Research Article

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Mango phenolics increase the serum apolipoprotein A1/B ratio in rats fed high cholesterol and sodium cholate diets

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ABSTRACT: BACKGROUND: Serum lipoproteins are in dynamic equilibrium, partially controlled by the apolipoprotein A1 to apolipoprotein B ratio (APOA1/APOB). Freeze-dried mango pulp (FDM) is a rich source of phenolic compounds (MP) and dietary fiber (MF), although their effects on lipoprotein metabolism have not yet been studied.

RESULTS: Thirty male Wistar rats were fed with four different isocaloric diets (3.4 kcal g^{-1}) for 12 weeks: control diet, high cholesterol (8 g kg^{-1}) + sodium cholate (2 g kg^{-1}) diet either alone or supplemented with MF (60 g kg^{-1}), MP (1 g kg^{-1}) or FDM (50 g kg^{-1}). MP and FDM reduced food intake, whereas MF and MP tended to increase serum APOA1/APOB ratio, independently of their hepatic gene expression. This suggests that lipoprotein metabolism was favorably altered by mango bioactives, MP also mitigated the non-alcoholic steatohepatitis that resulted from the intake of this diet.

CONCLUSION: We propose that phenolics are the most bioactive components of mango pulp, acting as anti-atherogenic and hepatoprotective agents, with a mechanism of action tentatively based on changes to the main protein components of lipoproteins.

Keywords: cholesterol; lipoprotein; mango; liver; rat; hepatoprotection

INTRODUCTION

The long-term consumption of Western diets alters several metabolic and genetic processes involved in cholesterol homeostasis. Hypercholesterolemia is mostly characterized by an increased serum concentration of low-density lipoprotein (LDL) concurrent with a decrease in high-density lipoproteins (HDL),[1] altering the well-defined threshold for total cholesterol (200 mg dL^{-1}).[2] However, the serum cholesterol concentration is not a static value because the complex pool of apolipoproteins is constantly modified by the amount of dietary cholesterol, its intestinal absorption/excretion and its hepatic/peripheral metabolism. Hypercholesterolemia is a risk factor for cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD)[3] and non-alcoholic steatohepatitis (NASH).[4,5] However, the analytical assessment of total cholesterol and lipoprotein concentration should be complemented with that of apolipoproteins A1(APOA1) and B (APOB) because their ratio is a valuable short-term[6] and long-term[7] predictor for many cardiometabolic illnesses, even beyond the clinical value of the HDL/LDL ratio.

An adequate diet that favors a high daily intake of fruits and vegetables can exert significant effects on cholesterol homeostasis. Hypercholesterolemia and other dyslipidemias can be mitigated with a daily intake of phytochemicals, including dietary fiber (soluble and insoluble) and phenolic compounds (monomeric and polymeric). The role of dietary fiber with respect to preventing and even treating CVD has been documented for many years,[1,2] although recent studies have also recognized an inverse correlation between a consistent intake of dietary phenolics and the development/progression of chronic diseases, such as CVD[8] and NAFLD.[9]

Mango (*Mangifera indica* L.) is a tropical fruit that is grown and consumed in many countries worldwide. Certain varieties (e.g. 'Ataulfo') are rich sources of dietary fiber and antioxidant phytochemicals (e.g. phenolic compounds) whose bioactivity has been demonstrated *in vitro*, *ex vivo* and *in vivo*.[10,11] As hypolipidemic agents, we have previously reported that a daily intake (200 g day^{-1}) of fresh-cut 'Ataulfo' mango for 1 month reduces serum triacylglycerol (TAG) and very low-density lipoprotein (VLDL) levels in healthy adults[12]; other benefits of mango

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phytochemicals include anti-inflammatory effects,[13] as well as others that mitigate obesity, diabetes and CVD, and induce improvements in brain, skin and intestinal health.[14] The selective bioaccessibility/bioavailability of mango phenolics[15,16] and the fermentative behavior[17] of both phenolics and fiber may have synergistic effects with respect to improving gastrointestinal and overall health.

However, the effects of mango consumption have not been documented on lipoprotein metabolism in organisms fed diets rich in cholesterol; furthermore, the precise effects of isolated fiber and phenolics are also lacking. The present study aimed to evaluate the effects of isolated mango fiber (MF), mango phenolics (MP) and freeze-dried mango pulp (FDM) on the serum lipid profile, serum apolipoproteins and hepatic mRNA expression of genes involved in the lipoprotein metabolism of male Wistar rats fed a high cholesterol/sodium cholate diet (HCC) during a 12-week period.

MATERIALS AND METHODS

Extraction and characterization of mango fractions

Ripe mangoes cv 'Ataulfo' (maturity stage 4[10]) were obtained from a local distributor in Hermosillo, Mexico (29.07°N, 110.95°W), then washed with a mild soap solution to remove excess dirt, and dried at room temperature for 15 min. The fruits were peeled with a sharp knife, and the edible pulp was separated from the skin and seed, both of which were properly discarded. The pulp was frozen at −80 °C for 24 h and freeze-dried for 72 h (FreeZone 6; Labconco, Kansas City, MO, USA) with a pressure of 0.03 mbar, and collector temperature of −50 °C. The recovered material was blended in a commercial blender to obtain a fine powder (FDM).

MF was obtained from FDM as reported previously.[18] To eliminate soluble matter, FDM was dissolved (1:5 w/v) in ethanol/water (80/20 v/v) and vacuum-filtered. The retained material was re-extracted twice under the same conditions, and left to dry overnight in open containers, in the dark at room temperature. Hydrophobic substances were then extracted with *n*-hexane (1:3 w/v) and vacuum-filtered; the process was repeated twice. MF was left to dry overnight in open containers, in the dark at room temperature. MF was then blended to obtain a fine powder, and stored at −35 °C in dark bags until analysis. The percentage of soluble and insoluble fiber was determined using a commercial kit (Megazyme, Wicklow, Ireland) in accordance with the manufacturer's instructions, whereas the neutral sugar composition of each fiber fraction was determined by gas chromatography as described previously.[19]

Extractable MP was obtained as previously described.[20] As a result of the high sugar content of FDM (approximately 90%), it was washed three times with aqueous ethanol as described for MF. Sugar-free matter was dissolved in aqueous ethanol in a 1:5 w/v ratio and sonicated for 20 min, maintaining a low temperature (5–10 °C) by adding ice to the sonicator apparatus. The mixture was filtered under vacuum, and the procedure was repeated a second time. Ethanol was eliminated by rotary evaporation (Yamato RE-200; Yamato Inc., Tokyo, Japan) at 40 °C. The ethanol-free samples were pooled and freeze-dried as described for FDM. The dry extracts were stored at −35 °C in dark bags until their incorporation into the diets.

Diets and bioassay

The research protocol was reviewed and approved by the Animal Experimentation Ethics Committee of the Research Centre for Food and Development (CIAD; Case Nº. CE/004/2016). Animals were handled in accordance with the NIH guide for the care and use of laboratory animals.[21] Five isocaloric diets (3.4 kcal g^{-1}) were prepared: a control diet; a high-cholesterol (8 g kg^{-1}) + sodium cholate (2 g kg^{-1}) diet alone (HCC), or supplemented with 60 g kg^{-1} MF (HCC + MF); 1 g kg^{-1} MP (HCC + MP); or 50 g kg^{-1} FDM (HCC + FDM). The doses of phenolics, fiber and mango pulp were chosen based on previous studies,[22,23] and the final amount of FDM incorporated into HCC + FDM diet was carefully chosen to avoid macronutrient (namely fiber, monosaccharides and dextrin) imbalances beyond optimal requirements for albino rodents of this age, whereas the individual concentrations of MP and MF in their corresponding diets were adjusted to that level reached in the HCC + FDM diet for the purpose of inter-diet comparisons.

The concentration of phenolics in the HCC + MP diet was 300, 100, 24 and 1.1 mg kg^{-1} of diet for chlorogenic acid, gallic acid, vanillic acid and protocatechuic acid, respectively.[10,24] Diets were prepared a few days before the arrival of the animals under strict hygienic conditions, and stored in individual bags at −20 °C until use. Table 1 provides further details of the composition of all experimental diets.

Thirty male Wistar rats (166 g) were individually housed in steel cages under standard environmental conditions (12/12 h light/dark cycle at 25 ± 1 °C), with *ad libitum* access to food and water. The animals consumed the control diet during the acclimatization period (1 week) and were then randomly assigned to one of five experimental diets ($n = 6$) for another 12 weeks. Food that was not consumed was recovered, weighed and replaced daily. The animals were weighed weekly in a three-beam balance with a metal cage. After the experimental period concluded, all animals were fasted overnight and anesthetized with an intraperitoneal dose of sodium pentobarbital (200 mg kg^{-1} body weight; Pisabental, PISA Agropecuaria, Atitalaquia, Hidalgo, Mexico). Once fully sedated, an incision was performed to expose the thoracic and abdominal cavities, and blood was withdrawn by cardiac puncture. Heart, liver, kidneys and testicles were collected, weighed and stored at −80 °C. Organosomatic indices were calculated by dividing the weight of each organ (liver, heart, kidneys and testicles) by the final body weight of the animal. A small section of the liver was separated for further histological analyses.

Serum biochemistry

A drop of whole blood was used to determine glycemia with a commercial glucose meter (Onetouch Ultra Mini; LifeScan Inc., Milpitas, CA, USA) and the remaining blood collected was centrifuged at 3000 × *g* for 10 min. Serum was recovered for quantification of the concentrations of total cholesterol (TC), TAG and HDL using commercially-available colorimetric kits (#1010-430 for TC; #2100-430 for TAG; and #0590-040 for HDL; Stanbio, Boerne, TX, USA). LDL was calculated with the Friedewald equation[25]: LDL TC − HDL (TAG/5).

Serum was also used to quantify enzyme activity of aspartate and alanine transaminases [AST (EC number 2.6.1.1) and ALT (EC number 2.6.1.2), respectively], γ-glutamyl transpeptidase [GGT (EC number 2.3.2.2)] and alkaline phosphatase [ALP (EC number 3.1.3.1)], using colorimetric kits (#2930-430 for AST; #2920-430 for ALT; #2960-430 for GGT; and #2900-430 for ALP; Stanbio). All parameters were determined in triplicate, whereas the serum concentrations of apolipoprotein A1 (APOA1) and B (APOB) were determined in duplicate using enzyme-linked immunosorbent assay (ELISA)-based kits (LS-F4180 for APOA1 and LS-F4843 for APOB; LifeSpan BioSciences, Seattle, WA, USA). Apolipoprotein

Table 1. Composition of the experimental diets (g kg^{-1})

Ingredient	Diet				
	Control	HCC	HCC + MF	HCC + MP	HCC + FDM
Corn starch	520	520	520	520	520
Sucrose	50	50	50	50	–
Cholesterol[1]	–	8	8	8	8
Sodium cholate[2]	–	2	2	2	2
Vegetable lard	30	30	30	30	30
Corn oil	30	30	30	30	30
Casein[3]	210	210	210	210	210
Vitamin mix[4]	18	18	18	18	18
Salt mix[5]	60	60	60	60	60
Cellulose[6]	60	60	–	60	59.5
Choline chloride[7]	2	2	2	2	2
Mango fiber (MF)[8]	–	–	60	–	–
Mango phenolics (MP)[8,9]	–	–	–	1	–
Freeze-dried mango pulp (FDM)[8,10]	–	–	–	–	50
Water	20	10	10	9	10.5

All diets provided 64% of gross energy from carbohydrates, 16% from lipids and 20% from protein. Bio-Serv (5160[1]; 1100[3]; F800[4]; F8505[5]; 3425[6]; 6105[7]),[2] Sigma-Aldrich (C1254),[8] obtained as described in the Materials and methods,[9] contained chlorogenic acid (300 mg kg^{-1} of diet), gallic acid (100 mg kg^{-1} of diet), vanillic acid (24 mg kg^{-1} of diet) and protocatechuic acid (1.1 mg kg^{-1} of diet).[10] FDM substituted sucrose as a result of its high concentration of sugars (93% gross energy). HCC, high cholesterol/sodium cholate; MF, mango fiber; MP, mango phenolics.

concentration was calculated with the five-parameter logistic regression method.

Hepatic histology

Individual liver samples ($n = 5–6$ per diet) were divided into two sections, fixed in 10% formaldehyde in phosphate-buffered saline and embedded in paraffin blocks. The blocks were sliced into semi-serial sections of 3 µm in thickness, and stained with hematoxylin and eosin. All evaluations were performed in a fluorescence microscope (DM2000; Leica Microsystems Inc., Chicago, IL, USA), images (20× and 63× magnification) were captured with a charge-coupled device camera and processed in the LEICA V2-program.

Hepatic gene expression

Total RNA was extracted from a liver sample with the TriReagent (Sigma-Aldrich, St Louis, MO, USA) in accordance with the manufacturer's instructions. The RNA obtained was treated with RNAse-free DNAse (Promega, Madison, WI, USA) and reverse transcribed (Promega) using oligo(dT)$_{15}$ in accordance with the manufacturer's instructions. cDNA was used to evaluate the relative expression of four genes by a real-time polymerase chain reaction using the probes (gene; GenBank; TaqMan™ assay; fluorophore): low-density lipoprotein receptor (LDLR; X13722.1; Rn00598442_m1; FAM), scavenger receptor class B member 1 (SCARB1; NM_031541.1; Rn00580588_m1; FAM), apolipoprotein A1 (APOA1; NM_012738.1; Rn00562483_g1; FAM), apolipoprotein B (APOB; NM_019287.2; Rn01499054_m1; FAM) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; NM_017008.4; Rn99999916_s1; VIC). All reactions were performed in triplicate in a StepOne thermal cycler (Applied Biosystems, Waltham, MA, USA), using 2.5 ng of cDNA in a total reaction volume of 10 µL. The gene of interest and the reference gene (GAPDH) were simultaneously amplified in each well (duplex reactions). The relative expression of each gene was calculated using the $2^{-\Delta\Delta CT}$ method.[26]

Statistical analysis

All data were tested for normality with an Anderson–Darling test; parametric data were subjected to analysis of variance (ANOVA) with Tukey's test, whereas non-parametric data were analyzed with the Kruskal–Wallis test, with both employing Minitab, version 18 (Minitab Inc., State College, PA, USA). $P < 0.05$ was considered statistically significant. Data are expressed as the mean ± SEM.

RESULTS AND DISCUSSION

Mango pulp and sub-fractions

FDM usually contains 26 (45%) and 32 (55%) g kg^{-1} DW of soluble and insoluble dietary fiber, respectively.[16] In the present study, MF isolated from FDM was predominantly soluble (approximately 69%) made up of arabinose and galactose, whereas its insoluble fraction (approximately 31%) was composed of xylose > arabinose > galactose (Fig. 1). These differences in composition are a result of not only differences in the analytical methods used, but also the presence of other non-digestible polysaccharides (e.g. resistant starch), which is also common in FDM.[16] Nevertheless, similar results have been reported for MF extracted from mango paste,[27] a by-product of the 'Ataulfo' mango juice industry,[15] whereas the MF composition was also reported previously.[19] The arabinogalactan-type and other α-/β-type hetero-polysaccharides, with or without covalently bound small sugars, are typical of mango pectins.[28] Both fractions, soluble and insoluble, bind cholesterol and bile salts, lessening their intestinal absorption, comprising a mechanism associated with the protective effect of mangoes against dyslipidemias.[29]

The content of methanol:water-extractable (80:20 v/v) and non-extractable (hydrolyzable tannins) phenolics in FDM is 144 and 130 mg EAG 100 g^{-1}.[16] According to previous studies,[10,24] the isolated MP fraction used in the present study is composed of four major phenolic acids (chlorogenic, gallic, vanillic and protocatechuic acids), as analyzed by high-performance liquid

Table 2. Final parameters of rats fed experimental diets for 12 weeks

Variable	Control	HCC	HCC + MF	HCC + MP	HCC + FDM
Daily food intake (NP)	23.0 ± 0.2 a	22.0 ± 0.1 b	22.2 ± 0.1 b	21.4 ± 0.1 c	21.1 ± 0.2 c
Final body weight (P)	377.8 ± 21.4	375.3 ± 5.8	373.7 ± 19.3	370.1 ± 13.4	360.6 ± 14.1
Liver weight (P)	10.3 ± 0.7 b	16.4 ± 0.9 a	13.7 ± 0.9 ab	14.8 ± 1.4 a	15.2 ± 1.0 a
Hepatosomatic index (1, P)	28.1 ± 3.0 b	43.6 ± 2.5 a	36.3 ± 1.7 ab	39.7 ± 2.6 a	41.9 ± 1.7 a
Heart weight (P)	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Cardiosomatic index (1, P)	3.1 ± 0.1	3.2 ± 0.0	3.2 ± 0.0	3.2 ± 0.2	3.4 ± 0.2
Kidney weight (P)	2.1 ± 0.2	2.4 ± 0.0	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.1
Nephrosomatic index (1, P)	5.6 ± 0.5	6.3 ± 0.2	6.5 ± 0.3	6.4 ± 0.5	6.6 ± 0.2
Testicle weight (NP)	3.3 ± 0.3	3.5 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	3.3 ± 0.1
Gonadosomatic index (1, NP)	8.9 ± 0.1	9.2 ± 0.0	9.3 ± 0.4	9.1 ± 0.4	9.2 ± 0.2

Data are expressed as the mean ± SEM (g). Different lowercase letters within the same row indicate significant differences ($P < 0.05$, $n = 6$ per group), as determined by a one-way ANOVA test for parametric data (P) or with the Kruskal–Wallis for non-parametric data (NP). FDM, freeze-dried mango pulp; HCC, high cholesterol/sodium cholate; MF, mango fiber; MP, mango phenolics. [1]Value × 10^{-3}.

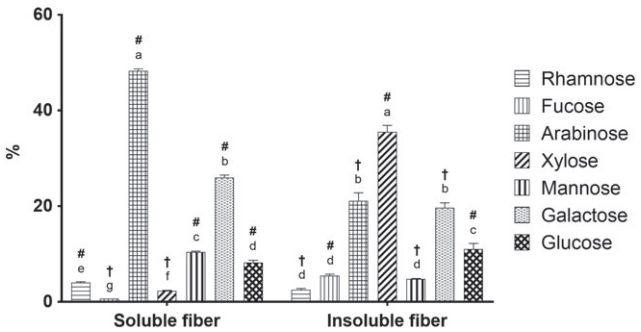

Figure 1. Neutral sugar composition of mango soluble and insoluble fiber. Different symbols indicate significant differences ($P < 0.05$, $n = 9$) for a particular sugar between fiber fractions, as determined by one-way ANOVA for parametric data (rhamnose, galactose and glucose) or the Kruskal–Wallis test for nonparametric data (fucose, arabinose, xylose and mannose). Different lowercase letters indicate significant differences ($P < 0.05$, $n = 9$) between sugars for the same fiber fraction as determined by the Kruskal–Wallis test.

chromatography with diode-array detection and mass spectrometry, after eliminating free sugars. It is well-known that fruit phenolics play key roles with respect to preventing and even treating several inflammatory- and dyslipidemia-related diseases,[30] although gallic acid, the most potent anti-oxidant molecule in both FDM and MP, has its own hypolipidemic[31] and hepatoprotective[32] mechanisms, including radical scavenging, lipid peroxidation inhibition and enhancement of bile acid metabolism.

Bioassay performance

The non-supplemented HCC diet intended to develop an atherogenic/NASH rodent model, whereas the concurrent supplementation of HCC diet with different mango fractions is useful for discriminating between the specific bioactivity of MF (intestinal level), MP (hepatic/systemic level), or both (FDM), with respect to lipoprotein metabolism in rats. Table 2 shows that rats fed HCC + MP and HCC + FDM diets consumed 0.9 g day^{-1} less food than HCC or HCC + MF fed rats ($P < 0.05$), which also means a lower intake of cholesterol (7.2 × 10^{-3} mg) and sodium cholate (1.8 × 10^{-3} mg) in both groups. Such an anorectic effect was also reported by Natal *et al.*,[33] who administered two mango cv 'Uba' juice to obese/hypercholesterolemic rats.

Dietary fiber and phenolic compounds are known to induce satiety by different mechanisms. Short-term satiety is exerted through delayed gastric emptying (mainly by soluble fiber), by altering the viscosity of the alimentary bolus at gastric level.[34] Satiety also occurs by modulating secretion of gastric inhibitory polypeptide and glucagon-like peptide-1 (short-term),[35] as well as leptin (long-term).[36] Modifying the gut–brain axis signals by actions of the gut microbiota is a complementary method that can exert satiety.[37] All of these mechanisms have been reported previously, and may be responsible for the described changes observed in the present study.

Hepatic functionality/histology

The liver of control (no HCC) rats presented an even brick-red color, healthy appearance and weight, with a hepatosomatic index (HSI) of 30 × 10^{-3} at the end of the study. Conversely, HCC-fed rats showed hepatomegaly (HSI = 40–50 × 10^{-3}), inflammation and a grey hue; in addition, although not statistically significant, a mild effect on improving the HSI was observed in rats fed HCC + MF compared to those fed with HCC + FDM and HCC + MP diets. Rodriguez-Gonzalez *et al.*[38] reported a lower nephrosomatic index in diabetic (streptozotocin-induced) Wistar rats when given a mixed mango 'Ataulfo' by-product (peel + pulp) for 3 weeks.

Hepatotoxicity and hepatomegaly are often seen in albino rats fed high-cholesterol and/or high-fat diets, which ultimately progresses to NASH, when increasing the percentage of cholesterol or bile salts; liver fibrosis increases even more leading to hepatic cirrhosis[4] and biliary tree dysfunction.[39,40] Evidence from the present study confirms the development of NASH and hepatic dysfunction after the sub-chronic intake of an HCC diet, whereas mixed ameliorating effects on hepatic enzymes occurred when this diet was supplemented with MF, MP and FDM (Table 3).

Hepatic histology (Fig. 2) revealed distorted lobules, fat vacuolation (macro- and micro-vesicular), hepatocyte ballooning and inflammatory cell infiltrates in a diet-specific manner (HCC > supplemented diets). All of these signs are consistent with different grades of NASH,[41] which was further confirmed by the increased serum activity of hepatic enzymes shown in Table 3, except for the AST/ALT ratio, which is indicative of drug-induced liver injury.[42] Jeong *et al.*[4] fed Wistar rats (weighing approximately 155 g) a similar HCC diet (10 g kg^{-1} cholesterol; 3 g kg^{-1} sodium cholate) and feeding protocol to that used in the present study, and reported the following signs of hepatic injury: high plasma

Table 3. Serum biochemistry of rats fed high cholesterol/sodium cholate diets for 12 weeks

Variable	Control	HCC	HCC + MF	HCC + MP	HCC + FDM
Glucose (mg dL^{-1}) (P)	178.7 ± 20.2	134.5 ± 16.1	215.0 ± 25.8	155.5 ± 14.3	168.5 ± 21.7
Total cholesterol (TC; mg dL^{-1}) (P)	77.6 ± 4.7	89.1 ± 6.0	79.2 ± 6.3	72.7 ± 5.5	95.4 ± 8.9
LDL-cholesterol (mg dL^{-1}) (P)	43.9 ± 2.9	47.4 ± 4.8	48.1 ± 6.8	43.0 ± 5.6	66.9 ± 11.3
HDL-cholesterol (mg dL^{-1}) (NP)	14.5 ± 1.5	26.1 ± 10.4	14.3 ± 1.0	39.7 ± 19.5	18.6 ± 3.2
TC/HDL ratio (P)	5.6 ± 0.5	4.9 ± 1.4	5.6 ± 0.6	3.6 ± 0.9	4.3 ± 0.6
HDL/LDL ratio (P)	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
Triacylglycerols (TAG; mg dL^{-1}) (NP)	119.9 ± 16.9	127.2 ± 13.3	106.2 ± 9.7	90.3 ± 8.4	94.2 ± 8.2
Aspartate aminotransferase (AST; IU L^{-1}) (P)	86.1 ± 11.4^{b}	128.1 ± 11.5^{ab}	128.2 ± 10.4^{ab}	135.7 ± 10.4^{a}	141.5 ± 9.1^{a}
Alanine aminotransferase (ALT; IU L^{-1}) (NP)	44.2 ± 9.2^{c}	65.4 ± 6.0^{ab}	103.8 ± 19.5^{a}	52.5 ± 14.6^{bc}	94.5 ± 11.3^{a}
AST/ALT ratio (NP)	3.71 ± 1.06^{ab}	2.22 ± 0.30^{b}	2.35 ± 0.36^{b}	4.64 ± 0.57^{a}	3.55 ± 1.16^{ab}
Alkaline phosphatase (ALP, IU L^{-1}) (P)	38.2 ± 1.4^{b}	66.1 ± 2.9^{a}	61.4 ± 4.6^{a}	58.7 ± 2.8^{a}	68.1 ± 2.4^{a}
γ-Glutamyl transpeptidase (GGT, IU L^{-1}) (NP)	7.0 ± 0.7^{ab}	10.9 ± 3.9^{ab}	5.4 ± 0.9^{ab}	7.4 ± 0.7^{a}	4.4 ± 0.9^{b}

Data are expressed as the mean ± SEM (g). Different lowercase letters within the same row indicate significant differences ($P < 0.05$, $n = 6$ per group), as determined by a one-way ANOVA test for parametric data (P) or with the Kruskal–Wallis for non-parametric data (NP). FDM, freeze-dried mango pulp; HCC, high cholesterol/sodium cholate; MF, mango fiber; MP, mango phenolics.

levels of hepatic enzymes (AST and ALT), hypercholesterolemia, macro-vesicular lipid droplets and mild hepatic fibrosis. It is noteworthy that hepatic histology of the HCC + MP group (Fig. 2E) was more similar to the control (Fig. 2F) than that of the HCC + FDM (Fig. 2C) group or the HCC + FM (Fig. 2D) group, indicating a partial amelioration of hepatic injury. Similar results have been reported by Natal *et al.*[33] in hypercholesterolemic-obese rats fed with mango cv 'Uba' juices rich in phenolics, as well as by Leontowicz *et al.*,[43] who co-administered 5% freeze-dried kiwifruit (different cultivars) in high-cholesterol (10 g kg^{-1})/moderate fat (100 g kg^{-1}) diets to Wistar rats.

Serum biochemistry

According to Table 3, serum lipids and glucose levels did not differ significantly ($P > 0.05$) among all of the dietary groups because the organosomatic (other than hepatosomatic) and atherogenic (TC/HDL and HDL/LDL) ratios did not differ either, which suggests that the liver played an important role in protecting peripheral organs from a potential cholesterol/sodium cholate overdose, possibly by controlling hepatic influx/efflux, deposition and phase I/II metabolism of cholesterol. In support of this, C57BL/6 mice fed laboratory chow (14% kcal from fat), high-fat (approximately 60% kcal from fat), western-type (42% kcal from fat/2 g kg^{-1} cholesterol) and atherogenic (43% kcal from fat/13 g kg^{-1} cholesterol/5 g kg^{-1} cholic acid) diets, for 3 weeks, presented diet-specific patterns of expression of phase I drug metabolizing[44] and phase II conjugation[45] enzymes, as well as certain xenobiotic transporters,[46] demonstrating the delicate plasticity of this organ with respect to the noted dietary offences.

Although the differences in serum lipids were not statistically significant, the HCC + MP group tended to increase HDL (Table 3) and reduce TC, LDL-cholesterol and TAG levels. Accordingly, we have previously reported that a short term (1 month) daily intake (200 g day^{-1}) of fresh-cut mango cv 'Ataulfo' pulp reduces serum TAG (−38%) and VLDL (−35%), with no apparent effects on TC in normolipidemic adults.[12] Selective hypolipidemic effects have been also observed in rats fed high-cholesterol diets supplemented with an aqueous garlic extract,[47] or with an acidic-methanol yerba mate extract.[48] We theorized at this point that MP may have exerted an effect on serum lipoprotein turnover and clearance (e.g. HDL functionality), although this was not revealed by evaluating the overall serum lipid concentrations.

From an analytical stand point, ELISA-based apolipoprotein assays are by far more specific and sensitive than colorimetric-based lipoprotein assays. Furthermore, direct apolipoprotein quantification is more closely related to the number of discrete lipoprotein particles, considering that non-HDL contains a single copy of APOB,[49] whereas HDL contains two to four copies of APOA1.[50,51] We therefore evaluated the serum protein concentrations of APOA1 and APOB, as well as their ratio (Fig. 3). The HCC + MP group had a significantly higher APOA1 concentration, a lower APOB concentration and a higher APOA1/APOB ratio compared to that of the control. The HCC, HCC + MF and HCC + FDM groups showed no statistical differences in these variables, and their values were intermediate to the control and HCC + MP groups. APOA1 has been classically considered the main structural and functional HDL-related apolipoprotein, with a particular role on reverse cholesterol transport (RCT)[52] and innate immunity.[53] Accumulated evidence suggests that the size and composition of HDL particles is not as rigid as previously assumed. For example, there is a wide range of HDL particle sizes with different APOA1-to-lipid ratios (cholesteryl esters, TAGs, phospholipids) and certain HDL populations that lack APOA1 and, instead, contain APOE. Furthermore, increasing the number of APOA1-containing HDL particles is a novel paradigm for improving HDL-mediated RCT,[54] instead of relying only on the total HDL cholesterol concentration or the HDL/LDL ratio, as has been performed classically. Accordingly, changes in the APOA1/APOB ratio exerted by MP may indicate that they increased the number of HDL particles and, theoretically, decreased their lipid-to-protein ratios, comprising a potential anti-atherogenic effect.

Lipoprotein production and assembly and its role in RCT are complex processes that are intimately orchestrated at several metabolomic levels. Serum cholesterol is transported within non-HDL lipoproteins, including LDL, from the liver to the periphery, whereas HDL transports it from peripheral tissues to the liver (RCT), where it is metabolized into bile salts to be eliminated through the biliary tree. It is likely that MP may have promoted a more efficient HDL turnover (e.g. by exerting effects on HDL assembly or peripheral uptake), although this needs to be confirmed in a follow-up study.

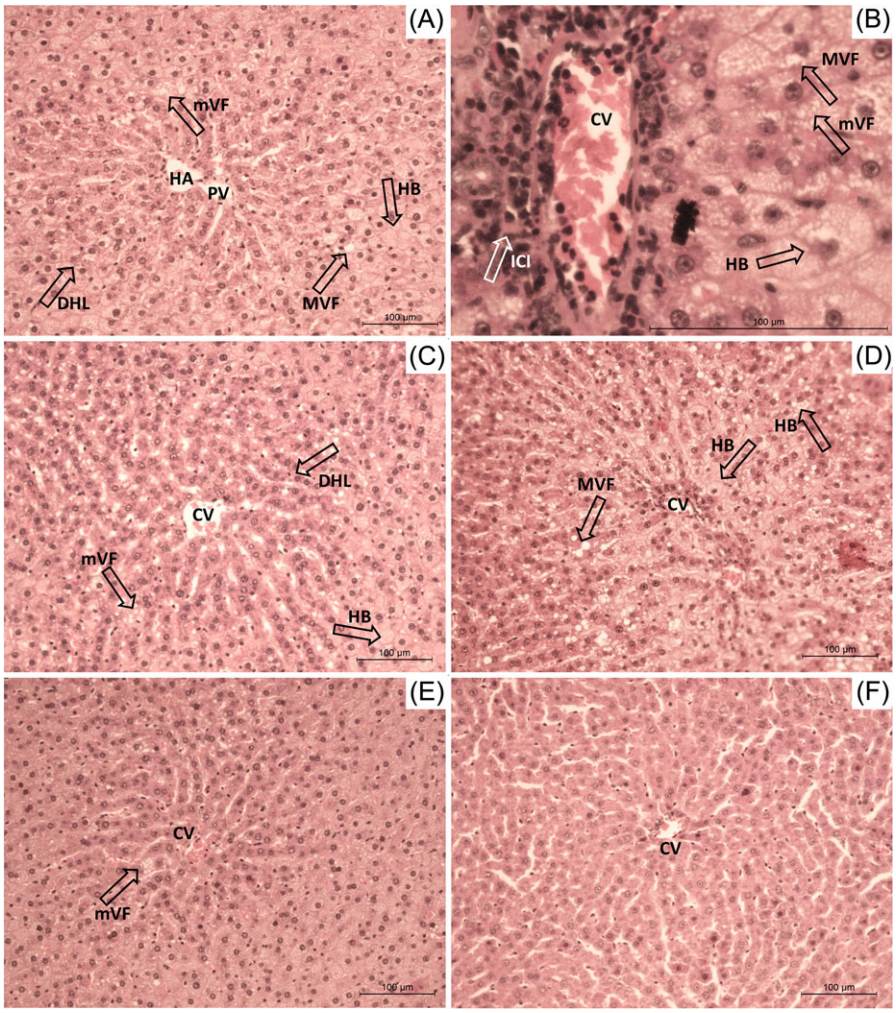

Figure 2. Histological evaluation of liver tissue. Photographs at 63× (B) and 20× (rest) magnification. Diets: high cholesterol/sodium cholate (HCC; A, B); HCC + freeze-dried mango pulp (HCC + FDM; C), HCC + mango fiber (HCC + MF; D), HCC + mango phenolics (HCC + MP; E) and control (F). Central vein (CV), hepatic portal vein (PV), hepatic artery (HA), distorted hepatic lobules (DHL), microvesicular fat vacuoles (mVF), macro-vesicular fat vacuoles (MVF), hepatocyte ballooning (HB) and inflammatory cell infiltrate (ICI).

Gene expression

The identity, physiological role and control of each lipoprotein particle partially depends on its protein composition: apolipoprotein A1 (APOA1) in HDL and B (APOB) in non-HDL particles are the most important drivers of cholesterol homeostasis. APOB allows LDL particles to interact with the LDL receptor (LDLR) in peripheral tissues, a potentially atherogenic process that deposits cholesterol in the arterial intima, or allows hepatic uptake to be metabolized and excreted. Similarly, APOA1 allows HDL particles to interact with the scavenger receptor class B member 1 (SCARB1) on peripheral tissues and the liver, promoting RCT.

To determine whether the observed changes in serum APOA1/APOB protein ratio were regulated at the transcription level, we further analyzed their relative (to GAPDH) hepatic mRNA expression, as well as that of LDLR and SCARB1. No significant changes were observed with respect to the mRNA expression of APOB, LDLR or SCARB1, although the relative expression of APOA1 was decreased in the HCC and HCC + FDM groups, whereas only the HCC + MF and HCC + MP fed rats reached the same mRNA level as that observed for the control group (Fig. 4). However, when expressed as the APOA1/APOB mRNA ratio, there were no significant differences ($P > 0.05$) among HCC-based diets compared to that found for their corresponding serum protein ratio (Fig. 3B).

According to previous research, changes exerted by MP on APOA1 may occur for a number of reasons. For example, polyphenols from pomegranate juice increase the serum activity and hepatic mRNA expression of paraoxonase 1 (PON1), an enzyme that closely associates with HDL particles, which results in decreased serum lipids in mice that are fed diets rich in fat, cholesterol and sodium cholate.[55] Cyanidin has been shown to promote RCT by increasing mRNA expression of ABCA1 and ABCG1 transporters in human aortic endothelial cells, and both transporters play important roles in preventing cholesterol overload by exporting it to HDL.[56] An anthocyanin-rich black elderberry extract has been shown to improve HDL function and decrease aortic cholesterol in hyperlipidemic mice by modulating the hepatic mRNA expression of various HDL-related genes, without significant changes to the serum HDL or APOA1 concentration.[57] These studies suggest that HDL-mediated RCT can be improved by dietary phenolics from different sources, and that the effects may not require a change in serum HDL or APOA1 concentration. In the case of mango, an increase in APOA1 was evident, although other effects that complement this increase cannot be ruled out, such as changes

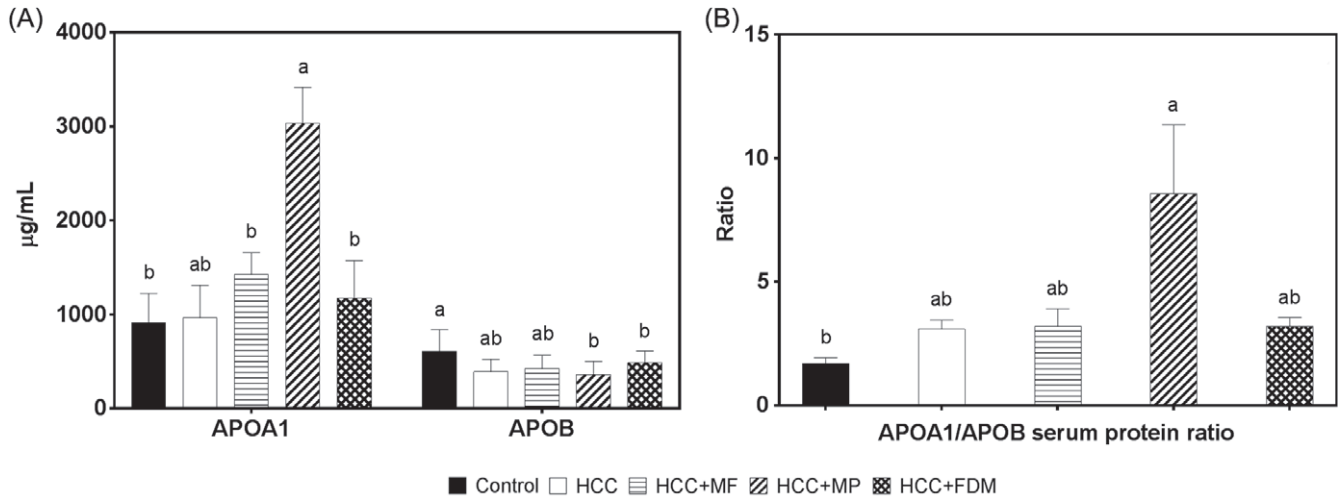

Figure 3. (A) Serum apolipoprotein A1 (APOA1) and apolipoprotein B (APOB) levels, and (B) their ratio. Different lowercase letters indicate significant differences ($P < 0.05$, $n = 6$ per group) between dietary treatments, as determined by the Kruskal–Wallis test. High cholesterol/sodium cholate (HCC), mango fiber (MF), mango phenolics (MP) and freeze-dried mango pulp (FDM).

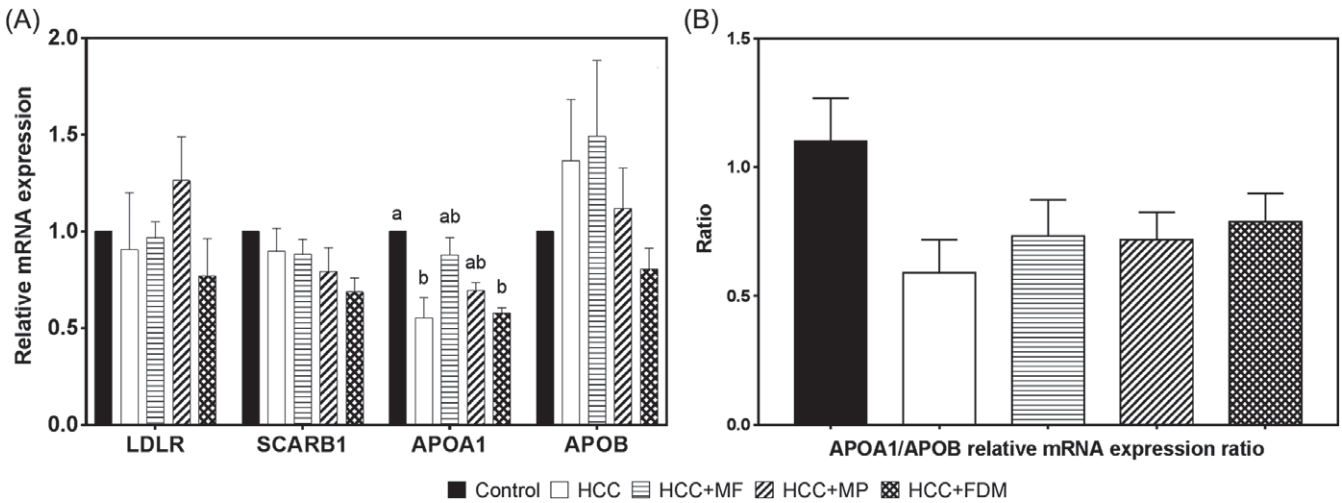

Figure 4. (A) Relative mRNA expression of hepatic genes involved in lipoprotein metabolism and (B) relative mRNA expression ratio of main apolipoproteins. Different lowercase letters indicate significant differences ($P < 0.05$, $n = 6$ per group) as determined by a one-way ANOVA for parametric data (LDLR, SCARB1 and APOA1/APOB ratio) or with the Kruskal–Wallis test for non-parametric data (APOA1 and APOB). Low-density lipoprotein receptor (LDLR), scavenger receptor class B member 1 (SCARB1), apolipoprotein A1 (APOA1) and apolipoprotein B (APOB). Normalizing gene was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). High cholesterol/sodium cholate (HCC); HCC + mango fiber (HCC + MF), HCC + mango phenolics (HCC + MP); and HCC + freeze-dried mango pulp (FDM).

to lipoprotein assembly, release into the circulation and peripheral uptake.

CONCLUSIONS

The bioactivity of FDM and the isolated MP and MF fractions were analyzed in rats fed HCC diets. MF exerted a modest normalization effect on liver biometry. FDM showed a lesser hepatoprotective effect than MF (e.g. attenuating hepatic enzyme activity). The most anti-atherogenic and anti-NASH effects were exerted by the MP fraction (increased serum APOA1/APOB protein ratio). The effects of MP suggest that they regulated HDL metabolism at the protein level, although changes to lipoprotein assembly, release into the circulation and peripheral uptake are also possible. MP were the most bioactive fraction of FDM, and their continued study with respect to HDL metabolism and liver health is warranted and is currently in progress.

Diets rich in fruits and vegetables are anti-atherogenic, and phenolic compounds are one of the molecular species responsible for modulating the hepatic mRNA and/or peripheral protein levels of several transcription factors, enzymes and membrane transporters.[58] Because MP were more bioactive than FDM, a dose-dependent action of MP is plausible; consequently, MP as a nutraceutical may have an even greater effect than the freeze-dried fruit. However, further experiments and strict clinical trials are required to evaluate any potential side effects because nutrient–nutrient or drug–nutrient interactions may take place in human subjects that could affect RCT and other related processes.

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