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Original article

Ethanolic extract of Lippia graveolens stem reduce biochemical markers in a murine model with metabolic syndrome

Maria Estela Frías-Zepeda [a], Martha Rosales-Castro [a], Gerardo. Norberto Escalona-Cardoso [b], Norma Paniagua-Castro [b,*]

[a] Instituto Politécnico Nacional-CIIDIR Durango, Biotechnology Group, Mexico
[b] Instituto Politécnico Nacional-ENCB- Ciudad de México, Physiology Department, Mexico

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ABSTRACT

Metabolic Syndrome (MetS) is a risk to develop metabolic-chronic degenerative disease, it is important to find natural alternatives to help decrease the risk. Mexican oregano has a traditional use in Mexican food, moreover, has pharmacologic effects that can help to reduce risk the metabolic syndrome. The aim of this work was to determine the effect of Mexican oregano ethanolic extract in metabolic syndrome in murine model.

Ethanolic extract of Mexican oregano (*Lippia graveolens*) stem (Ext) had a favorable effect on biochemical markers in a murine model of MetS, induced by injection of monosodium glutamate (MSG). From newborn female mice, two groups were formed: control and the MSG groups, which received a dosage of 2 mg/kg of MSG via subcutaneous injection at the second and fourth postnatal day (PD 2,4), and 4 mg/kg at the PD 6, 8, 10 to induce obesity. On week 13, a part of the MSG group received Ext (group MSG + Ext) at 300 mg/kg, administered orally daily from week 13 to week 18. The results indicated that ethanolic extract of *Lippia graveolens* stem decreases the percentage of body fat, waist circumference, and body weight gain as well as cholesterol, serum triglyceride concentrations and systolic and diastolic pressure. Insulin and leptin hormone values showed a significant effect with the Ext administration. However, hepatic lipoperoxidation levels of MSG and MSG + Ext groups did not show any statistically significant differences between them, both being higher than the control group. Taking in consideration the results obtained in this study, it is concluded that the administration of Ext had a beneficial effect in the murine model with MetS. This is the first study demonstrating the potential of the polar fraction *Lippia graveolens* stem in MetS.

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1. Introduction

Metabolic Syndrome (MetS) is defined as a complex metabolic disorder product of multiple risk factors, including central obesity, disturbance of glucose metabolism (insulin resistance, high blood glucose), arterial hypertension, and dyslipidemia. Concurrence of at least three of these factors is the cornerstone to diagnose an individual with MetS (O'Neill et al., 2016). The main long-term complications of MetS are diabetes mellitus Type 2, cardiovascular diseases, and death (Aguilar-Salinas and Viveros-Ruiz, 2019; Rabadán-Chávez et al., 2016).Table 1..

In developed countries, MetS affects around 25 % of the population, moreover, its prevalence is increasing rapidly throughout the world in parallel with the increasing prevalence of diabetes and obesity (Condorhuamán-Figueroa et al., 2019), in Mexico, the last Nutritional Survey reported that MetS had increased to 35 % in the last six years (ENSANUT, 2018). Currently, severe acute respiratory syndrome coronavirus (SARS-Cov) causes high morbidity and mortality worldwide, and obesity has been related with major complications in patients with COVD-19 disease (Ritter et al., 2020).

* Corresponding author at: Instituto Politécnico Nacional-ENCB- Ciudad de México, Physiology department Av Wilfrido Massieu s/n, Col. Planetario Lindavista, Ciudad de México, CP, Mexico.
E-mail addresses: mfriasz1100@alumno.ipn.mxmfri, asz1100@alumno.ipn.mx (M.E. Frías-Zepeda), mrosalesc@ipn.mx (M. Rosales-Castro), gescalonac@ipn.mx (G.N. Escalona-Cardoso), npaniagc@ipn.mx (N. Paniagua-Castro).

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Table 1
Chemical composition of Ethanolic extract of Mexican oregano.

Compound	Content (%)
Naringenin	27
Taxifolin	21.12
Eriodictyol	18.15
Caffeic acid	10.64
Luteolin	8.58
Cummaric acid	3.83
Quercetin 3-O-glycoside	2.06
2-Hydroxybenzoic acid	1.67
Apigenin	1.47
Quercetin	0.96
Floridzin	0.85
Acacetin	0.74

Data obtained by UPLC-MS.

The annual increase in the offer of new natural products with preventive, therapeutic, or adjuvant purposes in the treatment of some diseases has increased the consumption of medicinal plants. Herbs and spices have been used to prevent and treat chronic health disorders aimed to restore metabolic balance (Hassani et al., 2016). Therefore, identifying herbs and their active compounds with these effects can be a good alternative in the treatment of MetS. A single plant can contain diverse secondary metabolites (Francini-Pesenti et al., 2019). Epidemiological, clinical, and nutritional studies strongly support the evidence that dietary phenolic compounds enhance human health by lowering the risk and preventing the onset of degenerative diseases including cancer, cardiovascular diseases, and metabolic disorders (Zhang and Tsao, 2016). Due to this great phytochemical heterogeneity, it is important to applied proper extracting methods for the compounds of interest, this is the case of oregano stem research in relation to MetS.

Oregano is an aromatic plant whose principal species are: *Origanum vulgare* (Lamiaceace) native to Europe, *Lippia graveolens,* and *Lippia berlandieri Shauer* (Verbenaceae) native to America (García-Pérez et al., 2012), Mexican Oregano *Lippia graveolens* is a wild plant that grows in arid and semi-arid zones in Mexico and has been used for many centuries because of its multiple culinary, cosmetic, and medicinal properties (Long-Ze et al., 2007). Most of the studies are focused on the oil extracted from its leaves (Leyva-López et al., 2017). Although the stem represents a high percentage of the plant (50 %−70 %), its use in culinary are restricted, and it is considered like garbage, moreover studies on its therapeutic properties are limited (González-Güereca et al., 2007). It is important to take advantage of the oregano stem for its bioactive compounds, which can potentially be useful in the treatment of chronic diseases.

The objective of this study was to evaluate the effect of Mexican oregano stem ethanolic extract on biochemical markers (hepatic lipid peroxidation, blood glucose, insulin, leptin, cholesterol, triglycerides, HDL cholesterol), systolic and diastolic arterial blood pressure in murine model of MetS, as well as its toxicological effect.

2. Material and methods

2.1. Plant materials

Oregano samples were collected in Cuencamé, Durango, México, in October 2017, and taxonomically identified as *Lippia Graveolens* by Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Durango, Instituto Politécnico Nacional (CIIDIR-IPN) Durango's herbarium, with catalog numbers 35542, 37563 and 40073. The used study material was only the stem part, dried under a controlled temperature of 23 °C and powdered to 60 mesh screen.

2.2. Reagents

Hexane (CAS 110-54-3, Merck® México), Ethanol (CAS -64-17-5, Merck® México), isotonic saline solution (PisA® Mexico), monosodium glutamate (CICARELLI, CAS: 6106-04-03), 0.9 % NaCl (Sigma-Aldrich, St. Louis, MO. USA), food (Rodent Lab Chow 5001, Purina, St. Louis, MO, USA). (+)- Glucose (CAS 50-99-7, Merck®USA), Total cholesterol (TC) (RANDOX, LLD, 373451, Wallo chemical Neus Germany), triglycerides (TG) (RANDOX LLD, 359113 Wallo chemical Neus Germany), leptin hormone (Rat Leptin 96-Well Plate Assay, cat. #EZRL-83K), and insulin hormone (Rat Insulin 96-Well Plate Assay cat. #EZRM1-13K), Dimethyl Carboxymethyl Cellulose (DCC) (Veken® USA), thiobarbituric acid and reactive substances (TBARS). Chemicals and reagents used for the experimentation were all analytical grade.

2.3. Oregano stem extract

Pulverized material was defatted in hexane, 100 g of stem with 300 ml hexane, macerated under a controlled temperature of 23 °C for 24 h and agitated, and this process was performed in duplicate. The resulting material was then filtered with Whatman paper No. 4 and the vegetal material dried at 25 °C. An ethanolic extract was prepared from the stem product, with a mixture ethanol/water 50/50 v/v, and a mass/volume ratio of 1/30. Preparation consisted of macerating the samples under a controlled temperature of 23 °C for 24 h and then filtered. Fresh solvent was added, and the procedure was repeated. The extracts obtained in the first and second maceration were combined and concentrated in a rotary evaporator at 37 °C due to ethanol evaporation, obtaining a concentrated aqueous extract which was dried in a fume hood to obtain a dry pulverized extract (Ext).

2.4. Identification of phenolic compounds by UPLC-MS

ET50 1:30 stem sample was analyzed using the methodology proposed by Villegas-Novoa et al. (2019). Extracts were dissolved at 1 000 mg•L-1 in 50 % ethanol. An Acquit UPLC-MS (mass spectrometry coupled to high-performance liquid chromatography) system consisting of an automatic injector, pumps, and a Xevo TQ-S tandem triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) was used. Phenolic compounds were separated on a C18 100 × 2.1 mm, 1.7 µm column employing 7.5 mM formic acid mobile phase (phase A) and acetonitrile (phase B) with 210 µL•min-1, flow rate. Compounds were identified by interpreting mass spectra, through MS/MS array and using standards (Sigma-Aldrich) of various phenolic compounds (phenolic acids and flavonoids) by negative ionization.

Quantification was done from the standard curve of each of the compounds (standards) and results were reported as relative percentage of the response of each compound compared to the total response in a UPLC chromatogram.

2.5. Animals

Twenty-seven female CD1 mice were provided by Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Zacatenco, México. All testing complied with the guidelines set by the Bioethics Committee of the Escuela Nacional de Ciencias Biológicas IPN (CONBIOETICA09CEI03720130520) and were conducted in conformity with the Mexican Official standard (NOM-062-ZOO-1999). Test subjects were placed in polycarbonate cages

and kept under controlled conditions of 60 ± 10 % relative humidity and 21 ± 2 °C with 12 h light/darkness cycles, access to food and purified water *ad libitum*.

2.6. Metabolic syndrome murine model

Nine female and three male CD1 mice weighting 25 ± 5 g were housed in appropriate coupling cages, three females per male each unit to induce pregnancy and obtain proper testing subjects. Eighteen newborn female mice were separated in two groups: one negative control (n = 6), and MSG group (n = 12), the negative control group received subcutaneously 0.02 ml/kg of body weight of isotonic saline solution at the second and fourth postnatal day (PD2 and PD4), and 0.04 ml/kg of weight at the PD6, PD8 and PD10. The MSG group received subcutaneously monosodium glutamate (MSG) with a dosage of 2 mg/kg of weight at the PD2 and PD4, and 4 mg/kg of weight at PD6, PD8 and PD10 to induce obesity. On week twelve (W12), mice were randomly asigned in three groups of six mice each (n = 6), one control, one with MSG, and the other group with MSG, which received the extract MSG + Ext.

On W13, treatment started, the control group and MSG group received 0.9 % NaCl, and the MSG + Ext group received extract with a dose of 300 mg/kg of body weight. Doses were administered orally for a period of 30 days and all groups received a normal diet during the experimental period.

2.7. Glucose tolerance curve

Glucose tolerance curve (GTC) was measured on W17 using a digital glucometer (ACCU-CHEK® Performa, Roche Diagnostics, Indianapolis, IN USA). Mice were subjected to fasting for 12 h, later, 2 g/kg of weight of glucose was administered orally, and blood glucose were registered at 0, 30, 60, 90, and 120 min, for Control group, GMS and GMS + Ext. The results were expressed in mg/dL. Area Under Curve (AUC) was determined for each group.

2.8. Blood pressure

Both systolic and diastolic blood pressure values were measured on W17 using the tail-cuff technic (indirect blood pressure) with the IITC 5002 machine (Letica®) equipped with a microprocessor wrapped in a small bracelet, which detects blood pressure in the caudal artery located in the mouse's tail. The animals were located inside acrylic containers in which only the tail was exposed to avoid lecture variability; they were also exposed to vasodilation induced by convection through a thermal chamber five min before the test. Measurements were performed in triplicate on every mouse and the results were averaged (mm Hg).

2.9. Biochemical values

Blood was obtained via *retro*-orbital puncture before sacrifice and was centrifuged at 3500 rpm for 10 min to obtain serum that was kept frozen at −20 °C until use. RANDOX kits evaluated total cholesterol (TC) and triglyceride (TG) levels; Insulin hormone and leptin hormone concentration through ELISA kits (cat. EZRMI-3 K; Millipore, St. Charles, MO, EE. UU; cat. EZRL-83 K; Millipore, St. Charles, MO, EE. UU., respectively), according to the manufacturer's instructions.

2.10. Body weight

Body weight (BW) was measured (g) weekly. Weight increase in each mouse was calculated by subtracting the measurement of the final BW (W18) from the initial BW (W13) of treatment. *Mouse length and fat measurement.* Mice length was measured from the nasal tip to the anal region, and lower abdominal surface (waist) in cm, using a metric ruler. Retroperitoneal and perimeter fat were extracted and weighted, and their percentage measured against body weight. Each measurement was carried out immediately after euthanized by cervical dislocation on W18.

2.11. Hepatic lipid peroxidation assays

Once the liver was extracted, it was rinsed in 0.09 % saline solution, and the weight was registered. The hepatic lipid peroxidation assay was performed measuring malondialdehyde (MDA), by reaction of samples with thiobarbituric acid. A total of 50 mg of liver were homogenized in 0.5 ml of cold phosphate buffer (pH 7.0), then 800 μL of homogenate were added with TBAR's reactive (TBA, TCA, HCl). The sample was then brought to boil for 30 min in a water bath. After cooling, the flocculating precipitate was separated through centrifugation at 5000 rpm for 15 min. Sample absorbance was determined with a spectrophotometer at 532 nm (Human Corporation, X-ma1000). MDA was measured using an extinction coefficient of $1.56x10^5$ $M^{-1}cm^{-1}$. Measurements were registered as nM MDA/mg of liver tissue.

2.12. Acute Toxicity of Ext in Healthy Mice

For acute toxicity measurements, the methodology proposed by the OCDE-423-1991 was followed. Nine healthy mice were used, which were randomly distributed in three testing groups with three mice each (n = 3). The subjects weighted 30 ± 5 g and were subjected to a 12 h fasting period before testing. The animals were administered with a single dose 500, 1500, and 2000 mg/kg BW of Ext, respectively, dissolved in 0.05 % DMC solution, doses were administered orally. The groups were kept under observation for the next 4 h to look for any intoxication, symptoms, behavioral and physical abnormalities or mortality. If after 24 h no signs of poisoning or death were detected. All groups were kept under observation for 14 days. Once this period concluded, the number of live/dead animals was registered. Toxicological effects were registered in terms of mortality, defined as LD_{50}.

2.13. Statistical analysis

The results were expressed as an average ± standard error, the data normality tests were made by Shapiro-Wilk. The differences between groups for the blood pressure, TC, TG, Leptin hormone, Insulin hormone and peroxidation assay were determined with one-way ANOVA variance analysis, followed by a post hoc Holm-Sidak with a significance level of $p < 0.05$.

Data was examined with two-way ANOVA for repeated measurements followed by a post hoc Student-Newman-Keuls for body weight and blood glucose with a significance level of $p < 0.05$ Statistical analysis was performed with Sigma Plot v.12.0 software by Systat Software, San Jose CA. USA.

3. Results

3.1. Identification of phenolic compounds by UPLC-MS

Table 1 show the compounds identified in the ET50- 1:30 stem extract and their concentration. The main compounds are naringenin (27.00 %), taxifolin (21.12 %), eriodictyol (18.15 %), caffeic acid (10.64 %), luteolin (8.58 %), coumaric acid (3.83 %), quercetin 3-O-glycoside (2.06 %), 2-Hydroxybenzoic acid (1.67 %), apigenin (1.47 %), quercetin (0.96 %), floridzin (0.85 %) and acacetin

Table 2
Measurements of weight, length, and waist in female mice at W18.

Measurements	Control	MSG	MSG + Ext.
Weight (g)	34.66 ± 1.67[a]	33.36 ± 1.28 [a]	28.05 ± 1.43[b]
Length (cm)	10.11 ± 0.15 [a]	9.16 ± 0.13[b]	9.51 ± 0.27[c]
Waist (cm)	4.00 ± 0.11 [a]	4.46 ± 0.13[b]	4.05 ± 0.10 [a]

All values represent the Mean ± SE, (n = 6 in each group), in one-way ANOVA. Post hoc Holman–Sirak test, different literals by row represent significant differences $p < 0.05$.

(0.74 %). Compounds coincide with those identified for stem extracts obtained with hexane/ethyl acetate/methanol mixture (González-Güereca et al., 2007). Some other compounds reported for L. graveolens were not identified in this study, because reference standards for their identification and quantification were not available.

3.2. Effect of Ext on weight, adipose tissue length, and waist circumference

Weight gain in mice from week 13 to 18 is shown in Fig. 1A. Every group showed an increase in BW, however, MSG group has de highest increase (5.93 g ± 1.11) while MSG + Ext group remained relatively stable and low (3.65 g ± 1.15) with respect to the Control group (4.56 g ± 0.85). On W18 there was significant differences in weight between MSG versus MSG + Ext and Control groups ($p < 0.05$).

About the body fat percentage at the end of treatment, MSG group increased significantly (6.12 ± 0.72 g) compared with Control (1.87 g ± 0.36) and MSG + Ext (3.14 g ± 0.63), Fig. 1B. Treatment with mexican oregan extract decreased body fat but not its similar with the control group.

The results of body weight, length, and waist circumference measurements on W18 are described in Table 2. Regarding weight, no significant differences between groups were observed in Control and MSG, but MSG + Ext are less than the others ($p < 0.05$). Respect to the length of the mice, significant differences were shown between the three groups, with the MSG group showing the lowest growth (9.16 cm ± 0.13) compared to the control group, the MSG + Ext get intermedial value between both groups. For waist circumference measurements, the MSG group showed the highest value (4.46 cm ± 0.13) compared to the other groups ($p < 0.05$).

3.3. Effect of Ext on glucose tolerance

The results are presented in Fig. 2. In Fig. 2A, the comparison of the blood glucose curves between groups can be appreciated, no differences at 30 min between Control (249.5 ± 15.8 mg/dL) and the MSG (225.0 ± 17.2 mg/dL) groups were observed, but significant differences ($p < 0.05$) between both and the MSG + Ext group (184.0 ± 18.0 mg/dL). At 120 min, no differences were shown between MSG + Ext (101.3 ± 8.5 mg/dL) and Control (80.0 ± 7.7 m g/dL) groups, which were significantly different compared to MSG group (152.0 ± 9.1 mg/dL).

These results expressed as Area Under the Curve (AUC) are shown in Fig. 2B, the MSG + Ext group (15645.0 ± 78.7 mg/dL min) presented a smaller AUC showing a significant difference ($p < 0.05$) with respect to the other groups, GMS (18825.0 ± 96.9 mg/dL min) and Control (17362.5 ± 88.8 mg/dL min), which showed no significant differences between them.

3.4. Effect of Ext on blood pressure and biochemical markers

The results of blood pressure and biochemical markers are shown in Table 3, and Fig. 3 for insulin and leptin hormones. The MSG + Ext and Control groups showed no differences regarding cholesterol, triglycerides, systolic, and diastolic pressure, which were significantly lower than the values observed in MSG group ($p < 0.05$). Hepatic Lipoperoxidation, MSG and MSG + Ext groups not shown statistically differences between them, but both were higher than the Control group ($p < 0.05$). Insulin and leptin values showed significant differences between the three groups; the MSG group presented the highest and the MSG + Ext group a lower value ($p < 0.05$).

3.5. Acute toxicity of Extract in healthy mice

Administration of the extract to determine DL_{50} did not provoke any sign of intoxication and no mice died after a 14-days observation period. $DL_{50} > 2000$ mg/kg. This extract is inside the DL_{50} category 5 according to the stipulations required by the OECD-403–1991.

4. Discussion

MetS constitutes a worldwide public health problem, not only due to its high prevalence, but also because it is a risk factor that

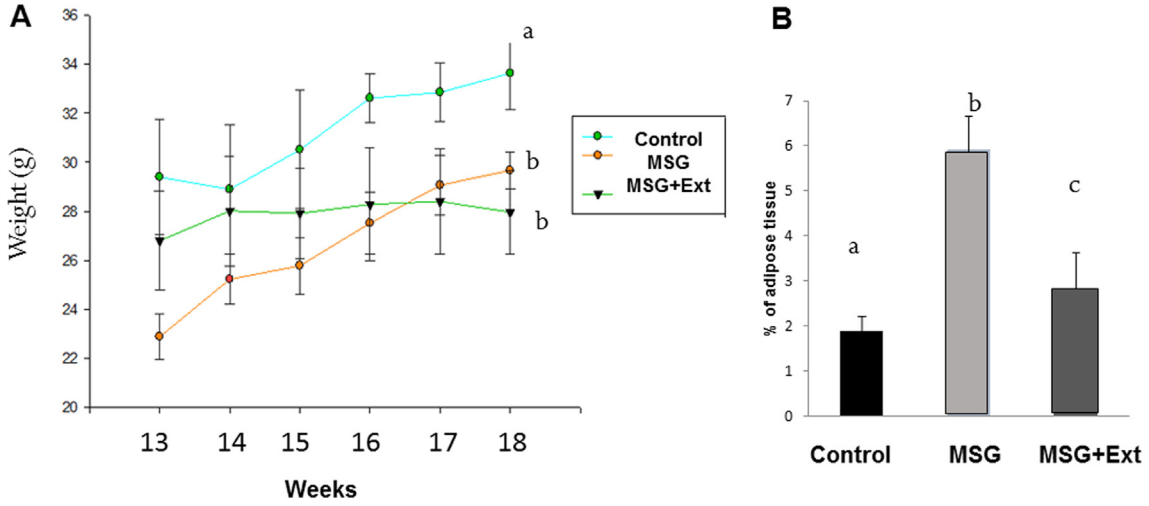

Fig. 1. A. Body weight of mice during treatments (n = 6). Mean ± SE, two-ways ANOVA, $p < 0.05$. **1B.** For the percentage of adipose tissue on week 18, was used ANOVA one via post hoc Tukey. Different literals represent significant difference between groups.

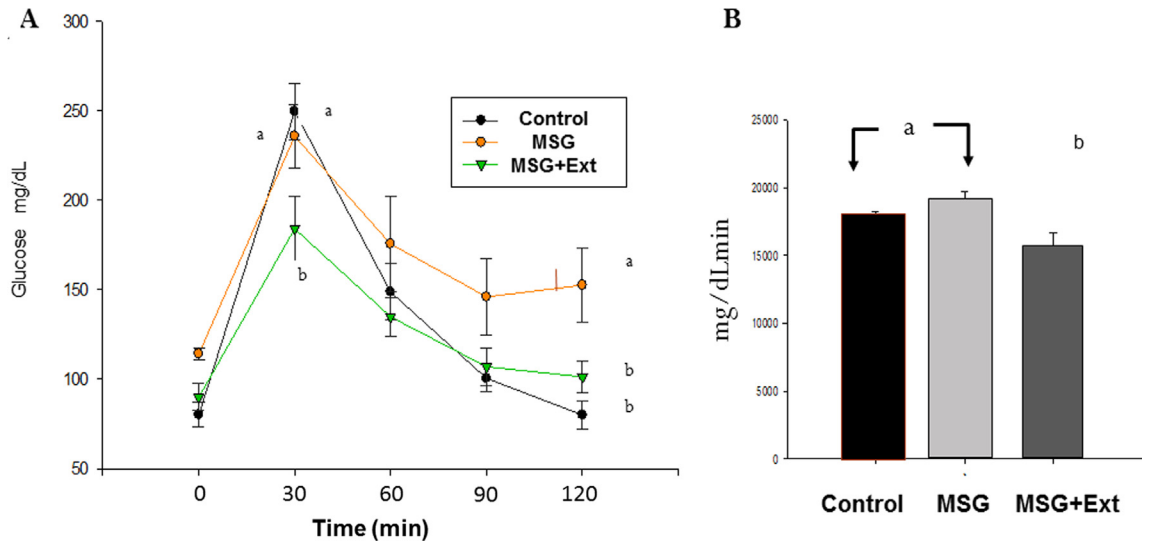

Fig. 2. A Blood glucose tolerance curve for female mice treated with *Lippia graveolans* extract. *Mean* ± SE two-way ANOVA. **2B**.- Area Under the Curve in one-way glucose ANOVA. Post hoc Tukey test, different literals represent significant differences, $p < 0.05$.

Table 3
Blood pressure and biochemical marker values in female mice.

Test	Control	MSG	MSG + Ext
Systolic pressure (mm Hg)	162.7 ± 6.5^{a}	176.01 ± 3.7^{b}	158.6 ± 2.1^{a}
Dystolic pressure (mm Hg)	145.6 ± 5.6^{a}	164.4 ± 3.0^{b}	145.8 ± 2.9^{a}
Cholesterol (mg/dL)	89.4 ± 3.5^{a}	120.2 ± 9.8^{b}	85.8 ± 6.3^{a}
Tryglicerides (mg/dL)	121.7 ± 8.2^{a}	188.0 ± 21.7^{b}	123.8 ± 6.3^{a}
Col-HDL (mg/dL)	51.8 ± 14.1^{a}	33.7 ± 2.4^{b}	56.5 ± 2.2^{c}
Hepatic Lipoperoxidation (mMMDA/mg)	8.12 ± 1.9^{a}	16.23 ± 2.6^{b}	13.3 ± 1.4^{b}

All values represent the Mean ± SE (n = 6 in each group), in one-way ANOVA. Post hoc Holman–Sirak test, different literals by row represent significant differences $p < 0.05$.

derives from other diseases producing morbidity and death, such as cardiovascular disease and type II diabetes mellitus. There is great interest in the search for secondary metabolites obtained from vegetal species for the treatment of these pathologies that affect humans.

Administration of the Mexican oregano stem extract in the MGS + Ext female mice group reduce metabolic alterations of MetS, decrease circumference and adipose tissue compared to the MGS group, since central obesity is the main trigger for other metabolic disorders related to MetS (Reshidan et al., 2019).

The induction of MetS by subcutaneous administration of MSG in newborn mice, produces hypothalamic arcuate nucleus destruction provoking metabolic and neuroendocrine dysfunction, developing abdominal adiposity and growth delay (Suárez-Román et al., 2013). The same results were observed in this study, since mice treated with MSG showed less growth and greater waist circumference with respect to both control and MGS + Ext groups.

Administration of Mexican oregano stem extract showed a favorable effect by decreasing the percentage of adipose tissue in the MSG + Ext group with respect to the MGS group ($p < 0.05$). This results coincide with those obtained by Meydani and Hasan (2010), in that polyphenolic compounds obtained from medicinal plans, vegetables, fruits and spices, have the ability to modulate physiological and molecular pathways involved in energetic metabolism, adiposity and obesity through various mechanisms: intestine fat absorption suppression, skeletal muscles glucose absorption, anabolic pathways suppression, adipose tissue catabolic pathways stimulation, adipose tissue angiogenesis inhibition, and reduction of chronic inflammation associated with adiposity. In this context, polyphenolic compounds as naringenin, taxifolin, apigenin, floridzine, quercetin among others have been found in *Lippa graveolens* leaves extract (Pérez-Gutierrez, 2014; Long-Ze et al., 2007; Gutiérrez-Grijalva et al., 2017), to which beneficial effects to these compounds related to inflammation, respiratory and digestive

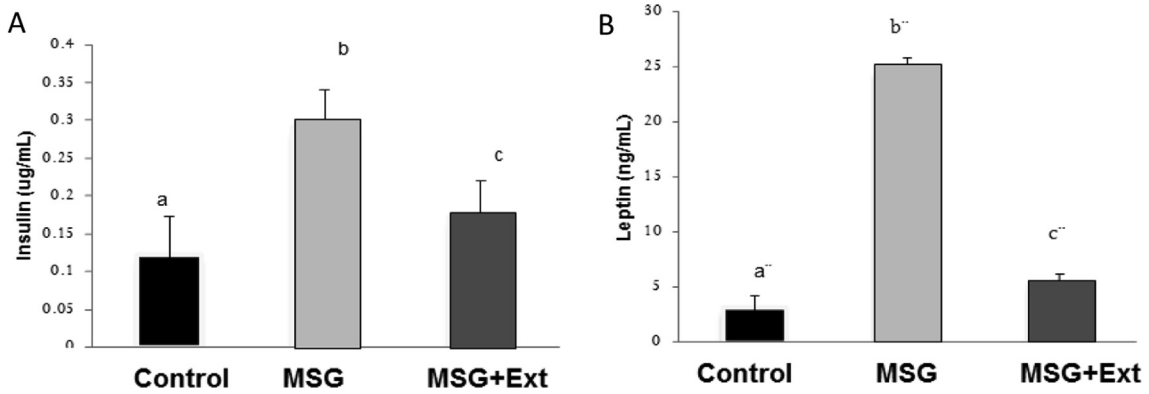

Fig. 3. Effect of Oregano Stem Extract on blood **(A)** insulin and **(B)** leptin hormone concentrations in female mice with monosodium glutamate-induced metabolic syndrome. One-Way ANOVA. Post hoc Holm Sidak test, different literals represent significant differences, $p < 0.05$.

disorders, headaches, rheumatism, and diabetes, due to their antioxidant and anti-inflammatory properties are attributing. Thus, polyphenolic compounds such as naringenin, quercetin, hexoside, kaempferol, pylosin, cirsimaritin, were isolated from Mexican oregano stem (González-Güereca et al., 2007).

With respect to Ext on glucose regulation, it showed a favorable effect, mainly in AUC accumulation, in which the MSG + Ext group value was the lowest compared to MGS and control groups. Mueller et al., (2008) obtained the inhibition of α-glucosidase enzyme by the action of polyphenols such as caffeic acid, naringenin, quercetin, luteolin and luteolin 7-O-glucoside, which provokes a decrease in carbohydrates absorption, lowering blood glucose concentration. In another study, extracts of Mexican oregano leaves, showing a better effect than acarbose to inhibit the α-glucosidase enzyme (Gutiérrez-Grijalva et al., 2019).

Another important finding was related to markers, MSG + Ext group presents a decrease in diastolic and systolic pressure. The reducing effect shown in arterial pressure can be explained due to the high flavonoids content, these compounds induce relaxation of blood vessels by promoting the presence of some vasodilator molecules such as acetylcholine, adenosine triphosphate (ATP) and adenosine diphosphate (ADP), substance P, bradykinin, histamine, thrombin, serotonin, and others (Condorhuamán-Figueroa et al., 2019).

Pérez-Gutierrez (2014), used extracts of hexane, chloroform, and methanol from the Mexican oregano leaf, administered in different doses. The methanol extract at 400 mg/kg of mouse body weight was the most effective dose for lowering biochemical levels of total cholesterol, triglycerides, col-LDL, and insulin hormone. Therefore, MetS associated dyslipidemia has been attributed to the insulin incapacity to inhibit adipose tissue level lipolysis, which induces apolipoprotein B, increase, the main low and very low-density lipoprotein (col-LDL and VLDL) protein compound, mainly characterized by hypertriglyceridemia (Pereyra-Rodriguez et al., 2016). Treatment with oregano stem extract to mice with MSG reduce cholesterol, triglycerides, col-LDL, and insulin hormone like Control levels ($p > 0.05$).

In this study, leptin and insulin hormone levels showed significant differences among the three groups, where MSG group had the highest value and MGS + Ext was only slightly higher than the control, showing the stem extract a favorable effect. This result has been related to the adiposity and hyperinsulinemia levels, which lead to the concept of hyperleptinemia (Acosta-Hernández et al., 2015).

According to the results of the chemical analysis of the ethanolic extract of oregano, the component in greater quantity is narigenin, this flavonoid has several pharmacological effects, such as anti-inflammatory, cardioprotective, modulation of insulin-glucose signaling and lowering cholesterol concentrations (Yoshida et al, 2013; Silver et al, 2011; Ren et al., 2016; Den-Hartoghand and Tsiani, 2019).

Other components of the extract are Taxifolin, eriodictyol, caffeic acid, and other flavonoids have antioxidant properties, can neutralize free radicals produced in the mitochondria and inhibit enzymes such as peroxidase (Chobot et al, 2016; Vladimirov et al., 2009). These antioxidant properties are also important in the treatment of MS, since the increase in adipose tissue generates a proinflammatory and prooxidant state that is related to insulin resistance and dyslipidemia (Sourav et al., 2011).

Therefore, the results obtained in this study from Mexican oregano stem extract are due to its antioxidative properties, caused by its flavonoids (González-Güereca et al., 2007).

In the determination of acute toxicity, there was no toxicity manifestation, mild or severe, during the 14 days testing period, which places the stem in category 5 as nontoxic according to the guidelines set by the OECD-423.

In Mexico, oregano is used in traditional Mexican cuisine, but only its leaves are used, and the stem is used to form compost for the field since it is considered an agro-industrial waste. However, the components such as flavonoids present in the stem would be an important source to prevent diseases. The importance of this work is to demonstrate that naringenin and other flavonoids are potential nutraceuticals that can help in the treatment of metabolic syndrome, highlighting the importance of not discarding the stem of Mexican oregano, and represents an opportunity of economic profit that is rarely explored, which could improve the exploitation of easily available vegetal material.

This study is limited in that it was tested only a dose of 300 mg/kg of mouse body weight of oregano stem extract, carrying out tests with higher doses of 400 and 500 mg/kg of body weight would be recommended; a comparison of studies using leaf residue extract, male mice could be made as well, to determine if these variables represent significant differences if any.

5. Conclusion

Our results suggest that Mexican oregano *Lippia graveolens* stem extract has the potential to prevent MetS related metabolic alterations due to its polyphenol mediated activity, and it represents an important opportunity for not delete de stem like a waste agro-industrial.

CRediT authorship contribution statement

Maria Estela Frías-Zepeda: Methodology. **Martha Rosales-Castro:** Conceptualization, Writing – original draft. **Gerardo. Norberto Escalona-Cardoso:** Writing – original draft. **Norma Paniagua-Castro:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

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.

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Further Reading

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