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Research Paper

Anti-hyperglycemic effect, inhibition of inflammatory cytokines expression, and histopathology profile in streptozotocin-induced diabetic rats treated with *Arracacia tolucensis* aerial-parts extracts

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ABSTRACT

Ethopharmacological relevance: *Arracacia tolucensis* is a medicinal plant used in northeast of Mexico as a remedy to treat people with Diabetes mellitus (DM); however, there are no scientific studies that support this information. Thus, we evaluated the anti-hyperglycemic effect of the hexane, ethyl acetate and ethanol extracts from aerial parts in streptozotocin-induced diabetic rats.

Materials and methods: DM was induced in Wistar male rats by single intraperitoneal injection of streptozotocin (STZ 50 mg/kg). After STZ-induction, hyperglycemic rats were treated with all three extracts orally at a single dose (250 mg/kg) each 48 h for 21 days. Glibenclamide (1 mg/kg) was used as a reference drug. The fasting blood glucose levels, the hematic biometry and biochemical profiles, and the inhibition of inflammatory cytokines expression were estimated. Histopathology analysis of pancreas, liver, spleen, and kidney tissue was carried out.

Results: Ours results showed that ethyl acetate extract decreased blood glucose levels significantly (75%, $p < 0.05$) when compared to diabetic rats and controlled the body weight loss; the lipids level did not change, but the enzyme levels of aspartate aminotransferase and alanine aminotransferase decreased significantly (60.83% and 66.16%, respectively, $p < 0.05$) and inhibited the expression of inflammatory cytokines,with respect to diabetic rats. Histopathology injury was not observed; by contrast repair of islet of Langerhans was exhibited.

Conclusion: These results validate the use of *Arracacia tolucensis* as a treatment against DM and suggests it is suitable to continue studies for its safe therapeutic use.

1. Introduction

Diabetes mellitus (DM) is a chronic disease that occurs when the pancreas do not produce enough insulin or alternatively, when the body cannot effectively use the insulin it produces (ADA, 2011). The World Health Organization estimates that more than 346 million people worldwide have diabetes, but this number is likely to increase to more than double by 2030 (WHO, 2013).

- Currently, several mechanisms have been identified in the pathophysiology of diabetes; one of them leads to the production of cytokines such as tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and interleukin 1β (IL-1β), which modulate the insulin response in liver and muscles (Chang and Chuang, 2010). Other ways are associated with alterations in metabolism; among them are disturbances in the production and clearance of plasma lipoproteins such as high density lipoprotein (HDL), and triglycerides (Goldberg, 2001). Moreover, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum are likely increased by cellular damage of metabolic organs (McAnuff et al., 2003).

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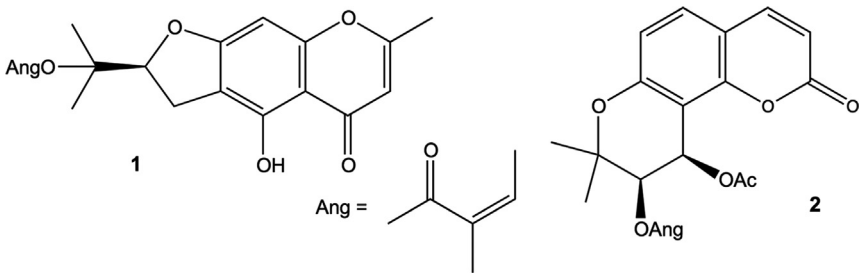

Fig. 1. Structure of isolated compounds from *Arracacia tolucensis* EtOAc extract: (S)-(+)-4′-O-Angeloylvisamminol (1) and (3′R, 4′R)-(−)-4′-O-acetyl-3′-O-angeloylkhellactone (praeruptorin A) (2) (Burgueño-Tapia et al., 2012).

- Although, there is treatment available for diabetes, the drugs used are associated with undesirable side effects and high cost for patients, which in recent years has led to intense research in alternative therapies such as medicinal plants that provide an effective, reliable and cheap treatment option. In this context, *Arracacia tolucensis* is a herbal medicine used in Mexico as a remedy to treat people with DM, cough and bronchitis. *Arracacia tolucensis* var. *multifida* Hemsley (S. Watss) Mathias & Constances (*Umbelliferae/Apiaceae*) is one of the 38 species found in the American Continent. Local population has known this herb by its Náhuatl name "Acocotli", or the common Spanish names of "Comino rústico", "Hierba del oso" and "Neldo" (Figueroa et al., 2007). This specie has been little studied, previous reports have demonstrated that the crude extract (MeOH–CH_2Cl_2 1:1) of this herb was not toxic or mutagenic for mice (Déciga-Campos et al., 2007), also showed action against *Mycobacterium tuberculosis*; while the organic extract and essential oil showed significant spasmolytic activity (Figueroa et al., 2007).
- From chemical point of view, the CH_2Cl_2–MeOH extract was found to be composed of 11 coumarins which were isoimperatorin, 8-methoxypsoralen (8-MOP), isoscopoletin, scopoletin, osthol, suberosin, herniarin, scoparone, umbelliferone, dihydroxypeucedanin and 5-methoxypsoralen (5-MOP); whereas, the essential oil composition displayed mainly osthol, suberosin, 8-MOP, 5-MOP, benzyl alcohol, terpinen-4-ol and α-cadinene (Figueroa et al., 2007). Our group recently reported two major compounds isolated and identified from the EtOAc extract, (S)-(+)-4′-O-Angeloylvisamminol (**1**, dihydrofurochromone) and (3′R, 4′R)-(−)-4′−O-acetyl-3′-O-angeloylkhellactone or praeruptorin A (**2**, pyranocoumarin) (Burgueño-Tapia et al., 2012).
- Hence, our aim was to evaluate the anti-hyperglycemic effect, inhibition of inflammatory cytokines expression, hematological, biochemical and histopathology profile in STZ-induced diabetic rats treated with *Arracacia tolucensis* aerial-part extracts.

2. Materials and methods

2.1. Plant material and preparation of extracts

Aerial parts of *Arracacia tolucensis* were collected from Alta Cima at Gomez Farias, Tamaulipas, northeastern of Mexico, on September 2011. A voucher specimen (No. 1769) was deposited at the Herbarium of Facultad de Estudios Superiores Iztacala-UNAM.

Aerial parts of *Arracacia tolucensis* were dried at room temperature and then powdered (500 g). The extracts were prepared by maceration with hexane (Hx), ethyl acetate (EtOAc), and ethanol (EtOH), the latter has a similar polarity to an aqueous extract, which is mostly used in traditional medicine, the solvents were purchased from J.T. Baker Chemical Co. (Jackson, TN., USA).

The maceration with each solvent lasted five days, and the samples were filtered and extracted again with each solvent for three times. The organic extracts prepared with each solvent were concentrated '*in vacuo*' in a rotary evaporator and the residual solvent was evaporated to dryness at room temperature, and only EtOH extract was lyophilized. The yields of the extracts were 4.99% (Hx), 5.59% (EtOAc) and 3.05% (EtOH). Since *Arracacia tolucensis* EtOAc extract showed the best anti-hyperglycemic activity, the isolation and identification of the constituents was carried out. As we previously noted, two major compounds were isolated (Fig. 1), (S)-(+)-4′-*O*-Angeloylvisamminol (**1**) and (3′R, 4′R)-(−)-4′-*O*-acetyl-3′-*O*-angeloylkhellactone or praeruptorin A (**2**) (Burgueño-Tapia et al., 2012). Nowadays, the chemical synthesis and biological assays of praeruptorin A are in progress to determine the anti-hyperglycemic effect and its action mechanism on STZ-induced diabetic rats and these will be reported in an independent paper at a later date.

2.2. Streptozotocin-induced diabetic rats

Healthy adult male albino Wistar rats weighing 250 ± 50 g were provided by the biotery of the Facultad de Estudios Superiores Iztcala-UNAM. The animals were housed at the biotery of the Escuela Nacional de Medicina y Homeopatia-IPN and kept under standard conditions in ventilated boxes (12 h light/dark and 22 ± 2 °C) and fed with Rodent Diet™ and water *ad libitum*. The DM was induced by a single dose of streptozotocin (STZ) from Sigma Chemical Co. (St Louis, MO., USA) at 50 mg/kg administered to each rat intraperitoneally (Szkudelski, 2001). After 48 h and 5 day, blood was collected by puncturing the tail, and fasting blood glucose level was estimate by using an Optium Xceed glucometer™. The rats showing blood glucose level ≥ 200 mg/dL on the 5th day were used for the present investigation. All animal procedures were adjusted to the "Mexican Official Norma" NOM-033 (1995) and NOM-062 (1999), and approved by the Ethical Animal Committee of the Escuela Nacional de Medicina y Homeopatía-IPN act ENMH-CB-057, which comply with international rules and policies.

2.3. Anti-hyperglycemic activity of Arracacia tolucensis extracts

The treatment was started on the 7th day after STZ-induction. The rats were divided into seven groups each with ten animals. Group I served as healthy control (0.5 ml H_2O sterile, orally). Group II served as healthy-vehicle control (100 μl DMSO + 200 μl H_2O sterile, orally) plus 0.5 ml H_2O sterile. Group III served as diabetic (STZ) control. Group IV received the glibenclamide (Roche) reference drug (1 mg/kg, orally). Group V, VI, VII received Hx, EtOAc and EtOH extracts at the dose of 250 mg/kg. This dose was selected considering that the LD_{50} previously reported for this species was 2852 mg/kg (Déciga-Campos et al., 2007). In addition, other references have used extracts concentrations ranges from 100 to 500 mg/kg (Pari and Amarnath, 2004; Shirwaikar et al.,

2006). All groups except I and III were administered one dose orally, each 48 h during 21 days. The changes in the body weight of animals were monitored on the 1st day (before the start of the experiment) and 21st day (after treatment). Blood was collected from the tail vein after 10 h of fasting, blood glucose level of each rat was estimated once a week by using a glucometer (Optium Xceed™).

2.4. Hematic biometry and biochemical profile

Blood was obtained by cardiac-puncture after euthanization. Samples were collected and deposited with anticoagulant (EDTA) and without anticoagulant. The blood with EDTA was processed in a Beckman Coulter AC-T instrument and the following blood parameters were estimated: lymphocytes, monocytes, granulocytes, hemoglobin and hematocrit. The blood without anticoagulant was centrifuged at 570*g* for 10 min at 4 °C and processed in a Cobas Mira Roche instrument. The serum was analyzed for the estimation of the following biochemical parameters: urea, creatinine, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin.

2.5. Detection of inflammatory cytokines expression by reverse transcription – polymerase chain reaction (RT-PCR)

2.5.1. RNA isolation

Samples of 0.5–1 cm obtained from the liver of rats of the experimental groups were deposited in *RNAlater*™ solution (Sigma). Then it was stored at −70 °C for further processing . The samples were processed by the *TRizol LS Reagent*™ (Sigma) technique. The assay was performed according to the protocol provided by the manufacturer. Briefly, 250 μl of *TRIzol LS Reagent* was added for each 50 mg of tissue and they were homogenized . RNA was extracted from each homogenate by adding 50 μl chloroform (J.T. Baker 99.98%) per 250 μl of the *TRIzol LS Reagent* used. Shake tubes vigorously by vortexing (15 s) and incubate them at room temperature for 2 min. Centrifuge the samples at 13,200*g* for 10 min at 4 °C. Transfer the aqueous phase to a clean tube. Precipitate the RNA from the aqueous phase by adding 100 μl of isopropyl alcohol (Sigma I9516) per 250 μl of the *TRIzol LS Reagent* used for the initial homogenization followed by vigorous vortexing (15 s) and incubate at room temperature for 10 min. Centrifuge at 13,200*g* for 10 min at 4 °C. Remove the supernatant. Wash the RNA pellet by adding 100 μl of 75% ethanol per 250 μl of the *TRIzol LS Reagent* used for the initial homogenization. Mix the samples by vortexing (15 s) followed incubation at room temperature (2 min). Then samples were centrifugated at 90*g* for 10 min at 4 °C. Decant the supernant, remove it as much as possible without disturbing the pellet. Dry the RNA pellet at room temperature. Finally, resolubilize the pellet in 50 μl of H_2O with diethyl pyrocabonate (DEPC). The expected values of the $A_{260}/_{280}$ ratio of the total RNA isolated were obtained by the Beckman D.U. 7500 spectrometer. RNA extraction was evaluated by 1% agarose gel electrophoresis (BioRad 161-3101). Bands (RNA 18s and 28s) of approximately 2 and 5 kilo base (kb) length were visualized under light UV (transilluminator BioRad®).

2.5.2. Amplification of inflammatory cytokine genes by RT-PCR

Reverse transcription (RT) was performed according to the following protocol with Invitrogene™ regents (Carlsbad, CA., USA): the samples were treated with DNase-I (1 μg per 10 μg of RNA). The reaction conditions were the following: 37 °C for 10 min, after that add 1 μl (0.5 M) of EDTA for the inactivation of DNAase at 75 °C for 10 min. For the RT reaction mix we used 4 μl of Buffer First-Strand 5X (250 mM Tris–HCl, pH 8.3 at room temperature; 375 mM KCl; 15 mM $MgCl_2$), 1 μl (500 μg/ml) of Oligo $(dT)_{12-18}$, 1 μl (10 mM) of dNTP mix, 2 μl (0.1 M) of DTT, 1 μl (40 U/μl) of RNAase inhibitor, 1 μl (200 U) of Super Script™ II Reverse Transcriptase and 5 μl (5 μg) of RNA of the sample. RT was carried out under the following conditions: 42 °C for 5 min, after that 70 °C for 15 min and finally 4 °C for 5 min. After that add 1 μl (2 U) of RNAase H and incubate at 37 °C for 30 min followed by inactivation at 65 °C for 20 min.

Table 1
Primer sequences from Kit Rat Inflammatory Cytokine Genes Set-1.

Rat gen	Amplicon size (bp)	5′/3′ Tm (°C)	Accession number
TNF-α	351	69/70	X66539
IL-1β	294	66/69	M98820
IL-6	453	68/70	M26744
GAPDH	532	67/68	M17701

The polymerase chain reaction (PCR) was performed according to the protocol provided by the manufacturer for the MPCR Kit for Rat Inflammatory Cytokine Genes Set-1™ (Maxim Biotech, Inc. San Francisco, CA., USA). Briefly, reaction mix used consisted of 12.5 μl of MPCR Buffer Mixure 2× (containing chemicals, enhancer, stabilizer and dNTPs), 2.5 μl of MPCR Primers 10×, 0.5 μl of Taq DNA polymerase (5 U/μl), 5 μl of cDNA (50 ng) and 4.5 μl of ddH_2O (DNase free). The PCR was carried out under the following conditions: 94 °C for 1 min followed by 59 °C for 4 min (2 cycles), 94 °C for 1 min followed by 59 °C for 2 min (35 cycles) and finally 70 °C for 10 min (1 cycle). The amplified genes were evaluated by 2% agarose gel electrophoresis. Expected bands of approximately 210–532 base pair (bp) length (Table 1) were visualized under light UV (transilluminator BioRad®) and analyzed by the Sigma Plot software.

2.6. Morphology and histopathology analysis

Rats were euthanized according to the International Standards and as previously mentioned NOM-033 (1995). Pancreas, liver, spleen and kidney tissue were macroscopically examined. Samples (0.5–1 cm) of each organ were fixed in 10% neutral buffered formalin (J.T. Baker) with PBS. After usual processes of dehydration, clearing and infiltration, the organs were embedded in paraffin wax and sectioned into 6–10 μm slices through a Microtome. The tissues were stained with hematoxylin and eosin. The slides were examined and photographed microscopically using achromatic plane objective (10×, 40× and 100×).

2.7. Statistic analysis

The experimental data were analyzed by one-way analysis of variance (ANOVA) followed by Newman–Keuls *post hoc* test of significance. P values <0.05 were considered as statistically significant. GraphPad Prism 5.0 (GraphPad Software, Inc.) was used for statistical analysis.

3. Results and discussion

3.1. Body weight

Induction of DM with STZ has been associated with a characteristic loss of body weight gain, which is due to increased catabolism of fats and proteins leading to muscle wasting (Kamalakkannan and Prince, 2006; Mondal et al., 2012). In this

experiment, the body weight gain was significantly decreased in diabetic rats when compared to the healthy group. However, diabetic rats after treatment with EtOAc or EtOH extracts of *Arracacia tolucensis* showed a lower loss in the body weight; this loss is of the same magnitude as the animals treated with glibenclamide (Table 2), this effect, could be due to a better control of the hyperglycaemic condition in diabetic rats. Decreased levels of blood glucose could improve body weight in streptozotocin-diabetic rats (Kamalakkanan et al. 2003; Kamalakkannan and Prince, 2006).

Table 2
Body weight (g) of experimental rats.

Group	Animal status	Day 1	Day 21	Day 21 (Weight gain)
I	Healthy	302.9 ± 11.1	388.4 ± 11.1	85.5 ± 9.0
II	Healthy + vehicle	293.3 ± 12.4	380.8 ± 13.7	87.5 ± 10.6
III	Diabetic	306.5 ± 11.7	324.1 ± 13.6	17.6 ± 10.3
IV	Diabetic + Glibenclamide	289.9 ± 4.1	339.3 ± 13.6	49.4 ± 8.2
V	Diabetic + Hx extract	321.1 ± 13.5	322.9 ± 15.2	1.8 ± 11.7
VI	Diabetic + EtOAc extract	327.5 ± 11.1	365.5 ± 19.4	38.0 ± 12.9
VII	Diabetic + EtOH extract	290.9 ± 8.8	335.1 ± 10.4	44.2 ± 7.8

Values are expressed as the mean ± S.E.M. of 10 rats in each group.

Table 3
Glucose level (mg/dL) of experimental rats.

Group	Animal status	Day 1	Day 21	Day 21 (Increased glucose)
I	Healthy	74.7 ± 6.8	76.4 ± 5.0	1.6 ± 4.8
II	Healthy + vehicle	83.2 ± 8.0	84.8 ± 4.6	1.5 ± 5.3
III	Diabetic	84.5 ± 6.1	361.7 ± 27.0	277.2 ± 15.9
IV	Diabetic + Glibenclamide	65.9 ± 3.8	252.3 ± 31.7	186.4 ± 18.4*
V	Diabetic + Hx extract	84.8 ± 10.0	260.7 ± 49.4	175.9 ± 29.1*
VI	Diabetic + EtOAc extract	91.6 ± 5.9	158.6 ± 25.0	66.9 ± 14.8*, **
VII	Diabetic + EtOH extract	58.6 ± 3.0	226.1 ± 30.6	167.5 ± 17.7*

Values are mean ± S.E.M. of 10 rats in each group, before induced DM and 21th day after treatment.

* Statistically different when compared with diabetic group, $p < 0.05$.

** Statistically different when compared with diabetic group + Glibenclamide, $p < 0.05$.

3.2. Anti-hyperglycemic activity of Arracacia tolucensis organic extracts

Arracacia tolucensis is a medicinal herb widely used in the northeast of Mexico for the treatment of DM and other diseases. We corroborated the traditional use with interviews with people from different communities and evaluated the anti-hyperglycemic effect of Hx, EtOAc and EtOH extracts from aerial parts through cellular, molecular and biochemical parameters. The results showed effective anti-hyperglycemic activity in STZ-induced diabetic rats (Table 3).

The glucose level of normoglycemic rats (groups I and II) were < 100 mg/dL, which increased to < 2 mg/mL after 21 days of treatment, while the diabetic rats showed an average of 361.7 ± 27.0 mg/dL (increased to 277.2 ± 15.9 mg/dL on day 21), and the diabetic animals treated with glibenclamide, Hx, EtOAc or EtOH extracts (groups IV–VII) showed values of 252.3 ± 31.7, 260.7 ± 49.4, 158.6 ± 25.0 and 226.1 ± 30.6 mg/dL respectively, with smaller increments at 200 mg/dL in all cases.

The glucose level does not come back to normal level, however the anti-hyperglycemic effect of the Hx and EtOH extracts at 250 mg/kg was comparable to the effect exerted by glibenclamide at 1 mg/kg (35% reduction of increased glucose level with respect to STZ rats). The *Arracacia tolucensis* EtOAc extract was found be the most active, showing a statistically significant reduction of 75% of the increased glucose level when compared to the diabetic rats, and a statistically significant reduction of 65% of the increased glucose compared to the group treated with glibenclamide. As previously cited, the EtOAc extract has pyranocoumarin and dihydrofurochromone as major compounds (Burgueño-Tapia et al., 2012). In relation to this, there are no previous reports describing the anti-hyperglycemic activity of dihydrofurochromones, however some authors have described the coumarins, a group of compounds based on a common skeleton of benzo-α-pyrone (Wójtowicz, 2008), have anti-diabetic properties (Lee et al., 2004; Ramesh and Pugalendi, 2005, 2007; Kumar et al., 2009). In the study published by Lee et al. (2004), the natural coumarin, peucedanol-7-O-β-D-glucopyranoside at 5.8 mg/kg dose showed 39% inhibition of postprandial hyperglycemia. While, Ramesh and Pugalendi (2005, 2007) describedthe anti-hyperglycemic properties of umbelliferone (7-hydroxycoumarin), a natural benzopyrone, which at 30 mg/kg/day dose reversed the glucose level 60% (114.28 ± 5.71 mg/dL) with respect to diabetic rats (289.28 ± 3.18 mg/dL) after 45 days. Recently, Kumar et al. (2009) have reported a series of pyranocoumarin derivates that have shown promising anti-hyperglycemic activity in a STZ-induced diabetic rat model lowering blood glucose level between 18.7% and 37.7% at 100 mg/kg dose after 24 h treatment. So, this suggests that pyranocoumarin is likely responsible for the anti-hyperglycemic activity of the *Arracacia tolucensis* EtOAC extract. Right now, our

Table 4
Hematic biometry profile of experimental rats.

Day 21 after treatment								
Group	Animal status	Leukocyte × 10^3/μL	Granulocyte (%)	Lymphocyte (%)	Monocyte (%)	Erythrocyte × 10^6/μL	Hemoglobin (g/dL)	Hematocrit (%)
I	Healthy	8.3 ± 1.3	65.4 ± 11.7	12.3 ± 2.4	6.2 ± 0.9	7.0 ± 0.4	14.0 ± 1.2	40.9 ± 2.2
II	Healthy + vehicle	9.7 ± 0.6	64.2 ± 6.4	10.3 ± 1.2	8.3 ± 1.2	6.6 ± 0.6	14.2 ± 1.2	39.2 ± 3.5
III	Diabetic	6.6 ± 0.7	79.7 ± 6.0	12.2 ± 1.9	7.7 ± 1.0	7.0 ± 0.7	15.3 ± 0.9	41.8 ± 5.0
IV	Diabetic + Glibenclamide	9.0 ± 0.6	72.5 ± 6.4	12.6 ± 0.9	10.3 ± 2.0	6.0 ± 0.5	12.1 ± 1.1	35.7 ± 3.1
V	Diabetic + Hx extract	9.0 ± 0.6	72.5 ± 6.5	13.6 ± 0.9	10.3 ± 2.1	7.8 ± 0.1	16.1 ± 0.6	44.0 ± 0.7
VI	Diabetic + EtOAc extract	9.1 ± 1.0	80.1 ± 6.8	12.2 ± 1.0	6.9 ± 1.4	6.9 ± 0.9	15.9 ± 1.0	38.2 ± 5.1
VII	Diabetic + EtOH extract	9.6 ± 1.1	77.0 ± 7.5	12.7 ± 1.0	7.5 ± 0.9	6.5 ± 0.4	13.4 ± 0.8	37.8 ± 2.7

Values are mean ± S.E.M. of 10 rats in each group. Not found difference statistically ($p < 0.05$) when compared with healthy control and diabetic group.

Table 5
Lipid profile of experimental rats.

21th day after treatment						
Group	Animal status	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	Healthy	65.1 ± 5.9	117.4 ± 22.2	12.8 ± 1.1	31.5 ± 7.4	20.5 ± 5.2
II	Healthy + vehicle	65.8 ± 5.9	159.1 ± 26.8	13.0 ± 1.1	26.0 ± 5.3	32.8 ± 5.4
III	Diabetic	70.8 ± 4.0	155.0 ± 30.7	14.4 ± 0.8	26.5 ± 7.2	31.7 ± 6.2
IV	Diabetic + Glibenclamide	68.2 ± 3.8	199.5 ± 30.6	13.7 ± 0.7	20.1 ± 2.3	40.9 ± 6.2*
V	Diabetic + Hx extract	73.7 ± 4.8	177.4 ± 24.5	14.8 ± 0.9	26.6 ± 3.3	36.2 ± 5.0
VI	Diabetic + EtOAc extract	66.8 ± 4.9	167.6 ± 15.3	13.3 ± 1.0	19.0 ± 4.1	34.3 ± 3.1
VII	Diabetic + EtOH extract	66.1 ± 2.5	188.6 ± 16.0	13.2 ± 0.4	18.3 ± 2.2	38.6 ± 3.2

Values are mean ± S.E.M. of 10 rats in each group. HDL (High density lipoprotein); LDL (low density lipoprotein); VLDL (Very low density lipoprotein).

* Statistically different when compared with healthy control, $p < 0.05$. Not found difference statistically ($p < 0.05$) when compared with diabetic group.

Table 6
Kidney and liver profile of experimental rats.

21th day after treatment						
Group	Animal status	Urea (mg/dL)	Creatinine (mg/dL)	Bilirrubin (mg/dL)	AST (U/L)	ALT (U/L)
I	Healthy	54.6 ± 2.5	0.71 ± 0.06	0.65 ± 0.30	152.8 ± 13.3	112.3 ± 33.7
II	Healthy + vehicle	52.5 ± 2.7	0.54 ± 0.06[a]	0.42 ± 0.10	149.2 ± 15.3	77.8 ± 10.4[a]
III	Diabetic	71.5 ± 7.6	0.77 ± 0.04	0.46 ± 0.04	354.9 ± 78.7	232.0 ± 61.6
IV	Diabetic + Glibenclamide	52.2 ± 2.5	0.49 ± 0.07[a]	0.81 ± 0.10	218.5 ± 34.7	140.3 ± 22.6
V	Diabetic + Hx extract	72.0 ± 10.8	0.62 ± 0.04	0.46 ± 0.06	209.4 ± 42.8	139.3 ± 36.3
VI	Diabetic + EtOAc extract	59.1 ± 5.8	0.70 ± 0.06	0.51 ± 0.10	139.0 ± 13.6[a]	78.5 ± 13.5[a]
VII	Diabetic + EtOH extract	48.4 ± 1.9[a]	0.54 ± 0.02[a]	0.36 ± 0.10	239.6 ± 74.8	167.4 ± 45.0

Values are mean ± S.E.M. of 10 rats in each group. AST (aspartate aminotransferase); ALT (alanine aminotransferase).

[a] Statistically different when compared with diabetic group, $p < 0.05$. Not found difference statistically ($p < 0.05$) when compared with healthy control.

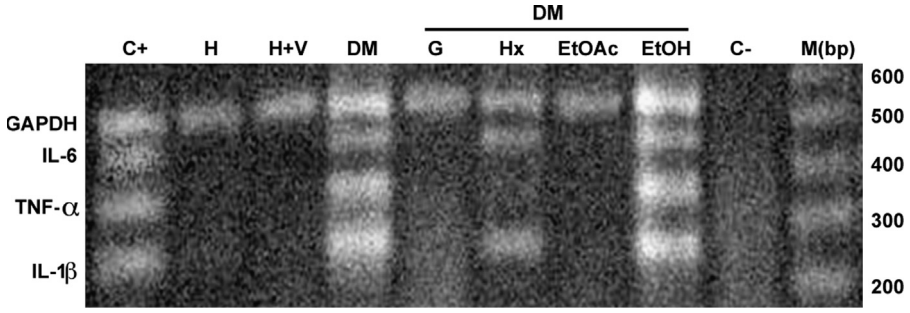

Fig. 2. Inflammatory cytokines expression of rat liver tissue. The amplified genes were evaluated by 2% agarose gel electrophoresis. Bands of approximately 210–532 base pair (bp) length expected were visualized under light UV. Positive control (C+), healthy (H), healthy plus vehicle (H+V), Diabetic (DM), and diabetic animals treated with glibenclamide (G), hexane (Hx), ethyl acetate (EtOAc) or ethanol (EtOH), negative control (C−), molecular mark (M, bp).

research group is studying the anti-hyperglycemic effect of pyranocoumarin.

3.3. Hematic biometry and biochemical profile

In general, the data of hematic biometry and lipid profile showed that the blood level of lymphocytes, monocytes, granulocytes, hemoglobin, hematocrit and serum level of cholesterol, triglycerides, HDL, LDL and VLDL were not statistically different with respect to healthy and diabetic rats (Tables 4 and 5), but the liver (AST, ALT and bilirubin) and kidney (urea and creatinine) profiles showed some changes (Table 6). Only the diabetic animals treated with EtOAc extract showed a significant reduction of the enzyme levels AST and ALT when compared to diabetic rats (61% and 66%, respectively). With respect to this, enzymes are parameters of hepatic and myocardial functionality; its increase involves necrosis processes but its decline is result of clinical improvement. Similar observations were reported for other plant extracts, which showed a significant recovery in the levels of these parameters (20–35%) in STZ-induced diabetic rats at 100 and 250 mg/kg dose, correcting the activities of these enzymes towards the control levels (Haldar et al., 2010; Jana et al., 2010). The diabetic rats treated with EtOH extract displayed a statistically significant reduction in the urea and creatinine levels with respect to diabetic rats (32.32% and 30.22%, respectively). Also, glibenclamide and vehicle groups decreased the creatinine levels. In this sense, kidney failure is manifested by increasing urea and creatinine but a decrease indicates clinical improvement. The bilirubin levels were not statistically different for all treatments and remained inside the reference range.

3.4. Inhibition of inflammatory cytokines expression

A feature of DM is the chronic inflammation, due to inflammatory cytokines expression such as TNF-α, IL-6 and IL-1β (Schenk et al., 2008; Chang and Chuang, 2010) generating DNA strand break in β-cells of pancreatic islets (Rabinovitch et al., 1994; Delaney et al., 1997), endothelial dysfunction (Gao et al., 2007)

and causing vascular injury such as neuropathy, retinopathy, and nephropathy (Navarro-González and Mora-Fernández, 2008). So we determined the expression of inflammatory cytokines from liver (Fig. 2) with the MPCR Kit for Rat Inflammatory Cytokine Genes Set-1™ (Maxim Biotech, Inc. San Francisco, CA., USA). The kit used in our experiments has been used by Cheng et al. (2003) and Matsuda et al., (2005) which gives us assurance for its use. All treatments expressed the constitutive GAPDH gen. The healthy rats did not show expression of inflammatory cytokines due to the absence of this condition and the group treated with glibenclamide showed similar results; by contrast the diabetic rats expressed all three inflammatory cytokines. With respect to groups treated with *Arracacia tolucensis* extracts, the EtOAc extract inhibited expression of the inflammatory cytokines and it was found be comparable with the healthy animals and the effect exerted by the reference drug, glibenclamide at 1 mg/kg; while the Hx extract showed expression of IL-6 and IL-1β, and the EtOH extract displayed all three cytokines involved in the inflammatory process. Concerning this, only the *Arracacia tolucensis* EtOAc extract showed anti-hyperglycemic activity and protective effect against inflammation such as curcumin, which not only decreased hyperglycemia, but also inhibited the expression of IL-6, MCP-1 and TNF-α (Jain et al., 2009). Recently, Yu et al., reported that pyranocoumarins praeruptorine A (Yu et al., 2011) C, D and E (Yu et al. 2012) extracted from *Peucedanum praeruptorum* (Umbelliferae) suppressed the inflammatory response in LPS-stimulated Murine macrophages by inhibiting the expression of TNF-α and IL-6 through inhibition of the nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) signal pathway activation. Therefore, our results suggest that praeruptorine A, one

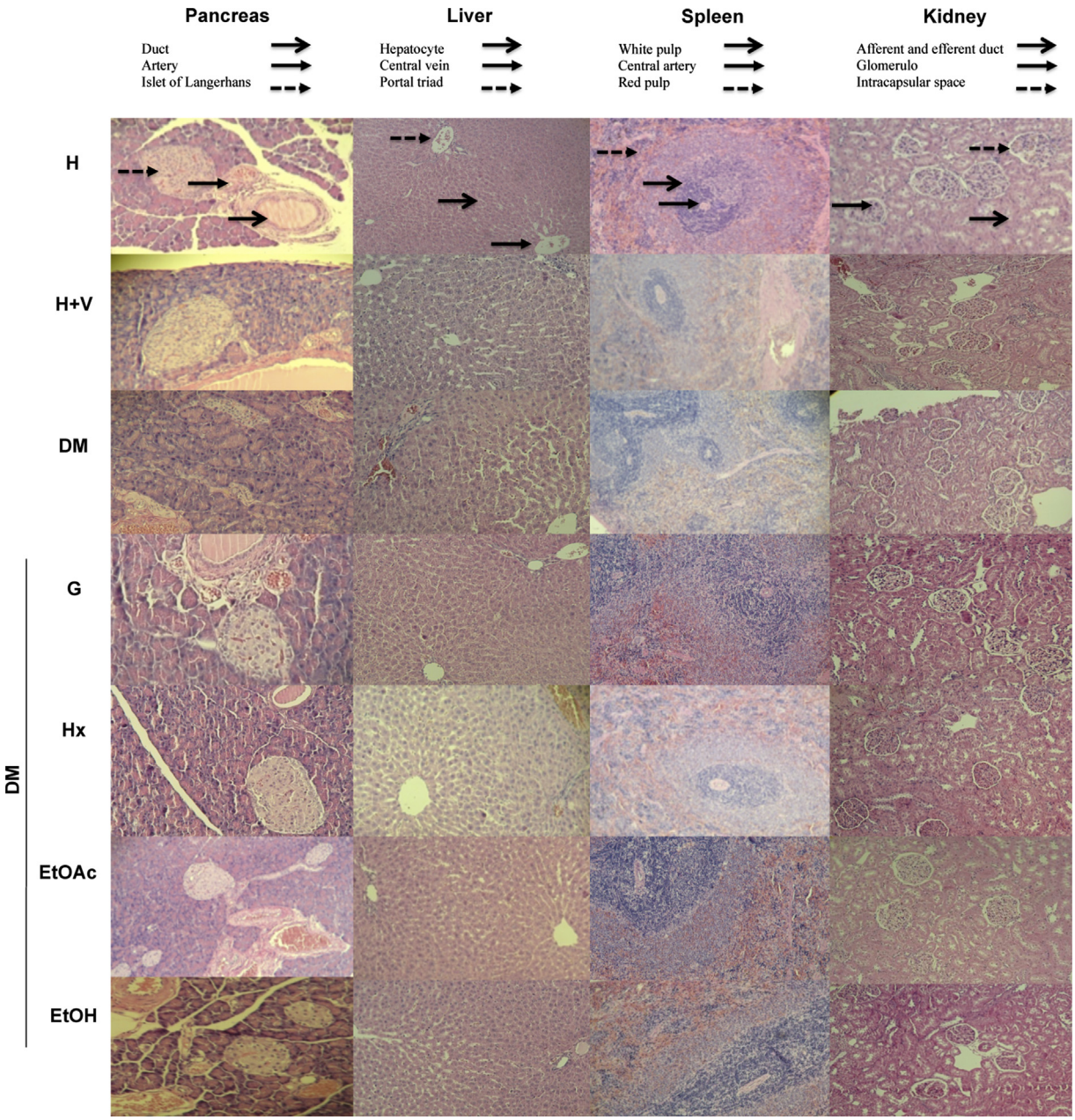

Fig. 3. Histopathology analysis of pancreas, liver, spleen, and kidney rat tissue (10×, 40× and 100×). Healthy (H), healthy+vehicle (H+V), diabetic (DM), after treatment with 1 mg/kg glibenclamide (G), 250 mg/kg hexane (Hx), ethyl acetate (EtOAc) or ethanol (EtOH) extract. Note that the architecture of all organs is preserved after treatment with *Arracaua. tolucensis* extracts.

of the major compounds of the EtOAc extract, is likely responsible for the repression of cytokines production.

3.5. Morphology and histopathology analysis

As part of the safety preclinical studies, the effect of the organic extracts were studied on pancreas, liver, spleen and kidney tissue of the diabetic rats treated. Healthy rats (group I and II) remained unchanged and showed normal histological characteristics (Fig. 3). The STZ-induced diabetic rats displayed partial injury to pancreas exhibiting reduced islet of Langerhans and vascularization, mild liver damage, and normal morphology in spleen and kidney (Fig. 3) in comparison with the healthy rats. Diabetic rats treated with glibenclamide showed normal architecture of all organs (Fig. 3). The histopathology study of all organs of diabetic rats treated with *Arracacia tolucensis* extracts revealed that the structure is preserved (Fig. 3). The pancreatic tissue exhibited lobules and islet of Langerhans, the blood vessels and arteries were normal. Thus, the damage caused by STZ was reversed and the normalization of pancreatic architecture was observed when diabetic rats were treated with *Arracacia tolucensis* extracts, similar to the reportings by other authors for some other plant extracts (Shirwaikar et al., 2006; Ahmed and Urooj, 2008; Kumar et al., 2011; Sharma et al., 2012). The hepatic tissue analysis showed intact architecture and normal hepatocytes. Similar observations were reported with *Petroselinum crispum* (Umbelliferae) aqueous extract, which reduced degenerative changes in the hepatocytes of diabetic rats (Bolkent et al., 2004). No injuries on spleen and kidney tissue were observed. Finally, it has been reported that the crude extract (MeOH–CH_2Cl_2 1:1) of this herb was not mutagenic, by the Ames test, or toxic for mice in acute administration (Déciga-Campos et al., 2007). These features indicate that the *Arracacia tolucensis* extracts may be good candidates in the treatment of diabetes.

It has been suggested that the reduction of hyperglycemia by most bioactive compounds from medicinal plants might act by one of the several mechanisms such as stimulation of insulin secretion, repair of proliferation of β-cells and enhancing the effects of insulin and adrenalin (Fayed et al., 1998). Thus, the possible biochemical mechanism by which the *Arracacia tolucensis* EtOAc extract reduced the blood glucose level of diabetic rats could be by acting on the β-cells of pancreatic islets. It may be either by increasing the secretion of insulin from the existing β-cells of pancreas, such as it was proposed for other plants that have been studied (Pari and Amarnath, 2004; Shirwaikar et al., 2006; Ahmed and Urooj, 2008; Iweala and Oludare, 2011; Sharma et al., 2012), because of the similarity observed after sulphonyl urea administration such as glibenclamide, (Pari and Amarnath, 2004) or by promoting the differentiation and proliferation of residual pancreatic β-cells and consequently the repair of atrophied islets Langerghans and pancreatic release of insulin, such as what was proposed for alkaloid ephedrine (Xiu et al., 2001). In addition, since the cytokines can damage DNA in pancreatic islets and islet β-cells lines (Rabinovitch et al., 1994), our findings indicate that the *Arracacia tolucensis* EtOAc extract could reduce the DNA fragmentation in β-cells of pancreas by a mechanism of cytokines production inhibition contributing to the normalization of the Langerhans islets function.

In conclusion, our results show that the EtOAc extract of the aerial parts from *Arracacia tolucensis* (250 mg/kg) in STZ-induced diabetic rats has anti-hyperglycemic activity. Furthermore, it controlled the body weight loss, the enzyme levels, AST and ALT were decreased, the expression of inflammatory cytokines was inhibited, and it was not affected by the cellular morphology of the organs, even the islet of Langerhans were repaired and did not show toxicity of liver, spleen or kidney. This is the first time that the anti-hyperglycemic effect from *Arracacia tolucensis* was evaluated in an animal model of diabetes. The results confirm the traditional use of *Arracacia tolucensis* for treatment against DM and place it as good candidate for future preclinical evaluations.

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