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Hypoglycemic potential of *Trixis angustifolia* aqueous extract in alloxan-induced diabetic mice

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Abstract

The aim of the present study was to evaluate the hypoglycemic potential of *Trixis angustifolia* in alloxan-induced diabetic mice. An intragastric administration of the aqueous extract (50, 100 and 200 mg/kg) prepared from the aerial parts of *T. angustifolia* was evaluated. The treatment with the extract at 100 mg/kg produced a significant lowered (30.5%) of the blood glucose levels in diabetic mice after 15 days of daily oral administration. In addition, the extract induced a significant decrease in serum total cholesterol, low density lipoprotein whereas increased the high-density lipoprotein level. Additionally, the presence of alkaloids, coumarins, saponins, flavonoids and reducing sugars were identified in the extract. These findings provide a basis explaining the traditional folk medicine use of this plant as a hypoglycemic agent by the Mexican people.

Introduction

Diabetes mellitus is a chronic metabolic disease, characterized by alteration in the carbohydrate, protein and lipid metabolism (American Diabetes Association, 2014). Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for new therapeutic drugs continues due to several adverse effects by using most of the existing synthetic drugs (Chaudhury et al., 2017). Medicinal plants, such as *Gaultheria trichophylla* (Alam and Saqib, 2017), *Ipomoea batatas* (Ludvik et al., 2004), *Momordica charantia* (Raman and Lau, 1996), *Nigella sativa* (Al-Hader et al., 1993), *Rosmarinus officinalis* (Bakirel et al., 2008), *Trigonella foenum-graecum* (Khosla et al., 1995), among others, represent an important source of potentially useful bioactive compounds for improving blood glucose control and preventing long term complications in diabetes mellitus (Ríos et al., 2015).

In Mexico, around 3000 vascular plants species described provide healing properties, including hypoglycemic activity (Alonso-Castro et al., 2017). However, many of them have not been studied and scientific bases are needed to understand their phytochemical characteristics and their potential pharmacological applications. The *Trixis* genus comprises more than one hundred species of the Compositae (Asteraceae) family (Borges and Saavedra, 2010). Species belonging to this genus such as *Trixis radialis*, *Trixis californica* and *Trixis angustifolia*, have been employed in Mexican traditional medicine as the treatment for metabolic disturbances (Hirschhorn, 1981; Marles and Farnsworth, 1995; Rodríguez-Chávez et al., 2017). Although, there is only ethnomedical information about its uses related to metabolic diseases.

T. angustifolia (Compositae), better known as "Hierba del viento", is a plant spread in the state of Durango,



México. The aerial part of this species is commonly used for medicinal purposes in the treatment of rheumatism, wounds, headaches, metabolic disturbances, and as an antipyretic agent (Gonzalez-Elizondo et al., 2004). Nevertheless, there are not systematic *in vivo* studies about this species. The aim of this research was to evaluate the hypoglycemic effect of *T. angustifolia* aqueous extract in alloxan-induced diabetic mice to support one of several further folkloric uses.

Materials and Methods

Chemicals

Alloxan monohydrate was procured from the Sigma Chemical Co., USA. Total cholesterol, high-density lipoprotein and triglyceride commercial kits were purchased from the Randox.

Plant collection and authentication

T. angustifolia was collected from its natural habitat in Durango, Mexico in April 2015. The botanical identification and authentication of the plant samples were performed by Quintos Escalante of CIIDIR-IPN. A specimen was deposited in the herbarium of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional with reference code: Col. M. González y S. Acevedo 2144.

Preparation of extract

T. angustifolia aqueous extract was prepared following the traditional medicine used at Durango State based on the volume of macerate with water administered to a young human adult of 70 kg per day as a treatment of several diseases. In this sense, the aerial part of the plant was dried and crushed into powder using a mortar and pestle. The dried powder (200 mg) of the aerial part was mixed with 5 mL of water. The resulting macerate was decanted into dry clean conical flask through folded cotton gauze stuffed into 10-mL BD Plastipak® syringe.

Experimental animals

Male and female ICR mice weighing 25–30 g were used for this study. The animals were housed in a room at a constant temperature of $22 \pm 1^\circ\text{C}$ and a 12 hours light/dark cycle and fed with standard rodent diet and water *ad libitum*. Mice were distributed into groups and treatments were randomized.

Acute toxicity study

T. angustifolia aqueous extract was administered intragastrically at dose 2,000 mg/kg body weight to three female and three male mice in each group. The control group received only water. The general behavior of mice was observed 4 hours after treatment and then every 24 hours for 14 days. At the end of experiment, all animals were euthanized by cervical dislocation, and

gross pathological changes in vital organs (liver, kidneys, heart, lung, spleen, ovaries and testes) were identified.

Induction of diabetes

Male mice were fasted for 18 hours and diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 200 mg/kg (Issa and Hussien, 2015). The alloxan was dissolved in 0.9% normal saline immediately before use. After 5 days, hyperglycemia was confirmed with OneTouch Ultra 2 (Johnson and Johnson, USA) blood glucosimeter. The blood sampling was done by sterilizing the tail with 10% ethanol and then nipping the tip tail. A drop of blood was placed on a blood glucose test strip and was inserted into a glucometer. The mouse with fasting blood glucose level greater than 400 mg/dL was considered as diabetic.

Experimental design

A total of 35 mice (28 diabetic surviving mice, seven normal mice) were used for the experiment. The animals were divided into five groups, comprising a minimum of seven animals in each group as follows: control: Normal mice received only water; diabetic: Diabetic mice received only water; Diabetic mice treated with *T. angustifolia* aqueous extract (50, 100 and 200 mg/kg/day).

Fasting glycaemia was determined after diabetic mice confirmed on days 4, 7, 11 and 15 as described above. At the end of the experiment, the animals were fasted overnight and euthanized by cervical dislocation. The blood samples were collected for blood lipid profile analysis and then centrifuged at $13,000 \times g$ for 15 min at 4°C to separate the serum.

Lipid profile analysis

The serum samples were subjected to lipid analysis (Mendieta et al., 2014). Dry tubes containing collected blood were centrifuged at $13,000 \times g$ for 15 min to obtain serum, which was stored at -20°C until the measurement of biochemical parameters. Total cholesterol, high density lipoprotein, and triglyceride were quantified using the corresponding reaction kits for the automatic Vitalab selectra 2 instrument (Wiener Lab, Netherlands).

Low-density lipoprotein cholesterol was calculated using the formula 1 and 2 (Friedewald et al., 1972):

$$\text{Very low-density lipoprotein (VLDL)} = \text{TG}/2.21 \dots\dots(1)$$

$$\text{Low-density lipoprotein} = \text{TC} - (\text{VLDL} + \text{HDL}) \dots\dots(2)$$

Where TG means triglyceride, TC means total cholesterol, HDL means high density lipoprotein

Preliminary phytochemical screening

The preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites pre-

sent in *T. angustifolia* aqueous extract in accordance with the methods described elsewhere (Salazar-Gómez et al., 2018), with some modifications. The tests were based on the visual observation, and visible color change, or precipitate formation was taken into consideration for the presence (+), moderate (++), abundant (+++) or absence (-) of particular active constituents.

Statistical analysis

All data are expressed as mean \pm SEM (standard error of the mean). To perform statistical analyses, Sigma-Plot® 11.0 software was used. Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple range median test. Significant difference was set at p values less than 0.05.

Results

Acute oral toxicity

Acute toxicity study revealed that the *T. angustifolia* aqueous extract was safe up to a dose level of 2,000 mg/kg body weight. The mortality and abnormal behavior were not observed in mice during the 14-day acute toxicity test. In addition, no significant pathological changes in the vital organs including color and texture were observed by macroscopic examination.

Effect on diabetic mice model

In alloxan-induced diabetic mice, the blood glucose level showed significant increase compared to the non-diabetic mice (Figure 1). Hypoglycemic effects of the aqueous extract showed to be on dose-independent manner with the mid dose producing a pronounced hypoglycemic effect. The aqueous extract treatment at 100 mg/kg significantly reduced the blood glucose level by 24.3% on day 11 of the experiment reaching the maximum decline on day 15 (-30.4%), whereas hyperglycemia was sustained in the diabetic mice.

Effect on lipid profile

The plasma levels of LDL were significantly increased

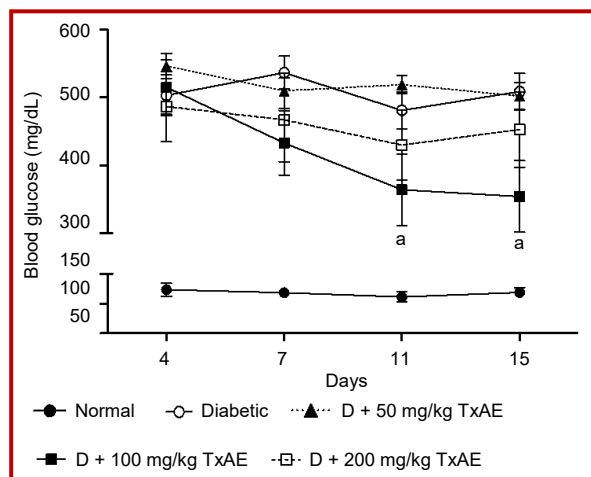


Figure 1: Effect of *T. angustifolia* aqueous extract administration blood glucose levels (mg/dL) of alloxan-induced diabetic mice (data are mean \pm SE; $n=7$ mice/group). ^a $p<0.05$ as compared with the diabetic group. D: Diabetic; TxAE: *T. angustifolia* aqueous extract

in the diabetic mice, contrariwise, the level HDL and triglyceride were significantly diminished in comparison to the control ($p<0.05$). The 15-day treatment with aqueous extract at dose 200 mg/kg showed a significant decrease in plasma level of LDL and total cholesterol. However, only at dose 50 mg/kg increased in the plasma level of HDL and triglyceride (Table I).

Preliminary phytochemical screening

Results of phytochemical tests of *T. angustifolia* aqueous extract revealed the presence of alkaloids, cumarins, saponins, flavonoids and reducing sugars (Table II).

Discussion

The present study demonstrates the potential of *T. angustifolia* aqueous extract as a hypoglycemic agent in alloxan-induced diabetic animals. As far as we know, this is the first work that analyses *T. angustifolia* hypoglycemic activity. The hypoglycemic activity of *T.*

Table I

Effect of *T. angustifolia* aqueous extract on serum lipid profile in alloxan-induced diabetic mice

	Total cholesterol (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	Triglyceride (mmol/L)
Control	3.1 \pm 0.2	0.9 \pm 0.1	1.9 \pm 0.2	1.4 \pm 0.1
Diabetic	3.1 \pm 0.3	1.3 \pm 0.1 ^a	1.1 \pm 0.1 ^a	1.0 \pm 0.3 ^a
Diabetic plus extract (50 mg/kg)	3.6 \pm 0.4	1.7 \pm 0.2	1.7 \pm 0.1 ^b	1.1 \pm 0.2 ^b
Diabetic plus extract (100 mg/kg)	2.9 \pm 0.2	1.6 \pm 0.2	1.2 \pm 0.1	0.8 \pm 0.1
Diabetic plus extract (200 mg/kg)	2.2 \pm 0.2 ^b	1.0 \pm 0.1 ^b	1.1 \pm 0.1	0.4 \pm 0.1

Data expressed as a mean \pm SEM; $n = 7$ mice/group; ^a $p<0.05$: the significant difference with respect to control group; ^b $p<0.05$: the significant difference with respect to diabetic group

Table II		
Phytochemical constituents of <i>T. angustifolia</i>		
Constituents	Test	Result
Alkaloids	Dragendorff test	++
	Mayer test	-
	Wagner test	-
	Sonnenschein test	+
Coumarins	Erlich reaction	+
Tannins	Ferric chloride	-
Saponins	Frothing	++
Reductor sugar	Fehling	+
	Benedict	+
Quinones	Bornträger	-
Cardiac glycosides	Kedde, Legal's and Baljet's reactions	-
Flavonoids	Shinoda	+++
Key: (-) Absence, (+) Poor, (++) Moderate, (+++) Abundant		

angustifolia aqueous extract at 100 mg/kg may be due to the regeneration of β -cells that were partially destroyed by alloxan, like the effect reported for other plants extracts based on polar solvents (Oh, 2015). A higher hypoglycemic effect at doses >100 mg/kg was expected, but when a dose of 200 mg/kg was administered a lower reduction in blood glucose levels was observed. This dose-independent effect could be linked to the presence of some other constituents in the aqueous extract which at high concentration could be acting as antagonist agents of the hypoglycemic effect as described in other aqueous plants extracts (Rangika et al., 2015). In phytochemical screening of aqueous extract, we observed the moderate presence of saponins and alkaloid. High concentration of these secondary metabolites and their interaction have showed to decrease the bioactive properties of each one (Milugo et al., 2013). The aqueous extract dose of 200 mg/kg, saponins and alkaloid would be administered in high concentration reducing the hypoglycemic effect observed at lower dose. However, further specific investigations are required to clarify hypoglycemic effects produced by this plant.

In alloxan-induced diabetes model, increased levels of TC, LDL and reduced level of HDL along with altered composition of TG particles are commonly reported (Alam and Saqib, 2017). In agreement with these previous reports, our model based on the administration of alloxan showed alterations in normal lipid profiles compared to control mice. It is well established that activity of lipoprotein lipase is reduced in β -cells destructive diabetes models resulting in over production of LDL by the liver (Taskinen and Borén, 2015).

Although no hypoglycemic effect was observed at a dose of 200 mg/kg, a decreased level of TC and LDL were observed in diabetic mice at the indicated dose. This finding suggests that *T. angustifolia* aqueous extract not only possesses a significant hypoglycemic effect, but also hypolipidemic activity in alloxan-induced diabetic mice. If the extract has the capacity to help on pancreatic β -cells regeneration due to its hypoglycemic effect, as was proposed above, it is possible that lipid metabolism could be restored leading to a reduced LDL synthesis.

To the best of our knowledge, there are no phytochemical studies regarding the use of water as solvent for the extraction of *T. angustifolia* constituents. This is important to be noted since popular use occurs more frequently with infusions, decoctions or macerations, and thus, little is known about the constitution of this kind of extract. Phytochemical screening revealed the presence of alkaloids, coumarins, saponins, flavonoids and reducing sugars. The relationship between some of these compounds on glucose and lipid catabolism has been studied previously (Bahmani et al., 2014; Marrelli et al., 2016). The hypoglycemic ability has been described for alkaloids, e.g. trigonelline, by the improvement in insulin resistance and β -cell regeneration (Zhou et al., 2012). Saponins have been suggested to be involved in pancreatic lipase and adipogenesis inhibition (Marrelli et al., 2016). Flavonoids are phenolic compounds highly distributed in plants and exhibit several biological activities (Hügel et al., 2016) such as hypolipidemic properties due to plasmatic lipid lipolysis (El-Newary, 2016) or hypoglycemic activity by regeneration of damaged pancreatic islets, stimulate calcium and glucose uptake (Vinayagam and Xu, 2015; Zeka et al., 2017). Recently, a report on secondary metabolites of *T. angustifolia* was published, in which flavones are the flavonoids with biological effect (Sánchez-Chávez et al., 2017). Based on these properties reported in the present work, the hypoglycemic and hypolipidemic effects of *T. angustifolia* aqueous extract may be linked to the presence of its most characteristic phytoconstituents especially flavonoids which are the most abundant in the aqueous extract. The specific secondary metabolites responsible for the hypoglycemic activity are needed to be identified by fractionation of the extract.

Conclusion

This study is the first evidence that supports the use of *T. angustifolia* as a hypoglycemic agent, also without any immediate unwanted effect.

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Ethical Issue

All experiments were conducted in compliance with the Mexican Official Standard (NOM–062-Z00-1999) technical specifications for the production, care, and use of laboratory animals, and the protocol and use of mice were approved by our institutional committee on animal care and use.

Oral acute toxicity was carried out according to the guideline No. 423 provided by the Organization of Economic Co-operation and Development (OECD) with slight modifications. This procedure was used to minimize the number of animals required for acute oral toxicity testing.

Conflict of Interest

Authors declare no conflict of interest

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