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HYPOGLYCEMIC EFFECT OF *HAMELIA PATENS* JACQ., AERIAL PART IN STZ-NA-INDUCED DIABETIC RATS

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Abstract

Type 2 diabetes (T2D) is a public health problem in Mexico and worldwide. In 2012, the Mexican health services report found that 9.2% of the Mexican adult population has been diagnosed with diabetes. Type 2 diabetes is characterized by tissue resistance to the action of insulin, combined with a relative deficiency in insulin secretion. A given individual may exhibit either increased insulin resistance or increased b cell deficiency, and these abnormalities may be mild or severe. In our country, the use of medicinal plants is a common practice among people with the disease. In the towns of Tlanchinol and San Salvador hidalgo, *Hamelia patens* L. is in traditional use for the treatment of T2D. In this study, we tested the hypoglycemic effect of the water (W) and ethanol-water (E) extracts of *Hamelia patens* L. on streptozotocin nicotinamide hyperglycemic rats and found that both extracts exert a hypoglycemic effect at the doses tested. The water extract produces a statistically significant effect at 120 minutes, while the ethanol-water extracts produce a hypoglycemic effect at 60 min. We demonstrated the presence of at least three active compounds in the E extract: chlorogenic acid, caffeic acid and quercetin, and at least two active compounds in the W extract: chlorogenic acid and quercetin, which may be involved in the hypoglycemic effect. Rutin was not detected in the extracts but may be involved in the hypoglycemic effect.

In conclusion, this study supports the traditional use of *Hamelia patens* as a hypoglycemic plant and correlates the main compounds (quercetin, chlorogenic and caffeic acids) with the effect.

Keywords: Type 2 diabetes, hypoglycemic agent, medicinal plant, *Hamelia patens.*
Abbreviations: T2D, Type 2 diabetes; W, water extract; E, ethanol extract

Introduction

Diabetes mellitus is defined as an elevated blood glucose level associated with absent or inadequate pancreatic insulin secretion, which may occur with or without impairment of insulin signaling. Type 2 diabetes is characterized by tissue resistance to the action of insulin, combined with a relative deficiency in insulin secretion. A given individual may exhibit either increased insulin resistance or increased b cell deficiency, and these abnormalities may be mild or severe. Although insulin is produced by the b cells in these patients, the production is inadequate to overcome insulin resistance, and therefore blood glucose rises. Impaired insulin signaling also affects the fat metabolism, resulting in increased free fatty acid flux and elevated triglyceride levels as well as reciprocally low levels of high-density lipoprotein (HDL) [1].

According to the International Diabetes Federation (IDF) [2], more than 387 million people worldwide were affected by T2D in 2014; this number will increase to 592 million in 2035. One of two people with diabetes do not know they have it and are progressing towards complications. Consequently, the disease is a major cause of mortality, providing a worrying indication of the future impact of diabetes as a major threat to global development. In 2014, the IDF identified Mexico as the country with the 6th highest number of people living with diabetes. The IDF estimates that nearly 12 million people in the country are diabetic. In 2012, the Mexican health services report found that 9.2% (6.4 million) of the Mexican adult population has been diagnosed with diabetes, and the true number could be double the reported number because many patients do not know their condition and have not been diagnosed [3]. This estimate would mean that in 2012, at least 12.8 million people were living with diabetes in Mexico, which correlates with the IFD data. In our country, diabetic people commonly use medicinal plants to treat type 2 diabetes, with or without the concomitant use of medically prescribed hypoglycemic agents [4].

In the municipality of Tlanchinol in the Mexican state of Hidalgo, especially in the towns of San Salvador and Tlanchinol, the aerial part of *Hamelia patens* Jacq. (Rubiaceae) is used to treat type 2 diabetes. The plant, known by the common name of "Madura Zapote", is a shrub up to 3 m tall with 3 to 5 leaves, opposite or grouped, and red or orange tubular flowers located in the terminal part. The fruits are globular or elongated, changing from red to black [5]. The plant can grow in various soils, from heavy clays to high alkalinity; however, it prefers soil or sandy clay [6]. In traditional Mexican medicine, it has at least 42 different uses, including use as a diuretic and for gastritis, inflammation, diarrhea, stomach cancer, gynecological disorders, skin lesions and anemia [7], [8]. In different states of the Mexican Republic, including Oaxaca and San Luis Potosi, its use to treat diabetes has been reported [9]. A recent review of the phytochemical composition of the plant [6] reports the following components: the pentacyclic oxindole alkaloids isopteropodine, rumberine, palmirine and maruquine, along with other constituents such as apigenin, ephedrine, flavanones, isomaruquine, narirutins, pteropodine, rosmarinic acid, narirutin, seneciophylline, speciophylline, and tannins.

The aims of this study were to evaluate the hypoglycemic activity of the water and ethanol-water extracts of *Hamelia patens* in hyperglycemic Stz-Na rats and detect the main group of components present in the active extracts.

Methods

Ethnobotany

With the help of the healer; Don Isabel Escalante, direct interviews were conducted with type 2 diabetic people in the town of San Salvador regarding the use of the plant in the treatment of type 2 diabetes. A sample was collected, the identity of the plant was confirmed, and a voucher specimen was deposited at the "Instituto Mexicano del Seguro Social" Herbarium in Mexico City (FVXX). Six kilograms of the aerial part of *Hamelia patens* was collected from different shrubs. The plant material was dried under constant conditions at 40º C, ground in an IKA Mf10 mill, and stored at room temperature.

Extracts

Plant extracts were prepared to study their hypoglycemic effects and to investigate the plant's basic phytochemical composition. The water extract (W), similar to the traditionally used tea, was made by boiling 50 g of the dry plant material with 500 ml water, followed by filtration and lyophilization. The yield of 20 g plants was 30 mg extract. The ethanolic extract (E) was prepared by adding 50 g of the plant material to 500 ml of an ethanol and water mixture (50:50); the extract was then heated at 40º C for four hours and filtered three times, followed by evaporation in a Buchi rotary evaporator. All extracts were kept at -4º C until they were used. The yield of 20 g plants was 60 mg extract.

TLC Analysis.

The aqueous (W) and the hydroalcoholic (E) extracts

were analyzed by standard TLC methods. Concentrated solutions of the standards and extracts (10 µL each extract, 1 µL each standard) were applied to a Merck 20 x 20 mm 60 F254 Silica Gel plate, using a nanomat 4 and capillary dispenser. The plates were developed using two solvent systems: for phenolic aglycones, a mixture of dichloromethane:methanol (9:1 V/V), and for phenolic glycosides, a mixture of ethyl acetate:acetic acid:formic acid:water (68:7:7:18 V/V). Both were revealed using diphenylborinic acid. The developed plates were dried at room temperature to remove solvents. UV-active compounds were detected under ultraviolet light at 254 and 365 nm. The plates were photographed, saved in jpg mode and processed by the CAMAG VideoScan software ® to identify the metabolites; standards of quercetin (6151-25-3), caffeic acid (331-39-5), rutin (207671-50-9) and chlorogenic acid (327-97-9) were purchased from Sigma-Aldrich Co. (Germany).

Animals and Induction of Experimental Diabetes

Eight-week-old Wistar rats weighing 200-250 g were obtained from the Bioterium of the Science School, UNAM, and were acclimated with free access to food and water for at least one week in an air conditioned room (25 °C with 55% humidity) on a 12 h light-dark cycle prior to performing the experiments. The animals were handled according to the National Institute of Health Guide for the Care and Use of Laboratory Animals [10]. Experimental diabetes was induced as described by Masiello [11]. The rats were fasted overnight and injected intraperitoneally with 150 mg/kg nicotinamide (NA; Sigma, N3376) 15 min before an intravenous injection of 65 mg/kg streptozotocin (Stz) in citrate buffer (Sigma, S0130), Stz-Na model.

Experimental Groups

The diabetic animals were placed into 7 groups (1-7) of eleven rats each. Group 1 was the non-diabetic control group. Group 2, the diabetic control group, received 1.5 ml of a physiological NaCl solution. Group 3 was given 5 mg/kg glibenclamide, a standard oral hypoglycemic agent, in the same vehicle. Groups 4 and 5 received W (30 mg/kg and 300 mg/kg, respectively), and groups 6 and 7 received E (60 mg/kg and 600 mg/kg, respectively). The extracts were dissolved in 1.5 ml of physiological NaCl solution. Plasma glucose concentrations were measured using an Accutrend ® Roche instrument and confirmed in duplicate. All the extracts were orally administered using a cannula. The administered doses were calculated according to the native herbal drug preparation ratio (DER native), based on the fact that one person of 70 kg consumes an average of 20 g dry plant [12].

Data analysis

The data were analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean ± S.E.M.

Results

Ethnobotany

We confirmed that diabetic people use the aerial part of the plant in the treatment of diabetes. For this purpose, the healer "Isabel Escalante" recommends drinking the infusion of the plant during the day. It is prepared by boiling approximately 20 g of dry plant in 500 ml of water, which is then cooled, strained, and kept refrigerated until consumption.

TLC Analysis

The chromatogram shown in figure 1 indicates that almost all extract constituents were clearly separated. It was evident that in 10 µL, the E extract has a greater presence of compounds than the aqueous extract. The TLC fingerprint of the standards and the extracts is shown in table 1 and figures 1, 2.

Table 1 and figure 2a suggest the presence of chlorogenic acid (Rf = 0.624) as the main component in the E extract and as the second major component in the W extract. The data indicate that when the extracts are compared with the standard quercetin (Rf = 0.568), both extracts have this flavonoid as one of their components. Caffeic acid (Rf = 0.260) was identified as one of the major compounds in the E extract (figure 2b), while rutin was not observed in either of the extracts.

Acute hypoglycemic effect

We confirmed that the Stz-Na model is a useful model to test hypoglycemic agents. The diabetic control group showed higher glucose levels than the non-diabetic group, and in the Stz-Na induced rats, the blood glucose levels were higher than the non-diabetic group at all the tested times. Furthermore, the glucose levels were stable compared with time 0 of the same group (table 2). The standard hypoglycemic agent glibenclamide produces a hypoglycemic effect 60 minutes after administration. This effect continues until 180 min, which means that the Stz-Na induced rats respond to insulin secretagogues such as glibenclamide. The tested extracts of *Hamelia patens,* water and ethanol-water, produce a hypoglycemic effect.

Both doses of the water extract produce a statistically significant effect at 120 minutes, while the ethanol-water extracts produce the effect at 60 min; see table 2.

Discussion

Hamelia patens is a plant that is used substantially in Mexican traditional medicine as well as in other traditional systems, as reported by Potosi [6]. We recently learned of its use for the treatment of type 2 diabetes in Tlanchinol Hidalgo. This use is also supported by the literature [9].

In this work, we confirm that the Stz-Na hyperglycemic Wistar rat model is suitable for testing plants and extracts with hypoglycemic activity because the pancreas in this animal model is still able to produce insulin, and it has a certain degree of insulin resistance [13]. The results presented here show that the standard hypoglycemic agent glibenclamide can reduce the blood glucose levels from 60 min until 180 min, which has statistical significance when compared with the control group. Both extracts W and E also exert a statistically significant hypoglycemic effect in this rat model. The effect of the E extract is observed earlier than the effect of the W extract, and we observe no difference between the tested doses. Of the chromatographic methods currently available, thin-layer chromatography (TLC) is widely used for the rapid analysis of plant extracts because it is a simple and readily available technique. Several studies have reported the recognition of phenolic compounds such as chlorogenic acid, caffeic acid, rutin and quercetin [14], [15], [16]. Urakova *et al*. demonstrated that there were no statistically significant differences between HPLC and HPTLC for the quantitative determination of chlorogenic acid in green coffee bean extracts (by TLC densitometry using CAMAG TLC Scanner 3 and WinCATS software version 1.3.4), concluding that this method could be used for the routine determination of chlorogenic acid in such extracts as an alternative to HPLC in rapid screening. [17].

In this manuscript, we use this technique to recognize the main compounds present in the *Hamelia patens* extracts. According to the results (figures 1, 2 and table 1), the chromatographic profile of the extracts is similar to the standards, quercetin, caffeic acid and chlorogenic acid. Furthermore, the phenolic constituents of the extract had R_f values corresponding to the values of the tested compounds. This result indicates the presence of at least three active compounds in the E extract: chlorogenic acid, caffeic acid and quercetin, and at least two active compounds in the W extract: chlorogenic acid and quercetin, which may be involved in the hypoglycemic effect. Rutin was not detected in the extracts.

Previous studies showed that chlorogenic acid decreases fasting blood glucose in hypoglycemic mice under a glucose tolerance test. Additionally, chlorogenic acid stimulates glucose transport in skeletal muscle through the translocation of GLUT 4, mediated by the activation of AMPK [18]. Chlorogenic acid is one of the major compounds present in *Hamelia patens*, suggesting that it may contribute, at least in part, to the hypoglycemic effect of the extracts. Quercetin, one of the constituents present in the extracts, potentiates insulin secretion and protects β-cell function and viability against oxidative damage. These effects are correlated with a major increase in extracellular signal-regulated kinases 1/2 phosphorylation [19]. Pharmacological studies of caffeic acid as a hypoglycemic agent suggest an anti-degenerative effect on islets from the pancreatic b in mice, the up-regulation of GLUT-4 in adipocytes, inhibition of the activity of alpha-amylase and alpha-glucosidase, increased mRNA expression of glucokokinase, decreases in glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activities, and regulation of b cell function [20], [21], [22], [23]. Azuma et al demonstrated by a pharmacokinetic trial in rats that caffeic acid is absorbed faster than chlorogenic acid in the digestive tract [24]; this difference may explain why E extract lowers blood glucose levels at 60 minutes after administration, whereas the effect of the W extract begins at 120 minutes.

In conclusion, this study supports the traditional use of *Hamelia patens* as a hypoglycemic plant and correlates the main compounds (quercetin, chlorogenic and caffeic acids) with the effect. Further studies are needed to clarify the mechanism of action.

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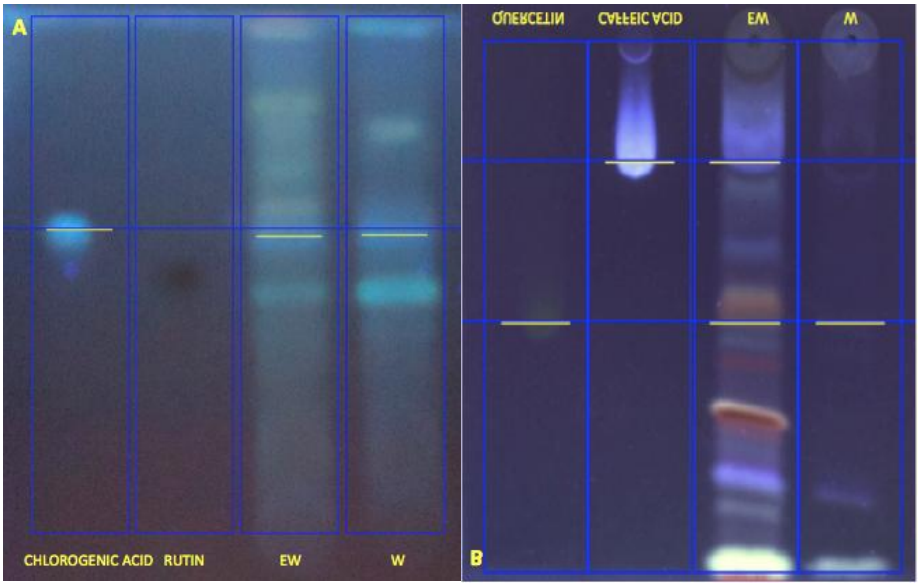

Figure 1. TLC. A) phenolic glycosides of H. patens. Solvent system: ethyl acetate : acetic acid : formic acid : water (68:7:7:18 V/V). B) phenolic aglycones. Solvent system: dichlorometane:Methanol (9:1 V/V).

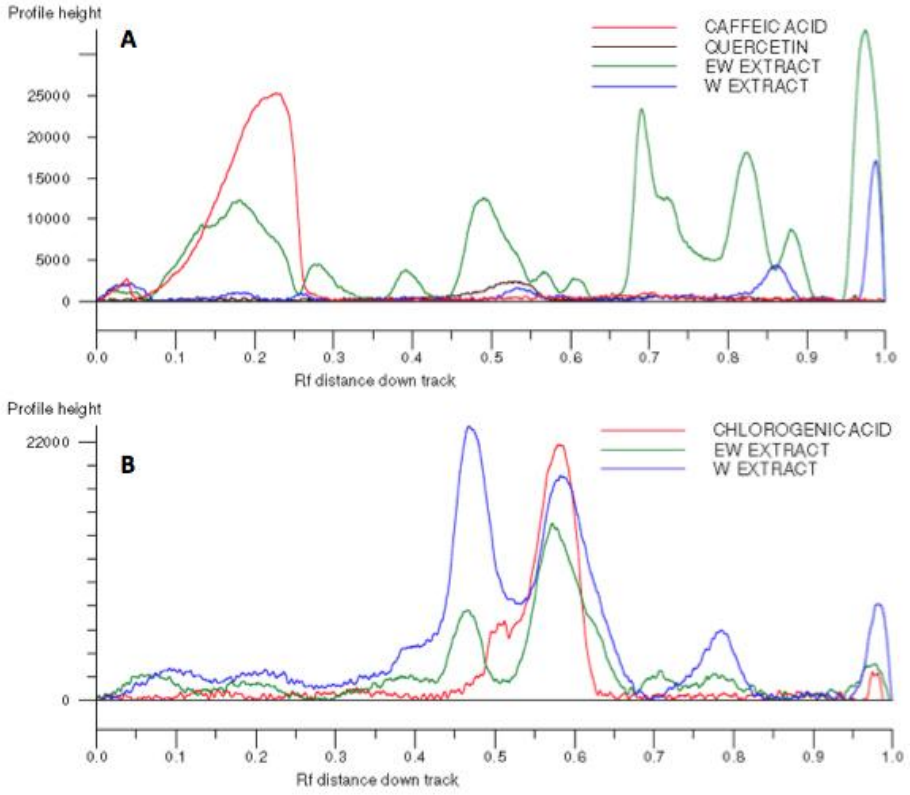

Figure 2.. TLC fingerprint of the standard and extracts. A) Caffeic acid, quercetin, EW extract and W extract. B) Chlorogenic acid, EW extract and W extract.

Table 1. Comparison of Rf of standards and identified peaks in the extracts.

STANDARD	R_f calculated	E extract	W extract
Caffeic acid	0.260	0.251	-
Quercetin	0,568	0.550	0.568
Chlorogenic Acid	0.624	0.614	0.617

Glucose Groups	***(mg/dl) T0***	***(mg/dl) T60***	***(mg/dl) T120***	***(mg/dl) T180***
1 ND	125 ± 2	131 ± 4	126 ± 2	124 ± 3
2 D	197 ± 7^{3}	188 ± 9^{3}	189 ± 11^{3}	194 ± 10^{3}
3 G 5 mg/kg	203 ± 9	153 ± $11^{1,a}$	124 ± $5^{3,c}$	112 ± $4^{3,c}$
4 W 30mg/Kg	202 ± 4	190 ± 10	161 ± 8^{c}	152 ± $4^{3,c}$
5 W 300mg/Kg	201 ± 5	187 ± 6	163 ± 5^{c}	151 ± $5^{3,c}$
6 E 60mg/Kg	191 ± 5	164 ± $6^{1,b}$	149 ± $3^{3,c}$	137 ± $2^{3,c}$
7 E 600mg/Kg	188 ± 3	163 ± $3^{2,c}$	153 ± $5^{2,c}$	144 ± $3^{3,c}$

Table 2. Acute hypoglycemic effects of the *Hamelia patens* aerial part on Stz-Na induced diabetic rats. The values represent the mean ± SEM. Superscripted letters in the same row indicate statistically significant differences compared with time 0. Superscripted numbers in the same column indicate statistically significant differences compared with the respective control group. a,1 ($p < 0.05$), b,2 ($p < 0.01$) and c,3 ($p < 0.001$). Gl, glucose; ND, non-diabetic; D, diabetic; G, glibenclamide; W, water extract, E, ethanol-water extract.