



Prosopis alba seed flour improves vascular function in a rabbit model of high fat diet-induced metabolic syndrome



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ABSTRACT

Aims: *Prosopis alba* flour is a natural source of nutrient and phytochemicals with potential effects on cardiovascular risk factors. The aim of this work was to examine the effects of dietary supplementation with *Prosopis alba* seed flour (Pr-Feed) on a high fat diet (FD)-induced rabbit model of metabolic syndrome.

Main methods: Rabbits were separated in four groups: fed regular diet (CD); CD supplemented with Pr-Feed; fed on 18 % FD; FD supplemented with Pr-Feed. All diets were administrated for 6 weeks. After the feeding period body weights, mean blood pressure, heart rate and visceral abdominal fat (VAF) were determined; glucose tolerance test (GTT) was performed; total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, triglycerides (TG), fasting glucose (FG), aspartate amino transferase, alanine amino transferase, bilirubin and creatinine were measured in serum. Abdominal aorta was excised and vascular function was assessed by acetylcholine relaxation and contractile response to KCl, norepinephrine and angiotensin II.

Key findings: Phytochemical analyses showed that the main compounds of Pr-Feed were apigenin C-glycosides. FD increased VAF, FG, TG, reduced HDL-cholesterol and induced abnormal GTT. Pr-Feed addition to FD did not modify these alterations. Aortic rings from rabbits fed on FD exhibited an impaired relaxation-response to acetylcholine and increased agonist vasoconstrictor responses. Pr Feed-supplemented FD improved the response to acetylcholine, and prevented the increase of the contractile response to KCl, norepinephrine and angiotensin II.

Significance: Results suggest that dietary supplementation with Pr-Feed, rich in apigenin C-glycosides, has vascular protector properties and could be used to prevent vascular alterations characterizing the metabolic syndrome.

1. Introduction

Prosopis alba trees are widely distributed in dry areas of the Argentinean North West. This specie, commonly known as algarrobo blanco, is very valuable for economies of native people [1]. Algarrobo trees have traditionally used as a source of hard wood for furniture manufacture, and their pods for mesocarp flour preparation. However, in the process of flavor elaboration, seeds are generally discarded as waste materials. Previously, we reported that *P. alba* cotyledons are a source of macronutrients and biologically active molecules. Protein hydrolysate obtained from seed flour showed antioxidant and anti-inflammatory properties [2]. Phenolic compounds were characterized in cotyledons flour and apigenin C-glycosides have been described as the main polyphenols [3]. These properties of *P. alba* seeds flour render it a potential ingredient to develop functional foods for preventing chronic diseases associated to

inflammatory process.

Metabolic syndrome (MS) represents a conjunction of several metabolic disturbances such as abdominal obesity, insulin resistance, hypertriglyceridemia, decreased levels of high density lipoprotein-cholesterol (HDL-C) and hypertension [4]. Several studies support to the concept that a pro-inflammatory status is a component of the MS [5]. Certain dietary components and over 800 plants help prevent or moderate MS [6]. Recently, much attention has been focused on plant flavonoids that might be beneficial in reducing the risk of obesity and obesity associated metabolic disorders. It has been revealed that several flavonoids could induce neutral lipid hydrolysis from lipid stores in adipose tissues and the liver [7]. Furthermore, several flavonoids inhibit intestinal glucose and fructose transport by glucose transporter 2 [8]. Indeed, dietary catechins and anthocyanins significantly decrease the weight of intraperitoneal adipose tissues [9, 10]. Investigation of the metabolic effects of

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flavonoids might lead to more effective strategies for the treatment of obesity and obesity-associated metabolic disorders.

The quality and quantity of fat consumed in the diet are strongly associated with prevention or improvement of metabolic abnormalities characterizing the MS [11]. A valid animal model is required to identify treatment options for MS in humans. In such sense, we have recently characterized a rabbit model of high fat diet induced MS [12]. This model showed a pro-inflammatory status associated to functional alterations of blood vessels (endothelial dysfunction and increased vascular reactivity).

The aim of this work was to evaluate the potential effects of *P. alba* seed flour as functional food to prevent metabolic and vascular alterations in a rabbit model of high fat induced-MS.

2. Materials and methods

2.1. Seed flour preparation

To obtain seed flour from *P. alba*, pods were dried at 50 °C until constant weight and grounded. Then, they were sieved to separate mesocarp flour and seeds. Dried seeds were grounded in an ultra-mill (Numak, F100-Argentina) to obtain seed flour. Flour was stored in plastic screw-capped bottles at -20 °C until further use.

2.2. Food preparation

P. alba seed flour was incorporated to regular rabbit chow (Ganave, Pilar, Argentina) to obtain *P. alba* seed flour supplemented chow (Pr-feed). Briefly 15.6 g of *P. alba* seed flour, 10 g corn starch and 100g of regular chow (56 % total carbohydrates, 15% total protein, 15% fiber, 3% total fat and 11% ash, 250 Cal/100 g) were wet mixed, re-pelleted, freeze-dried and then were maintained at room temperature. Fat diets were prepared by mixing 100 g of regular chow with 8 g lard, 10 ml of corn oil and 10 g of corn starch, re-pelleted and dried (412 Cal/100g).

2.3. Animals and study design

Experiments were reviewed and approved by our Institutional Animal Care and Use Committee (Bioethics Committee of the School of Medicine of the National University of Tucuman, Argentina). Twenty four male Flanders hybrid rabbits, weighing 900–1000 g on arrival, were individually housed in metal cages in a room with controlled temperature, humidity, and a 12-h light cycle. Animals were randomly assigned to four groups of six animals. Animals were fed on its diet for 6 weeks. The four groups included: 1- control diet (CD): 150 g/day of regular rabbit chow, 2- CD and Pr-feed (CD-Pr feed): 8 g/day Pr-feed and 150 g/day of CD, 3- Fat diet (FD): fat-supplemented chow *ad libitum*, and 4- FD with Pr-feed (FD-Pr feed): 8 g/day Pr-feed and FD *ad libitum*. The total intake of *P. alba* seed flour was 1 g day/animal. Water was given *ad libitum*. The animals were weighed before dietary manipulation and every day throughout the period of the experiment. CD group represented the metabolic and vascular state of healthy rabbits. FD group was characterized by vascular dysfunction and metabolic alterations compatible with MS definition [12].

2.4. Samples

At the end of the feeding period, after an overnight fast, rabbits were weighed and anesthetized under ketamine (20 mg/kg) and diazepam (0.5 mg/kg). Mean blood pressure (MAP) and heart rate were measured directly in carotid artery through a catheter connected to a pressure transducer (Gould Instruments, Cleveland, Ohio, USA) and recorded using a data acquisition system (Biopac MP100, Aero Camino Goleta, California, USA). After MAP measurement, blood samples were collected in prechilled glass tubes containing EDTA 10^{-7} M through a catheter inserted in carotid artery. Using surgical techniques a midline incision was made in the rabbit. Adipose tissues from abdominal areas were

collected and weighted. The visceral abdominal fat (VAF) was expressed as a percentage of the total body weight: (fat weight/animal weight) x 100. Plasma total cholesterol (TC) and triglycerides (TG) were determined using colorimetric reactions with commercial kits (Wiener, Rosario, Santa Fe, Argentina). The heart, liver and kidneys were weighed.

2.5. Glucose tolerance test

An intraperitoneal glucose tolerance test (GTT) was performed as was previously described [13]. After an overnight fasting period of 12 hs, an injection of 2 g/kg glucose solution was administrated intraperitoneally. Blood samples were collected from the ear vein before (0 min) and 120 min after the glucose injection. The glucose concentrations were measured immediately after blood collection with an enzymatic kit (Wiener Lab® Rosario, Argentina) based on the glucose oxidase method.

2.6. Phenolic quantification by HPLC-DAD

The phenolic quantification of *P. alba* seed flour was determined by methanol/water 70:30 extraction and analyzed by HPLC-DAD. The HPLC system was a Waters equipment (Water Corporation, Milford, Massachusetts) consisting of a binary pump 1525, an ultraviolet (UV) diode array detector 2998, Water X-bridge C18 column (250 × 4.6 mm i. d.; 4,6µm). The HPLC-DAD analyses were performed using a linear gradient solvent system consisting of acetic acid/water (0.1/99.9) (A) and methanol/water (99.9/0.1) (B) as follow: 10 % B initial, followed by 57% B 45 min, 60% B until the end. The flow rate was 0.5 ml/min and the volume injected was 20µl. The compounds were monitored at 280 and 340 nm and UV spectra from 200 to 600nm were recorded for peak characterization. The Empower 2TM software was used [3].

2.7. Assessing of vascular reactivity

After rabbits were euthanized thoracic aortas were excised. They were cleaned of adventitial tissue and immediately placed in cold Krebs buffer (128 mM NaCl, 14.4 mM NaHCO₃, 1.2 mM NaH₂PO₄, 4.7 mM KCl, 0.1 mM disodium salt of ethylene diamine tetraacetic acid, 2.5 mM CaCl₂, 11.1 mM glucose, pH 7.2). Then, five millimeters wide rings were cut, and mounted in a 10 ml organ bath containing the Krebs solution, aerated with 95% O₂ and 5% CO₂, and equilibrated at 37 °C and pH 7.4. Some aortic rings were mechanically deprived of endothelium to analyze its role in the contractile response to vasoactive agonists. Isometric contractions were measured using force-displacement transducers (Grass Technologies, West Warwick, USA) and recorded under an initial tension of 2 g, which was found to be the optimal tension for KCl 96 mM induced contraction). Initially to check the endothelial function, aortic rings were contracted with phenylephrine (Phe) 5×10^{-6} M and exposed to the endothelium-dependent vaso relaxant acetylcholine (Ach, 10^{-8} M to 5×10^{-6} M). Thus, a concentration-response curve (CRC) was constructed. To check contractile response to vasoconstrictors, aortic rings with intact endothelium (E+) or endothelium removed (E-) were exposed to increasing doses either of angiotensin II (Ang II, 10^{-10} to 10^{-6} M) or norepinephrine (10^{-8} to 10^{-4} M) to construct CRCs. KCl-contractile response was checked by exposing aortic rings to a single dose (96 mM).

2.8. Statistical analysis

All data are expressed as mean ± SE. The pEC₅₀ (negative log of molar concentration of agonist inducing 50% of the maximal contraction) and the maximal contractile response (R_{max}) were calculated using a curve-fitting analysis program (Graph Pad Prism 3.0; GraphPad Software Inc., San Diego, CA). Differences between weight and CRCs for the different groups were examined with two ways ANOVA for repeated measures followed by a Duncan's posttest. Differences between the four diet groups were tested by one way ANOVA followed by a Duncan's posttest. Pearson's correlation coefficients were used to assess relationships between

normally distributed variables. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Nutritional and phytochemical characterization of *Prosopis alba* seed flour

Nutritional and phytochemical composition of *P. alba* seed flour (Table 1) were analyzed. Carbohydrates and proteins were the main macronutrient in *P. alba* flour. The principal minerals were potassium and calcium. As phytochemical, the main compounds were polyphenols. Each phenolic compound was identified by HPLC-MS/MS and quantified by HPLC-DAD by using commercial standards. *P. alba* seed flour contained principally apigenin C-glycosides such as isovitexin (0.89 mg/g flour), isoschaftoside (0.42 mg/g flour), vicienin II (0.37 mg/g flour), schaftoside (0.34 mg/g flour) and vitexin (0.25 mg/g flour) (Fig. 1). Similar compounds were previously reported to *P. alba* cotyledons flour [3, 14], and *P. alba* mesocarp flour [15, 16]. Isovitexin has been reported as antioxidant and anti-inflammatory [17, 18], properties that may contribute to its protective effects on cardiovascular diseases [19]. Iso-schaftoside has been reported as a potential antihypertensive [20].

High fiber content was also found in *P. alba* seed flour (10.28%) (Table 1). Fibers have special properties as hypolipidemic agents. Their beneficial effects on cardiovascular health, glucose tolerance and insulin sensibility by reducing lipid digestion were widely reported [21, 22].

3.2. Biochemical, clinical and hemodynamic parameters

Pr-feed used to supplement rabbit diet in CD and FD models contain 12.42 % of *P. alba* seed flour. In both treatments, rabbits were fed with 8 g Pr-feed/day (25 Cal extra).

Animal body weights increased gradually during the six weeks of treatment (two fold). Significant differences between the four groups were not observed before, during or at the end of the feeding trials (5–6 weeks) (data not shown).

After 6 weeks of dietary treatment, TG plasma levels and VAF were increased in animals fed on a FD and FD-Pr Feed as compared with animals fed on a CD or a CD-Pr Feed. The addition of fat did not modify plasma levels of TC [23] and reduced HDL-C. Pr-Feed was able to reduce significantly TC levels in both diet groups (Table 2). Alterations in the glucose metabolism, previously characterized in the MS model (increase in fasting glucose and abnormal glucose tolerance test), were not prevented by Pr-Feed intake. Even more, Pr-Feed intake increased fasting glucose and induced an abnormal glucose tolerance test in rabbits fed a CD.

MAP and heart rate did not show differences between the four groups (Table 2).

Hepatic and renal-biochemical parameters as bilirubin (total, direct and indirect), AST, ALT and creatinine levels showed that *P. alba* seed flour incorporation to the diet did not modify liver and kidney function. Moreover, no changes were found in liver, kidney and heart weight (Data

Table 1

Macronutrient, phytochemical and mineral composition of *Prosopis alba* seed flour.

Component	Seeds flour	Minerals	g/100g of seeds flour
Total carbohydrates (g/100 g seed flour)	76.05 ± 2.94	sodium	0.171 ± 0.012
Total proteins (g/100 g seed flour)	11.33 ± 1.00	calcium	2.157 ± 0.119
Fat (g/100 g seed flour)	1.84 ± 0.75	iron	0.022 ± 0.002
Fiber (g/100 g seed flour)	10.28 ± 1.51	magnesium	0.777 ± 0.034
Ash (g/100g seed flour)	0.15 ± 0.01	potassium	11.563 ± 0.563
Total Phenolic (mg GAE/100 g DW)	216.06 ± 17.94	Copper	0.0054 ± 0.0003
Anthocyanins (mg C3GE/100 g DW)	<DL	zinc	0.020 ± 0.001
Ascorbic acid (mg L-AA/100 g DW)	<DL		
Carotenoids (mg β-CE/100 g DW)	7.32 ± 0.70		

DW: dry weigh. GE: glucose equivalent. < DL: lower than detection limit. GAE: gallic acid equivalents; QE: quercetin equivalents. C3GE: cyanidin-3-glucoside equivalents; L-AA: L-ascorbic acid; β-CE:β-carotene equivalents; Pβ₂E: procyanidin β₂ equivalents APO: apomorfine equivalents.

Table 2

Biochemical, clinical and hemodynamic parameters from rabbits fed on control diet (CD), fat diet (FD), CD supplemented with *Prosopis alba* seed flour (CD-Pr Feed), FD supplemented with *Prosopis alba* seed flour (FD-Pr Feed).

	CD	FD	CD-Pr Feed	FD-Pr Feed
Body Weight (g)	2138 ± 82	2043 ± 46	1837 ± 59	1941 ± 114
Visceral abdominal fat (%)	0.93 ± 0.06	2.24 ± 0.14*	1.22 ± 0.21	3.05 ± 0.27*
Fasting Glucose (mg/dl)	113.2 ± 2.7	126.1 ± 5.8*	125.9 ± 5.3*	131.7 ± 6.8*
Glucose 120'	138.1 ± 2.7	163 ± 4.6*	163.8 ± 8.5*	172.8 ± 8.0*
Total Cholesterol (mg/dl)	70.5 ± 6.0	67.08 ± 4.8*	35.9 ± 3.9*	48.3 ± 3.6*
HDL-C (mg/dl)	51.4 ± 4.1	23.2 ± 2.8*	11.2 ± 7.4	12.3 ± 5.0
LDL-C (mg/dl)	21.8 ± 3.1	42.1 ± 7.2	9.5 ± 6.3	6.3 ± 5.3
Triglycerides (mg/dl)	104.9 ± 14	191.8 ± 22.0*	101.1 ± 12.8	171.6 ± 14.5*
Blood pressure (mmHg)	57.2 ± 2.7	56.7 ± 5.3	54.3 ± 7.4	51.8 ± 4.3
Heart rate	277.6 ± 29	281.8 ± 27	264.0 ± 24.0	242.7 ± 13.8

* $p < 0.05$ indicates statistically differences between rabbits fed on CD and other diet groups (one way ANOVA and Duncan's posttest).

not shown).

3.3. Effects of dietary *Prosopis alba* seed flour on vascular reactivity

3.3.1. Response to acetylcholine

Endothelial function was assessed by checking relaxation responses to Ach in aortic rings contracted with Phe 5×10^{-6} M as function of the

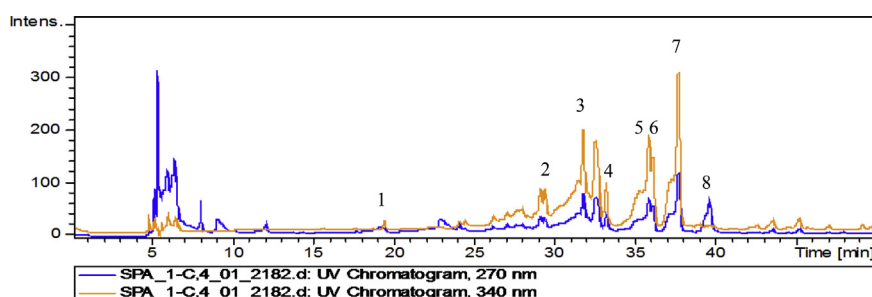


Fig. 1. Fingerprint of *P. alba* seed aquos:metanol (30:70) extract

dietary interventions. Ach (10^{-8} to 5×10^{-6} M) caused endothelium-dependent relaxation in a concentration-response manner in all diet groups. Aortic rings from the FD group exhibited lower Ach-endothelium-dependent relaxation than the CD group (Fig. 2). Pr-Feed intake improved Ach relaxation significantly in aortic rings from rabbits fed on FD.

3.3.2. Response to vasoconstrictors agonists

Vascular reactivity of different diet groups was investigated as function of the following contractile agonists: KCl (receptor-independent vasoconstrictor), Ang II (renin-angiotensin system agonist) and norepinephrine (sympathetic system agonist). Differences were observed in KCl-induced vasoconstriction after 5–6 weeks of dietary treatment (Fig. 3). Aortic rings from rabbits fed on a FD showed higher contraction response than those of rabbits fed on a CD independently of endothelium presence. Treatment with Pr-Feed reduced significantly KCl contractile response in E-arteries (Fig. 3).

Endothelium removal improves $R_{\max}$ and pEC_{50} to Ang II in the CD group. This effect was not observed in the FD group. Comparison of CD and FD groups showed no changes of Ang II- $R_{\max}$ both in E+ and E-arteries. However, FD sensitized Ang II contractile response in E+ aortic rings (there was a leftward shift of the CDR). These results agree with previous work [12]. Treatment with Pr-Feed prevented Ang II sensitization induced by endothelium removal in arteries from CD group and blunted significantly the $R_{\max}$ in E-arteries from both diet groups (Table 3).

Contractile response and sensitivity to norepinephrine was reduced by endothelium removal in arteries from CD group. This effect was opposite to those of Ang II and disappeared in arteries from FD group (Table 4). The effect of Pr-Feed-treatment on norepinephrine response was endothelium and diet dependent. In E+ aortic rings from rabbits fed on CD, Pr-Feed did not change $R_{\max}$ and reduced significantly sensitivity (induced a rightward shift of the CDR), while in E-aortic rings from rabbits fed on FD, Pr Feed significantly blunted the $R_{\max}$ to norepinephrine.

4. Discussion

A meta-analysis performed with data from 21 studies report that MS is an important risk factor for cardiovascular disease incidence and mortality, as well as all-cause mortality. Therefore, the prevention of the MS should become an important approach for the reduction of the cardiovascular disease burden in the general population [24]. Changes in lifestyle including healthy dietary regimens and increased physical

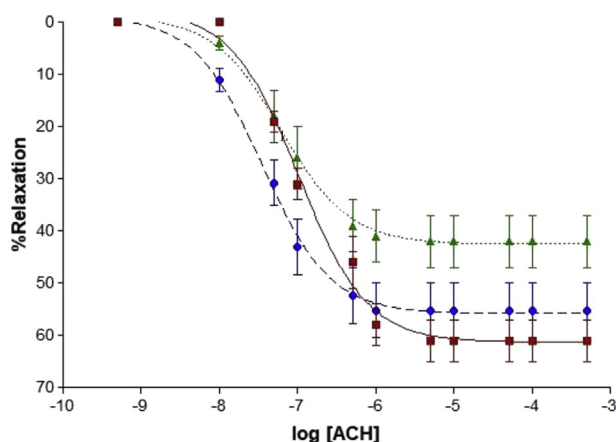


Fig. 2. Effect of different diet treatments on acetylcholine induced relaxation. (Red square) aortic rings from rabbits fed on regular diet; (Green triangle) aortic rings from rabbits fed on 18 % high fat diet; (Blue circle) aortic rings from rabbits fed on high fat diet supplemented with *Prosopis alba* seed flour.

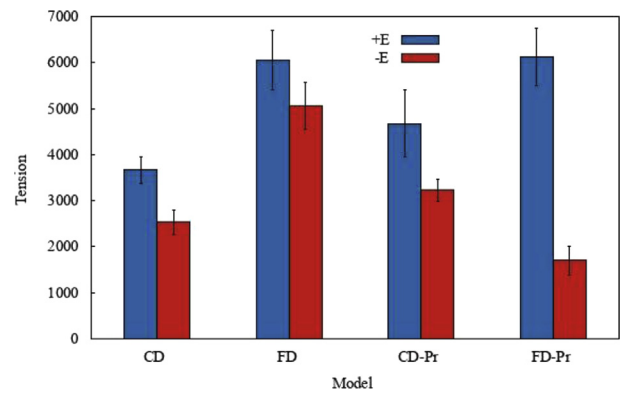


Fig. 3. Contractile response to KCl in rabbit aortic rings endothelium intact (+E) and endothelium removed (-E) from rabbits fed on different diets. CD: rabbits fed on regular diet. FD: rabbits fed on 18 % high fat diet; CD-Pr: rabbits fed on CD supplemented with *Prosopis alba* seed flour; FD-Pr: rabbits fed on CD supplemented with *Prosopis alba* seed flour.

Table 3

Maximal contractile response ($R_{\max}$) and pEC_{50} to Angiotensin II in endothelium intact (E+) and endothelium removed (E-) rabbit aortic rings.

	E+		E-	
	$R_{\max}$	pEC_{50}	$R_{\max}$	pEC_{50}
CD	4117 ± 414	7.82 ± 0.08	5112 ± 298 ^b	8.46 ± 0.07 ^b
FD	3880 ± 542	8.15 ± 0.06 ^b	4352 ± 873	8.00 ± 0.09
CD-Pr Feed	2845 ± 268 ^a	8.08 ± 0.01	2540 ± 683 ^a	7.64 ± 0.12 ^a
FD-Pr Feed	3201 ± 561	8.14 ± 0.06	2489 ± 247 ^a	7.77 ± 0.07 ^{a,b}

CD: rabbits fed on regular diet; FD: rabbits fed on 18 % high fat diet; CD-Pr Feed: rabbits fed on CD supplemented with *Prosopis alba* seed flour; FD-Pr Feed: rabbits fed on FD supplemented with *Prosopis alba* seed flour.

^a $p < 0.05$ indicates statistically differences between rabbits fed on CD or FD and rabbits fed on a Pr-Feed supplemented diet (CD-Pr Feed or FD-Pr Feed).

^b $p < 0.05$ indicates statistically differences between endothelium intact (E+) and endothelium removed (E-) arteries.

Table 4

Maximal contractile response ($R_{\max}$) and pEC_{50} to norepinephrine in endothelium intact (E+) and endothelium removed (E-) rabbit aortic rings.

	E+		E-	
	$R_{\max}$	pEC_{50}	$R_{\max}$	pEC_{50}
CD	11572 ± 1104	6.25 ± 0.06	7954 ± 700 ^b	5.98 ± 0.12 ^b
FD	11080 ± 573	6.31 ± 0.17	11490 ± 1051	6.21 ± 0.19
CD-Pr Feed	9834 ± 1310	5.80 ± 0.1 ^a	7380 ± 735 ^b	5.91 ± 0.12
FD-Pr Feed	10580 ± 1235	6.19 ± 0.28	3258 ± 726 ^{a,b}	5.86 ± 0.07 ^{a,b}

CD: rabbits fed on regular diet; FD: rabbits fed on 18 % high fat diet; CD-Pr Feed: rabbits fed on CD supplemented with *Prosopis alba* seed flour; FD-Pr Feed: rabbits fed on FD supplemented with *Prosopis alba* seed flour.

^a $p < 0.05$ indicates statistically differences between rabbits fed on CD or FD and rabbits fed on a Pr-Feed supplemented diet (CD-Pr Feed or FD-Pr Feed).

^b $p < 0.05$ indicates statistically differences between endothelium intact (E+) and endothelium removed (E-) arteries.

activity should be the first lines of work. Successful dietary strategies include energy restriction and weight loss, manipulation of dietary macronutrients, incorporation of functional foods and bioactive nutrients. Dietary components such as functional fats, digestive enzymes inhibitors, various beverages, different fruits, specific vegetables, grains, legumes, herbs and spices were demonstrated to have potential health benefits for the treatment of MS [6]. Likewise, flavonoid-rich beverages, foods, and extracts, as well as pure flavonoids are studied for the prevention and/or amelioration of MS and its associated diseases [25]. Previously, we demonstrated that cotyledon flour from *P. alba* is rich in

C-glycosides flavonoids with anti-inflammatory and antioxidant properties [3]. In the present study we examined the effects of *P. alba* seed flour on metabolic and vascular alterations characteristics of MS using a rabbit model.

Results showed that FD increased VAF and fasting glucose, induced glucose intolerance, increased TG and reduced HDL-C. There was no change in body weight along the treatment. In population there are people with normal body weight and metabolic alterations such as insulin resistance and increased VAF [26], known as metabolically obese with normal weight (MONW). Therefore, our rabbit model of high fat induced-MS had metabolic disorders imitating situations in MONW individuals when reproducing the main clinical manifestations of the MS in human beings. Pr-Feed did not improve these alterations characterizing the MONW model. These results were unexpected taking into account that the major flavonoids found in *P. alba* flour were apigenin C-glycosides, whose anti-obesity effects were reported [27]. In such sense, Kim et al. [28] demonstrated reduction of lipid droplet accumulation and inhibition of TG accumulation, together with decreased PPAR γ protein expression levels in 3 T3-L1 cells treated with apigenin C-glycosides. However, Galleano et al [25] reported no enough evidence attributing an effect of flavonoids on major end-points of obesity. In this regard, a glycoside flavonoid present in strawberries (tiliroside) increased whole-body fatty acid oxidation but failed to prevent body weight gain and visceral fat accumulation in obese-diabetic mice [29]. In the same way, Almoosawi, Fyfe, Ho, & Al-Dujaili [30] found no modification of body mass, composition, or waist circumference after consumption of dark chocolate rich in catechin and epicatechin (1.0 g/day) for two weeks in overweight/obese subject. Likewise, the administration of licorice flavonoid oil (300 mg/day) for eight weeks [31] or flavonol quercetin supplementation (500 and 1000 mg/day) for 12 weeks [32] had no effects on body mass and body composition. Considering all these data as a whole, it should be noted that changes in body mass in obese/overweight individuals need strong and long-term interventions to result in a significant change in body weight or waist circumference. Indeed, VAF is strongly associated with metabolic disorders [33] and Pr-Feed had no effects on VAF. Therefore, this would be the reason why Pr Feed did not modify risk factors in our MONW model. Although the feeding of Pr-Feed rich in apigenin C-glycosides had no effect on TG levels, TC was reduced in both diet groups. According to that, Belguith-Hadriche et al. [34], found hypocholesterolemic effect of vitexin enriched extract from Tunisian *Ficus carica* fruit in high-fat diet-induced hyperlipidemic rats. Moreover, Brown, Rosner, Willett, & Sacks [35] showed that soluble fibers reduced TC. Taking these data from the literature together with the high fiber content found in Pr-Feed, reduced cholesterol absorption may account for the TC levels reduction.

Diet polyphenols have been shown to possess anti-diabetic property, suggesting its use as therapy for the prevention and management of Type 2 diabetes [36]. In such sense, vitexin and isovitexin orally administered significantly reduced postprandial blood glucose both in sucrose loaded normoglycemic mice and sucrose induced diabetic rats [37]. However, Pr-Feed administration did not only change glucose metabolism of rabbits fed on FD but increased basal glucose and induced abnormal glucose tolerance curve in rabbits fed on CD. Pr-Feed incorporated 4.7 g carbohydrates to the CD (0.76 g from *P. alba* flour). Even though the quantity may seem scarce, younger rabbits are very sensitive to carbohydrates from the diet [38]. Therefore, the increase in carbohydrate percentage would account for the unexpected and contradictory effect of Pr-Feed administration on glucose metabolism. Further studies will be necessary to elucidate this point.

While Pr-Feed treatment did not modify metabolic alterations, beneficial effects on the vascular function were found. Relaxation to acetylcholine was normalized and Ang II, norepinephrine and KCl contractile response was attenuated in aortic rings from rabbits fed on FD after 6 weeks of Pr-Feed administration. The beneficial effect of flavonoids on vascular function has been widely demonstrated [39]. In previous work we demonstrated the vasorelaxant and Ang II-antagonist

effects of chalcones from *Zuccagnia punctata* in aorta from hypercholesterolemic rabbits [40]. Recent research showed that flavonoids such as apigenin C-glycoside can modulate the vascular contractility in an agonist-dependent manner. The mechanism involved seems to be not only endothelium-dependent but also to involve the inhibition of MAP-K/ERK pathway and the partial inhibition of Rho-kinase [41]. The effect of vitexin can, at least partly, be attributed to its effect in blocking Ca²⁺-mediated pathways. Lu et al [42] demonstrated, both *in vitro* and *in vivo*, that Vitexin inhibited the isoproterenol-induced increase in resting intracellular free Ca²⁺ as well as the expression of the Ca²⁺ downstream effectors calcineurin-NFATc3 and phosphorylated calmodulin kinase II (CaMKII). Considering that KCl induces contraction by opening voltage dependent Ca²⁺-channels and increase of the intracellular Ca²⁺ levels, inhibition of Ca²⁺-mediated pathways may explain the effects of Pr-feed on E-arteries. Indeed, contractile response to Ang II and norepinephrine also involves Ca²⁺ influx in smooth muscle [43]. Therefore, the fact of Pr-Feed intake has had effect on the contractile response to Ang II and norepinephrine in only E-arteries reaffirms the view that Pr-feed intake may modify intracellular Ca²⁺ homeostasis. Moreover, the vitexin-containing *Lagenaria siceraria* fruit, exhibits anti-hypertensive and cardio protective effects in rat model of L-NAME-induced hypertension and myocardial necrosis and inflammation [44]. More research is necessary to determine whether cardioprotector effects of apigenin C glycosides are related to Ca²⁺ movement mechanisms.

5. Conclusion

Pr-Feed has beneficial effects on vascular function in a rabbit model of MONW despite no effects on metabolic alterations. Therefore, Pr-Feed would have vascular protector properties and might be used to prevent vascular alterations induced by MS. At this point, we would like to highlight that the difference between prevention and therapy, prevention implies low doses, but long duration of the treatments (years), while therapy is associated with high doses (and potentially side effects), but short time of administration. *P. alba* dietary supplementation together with other nutraceuticals with recognized properties on metabolic alterations (i.e *Salvia hispanica*, chia oil on hypertriglyceridemia or *Smallanthus sonchifolius*, Yacon roots on insulin resistance) may be promising. The major compounds found in the Pr-Feed were apigenin C-glycosides. Therefore, they may account for these beneficial effects. Mechanisms involved will be goal of further studies.

Declarations

Author contribution statement

Florencia Cattaneo: Performed the experiments; Analyzed and interpreted the data.

Gabriela Alarcon y Julieta Roco: Performed the experiments.

Maria Ines Isla: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Susana Jerez: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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