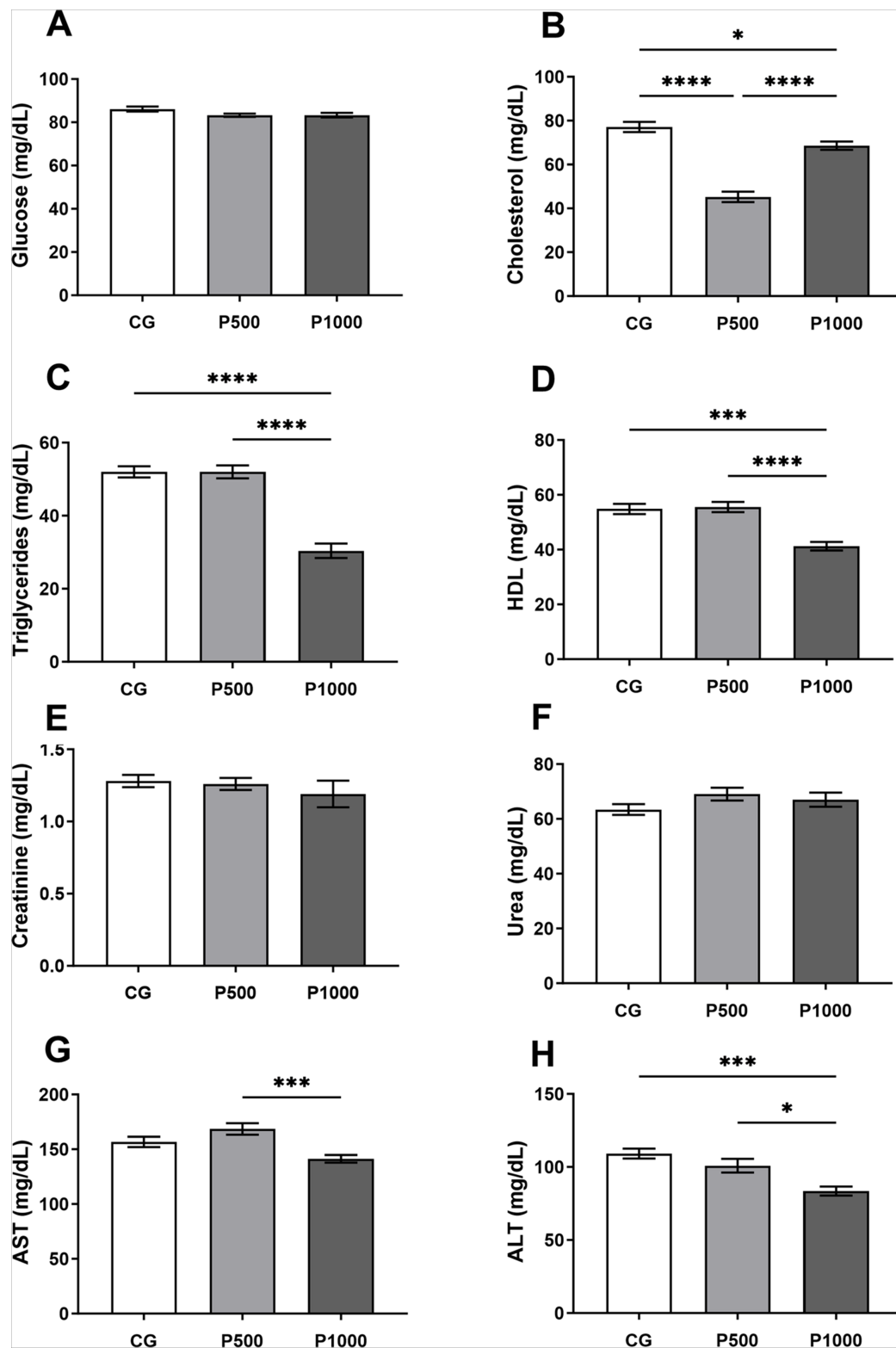


Figure 3

Biochemical parameters.

Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

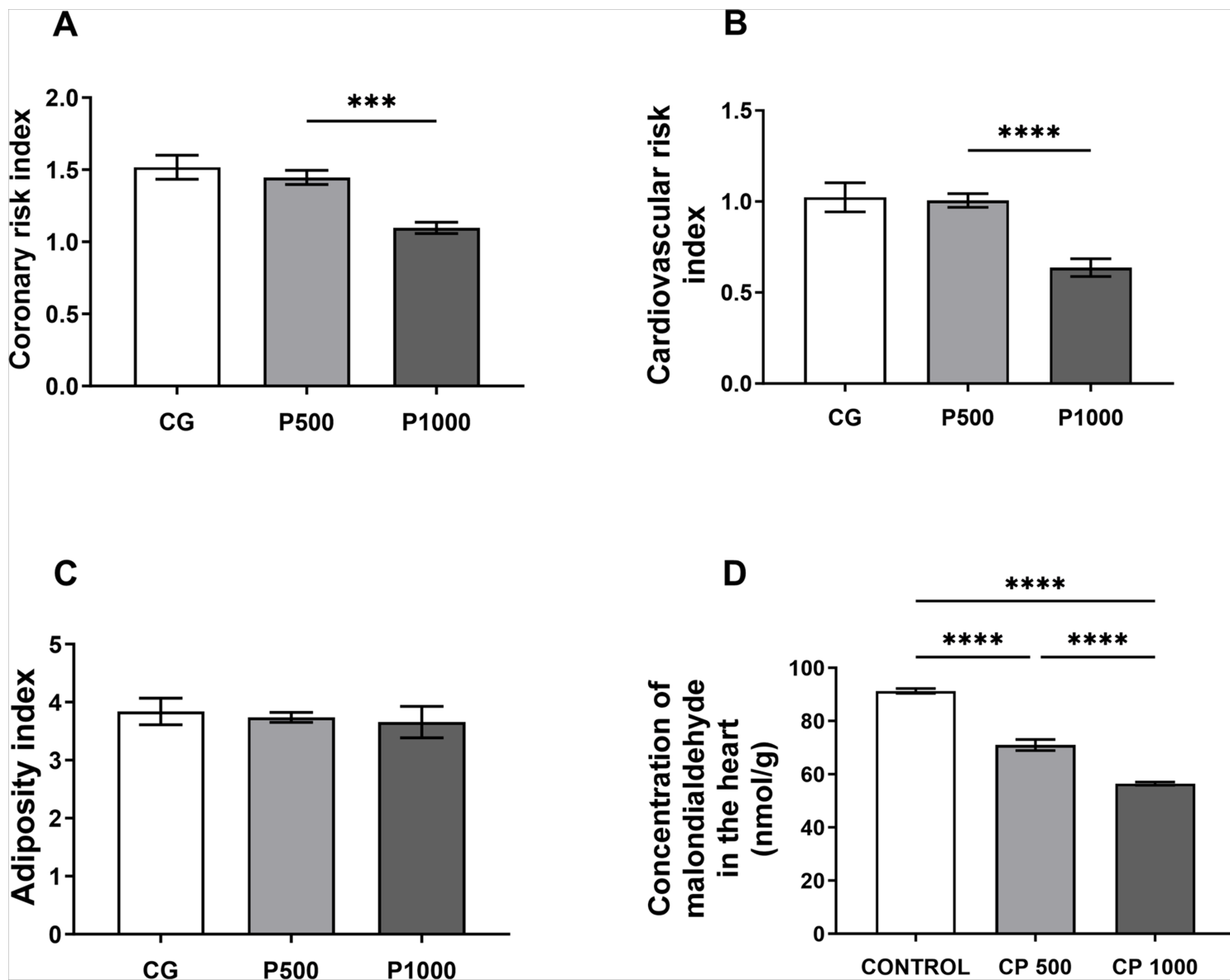


Figure 4

Adiposity index (ADI), Coronary risk index (CRI), Cardiovascular risk index (CVRI), and Malondialdehyde (MDA) levels in coronary tissue.

Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test (p < 0.05). MDA levels are expressed in nmol/dl.

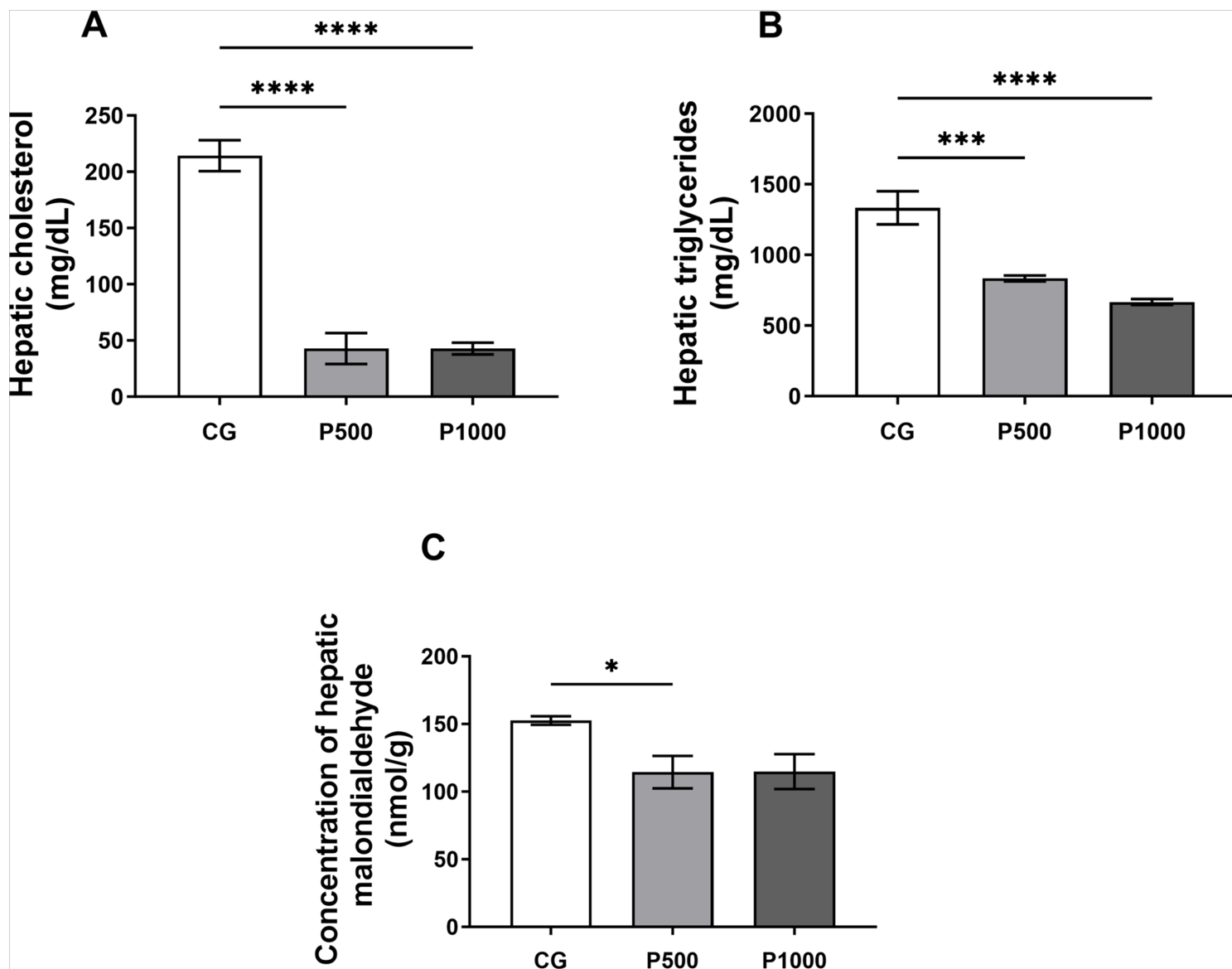


Figure 5

Hepatic cholesterol, triglycerides, and malondialdehyde (MDA).

Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test ($p < 0.05$). MDA levels are expressed in nmol/dl.

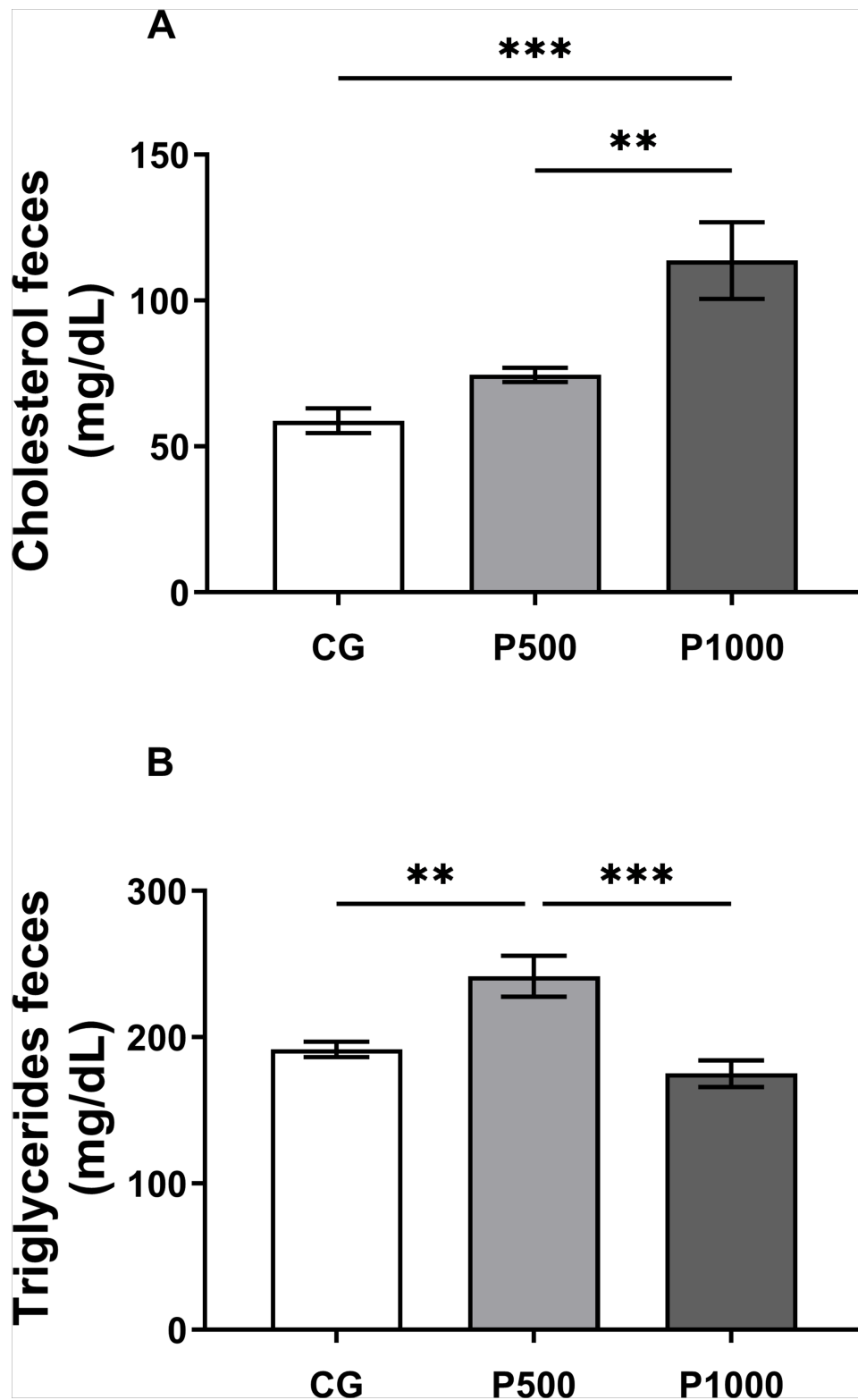


Figure 6

Feces cholesterol and triglycerides.

Data are expressed as mean $\pm$ standard error. CONTROL (n = 11); P500 (n = 11); P1000 (n = 11). Data were analyzed using One-Way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Supplementary Files

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