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Effects of the multicomponent nutraceutical (Reverse®) in Wistar rats with atherogenic obesity induced by saturated fat from *Cocos nucifera* L.: A preclinical cardiometabolic evaluation

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Abstract

Obesity is a chronic, multifactorial, and relapsing disease associated with inflammation, oxidative stress, dyslipidemia, insulin resistance, and increased cardiovascular risk, prompting investigation of safe plant-based nutraceutical formulations containing phenolic compounds, flavonoids, and isoprenoids as complementary strategies to modulate metabolic alterations associated with excess adiposity. This study evaluates the phytochemical profile of the multicomponent nutraceutical Reverse® and its therapeutic effects on male Wistar rats subjected to a short-term dietary challenge model with saturated fat from *Cocos nucifera* L., which induces acute metabolic stress and dyslipidemia. The formulation was characterized by spectrophotometric determination of total phenols and total flavonoid contents and by gas chromatography–mass spectrometry determination of δ-tocotrienol and geranylgeraniol. The animals were divided into six experimental groups (n = 5/group): negative control (CNE), metformin (MET), Reverse® with *Bixa orellana* at 300 mg/kg (CBA) and 600 mg/kg (CBB), and Reverse® without *B. orellana* at 300 mg/kg (SBA) and 600 mg/kg (SBB). We evaluated weight gain, abdominal fat, organ weights, biochemical parameters (lipid profile, alanine aminotransferase [ALT], aspartate aminotransferase), histopathologic findings, and aortic scanning electron microscopy findings. Reverse® presented 22.08–28.72 mg GAE/g of total phenols, 16.53–18.80 mg EQ/g of total flavonoids, 32.0 mg/g of δ-tocotrienol, and 104.0 mg/g of geranylgeraniol. In the treated groups, weight gain, abdominal fat, total and LDL cholesterol levels, and ALT were reduced; triglyceride levels were modulated; and hepatic, cardiac, and vascular histomorphology was preserved compared with CNE. The findings demonstrate that Reverse® has a phytochemical composition consistent with cardiometabolic protective activity and exhibits significant potential as an adjuvant nutraceutical to modulate diet-induced acute metabolic dysfunctions by improving lipid profiles, reducing adiposity, and preserving organ morphology.

However, due to limited sample size, further dose–response curve, bioavailability, safety, and clinical validation studies are needed before making therapeutic inferences in humans.

Keywords: Obesity; Plant-based nutraceuticals; Phenolic compounds; Flavonoids; Tocotrienols; Geranylgeraniol; Wistar rats

1. Introduction

Obesity is recognized as a multifactorial, progressive, and relapsing chronic disease, characterized by the excessive accumulation of adipose tissue and systemic metabolic alterations that increase the risk of cardiovascular diseases, type 2 diabetes mellitus, metabolic steatosis, liver disease, and other chronic non-communicable diseases.[1,2] In 2022, approximately 2.5 billion adults were overweight, including more than 890 million with obesity, highlighting the epidemiological magnitude of the problem and the need for science-based preventive, therapeutic, and adjuvant strategies.[2] Recent projections also indicate substantial growth in the global prevalence of obesity by 2030, underscoring the disease's health, economic, and social impacts.[3] Beyond a positive energy balance, obesity pathophysiology involves adipocyte dysfunction, low-grade systemic inflammation, oxidative stress, insulin resistance, altered gut microbiota, and dysregulated lipid metabolism.[4,5]

Chronic inflammation associated with obesity results from adipose tissue expansion, immune cell infiltration, and the release of cytokines and adipokines that interfere with insulin signaling and hepatic and vascular metabolism.[4,5] This environment favors dyslipidemia, increased atherogenic lipoproteins, endothelial damage, and a higher cardiometabolic risk. Although dietary interventions, physical activity, pharmacological therapies, and, in selected cases, bariatric surgery are cornerstones of clinical management, there is growing interest in bioactive compounds of plant origin that can act as adjuvants, not therapeutic substitutes, in modulating oxidative, inflammatory, lipid, and glycometabolic pathways.[6-9]

Multicomponent nutraceuticals are of scientific interest because they combine distinct phytochemical classes, such as phenolic acids, flavonoids, carotenoids, methylxanthines, tocotrienols, and isoprenoids, which can act on complementary targets. Recent reviews highlight that phytochemicals can modulate adipogenesis, lipid metabolism, energy expenditure, inflammation, oxidative stress, glycemic homeostasis, and gut microbiota, but also emphasize limitations related to bioavailability, dose standardization, matrix quality, and the need for clinical validation.[8-11] Thus, biological plausibility does not equate to direct demonstration of clinical efficacy; therefore, chemical characterization, marker standardization, preclinical evaluation, and critical analysis of translational limitations are necessary steps to support the scientific development of these products.

Reverse® is a plant-based nutraceutical formulation composed of *Astrocaryum aculeatum* oil, *Bixa orellana* L. oil, *Ilex paraguariensis* extract, *Passiflora incarnata* L. extract, and *Myrciaria dubia* (Kunth) extract, as specified in the evaluated product. Therefore, it is a plant-derived product used as an adjuvant to modulate obesity and associated cardiometabolic alterations. These ingredients have diverse chemical profiles, and the literature describes them as sources of compounds with antioxidant, anti-inflammatory, and lipid-metabolism-modulating potential. *I. paraguariensis* contains phenolic acids, flavonoids, methylxanthines, and saponins, although its gastrointestinal stability and bioavailability depend on processing, mode of consumption, and interactions with the food matrix.[12] *Passiflora* species contain phenolic compounds and flavonoids of nutritional and pharmacological interest,[13] while *M. dubia* is recognized for its content of vitamin C, polyphenols, carotenoids, and biofunctional compounds associated with antioxidant, antihyperglycemic, and anti-obesity activities in *in vitro* and *in vivo* studies.[14]

Recent evaluations of multicomponent formulations have shown significant improvements in lipid metabolism and gut microbiota diversity, and reductions in systemic inflammation in obese animal models.[8,15,16] These findings emphasize the need to evaluate complex botanical matrices, as they often exhibit synergistic effects beyond those of isolated compounds. Notably, a research gap persists regarding the preclinical evaluation of nutraceuticals combining hydrophilic polyphenols with lipophilic isoprenoids (tocotrienols and geranylgeraniol) in a single formulation, which the present study addresses.

Among the lipophilic components, tocotrienols and geranylgeraniol stand out and are frequently associated with derivatives of *B. orellana*. Tocotrienols are isoforms of vitamin E that have been investigated for potential

antioxidant, anti-inflammatory, hypocholesterolemic, and adipogenesis-modulating effects, although clinical evidence remains heterogeneous and depends on dose, isoform, formulation, and study population.[17-19] Geranylgeraniol, in turn, is a C20 isoprenoid implicated in the mevalonate pathway, protein prenylation, and isoprenoid homeostasis, with preclinical evidence suggesting metabolic and anti-inflammatory effects in obesity models.[20,21]

Despite the increasing availability of pharmacological treatments for obesity, there remains a significant clinical gap in finding safe, long-term adjuvant therapies that can simultaneously target dyslipidemia, oxidative stress, and low-grade inflammation without severe adverse effects. Plant-based multicomponent nutraceuticals offer a promising alternative, yet many lack rigorous preclinical validation regarding their phytochemical standardization and physiological impacts in diet-induced obesity models. In this context, the present study aims to characterize the phytochemical markers of Reverse® and evaluate their effects on weight, biochemical, histopathological, and vascular parameters in Wistar rats subjected to a short-term high-fat diet with saturated fat derived from *Cocos nucifera* L. The central hypothesis was that the formulation, by combining hydrophilic and lipophilic plant-derived compounds with bioactive potential, could modulate cardiometabolic alterations associated with the acute diet-induced metabolic stress model, thereby supporting its investigation as an adjuvant nutraceutical. The main contributions of this work include: the comprehensive chemical profiling of Reverse®, highlighting the quantification of δ-tocotrienol and geranylgeraniol using gas chromatography–mass spectrometry (GC–MS); the demonstration of its *in vivo* efficacy in mitigating weight gain, visceral adiposity, dyslipidemia, and vascular endothelial damage in a robust saturated fat-induced acute metabolic dysfunction model; and the comparative evaluation of formulations with and without *B. orellana* to assess the contribution of tocotrienol-rich fractions.

2. Materials and methods

2.1. Material

Reverse® samples were provided by Ages Bioactive Compounds Co. (São Paulo, Brazil). The evaluated product was a multicomponent plant-based nutraceutical granule developed for encapsulation in hard gelatin capsules. According to the certificate of analysis for batch 002/2025 (expiry date 03/2028), the formulation contains *A. aculeatum* oil, *B. orellana* L. oil, *I. paraguariensis* extract, *P. incarnata* L. extract, and *M. dubia* (Kunth) extract. The saturated fat from *C. nucifera* L. used for model induction was obtained from the Drug Research Laboratory of the Federal University of Amapá, Macapá, Amapá, Brazil.

2.2. Quantification of total phenols

Total phenol quantification was performed using a spectrophotometric method (UV 1280, Shimadzu, Japan) based on the Folin–Ciocalteu reagent, using gallic acid as a reference standard. The analytical curve was constructed using gallic acid standard solutions at concentrations of 0.05, 0.10, 0.12, 0.14, 0.16, 0.18, and 0.20 mg/mL, with absorbance readings at 765 nm, adjusted using ordinary least-squares linear regression. Reverse® samples were analyzed at concentrations of 10.0, 5.0, and 2.5 mg/mL, with three absorbance readings per concentration. The results were expressed as gallic acid equivalents per gram of sample (mg GAE/g). This method estimates the overall reducing capacity of total phenols and accounts for potential interference from other reducing agents in complex plant matrices.[22,23]

2.3. Quantification of total flavonoids

Total flavonoid quantification was performed using a colorimetric spectrophotometric method based on complexation with aluminum chloride ($AlCl_3$), using quercetin as a reference standard. This method relies on the principle that $AlCl_3$ forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Additionally, aluminum chloride forms acid-labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids, resulting in a bathochromic shift and hyperchromic effect that can be measured spectrophotometrically. The formation of these colored Al^{3+}–flavonoid complexes allows selective estimation of flavonoid content in the presence of other phenolic compounds.[24,25] The analytical curve was constructed with quercetin solutions at concentrations of 0.05, 0.10, 0.20, 0.30, and 0.40 mg/mL. Samples were prepared at concentrations of 10.0, 5.0, and 2.5 mg/mL and analyzed in triplicate. After reaction with $AlCl_3$, absorbance was measured at 415 nm using a UV–Vis spectrophotometer. The results were expressed as quercetin equivalents per gram of sample (mg EQ/g).

2.4. Determination of δ-tocotrienol and geranylgeraniol

The determination of δ-tocotrienol and geranylgeraniol was performed using GC–MS (GC-2010 system, Shimadzu, Japan) equipped with an AOC-5000 autosampler and an MS2010 Plus detector in electron-impact mode at 70 eV. Analyses were conducted on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, United States of America [USA]), with helium as the carrier gas, an operating pressure of 65 kPa, an injection volume of 5.0

μL, and a flow split ratio of 5:0. The injector and detector temperatures were maintained at 210 °C and 250 °C, respectively. The oven temperature program consisted of 130 °C for 3 min, followed by heating at 5 °C/min to 300 °C, for a total chromatographic run time of 46 min.

Reverse® samples were prepared in hexane at 5 mg/mL. 1 mL aliquots were derivatized with 50 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and analyzed. Quantification was performed using analytical curves prepared in hexane (10 mg/mL) containing δ-tocotrienol and geranylgeraniol at 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. All standards were derivatized with BSTFA before injection, and quantification was performed using ordinary least-squares linear regression of concentration versus detector response. The free-intercept specification was retained after comparison with an origin-constrained sensitivity model because the series did not include a zero-concentration standard and the detector baseline was not established as exactly zero, according to a previously described methodology.[26]

2.5. Animals and ethical considerations

A total of 30 adult male albino Wistar rats, with an initial body weight between 250 and 400 g, obtained from the Animal Facility of the Multidisciplinary Center for Biological Research of the State University of Campinas (CEMIB-Unicamp), were used. The animals were acclimatized in polyethylene cages, under a controlled temperature of 22 ± 3 °C, a 12-hour light/dark cycle, and free access to water and standardized rodent feed. The Research Ethics Committee/Ethics Committee on the Use of Animals of the Federal University of Amapá approved the study under protocol 0012/2024, in accordance with ethical principles for animal experimentation.

2.6. Experimental design and induction of obesity

The animals were divided into six experimental groups (n = 5 per group), treated by gavage (2 mL per os), and monitored for 40 days. The negative control group (CNE) received distilled water, the positive control group (MET) received metformin hydrochloride 300 mg/kg/day, and CBA group received Reverse® with *B. orellana* and other bioactive compounds at a dose of 300 mg/kg, the CBB group received Reverse® with *B. orellana* and other bioactive compounds at a dose of 600 mg/kg, the SBA group received Reverse® without *B. orellana* and other bioactive compounds at a dose of 300 mg/kg, and the SBB group received Reverse® without *B. orellana* and other bioactive compounds at a dose of 600 mg/kg.

Acute metabolic stress was induced by daily gavage administration of 2 mL of saturated fat from *C. nucifera* L., from the 21st to the 40th experimental day. The use of saturated fat from *C. nucifera* L. (virgin coconut oil) as an obesogenic agent is a well-established and validated method to induce metabolic dysfunctions in Wistar rats, including visceral adiposity, hepatic lipid accumulation, dyslipidemia, and low-grade systemic inflammation, serving as a short-term dietary challenge model rather than a chronic obesity model.[27,28] This hypercaloric overload rapidly promotes an atherogenic lipid profile characterized by elevated total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides. The 20-day induction period was selected based on previous studies demonstrating that this timeframe is sufficient to establish significant metabolic alterations in this strain.[27] Body weight was measured on day 0 and every seven days until day 40. Weight variation and the area under the curve (AUC) were used to assess weight-gain progression.

2.7. Biochemical evaluation

In the final experimental phase, 1.5 mL of blood was collected from the retro-orbital plexus for analysis of total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, triglycerides, aspartate aminotransferase (AST/SGOT), and alanine aminotransferase (ALT/SGPT). The samples were centrifuged for 10 min at 5,000 rpm, and analyses were conducted using standardized colorimetric/enzymatic methods according to the instructions for the laboratory kits used (AST: 1025; ALT: 1928; Gold Analisa Diagnóstica Co., Brazil).

2.8. Euthanasia, organ harvesting, histopathology, and scanning electron microscopy

After 41 days, the animals were fasted for 12 h and anesthetized with an overdose of sodium thiopental 150 mg/kg via intraperitoneal injection. Heart, liver, kidneys, and abdominal fat were collected. The organs were weighed on an electronic analytical balance (ATY224 R, SHIMADZU, Japan), and fragments of heart and liver were fixed in 10% buffered formalin, dehydrated in an alcohol series, cleared in xylene, embedded in paraffin, sectioned using a rotary microtome (HIS-2268, MRC Laboratory Instruments, Israel) to a thickness of 4–5 μm, and stained with hematoxylin and eosin (H&E; 1039, Gold Analisa Diagnóstica Co., Brazil). The thoracoabdominal aorta was collected for ultrastructural evaluation using scanning electron microscopy (SEM), focusing on endothelial surface integrity and morphological alterations indicative of vascular injury. For SEM preparation, the aortic segments were longitudinally opened and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h, post-fixed in 1% osmium tetroxide for 2 h, and dehydrated through a graded ethanol series (30%–100%).

The specimens were then critical-point dried using CO_2, mounted on aluminum stubs, and sputter-coated with a thin gold layer before examination under the scanning electron microscope (FlexSEM 1000 II VP-SEM, Hitachi, Japan). The integrity of the endothelial surface is a primary indicator of vascular health; morphological alterations such as endothelial cell retraction, focal denudation, exposure of the subendothelial matrix, crater-like lesions, and adhesion of platelets or leukocytes are direct markers of early atherosclerotic vascular injury and endothelial dysfunction.[29-31]

2.9. Statistical analysis

Quantitative results were expressed as mean ± standard deviation. Before analysis, we assessed data normality using the Shapiro–Wilk test and verified homogeneity of variances using Levene's test, confirming that parametric assumptions were met for all primary endpoints. To evaluate statistical differences among the six independent groups, we conducted a one-way analysis of variance (ANOVA) with Tukey's post hoc test to control the family-wise error rate and identify which pairwise comparisons were statistically significant (e.g., CNE vs. treated groups). The sample size (n = 5 per group) was determined based on prior power analyses for similar preclinical metabolic studies, considering an effect size (Cohen's d) of 1.5–2.0, $\alpha = 0.05$, and power $(1-\beta) \geq 0.80$, which is consistent with recommendations for exploratory pharmacological studies in rodents.[32,33] Effect sizes were also calculated post hoc to confirm the magnitude of the observed differences. For the analytical calibration data, absorbance or chromatographic peak area was modeled as a function of standard concentration using ordinary least-squares linear regression with a freely estimated intercept. We also examined an origin-constrained model ($y = \beta_1 x$) as a sensitivity analysis. Because the fitted standard series did not include a zero-concentration standard and the experiments did not establish that the blank response was exactly zero, the intercept was not constrained a priori. Model adequacy was assessed from the fitted equation, residual error, and residual pattern across the working range; R^2 was used as a descriptive measure, not the sole criterion, for model selection. We used the selected free-intercept equations only within their corresponding calibration ranges. P-values < 0.05 were considered statistically significant. We performed analyses using GraphPad Prism version 5.05 (GraphPad Software, USA).

3. Results

3.1. Phytochemical characterization: total phenols and total flavonoids

The analytical curves for quantifying total phenols and total flavonoids showed adequate linearity across the evaluated concentration ranges. Ordinary least-squares models with freely estimated intercepts were used as the primary fits, while origin-constrained models were examined as sensitivity analyses. The free-intercept specification was retained because the standard series began above zero concentration and the experiments did not demonstrate that the response at zero concentration was exactly zero. Thus, the negative intercepts observed for the gallic acid and quercetin curves represent extrapolated background terms of the calibration equations and do not indicate a negative analytical response within the working ranges. Model adequacy was evaluated using the equation, residual error, and residual pattern across the range, with R^2 as a descriptive statistic rather than the sole basis for selection. The gallic acid curve was fitted with the equation $y = 2.885836x - 0.016649$, with $R^2 = 0.999083$. The quercetin curve was fitted with the equation $y = 1.920082x - 0.019151$, with $R^2 = 0.996244$. These results indicate satisfactory linear fits for interpolating the samples under the experimental conditions used (Figures 1 and 2).

The total phenolic content in the Reverse® solutions at 10.0, 5.0, and 2.5 mg/mL was 0.2208, 0.1190, and 0.0718 mg/mL, respectively. After normalization by sample mass, the values corresponded to 22.08, 23.80, and 28.72 mg GAE/g, indicating a measurable presence of reducing substances expressed as gallic acid equivalents (Tables 1 and 2).

The total flavonoid content in the Reverse® solutions at 10.0, 5.0, and 2.5 mg/mL was 0.188011, 0.086627, and 0.041317 mg/mL, respectively. After normalization, the values were 18.80, 17.33, and 16.53 mg EQ/g, indicating a quantifiable presence of equivalent flavonoid compounds in the formulation (Table 3).

3.2. Determination of δ-tocotrienol and geranylgeraniol

The analytical curves for δ-tocotrienol and geranylgeraniol showed adequate linearity over the evaluated range. The curves were fitted using ordinary least-squares linear regression with freely estimated intercepts, and origin-constrained models were examined as sensitivity analyses but were not imposed because the standard series did not include zero concentration and the detector baseline was not established as exactly zero. For δ-tocotrienol, the fitted line was $y = 1 \times 10^8 x + 3 \times 10^6$, with $R^2 = 0.998$. For geranylgeraniol, the fitted line was $y = 2 \times 10^8 x + 6 \times 10^6$, with $R^2 = 0.999$. The small positive intercepts are consistent with a nonzero baseline/instrument response and were not extrapolated beyond the validated range. Chromatographic analysis of the Reverse® sample in hexane at 5 mg/mL

Table 1. Parameters of the analytical curves for total phenols and total flavonoids

Assay	Standard	Concentration range (mg/mL)	Equation of a line	R^2	Regression-model assessment
Total phenols	Gallic acid	0.05–0.20	y = 2.885836x − 0.016649	0.999083	Ordinary least squares; free intercept retained after origin-constrained sensitivity check; adequate linearity
Total flavonoids	Quercetin	0.05–0.40	y = 1.920082x − 0.019151	0.996244	Ordinary least squares; free intercept retained after origin-constrained sensitivity check; adequate linearity

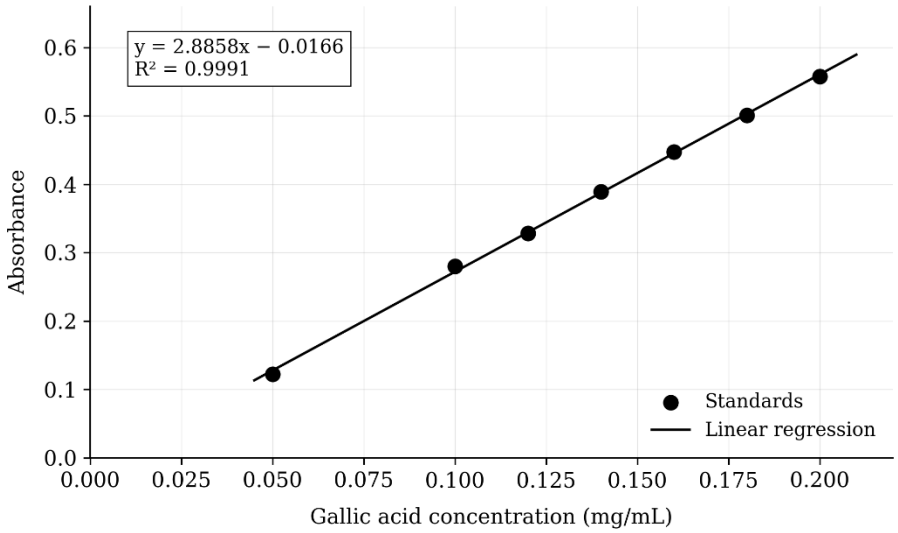


Figure 1. Calibration curve for the determination of total phenols using the Folin–Ciocalteu method, with gallic acid as the reference standard. The points were fitted using ordinary least-squares linear regression with a freely estimated intercept. The curve showed a satisfactory linear fit in the range of 0.05–0.20 mg/mL, with R^2 = 0.999083.

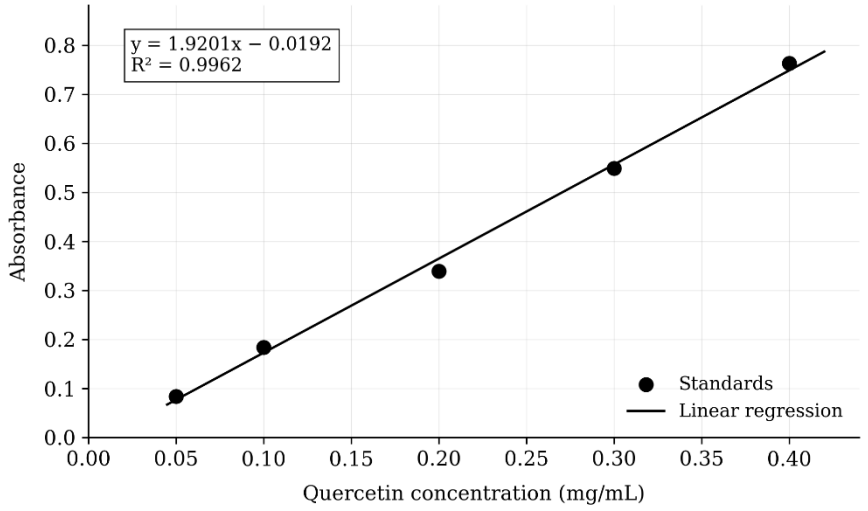


Figure 2. Calibration curve for the determination of total flavonoids using the $AlCl_3$ complexation method, with quercetin as the reference standard. The points were fitted using ordinary least-squares linear regression with a freely estimated intercept. The curve showed an adequate linear fit in the range of 0.05–0.40 mg/mL, with R^2 = 0.996244.

showed peaks corresponding to geranylgeraniol and δ-tocotrienol, with retention times of 22.5 min and 33.0 min, respectively (Figure 3).

Table 2. Quantification of total phenols in Reverse®

Sample concentration (mg/mL)	Content in the solution (mg/mL)	Normalized content (mg EAG/g)
10.0	0.2208	22.08
5.0	0.1190	23.80
2.5	0.0718	28.72

Table 3. Quantification of total flavonoids in Reverse®

Sample concentration (mg/mL)	Content in the solution (mg/mL)	Normalized content (mg EQ/g)
10.0	0.188011	18.80
5.0	0.086627	17.33
2.5	0.041317	16.53

The concentrations determined in the analyzed solution were 0.16 mg/mL for δ-tocotrienol and 0.52 mg/mL for geranylgeraniol. Based on the extract's initial concentration, the calculated contents were 32.0 mg/g of δ-tocotrienol (3.2% w/w) and 104.0 mg/g of geranylgeraniol (10.4% w/w). Mass spectrometry corroborated the marker identities, with fragmentation profiles compatible with the analytes evaluated (Figures 4–6).

3.3. Weight evolution, abdominal fat, and organ weight

Weight evolution showed that groups treated with Reverse® gained less weight than the CNE group. Analysis of weight variation and AUC showed significant reductions in the CBA, CBB, SBA, and SBB groups compared with the CNE group ($p < 0.05$), with responses similar to or greater than those observed in the MET group.

Abdominal fat analysis corroborated the weight findings. The Reverse®-treated groups showed a significant reduction in visceral adiposity compared with the CNE group ($p < 0.001$). Among the treated groups, the SBA group had the lowest mean abdominal fat, followed by the SBB, CBB, and CBA groups. The MET group also showed reduced adiposity compared with the CNE group. This is consistent with metformin's known mechanisms of action, including the activation of AMP-activated protein kinase (AMPK), which enhances fatty acid oxidation and suppresses lipogenesis in adipose tissue.

Organ weights showed reduced liver weight in the treated groups compared with CNE. The SBB group had the lowest liver weight among the evaluated groups, while kidney weights remained relatively homogeneous, with no differences indicating macroscopic renal alteration by organ weight. For the heart, the CBB, SBA, and SBB groups showed values lower than CNE (Table 4; Figures 7 and 8).

3.4. Biochemical parameters

The AST levels remained similar across the evaluated groups. The CNE group showed a mean of 69.4 ± 7.82 U/L, while the CBA, CBB, SBA, SBB, and MET groups showed means of 66.0 ± 5.68, 68.83 ± 4.70, 77.8 ± 6.76, 81.83 ± 21.59, and 71.0 ± 22.62 U/L, respectively. For ALT, the CNE group had a mean of 65.4 ± 5.22 U/L, whereas all groups treated with Reverse® had lower values, ranging from 29.83 ± 2.99 to 32.4 ± 4.21 U/L. The MET group showed an intermediate value of 45.5 ± 10.5 U/L. These values were compared with established laboratory benchmarks for healthy male Wistar rats, where normal ALT ranges from 10 to 40 U/L, and AST ranges from 50 to 150 U/L.[34] The CNE group showed elevated ALT (65.4 ± 5.22 U/L), indicating mild hepatocellular injury from the

Table 4. Organ weights of animals across groups

Organ	CBA	CBB	SBA	SBB	CNE	MET
Heart (g)	1.17 ± 0.98	1.17 ± 0.90*	1.07 ± 0.20*	1.08 ± 0.61*	1.44 ± 0.20	1.40 ± 0.17
Liver (g)	13.77 ± 0.81*	14.06 ± 0.28*	12.14 ± 1.62*	11.12 ± 0.73*	16.47 ± 0.72	14.00 ± 0.70*
Kidneys (g)	1.36 ± 0.58	1.44 ± 0.21	1.30 ± 0.13	1.18 ± 0.10	1.30 ± 0.19	1.37 ± 0.21

Notes: Data are expressed as mean ± standard deviation. *$p < 0.05$ versus CNE.

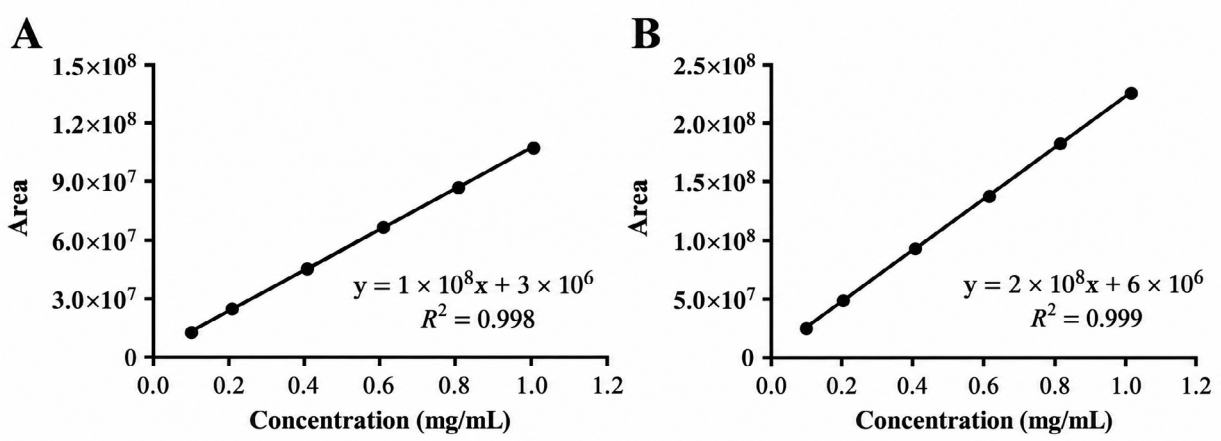


Figure 3. Calibration curves for (A) δ-tocotrienol and (B) geranylgeraniol obtained using gas chromatography–mass spectrometry. The points were fitted using ordinary least-squares linear regression with freely estimated intercepts.

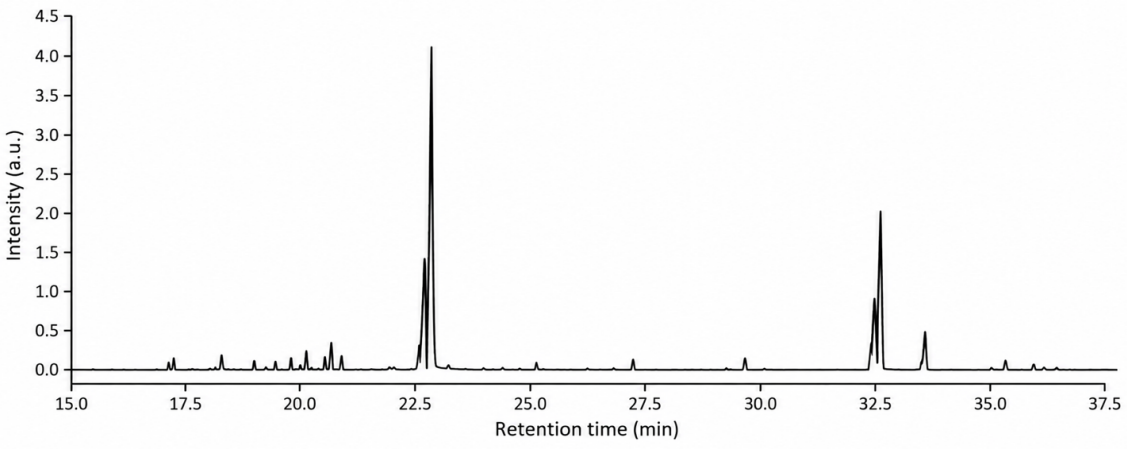


Figure 4. Chromatographic analyses of the calibration curve. The peaks correspond to geranylgeraniol (22.5 min) and δ-tocotrienol (33.0 min).

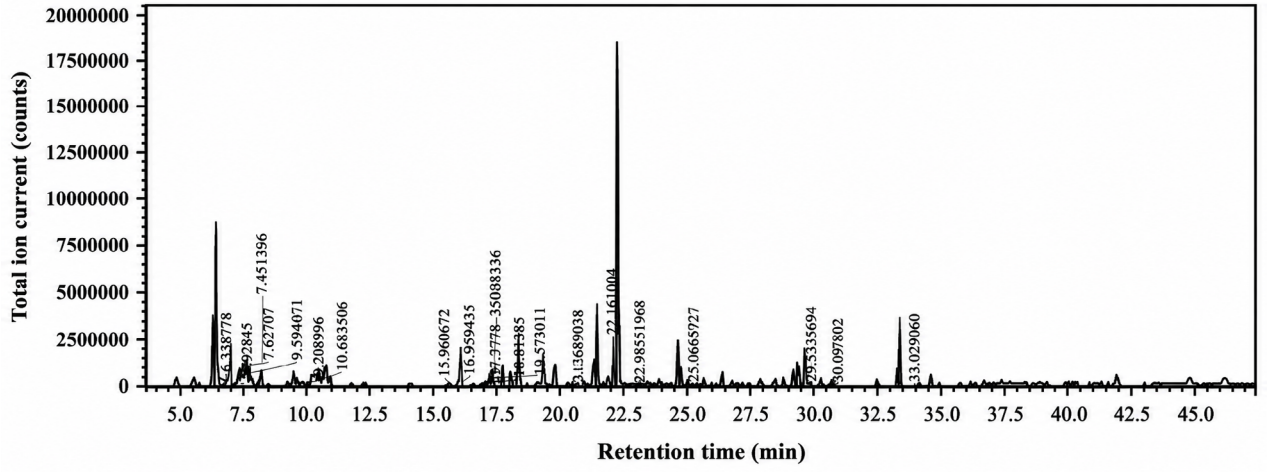


Figure 5. Representative chromatogram of the Reverse® sample highlighting the peaks corresponding to geranylgeraniol (22.5 min) and δ-tocotrienol (33.0 min)

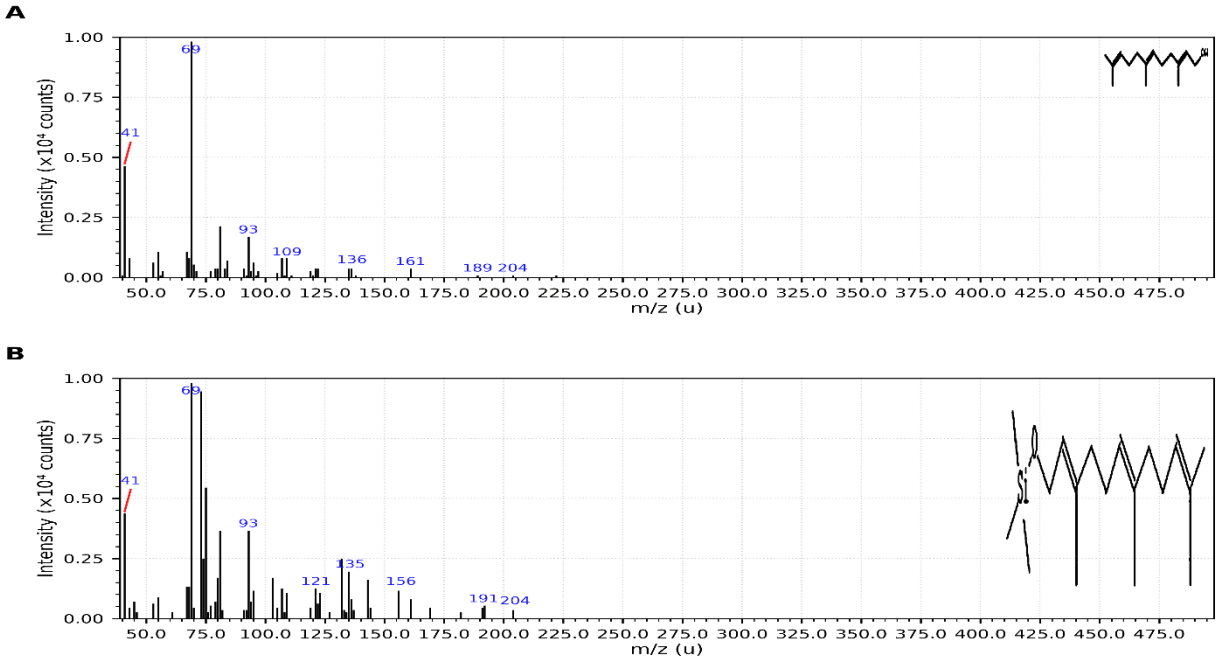


Figure 6. Mass spectrometry results. (A) Mass spectrum of δ-tocotrienol, showing characteristic fragments consistent with its chemical structure. (B) Mass spectrum of geranylgeraniol, showing diagnostic ions typical of isoprenoid compounds.

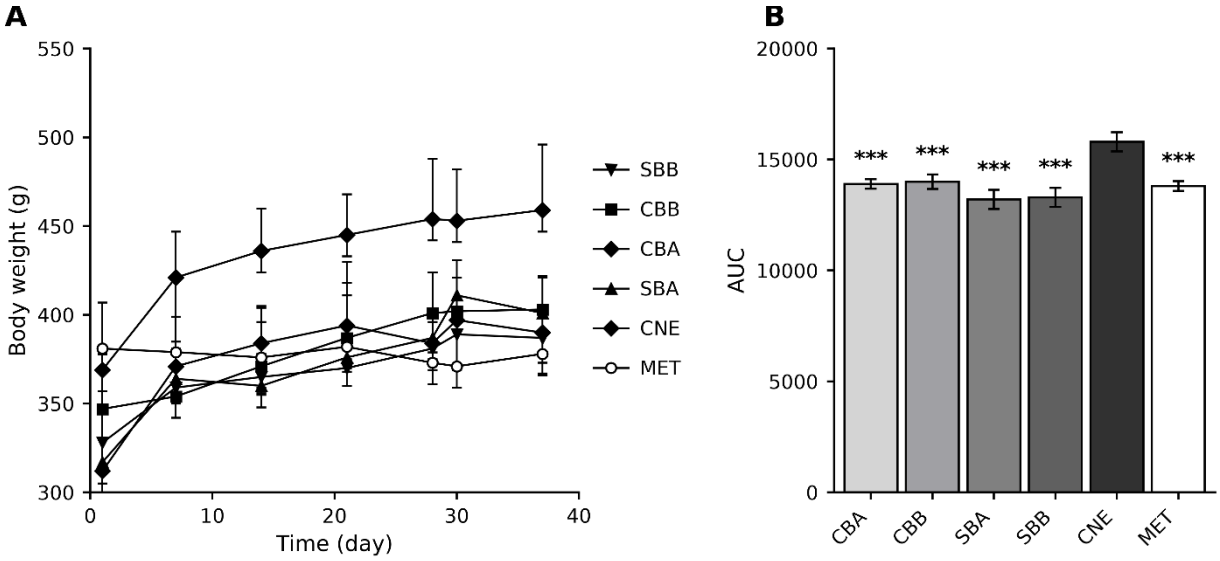


Figure 7. Weight changes in animals during the experimental period. (A) Variation in body weight throughout the treatment. (B) Area under the curve (AUC) of weight variation.
Notes: Data are presented as mean ± standard deviation. Statistical differences were evaluated using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ versus CNE.

high-fat diet, whereas the treated groups maintained ALT within the normal physiological range.

The lipid profile showed lower total cholesterol and LDL cholesterol in the treated groups than in CNE. Total cholesterol was 179.0 ± 21.6 mg/ dL in the CNE, while the CBA, CBB, SBA, SBB, and MET groups had mean values of

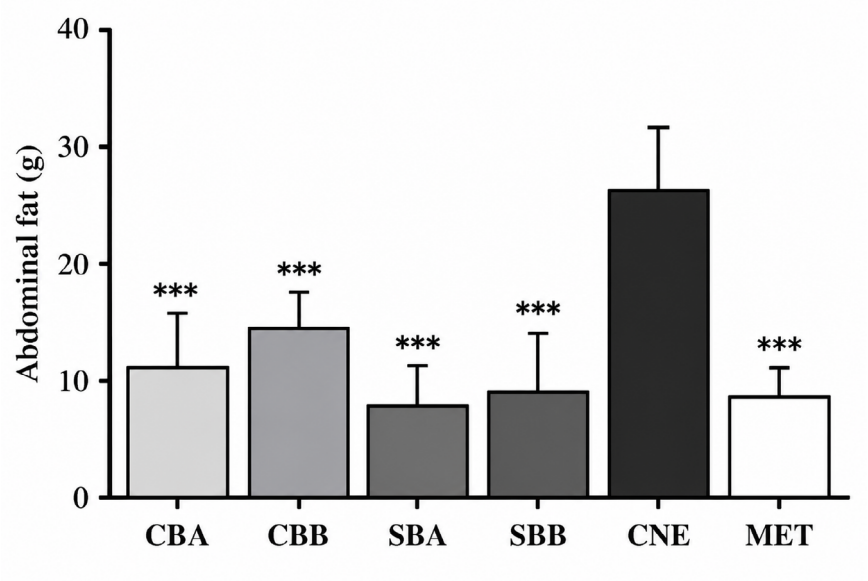


Figure 8. Abdominal fat of animals across groups
Notes: Data are presented as mean ± standard deviation. Statistical differences were assessed using one-way ANOVA followed by Tukey's post hoc test; ***$p < 0.001$ versus CNE.

64.4 ± 4.15, 62.33 ± 5.46, 70.2 ± 2.38, 58.83 ± 6.70, and 81.5 ± 14.84 mg/ dL, respectively ($p < 0.05$ versus CNE). LDL cholesterol was 153 ± 32.58 mg/dL in the CNE and ranged from 12.5 ± 9.80 to 27.4 ± 19.7 mg/dL in the groups treated with Reverse® ($p < 0.05$ versus CNE).

Meanwhile, HDL cholesterol levels were maintained or increased in the groups treated with Reverse®, with the SBA group showing the most significant increase, with a mean of 55.4 ± 2.07 mg/ dL. The MET group had the lowest mean HDL cholesterol (22.5 ± 2.12 mg/dL). Regarding triglycerides, the CNE group had a mean of 325.2 ± 54.5 mg/dL. The CBA, CBB, SBA, SBB, and MET groups showed mean values of 213.2 ± 58.0, 205.83 ± 97.5, 80.8 ± 44.58, 202.16 ± 90.19, and 99.0 ± 12.72 mg/ dL, respectively, with the greatest reduction observed in the SBA group (Table 5).

3.5. Histopathological evaluation and scanning electron microscopy

Histopathological evaluation showed mild hepatic steatosis in the CNE group, whereas the Reverse® and metformin groups showed preserved hepatic architecture on the evaluated slides. In the treated groups, hepatocytes and sinusoids maintained their histological organization, with no evidence of necrosis, inflammatory infiltrate, or vacuolar accumulation compatible with marked steatosis (Figure 9).

In cardiac tissue, the slides showed organized muscle fibers and no significant morphological alterations in the evaluated groups. The examined images showed no evident areas of necrosis, myocardial degeneration, or significant inflammatory infiltrate (Figure 9).

Ultrastructural evaluation of the aorta using SEM demonstrated changes compatible with atherosclerotic plaque in the CNE group. In the Reverse®-treated and MET groups, the vascular surface showed morphological preservation, with no evident lipid deposition, erosive lesions, or gross alterations in endothelial organization in the images analyzed (Figure 10).

4. Discussion

This study showed that Reverse® is a multicomponent plant-based nutraceutical composed of total phenols, total flavonoids, δ-tocotrienol, and geranylgeraniol. The product was associated with favorable effects on weight gain, visceral adiposity, lipid profile, ALT, and hepatic and vascular morphology in Wistar rats subjected to a short-term dietary challenge model with saturated fat from *C. nucifera* L., which induces acute metabolic stress and dyslipidemia. These findings are relevant because they integrate, in the same investigation, phytochemical markers of the formulation and biological outcomes of experimental obesity, allowing discussion of Reverse® as an adjuvant nutraceutical candidate for modulating cardiometabolic alterations. The interpretation of the results should consider two complementary levels of evidence: chemical characterization, which supports the presence of bioactive markers, and the biological response in the animal model, which indicates modulation

Table 5. Biochemical parameters of animals across groups

Parameter	CBA	CBB	SBA	SBB	CNE	MET
AST (U/L)	66.0 ± 5.68	68.83 ± 4.70	77.8 ± 6.76	81.83 ± 21.59	69.4 ± 7.82	71.0 ± 22.62
ALT (U/L)	32.0 ± 3.53	30.66 ± 3.98	32.4 ± 4.21	29.83 ± 2.99	65.4 ± 5.22	45.5 ± 10.5
Total cholesterol (mg/dL)	64.4 ± 4.15*	62.33 ± 5.46*	70.2 ± 2.38*	58.83 ± 6.70*	179.0 ± 21.6	81.5 ± 14.84*
HDL (mg/dL)	49.4 ± 5.94	46.3 ± 4.80	55.4 ± 2.07	48.33 ± 4.27	45.4 ± 5.90	22.5 ± 2.12
LDL (mg/dL)	27.4 ± 19.7*	25.0 ± 18.74*	12.5 ± 9.80*	23.5 ± 17.37*	153.0 ± 32.58	27.0 ± 11.59*
Triglycerides (mg/dL)	213.2 ± 58.0*	205.83 ± 97.5	80.8 ± 44.58*	202.16 ± 90.19	325.2 ± 54.5	99.0 ± 12.72*

Notes: Data are expressed as mean ± standard deviation. *$p < 0.05$ versus CNE.
Abbreviations: HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

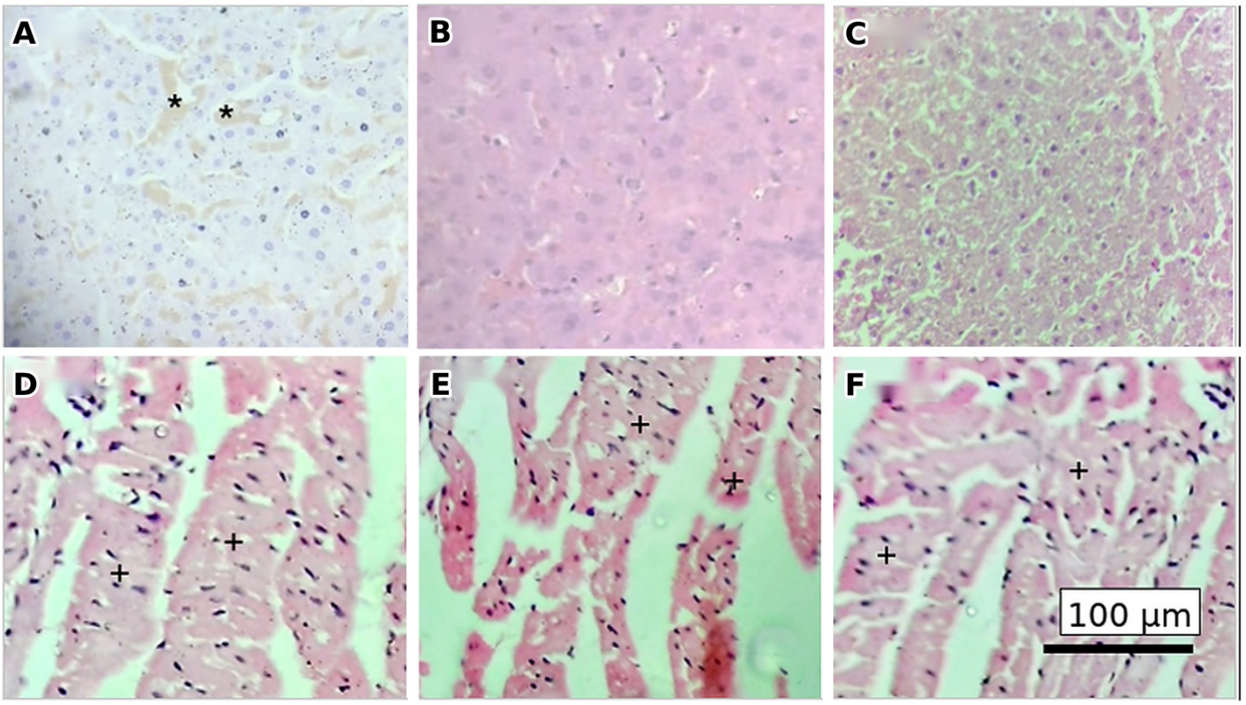


Figure 9. Representative photomicrographs of liver and heart stained with hematoxylin and eosin. Liver tissue from the (A) CNE, (B) SBB, and (C) MET groups. In CNE, histopathological evaluation demonstrates mild hepatic steatosis, characterized by small intracellular lipid droplets (microvesicular steatosis) occupying less than 33% of the hepatic parenchyma (Grade 1 according to Brunt criteria), without significant lobular inflammation. Heart tissue from the (D) CNE, (E) CBB, and (F) MET groups, with preserved cardiac fibers and cardiomyocytes (+), demonstrating organized, striated muscle fibers with centrally located nuclei and no significant morphological alterations such as hypertrophy, necrosis, or inflammatory infiltrates in any of the evaluated groups. Scale bar: 100 µm; magnification: 40×.

of metabolic and morphological outcomes under the experimental conditions used.

The presence of total phenols and flavonoids aligns with Reverse®'s plant composition, particularly its inclusion of *I. paraguariensis*, *P. incarnata*, and *M. dubia*. Polyphenols are a broad class of plant secondary metabolites, including phenolic acids, flavonoids, stilbenes, and lignans, and are frequently associated with antioxidant and anti-inflammatory activity and modulation of metabolic pathways linked to obesity.[8,9,11,35] In adipocytes and metabolically active tissues, these compounds can interfere with adipogenesis, lipogenesis, lipolysis, inflammatory signaling, oxidative stress, and insulin sensitivity, although their effects depend on chemical structure, dose, food

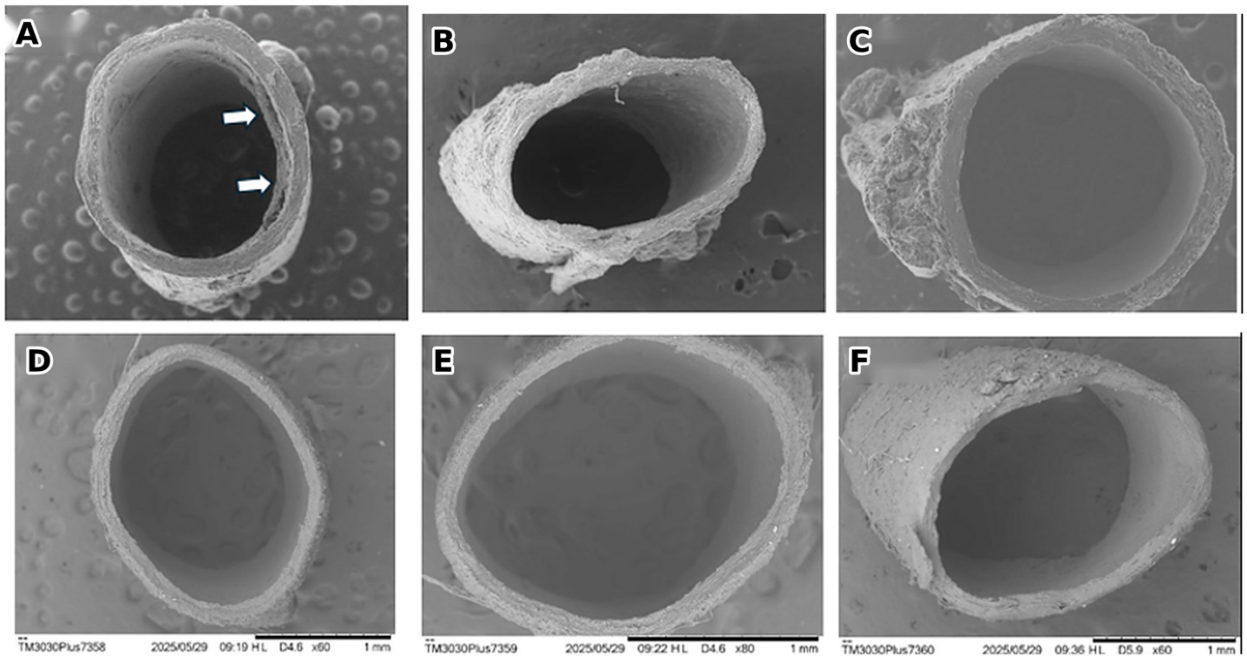


Figure 10. Scanning electron microscopy of cross-sections of the aorta from experimental groups: (A) CNE, (B) SBA, (C) SBB, (D) CBA, (E) CBB, and (F) MET. In the CNE group, white arrows indicate changes compatible with atherosclerotic plaque. In the SBA, SBB, CBA, CBB, and MET groups, the representative images show morphological preservation of the vascular wall. Scale bar: 1 mm; magnification: (A, C, D, F) 60×, (B, E) 80×. Acquisition: 15 kV.

matrix, intestinal metabolism, and bioavailability. Therefore, one should interpret the spectrophotometric results in this study as global markers of phytochemical composition and reducing capacity, not as isolated evidence of therapeutic activity.

This caution is particularly important in the Folin–Ciocalteu assay, as the method estimates total reducing capacity and can be affected by non-phenolic substances, such as ascorbic acid and other reducing agents.[22,23] The presence of *M. dubia*, a fruit known for its high levels of vitamin C and phenolic compounds, underscores the need to interpret total phenol values critically.[14] Similarly, quantifying total flavonoids with $AlCl_3$ is useful for screening and standardization but does not replace individual flavonoid identification using chromatographic methods. Nevertheless, total phenols (22.08–28.72 mg EAG/g) and total flavonoids (16.53–18.80 mg EQ/g) indicate that the matrix contains a bioactive, hydrophilic fraction consistent with the proposed modulation of oxidative stress and inflammation in experimental obesity.

The determination of δ-tocotrienol and geranylgeraniol using GC-MS is a strength of the study, as it confirms the presence of relevant lipophilic markers in a matrix that also contains hydrophilic compounds. The 32.0 mg/g δ-tocotrienol content is particularly important, as tocotrienols have been investigated for their effects on inflammation, oxidative stress, adipogenesis, and lipid metabolism, including modulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and inflammatory signaling pathways.[18,19] In an obesity model, tocotrienols extracted from annatto reduced markers of inflammation and oxidative stress and improved macronutrient metabolism in obese mice.[19] However, clinicians should still interpret clinical evidence with caution. In a systematic review and meta-analysis of randomized clinical trials, Khor *et al.*[17] observed reduced C-reactive protein after tocotrienol supplementation, but the results were less consistent for interleukin -6 (IL-6), tumor necrosis factor-alpha (TNF-α), and malondialdehyde. Therefore, the presence of δ-tocotrienol in Reverse® supports mechanistic plausibility, but does not, in isolation, authorize definitive clinical inferences.

The geranylgeraniol content of 104.0 mg/g also supports the formulation's metabolic plausibility. This isoprenoid participates in the mevalonate pathway and can influence protein prenylation, ubiquinone synthesis, and isoprenoid homeostasis.[21] In an experimental obesity model, Chung *et al.*[21] observed that dietary geranylgeraniol improved glycemic homeostasis parameters and reduced pro-inflammatory mediators, in association with changes

in the gut microbiota. In the present study, the quantitative presence of this lipophilic marker should be integrated with the observed effects on adiposity, lipid profile, and vascular preservation, suggesting that the formulation's isoprenoid fraction may contribute to cardiometabolic modulation. However, these findings remain preclinical and do not replace specific assays with the complete formulation, bioavailability markers, and evaluation of molecular pathways.

From a biological standpoint, the reduction in weight gain and abdominal fat in the treated groups was a central result and should be highlighted. The decrease in visceral adiposity relative to CNE suggests modulation of abdominal lipid accumulation, a relevant outcome given the associations among visceral fat, systemic inflammation, insulin resistance, and cardiometabolic risk.[34,36] The phenolic/flavonoid fraction may contribute through antioxidant and anti-inflammatory mechanisms, while tocotrienols, carotenoids, and geranylgeraniol may act on lipid metabolism, mitochondrial function, and cell signaling. Recent reviews indicate that phytochemicals can modulate adipogenesis, lipogenesis, thermogenesis, energy expenditure, and intestinal metabolism, offering a mechanistic basis for interpreting the observed response.[8,9] However, the current design does not allow us to identify which component drives each effect or establish a direct causal relationship between a specific chemical marker and the physiological response.

The lipid profile also improved. The groups treated with Reverse® showed lower total cholesterol and LDL-cholesterol levels than the CNE group, with a significant reduction in LDL cholesterol. Dyslipidemia in obesity is related to greater availability of free fatty acids, increased hepatic production of triglyceride-rich lipoproteins, reduced HDL function, and a higher atherogenic risk.[37] In this context, the results suggest a potential hypolipidemic action in the experimental model. The maintenance or increase of HDL in the groups treated with Reverse® is also relevant, as this fraction participates in reverse cholesterol transport and has antioxidant and anti-inflammatory functions.[38] Integrating these findings with phytochemical markers is biologically coherent, since tocotrienols can modulate cholesterol synthesis, phenolic compounds and flavonoids can attenuate lipid oxidation and inflammation, and geranylgeraniol can participate in isoprenoid pathways related to metabolic homeostasis.

The results for ALT and liver weight indicate potential liver protection under the conditions evaluated. Experimental obesity and high-fat diets can promote hepatic lipid accumulation, inflammation, and altered aminotransferase levels, phenomena associated with metabolic steatosis and liver disease.[39] The reduction in ALT in the treated groups, along with histological preservation of the liver, suggests reduced hepatocellular damage or lipid accumulation compared with CNE. This result should be discussed alongside the presence of phenols, flavonoids, tocotrienols, and geranylgeraniol, as these classes have been linked to modulation of oxidative stress, inflammation, and hepatic lipid metabolism.[8,9,18,19] However, the absence of histomorphometric quantification, hepatic inflammatory markers, hepatic triglyceride content, and molecular analysis limits the strength of the inference.

The SEM evaluation showed morphological preservation of the aorta in the treated groups and alterations compatible with atherosclerotic plaque in CNE. This finding should be highlighted because it aligns with reductions in total cholesterol and LDL cholesterol and suggests a potential vasculoprotective effect in the evaluated model. Mechanistically, the combination of phenolic/flavonoid compounds with antioxidant activity, tocotrienols with anti-inflammatory and hypolipidemic potential, and geranylgeraniol, which is associated with isoprenoid homeostasis, may contribute to reduced endothelial damage and vascular lipid deposition. However, the interpretation should remain descriptive, as SEM, although useful for visualizing ultrastructural changes, does not replace quantitative analyses of lesion area, endothelial immunostaining, vascular inflammatory markers, or functional assessment of vasoreactivity. Thus, the most appropriate expression is that Reverse® was associated with vascular morphological preservation under the study conditions, and not that it clinically prevented atherosclerosis.

The comparison between formulations with and without *B. orellana* indicates that the effects did not depend exclusively on the presence of this component. In some parameters, the SBA and SBB groups showed particularly favorable responses, including reductions in triglycerides, LDL cholesterol, and ALT. This pattern may reflect non-linear interactions among constituents, differences in bioavailability, differences in formulation composition, or a non-monotonic, dose-dependent response. Complex nutraceutical formulations may exhibit synergism, antagonism, or modulation of absorption among their components, underscoring the need for specific mechanistic and pharmacokinetic studies.[10-12,39] In this sense, the most robust interpretation is that the set of plant raw materials and phytochemical markers of Reverse® acted as an integrated matrix rather than a simple sum of isolated compounds.

The study has limitations worth noting. The sample size was small (n = 5 per group), limiting the generalizability

of the findings and increasing sensitivity to individual variation. The experimental model used male rats, so inferences about effects in females or humans are not warranted. The study did not evaluate food intake, energy expenditure, systemic inflammatory markers, insulin, glycemia, homeostatic model assessment of insulin resistance (HOMA-IR), microbiota profile, marker bioavailability, or long-term toxicity. Furthermore, the multicomponent formulation makes it difficult to attribute effects to isolated compounds. These limitations are relevant because recent reviews on nutraceuticals highlight the need for standardization, safety assessment, analysis of compound interactions, and investigation of potential drug interactions in the context of chronic use.[9,10] Despite these limitations, the study provides relevant preclinical evidence that Reverse® has a bioactive phytochemical profile and can modulate cardiometabolic parameters in the evaluated model.

Given its exploratory nature and the specific 20-day dietary challenge model, we interpret this investigation as a preclinical case study evaluating the acute response to a multicomponent formulation. The literature emphasizes that short-term rodent models primarily reflect acute metabolic stress rather than the complex, relapsing neurohormonal adaptations seen in chronic human obesity.[39,40] Taken together, the data support the potential of Reverse® as a plant-based nutraceutical adjuvant for research in experimental obesity and associated cardiometabolic alterations. The formulation integrates hydrophilic and lipophilic compounds with mechanistic plausibility: phenols and flavonoids as markers of reducing capacity and oxidative-inflammatory modulation; δ-tocotrienol as a marker associated with lipid metabolism, inflammation, and oxidative stress; and geranylgeraniol as an isoprenoid related to the mevalonate pathway and metabolic homeostasis. The convergence of these markers and the results—less weight gain, less abdominal fat, improved lipid profile, reduced ALT, and liver/vascular preservation—strengthens the hypothesis that the product can act as an adjuvant in modulating obesity. To advance toward translational application, studies with greater statistical power, dose–response evaluation, comparison between formulations, identification of individual chemical markers, validation of analytical methods, safety studies, bioavailability, and controlled clinical trials will be necessary.

5. Conclusion

The plant-based nutraceutical Reverse® presented a multicomponent phytochemical matrix, with measurable levels of total phenols, total flavonoids, δ-tocotrienol, and geranylgeraniol. In Wistar rats subjected to a short-term high-fat dietary challenge using saturated fat from *C. nucifera* L. in an acute diet-induced metabolic stress model, the treatment was associated with reduced weight gain, decreased abdominal fat, improved lipid profile, reduced ALT, and preserved hepatic, cardiac, and vascular histomorphology compared with the negative control. Integrating these results with phytochemical markers supports the plausibility of short-term cardiometabolic protection. Therefore, while Reverse® effectively mitigates acute dietary stress, it cannot yet be extrapolated to chronic obesity. Strengths of this study include a comprehensive, multidisciplinary approach that combines phytochemical characterization (spectrophotometry and GC–MS) with robust *in vivo* morphological (histopathology and SEM) and biochemical assessments, as well as a comparative evaluation of formulations with and without *B. orellana*. However, limitations include the small sample size (n = 5 per group), the absence of molecular pathway analyses (e.g., gene expression of inflammatory markers, HOMA-IR, insulin quantification), the lack of food intake monitoring, and the restriction to male animals. The findings imply that Reverse® can effectively mitigate key cardiometabolic complications associated with high saturated fat intake in this experimental model, including dyslipidemia, hepatic stress, and vascular endothelial damage. We recommend further long-term dose–response studies, evaluation of bioavailability and interactions with the gut microbiota, safety assessments, and randomized controlled clinical trials to confirm these promising preclinical outcomes in human populations. These findings support the preclinical potential of Reverse® as a nutraceutical composed of plant-based raw materials, developed for mitigating acute diet-induced metabolic dysfunctions. However, they do not allow for definitive clinical inferences. Further studies on analytical validation, safety, bioavailability, dose-response, and clinical efficacy are needed to confirm its applicability in chronic metabolic diseases.

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Conflict of interest

José Carlos Tavares Carvalho is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Ethics approval and consent to participate

The animal study protocol was approved by the Research Ethics Committee of the Federal University of Amapá under protocol no. 012/2024.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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