

Isolation and identification of anti-obesity and anti-adipogenic compounds from *Thevetia peruviana*

[Aislamiento e identificación de compuestos con actividad antiobesidad y antiadipogénica de *Thevetia peruviana*]

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Abstract: *Thevetia peruviana* has been used in Mexican traditional medicine for its slimming effects. Active fractions and compounds from *T. peruviana* were isolated and identified by means of gravitational open-column chromatography, and the fractions were evaluated on mice with MonoSodium Glutamate-induced obesity. Orlistat was used as positive control, and treatments were administered, orally, once a day for 8 weeks. The fraction with higher activity was sub-fractioned and, with the intention of avoiding using more mice, the fractions were analyzed by evaluating their capability to inhibit adipogenesis in the 3T3-L1 cell line. Animals treated with the F-B fraction revealed a significantly smaller weight increase and maintained adequate insulin sensitivity, and significantly lower blood levels of cholesterol and triglycerides. The active compounds that demonstrated greatest adipogenesis inhibition were palmitic acid (1), oleic acid (2), octadecanoic acid (3), 2-palmitoyl glycerol (4), 2-oleoyl glycerol (5) and stigmastene (6).

Keywords: Obesity; *Thevetia peruviana*; Adipogenesis inhibition; Decanoic acids; 3T3-L1 cell line.

Resumen: *Thevetia peruviana* ha sido utilizada en la medicina tradicional mexicana por sus efectos adelgazantes. Para la identificación de las fracciones y los compuestos activos de *T. peruviana* se utilizó cromatografía en columna abierta y las fracciones obtenidas se evaluaron en ratones con obesidad inducida con glutamato monosódico. Como control positivo se utilizó Orlistat y los tratamientos se administraron oralmente una vez al día durante 8 semanas. La fracción con mayor actividad fue sub-fraccionada y, con la intención de evitar el uso de más ratones, los productos fueron seleccionados por su capacidad para inhibir la adipogénesis en la línea celular 3T3-L1. Los animales tratados con la fracción F-B mostraron un aumento de peso significativamente menor y mantuvieron la sensibilidad a la insulina, así como, niveles significativamente más bajos de colesterol y triglicéridos. El análisis de GC-MS indicó que esta fracción activa está compuesta principalmente por los ácidos palmítico (1), oleico (2) y octadecanoico (3), 2-palmitoil glicerol (4), 2-oleoil glicerol (5) así como la estigmasteno (6).

Palabras clave: Obesidad; *Thevetia peruviana*; Inhibición de la adipogénesis; Acido decanoico; Linea celular 3T3-L1.

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INTRODUCTION

In patients with obesity, there is a significant increase in visceral and subcutaneous (s.c.) adipose tissue, distributed mainly in the lower extremities and as abdominal fat. It has been proposed that the increase in visceral adipose tissue is due to the incapacity of the s.c. tissue to handle the excess of triglycerides, which caused their ectopical deposition (Halberg, 2008). After the identification of leptin, a protein factor (produced in adipose tissue) that exerts an effect on the central nervous system, the search of other factors produced in this tissue and with long-distance action, has been stimulated (Hallaas *et al.*, 1995; Halberg *et al.*, 2008). For this reason, adipose tissue is considered an endocrine organ and, in persons with obesity, insulin resistance could be considered a consequence of adipose-tissue malfunction, secondary to its hyperplasia and/or hypertrophy (Wellen & Hotamisligil, 2003). Some scientific reports confirm that the functions of an adipocyte are similar to those of immune cells; thus, they are capable of producing cytokines and complementing activation (Charrieri *et al.*, 2003). Another pathology that is linked to the increase of adipose tissue is dyslipidemia, especially when the increase in fatty tissue is located in the center of the body (Nelsen *et al.*, 2004).

The increase of adipose tissue in the organism is identified as an alteration with a negative impact on the human health, since it increases the risk of presenting insulin resistance, glucose intolerance, hypertension, dyslipidemia, and cardiovascular problems (Kim *et al.*, 2007; Maritza, 2007; Ibrahim, 2010). It has been demonstrated that the reduction of body weight (in patients with obesity) and, consequently, the reduction in adipose tissue (between 5 and 10%) decreases the risk of developing the previously mentioned pathologies or facilitates their medical treatment (Mayor, 2006; Andersson *et al.*, 2009).

The species *Thevetia peruviana* (Pers.) K. Schum (of the Apocynaceae family), popularly known as “friar’s elbow”, is a native plant of tropical America that is distributed around the world, but grows mainly in Mexico and Brazil. The fruit of this plant has in its interior 2-4 seeds (Eddleston *et al.*, 2000). *T. peruviana* seeds have been widely used in Mexican Traditional Medicine for reducing body weight (Villavicencio & Pérez, 2006; Torres, 2010). The seeds contain a large amount of oil and, at a

smaller proportion, proteins, but with some variations depending on the season (Alexander, 1964; Standley & Williams, 1969; Obasi *et al.*, 1990). Recent work has demonstrated that the hexanic extract obtained from the seeds of *T. peruviana* has the capability to reduce body weight in mice with MonoSodium Glutamate (MSG)-induced obesity (Pérez *et al.*, 2017).

The objective of the present study was to isolate (by means of chromatography techniques) and identify the fractions and active compounds contained in the hexanic extract of *T. peruviana* with the ability to inhibit adipogenesis in 3T3-L1 cells.

MATERIALS AND METHODS

Materials used in the pharmacological experiments

MonoSodium Glutamate (MSG) (Aldrich, >98% pure) was utilized as obesity inducer. For determining blood glucose, an AccuChek Performa Roche Glucometer was employed while, for measuring cholesterol and triglycerides, an Accutrend Performa Roche was used. For evaluating the anti-adipogenic effect, DMEM medium with 10% Calf Serum (ATCC, USA) was used. Insulin, dexamethasone, and 3-isobutyl-1-methyl xanthine (Sigma-Aldrich) were used to induce differentiation of 3T3-L-1 cells. Oily red was used to stain the lipids, while KOH was utilized as saponificant.

Plant material

The fruits with seeds of *T. peruviana* were collected in their natural habitat in the state of Morelos, Mexico. For identification of the species, a herbarium specimen was prepared with the aerial parts and seeds, and deposited for safekeeping and registration in the IMSSM herbarium. Identification of the species was in charge Abigail Aguilar-Contreras, Director of the Herbarium, and the registry number assigned was IMSSM-16000.

Fractionation and purification of the extract

The seeds were dried at room temperature protected from light. Later, they were milled until to obtain small particles (< 2-mm, 2,769 g) and extracted by maceration in *n*-hexane (39.7% yield). The product was filtered, and the solvent was removed by low-pressure distillation using a rotary evaporator (LABOROTA 400, Heidolph, Germany). Afterward, the *n*-hexane extract (300 g) was subjected to a column chromatography to isolate and identify active

fractions and compounds. Open-column gravitational chromatography with silica gel normal phase (Merck) as stationary phase and hexane: EtOAc gradient as mobile phase was used. The separation procedure was started with 100% of hexane and the EtOAc was increased gradually. This process allowed to obtain three groups of chemical constituents: F-A (colorless oil, 214.2 g); F-B (white semi-solid, 4.8 g), and F-C (yellow oil, 1.6 g).

Sub-fractioning of the most active fraction

A total of 3.18 g of the most active fraction (F-B) was subjected to chromatographic separation. The mixture was absorbed in 5 g of silica gel normal phase. Column chromatography was used, which was packed with the same support described previously and eluted with the same solvent gradient. The products obtained from this separation procedure were analyzed with TLC and, based on the resolution observed in each of the sub-fractions, the latter were grouped into three sub-fractions: F-1 (490.0 mg); F-2 (574.6 mg), and F-3 (532.7 mg). These products were subjected to an *in-vitro* test in the 3T3-L1 cell line.

Animal models

MSG-induced obesity in mice

Male CD-1 strain mice (acquired in ENVIGO RMS, S.A. de C.V.) were paired. Offspring obtained from pairings were used to induce obesity. On postnatal days 2 and 4, they were subcutaneously (s.c.) administered with 2 mg/kg of MSG, while on days 6, 8 and 10, they received 4 mg/kg of MSG by the same route.

Once the animals with MSG-induced obesity reached 2 months of age and achieved a weight higher than 31 g, treatment was initiated. Three experimental groups were formed ($n = 10$) that received a daily oral dose (25 mg/kg) of the fractions (F-A, F-B, F-C) of the hexanic extract of *T. peruviana*, during 8 weeks. The positive control group was treated with Orlistat (10 mg/kg) by the same route and the same administration scheme. The negative control group was treated with the vehicle (10% Tween 20). In addition, a witness group was included, which consisted of normal animals that were not administered MSG.

Differentiation of 3T3-L1 preadipocytes

Fractions F-1, F-2, and F-3, obtained by means of the chemical separation of F-B, were evaluated in an *in-*

vitro model in order to identify their ability to inhibit the differentiation of 3T3-L1 into adipocytes.

The fibroblasts (3T3-L1) were cultivated in DMEM medium at 37°C in a 5% CO₂ environment. Glucose (25 mM) was added to this medium and supplemented with 10% Fetal Bovine Serum (FBS). After achieving a cell confluence higher than 80%, induction of cell differentiation was initiated, and it was considered as day 0. The proliferation medium was changed for the differentiation medium by adding insulin (80 mM), dexamethasone (50 µM, Sigma-Aldrich), and 3-IsoButyl-1-MethylXanthine (IBMX, 100 mM). With this procedure, the cells should be totally differentiated by day 10. The experimental treatments (F-1, F-2, and F-3) were added to the medium at the beginning of the differentiation at a concentration of 25, 37.5, 50, and 62.5 µM. Gemfibrozil (Lopid®) and the vehicle were used as positive and negative control group, respectively.

Qualitative evaluation of the capability of the treatments to inhibit the differentiation of the fibroblasts into adipocytes was carried out by means of microscopic observation (orange color inside of the adipocyte), due to the oily-red staining. Subsequently, with the staining, it was possible to perform the quantification of the intracellular lipid content through spectrophotometry at 492 nm. The results were reported as the percentage of lipid accumulation in relation to the negative control group, included in the differentiation, that was not treated with any of the fractions.

Chemical analysis of bioactive fraction

Fraction F-3 was analyzed by means of a Gas Chromatography-Mass Spectrometric (GC-MS) analysis using 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. Chemical identification was performed,

comparing F-3 mass spectra with those of the National Institute of Standards and Technology (NIST) 1.7 Library. This procedure allowed us to identify six compounds (see results).

Statistical analysis

For statistical analysis, the IBM SPSS ver. 20 statistical program was used. Results were expressed

as mean \pm Standard Deviation (SD). A value of $p < 0.05$ was considered to identify significant statistical differences between the groups. Values were analyzed by means of Student *t* test, ANOVA, and Tukey post-test.

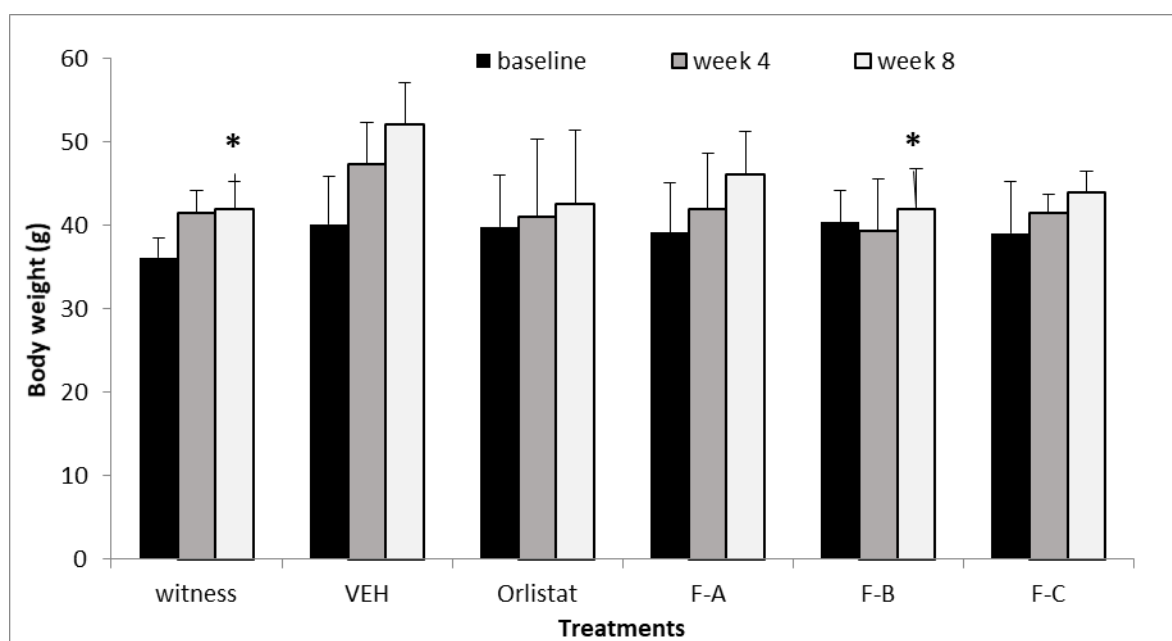


Figure No. 1

Effect produced after 4 and 8 weeks of daily administration (p.o.) of 10 mg/kg of Orlistat (positive control), Vehicle (VEH, negative control), and the F-A, F-B, F-C fractions (25 mg/kg) obtained from the hexanic extract of *Thevetia peruviana* seeds 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 (VEH) in the Tukey test.

RESULTS

Effect produced by fractions obtained from the hexanic extract of *T. peruviana* seeds in mice with MSG-induced obesity

As shown in Figure No. 1, animals of the negative control group (VEH), those who only received MSG and vehicle, presented a gradual weight increase, but one that was significantly higher when compared with that of the witness group (which was not administered MSG). Fractions F-A, F-B, and F-C (25 mg/kg) successfully decreased weight in animals that received MSG; the latter is appreciated when compared with the negative control group. Likewise,

animals that were treated with Orlistat (10 mg/kg orally) maintained their weight during the 8 weeks of administration. The group of animals that was treated with F-B was the one that accomplished greatest weight reduction and the sole group that showed a significant difference ($p < 0.0005$) when compared to the negative control group. At the end of the 8 treatment weeks, it was observed that the weight increase in the negative control group was on average 12 g, while the group treated with Orlistat showed an increase of 4 g, and the group treated with F-B only increased 1.0 g.

Effect produced by fractions obtained from the hexanic extract of *T. peruviana* seeds on the glucose-tolerance test in mice with MSG-induced obesity

At the end of administration of the treatments (8 weeks), a glucose-tolerance test was performed. Figure No. 2 presents the blood-glucose level of the animals. Time zero corresponds to the determination

of fasting blood glucose. In all cases, 15 min after the administration of glucose, a consistent elevation of the blood glucose could be appreciated, values that are gradually reduced with more time. The groups treated with F-A and VEH exhibited a higher increase in glycemia and demonstrated a late decrease of this parameter.

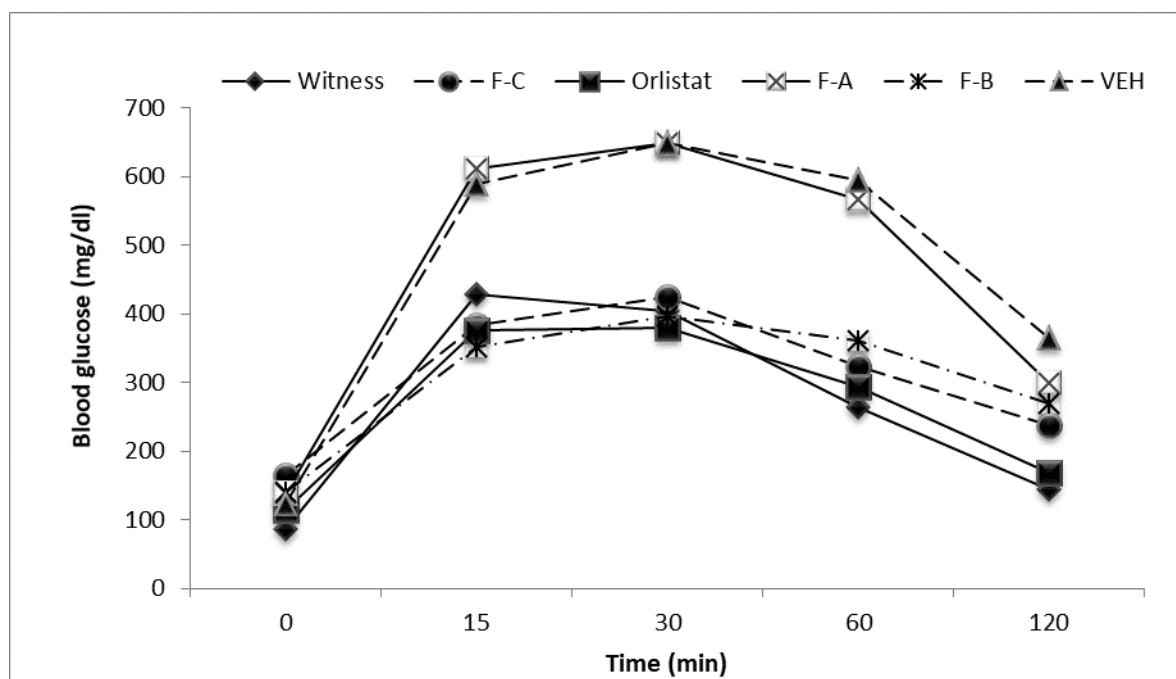


Figure No. 2

Glucose-tolerance test carried out at the end of week 8 of treatment (p.o.) with 10 mg/kg of Orlistat (positive control), Vehicle (VEH, negative control) and the F-A, F-B, and F-C fractions (25 mg/kg) obtained from the hexanic extract of *Thevetia peruviana* seeds in mice with MonoSodium Glutamate (MSG)-induced obesity.

Witness group was not treated with MSG. No statistical difference was observed

Effect produced by fractions obtained from the hexanic extract of *T. peruviana* seeds on the insulin-resistance test in mice with MSG-induced obesity

An insulin-resistance test was performed at the end of treatment administration. As revealed in Figure No. 3, blood glucose levels at time zero were high,

especially in the negative-control (MSG alone) and F-A groups. These same groups did not respond to the administration of insulin, showing low sensitivity to the hormone, while the rest of the groups, and especially the F-B group, showed a consistent response to the administration of insulin, evidencing a preserved insulin sensitivity.

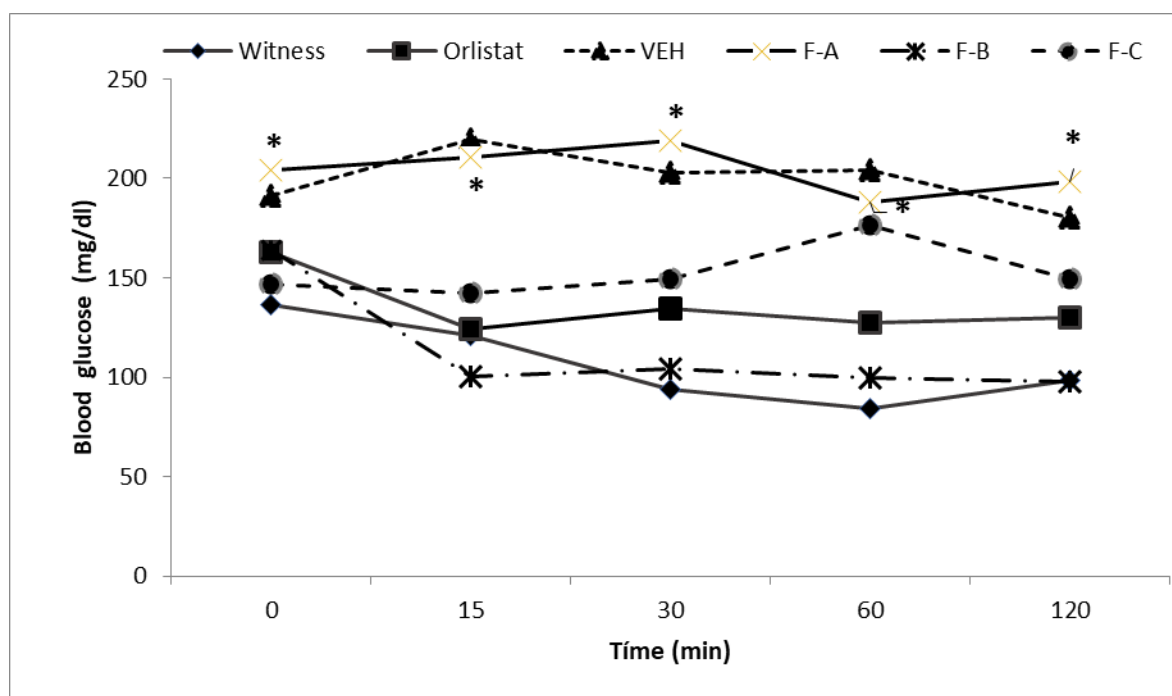


Figure No. 3

Insulin-resistance test carried out at the end of week 8 of treatment (p.o.) with 10 mg/kg of Orlistat (positive control), Vehicle (VEH, negative control), and the F-A, F-B, and F-C fractions (25 mg/kg) obtained from the hexanic extract of *Thevetia peruviana* seeds in mice with MonoSodium Glutamate (MSG)-induced obesity.

Witness group was not treated with MSG. * = $p < 0.05$ with regard to the Witness group in the Tukey test.

Effect produced by fractions obtained from the hexanic extract of *T. peruviana* seeds on blood cholesterol and triglycerides in mice with MSG-induced obesity

The blood-cholesterol level in animals with MSG-induced obesity, and after administration of the 8-weeks of treatment, was notably higher in the negative control group (VEH). The experimental groups, the positive control (Orlistat), and the group treated with F-B (despite having been pretreated with MSG) exhibited significantly lower levels ($p < 0.005$) of plasma cholesterol (Figure No. 4). Meanwhile, in the case of triglyceride determination (Figure No. 5), this parameter was found high only in the negative control group, and in that treated with Orlistat. In this evaluation, F-B was the group that showed greatest similarity with the witness group ($p = 0.01$).

Effect produced by sub-fractions obtained from F-B that is derived from the hexanic extract of *T. peruviana* seeds on the differentiation of 3T3-L1 cells

On day 9 after having induced differentiation and having administered the treatments, the capability of sub-fractions F-1, F-2, and F-3 (obtained from F-B) to inhibit the differentiation of the fibroblasts of 3T3-L1 cells into adipocytes was evaluated. Figure No. 6 illustrates that the fraction with greatest capability to inhibit differentiation was F-3, also revealing dose-dependent behavior.

The total lipid content that was quantified by means of spectrophotometry in adipocytes treated with different sub-fractions obtained from F-B is presented in Figure No. 6.

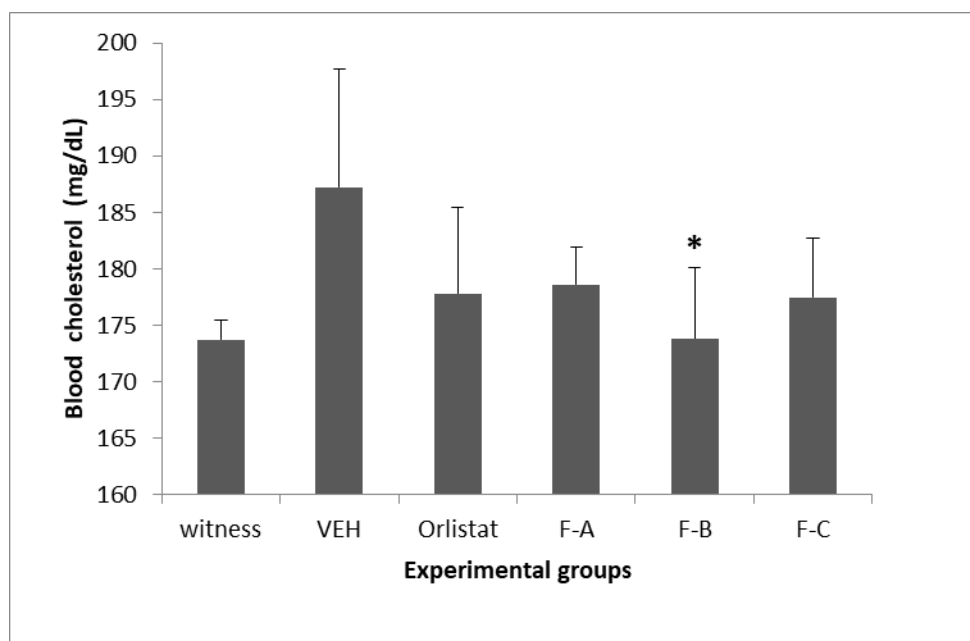


Figure No. 4

Effect produced by the administration (p.o.) of the vehicle (negative control group, VEH), Orlistat (10 mg/kg, positive control), and the fractions F-A, F-B, and F-C (25 mg/kg) obtained from the hexanic extract of *Thevetia peruviana* seeds on the blood levels of cholesterol in mice with MSG-induced obesity. *= $p < 0.05$ with regard to the negative control group (VEH) in the Tukey test.

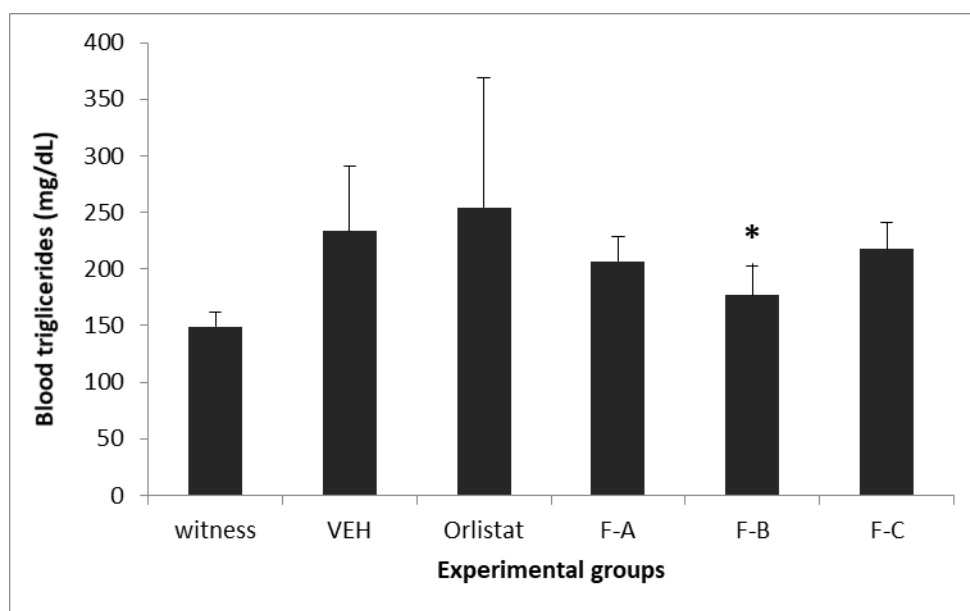
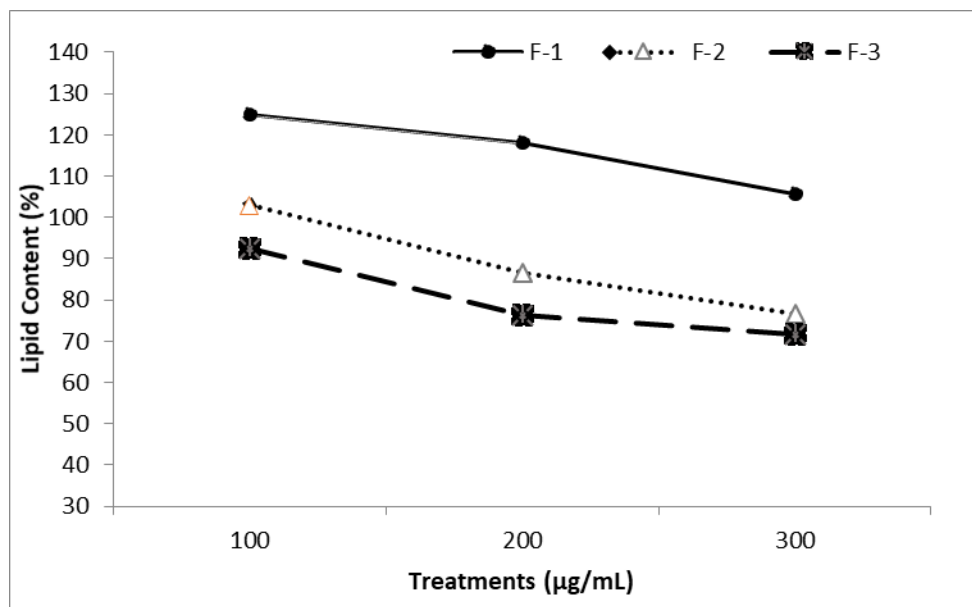


Figure No. 5

Effect produced by the administration (p.o.) of the vehicle (negative control group, VEH), Orlistat (10 mg/kg, positive control), and the fractions F-A, F-B, and F-C (25 mg/kg) obtained from the hexanic extract of *Thevetia peruviana* seeds on the blood levels of triglycerides in mice with MSG-induced obesity. *= $p < 0.05$ with regard to the negative control group (VEH) in the Tukey test.

A)



B)

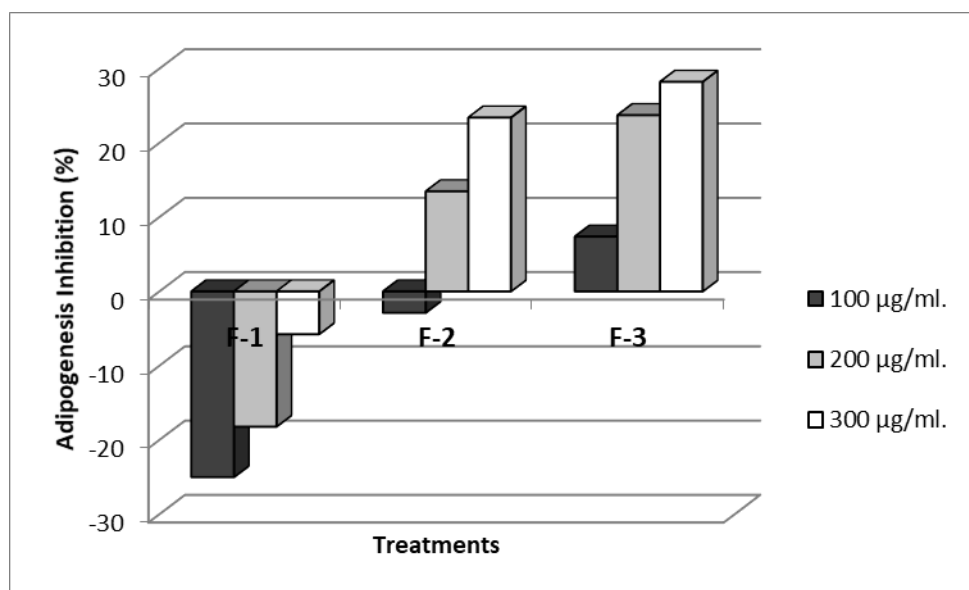


Figure No. 6

Effect produced by sub-fractions F-1, F-2, and F-3 obtained from F-B of the hexanic extract of *Thevetia peruviana* seeds on: A) the amount of lipids contained in the adipocyte product of the differentiation (on day 9) of 3T3-L1 cells, and B) adipogenesis induced in 3T3-L1 cells.

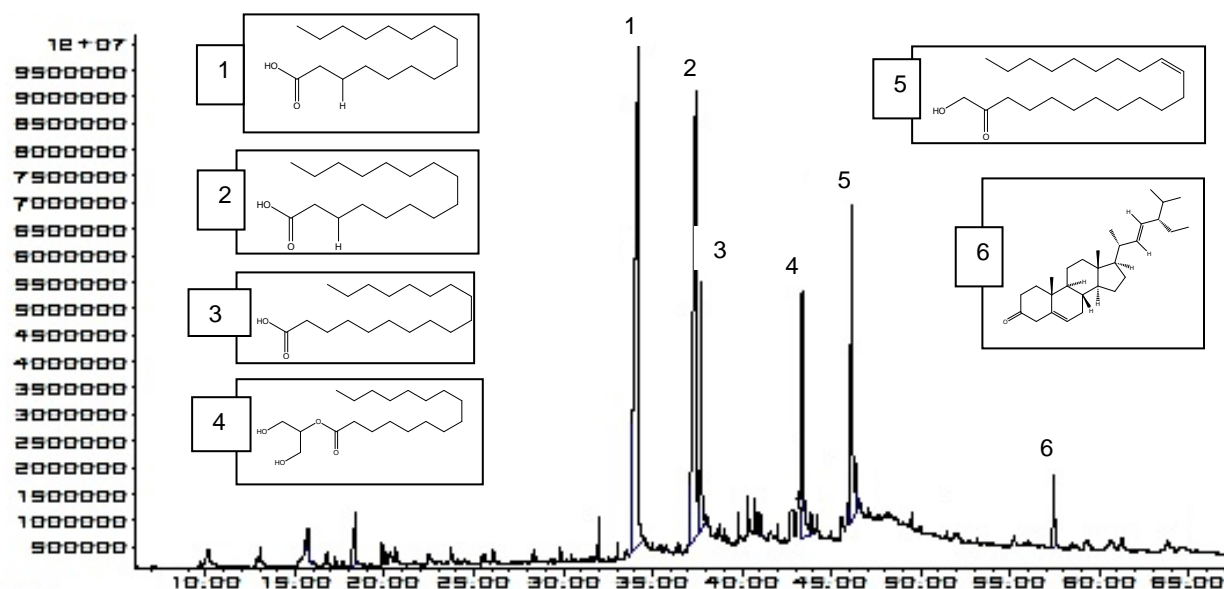


Figure No. 7

Chromatographic profile of F-3, the sub-fraction obtained from F-B, derived from the hexanic extract of *T. peruviana* seeds. 1 = Palmitic acid; 2 = Oleic acid; 3 = Octadecanoic acid; 4 = 2-palmitoyl glycerol; 5 = 2-oleoyl glycerol; 6 = Stigmastanone.

Structural elucidation of compounds contained in the F-3 sub-fraction obtained from *T. peruviana*

Figure No. 7 presents the chromatographic profile of

F-3, which demonstrated greatest ability to inhibit the differentiation of 3T3-L1 cells into adipocytes, as well as the chemical structures of the compounds.

Table No. 1
Chemical composition by GS-MS of F-3 sub-fraction from *T. peruviana*

Number	Name	Time (min)	Molecular weight (g/mol)
1	palmitic acid	34.22	256.43
2	Oleic acid	37.42	282.46
3	Octadecanoic acid	37.75	284.48
4	2-palmitoyl glycerol	43.37	330.50
5	2-oleoyl glycerol	46.12	356.54
6	Stigmastanone	57.45	412.70

DISCUSSION

Due to the dramatic increase in the prevalence of obesity and Type 2 Diabetes Mellitus (DM2) worldwide, it is considered imperative to promote strategies to confront the epidemiological growth of these diseases. In this effort, the plant species

employed in traditional medicine have also played an important role (Zaid *et al.*, 2015). Publications available in recent years have permitted the identification of medicinal plants extracts as possessing a very important potential in the prevention and treatment of obesity. Huang *et al.*

(2016), demonstrated that the combined extract of cocoa coffee, green tea and garcinia was able to stimulate lipid metabolism in high energy diet-induced obesity in rats, which is attributable to fat mobilization from adipose tissue. A recent ethnobotanical review identified a total of 139 plant species native to Mexico, Central America, and the Caribbean that are utilized empirically for the treatment of obesity. Only thirty-three of these species have been researched, thus leaving 106 that have not yet been studied. From these plants, four compounds have been identified, but none of these has been included in clinical trials (Alonso *et al.*, 2015).

In Mexico, the plant species *T. peruviana* has been empirically used for many years to reduce weight. Extracts obtained from this species are employed in the elaboration of herbal supplements that are commercialized with the purpose of reducing weight but, they are not validated for their quality, efficacy and safety. In the present work, mice pretreated during neonatal stage with MSG were used. The fractions obtained from the hexanic extract of *T. peruviana* seeds, orally administered during 8 weeks, were able to decrease weight gain in the mice. Fraction F-B maintained an even lower weight in mice than that shown by the group treated with Orlistat (positive control). Other relevant aspects that were identified in this study was that the group of mice with MSG-induced obesity treated with F-B exhibited a fasting blood-glucose level lower than that of the negative control group. Additionally, this group showed good response to the glucose-tolerance test and was able to prevent evolution to an insulin-resistance stage. Also, serum-cholesterol and triglyceride levels in this group of animals were similar to that of the witness group and lower than that of the group treated only with the vehicle (negative control group).

The results found in this work are comparable with those that have been identified in other species with significant effects on obesity. For example, the species *Psacalium decompositum* accomplished the reduction of body weight, cholesterol, and triglycerides with an associated anti-inflammatory effect, by means of intragastric administration of 150 mg/kg of a fructooligosaccharide fraction obtained from its seeds (Merino *et al.*, 2014). While the plant species *Garcinia cambogia* could be the one most frequently

referred (in scientific literature) due to its important effect against obesity. This species has shown the capability to reduce visceral fat and adipocyte size, as well as to reduce glucose intolerance and resistin levels in Plasma of mice fed a high-fat diet (Kim *et al.*, 2013).

Administration (i.p.) of a saponin crude extract obtained from the roots of "Red Ginseng" (of Korean origin) (200 mg/kg, intraperitoneally [i.p]) during 3 weeks, was able to reduce, up to 20%, the weight in rats fed a high-fat diet (Kim *et al.*, 2005). Over the last decade, essential oils have gained popularity as a source of bioactives, with many potential health benefits (Burt, 2004). A good example could be the species *Coriandrum sativum* L. commonly known as coriander. Seeds and pericarp are the most widely used components of coriander; while the most important constituent has been the essential oil and the fatty oil. Administration of coriander seed oil has been capable of decreasing the levels of total lipids, total cholesterol, triacylglycerols and LDL-cholesterol in rats fed on a high cholesterol diet (Najla-Gooda *et al.*, 2012). Sterols isolated from brown, red and green macroalgae exhibit significant anti-obesity properties. Fucosterol was evaluated for its potential to inhibit adipocyte differentiation and lipid formation. Fucosterol decreases the expression of the adipocyte marker proteins PPAR and CCAAT/enhancer-binding protein (C/EBP) in a concentration-dependent manner (Seca & Pinto, 2018). Lee *et al.* (2017), showed that fucosterol, isolated from *Ecklonia stolonifera* Okamura, exhibits the ability to inhibit adipogenesis of 3T3-L1 preadipocytes through downregulation of SREBP1 and modulation of multiple signaling pathways including PI3K/Akt and ERK-dependent FoxO signaling pathway.

Column chromatography allowed to obtain three sub-fractions: F-1; F-2, and F-3, of which, sub-fraction F-3 was the most active when evaluated in a model that measures their capability to inhibit adipogenesis in 3T3-L1 cells.

Chemical analysis of this active fraction demonstrated the presence of low-polarity compounds belonging to the decanoic acid and phytosterol groups. These compounds were identified as palmitic acid (1), oleic acid (2), octadecanoic acid (3), 2-palmitoyl glycerol (4), 2-oleoyl glycerol (5) and stigmastene (6). The identified compounds are characterized by being of low polarity, then in future

investigations it will be necessary to identify a probable action mechanism. In this work it was identified the capability of F-3 to inhibit de adipocytes differentiation, nevertheless, these types of molecules could participate in the following modes of action: increase energy expenditure, decrease carbohydrates absorption, decrease lipid absorption, or increase lipolysis.

The genus *Thevetia*, among other members of the Apocynaceae Family, is identified as a natural source of cardiac glycosides (Wen *et al.*, 2016). Regarding *Thevetia peruviana*, and particularly with the cardiac glycosides that it contains, different scientific reports refer their toxic effects. There are even articles that mention intentional ingestion of the seeds for the infliction of self-harm, probably with suicidal purposes. These same authors also note that the toxicity is due to its content of cardiac glycosides (Bandara *et al.*, 2010). Medicinal plants are generally considered as innocuous, relatively free of severe adverse effects, but it is important to consider the presence of some species that produce toxic effects. Cardiac glycosides are normally obtained by means of extraction with polar solvents such as, ethanol, methanol or hydroalcoholic. Nevertheless, it is important to perform an exhaustive chemical analysis, in order to demonstrate the absence of these toxic compounds in the hexane extract and the derived fractions. The objective of the present work was to isolate and identify the fractions and compounds contained in the hexane extract of *T. peruviana* with the ability to inhibit adipogenesis in 3T3-L1 cells. However, it will be important to evaluate in detail the presence of possible toxic effects in the fractions or compounds.

CONCLUSION

The chromatographic separation of the hexanic extract from *T. peruviana* seeds permitted us to identify that the F-B fraction was that possessing greatest capacity to reduce weight in mice with obesity induced with MSG, in addition to lowering blood-cholesterol levels and maintaining, in the animals, sensitivity to insulin, observed through a glucose-tolerance test and an insulin-resistance test. Fraction F-B maintained an even lower weight in mice than that shown by the group treated with Orlistat (positive control). The group of mice with MSG-induced obesity treated with F-B exhibited a fasting blood-glucose level lower than that of the negative control group. The separation of the active fraction (from the hexane extract) and the pharmacological evaluation of sub-fractions permitted us to identify the ability of F-3 to inhibit the differentiation of fibroblasts of 3T3-L1 cells into adipocytes. The compounds that were identified as active, and to which the pharmacological activity is attributed, included palmitic acid, oleic acid, octadecanoic acid, 2-palmitoyl glycerol, 2-oleoyl glycerol, and stigmastenone. It will be important to evaluate the presence of possible toxic effects in fractions or compounds obtained from this plant species, and perform a more detailed experiments in order to elucidate the possible action mechanism.

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