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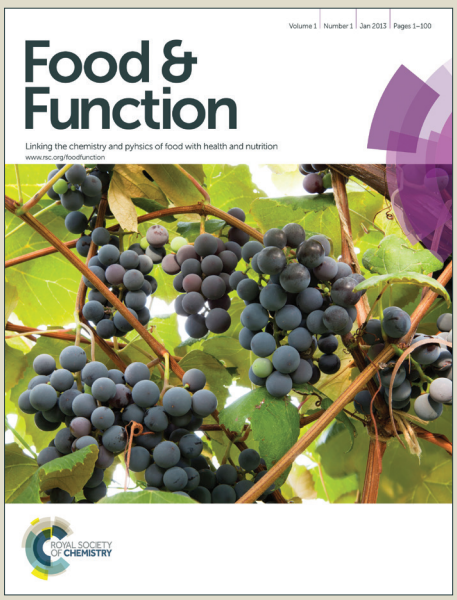

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Dietary Rosa mosqueta (Rosa rubiginosa) oil prevents high diet-induced hepatic steatosis in mice

Amanda D'Espessailles[a], Camila G. Dossi[a], Alejandra Espinosa[b], Daniel González-Mañán[a], Gladys S. Tapia[a]

[a]Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile

[b]Department of Medical Technology, Faculty of Medicine, University of Chile, Santiago, Chile

Corresponding author:

Dr. Gladys S. Tapia

E-mail: gtapia@med.uchile.cl

Postal adress: Independencia 1027, Independencia, Santiago Chile.

Fax: 56-2-7372783

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Abbreviations:

ACOX-1, acyl-CoA oxidase 1; **ALA**, α-linolenic acid; **LA**, alinoleic acid; **DHA**, docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **FA**, fatty acid; **FAME,** fatty acid methyl ester; **HFD**, high-fat diet; ***n*-3 LCPUFA**, *n*-3 long-chain PUFAs; **NAFLD**, nonalcoholic fatty liver disease; **NF-κB**, nuclear factor-κB; **PPAR- α**, peroxisome proliferator activated receptor alpha; **PUFA**, polyunsaturated fatty acid; **RM,** Rosa mosqueta; **SREBP-1c**, sterol regulatory element binding protein 1c; **TG,** triacylglycerol

Abstract

The effects of dietary Rosa mosqueta *(*RM, *Rosa rubiginosa)* oil, rich in α-linolenic acid, in the prevention of liver steatosis were studied in mice fed a high fat diet (HFD). C57BL/6j mice were fed either control diet or HFD, with or without RM oil for 12 weeks. The results indicate that RM oil supplementation decreases fat infiltration of the liver from 43.8% to 6.2%, improving the hepatic oxidative state, insulin levels, HOMA index, and both body and adipose tissue weight of HFD plus RM treated animals compared to HFD without supplementation. In addition, DHA concentration in liver was significantly increased in HFD fed mice with RM oil compared to HFD (3 v/s 1.6 g/100 g FAME). The n-6/n-3 ratio was not significantly modified by treatment with RM. Our findings suggest that RM oil supplementation prevents the development of hepatic steatosis and the obese phenotype observed in HFD fed mice.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical-pathological term encompassing a wide range of diseases characterized by intrahepatic triacylglycerol (TG) content higher than 5% of liver weight (hepatic steatosis) in absence of significant alcohol consumption (20-30 g/day in man; 10-20 g/day in woman), alongside with negative viral and autoimmune liver disease markers[1, 2]. NAFLD is being increasingly recognized as a major chronic liver disease and a public health problem in western population due to its strong association with obesity and related comorbidities as insulin resistance, hyperglycemia, atherogenic dyslipidemia, hypertension and other risk factors related to metabolic syndrome[3, 4], therefore NAFLD contributes to both adverse hepatic and metabolic outcomes. The mechanisms underlying excessive lipid accumulation on hepatocytes are not completely understood, but it is known that it results from an imbalance between lipid availability (enhanced blood uptake of fatty acids derived from adipose tissue and/or de novo lipogenesis) and lipid disposal (decreased fatty acid β-oxidation and diminished hepatic lipoprotein synthesis)[5, 6], together with insulin

resistance, oxidative stress and liver inflammation are critical factors for hepatic steatosis development[5]. NAFLD is characterised by alterations in n-6 and n-3 long-chain polyunsaturated fatty acids (LCPUFAs) status in liver, which is reflected in a significant depletion of n-3 LCPUFAs levels and enhancement of n-6/n-3 LCPUFAs ratio, existing a positive correlation between these variables and increased liver oxidative stress markers, alongside with decreased Δ-6 and Δ-5 desaturase activity both in murine model[7] and humans[8]. Most of NAFLD subjects consume high n-6 fatty acid levels in relation to n-3 LCPUFAs, due to very low fish consumption and high intake of sugar-based beverages and red meat compared to the general population[9]. Even though consumption of marine n-3 LCPUFAs sources is recommended to prevent NAFLD, there are complications frequently associated to these products (availability, price, palatability and overharvest of fish resources), making it necessary to seek other alternatives for n-3 LCPUFAs intake[10, 11]. Vegetable oils of accessible consumption are the most important sources of essential n-3 α-linolenic acid ALA (C18:3 n-3, ALA), among which rosa mosqueta, chia, flaxseed and other oils are included.[12, 13]

Rosa mosqueta (*Rosa rubiginosa*) is a wild shrub that grows in some specific areas of Central Europe, western Asia and the Andean region of Chile. One of the products derived from the seeds is the Rosa mosqueta (RM) oil, which is characterized by a high ALA concentration (about 30% of total fatty acid content) and a n-6:n-3 ratio of 1, which makes it a nutritional alternative to providing ALA for its hepatic bioconversion to eicosapentaenoic (C20:5 n-3, EPA) and docosahexaenoic (C22:6 n-3, DHA) acids[14]. EPA and DHA have several roles in different physiological contexts, leading to positive health benefits that support their use in prevention of non-transmissible chronic diseases. EPA and DHA participate in the regulation of hepatic lipid metabolism, decreasing de novo lipogenesis through reduction in lipogenic genes transcription, and inducing gene expression of fatty acid oxidation components[15], alongside to cytoprotective actions through both upregulation of antioxidant enzyme as

77 downregulation of pro-inflammatory gene expression[16, 17]. In relation to NAFLD, our
78 group has demonstrated that dietary EPA and DHA supplementation prevents and
79 reverses the steatotisis and the pro-inflammatory and pro-oxidative status induced by a
80 high-fat diet (HFD) in mice[18-20]. Recently, it has been demonstrated that oral RM oil
81 administration in rats significantly increases hepatic levels of ALA, EPA and DHA and
82 decreases n-6/n-3 ratio. Moreover, RM activates peroxisome proliferator-activated
83 receptor alpha (PPAR-α), increasing expression of PPAR-α related lipolytic genes,
84 without changes in liver damage parameters[11, 21]. For all these reasons, RM has
85 potentiality to be used clinically to prevent both hepatic steatosis and metabolic
86 syndrome induced by unhealthy nutrition, which will add to their current use as
87 cosmetic product[11].

88 The aim of this study was to test whether oral administration of RM oil prevents both
89 steatosis and oxidative stress in liver from mice HFD fed. Parameters related to liver
90 morphological characteristics (lipid vesicles), metabolic syndrome (visceral adipose
91 tissue, serum glucose, insulin, HOMA index, cholesterol and tryacilglicerides levels),
92 oxidative stress (TBARS and protein carbonylation), liver total fat content and fatty acid
93 composition in relation to ALA, EPA and DHA were determined.

94

2. Materials and Methods

2.1 Ethics statement

Experimental animal protocols and animal procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, NIH Publication 6-23, revised 1985) and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (CBA 0386 FMUCH).

2.2 Animal preparation and supplementation with *Rosa rubiginosa oil*

Weaning male C57BL/6J mice weighing 12 to 14 g were obtained from the Animal Facility at the Faculty of Medicine, University of Chile, Chile. Room temperature was kept constant at 21°C and light was maintained on a 12:12-h light-dark cycle. At 20 days of age, mice were randomly divided into four groups: a) control diet (CD) containing 10% fat, 20% protein, and 70% carbohydrate; b) control diet plus *Rosa rubiginosa* oil; c) high-fat diet (HFD) containing 60% fat, 20% protein, and 20% carbohydrate (D12492, Research Diets, NJ, USA) and c) high-fat diet plus *Rosa rubiginosa* oil from days 1 to 84 (12 weeks). After 12 weeks, the animals were fasted (6-8 h) and then anesthetized with Zoletil® (Tiletamine hydrochloride and Zolacepam hydrochloride, 20-40 mg/Kg intraperitoneally). Weekly controls of body weight and diet intake were performed through the whole period.

The *Rosa rubiginosa* oil supplemented groups received 1.94 mg ALA/ g animal weight/ day (Coesam, Chile) through oral administration; control groups were given isovolumetric amounts of saline solution.

The fatty acid composition of the RM oil used is as follows: (i) total saturated fatty acids were 6.281 g in wich 0 g are decanoic acid, dodecanoic acid, tetradecanoic acid; 3.489 g is palmitic acid; 1.778 g stearic acid; 0.746 is eicosanoic acid; docosanoic acid is 0.159; tetracosanoic acid is 0.067; (ii) monounsaturated fatty acids 14.886 g in which

120 0.117 g is palmitoleic acid; 14.416 g is oleic acid; 0.352 g is eicosaenoic acid and 0 g
121 are erucic and tetracosaenoic acid; (iii) polyunsaturated fatty acids (PUFA) 76.652 g in
122 which 43.131 g is linoleic acid; 33.520 g is α-linolenic acid and 0 g are γ- linolenic,
123 eicosadienoic, eicosatrienoic, eicosatetraenoic, eicosapentaenoic, docosapenaenoic
124 and docosahexaenoic acid. The RM oil has 0 % of either EPA or DHA and a n-6:n-3
125 ratio of 1.3. Values are expressed as g per 100 g of *Rosa rubiginosa* oil and were
126 obtained using a Hewlett Packard gas chromatograph (model 7890A).

127 **2.3 Tissue and blood samples**

128 Liver samples were frozen in liquid nitrogen and stored at -80^{o}C, or fixed in phosphate-
129 buffered formalin, embedded in paraffin, sectioned by microtome and stained with
130 hematoxylin-eosin (HE). Blood samples were taken by cardiac puncture and then
131 centrifuged, and serum was stored at -20°C. Liver slides stained with HE were
132 assessed by optical microscopy (Olympus CX31, Japan) for morphology analysis in a
133 blind fashion. Presence of both steatosis and inflammation were both graded as
134 absent, mild, moderate or severe[22].

135 **2.4 Liver total fat content and fatty acids analysis**

136 Total lipids were extracted from whole-liver homogenates using a modified Bligh and
137 Dyer extraction procedure[23]. Liver samples were homogenized in distilled water and
138 the lipid components were extracted with a 1:2 chloroform:ethanol solution, followed by
139 centrifugation (2.000 g for 10 min at room temperature). After extraction of the
140 chloroformic phase, the solvent was allowed to evaporate and the samples were stored
141 at -20°C [23]. Previous to the gas-liquid chromatography assay, fatty acids and
142 phospholipids from liver were methylated by incubation (100°C) with BF3 methanol
143 (14%) and the fatty acid methyl esters (FAME) were extracted with hexane. After
144 evaporation with nitrogen and the resuspension in dichloromethane, samples were

stored at -20°C until the gas-liquid chromatography assay[24]. Values were expressed as g of fat/ 100 g of liver. A Hewlett Packard gas chromatograph (model 7890A), equipped with a capillary column (J and WDB-FFAP, 30m 60.25mm; I.D. 0.25 mm), automatic injector and flame ionization detector, was used for FAME separation and detection. Identification of FAME was carried out by comparison of their retention times with those of individual purified standards, and values were expressed as g/100 g FAME.

2.5 Biochemical determinations (serum glucose, insulin, cholesterol and triacylglycerol)

Blood glucose concentrations were measured on a Johnson and Johnson OneTouch Glucometer following manufacturer's instructions. Plasma insulin concentration (μUI/mL) was determined by a commercially available immunoassay specific for mice (Mercodia, Uppsala, Sweden). Insulin resistance was estimated by the homeostasis model assessment method (HOMA) [fasting insulin (μUI/mL) x fasting glucose (mg/dL)/405][25]. Cholesterol (mg/100mL) and triacylglycerol levels were measured using specific diagnostic kits (Wiener Lab, Argentina).

2.6 Stress oxidative determinations: oxidative protein damage and TBARS assay

Liver oxidized proteins content was determined in frozen tissue, treated with 2.4-dinitrophenylhydrazine to form a Schiff base. Production of the corresponding hydrazone was measured spectrophotometrically between 350 and 390 nm to determine concentration of carbonyls, and at 280 nm to determine total protein concentration[26]. Values were expressed as nmol carbonyls/mg protein.

The measurement of liver thiobarbituric acid reactive substances (TBARS) was determined through an assay kit following the manufacturer's instructions (Cayman`s Chemical Company, MI, USA). Values were expressed as μ moles of malondialdehyde equivalents/L of tissue homogenate.

2.7 Statistical analyses

Statistical analysis was performed with GraphPad Prism™ version 5.0 (GraphPad Software, Inc. San Diego, CA, USA). Values shown represent the mean ± SEM for the number of separate experiments indicated. One-way ANOVA and Newman-Keuls test, with a *P<0.05*, were considered significant.

3. Results

3.1 Rosa mosqueta oil supplementation reduces body and visceral fat weight, glycemia, insulin and triacylglycerols levels altered by HFD, without changes in food intake.

The initial body weight among animal groups were not significantly different (Table 1). After 12 weeks of diet with or without the Rosa mosqueta (RM) oil supplementation, HFD fed mice with RM oil supplementation body weight was significantly lower (11.2%, *P<0.05*) compared to those given control diet HFD without supplementation; but higher (*P<0.05*) compared to control diet (CD; 17.6 %) and CD + RM (19.2%). In the animals subjected to control diet, RM oil supplementation had no effect on the body weight after 12 weeks of treatment (Table 1). Visceral fat weight, measured as adipose tissue/body weight ratio, was significantly decreased (17.1%, *P<0.05*) in the HFD fed mice with RM oil compared to the HFD without supplementation, although such HFD + RM values do not normalize to visceral adipose tissue weight observed in the animals subjected to control diets with or without RM oil supplementation (Table 1). Glycemia levels were 17% higher in the HFD fed mice animals than in the CD and the CD + RM fed group. The RM oil supplementation had no effect on glycemia levels in HFD fed mice. Insulin levels were significantly decreased (30%, *P<0.05*) in the HFD with RM oil group compared to HFD without supplementation, reaching similar values observed at the CD and CD + RM groups (Table 1). HOMA index showed a similar outcome. RM oil

supplemented HFD fed mice showed a decrease in 29% in the HOMA index compared to HFD fed mice without supplementation. In addition, RM is capable of decreasing HOMA in both control and HFD group (Table 1).

Table 1 also shows the levels of serum cholesterol and tryacilglicerides (TG) in all the experimental groups. Cholesterol level in RM oil supplemented group does not differ from the HFD group. A decrease of 49% ($P<0.05$) in the TG levels in HFD fed mice supplemented with RM oil was observed, compared to HFD fed mice without the supplementation. In addition, the values observed in the HFD with RM oil did not differ from the control groups.

No significant differences in food intake were observed during the 12 weeks of treatment in the different experimental groups. The food intake at first week was: CD group: 2.2 ± 0.3 g/day; CD plus RM oil supplementation group 1.9 ± 0.5 g/day; HFD group 2.3 ± 0.4 g/day and HFD plus RM oil group supplementation 2.0 ± 0.3 g/day. At the end of 12 weeks of treatment, the food intake was: CD group 5.0 ± 0.3 g/day; CD plus RM oil supplementation 4.9 ± 0.2 g/day; HFD group 5.0 ± 0.3 g/day and HFD plus RM oil supplementation 4.8 ± 0.3 g/day.

3.2 Rosa mosqueta oil supplementation prevents hepatic lipid infiltration induced by HFD.

In all groups, liver histology was characterized by the absence of arquitectural distortion, lobular inflammation, necrotic foci, or fibrosis (Fig. 1A). Animals given CD with or without RM oil did not show lipid vesicles in hepatocytes [Fig 1A (a, b and d)]. However, HFD fed mice without RM oil supplementation exhibited macro and microvesicular steatosis with a 43.8% of fat infiltration (Fig. 1A (c) and Fig. 1B) whereas HFD with RM group elicited 6.2% fat infiltration (Fig. 1B). The RM oil supplemented HFD group showed a diminution of 40% ($P<0.05$) in the liver fat content

(6.6 ± 1.08 g/100 g FAME) (Fig. 1C) respect to HFD without supplementation (11.01 ± 1.2 g/100 g FAME) group.

3.3 Rosa mosqueta oil supplement is bioconverted to EPA and DHA in the liver.

The Fig. 2 shows the hepatic contents of α-linolenic acid, eicosapentanoic acid and docosohexanoic acid. HFD fed mice subjected to RM oil supplementation presented α-linolenic levels (0.31 ± 0.02 g/100 g FAME) similar to HFD without supplementation (0.29 ± 0.02 g/100 g FAME) and CD (0.27 ± 0.02 g/100 g FAME) group. Interestingly, CD fed mice with RM oil supplementation (0.42 ± 0.04 g/100 g FAME) showed a significantly (*P<0.05*) higher concentration of α-linolenic acid than the CD group (Fig. 2A).

EPA and DHA bioconversion from RM oil's α-linolenic acid is shown in Fig. 2B and C. HFD fed mice subjected to RM oil supplementation presented EPA levels (0.24 ± 0.11 g/100 g FAME) similar to HFD without supplementation levels (0.21 ± 0.02 g/100 g FAME) and to the control group (0.29 ± 0.06 g/100 g FAME). EPA concentration was significantly increased (*P<0.05*) in the CD fed mice with RM oil supplementation (0.43 ± 0.02 g/100 g FAME) compared to CD, HFD and HFD plus RM (Fig. 2B). DHA concentration in liver was significantly increased (*P<0.05*) in HFD fed mice with RM oil supplementation (3.00 ± 0.25 g/100 g FAME) compared to HFD without supplementation (1.61 ± 0.15 g/100 g FAME), but not different than CD (3.48 ± 0.08 g/100 g FAME) and CD with RM oil supplementation (3.84 ± 0.18 g/100 g FAME) (Fig. 2C).

As an index of n-3 LCPUFA bioconversion we used a relationship between the total EPA and DHA content and α-linolenic levels. The HFD fed groups had a bioconversion index of 6.16 ± 0.68, statistically lower than the one observed in the control group with

(10.72 ± 1.53) or without (13.14 ± 0.93) RM oil supplementation and in the HFD fed group with RM oil (10.39 ± 0.74) as shown in Fig. 2D.

3.4 Rosa mosqueta oil supplementation does not improve n-6/n-3 ratio altered by HFD.

Figure 3 shows the n-6/n-3 ratio observed in all the experimental groups. The RM oil supplementation did not alter the n-6/n-3 ratio in both the CD and the HFD fed mice. HFD fed animals with (3.58 ± 0.09) or without (4.41 ± 0.55) RM oil showed a higher *(P<0.05)* n-6/n-3 ratio than control groups (1.76 ± 0.02 in the CD group versus 1.95 ± 0.21 in the CD+RM).

3.5 Rosa mosqueta oil supplementation decreases both hepatic protein and lipid oxidation induced by HFD.

Mice subjected to HFD and RM oil supplementation exhibited a significantly (*P<0.05*; 5.6 ± 0.3 nmol carbonyl/mg protein) decrease in liver protein carbonyl content in respect to HFD fed animals without RM oil supplementation (10.1 ± 1.4 nmol carbonyl/mg protein), but similar values of oxidized proteins than control groups: CD (5.1 ± 1.0 nmol carbonyl/mg protein) and CD with RM oil supplementation (5.8 ± 0.9 nmol carbonyl/mg protein) (Fig. 4A).

Malondialdehyde (MDA) is a lipid peroxidation product. HFD fed mice showed a increased MDA equivalents concentration *(P<0.05)* compared to CD with (2.9 ±0.6 μM/L) and without (2.98 ± 0.36 μM/L) RM oil supplementation. RM oil supplementation in HFD fed mice (3.8 ± 0.2 μM/L) decreases the MDA concentration in 18% compared to HFD group (4.6 ± 0.3 μM/L).

4. Discussion

It has been demonstrated that daily supplementation with n-3 LCPUFA (EPA plus DHA) can prevent and reverse the metabolic alterations induced by HFD intake in mice, improving the glucose intolerance and insulin resistance, decreasing the adipose tissue and the hepatic steatosis[19, 20]. In addition, n-3 LCPUFA produces the upregulation of antioxidant enzyme and downregulation of pro-inflammatory gene expression[18,20]. In this study, Rosa mosqueta oil, ALA enriched oil, was used as a dietary supplement to prevent the steatosis and associated metabolic alterations induced by a high fat diet in a mice model. We demonstrate that the RM oil supplementation can effectively prevent the development of hepatic steatosis, and that it could be by the EPA and DHA transformation. Moreover, RM improves hepatic oxidative stress observed in high fat diet fed mice.

While the metabolic effects of others ALA-rich oils have been investigated[27-29], it is not clear the mechanisms involved in their actions and moreover, such studies cannot ensure specific effects attributable exclusively to ALA or otherwise, to EPA and DHA generated from ALA or another compounds present in these vegetables oils, due to the high complex composition of these oils.

Here we demonstrate that RM oil supplementation significantly reduces body weight, visceral fat, insulin, and TG levels altered by the HFD model. In a similar approach, it has been shown that chia seeds, a rich source of ALA, improves insulin sensibility and glucose tolerance, reduces visceral adiposity, decreases hepatic steatosis and reduces cardiac and hepatic inflammation[30]. However, chia oil was not able to produce any change in the plasma lipids levels; in spite of this, another study has shown that dietary chia supplementation normalizes TG levels in dyslipaemic rats[29]. In addition, a human study showed that flaxseed consumption over 8 weeks improved the serum concentration of TG, total cholesterol, and LDL-c in patients with lipid abnormalities. Moreover, our results show that RM oil supplementation prevents hepatic infiltration

induced by HFD as was reflected in the hepatic lipid vesicles (Fig. 1A-B) and the lipid content of the liver. In this aspect, it has been shown that n-3 LCPUFA, especially EPA and DHA, can modulate the lipid metabolism in the liver modulating principal pathways: first, decreasing hepatic synthesis of fatty acids and consequently TG, suppressing gene expression of SREBP-1c; and second, by increasing their proteasomal degradation[30, 31], with the results of a higher expression of PPAR-α and downstream proteins. These changes could decrease VLDL formation and serum TG concentration. Even though the complete molecular mechanism of the RM oil actions has not been studied, our preliminary studies (data not shown) show an increase of mRNA PPAR-α expression and upregulation of ACOX-1, which are involved in the lipidic β-oxidation process and could explain in part the effect of this oil in lowering the lipid infiltration of the liver[20].

RM oil is one of the richest plant sources of omega-3 fatty acid α-linolenic which could be converted to n-3 LCPUFAs in the liver. The bioconversion of ALA to EPA and DHA is supported by several studies in animals and cells. Though the bioconversion in humans is controversial, it could be due to the limitation of the studies that usually analyze changes of fatty acids in the plasma, and it has been demonstrated that the bioconversion occurs in a tissue-dependent manner; thus there could be specific changes in the DHA and EPA concentration in specific tissues. In this respect, in an animal study of chronic supplementation with high-ALA chia seed it was observed an accumulation of DHA both in heart and liver without plasmatic changes[32]. In addition, we have previously demonstrated that oral RM oil administration in rats significantly increases hepatic levels of ALA, EPA and DHA and decreases n-6/n-3 ratio, without alterations in liver parameters[11, 21]. Furthermore, the bioconversion of ALA to EPA and DHA depends on the amount of dietary ALA and the ratio of dietary linoleic acid (LA) to ALA as a result of the competition between n-6 and n-3 fatty acids as substrates for desaturation by the Δ-6 desaturase enzyme[33] and because LA reduces Δ-6 desaturase

levels[34]. In agreement with these views, we observed that RM oil, high in ALA, was bioconverted to EPA and DHA in the liver in a dependent-treatment manner, as shown Fig. 2A and B. There were not any differences in the α-linolenic acid and EPA levels between HFD and HFD treated with RM oil groups[11]. This result could be explained by chronic oxidative stress induced in liver of HFD-fed mice, which can lead to enhanced ROS-mediated lipoperoxidation of PUFA molecules on account of its high susceptibility to this type of reactions, thereby contributing to drastic ALA decreased levels observed in HFD treated with RM group when compared to CD treated with RM oil group, in which pro-oxidative state is not observed [35]It also could be explained addressing two aspects: metabolization of these fatty acids and tissue specificity. Metabolism of these n-3 fatty acids generates several metabolites: E and D-series of resolvins[36], D1 protectin, 17S-hydroxy-DHA and formation of epoxyeicosaquatraenoic acid and epoxydocosapentanoic acid regiosomers[37]. These molecules are potent anti-inflammatory mediators and could be responsible in part for the improvement observed with RM oil supplementation. On the other hand, as the accumulation and bioconversion of the n-3 LCPUFA are tissue-dependent and we can only observe the hepatic response of the systemic effect of these fatty acids[18, 32]. However, when we observed the DHA levels and the bioconversion index, the ALA rich oil supplementation was significantly able to prevent the depletion of n-3 PUFA observed in HFD fed animals as shown in figure 2C and D. In recent studies, it has been demonstrated that positive effects in health associated with ALA administration are not due only with its bioconversion to EPA and DHA, but also with ALA biological activity itself[38]. Another component present in RM oil is the oleic acid (C18:1; 14.4g/100 g RM oil), that might have protective effects by stimulating antioxidative capacity and fatty acid oxidation in myocyte and adipocite cell cultures[39, 40], therefore it would be of particular interest to study its potencial actions at hepatic level.

In a molecular aspect, the biological actions of RM probably rely on its fatty acid composition and its antioxidant and anti-inflammatory capacity[16]. Whatever, as in the case of DHA and EPA, the mechanism of action of ALA is not completely clear. First, ALA could be beneficial, acting as the precursor of EPA and DHA as was mentioned before. Second, ALA consumption may be a good strategy to decrease elongation on n-6 fatty acids leading to a reduced araquidonic acid content[41], and could be reflected in an improvement in the n-6/n-3 ratio. And third, ALA may have beneficial actions directly, through interaction with ion channels[42] or nuclear receptors as PPAR or RXR[43]. In a study in a model of Δ-6 desaturase null mouse was demonstrated that ALA can act independently of its bioconversion to EPA and DHA on risk factors associated with the development of fatty liver disease[38].

We observed a decrease in the lipid and protein oxidation in the animals subjected to RM oil supplementation and HFD diet (Fig. 4). In according with these findings, it is possible that the n-3 LCPUFAs obtained by hepatic bioconversion had an important role in the oxidative stress reduction observed. It was postulated that the antioxidant response of n-3 LCPUFAs was ascribed to their spontaneous lipid peroxidation, with generation of cyclopentenone-containing J-ring isoprostanes that activate nuclear factor (erythroid-derived 2)-like 2 (Nrf2)[44], a factor controlling the expression of antioxidant enzymes and other cytoprotective proteins[45].

5. Conclusions

Using an animal model of HFD-induced liver steatosis we demonstrate that the dietary *Rosa rubiginosa* oil supplementation (i) significantly reduces body weight, glycemia, insulin, and TG levels altered by HFD; (ii) prevents the hepatic lipid infiltration observed in mild steatosis; (iii) recovers DHA levels in HFD fed mice livers; and (iv) decreases oxidative stress induced by HFD. These findings are the first to demonstrate the metabolic actions of *Rosa rubiginosa* oil against the health alteration induced by a high

fat diet in an animal model, providing rational basis for developing studies in the functional proprieties of this vegetal oil and the possible uses in steatosis and metabolic alterations treatment.

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Figure legends

462 **Figure 1.** Effect of Rosa mosqueta (RM) oil supplementation on hepatic lipid infiltration
463 induced by HFD in mice. (A) Liver histology 100X, (B) hepatocyte lipid infiltration and
464 (C) total liver fat content. Animals were given (a) control diet (CD), (b) control diet plus
465 RM oil (CD+RM), (c) high fat diet (HFD), or (d) HFD plus RM oil (HFD+RM). Values are
466 expressed as mean ± SEM for 4-9 animals per experimental group. Letters above the
467 bars indicate statistically significant differences between the groups (*P<0.05*; one-way
468 ANOVA and the Newman-Keuls test).

469 **Figure 2.** Effect of Rosa mosqueta (RM) oil supplementation on EPA and DHA
470 bioconversion in the liver. Hepatic levels of (A) α-linolenic, (B) EPA and (C) DHA; and
471 (D) bioconversion index. Animals were given (a) control diet (CD), (b) control diet plus
472 RM oil (CD+RM), (c) high fat diet (HFD), or (d) HFD plus RM oil (HFD+RM). Values are
473 expressed as mean ± SEM for 4-9 animals per experimental group. Letters above the
474 bars indicate statistically significant differences between the groups (*P<0.05*; one-way
475 ANOVA and the Newman-Keuls' test).

476 **Figure 3.** Effect of Rosa mosqueta (RM) oil supplementation on the hepatic n-6/n-3
477 ratio altered by high fat diet in mice. Animals were given (a) control diet (CD), (b)
478 control diet plus RM oil (CD+RM), (c) high fat diet (HFD), or (d) HFD plus RM oil
479 (HFD+RM). Values are expressed as mean ± SEM for 4-9 animals per experimental
480 group. Letters above the bars indicate statistically significant differences between the
481 groups (*P<0.05*; one-way ANOVA and the Newman-Keuls' test).

482 **Figure 4.** Effect of Rosa mosqueta (RM) oil supplementation on the hepatic oxidative
483 stress induced by high fed diet in mice. Hepatic levels of (A) liver protein carbonyl
484 content and (B) malondialdehyde. Animals were given (a) control diet (CD), (b) control
485 diet plus RM oil (CD+RM), (c) high fat diet (HFD), or (d) HFD plus RM oil (HFD+RM).
486 Values are expressed as mean ± SEM for 4-9 animals per experimental group. Letters

487 above the bars indicate statistically significant differences between the groups (*P<0.05*;
488 one-way ANOVA and the Newman-Keuls' test).

489

490

Table 1. General parameters in the different experimental groups: body and abdominal adipose tissue weight, glycemia, serum cholesterol and triacylglycerols.

	Experimental groups			
Parameters	**a) Control diet**	**b) Control diet + RM**	**c) High fat diet**	**d) High fat diet + RM**
Initial body weight (g)	14.5 ± 0.6	13.2 ± 0.6	13.9 ± 0.4	15.7 ± 0.7
Final body weight (g)	21.6 ± 0.9	21.3 ± 0.8	28.6 ± 1.0$^{a.b.d}$	25.4 ± 0.9$^{a.b}$
Adipose tissue/body weight ratio x100	1.3 ± 0.2	1.1 ± 0.08	4.1 ± 0.2$^{a.b.d}$	3.4 ± 0.3$^{a.b}$
Glycemia (mg/dl)	139 ± 8.6	132 ± 5.0	159 ± 8.4^{b}	162 ± 6.0^{b}
Insulin (µUI/ml)	19.2 ± 0.37	15.1 ± 1.7	23.7 ± 0.9$^{a.b.d}$	16.6 ± 1.1
HOMA (µUI/ml x mg/dl	6.6 ± 0.09^{b}	4.9 ± 0.06	9.3 ± 0.3$^{a.b.d}$	6.6 ± 0.4^{b}
Serum cholesterol (mg/dl)	161.4 ± 18.2	122.5 ± 11.6	258.6 ± 42.3^{b}	241.9 ± 40.4
Serum TAG (mg/dl)	79.0 ± 0.5	79.2 ± 2.2	118.9 ± 9.0$^{a.b.d}$	79.8 ± 2.1

Values represent means ± SEM for 4-9 mice per experimental group. Significant differences between the groups are indicated by the letters identifying each group *(P<0.05*; one-way ANOVA and the Newman-Keuls test). RM: Rosa mosqueta.

Figure 1

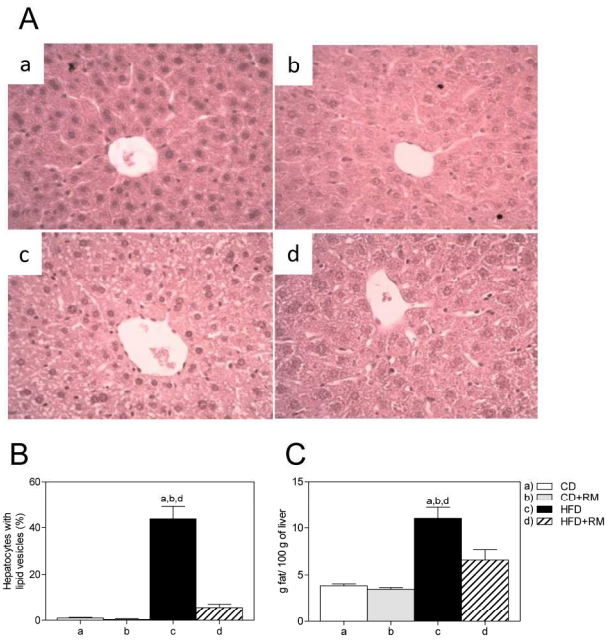

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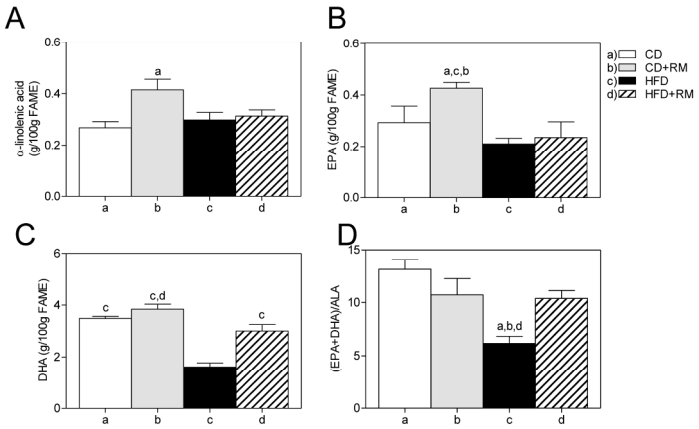
Figure 2



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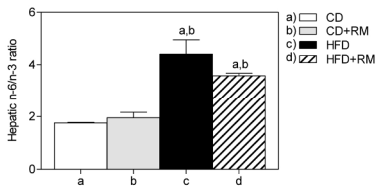

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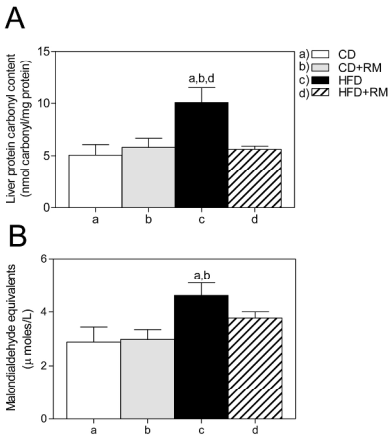
Figure 4



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