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RESEARCH ARTICLE

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Fiber and phenolic compounds contribution to the hepatoprotective effects of mango diets in rats fed high cholesterol/sodium cholate

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The present study evaluated the contribution of mango fiber (MF) and mango phenolic compounds (MP) to the hepatoprotective effect of freeze-dried mango pulp (FDM) cultivar (cv.) "Ataulfo" diets in high cholesterol/sodium cholate (HCC)–fed rats. Male Wistar rats were fed with a HCC diet for 12 weeks, either untreated, or supplemented with MF, MP, FDM, or a control diet (no HCC; n = 6/group). All mango treatments significantly decreased hepatic cholesterol deposition and altered its fatty acid profile, whereas MF and MP mitigated adipose tissue hypertrophy. MF caused a lower level of proinflammatory cytokines (IL-1α/β, IFN-γ, TNF-α) whereas FDM increased the anti-inflammatory ones (IL-4, 6, 10). Mango treatments increased catalase (CAT) activity and its mRNA expression; superoxide dismutase (SOD) activity was normalized by MF and FDM, but its activity was unrelated to its hepatic mRNA expression. Changes in CAT and SOD mRNA expression were unrelated to altered Nrf2 mRNA expression. Higher hepatic PPARα and LXRα mRNA levels were found in MP and MF. We concluded that MF and MP are highly bioactive, according to the documented hepatoprotection in HCC-fed rats; their mechanism of action appears to be related to modulating cholesterol and fatty acid metabolism as well as to stimulating the endogenous antioxidant system.

KEYWORDS

cytokines, gene expression regulation, mango, nonalcoholic fatty liver disease, polyphenols, PPARα

1 | INTRODUCTION

The etiology of nonalcoholic fatty liver disease (NAFLD) is heterogeneous and multifactorial, but an excessive cholesterol intake is critical for its rapid development and progression. Its pathophysiological spectrum extends from mild steatosis to hepatocellular carcinoma, passing through several intermediate states such as nonalcoholic steatohepatitis (NASH) and fibrotic/cholestatic liver disease (Rinella, 2015). Most patients with NAFLD/NASH have no symptoms at the time of diagnosis, but hepatomegaly can be determined during a physical examination, whereas laboratory analyses often show increased serum levels of liver enzymes, neutral lipids, and cholesterol (Rinella,

2015). Fatty liver is detectable by imaging studies, whereas hepatic steatosis, lobular inflammation, ballooning degeneration, and fibrosis are confirmed with histochemical analyses (Angulo, 2002). NASH develops from several environmental, physiological, and genetic factors, such as accumulation of fats, insulin resistance, and mitochondrial dysfunction, which cause oxidative stress and liver inflammation. It is noteworthy that mild steatosis usually has a benign prognosis, but NASH-associated mortality is very high, due to many clinical complications including cirrhosis and cardiovascular disease (Angulo, 2002; Huang, 2009; Rinella, 2015).

NASH represents a substantial economic burden for health systems worldwide; thus, several diagnostic tools and experimental research models have been developed to mimic its clinical manifestation in humans. Genetic (e.g., ob/ob, db/db, and *PPARα* knockout mice) and diet-induced (e.g., high-fat, high-fructose, and low methionine/choline) models have been particularly useful for not only elucidating the molecular pathogenesis of NASH, but also to evaluate the therapeutic effects of many drugs and phytochemicals (Angulo, 2002). A specific time- and dose-dependent manifestation of NASH occurs when rodents are fed with high cholesterol and sodium cholate (HCC) diets.

An effective NASH treatment should be focused on reversing hepatic lipotoxicity, inflammation, and fibrosis, which is why lifestyle modifications, such as increased physical activity, are essential to achieving these goals. In addition, a higher-than-normal fruit and vegetable intake (low fat, high dietary fiber), and avoiding foods rich in saturated fats and cholesterol (western diet) and highly processed ones (low methyl-donors), are common recommendations. As far as pharmacological intervention is concerned, GLP1 receptor agonists (e.g., liraglutide), PPARγ agonists (e.g., pioglitazone), and PPARα/δ agonists (e.g., elafibranor) have proven effects on reducing insulin resistance and hepatotoxicity, although their administration should be closely monitored because side effects may occur (Lassailly, Caiazzo, Pattou, & Mathurin, 2016).

Mango (*Mangifera indica* L.) cv. "Ataulfo" contains dietary fiber and phenolic compounds with proven benefits in controlling plasma lipids in healthy humans (Robles-Sanchez et al., 2011) and HCC-fed rats (Domínguez-Avila et al., 2019). Our group previously found that different fractions and doses of mango cv. Ataulfo controlled plasma lipids in HCC-fed rats (Domínguez-Avila et al., 2019). Consumption of 50 g kg^{-1} of FDM showed a lesser hepatoprotective effect than MF (60 g kg^{-1}, e.g., attenuating hepatic enzyme activity), whereas the most antiatherogenic and anti-NASH effects were exerted by the MP fraction (1 g kg^{-1}), increasingserum APOA1/APOB protein ratio. However, plausible mechanisms (synergistic/complementary) by which MF and MP act on ameliorating NASH have not been reported yet. Therefore, the present study aimed to evaluate the contribution of MP and MF on the hepatoprotective effect of mango pulp diets in HCC-fed rats. The NASH model used here is explained and summarized in Figure S1 (Arguello, Balboa, Arrese, & Zanlungo, 2015; Beynen, Lemmens, Debruijne, Katan, & Vanzutphen, 1986; Castoldi, Naffah de Souza, Câmara, & Moraes-Vieira, 2016; Charytoniuk, Drygalski, Konstantynowicz-Nowicka, & Chabowski, 2017; Chung & Parks, 2016; Enright, Joyce, Gahan, & Griffin, 2017; Ioannou, 2016; Zhong et al., 2015). Histology of the liver and epididymal adipose tissue, hepatic lipids, hepatic gene expression (PPARα, LXRα, Nrf2, catalase [CAT], superoxide dismutase [SOD]), hepatic antioxidant enzyme activity (CAT, SOD), and serum proinflammatory and anti-inflammatory cytokines were evaluated.

2 | MATERIALS AND METHODS

2.1 | Raw materials and chemicals

Ripe mango cv. Ataulfo pulp was obtained, frozen (−80°C, 24 hr) and freeze-dried (−50°C, 0.03 mbar, 72 hR); a subsample of freeze-dried mango pulp (FDM) was further treated to isolate mango fiber (MF) and mango phenolics (MPs) as previously described (Domínguez-Avila et al., 2019). Food-grade ingredients were used to prepare experimental diets were either from Bio-Serv (Flemington, NJ, USA) or purchased in a local market; ACS analytical- or GC-grade solvents, Tri-Reagent and Supelco® 37-fatty acid methyl ester mix were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used for histological analysis were from Hycel (Zapopan Jal., Mexico), whereas those used for molecular studies were from Promega (Madison, WI, USA) and Thermo-Fisher (Waltham, MA, USA).

2.2 | Experimental protocol

Thirty young male Wistar rats (166 g) were fed for 12 weeks with five experimental diets (six rats/diet): A control diet with no added cholesterol or sodium cholate (control), a high cholesterol/sodium cholate diet (0.8%/0.2% w/w) untreated (HCC), or supplemented with MF (6% w/w, HC + MF), MP (0.1% w/w, HCC + MP), or FDM (5% w/w, HCC + FDM). Each diet provided 64%, 16%, and 20% of total calories from carbohydrates (96% complex/4% sugars), lipids (vegetable oil: lard, 1:1), and protein (casein), respectively; their micronutrient composition was similar, and none of them were choline (0.2%) or sulfur-amino acid (0.98%) deficient.

Rats were individually housed in stainless steel cages under controlled environmental conditions (25°C ± 2°C, relative humidity 45%–60%, 12-hr light-dark cycles) throughout the experiment. The protocol was reviewed and approved by the Animal Experimentation Ethics Committee of the Research Center for Food and Development (CE/004/2016); the animals were cared for according to corresponding Mexican regulations (NOM-062-ZOO-1999) and the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health (NIH). After the experimental period, the rats were fasted overnight and anesthetized with an intraperitoneal injection of sodium pentobarbital (200 mg/kg BW, Pisabental, PISA Agropecuaria, Atitalaquia, Hidalgo, Mexico).

2.3 | Biological samples

Biological samples were processed as previously reported by Domínguez-Avila et al. (2015). Blood was collected by cardiac puncture to recover serum after centrifugation (3,000 *x g*, 10 min, 4°C). A 0.1-g liver sample was excised and stored in Tri-Reagent at −80°C for subsequent RNA extraction. A total of 0.5 g of liver and epididymal white adipose tissue (WAT) were conserved in 10% formalin/phosphate-buffered saline (>10-fold volume) for at least 48 hr, a 1-g sample was frozen to extract total lipids, and a final sample (0.5 g) was submerged in phosphate-buffered saline (PBS) and frozen to analyze the activities of antioxidant enzymes.

2.4 | Histological analyses

Formalin-fixed samples (liver and WAT) were embedded in paraffin, sliced (4–5 μm thick), deparaffinized, rehydrated, and stained with hematoxylin and eosin following the procedure reported by Mendoza-Sánchez et al. (2019) with some adaptations. Double-blind observations were performed by two trained pathologists, using a fluorescent microscope (LEICA DM2000, Leica Microsystems Inc., Chicago, IL, USA). Images were captured at 40× (liver) and 20× (WAT) optical magnification and processed in the LEICA V2 program.

2.5 | Hepatic lipids

Hepatic lipids were extracted from liver samples (1.0 g) with ice-cold chloroform:methanol (2:1 v/v), using the Folch method (Folch, Lees, & Sloane Stanley, 1957). Cholesterol and fatty acids (as fatty acid methyl esters, fatty acid methyl esters) were analyzed in triplicate by gas chromatography with the same protocol used in a recent report (Wall-Medrano et al., 2017), in an Agilent 6890 apparatus (Hewlett-Packard, Wilmington, DE, USA), with an SP-2560 capillary column (100 m × 0.25 mm; Supelco, Bellefonte, PA, USA), and a flame-ionization detector. Values were expressed as mg/g (cholesterol), g/100 g (saturated [SFAs], monounsaturated [MUFAs], poly-[PUFAs], and total unsaturated [UFAs] fatty acids) of total lipids, and as fatty acid ratios (MUFAs/SFAs, PUFAs/SFAs, UFAs/SFAs, n6/n3 UFAs).

2.6 | Antioxidant enzyme activity

Samples of 0.5 g of liver were homogenized in a total volume of 1.5 mL of PBS (50 mM, pH 7.0) and centrifuged (12,000 *x* g, 4°C, 15 min), following the adapted procedure reported by Domínguez-Avila et al. (2015). Supernatants were used to determine total protein content and antioxidant enzyme activity. Total protein content was determined using Bradford's reagent (Sigma-Aldrich) according to the manufacturer's protocol. CAT activity was measured by spectrophotometrically monitoring (λ = 240 nm) the disappearance of H_2O_2 (Aebi, 1984); one unit of CAT was defined as the amount of enzyme that catalyzes 1 μmol of H_2O_2/min. SOD activity was measured by inhibiting auto-oxidation of pyrogallol (Marklund & Marklund, 1974); one unit of SOD was defined as the amount of enzyme that inhibited pyrogallol autooxidation by 50%.

2.7 | Serum cytokine profile

Serum levels of proinflammatory and anti-inflammatory cytokines/chemokines were evaluated at the end of the study as described by Salazar-López et al. (2017) and the manufacturer's protocol, using a 12-target commercial cytokine enzyme-linked immunosorbent assay (Qiagen, Valencia, CA, USA). The kit detected interleukin (IL)-1α (IL-1α), IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), granulocyte macrophage colony-stimulating factor (GM-CSF), and chemokine ligand 5 (CCL5). Mean cytokine/chemokine response from each dietary group was expressed as arbitrary units relative to control group response (fold-change), and statistical differences between nonsupplemented (HCC) and supplemented (MF, MP, FDM) were reported.

2.8 | Hepatic gene expression

The Tri-Reagent was used to extract hepatic RNA as previously reported by Domínguez-Avila et al. (2019). Individual RNA samples were treated with RNAse-free DNAse and reverse transcribed as indicated by the manufacturer (Promega). Obtained cDNA was analyzed by real time polymerase chain reaction (PCR) using the following TaqMan probes (FAM fluorophore, Thermo-Fisher): peroxisome proliferator-activated receptor α (PPARα, GenBank: NM_013196.1; TaqMan assay: Rn00566193_m1), liver X receptor α (LXRα, GenBank: NM_031627.2; TaqMan assay: Rn00581185_m1), nuclear factor (erythroid-derived 2)-like 2 (Nrf2, GenBank: NM_031789.2; TaqMan assay: Rn00582415_m1), CAT (GenBank: NM_012520.2; TaqMan assay: Rn00560930_m1), and SOD (GenBank: NM_017050.1; TaqMan assay: Rn00566938_m1), using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference gene (VIC fluorophore, GenBank: NM_017008.4; TaqMan assay: Rn99999916_s1). All genes were amplified in duplex in a final volume of 10 μl, containing 2.5 ng of cDNA. Reactions were performed in triplicate, and the $2^{-\Delta\Delta CT}$ method was used to determine relative mRNA expression, as previously described (Schmittgen & Livak, 2008).

2.9 | Statistics

An Anderson-Darling test was used to establish data normality. Normal data were then submitted to one-way ANOVA followed by post hoc Tukey-Kramer test to evaluate the contribution of MF and MP to the effect of mango pulp on the liver responses. Nonparametric data were analyzed by Kruskal-Wallis ranking analysis in the statistical software Minitab v 18. Results were considered statistically different when $p < .05$.

3 | RESULTS

3.1 | Experimental feeding

Figure 1 shows weekly (a) food intake and (b) body weight gain of rats fed experimental diets. Rats fed HCC-based diets (supplemented or not) tended to consume less food (~1.0 g less), but statistical differences were only found on Weeks 3, 7, 10, 11, and 12. Food intake in HCC + MP and HCC + FDM groups was even lower (~1.0 g less), particularly from Weeks 7 to 11 when compared with the untreated HCC-fed group ($p < .05$). Despite this, differences in cholesterol (~0.007 mg/day) or sodium cholate (~0.001 mg/day) intake among HCC-fed groups was not considered different, assuming a similar hepatic overload of cholesterol (Table 1) because NASH-related features were quite similar (Figure 2). Mean weight gain was not different ($p > .05$) between dietary groups.

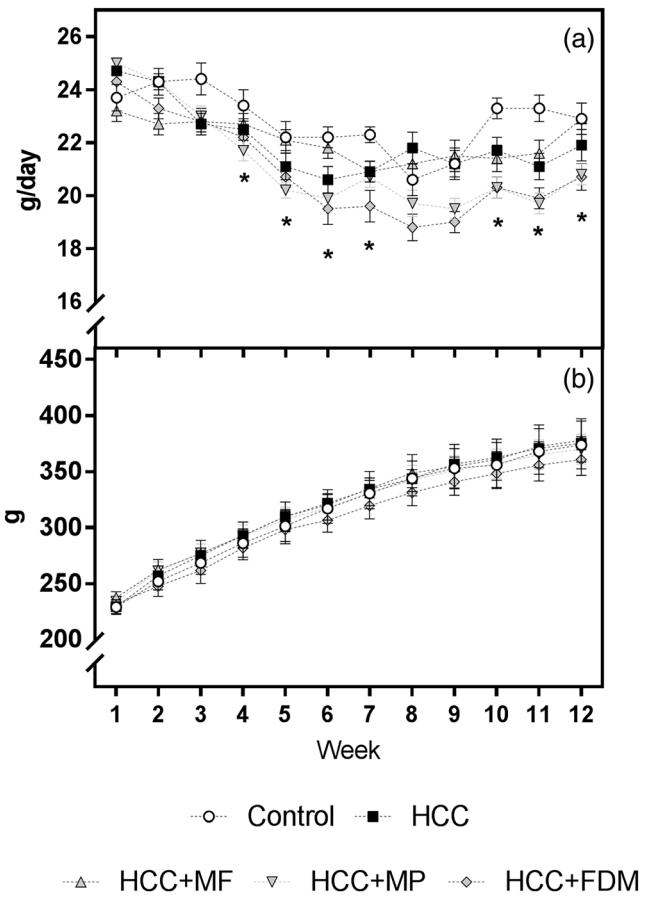

FIGURE 1 Twelve-week food intake and body weight gain of rats fed experimental diets. (a) Mean daily food intake and (B) body weight. Isocaloric (3.4 kcal/g) diets: Control, high-cholesterol (0.8% w/w)/sodium cholate (0.2% w/w) diet alone (HCC) or supplemented with 6% mango fiber (HCC + MF), 0.1% mango phenolics (HCC + MP), or 5% freeze-dried mango pulp (HCC + FDM). (*) Statistical differences ($p < .05$) between control diet and HCC + MP/HCC + FDM

3.2 | Histological analyses

HCC-fed rats exhibited a much larger liver (hepatosomatic index 44 × 10^{-3}) and epididymal fat pad than control rats. Hexagonal-shape hepatocytes (20–30 μm) arranged radially from the centrilobular vein, with no signs of steatosis, inflammatory infiltrates, or fibrosis were observed on animals fed with the control diet (Figure 2), whereas HCC caused typical features of NASH, such as hepatic lobule distortion. Adipocyte hyperplasia/hypertrophy (Figure S2) were observed in HCC-fed rats which were reduced in the HCC + MP group, but not in HCC + MF, or HCC + FDM groups. These results suggest that MP apparently modulate cholesterol absorption and accumulation, reducing liver inflammation.

3.3 | Hepatic lipids

Changes in hepatic fatty acid profile could be used as marker of liver injury associated with HCC diet and NASH development. Higher content of saturated fatty acids and lower MUFAs/PUFAs ratio was related with higher risk of liver alterations. Table 1 shows hepatic cholesterol concentration and fatty acid profile of rats fed experimental diets. All three supplemented diets reduced hepatic cholesterol deposition (30%–35%), as compared with untreated HCC-fed rats. However, this apparent reduction did not reach normal levels, as compared with the control group (7.2–7.9 times lower).

As compared with control rats, those fed the HCC-diet (untreated) had 12 and one times more cholesterol and MUFAs, 0.3–0.4 times lower PUFAs and SFAs and a higher MUFAs/SFAs, MUFAs/PUFAs (Figure S3) and n6/n3 (18 vs. 14) ratio. It is noteworthy that HCC reduced the hepatic content of palmitic (16:0), stearic (18:0), arachidonic (20:4 n6), and docosahexaenoic (22:6 n3) acids, whereas simultaneously increasing the content of palmitoleic (16:1 n7), oleic (18:1 n9c), and α-linolenic (18:3 n3) acids, as compared with control group. A normalization effect of the n6/n3 ratio was observed in rats fed HCC + MF and HCC + FDM diets (15.5 and 14.5), as compared with the control group (14.0). Supplemented groups also showed a different ability to modify the relative content of fatty acids as follows: HCC + MF > HCC + FDM > HCC + MP; however, HCC + MF and HCC + FDM groups showed a lower n6/n3 ratio when compared with the HCC group. As compared with HCC-fed rats, livers from HCC + MF-fed rats showed a lower relative content of 16:1 n7, 18:1 n9t, 20:4 n6 but a higher content of stearic 18:0, 20:2 n9, 22:4 n6, 22:6 n3. Lastly, HCC + MP-fed rats showed a higher MUFAs/PUFAs ratio but the same n6/n3 ratio observed in the HCC + MF group.

3.4 | Antioxidant enzyme activity and gene expression

As shown in Figure 3, liver antioxidant enzyme activity and gene expression after 12 weeks of treatment were affected when supplemented with MF, MP, and FDM. CAT activity and its gene

TABLE 1 Partial hepatic lipidome of rats fed experimental diets

Lipids	Control	HCC	HCC + MF	HCC + MP	HCC + FDM
Cholesterol[a]	30.6 ± 2.3*	384.0 ± 24.4*	251.2 ± 14.4*	270.8 ± 23.7*	270.9 ± 9.1*
Fatty acids[b]					
14:0	1.0 ± 0.1*	0.8 ± 0.1*	0.9 ± 0.1*	1.0 ± 0.0*	0.8 ± 0.0*
16:0	19.7 ± 0.2*	15.9 ± 0.5*	16.3 ± 0.4*	16.9 ± 0.3*	15.4 ± 0.4*
17:0	0.4 ± 0.1*	0.3 ± 0.0*	0.3 ± 0.0*	0.3 ± 0.1*	0.3 ± 0.0*
18:0	13.4 ± 0.4*	4.0 ± 0.1*	5.0 ± 0.2*	4.3 ± 0.2*	4.8 ± 0.1*
16:1 n7	2.0 ± 0.2*	9.4 ± 0.3*	7.9 ± 0.6*	8.1 ± 0.4*	8.4 ± 0.2*
20:1 n9	0.2 ± 0.0*	0.6 ± 0.0*	0.5 ± 0.1*	0.5 ± 0.0*	0.5 ± 0.0*
18:1 n9-*t*	3.0 ± 0.1*	2.2 ± 0.1*	2.7 ± 0.2*	2.5 ± 0.1*	2.5 ± 0.1*
18:1 n9-*c*	21.3 ± 1.0*	40.5 ± 0.5*	38.7 ± 0.8*	42.5 ± 0.4*	40.9 ± 0.5*
18:2 n6	16.6 ± 0.6*	19.1 ± 0.5*	17.8 ± 0.7*	17.8 ± 0.3*	18.4 ± 0.4*
18:3 n6	0.2 ± 0.0*	0.3 ± 0.0*	0.3 ± 0.0*	0.2 ± 0.0*	0.2 ± 0.0*
18:3 n3	0.2 ± 0.0*	0.9 ± 0.0*	0.9 ± 0.1*	1.1 ± 0.1*	1.2 ± 0.1*
20:2 n9	0.0 ± 0.0*	0.3 ± 0.0*	0.1 ± 0.0*	0.2 ± 0.0*	0.2 ± 0.0*
20:3 n6	0.2 ± 0.1*	0.6 ± 0.0*	0.5 ± 0.0*	0.4 ± 0.1*	0.6 ± 0.1*
20:4 n6	19.0 ± 0.8*	4.6 ± 0.1*	7.4 ± 0.5*	3.8 ± 0.3*	5.1 ± 0.3*
22:4 n6	0.4 ± 0.0*	0.4 ± 0.1*	0.2 ± 0.1*	0.1 ± 0.0*	0.1 ± 0.0*
22:6 n3	2.4 ± 0.1*	0.5 ± 0.0*	0.8 ± 0.1*	0.3 ± 0.0*	0.5 ± 0.0*

Note. Fatty acids include saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Dietary groups: Control, high-cholesterol (0.8% w/w)/sodium cholate (0.2% w/w) diet alone (HCC) or supplemented with 6% mango fiber (HCC + MF), 0.1% mango phenolics (HCC + MP) or 5% freeze-dried mango pulp (HCC + FDM).

Abbreviations: FDM, freeze-dried mango pulp; HCC, high cholesterol/sodium cholate; MF, mango fiber; MP, mango phenolics.

[a]Values are expressed as mg/g (±SEM) of total extracted lipids (Folch method).

[b]Values are expressed as g/100 g (±SEM) of total extracted lipids (Folch method).

*Significant differences ($p < .05$).

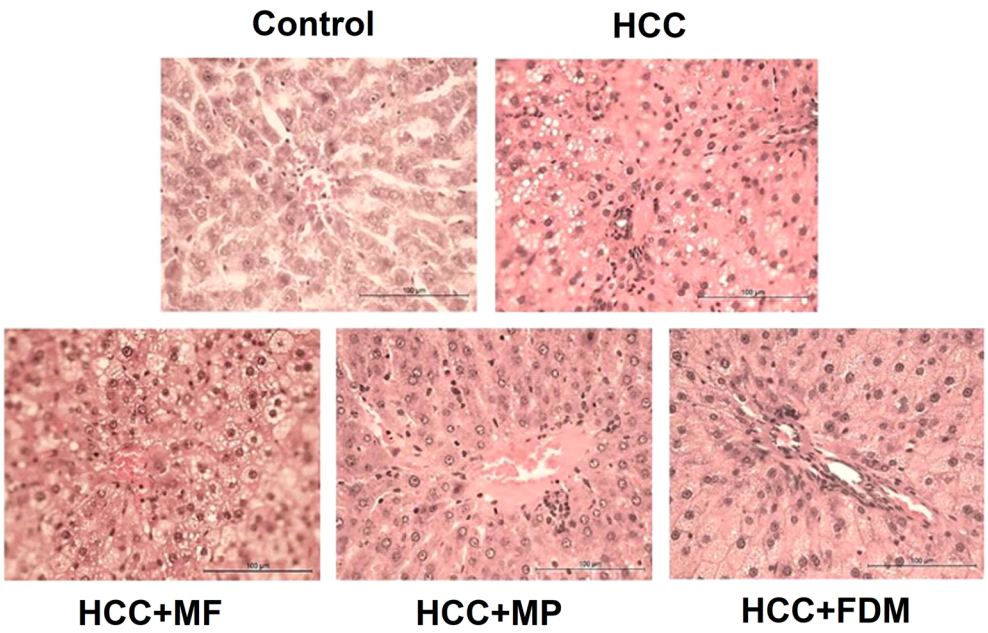

FIGURE 2 Hematoxylin and eosin staining (×40) of hepatic tissues from rats fed experimental diets. Diets: control, high-cholesterol/sodium cholate diet alone (HCC) or supplemented with mango fiber (HCC + MF), mango phenolics (HCC + MP), or freeze-dried mango (HCC + FDM). Neutral lipid droplets, central vein, peri-sinusoidal, peri-portal and bridging fibrosis, hepatocyte ballooning, lobular, and portal inflammation [Colour figure can be viewed at wileyonlinelibrary.com]

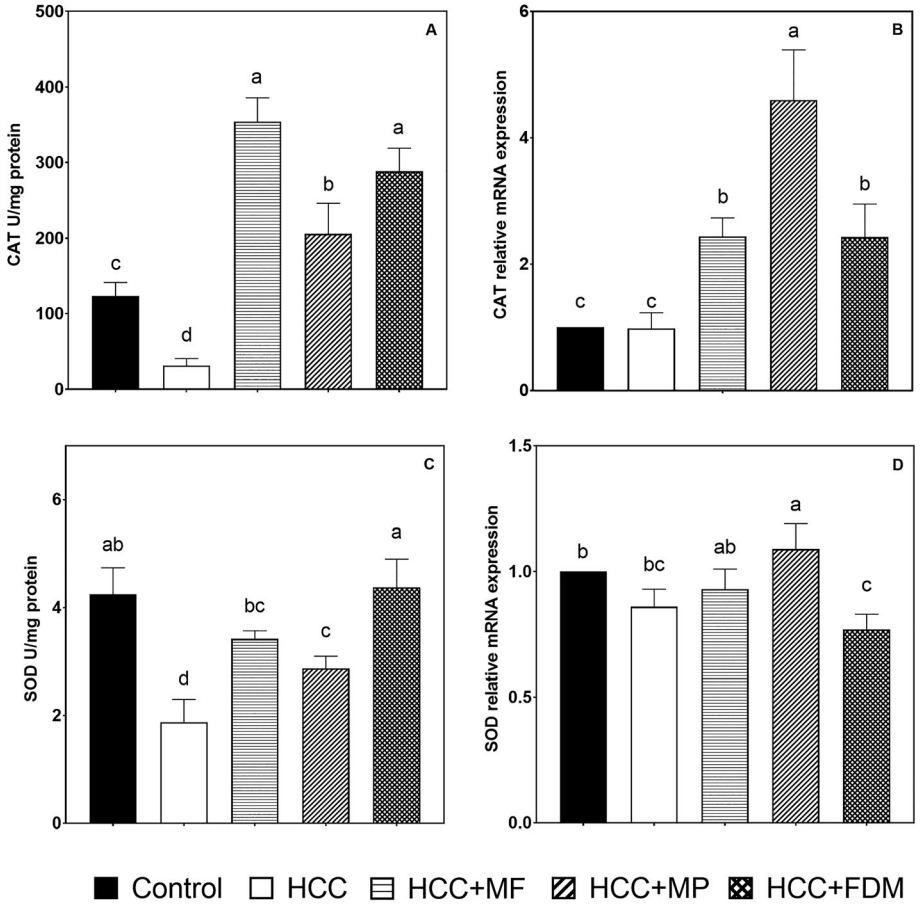

FIGURE 3 Antioxidant enzyme activity and relative mRNA expression. (a) Hepatic catalase (CAT) enzyme activity and (b) its relative mRNA expression. (c) Hepatic superoxide dismutase (SOD) enzyme activity and (d) its relative mRNA expression. Different letters indicate significant differences ($p < .05$)

expression were significantly increased in response to mango-supplemented diets, as compared with untreated (HCC) and control groups. SOD activity increased in treated groups, as compared with the HCC group, and a similar tendency was found in its gene expression. NASH is related to higher lipid peroxidation and mitochondrial dysfunction, which generates highly reactive molecules, such as peroxides, superoxide, and hydroxyl radicals, altering the redox homeostasis. HCC diet apparently promoted an overproduction of reactive oxygen species (ROS), whereas a higher activity of CAT and SOD in MF, MP, and FDM groups, as compared with HCC group, indicates a protective effect against oxidative stress in hepatocytes, which could prevent the development of steatosis. Furthermore, MP significantly stimulated CAT and SOD gene expression, as compared with MF and FDM. In previous results (Domínguez-Avila et al., 2019), MP fraction was able to exert higher protection against nonalcoholic steatosis by altering the APOA1/APOB protein expression and reducing food intake. Polyphenols have been considered signaling molecules that mitigate oxidative changes due to activation of transcription factors associated with inflammatory processes such as nuclear factor-kappa B, mitogen-activated protein kinase (MAPK), and nuclear factor-erythroid derived 2 (Nrf2), making them key components to prevent/ameliorate noncommunicable diseases.

3.5 | Serum cytokine profile

Plasma proinflammatory and anti-inflammatory cytokine/chemokine response after the 12-week experimental period is depicted in Figures 4 and 5, respectively. For comparison purposes between HCC-fed groups, data were expressed as arbitrary units relative to the response of control group (fold-change). The HCC group had an increased level of IL-2, IL-6, IL-10, IL-12, IL-13, IFN-γ, TNF-α, and CCL5 but a decreased concentration of IL-1α and IL-4, with no apparent modifications on IL-1β and GM-CSF. As compared with the HCC (untreated)–fed group, the pattern of cytokine/chemokine secretion in supplemented diets was diet-specific: although HCC + MF (IL-1α/β, IFN-γ, TNF-α) caused a lower plasma level of proinflammatory cytokines, HCC + FDM increased anti-inflammatory ones (IL-4, IL-6, IL-10), and mixed effects were observed with the HCC + MP diet. HCC + MF was the only supplemented diet capable of reducing GM-CSF, but serum levels of IL-2 and TNF-α (proinflammatory) and IL-13 and CCL-5 (anti-inflammatory) were not modified by supplemented diets, as compared with untreated HCC-fed rats. Lastly, most of the aforementioned statistical differences described were changes of <10%, whereas the most noticeable was that of IFN-γ, which increased by 70% in the HCC group but was almost normalized to control levels in the HCC + MF group (just 6% increase).

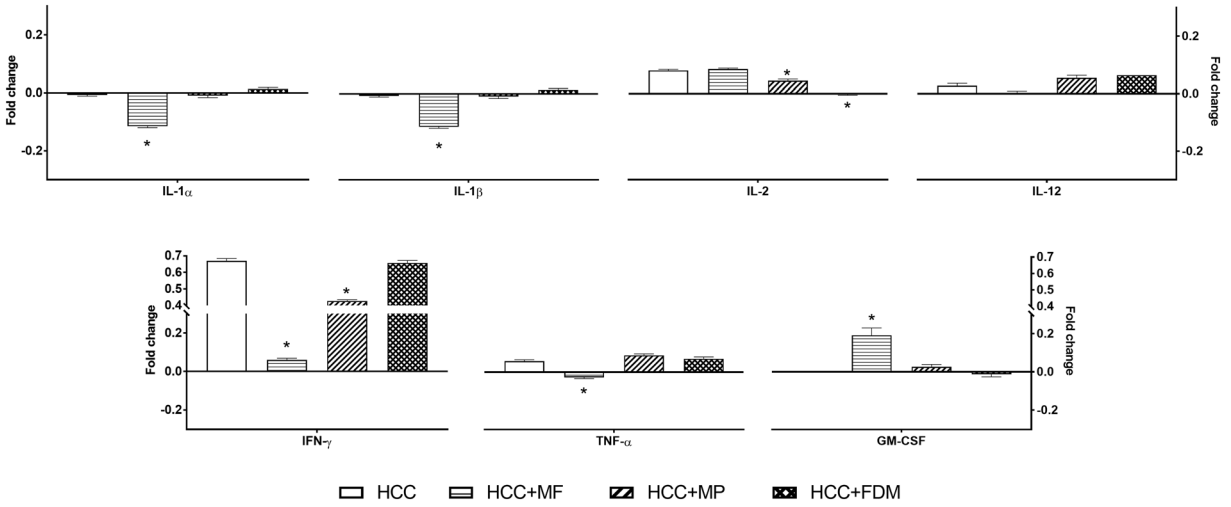

FIGURE 4 Plasma proinflammatory cytokine/chemokine response from rats fed experimental diets. High-cholesterol/sodium cholate diet alone (HCC) or supplemented with mango fiber (HCC + MF), mango phenolics (HCC + MP), or freeze-dried mango (HCC + FDM). Cytokine/chemokine response of each dietary group is expressed as arbitrary units relative to control group response (fold-change). Asterisks indicate statistical differences between any supplemented dietary group versus the nonsupplemented one (HCC)

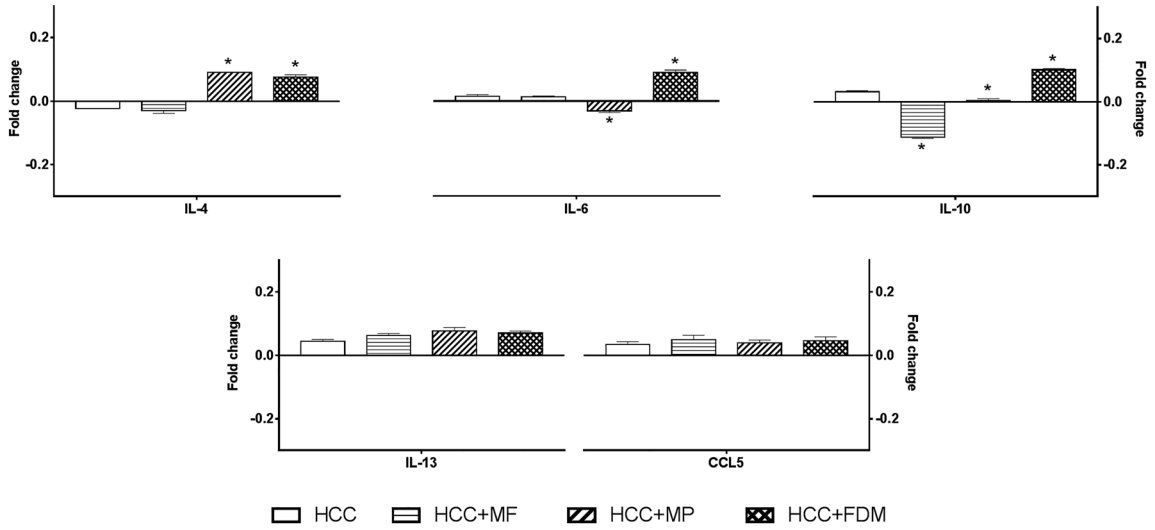

FIGURE 5 Plasma anti-inflammatory cytokine/chemokine response from rats fed experimental diets. High-cholesterol/sodium cholate diet alone (HCC) or supplemented with mango fiber (HCC + MF), mango phenolics (HCC + MP), or freeze-dried mango (HCC + FDM). Cytokine/chemokine response of each dietary group is expressed as arbitrary units relative to control group response (fold-change). Asterisks indicate statistical differences between any supplemented dietary group versus the nonsupplemented one (HCC)

3.6 | PPARα, LXRα, and Nrf2 gene expression

Relative mRNA expression of genes involved in cholesterol/fatty acid handling (PPARα, LXRα) and oxidative stress (Nrf2) is shown in Figure 6. PPARα and LXRα expression was modulated in all treated groups, but MP significantly increased ($p < .05$) the expression as compared with HCC group. In this sense, MP could be associated with a better regulation of cholesterol metabolism to mitigate NASH progression. Nrf2 expression was significantly higher in HCC and HCC + MF groups, as compared with the control group. Nrf2 promotes gene transcription through the antioxidant response element (ARE) sequence, which could be activated as a protection mechanism against radical production induced by high HCC diet.

4 | DISCUSSION

Prolonged and excessive intake of cholesterol is associated with overproduction of ROS, followed by inflammation and cytotoxicity of the liver that progresses to NASH, and ultimately results in cirrhosis and cardiovascular disease (Angulo, 2002). When rodents are given diets high in cholesterol with (Beynen et al., 1986; Jeong et al., 2005; Wang et al., 2010) or without (Arguello et al., 2015; Charytoniuk et al., 2017; Ioannou, 2016; Musso, Gambino, & Cassader, 2013) bile salts, lower cholesterol biosynthesis, catabolism, and worse liver damage is generated in a well-defined, time- and dose-dependent manner (Figure S1). Our findings indicated that FDM mitigates the hepatotoxic effects of an HCC diet, as resulting

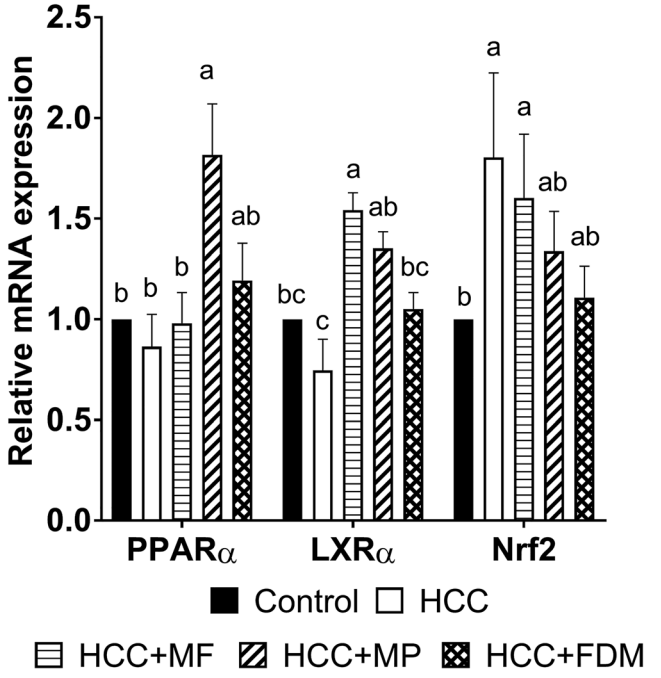

FIGURE 6 Relative hepatic mRNA expression of nuclear receptors of rats fed experimental diets. High-cholesterol/sodium cholate diet alone (HCC) or supplemented with mango fiber (HCC + MF), mango phenolics (HCC + MP), or freeze-dried mango (HCC + FDM). PPARα: peroxisome proliferator-activated receptor α; LXRα: liver X receptor α; Nrf2: nuclear factor (erythroid-derived 2)-like 2. Different letters indicate significant differences ($p < .05$)

from the action of its functional components (MF and MP); these effects included preserving the structural integrity of hepatocytes, tight-balance of hepatic fatty acids, and regulation of mRNA expression and activity of different transcription factors and antioxidant enzymes. The benefits found in our HCC-rat model are promising for NASH diet therapy in humans (Lassailly et al., 2016) because it closely matches the human disease trajectory (Brunt, Janney, Di Bisceglie, Neuschwander-Tetri, & Bacon, 1999; Caligiuri, Gentilini, & Marra, 2016; Rinella, 2015; Takahashi, Soejima, & Fukusato, 2012).

As compared with the control diet, HCC diet caused a mild anorexigenic effect, whereas HCC + MP and HCC + FDM, and HCC + MF to a lower extent, induced an even lower food intake. As described in Figure S1, appetite is largely modulated by the gut microbiota–brain axis, and dietary cholesterol by itself is capable of modifying the rat gut microbiota (Bo et al., 2017; Charytoniuk et al., 2017; Enright et al., 2017; Zhong et al., 2015), whereas hypercholesterolemia induces adipose dysfunction that alters the secretion of neurohormones that regulate appetite and satiety (Aguilar & Fernandez, 2014). As to the effect of MF (mainly pectin) and MP (mainly phenolic acids; Quiros-Sauceda et al., 2017), both are capable of inducing satiety by different mechanisms, accentuating the anorexic signals promoted by HCC. Natal et al. (Natal et al., 2017) reported a similar anorexic effect on weaning Wistar rats (21 days, 69 g) fed normal chow (AIN 93 M) or a high fat/low cholesterol (HFD; 21/0.15% w/w) with water (ad libitum) or with two cv. Ubá mango juices (35 mL/day) with different phytochemical composition. Although weight gain (Figure 1b), food efficiency ratio (weight gain/diet consumed), and specific organosomatic indices other than liver (heart, kidney, testicle) were similar in HCC fed rats, either untreated or supplemented (Domínguez-Avila et al., 2019), the anorexigenic signals could have been improved by adjusting the doses of treatments.

Increased adipocyte size and number are associated with many metabolic diseases including NASH/NAFLD (Osman et al., 2013). In this study, adipocyte hyperplasia and hypertrophy were observed in rats fed HCC-based diets, but not in control rats. As described in Figure S1, subchronic intake of HCC diets induces transient hypercholesterolemia, causing adipose inflammation and dysfunction and reproducing the typical biphasic process of NASH ([a] Insulin-dependent/independent WAT, liver accumulation of cholesterol and triacylglycerols; and, [b] liver inflammation) and mild fibrosis (Brunt et al., 1999; Jeong et al., 2005). It is known that hypercholesterolemia induces adipose dysfunction regardless of body weight (Aguilar & Fernandez, 2014) and that excessive accumulation of cholesterol and fatty acids in adipose tissue causes hyperplasia, hypertrophy, and inflammation (Chung & Parks, 2016), altering the adipocyte's membrane fluidity and endocrine function (Aguilar & Fernandez, 2014).

Hepatic cholesterol concentration was significantly decreased by all treatments, as compared with the HCC group. Comparable studies have shown that in rats fed high cholesterol diets, vegetable components (soybean; Jung & Kim, 2013) or phenolic compounds of vegetable origin (chlorogenic acid; Wan et al., 2013) decrease hepatic cholesterol deposition. Thus, FDM and its bioactive constituents (MF and MP) offered protection against hepatic cholesterol overload. The mechanism of action may be based on modulation of cholesterol absorption at the intestinal level. For example, Guo, Shu, and Yang (2016) determined that green tea (*Camellia sinensis* L.) fiber significantly decrease hepatic cholesterol concentration, due to its cholesterol- and bile-binding ability (particularly at intestinal pH values), which promoted fecal cholesterol excretion. On the other hand, Wang et al. (2015) administered 200 mg of polyphenols from adlay (*Coix lacryma-jobi* L. var. ma-yuen Stapf.) in the high cholesterol diet (1.0%) of male Wistar rats for 28 days, and found a modulation of microbiota, such as *Clostridium innocuum*, *Blautia luti*, and some strains of the order *Erysipelotrichales*, which are bacterial genera associated with lipid imbalance and metabolic disorders. These mechanisms could be associated with the reduction of hepatic adiposity and cellular damage exerted by mango cv. Ataulfo fractions.

Cholesterol is a known mediator of chronic inflammation, a process that hinders reverse cholesterol transport, and promotes cholesterol accumulation in a positive feedback loop (Tall & Yvan-Charvet, 2015). Our data showed that the most evident anti-inflammatory effects were exerted by MF, as evidenced by the significant decreases of most inflammatory cytokines, and particularly on IFN-γ. Previous evidence has shown that IFN-γ-mediated adaptive immunity contributes to the progression of NASH and atherosclerosis (Sutti et al., 2014). Because MF is nondigestible, its effects are likely related to

different factors at the gastrointestinal level. For example, supplementation with Tommy Atkins mango pulp was associated with improvement of gut microbiota, specifically, genus *Bifidobacteria*, *Akkermansia*, and *Aldercrutzia*, as well as modulation of short chain fatty acid production and gut inflammation (Ojo et al., 2016). Thus, MF consumption may be an interesting strategy to treat NAFLD, which is based on promoting beneficial microorganisms, whereas inhibiting pathogenic or harmful ones, resulting in a strengthened gut-liver axis (Kirpich, Parajuli, & McClain, 2015). Healthy gut microbiota can favorably modulate the influx of nutrients and bacterial metabolites to the liver, resulting in decreased inflammation and formation of fibrotic tissue, both of which promote liver fibrosis and NAFLD (Federico, Dallio, Godos, Loguercio, & Salomone, 2016).

Similar to cytokine regulation, supplementation of diet with FDM and its fractions (MF and MP) also had a modulatory effect on activity and gene expression of endogenous antioxidant enzymes, SOD and CAT. A normalization effect was observed in SOD activity and gene expression in MF-, MP-, and FDM-supplemented groups. Similar behavior was observed when supplementing HCC diet of rats with green and black olive extracts (Fki, Bouaziz, Sahnoun, & Sayadi, 2005) and rice bran extract (Wang, Li, Sun, Wang, & Yu, 2014). Regarding CAT, an increase was observed in its activity and gene expression, as compared with HCC and control groups. Similar findings were observed in serum and liver of hypercholesterolemic rats when their diets were supplemented with 2.5% green tea leaves and 5% tamarind extract (Lin et al., 1998; Martinello et al., 2006). A high-cholesterol diet is related to an overproduction of free radicals associated with lipid oxidation. Under this condition, tissues activate antioxidant mechanisms to recover redox homeostasis. Higher activity of CAT and SOD in the liver of treated groups was promoted by mango bioactive constituents, which apparently allowed an efficient quenching of superoxide radical and hydrogen peroxide. This prevented damage to the cell's macromolecules, in order to reestablish homeostasis, similar to the control group. This mechanism may be related to activation of transcription factors associated with the antioxidant response element sequence, such as Nrf2 (nuclear factor E2-related factor 2), which regulates transcription of Phase II detoxifying or antioxidant enzymes. Nrf2 is mainly activated by stress signals thought to result from dissociation of the complex Keap1/Nrf2 in the cytoplasm, releasing Nrf2 for translocation into the nucleus to exert its activity (Na & Surh, 2008; Nguyen, Nioi, & Pickett, 2009). In this sense, MF- and MP-supplemented groups showed higher Nrf2 expression than the control group, which could be due to a stress-induced response. Similar activations have been reported in response to apple pomace (Sharma et al., 2016) and green tea (Na & Surh, 2008) consumption, as well as from other fruits and vegetables (Orena et al., 2015). Nonetheless, activation of other signaling pathways could have been involved, such as mitogen activated protein kinases (Beltrán-Debón et al., 2011; Xu et al., 2015) to ameliorate adipose inflammation and dysfunction of hepatocytes during NASH development.

The hepatic fatty acid profile was quantitatively analyzed, showing that the concentration of SFAs (mainly 18:0) and PUFAs (mainly 20:4 n6) decreased, whereas MUFA concentration increased (mainly 18:1 n9c) in all HCC groups. This pattern has been documented in the liver of rats that developed NASH/NAFLD as a consequence of ingesting high-cholesterol diets (1.25%, 6 weeks; Serviddio et al., 2016) and in the serum of NASH and NAFLD patients (Puri et al., 2009). It has also been established that in mice, most of the changes to hepatic fatty acids are also evident in the serum (Eisinger et al., 2014). These changes are attributed to an increased Δ^9 desaturase activity, which metabolizes 16:0 → 16:1 n7 and 18:0 → 18:1 n9 and favors this metabolic route over the elongase pathway that generates longer PUFAs (Puri et al., 2009).

The changes to the fatty acid profile are fairly homogenous among all groups, tentatively suggesting that mango bioactives exerted no effects. However, a clear anti-inflammatory effect by the FDM is evident on the n6/n3 ratio. A higher ratio is considered hepatoprotective in rats fed high-carbohydrate high-fat diets (Poudyal, Panchal, Waanders, Ward, & Brown, 2012), whereas diets with low n6/n3 ratios have similar beneficial effects (Yang et al., 2016). According to this data, FDM exerted the most hepatoprotective effects, as compared with MF and MP, by preventing an increase in the n6/n3 ratio.

Cholesterol and overall lipid metabolism (e.g., de novo lipogenesis, and bile salt production) are highly influenced by the actions of different transcription factors, such as PPARα and LXRα. Phenolic compounds from various sources have been reported as modulators of PPARα and LXRα expression and activity, and some of their effects related to lipid and cholesterol metabolism are directly linked to these transcription factors. For example, supplementing a high-cholesterol diet with chlorogenic acid upregulated PPARα mRNA, which was correlated with an improved serum lipid profile and decreased hepatic lipid accumulation in Sprague Dawley rats (Wan et al., 2013). The flavonoid quercetin modulated the hepatic metabolism of cholesterol that yields bile acids in Wistar rats by upregulating the LXRα mRNA and protein levels (Zhang et al., 2016). These and similar findings have made it possible to suggest that some polyphenols like gallic acid (Chao et al., 2014), mangiferin (Wang et al., 2017), and chlorogenic acid (Wan et al., 2013) may be suitable options to prevent, mitigate, or treat NAFLD.

The bioactive constituents of FDM mitigated biochemical lesions to the liver, specifically, hepatic cholesterol concentration and n6/n3 ratio. Cholesterol and fatty acid metabolisms are governed by the expression and activity of the transcription factors PPARα and LXRα. The upregulating effects of the said transcription factors by the mango bioactives suggests that this may be the mechanism of action through which they exerted these changes. Numerous studies have shown that fiber and phenolic compounds from various sources are capable of inducing similar effects on the mRNA expression of PPARα and LXRα. For example, the previously documented effects of oat and wheat bran reported by Han et al. (2015) were related to hepatic changes on the PPARα and LXRα expression. Experimental models of HCC-fed animals have shown that different sources and types of phenolics exerted significant changes to PPARα and LXRα expression, such as green tea polyphenols (C57BL mice, 1.0% polyphenols, 1.0/0.5% cholesterol/sodium cholate), stilbenoids from *Morus alba*

(Sprague Dawley rats, 1-5 mg/kg/day of stilbenoids, 1.0/0.25% cholesterol/sodium cholate, four weeks), and chlorogenic acid (Sprague Dawley rats, 1 or 10 mg/kg/day of chlorogenic acid, 2.0/1.0% cholesterol/cholic acid, 28 days; Jo, Kim, & Lim, 2014; Tirosh et al., 2017; Wan et al., 2013). The importance of transcription factor upregulation at the hepatic level is highlighted by the fact that lipid-lowering pharmaceuticals act as PPARα agonists (fibrates). Furthermore, newer generations of LXRα agonists are currently being developed (T0901317, GW3965) and maybe commercially available after successful clinical trials (Hong & Tontonoz, 2014). If the effects and mechanisms of action of vegetable phenolics are comparable with pharmaceuticals, their consumption should be prioritized in order to preserve hepatic and overall health.

5 | CONCLUSIONS

All mango treatments mitigated hepatic cholesterol concentration by similar amounts, and the n6/n3 ratio was improved by MF and FDM. The inflammatory status of the animals was improved by all treatments, most notably MF, which may have been exerted through an improved intestinal microbiota or other actions at the gastrointestinal level, and MP, which could regulate the antioxidant endogenous system. Mango and its different bioactives exerted hepatoprotective actions through varied mechanisms of action, this data advocates for its increased consumption as part of a healthy diet that can promote liver and overall health.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

Aebi, H. (1984). [13] Catalase in vitro. In *Methods in Enzymology* (Vol. 105) (pp. 121–126). New York: Academic Press.

Aguilar, D., & Fernandez, M. L. (2014). Hypercholesterolemia induces adipose dysfunction in conditions of obesity and nonobesity. *Advances in Nutrition*, *5*(5), 497–502. https://doi.org/10.3945/an.114.005934

Angulo, P. (2002). Medical progress—Nonalcoholic fatty liver disease. *New England Journal of Medicine*, *346*(16), 1221–1231. https://doi.org/10.1056/NEJMra011775

Arguello, G., Balboa, E., Arrese, M., & Zanlungo, S. (2015). Recent insights on the role of cholesterol in non-alcoholic fatty liver disease. *Biochimica et Biophysica Acta-Molecular Basis of Disease*, *1852*(9), 1765–1778. https://doi.org/10.1016/j.bbadis.2015.05.015

Beltrán-Debón, R., Rull, A., Rodríguez-Sanabria, F., Iswaldi, I., Herranz-López, M., Aragonès, G., … Joven, J. (2011). Continuous administration of polyphenols from aqueous rooibos (Aspalathus linearis) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice. *Phytomedicine*, *18*(5), 414–424. https://doi.org/10.1016/j.phymed.2010.11.008

Beynen, A. C., Lemmens, A. G., Debruijne, J. J., Katan, M. B., & Vanzutphen, L. F. M. (1986). Interaction of dietary-cholesterol with cholate in rats: Effect on serum-cholesterol, liver cholesterol and liver-function. *Nutrition Reports International*, *34*(4), 557–563.

Bo, T., Shao, S., Wu, D., Niu, S., Zhao, J., & Gao, L. (2017). Relative variations of gut microbiota in disordered cholesterol metabolism caused by high-cholesterol diet and host genetics. *MicrobiologyOpen*, *6*(4), e00491. https://doi.org/10.1002/mbo3.491

Brunt, E. M., Janney, C. G., Di Bisceglie, A. M., Neuschwander-Tetri, B. A., & Bacon, B. R. (1999). Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. *American Journal of Gastroenterology*, *94*(9), 2467–2474. https://doi.org/10.1016/S0002-9270(99)00433-5

Caligiuri, A., Gentilini, A., & Marra, F. (2016). Molecular Pathogenesis of NASH. *International Journal of Molecular Sciences*, *17*(9). https://doi.org/10.3390/ijms17091575

Castoldi, A., Naffah de Souza, C., Câmara, N. O. S., & Moraes-Vieira, P. M. (2016). The macrophage switch in obesity development. *Frontiers in Immunology*, *6*(637). https://doi.org/10.3389/fimmu.2015.00637

Chao, J., Huo, T.-I., Cheng, H.-Y., Tsai, J.-C., Liao, J.-W., Lee, M.-S., … Peng, W.-H. (2014). Gallic acid ameliorated impaired glucose and lipid homeostasis in high fat diet-induced NAFLD mice. *PLoS ONE*, *9*(6), e96969. https://doi.org/10.1371/journal.pone.0096969

Charytoniuk, T., Drygalski, K., Konstantynowicz-Nowicka, K., & Chabowski, A. (2017). Alternative treatment methods attenuate the development of NAFLD: A review of resveratrol molecular mechanisms and clinical trials. *Nutrition*, *34*, 108–117. https://doi.org/10.1016/j.nut.2016.09.001

Chung, S., & Parks, J. S. (2016). Dietary cholesterol effects on adipose tissue inflammation. *Current Opinion in Lipidology*, *27*(1), 19–25. https://doi.org/10.1097/Mol.0000000000000260

Domínguez-Avila, J. A., Alvarez-Parrilla, E., López-Díaz, J. A., Maldonado-Mendoza, I. E., Gómez-García, M. d. C., & de la Rosa, L. A. (2015). The pecan nut (*Carya illinoinensis*) and its oil and polyphenolic fractions differentially modulate lipid metabolism and the antioxidant enzyme activities in rats fed high-fat diets. *Food Chemistry*, *168*, 529–537. https://doi.org/10.1016/j.foodchem.2014.07.092

Domínguez-Avila, J. A., Wall-Medrano, A., de la Rosa, L. A., Alvarez-Parrilla, E., Astiazaran-Garcia, H., & González-Aguilar, G. A. (2019). Mango phenolics increase serum apolipoprotein a1/b ratio in rats fed high cholesterol and sodium cholate diets. *Journal of the Science of Food and Agriculture*, *99*(4), 1604–1612. https://doi.org/10.1002/jsfa.9340

Eisinger, K., Krautbauer, S., Hebel, T., Schmitz, G., Aslanidis, C., Liebisch, G., & Buechler, C. (2014). Lipidomic analysis of the liver from high-fat diet induced obese mice identifies changes in multiple lipid classes. *Experimental and Molecular Pathology*, *97*(1), 37–43. https://doi.org/10.1016/j.yexmp.2014.05.002

Enright, E. F., Joyce, S. A., Gahan, C. G. M., & Griffin, B. T. (2017). Impact of gut microbiota-mediated bile acid metabolism on the solubilization capacity of bile salt micelles and drug solubility. *Molecular Pharmaceutics*, *14*(4), 1251–1263. https://doi.org/10.1021/acs.molpharmaceut.6b01155

Federico, A., Dallio, M., Godos, J., Loguercio, C., & Salomone, F. (2016). Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: Translational and clinical evidence. *Translational Research*, *167*(1), 116–124. https://doi.org/10.1016/j.trsl.2015.08.002

Fki, I., Bouaziz, M., Sahnoun, Z., & Sayadi, S. (2005). Hypocholesterolemic effects of phenolic-rich extracts of Chemlali olive cultivar in rats fed a cholesterol-rich diet. *Bioorganic & Medicinal Chemistry*, *13*(18), 5362–5370. https://doi.org/10.1016/j.bmc.2005.05.036

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509.

Guo, W. X., Shu, Y., & Yang, X. P. (2016). Tea dietary fiber improves serum and hepatic lipid profiles in mice fed a high cholesterol diet. *Plant Foods for Human Nutrition*, *71*(2), 145–150. https://doi.org/10.1007/s11130-016-0536-7

Han, S., Jiao, J., Zhang, W., Xu, J., Wan, Z., Zhang, W., … Qin, L. (2015). Dietary fiber prevents obesity-related liver lipotoxicity by modulating sterol-regulatory element binding protein pathway in C57BL/6 J mice fed a high-fat/cholesterol diet. *Scientific Reports*, *5*, 15256. https://doi.org/10.1038/srep15256

Hong, C., & Tontonoz, P. (2014). Liver X receptors in lipid metabolism: Opportunities for drug discovery. *Nature Reviews Drug Discovery*, *13*(6), 433–444. https://doi.org/10.1038/nrd4280

Huang, P. L. (2009). A comprehensive definition for metabolic syndrome. *Disease Models & Mechanisms*, *2*(5-6), 231–237. https://doi.org/10.1242/dmm.001180

Ioannou, G. N. (2016). The role of cholesterol in the pathogenesis of NASH. *Trends in Endocrinology and Metabolism*, *27*(2), 84–95. https://doi.org/10.1016/j.tem.2015.11.008

Jeong, W., Jeong, D. H., Do, S. H., Kim, Y. K., Park, H. Y., Kwon, O. D., … Jeong, K. S. (2005). Mild hepatic fibrosis in cholesterol and sodium cholate diet-fed rats. *Journal of Veterinary Medical Science*, *67*(3), 235–242. https://doi.org/10.1292/jvms.67.235

Jo, S. P., Kim, J. K., & Lim, Y. H. (2014). Antihyperlipidemic effects of stilbenoids isolated from *Morus alba* in rats fed a high-cholesterol diet. *Food and Chemical Toxicology*, *65*, 213–218. https://doi.org/10.1016/j.fct.2013.12.040

Jung, J. H., & Kim, H. S. (2013). The inhibitory effect of black soybean on hepatic cholesterol accumulation in high cholesterol and high fat diet-induced non-alcoholic fatty liver disease. *Food and Chemical Toxicology*, *60*, 404–412. https://doi.org/10.1016/j.fct.2013.07.048

Kirpich, I. A., Parajuli, D., & McClain, C. J. (2015). The gut microbiome in NAFLD and ALD. *Clinical Liver Disease*, *6*(3), 55–58. https://doi.org/10.1002/cld.494

Lassailly, G., Caiazzo, R., Pattou, F., & Mathurin, P. (2016). Perspectives on treatment for nonalcoholic steatohepatitis. *Gastroenterology*, *150*(8), 1835–1848. https://doi.org/10.1053/j.gastro.2016.03.004

Lin, Y.-L., Cheng, C.-Y., Lin, Y.-P., Lau, Y.-W., Juan, I. M., & Lin, J.-K. (1998). Hypolipidemic effect of green tea leaves through induction of antioxidant and Phase II enzymes including superoxide dismutase, catalase, and glutathione S-transferase in rats. *Journal of Agricultural and Food Chemistry*, *46*(5), 1893–1899. https://doi.org/10.1021/jf970963q

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, *47*(3), 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Martinello, F., Soares, S. M., Franco, J. J., Santos, A. C., Sugohara, A., Garcia, S. B., … Uyemura, S. A. (2006). Hypolipemic and antioxidant activities from Tamarindus indica L. pulp fruit extract in hypercholesterolemic hamsters. *Food and Chemical Toxicology*, *44*(6), 810–818. https://doi.org/10.1016/j.fct.2005.10.011

Mendoza-Sánchez, M., Pérez-Ramírez, I. F., Wall-Medrano, A., Martinez-Gonzalez, A. I., Gallegos-Corona, M. A., & Reynoso-Camacho, R. (2019). Chemically induced common bean (Phaseolus vulgaris L.) sprouts ameliorate dyslipidemia by lipid intestinal absorption inhibition. *Journal of Functional Foods*, *52*, 54–62. https://doi.org/10.1016/j.jff.2018.10.032

Musso, G., Gambino, R., & Cassader, M. (2013). Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Progress in Lipid Research*, *52*(1), 175–191. https://doi.org/10.1016/j.plipres.2012.11.002

Na, H.-K., & Surh, Y.-J. (2008). Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food and Chemical Toxicology*, *46*(4), 1271–1278. https://doi.org/10.1016/j.fct.2007.10.006

Natal, D. I. G., Rodrigues, K. C. D., Moreira, M. E. D., de Queiroz, J. H., Benjamin, L. D., dos Santos, M. H., … Martino, H. S. D. (2017). Bioactive compounds of the Uba mango juices decrease inflammation and hepatic steatosis in obese Wistar rats. *Journal of Functional Foods*, *32*, 409–418. https://doi.org/10.1016/j.jff.2017.03.023

Nguyen, T., Nioi, P., & Pickett, C. B. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *Journal of Biological Chemistry*, *284*(20), 13291–13295. https://doi.org/10.1074/jbc.R900010200

Ojo, B., El-Rassi, G., Perkins-Veazie, P., Clarke, S., Smith, B. J., & Lucas, E. A. (2016). Mango supplementation prevents gut microbial dysbiosis and modulates short chain fatty acid production independent of body weight reduction in C57BL/6 mice fed a high fat diet. *The FASEB Journal*, *30*(1_supplement), 1166-6. https://doi.org/10.1096/fasebj.30.1_supplement.1166.6

Orena, S., Owen, J., Jin, F., Fabian, M., Gillitt, N. D., & Zeisel, S. H. (2015). Extracts of fruits and vegetables activate the antioxidant response element in IMR-32 cells. *The Journal of Nutrition*, *145*(9), 2006–2011. https://doi.org/10.3945/jn.115.216705

Osman, O. S., Selway, J. L., Kępczyńska, M. A., Stocker, C. J., O'Dowd, J. F., Cawthorne, M. A., … Langlands, K. (2013). A novel automated image analysis method for accurate adipocyte quantification. *Adipocytes*, *2*(3), 160–164. https://doi.org/10.4161/adip.24652

Poudyal, H., Panchal, S. K., Waanders, J., Ward, L., & Brown, L. (2012). Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. *Journal of Nutritional Biochemistry*, *23*(2), 153–162. https://doi.org/10.1016/j.jnutbio.2010.11.011

Puri, P., Wiest, M. M., Cheung, O., Mirshahi, F., Sargeant, C., Min, H. K., … Sanyal, A. J. (2009). The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology*, *50*(6), 1827–1838. https://doi.org/10.1002/hep.23229

Quiros-Sauceda, A. E., Chen, C. Y. O., Blumberg, J. B., Astiazaran-Garcia, H., Wall-Medrano, A., & Gonzalez-Aguilar, G. A. (2017). Processing 'ataulfo' mango into juice preserves the bioavailability and antioxidant

capacity of its phenolic compounds. *Nutrients*, *9*(10), 1082. https://doi.org/10.3390/nu9101082

Rinella, M. E. (2015). Nonalcoholic fatty liver disease: A systematic review. *Journal of the American Medical Association*, *313*(22), 2263–2273. https://doi.org/10.1001/jama.2015.5370

Robles-Sanchez, M., Astiazaran-Garcia, H., Martin-Belloso, O., Gorinstein, S., Alvarez-Parrilla, E., de la Rosa, L. A., … Gonzalez-Aguilar, G. A. (2011). Influence of whole and fresh-cut mango intake on plasma lipids and antioxidant capacity of healthy adults. *Food Research International*, *44*(5), 1386–1391. https://doi.org/10.1016/j.foodres.2011.01.052

Salazar-López, N. J., Astiazarán-García, H., González-Aguilar, G. A., Loarca-Piña, G., Ezquerra-Brauer, J.-M., Domínguez Avila, J. A., & Robles-Sánchez, M. (2017). Ferulic acid on glucose dysregulation, dyslipidemia, and inflammation in diet-induced obese rats: An integrated study. *Nutrients*, *9*(7), 675. https://doi.org/10.3390/nu9070675

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, *3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73

Serviddio, G., Bellanti, F., Villani, R., Tamborra, R., Zerbinati, C., Blonda, M., … Iuliano, L. (2016). Effects of dietary fatty acids and cholesterol excess on liver injury: A lipidomic approach. *Redox Biology*, *9*, 296–305. https://doi.org/10.1016/j.redox.2016.09.002

Sharma, S., Rana, S., Patial, V., Gupta, M., Bhushan, S., & Padwad, Y. (2016). Antioxidant and hepatoprotective effect of polyphenols from apple pomace extract via apoptosis inhibition and Nrf2 activation in mice. *Human & Experimental Toxicology*, *35*(12), 1264–1275. https://doi.org/10.1177/0960327115627689

Sutti, S., Jindal, A., Locatelli, I., Vacchiano, M., Gigliotti, L., Bozzola, C., & Albano, E. (2014). Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. *Hepatology*, *59*(3), 886–897. https://doi.org/10.1002/hep.26749

Takahashi, Y., Soejima, Y., & Fukusato, T. (2012). Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World Journal of Gastroenterology*, *18*(19), 2300–2308. https://doi.org/10.3748/wjg.v18.i19.2300

Tall, A. R., & Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. *Nature Reviews Immunology*, *15*(2), 104–116. https://doi.org/10.1038/nri3793

Tirosh, O., Hirsch, N., Konstantinov, A., Anavi, S., Aronis, A., Hagay, Z., & Madar, Z. (2017). OP-17 - Tea extracts-induced liver injury: Lipotoxic interaction between lipids and polyphenols. *Free Radical Biology and Medicine*, *108*(Supplement 1), S8. https://doi.org/10.1016/j.freeradbiomed.2017.04.056

Wall-Medrano, A., De la Rosa, L. A., Vazquez-Flores, A. A., Mercado-Mercado, G., Gonzalez-Arellanes, R., Lopez-Diaz, J. A., … Molina-Corral, F. J. (2017). Lipidomic and antioxidant response to grape seed, corn and coconut oils in healthy wistar rats. *Nutrients*, *9*(1), 82. https://doi.org/10.3390/nu9010082

Wan, C. W., Wong, C. N. Y., Pin, W. K., Wong, M. H. Y., Kwok, C. Y., Chan, R. Y. K., … Chan, S. W. (2013). Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet. *Phytotherapy Research*, *27*(4), 545–551. https://doi.org/10.1002/ptr.4751

Wang, H., Zhu, Y.-Y., Wang, L., Teng, T., Zhou, M., Wang, S.-G., … Sun, Y. (2017). Mangiferin ameliorates fatty liver via modulation of autophagy and inflammation in high-fat-diet induced mice. *Biomedicine & Pharmacotherapy*, *96*, 328–335. https://doi.org/10.1016/j.biopha.2017.10.022

Wang, Q., Du, Z., Zhang, H., Zhao, L., Sun, J., Zheng, X., & Ren, F. (2015). Modulation of gut microbiota by polyphenols from adlay (Coix lacryma-jobi L. var. ma-yuen Stapf.) in rats fed a high-cholesterol diet. *International Journal of Food Sciences and Nutrition*, *66*(7), 783–789. https://doi.org/10.3109/09637486.2015.1088941

Wang, Y. M., Zhang, B., Xue, Y., Li, Z. J., Wang, J. F., Xue, C. H., & Yanagita, T. (2010). The mechanism of dietary cholesterol effects on lipids metabolism in rats. *Lipids in Health and Disease*, *9*, 4. https://doi.org/10.1186/1476-511x-9-4

Wang, Y.-X., Li, Y., Sun, A.-M., Wang, F.-J., & Yu, G.-P. (2014). Hypolipidemic and antioxidative effects of aqueous enzymatic extract from rice bran in rats fed a high-fat and -cholesterol diet. *Nutrients*, *6*(9), 3696–3710. https://doi.org/10.3390/nu6093696

Xu, Z.-R., Li, J.-Y., Dong, X.-W., Tan, Z.-J., Wu, W.-Z., Xie, Q.-M., & Yang, Y.-M. (2015). Apple polyphenols decrease atherosclerosis and hepatic steatosis in ApoE−/− mice through the ROS/MAPK/NF-κB pathway. *Nutrients*, *7*(8), 5324–7105. https://doi.org/10.3390/nu7085324

Yang, L. G., Song, Z. X., Yin, H., Wang, Y. Y., Shu, G. F., Lu, H. X., … Sun, G. J. (2016). Low n-6/n-3 PUFA ratio improves lipid metabolism, inflammation, oxidative stress and endothelial function in rats using plant oils as n-3 fatty acid source. *Lipids*, *51*(1), 49–59. https://doi.org/10.1007/s11745-015-4091-z

Zhang, M., Xie, Z. K., Gao, W. N., Pu, L. L., Wei, J. Y., & Guo, C. J. (2016). Quercetin regulates hepatic cholesterol metabolism by promoting cholesterol-to-bile acid conversion and cholesterol efflux in rats. *Nutrition Research*, *36*(3), 271–279. https://doi.org/10.1016/j.nutres.2015.11.019

Zhong, C.-Y., Sun, W.-W., Ma, Y., Zhu, H., Yang, P., Wei, H., … Song, Z.-Y. (2015). Microbiota prevents cholesterol loss from the body by regulating host gene expression in mice. *Scientific Reports*, *5*, 10512. https://doi.org/10.1038/srep10512

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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