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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

Mary Carmen García-Galicia [a], Eleuterio Burgueño-Tapia [b], Andrés Romero-Rojas [c], Julio César García-Zebadúa [a], Jorge Cornejo-Garrido [a], Cynthia Ordaz-Pichardo [a,*]

[a] *Laboratorio de Biología Celular y Productos Naturales, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera 239, Col. La Escalera, C.P. 07320 México, D.F., México*

[b] *Departamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomás, C.P. 11340 México, D.F., México*

[c] *Centro Universitario de Diagnóstico y Laboratorio de Inmunología de la Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Av. 1° de Mayo s/n, Col. Santa María Las Torres, C.P. 54740 Cuautitlán, Estado de México, México*

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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).

* Correspondence to: Laboratorio de Biología Celular y Productos Naturales, Edificio de Posgrado, Primer Piso, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera 239, C.P. 07320, Delegación Gustavo A. Madero, México, D.F., México. Tel.: 55 57296000x55535; fax: 55 57296000x55561.
E-mail addresses: dra_cynthia@hotmail.com, cynthiaop@yahoo.com.mx (C. Ordaz-Pichardo).

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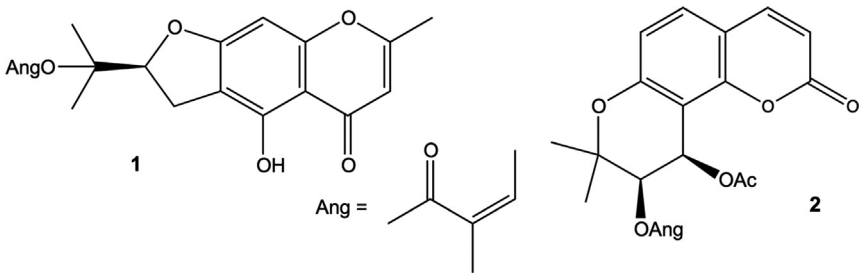

Fig. 1. Structure of isolated compounds from *Arracacia tolucensis* EtOAc extract: (S)-(+)-4′-O-Angeloylvisamminol (1) and (3′R, 4′R)-(−)-4′-O-acetyl-3′-O-angeloylkhellactone (praeruptorin A) (2) (Burgueño-Tapia et al., 2012).

- 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.,