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EFFECT OF *THEVETIA PERUVIANA* ON MURINE-INDUCED OBESITY

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Abstract

Background: Obesity is considered a multifactorial disease that has importantly increased the development of chronic degenerative diseases. Drugs available for treating obesity have the inconvenience of producing side effects of importance. In Mexican traditional medicine, the seeds of *Thevetia peruviana* have been widely employed for weight reduction.
Materials and Methods: The effect produced by different extracts of *T. peruviana* on MonoSodium Glutamate (MSG)-induced obesity in mice was evaluated. A chemical analysis oriented toward the identification of the chemical compounds contained in the active extract was carried out.
Results: Oral administration of the hexanic extract of *T. peruviana* (for 7 weeks) was capable of diminishing weight gain by up to 24.1% in the mice without observing the development of resistance to insulin. Median lethal dose of the hexanic and Ethyl Acetate (EtOAc) extracts was >2 g/kg. By utilizing bio-assay guided fractionation, eight secondary metabolites were purified and characterized.
Conclusion: The hexanic extract obtained from *Thevetia peruviana* seeds was capable of reducing weight gain in mice with induced obesity. In addition, this extract showed good response to the glucose tolerance test, was able to avoid the development of insulin resistance, and also substantially increased serum adiponectin levels. Eight low-polarity compounds were identified in the active fraction. This species could be considered for ongoing investigation as a potential option to reduce obesity

Key words: Obesity, *Thevetia peruviana*, medicinal plants, body weight, adiponectin, monosodium glutamate, sterols.

***Abbreviations*:** MSG, MonoSodium Glutamate; EtOAc, Ethyl Acetate; TLC, Thin-Layer Chromatography; BW, Body Weight; CNS, Central Nervous System; T2D, Type 2 Diabetes; ELISA, Enzyme-Linked ImmunoSorbent Assay; r.o., retro-orbitally; BMI, Body Mass Index; UPLC, Ultra Performance Liquid Chromatography; LD_{50}, median Lethal Dose.

Introduction

Nearly two decades ago (1997), obesity was declared a worldwide epidemic by the World Health Organization (WHO) (Dávila-Torres *et al*. 2015). It is considered a chronic disease that has as its main characteristic the increase of adipose tissue, which is manifested in Body Weight (BW) gain. The relationship between a person's weight and height is used to determine the degree of obesity (Barbany and Foz 2002).

Obesity is the result of the loss of equilibrium between the ingestion of food with a high-energy content and energetic expenditure (Rankinen and Bouchard 2008); 95% of patients with obesity have exogenous or simple obesity (International Obesity Task Force [IOFT] 2012). Thus, with the aim of diminishing obesity, the main strategies have been primarily based on lifestyle modification (Waxman 2004). In obesity, the increase of adipose tissue, which releases excessive amounts of free fatty acids and hormones, produces insulin resistance and promotes glucose release in liver. This tissue also produces glycerol and proinflammatory cytokines; the release of these compounds produces hyperglycemia,

hyperinsulinemia, hypercholesterolemia, and hypertriglyceridemia (Kopelman 2000; Stumvoll and Goldstein van Haeften 2008).

For the treatment of obesity, there are drugs, among which are included appetite suppressants, which act by modulating the Central Nervous System (CNS), and drugs that modulate the absorption of specific nutrients such as fat, both with the inconvenience of producing important side effects (Luque and Rey 1999; Heck et al. 2000). Among the uses of medicinal plants in traditional medicines, it is possible to find species that are employed for "weight loss" and, among the compounds contained by some of these plants, flavonoids, alkaloids, and saponins have been identified. These compounds usually produce antiobesity effects throughout different mechanisms of action (Yun 2010).

In an individual with obesity, the organism can generate insulin resistance that, in turn, can comprise a factor that allows the development of Type 2 Diabetes (T2D), a disease related with different disorders such as hyperlipidemia, atherosclerosis, and hypertension (Stumvoll and Goldstein van Haeften 2008).

MonoSodium Glutamate (MSG) is the sodium salt of the amino acid known as glutamic acid. This salt is obtained via the fermentation of sugar cane or from cereals (Arteaga-Sánchez 2012) and it is utilized as a condiment to potentiate food flavors; thus, it is a basic element in the food industry. Administration of MSG in early life stages, as in the case of neonatal mice, affects the development of the CNS and has repercussions, including that of affecting the satiety threshold, in this manner stimulating the appetite and increasing food consumption (Carbonero-Carreño 2013). Different studies conducted on mice and rats have proposed the relationship between the consumption of this compound and obesity (Arteaga-Sánchez 2012). Subcutaneous (s.c.) administration of MSG in neonatal mice induces obesity. Obesity is a multifactorial disease that has importantly increased the development of chronic-degenerative diseases, thus medical-care costs.

Due to the dramatic increase in the prevalence of obesity and T2D worldwide, it is urgent to promote novel strategies for combating the epidemiologic growth of these diseases; for this, the consideration of the potential of certain medicinal plants would be advantageous (Zaid *et al*. 2015). Recent publications highlight the potential that natural bioactive products possess in the prevention of chronic diseases such as cancer, cardiovascular diseases, and metabolic and inflammatory disorders and, in addition, their probable impact on obesity and insulin resistance (Huang *et al*. 2016). In an ethnobotanical study, it was identified that a total of 139 plant species native to Mexico, Central America, and the Caribbean are utilized empirically for the treatment of obesity. Thirty three of these species have been included in some scientific work, but 106 of these, to our knowledge, have not yet been evaluated (Alonso-Castro *et al*. 2015).

Among plants included in scientific reports, we can mention the following: *Momordica charantia*; *Centella asiatica*; *Morinda citrifolia*; *Annona muricata* (Gooda *et al*. 2012); *Camellia sinensis*; *Caralluma fimbriata*; *Citrus aurantium*; *Coleus forskohlii*; *Garcinia cambogia*; *Phaseolus vulgaris* (Astell *et al*. 2013); *Zingiber officinale*; *Caralluma fimbriata*, and *Hibiscus sabdariffa* (Hasani-Ranjabar *et al*. 2009).

The seeds from *Thevetia peruviana* (Pers.) K. Schum. of the Apocynaceae family, popularly known as "*Codo de fraile*" and "*Ayoyote*" (Argueta *et al*. 1994), are utilized in Mexican traditional medicine for weight reduction (Torres 2009). Other scientific works have identified antidiarrheic, antimicrobial, and cytotoxic properties (Hassan *et al*. 2011), while pesticide activity has been identified in *T. peruviana* bark and leaves (Singh *et al*. 2010).

The objective of the present work was to evaluate the effect produced by different extracts of *T. peruviana* in mice with MSG-induced obesity and, by means of bio-assay guided fractionation, to identify the chemical compounds contained in the active extract. Since adiponectin (produced exclusively by the adipose tissue) possesses anti-inflammatory and insulin-sensitizing effects (Messinis *et al*. 2013), and because resistin has been implicated in the pathogenesis of insulin resistance and T2D (Kusminski *et al*. 2005), both proteins were quantified in the plasma of mice at the end of treatment administration.

Materials and Methods

Plant material

Plant material of *Thevetia peruviana* (Pers.) K. Schum., of the Apocynaceae Family, was obtained (January 2015) in its natural habitat in the state of Morelos, Mexico. A herbarium sample was prepared for its identification and deposited for reference at the IMSSM Herbarium of the Mexican Institute of Social Security. Abigail Aguilar-Contreras, M.Sc., Herbarium Director, was charged with the identification of the plant species. The voucher sample was registered with the number IMSSM-16000. Seeds were extracted from the endocarp, crushed until obtaining a 2-mm particle size, and dried under dark conditions at room temperature.

Extract preparation

The dried and ground material (seeds, 2.76 kg) was extracted by sub-sequential maceration in the ascending polarity solvents *n*-hexane and Ethyl Acetate (EtOAc). The liquid extracts (10 L) were filtered and processed to total dryness by means of a rotary evaporator (Laborota 4000, Heidolph). These extracts, which included *n*-hexane (1.10 kg, 39.8%) and EtOAc (169.3 g, 6.12%), were stored at –20°C and protected from light.

Chemical separation of the *Thevetia peruviana* active extract

Based on the results obtained from the evaluation of the whole extracts, the *n*-hexane extract was subjected to bioassay-guided fractionation. The first chemical separation was performed by chromatographic open column previously packed with silica gel 60 employing a mixture of *n*-hexane/Ethyl Acetate (EtOAc) gradient system as mobile phase (starting with 100% of n-hexane and progressively increasing EtOAc until reaching 100% of the last solvent). Samples of 200 mL were taken and analyzed by Thin-Layer Chromatography (TLC). This chemical monitoring process allowed for the grouping of fractions according to their composition, obtaining a total of eight fractions: **C1F1**–**C1F8**.
The most active fraction (**C1F2**) was subjected to a chromatographic open column previously packed with 100 g of silica gel 60. The *n*-hexane/EtOAc gradient system was used as mobile phase, starting with 100% of the solvent of least polarity and finalizing with 100% EtOAc. One hundred eighty samples were obtained, which were grouped into five final fractions according to their chemical composition (**C2F1–C2F5**).

GC-MS analysis

The chemical composition of **C2F1, C2F2,** and **C2F3** fractions was analyzed by gas chromatography in a chromatograph equipped with a quadrupole mass detector in electron impact mode at 70 eV. Volatile compounds were separated on an HP 5-ms capillary column (25 m long, 0.2 mm i.d., 0.3-µm film thickness). Furnace temperature was set at 40°C for 2 min, then programmed from 40–260°C at 10°C/min, and maintained for 20 min at 260°C. Mass detector conditions were as follows: interphase temperature 200°C, and mass acquisition range, 20–550. Injector and detector temperatures were set at 250°C and 280°C, respectively. The splitless injection mode was carried out with 1 µL of each fraction (3 mg/mL solution). The carrier gas was helium at a 1-mL/min flow rate. Identification of volatiles was performed, comparing their mass spectra with those of the National Institute of Standards and Technology (NIST) 1.7 Library.

Evaluation of acute toxicology

To evaluate possible toxic effects, different doses (50, 100, 500, 1,000, and 2,000 mg/kg) of the hexanic and EtOAc extracts from *T. peruviana* seeds were administered orally (o.a.) to CD-1 mice (average weight, 41.0 g). Survival was measured during a 24-h period.

Material utilized for pharmacological experiments

We employed MSG (Aldrich, ≥98% pure) as obesity inducer. In order to quantify the concentrations of plasma resistin and adiponectin, kits including Enzyme-Linked Immunosorbent Assay (ELISA) (the Mouse adiponectin/Acrp30 kit) and the Mouse Resistin Quantikine kit (R&D Systems) were employed, while for glucose measurement, we used an AccuChek Performa Roche® Glucometer. Finally, for measurement of cholesterol and triglycerides, we utilized an Accutrend Performa Roche® Glucometer.

Obesity induction with MSG in mice

Male and female CD-1-strain mice were paired. Offspring obtained from the pairings were utilized in the experimentation as follows: on postnatal days 2 and 4, mice received 2 mg/kg of MSG subcutaneously (s.c.), while on postnatal days 6, 8, and 10, they were administered 4 mg/kg of MSG via the same route.

Once obese mice were obtained (at 2 months of age and with an average weight of 31 g), the animals were organized into four groups (with eight animals each) and administered daily for 7 weeks with treatments via oral route (through an orogastric [o.g.] cannula) as follows: the negative control group was treated with the vehicle (Tween 80) alone; the positive control group received Orlistat (10 mg/kg) plus vehicle; the first experimental group was treated with the hexanic extract of *T. peruviana* (50 mg/kg) plus vehicle, and the second experimental group, with the EtOAc extract of *T. peruviana* (50 mg/kg) plus vehicle. With the aim of obtaining a reference (no obese animals), a witness group was included (also composed of eight animals), which did not receive treatment with MSG.

The BW of the mice was measured weekly and, at the end of treatment, blood levels of cholesterol, triglycerides, adiponectin, and resistin were measured. In addition, a glucose tolerance test was performed, as well as an insulin resistance test.

The studies were carried out under Official Mexican Regulations for the Correct Use of Animal Experimentation (Norma Oficial Mexicana, http://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.pdf) and conducted in accordance with internationally accepted principles for laboratory animal use and care (e.g., European Community guidelines/EEC Directive of 1986 or US guidelines/NIH publication).

Glucose tolerance test

At the end of week 7 of administration and after a 12-h fasting period, we measured the plasma glucose level, in all experimental groups, and the result was considered as time zero. Later, we administered a 2-g/kg dose of glucose intraperitoneally (i.p.). For construction of the glucose tolerance curve, determinations of glycemia in caudal vein were performed at 15, 30, 60, and 120 min.

Insulin resistance test

At the end of week 7 of administration and without a previous fasting period, in all experimental groups, we administered a 0.75-International Unit (IU)/kg dose of insulin i.p. For construction of the insulin resistance curve, we carried out measurements of glycemia in caudal vein at 0, 15, 30, 60, and 120 min.

Determination of resistin and adiponectin

For quantification of resistin and adiponectin in plasma, at the end of week 7 of treatment administration, we selected four mice from each group for resistin and four for adiponectin determination.
We obtained blood samples retro-orbitally (r.o.) with previously heparinized capillary tubes (in Eppendorf tubes), which were centrifuged in order to obtain the plasma samples. In both cases, the manufacturer's instructions (of the ELISA kits) were followed.

Determination of body mass index and visceral fat

At the end of the administration period, we determined the Body Mass Index (BMI) in all of the animals. This was obtained by means of dividing the weight of the mouse in g by the length of the same mouse (defined as the length between the nose and anus in cm of the mouse). For measurement of visceral fat, the animals were sacrificed, the visceral fat was dissected, and, in all cases, with the aid of a standardized filter paper, the weight of the tissue was determined.

Statistical analysis

For statistical analysis, we employed the IBM SPSS ver. 20. statistical software program. To identify the difference between groups, we utilized ANalysis Of VAriance (ANOVA) and the Tukey post-hoc test. A statistically significant difference was considered between groups when p values were <0.05.

Results

Evaluation of Acute Toxicology

Evaluation of the different extracts of *T. peruviana* (doses of 0.05–2 g/kg orally [o.a.]) demonstrated that the LD_{50} of the hexanic and EtOAc extracts was >2 g/kg.

Effect Produced by the Extracts of *Thevetia peruviana* on Mice with Induced Obesity

In the MSG-induced obesity model, animals included in the witness, in the positive control, and in the negative control groups presented a progressive increase of BW during the 7 treatment weeks; however, as can be appreciated in **Figure 1**, the weight increase in obese animals that received the vehicle (negative control) was significantly greater. On the other hand, the extracts obtained from *T. peruviana* were capable of significantly reducing BW gain, especially in the group treated with the hexanic extract, which demonstrated minimal weight increase during the 7 treatment weeks. In this group, weight gain was 24.1% lower in comparison with that of the negative control group. From week 2 of administration, a statistically significant difference was identified ($p = 0.047$) between the group treated with the hexanic extract of *T. peruviana* and the group that received the vehicle (negative control). This difference was also identified in the remaining experimental group from week 3 of administration ($p = 0.047$ and $p = 0.001$).

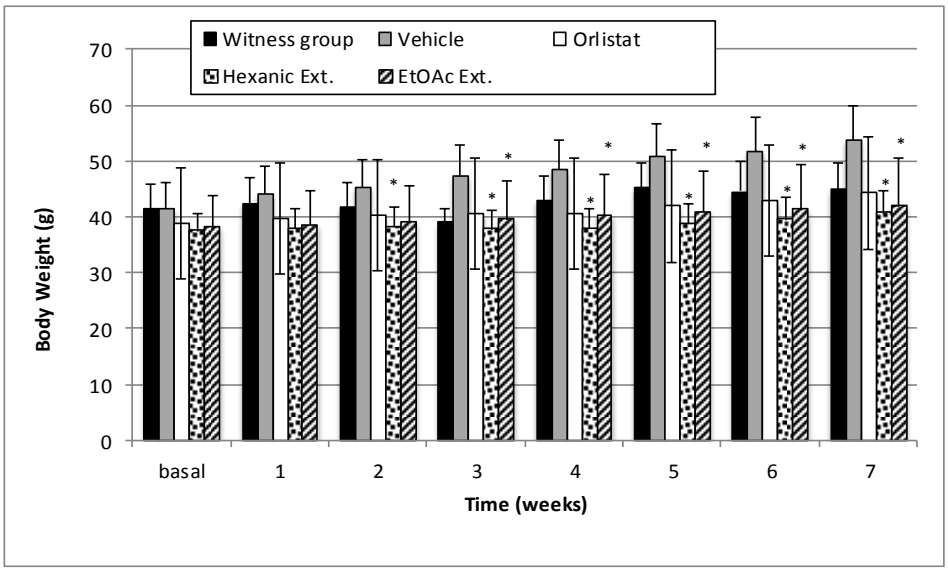

Figure 1: Effect produced by oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicle (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG. * = p <0.05 with regard to the negative control in the Tukey test.

Glucose Tolerance Test

Once the administration period of the treatments concluded (at 7 weeks), four animals from each group were selected randomly in order to perform a glucose tolerance test. We were able to observe that, in animals with obesity, the group that received the vehicle alone (negative control) was the only group that presented high rates of glycemia at time zero (>200 mg/dL) and, after 2 h of having been administered glucose, this same group again presented high rates of glycemia (**Figure 2**). The animals treated with the extracts of *T. peruviana* demonstrated an expected response to the glucose challenge and recovered the amounts of the glycemic baseline, evidencing the absence of glucose intolerance.

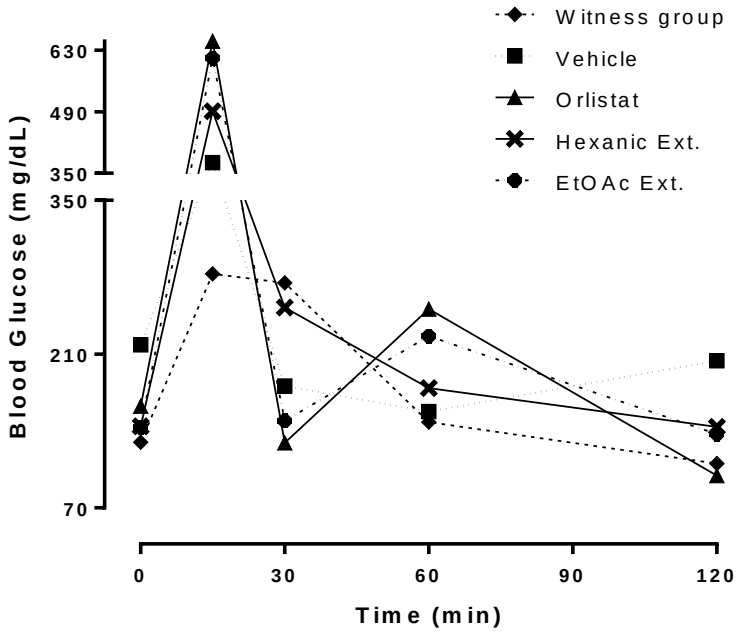

Figure 2: Glucose tolerance test carried out at the end of week 7 of treatment with the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of the seeds of *Thevetia peruviana*, Orlistat (positive control), and vehicle (negative control) in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG.

Insulin Resistance Test

Once the administration period was concluded (7 weeks), four animals of each group were selected randomly to perform an insulin resistance test. In this case, the animals were not submitted to a previous fast. At baseline time, a blood glucose concentration was identified within normal rates (for animals not submitted to a fast) in the group treated with the hexanic extract and in the group treated with Orlistat, as well as in the witness group. Negative-control-group animals and those of the experimental group treated with the EtOAc extract did not respond to the administration of insulin, evidencing a possible development of insulin resistance. In contrast, the witness group, the positive control group, and the group that received treatment with the hexanic extract of *T. peruviana* exhibited an expected response to administration of insulin, suggesting that sensitivity to insulin was preserved in these groups (**Figure 3**).

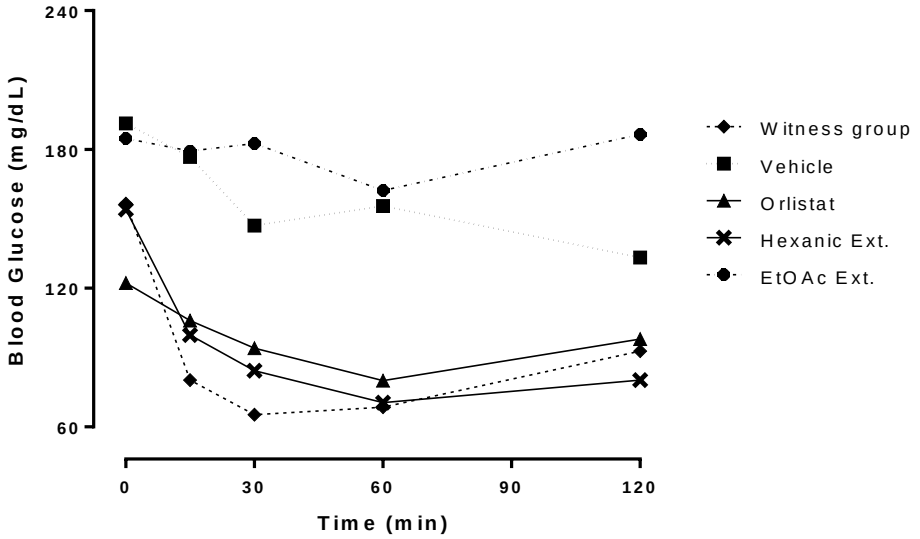

Figure 3. Insulin resistance test performed at the end of week 7 of treatment with the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of the seeds of *Thevetia peruviana*, Orlistat (positive control), and vehicle (negative control) in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG.

Effect Produced by *Thevetia peruviana* on the Plasma Levels of Cholesterol, Triglycerides, and Body Mass Index

At the end of the period of treatment administration (7 weeks), the blood cholesterol level was determined in all animals with caudal-vein blood. The greatest concentration of cholesterol was observed in the group treated with the vehicle (negative control), whose result was significantly ($p = 0.006$) higher than that of the witness group. The extracts of *T. peruviana* and Orlistat (positive control) lowered blood cholesterol levels, but the only group that achieved a significant difference ($p = 0.013$), and levels very similar to those of the witness group, was that administered the hexanic extract of *T. peruviana* (**Figure 4**).

In these same animals, we determined blood triglyceride levels. In all of the treatment groups, higher levels were observed than those exhibited by the witness group. Only the group of animals treated with the hexanic extract of *T. peruviana* evidenced a lower level of triglycerides (**Figure 5**), but without reaching a statistically significant difference.

Evaluation of BMI at the end of treatment administration permitted identifying that administration of MSG induced a considerable increase of this variable, which can be appreciated in the group administered the vehicle alone (negative control). Statistical analysis reflected a significant difference ($p = 0.001$) of this group of mice with regard to the witness group. In this case, the hexanic extract of *T. peruviana* achieved lowest BMI values and exhibited a significant difference ($p = 0.003$) with respect to the negative control, but did not reach the levels presented by the witness group. The groups of animals that received Orlistat (positive control) and the EtOAc extract of *T. peruviana* also demonstrated a lower BMI than the group treated with vehicle alone (**Figure 6**).

MSG administration substantially increased visceral fat in the mice. This effect could not have been inhibited by the administration (during 7 weeks) of Orlistat (positive control), nor by administration of the extracts of *T. peruviana* (data not shown).

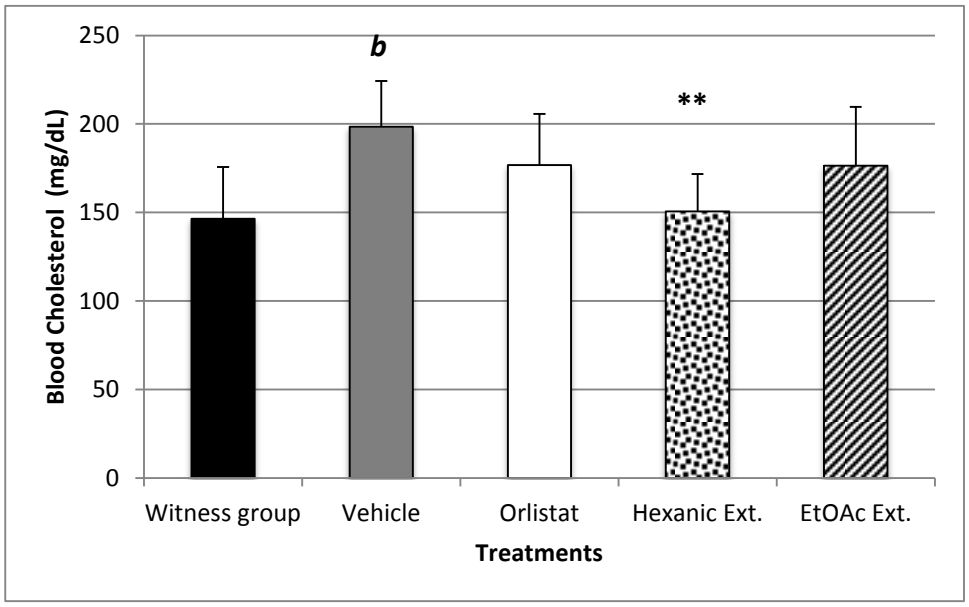

Figure 4: Effect produced by the oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicle (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* on plasma cholesterol levels in mice with MonoSodium Glutamate (MSG)-induced obesity. ** = p <0.05 with regard to the negative control group (Vehicle) in the Tukey test. ***b*** = p <0.05 with respect to the witness group in the Tukey test. Witness group was not treated with MSG.

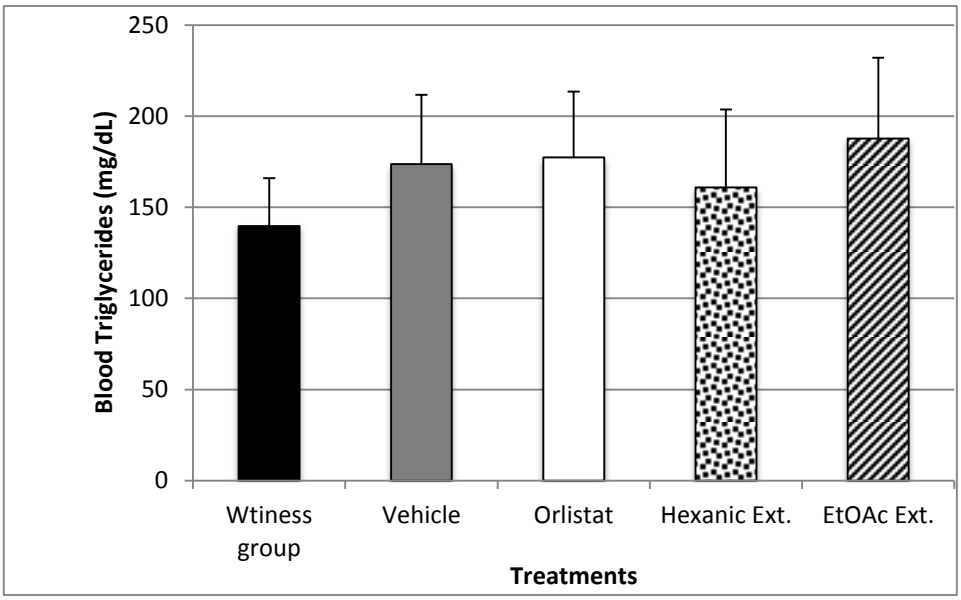

Figure 5: Effect produced by the oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicule (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* on the blood triglyceride levels in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG.

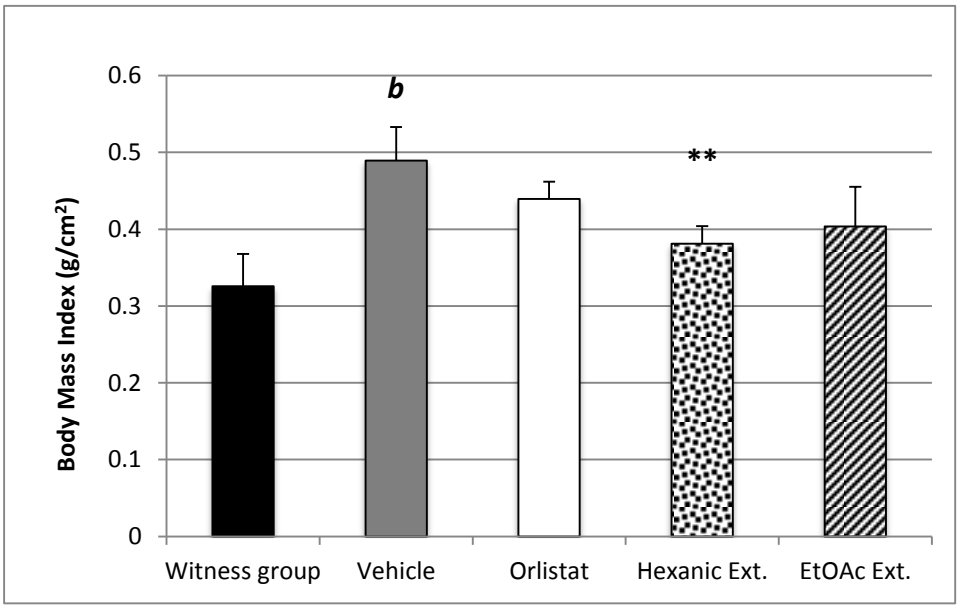

Figure 6: Effect produced by the oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicle (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* on Body Mass Index (BMI) in mice with MonoSodium Glutamate (MGS)-induced obesity. Witness group was not treated with MSG. ****** = p <0.05 with relation to the negative control group (Vehicle) in the Tukey test. ***b*** = p <0.05 with respect to the witness group in the Tukey test.

Effect Produced by *Thevetia peruviana* on Resistin and Adiponectin Levels

At the end of the administration period, animals were selected randomly to take blood samples for measurement of resistin and adiponectin plasma concentrations. Resistin levels were very similar in the different treatment groups; only animals treated with Orlistat alone (positive control) presented high levels of this cytosine (**Figure 7**). In the case of adiponectin, all of the animals treated with the plant extracts and Orlistat exhibited higher concentrations, while the hexanic extract of *T. peruviana* was that which presented the highest concentration of this cytosine (**Figure 8**).

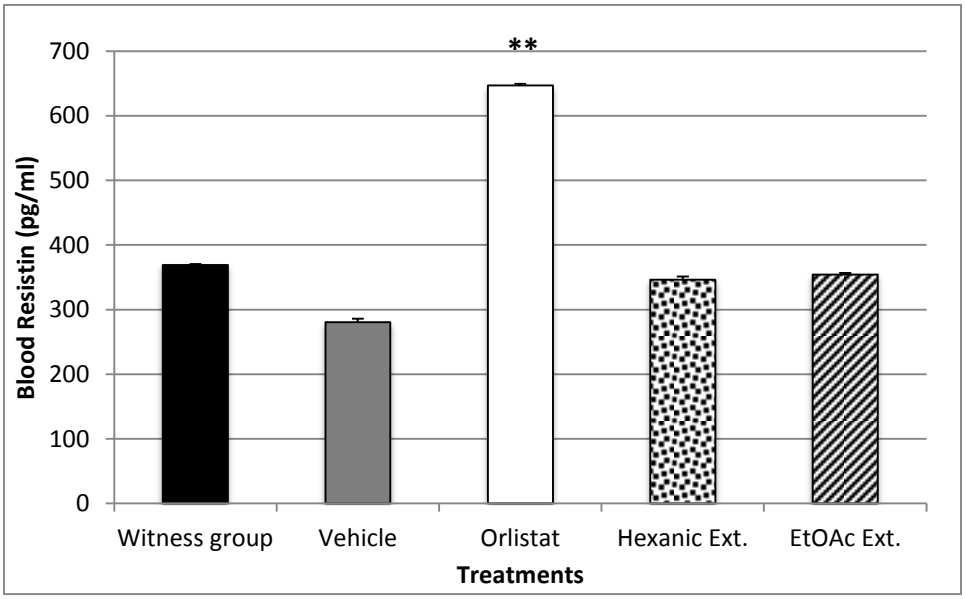

Figure 7: Effect produced by the oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicule (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* on blood resistin levels in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG. ** = p <0.05 with regard to the negative control group (Vehicle) in the Tukey test.

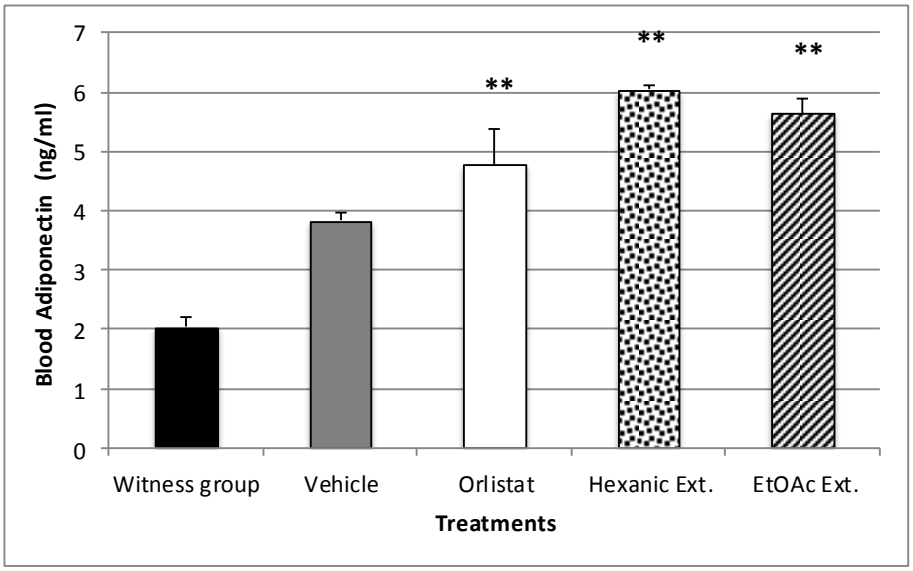

Figure 8: Effect produced by the oral administration (o.a.) of 10 mg/kg of Orlistat (positive control), vehicle (negative control), and the hexanic and Ethyl Acetate (EtOAc) extracts (50 mg/kg) of *Thevetia peruviana* in blood adiponectin levels in mice with MonoSodium Glutamate (MSG)-induced obesity. Witness group was not treated with MSG. ** = p <0.05 regarding the relationship with the negative control group (Vehicle) in the Tukey test.

Bio-assay Guided Purification and Structural Elucidation of Active Compounds

By means of gravitational open column chromatography followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis, identification was possible of the following non-polar compounds classified as sterols: stigmasta-3,5-dien-7-one (**1**); stigmasta-4-en-3-one (**2**); stigmasta-5,24(28)-dien-3ol (**3**); 24-methylenecycloartan-3-one (**4**); stigmasta-5,22-dien-3ol (**5)**; 13,27-cycloursane (**6**); 7-dehydrodiosgenin (**7**); β-sitosterol (**8**), and campesterol (**9**) (**Figure 9**).

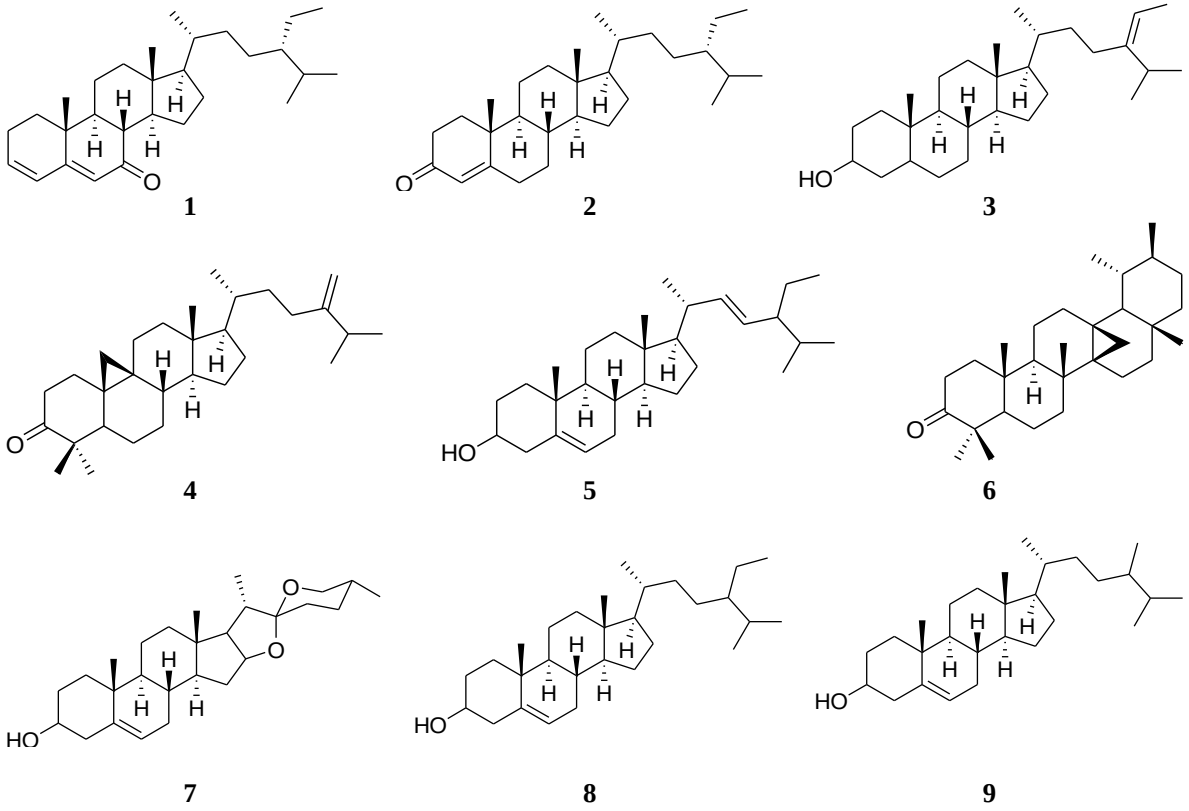

Figure 9: Chemical structure of compounds identified in the active fraction of *Thevetia peruviana.*

Discussion

The hexanic and EtOAc extracts obtained from *T. peruviana* seeds, that were administered orally during 7 weeks, reduced weight gain in the mice, especially the hexanic extract, which maintained an even lower weight than that demonstrated by the group treated with Orlistat (positive control). The inhibition of weight gain achieved by the hexanic extract of *T. peruviana* was 24.1%. Another aspect-of-interest was that observed in the group of mice treated with the hexanic extract, which demonstrated a lower fasting glycemic level, adequate response to the glucose tolerance test, and the extract was capable of avoiding the development of insulin resistance. Additionally, the levels of blood cholesterol and triglycerides exhibited by this group of animals were similar to that of the witness group and lower than those of the group treated with vehicle alone. The plasma adiponectin concentration in these animals was considerably higher to that found in the positive control group and in the group receiving vehicle alone (negative control). This datum merits special importance, due to that adiponectin is a hormone deriving from the adipocyte, whose reduction has been identified as a factor associated with T2D, coronary disease, hypertension, and ventricular hypertrophy, while adiponectin has been identified as a molecule-of-importance for preventing obesity-related diseases (López-Jaramillo 2016).

The results found in this work are comparable with those that have been identified in other species with significant effects on obesity: for example, for the species *Psacalium decompositum*, intragastric (i.g.) administration of 150 mg/kg (during 2 weeks) of a fructooligosaccharides fraction, obtained from the roots of this plant, achieved diminution of BW, cholesterol, and triglycerides, accompanied by associated anti-inflammatory effect (Merino-Aguilar *et al*. 2014). In an international scenario, it is probable that the plant species *Garcinia cambogia* is that which is most frequently referred in the scientific literature due to its potential effect against obesity. This species has demonstrated the capacity to reduce visceral fat and adipocyte size, in addition to being capable of reducing glucose intolerance and resistin levels in the plasma of mice fed with a fat-rich diet (Kim *et al*. 2013). Administration of a crude extract of saponins obtained from Korean Red Ginseng (200 mg/kg, *i.p.*) during 3 weeks achieved a reduction of up to 20% of weight in rats fed a diet high in fat (Kim *et al*. 2005). A recent review article cited that an interesting number of plant species have shown their effect on BW in animal models. Among these species, the following are noteworthy: *Camelia sinensis*; the administration of an aqueous extract (0.8, 1.6, and 3.2 g/L) in Wistar rats fed a diet rich in fat during 26 weeks attenuated visceral accumulation of fat in 40%; *Hibiscus sabdariffa*; the aqueous extract of the calyx, administered (120 mg/kg/day) during 60 days, diminished weight gain by up to 22%, and *Persea americana*; the administration of an aqueous and methanolic extract (10 mg/kg) during 8 weeks in hypercholesteremic rats produced a 25% reduction in BW gain in comparison with the control (Gamboa-Gómez *et al*. 2015).

By means of bio-assay guided fractionation, it was possible to isolate and identify different compounds in the active fraction; the majority of these corresponded to the phytosterol group. The latter is especially important because phytosterols have been considered very interesting chemical compounds due to their potential health benefits. Increased attention has been generated because they promote cardiovascular health, due to their hypocholesterolemic properties. These compounds are found in cell membranes and are structurally in close proximity to cholesterol. Stigmasta-5,22-dien-3-ol and β-sitosterol are the main phytosterol constituents of *Coxilachrymal-jobi* L., a crop widely used in traditional Chinese Medicine (Wu TT *et al*. 2007). This plant species, popularly known as Adlay, demonstrated the ability to inhibit adipocyte differentiation and to increase glucose uptake in 3T3-L1 cells (Ha do *et al*. 2010). The water extract from this plant has demonstrated anti-obesity effects through a neuroendocrine action mechanism (Kim *et al*. 2007)

The phytosterol compounds, stigmasta-3,5-dien-7-one, stigmasta 4-en-3-one, and β-sitosterol have also been identified in the plant species *Capsella bursa-pastoris*, a plant widely utilized in traditional medicine as an anti-inflammatory in many countries (Al-Snafi 2015).

Stigmasta-3,5-dien7-one and sitosterol were found in the extract of the fruits and leaves of *Vaccinium myrtillus* L., a plant native to Europe, North Asia, Western Canada, and the U.S., which is the most frequent ingredient of commercial antidiabetic products available on the market in the 20th century (Mohammed *et al*. 2015).

β-sitosterol has been found in the *Caralluma fimbriata* extract, which is employed as an anti-obesity agent and appetite suppressor (Dutt *et al*. 2012).

Bio-assay guided separation permitted the isolation and identification of stigmasta-4-en-3-one as the active compound from *Parkia speciosa* with a hypoglycemic effect (Jamaluddin 1995).

Daily administration of β-sitosterol at a dose of 25 mg for a prolonged time in patients was able to reduce serum total cholesterol with a reduction of serum total lipids (Best *et al*. 1955).

Conclusion

With the results obtained, it can be concluded that the hexanic extract from *Thevetia peruviana* seeds was capable of reducing weight gain (up to 24.1%) in mice with MSG-induced obesity. In addition, this extract showed good response to the glucose tolerance test, was able to avoid the development of insulin resistance, and also substantially increased serum adiponectin levels. The chemical separation procedure allowed for the identification of the following active chemical compounds: stigmasta-3,5-dien-7-one, stigmasta-4-en-3-one; stigmasta-5,22-dien-3-one; stigmasta-5,24(28)-dien-3ol; 24-

methylenecycloartan-3-one; stigmasta-5,22-dien-3ol; 13,27-cycloursane; 7-dehydrodiosgenin; β-sitosterol, and 3,27-cycloursane. This species could be considered for ongoing investigation as a potential option to reduce obesity.

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