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Mulinum spinosum root extract, rich in antioxidant compounds, mitigates harmful effects in mice with diet-induced metabolic syndrome

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ABSTRACT

Background: Metabolic syndrome (MetS) is a health issue with a high incidence in adult population. Using herbal medicines for the management of serious complications of MetS, such as dyslipidemia and hyperglycemia, is highly promising. An aqueous extract from Mulinum spinosum (M. spinosum), traditionally used as hyperglycemic and anti-inflammatory, could have beneficial effects on the treatment of MetS.

Purpose: The present study was aimed to characterize the composition of *M. spinosum* roots decoction, and to evaluate antidiabetic, antilipemic and antioxidant effects in an animal model of MetS.

Study design and methods: *M. spinosum* roots extract was characterized using high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) method. Total Phenolic Content (TPC) was spectrophotometrically measured and the antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) assay. To generate the MetS model, adult male C57BL/6 mice were fed with a 20% w/v fructose (Fru) in drinking water combined with 30% w/w high fat diet (HFD) over a period of 12 weeks. *M. spinosum* aqueous extracts (3 or 6 g/kg/day) were administered in drinking water together with Fru-HFD. At the end of the exposure period, mice were weighed and glucose tolerance test was determined. After sacrifice, adipose tissues were isolated and blood samples were collected to evaluate lipid profile, lipid peroxidation level (LPO) and ferric reducing antioxidant power (FRAP).

Results: The characterization of *M. spinosum* revealed the presence of caffeic acid, *trans*-resveratrol, kaempferol-3-glucoside, (-)-epicatechin, (-)-gallocatechin gallate, (-)-epigallocatechin and (+)-catechin. A high antioxidant capacity of the extract was revealed by ORAC test. Mice fed a Fru-HF diet and treated with *M. spinosum,* reduced diet-induced weight gain and significantly decreased mesenteric fat compared with Fru-HFD group ($p<0.01$). Post prandial glycaemia significantly diminished when mice were treated with *M. spinosum*, and also total cholesterol, LDL-cholesterol and triglycerides (TG) were reduced. Regarding the antioxidant effect of the consumption of *M. spinosum*, we found a decrease in plasmatic LPO, and a significant increase in plasmatic FRAP.

Conclusion: To our knowledge, the phenolic composition and the antioxidant capacity of *M. spinosum* roots decoction is reported for the first time. In addition, we provide evidence that *M. spinosum* root extract is a promising source of antioxidants able to alleviates Fru-HFD-induced metabolic alterations and slow the progression of MetS.

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Table 1
Total phenolic content and antioxidant capacity of M. Spinosum roots decoction.

	E1	E2
TPC FC (mg GAE L^{-1} extract)	287.4 ± 4.3	581.2 ± 9.6
TPC 280 (mg GAE L^{-1} extract)	624.4 ± 16.6	1018.5 ± 5.7
ORAC (μmol TE mL^{-1})	6.1 ± 0.5	7.4 ± 0.7

*Data are mean ± standard deviation. TPC FC: Total phenolic content, Folin Cicalteau; ORAC: oxygen radical absorbance capacity; E1: *M. spinosum* extract 2 g% w/v; E2: *M. spinosum* extract 4 g% w/v.

Table 2
Concentration of phenolic compounds in decoction of M. Spinosum roots.

	E1	E2
(-)-gallocatechin	561±24	955±43
Quercetin-3-galactoside	49±1	251±2
Quercetin-3-glucoside	903±84	2129±193
Kaempferol-3-glucoside	7612±424	11,994±924
Gallic acid	113±3	153±6
OH-Tyrosol	802±70	1412±113
Tyrosol	2091±93	3255±124
(-)-epigallocatechin	5781±53	12,962±101
(+)-catechin	816±64	3746±302
(-)-epicatechin	6338±501	10,920±77
(-)-epigallocatechin gallate	1026±78	1986±168
(-)-gallocatechin gallate	6157±333	15,179±938
Caftaric acid	1216±54	2491±103
Caffeic acid	38,078±1534	62,663±2334
p-coumaric acid	1198±45	2697±105
Ferulic acid	180±6	439±18
trans-resveratrol	7887±213	15,771±397
Sum	48,560±1123	84,063±1998

Average concentrations (μg/ L^{-1} of decoction) with their standard deviations, $n = 3$ replicates. E1: *M. spinosum* extract 2 g% w/v: E2: *M. spinosum* extract 4 g% w/v.

Abbreviations

(M. spinosum) Mulinum spinosum, (MetS) Metabolic Syndrome, (HPLC-DAD) high-performance liquid chromatography coupled with diode array detection, (TPC) Total Phenolic Content, (ORAC) oxygen radical absorbance capacity, (AAPH) 2,2′-azobis-2-methyl-propionamidine dihydrochloride, (MeCN) acetonitrile, (MeOH) methanol, (FA) formic acid, (IP) intraperitoneal, (HFD) high fat diet, (Fru) fructose, alkaline Phosphatase (ALP), Aspartate aminotransferase/Glutamic Oxaloacetic Transaminase (AST/GOT), Alanine aminotransferase/Glutamic Pyruvic Transaminase (ALT/GPT), (TBARS) Thiobarbituric Acid Reactive Substances, (MDA) malondialdehyde, (FRAP) ferric ion reducing antioxidant power, (LDL) low density lipoprotein, (FC) Folin–Ciocalteu, (TG) triglyceride, (LPO) lipid peroxidation, (ROS) Reactive Oxygen Species.

Introduction

Profound changes in the environment, human behavior and lifestyle have driven to increasing rates of Metabolic Syndrome (MetS) characterized by low physical activity and high-calorie diet consumption, which lead to earlier development of type 2 Diabetes (Ferreira et al., 2020; Povel et al., 2013; Saklayen, 2018). Oxidative stress refers to an imbalance between Reactive Oxygen Species (ROS) production and the antioxidant system leading to a reduction of peripheral insulin sensitivity and contributing to the development of Diabetes via several molecular mechanisms (Yaribeygi et al., 2020; Zhang et al., 2020).. Current management criteria for MetS risk factors involve changes in lifestyle and the use of pharmacological agents. In addition to approved pharmacological therapies, there is growing interest in the use of naturally occurring compounds, as alternative strategies that could be effective in counteracting multiple components of MetS, maybe avoiding the onset of side effects (Nyakudya et al., 2020). Since hyperglycemia is close related with oxidative stress, new antidiabetic agents, in addition to control hyperglycemia, should act reducing oxidative stress. Many natural medicines, which include crude or cooked extracts, are easily accessible and have interesting therapeutic potential in the treatment of patients with MetS (Kellogg et al., 2019). However, scarce knowledge is available on the way these natural extracts underscore or promote health. First, because there is a lack of characterization of natural extracts and, second, because each compound, isolate or in combination, can trigger a multitude of effects.

Medicinal plants still play an important role in primary healthcare in the communities of urban and suburban centers. Among the medicinal species of Patagonian steppe, *Mulinum spinosum* (*M. spinosum*), (Cav.) Pers. (Apiaceae), (accepted name of a species in the genus *Mulinum*, family Apiaceae) commonly known as 'neneo', is an endemic plant of the mountains of Patagonia region. *M. spinosum* is traditional used as anti-parasitic (Sulsen et al., 2006), antibacterial (Echenique et al., 2014), analgesic, and anti-inflammatory agent (Estomba et al., 2006; Molares and Ladio, 2014). Secondary metabolites, exclusive constituents of medicinal plants belonging to *Mulinum* genera, are used as hypoglycemic agents (Dzul-Beh et al., 2020), and previous experiments from our group showed that a decoction of *M. spinosum* root has a potential anti-hyperglycemic activity in alloxane-induced diabetic mice (data not published). Scientific studies demonstrating the chemical characterization of the root extract and the beneficial effects of *M. spinosum* roots on metabolic alterations associated with MetS, are not published yet. The present study focused on chemically characterize the decoction of *M. spinosum* roots and on evaluating the hypoglycemic, anti-lipemic and antioxidant activity of *M. spinosum* roots extract in an experimental animal model of MetS.

2. Materials and methods

Study design: First, HPLC-DAD chemical characterization, total phenolic content and antioxidant capacity of the aqueous extract of *M. Spinosum* root was estimated. Then, anti-glycemic, antilipemic and antioxidant effects were investigated using in vivo model of MetS in mice achieved by ingesting a rich fructose/high fat diet.

2.1. Standards, solvents and chemicals

All details of standards used, and solvents and chemicals employed in the study, are placed in supplementary material.

2.2. Plant sampling

Fresh roots of *M. spinosum* were collected at Paraje Collón Cura (Neuquén province; 40°25′00.5″S 70°34′24.7″W, Argentina), and were authenticated by a botanical advisor at the Department of Applied Biology, Universidad Nacional del Comahue (Project no. PIN04-N022). More details in supplementary material.

2.3. Aqueous extracts preparation

Plant material was prepared according to the traditional method used (decoction). Dried and ground root were weighed on a standard Ohaus brand scale, model CS 200. 15 g or 30 g were boiled in 900 ml of water for 20 min and filtered with Whatman® qualitative filter paper, Grade 1. Thereafter, two aqueous extracts were obtained: E1 (2 g% w/v) and E2 (4 g% w/v). Extracts were kept in amber bottles at 4 °C until use.

2.4. Chromatographic method

Phenolic compounds were determined using a Dionex Ultimate 3000 HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany) and a reversed phase Kinetex C_{18} column (3.0 mm × 100 mm, 2.6 μm) (Phenomenex, Torrance, CA, USA). As mobile

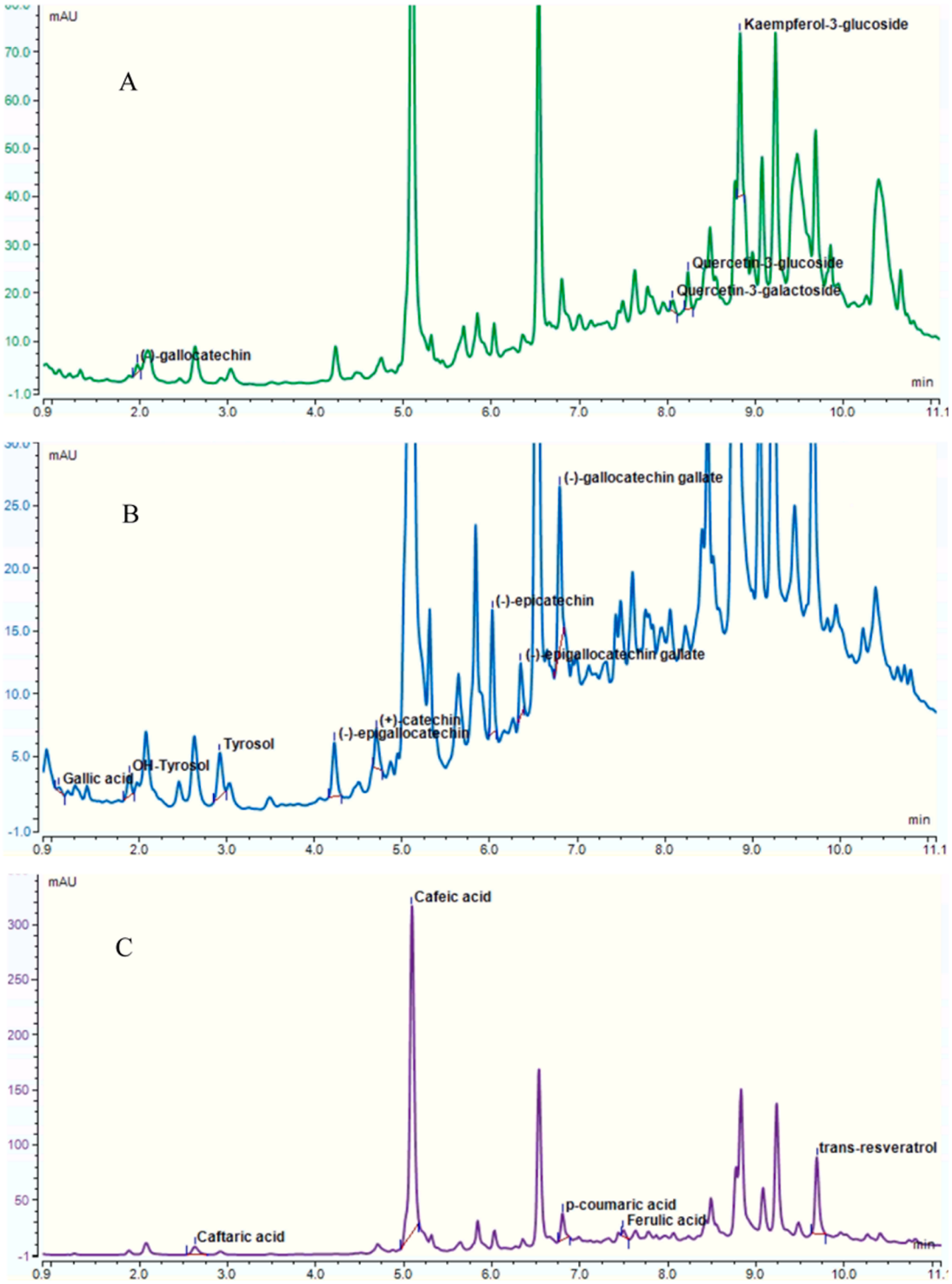

Fig. 1. Representative HPLC-DAD chromatograms obtained for phenolic compounds quantified in this work for decoction of *M. Spinosum* roots (sample E2, Table 2). A: 254 nm, B: 280 nm and C: 320 nm.

phases were used ultrapure water with 0.1% FA (A) and MeCN (B). Analytes were separated using a previously reported method (Fontana et al., 2016). The identification of PCs in samples was based on the comparison of the retention times (tR) of compounds in samples with those of authentic standards. An aliquot of decoction of *M. Spinosum* roots was also spiked with known concentrations of standards in order to ensure a correct quantification and identification of compounds. Sample quantification was done by an external calibration with pure standards.

2.5. Total phenolic content (TPC)

The TPC was spectrophotometrically measured with an UV–vis spectrophotometer Cary-50 (Varian Inc., Mulgrave, Australia) from an aliquot of the extract. TPC was quantify by Folin–Ciocalteu assay (FC) as reported by (Antoniolli et al., 2015).

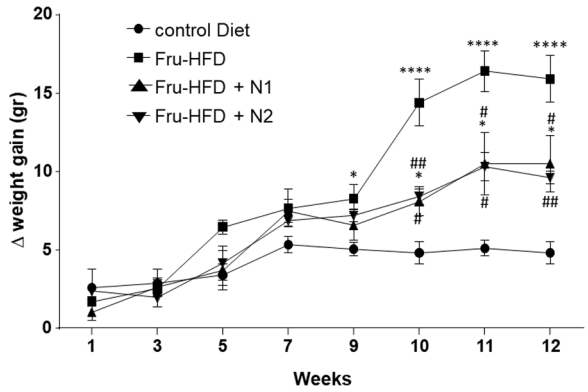

Fig. 2. *M. spinosum* reduces weight gain in mice fed a fructose/high fat diet (Fru-HFD). Body weight was monitored over 12 weeks after starting treatments and weight changes after dietary intervention, from the baseline value at week 0 for each group at each week, were analyzed ($n = 8$). Data are presented as means ± SEM, *$p<0.05$ and ****$p<0.0001$ vs control diet; # $p<0.05$; ## $p<0.01$ vs Fru-HFD fed mice. N1: Neneo3 g/kg/day; N2: Neneo6 g/kg/day.

2.6. Antioxidant capacity

The antioxidant capacity of the extracts was determined by the oxygen radical absorbance capacity (ORAC) assay, as previously reported (Ferreyra et al., 2020) with some modifications. All samples were analyzed in triplicate and results were expressed as the mean ± standard deviation (SD).

2.7. Animals and diet

All procedures were approved by the Animal Research Committee of the National University of Cuyo (CICUAL approval no. 178/2019, School of Medical Science, Mendoza, Argentina), in accordance with international Guidelines. Details of the animal protocol are in supplementary material.

2.7.1. Glucose tolerance test

Animals were fasted for 5 h, with free access to water. Fasted blood glucose levels were determined before a solution of glucose (2 gr/kg body weight) was administered via intra-peritoneal (IP). Subsequently, the blood glucose level was measured at 120 min post-injection. To measure glucose levels, blood from tail veins was collected and glycemia levels were determined by a reactive strip and a glucometer (Accuchek, Performa Roche Diagnostic)

2.7.2. Tissue collection

After sacrificing the animals, liver, kidney and adipose tissues (epididymal, retroperitoneal and mesenteric) were rapidly isolated, washed in ice-cold phosphate-buffered saline, patted dry and weighed.

2.7. Biochemical assays

The plasma triglycerides (TG), total Cholesterol and LDL-cholesterol (LDL-C) were determined using commercial kits by enzymatic colorimetric methods (Biosystem S.A, Barcelona, Spain). Hepatic enzymes, including serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), and serum alkaline phosphatase (ALP), were measured by a photometric method.

2.7.4. Lipid peroxidation

Lipid peroxidation (LPO), which is a marker of unsaturated cell membrane lipid damage by free radicals, was measured by the thiobarbituric acid reactive substances (TBARS) method in plasma samples.

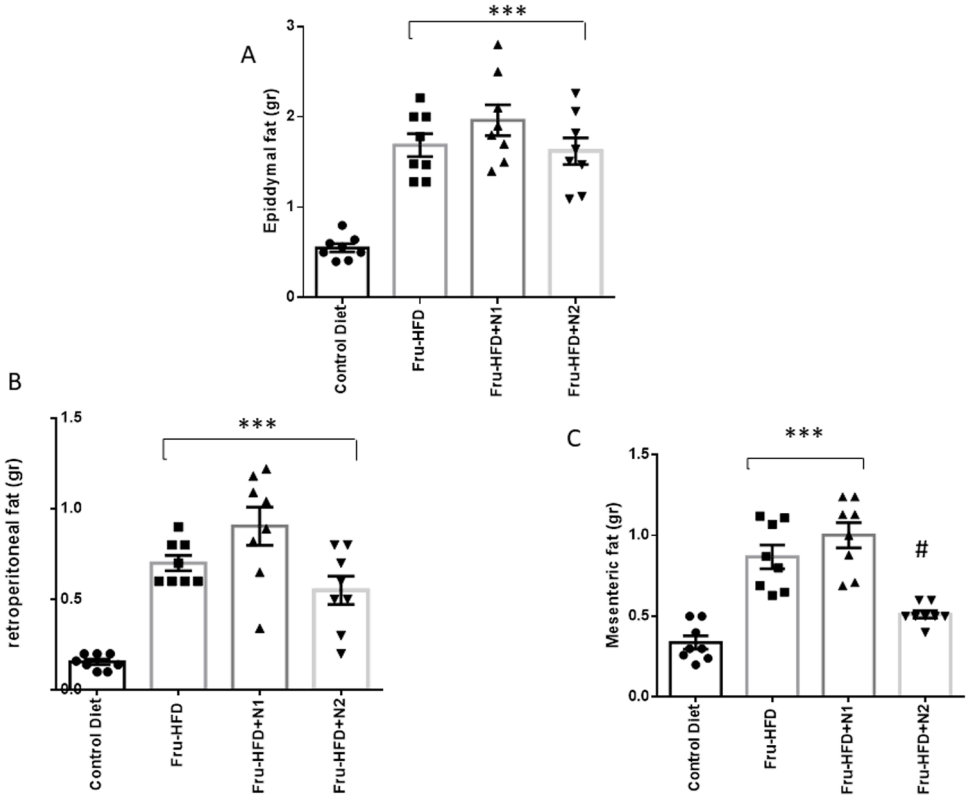

Fig. 3. *M. spinosum* decreases mesenteric fat in mice fed a Fructose/high fat diet (Fru-HFD). (A) Epididymal fat, (B) Retroperitoneal fat and (C) Mesenteric fat. Data are presented as means ± SEM ($n = 8$) ***$p<0.001$ vs control diet; #$p<0.05$ vs Fru-HFD fed mice. CD: Control diet; N1: Neneo3 g/kg/day; N2: Neneo6 g/kg/day.

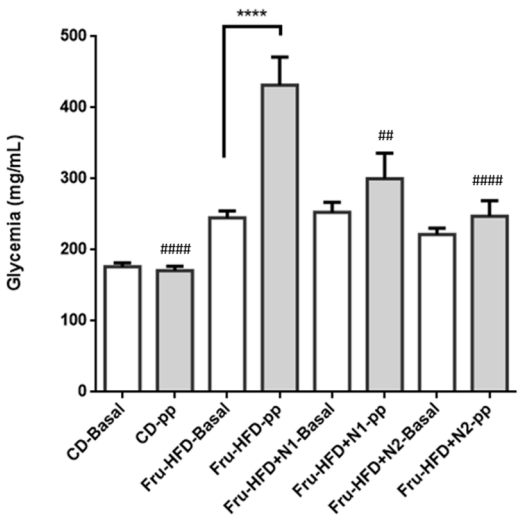

Fig. 4. *M. spinosum* diminishes glycaemia 2-hour after glucose overload in mice fed a Fructose/high fat diet (Fru-HFD). Fasting glycemia (basal) and glycemia after two-hour post glucose overload (pp). Data are presented as means ± SEM ($n = 8$). ****$p<0.0001$ vs Fru-HFD basal; ##$p<0.01$; ####$p<0.0001$ vs Fru-HFD pp. CD: Control diet; N1: Neneo3 g/kg/day; N2: Neneo6 g/kg/day; pp: post glucose overload.

Table 3
Average of lipid levels in mice fed control diet (CD), High fructose/High fat diet (Fru-HFD) with/without *M. spinosum* root decoction.

	CD	Fru-HFD	Fru-HFD +N1	Fru-HFD +N2
Total cholesterol [mg /dL]	74±5	142±4****	125±4****	113±5****###
LDL-Cholesterol [mg /dL]	20.7 ± 2.4	57.7 ± 1.4****	55±4****	42±3***##
Triglycerides [mg /dL]	54±2	82±5***	44±4###	43±4###
GOT(UI/l)	140 ± 14	217 ± 68	205 ± 93	193 ± 52
GPT(UI/l)	18 ± 4	17 ± 4	24 ± 4	18 ± 4
ALP (UI/l)	147 ± 8	125 ± 14	134 ± 7	162± 5
Liver mass (g)	1.46± 0.04	1.75 ± 0.05***	1.49 ± 0.02 ##	1.44± 0.06###

Values are mean ($n = 8$) ± SEM. ***$P<0.001$; ****$P<0.0001$ vs control diet (CD); ##$P<0.01$; ###$P<0.001$ vs Fru-HFD. N1: Neneo3 g/kg/day *M. spinosum*; N2: Neneo6 g/kg/day *M. spinosum*.

2.7.5. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability of plasma was estimated as previously described (Berker et al., 2010). The standard curve was made using Trolox (Cayman, USA) as standard. Results were expressed as mg (Trolox) L-1.

2.7.6. Statistical analysis

Results of phenolic compounds concentration are expressed as means ± SD. Animal experimental data are shown as means ± SEM. Statistical significance was determined using the one-way analysis of variance, followed by the Tukey multiple comparison test; p values < 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism version 7.0 statistical software (GraphPad Software Inc., CA, USA).

3. Results

3.1. Chemical characterization of M. spinosum roots decoction

The TPC and antioxidant capacity of *M. Spinosum* roots decoction are shown in Table 1. These parameters are important to understand possible association with individual phenolic composition. The levels of TPC and antioxidant capacity were dose dependent. The profiles and concentration of phenolic compounds are summarized in Table 2. A total of seventeen compounds of different families were identified and quantified in the analyzed sample at the two concentrations of decocted roots. To the best of our knowledge and based on the literature survey, all these phenolic compounds were first time reported from *M. Spinosum* roots decoction. The compounds corresponded to different non-flavonoids (hydroxybenzoic and hydroxycinnamic acids, stilbenes and phenylethanol derivatives) and flavonoids (flavanols, including different tannins and flavonols derivatives). The sum of phenolic compounds concentration was 48,560±1123 μg/ L^{-1} for 2 gr% and 84,063 ± 1988 μg/ L^{-1} for 4 gr% of *M. spinosum* root extract. The most abundant compounds were caffeic acid, the stilbene *trans*-resveratrol, the flavonol derivative kaempferol-3-glucoside and the tannin (-)-epicatechin. These compounds showed concentrations of 62,663, 15,771, 11,994 and 10,920 μg/ L^{-1} respectively when 4 gr% of *M. spinosum* were tested. Fig. 1 shows the representative chromatograms obtained at each detection wavelength of the decoction of *M. spinosum* roots (Sample E2).

3.2. Effects of M. spinosum on body weight and visceral fat weight

To address whether *M. spinosum* displays a metabolic protective effect, we used Fru-HFD-fed mice as a MetS model. Animals were monitored weekly and changes of the body weight were registered. Mice fed a Fru-HFD showed a significant increase in body weight compared to the control group from weeks 10 (*$p<0.05$) to 12; (****$p<0.0001$ Fig. 2). However, Fru-HFD-fed mice treated with 3 g/kg/day (N1) or 6 g/kg/day (N2) of *M. spinosum* extract, showed a significant reduction in body weight gain compared to the Fru-HFD group (#$p<0.05$; ##$p<0.01$ Fig. 2). Furthermore, Fru-HFD-fed mice resulted in a significant increase in epididymal, retroperitoneal and mesenteric fat compared with control group (Fig. 3A, 3B and 3C respectively). However, treatment with N2 significantly reduced mesenteric fat weight compared with Fru-HFD alone (#$p<0.05$; Fig. 3C). No significant difference in food intake was observed among any Fru-HFD-fed mice, suggesting *M. spinosum's* effects were not due to reduced food consumption.

3.3. Effect of M. spinosum on glycemia regulation

Fasting and two hour-post glucose load blood levels were carried out to check insulin resistance in Fru-HFD fed mice after twelve weeks. The results showed that Fru-HFD significantly increase basal blood glucose ($p < 0.001$; Fig. 4) compared with control diet-fed group. However, after 120 min of intraperitoneal injection of 2 g/kg body weight of glucose, blood glucose levels in Fru-HFD fed mice treated with 3 g/kg/day or 6 g/kg/day *M. spinosum* extract significantly diminished, compared with Fru-HFD group ($p < 0.05$ and $p<0.01$, respectively Fig. 4).

3.4. Effect of M. spinosum on plasma lipid levels and hepatic profile

Results of plasmatic lipid profile are presented in Table 3. Fru-HFD group presented a lipidic profile consistent with the MetS animal model (Leonardi et al., 2020), augmented total cholesterol ($p<0.0001$), LDL-cholesterol ($p<0.0001$) and triglyceridemia ($p<0.001$), compared with control diet (CD) group. Remarkably, mice fed Fru-HFD supplemented with *M. spinosum* extract 3 g/kg/day (N1) ameliorated plasmatic triglyceride levels ($p<0.001$), compared with Fru-HFD group, and mice fed with Fru-HFD supplemented with 6 g/kg/day (N2) *M. spinosum* extract, showed a significant decrease of all plasma lipid markers tested, total cholesterol level ($p<0.001$), LDL-cholesterol ($p<0.01$) and triglycerides ($p<0.001$), compared with mice on Fru-HFD. A plasmatic hepatic profile was performed, and we found that hepatic amino transferase enzymes, including ALP (alkaline Phosphatase), AST/GOT (Aspartate aminotransferase/Glutamic Oxaloacetic Transaminase),

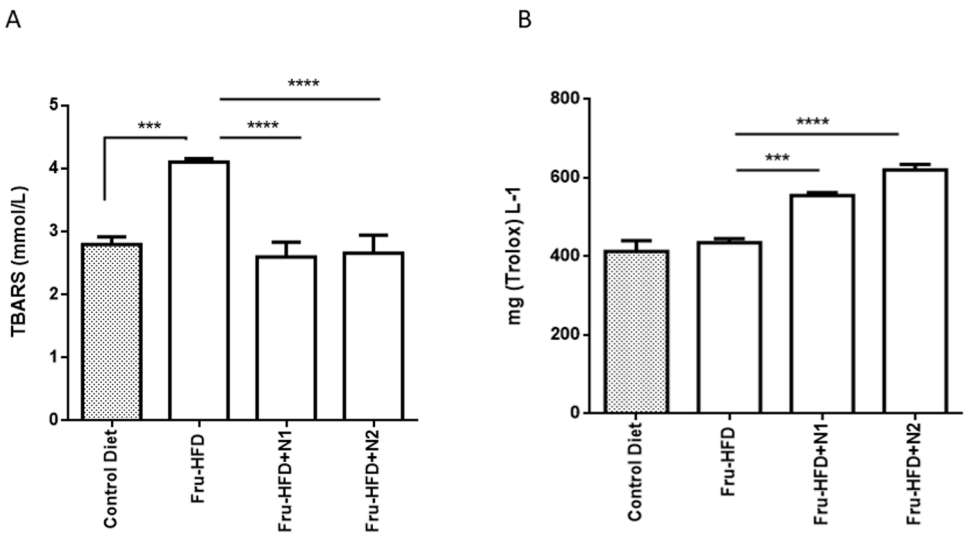

Fig. 5. *M. spinosum* alleviates oxidative stress in mice fed a Fructose/high fat diet (Fru-HFD). A. The plasma level of malondialdehyde (MDA), the major lipid peroxidation product, was evaluated as thiobarbituric acid reactive substances (TBARS). N1 and N2 significantly reduced Fru-HFD-induced TBARS. B. Ferric Reducing Antioxidant Power (FRAP) was determined on plasma samples. N1 and N2 significantly increased plasmatic total antioxidant capacity. Data are presented as means ± SEM. ***$p<0.001$; ****$p<0.0001$. CD: Control diet; N1: Neneo3 g/kg/day; N2: Neneo6 g/kg/day; TBARS: thiobarbituric acid reactive substance.

ALT/GPT (Alanine aminotransferase/Glutamic Pyruvic Transaminase) showed no significant differences between any groups, based on statistical analysis. Interestingly, we found *M. spinosum* extract intake prevented Fru-HFD-induce liver weight increase (Table 3).

3.5. Effect of M. spinosum on oxidative stress status

Regarding the assessment of oxidative stress markers of damage, systemic LPO, measure by end products such as TBARS, increased in mice fed Fru-HFD compared to control chow mice (Fig. 5A). But, there was a significant decrease in TBARS concentration in mice treated with N1 ($p < 0.001$) or N2 of *M. spinosum* extract ($p< 0.001$). FRAP was used as a marker of antioxidant status based on antioxidant principles to form a colored complex with potassium ferricyanide. After 12 weeks of Fru-HFD, no significant difference was observed in plasma activity of FRAP compared with control diet. However, treatment with either concentration (N1 or N2) of *M. spinosum*, significantly increased plasma antioxidant status compared to Fru-HFD group (Fig. 5B).

4. Discussion

The current study presented for the first time, the chemical composition of an aqueous extract from M. spinosum roots, and revealed a rich polyphenols mixture with a high antioxidant capacity. Studies have demonstrated that the chemical structure of plant polyphenols is appropriate for reactive species scavenging, and also, the effectiveness of polyphenols antioxidant activity has been deeply studied (Morvaridzadeh et al., 2020). So, instead of supplements, using naturally occurring phytonutrients for limiting or reducing oxidative stress could play a very important role in health protection and disease control. In our study particularly interesting was the presence at high levels of the antioxidant *trans*-resveratrol from stilbene family. The phenolic compounds, in general and specifically stilbenes, are produced by plants as a response to stress and/or defense to environmental conditions or pathogen infections (Dubrovina and Kiselev, 2017). *Trans*-resveratrol compound has been deeply studied over the last years due to its beneficial health effects, conferring strong protection against metabolic, cardiovascular and other age-related complications (Kulkarni and Canto, 2015). Up-to-date evidence strongly supports a contribution of polyphenols to hypoglycemic and hypocholesterolemic effects, maintaining good human health, as well as, preventing diseases or pathologies associated with oxidative stress. (Durazzo et al., 2019; Fraga et al., 2019). Another interesting finding of our study, regarding the characterization of *M. spinosum,* is the high content of caffeic acid that was found in the decoction of the root of this shrub. Caffeic acid is a natural antioxidant able to increase superoxide dismutase, catalase, and glutathione peroxidase activities (Jung et al., 2006), and is well-recognized as an antidiabetic agent (Chukwuma et al., 2019).

Oxidative stress and redox deregulation are typical features of numerous diseases and uncontrolled ROS generation represents a major source of pathological oxidative damage. In order to provide a scientific basis for the use of *M. spinosum* root extract in traditional medicine, we next study the effect of *M. spinosum* intake in a diet-induced MetS animal model. In general, diets with high levels of fructose associate with high fat content, promote weight gain, abdominal fat, hyperglycemia and insulin resistance in mice (Zhuhua et al., 2015) (Sato Mito et al., 2009). Our choice to use a high fat-high fructose diet was driven by its high similarity with the actual human western diet and by the panel of symptoms it causes (eg hyperinsulinemia, insulin resistance, impaired glucose tolerance, dyslipidemia, increase abdominal fat deposition) (Dissard et al., 2013). During our study, we observed that mice fed a Fru-HFD and treated with *M. spinosum* root decoction, reduce body weight, particularly mesenteric adipose tissue, and improved glucose tolerance. To our knowledge, the only report that mentions an antihyperglycemic effect of one of the components of *M. spinosum,* the mulinolic acid, was published by Fuentes et al. In this work, streptozotocin diabetic rats treated with mulinolic acid showed a reduction on glycemia level, and authors suggested this secondary metabolite from *M. spinosum* operated on glucose utilization or production in the liver (Fuentes et al., 2005). High fructose consumption promotes and complicates glucose metabolism, causing alterations in the lipid profile. Our data also demonstrated that *M. spinosum* extract has the potential to lower triglyceridemia and cholesterolemia levels. This lipid-lowering activity may be attributed to the phytoconstituents, such as phenolic compounds present in it, as has been reported for other root extracts (Chawda et al., 2014; Espindola et al., 2016). Polyphenols can modulate carbohydrate and lipid metabolism, lessen hyperglycemia, dyslipidemia and insulin resistance, improve fat metabolism and alleviate oxidative stress (Yaribeygi et al., 2020; Zhang et al., 2020). Thus, polyphenols can have a positive effect on the prevention and alleviation of the course of MetS.

The long-term consumption of high fat/high fructose diets, which leads to MetS, will inevitably end up to the appearance of non-alcoholic fatty liver disease. Our data showed *M. spinosum* prevented Fru-HFD-induced liver weight gain, so the use of *M. spinosum* root extract could yield hepatoprotective effect against excessive fat built-up in the liver.

The increase in oxidizing species formation in MetS has been accepted as a major underlying mechanism for the increase of protein

and lipid oxidation and the impairment of antioxidant systems (Vona et al., 2019). As the antioxidant effect of *M. spinosum* could be attributed to bioactive compounds such as phenolic compounds present in root decoction, we evaluated the plasmatic redox status in our model. Remarkably, we found that both concentration of *M. spinosum* strongly reduced Fru-FHD-induced LPO in part by increasing FRAP capacity. Oxidative stress directly interferes in lipid oxidation and leads to an increased cellular lipid accumulation that inhibits insulin signaling. This situation affects the translocation of GLUT-4 in insulin-sensitive tissues such muscle or fat, which leads to circulating glucose accumulation and consequently, to hyperglycemia (Garcia-Garcia et al., 2020). Based on our findings, it is possible to speculate that polyphenols present in *M. spinosum* root extract protect from LPO and would improve insulin sensitivity by facilitating the translocation of glucose transporters in target tissues. New studies should be carried out in order to test this hypothesis.

5. Conclusions

This study showed for the first time, the phenolic composition of *M. spinosum* roots decoction. Our results validate properties attributed to the traditional use of this shrub as anti-glycemic agent. Beside, we provide evidence that *M. spinosum* is a promising source of antioxidants able to modulate weight gain, glucose and lipid profile, plasmatic lipid peroxidation and antioxidant status.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author agreement

All authors certify that they have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

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Author Contributions

S.B., C. Cr and C. Ca formulated the hypothesis, applied for grant support, initiated and conducted the study; S. F. and A.F performed chemical characterization; M. d P. and C. S. collected the tissue samples and contributed relevantly to the manuscript; MB. P. performed FRAP analysis; S.B., A.F. and C. Ca contributed to the discussion of results and critically reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100169.

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