Phytopharmacology

Inforesights Publishing

Acute hypoglycemic effect of ethanolic extracts from *Marrubium vulgare*

Jorge Vergara-Galicia[1*], Francisco Aguirre-Crespo[1], Adrián Tun-Suarez[1], Alejandra Aguirre Crespo[1], Marisa Estrada-Carrillo[2], Irene Jaimes-Huerta[2], Angélica Flores-Flores[3], Samuel Estrada-Soto[3], Ortiz-Andrade Rolffy[4]

[1]División de Ciencias de la Salud, Universidad de Quintana Roo, Avenida Erick Paolo Martínez esq. 4 de Marzo, Col. Magisterial, 77039, Chetumal, Quintana Roo, México.
[2]Departamento de Biotecnología, Universidad Politécnica del Estado de Morelos, Boulevard Cuauhnáhuac 566, Colonia Lomas del Texcal, 62550, Jiutepec, Morelos, México.
[3]Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Colonia Chamilpa, 62209, Cuernavaca, Morelos, México.
[4]Facultad de Química, Universidad Autónoma de Yucatán, Mérida, Calle 421 No. 41 x 26 y 28 Col. Industrial, 97150, Mérida, Yucatán, México.

*Corresponding Author: vgjorge7@uqroo.mx

Received: 19 March 2012, **Revised**: 28 March 2012, **Accepted**: 28 March 2012

Abstract

Marrubium vulgare is used in Mexican traditional medicine for the treatment of diabetes mellitus. The hypoglycemic effects produced by the acute administration of various ethanolic extracts (root, leaf and stem) from *M. vulgare* (REE) on normoglycemic rats were investigated. Both extracts (root and stem) resulted in significant reductions of glycemia in healthy rat after intragastric administration at a dose of 100 mg/kg. The ethanolic root extract oral administration was conducted to determine oral glucose tolerance test using glucose as substrate. The increase in plasma glucose level was significantly suppressed by the extract after substrate administration. These results suggest that REE might exert its anti-diabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the postprandial increase of blood glucose. Therefore, *M. vulgare* is a source for obtaining lead compounds for designing therapeutic agents with potential antidiabetic effects.

Keywords: Antidiabetic; Glucose tolerance test; *Marrubium vulgare*

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In addition, the total number of people with diabetes is project to increase from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides (Goodman and Gilman, 2009). Unfortunately, apart from having a number of side effects, none of the oral

synthetic hypoglycemic agents has been successful in diabetes management and controlling long-term microvascular and macrovascular complications (Goodman and Gilman, 2009; Momin, 1987; Stenman et al., 1990). Moreover, the toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity and treatment (Spiller and Sawyer, 2006). In this context, alternative medicines particularly herbal medicines are available for the treatment of diabetes since they have advantages effectiveness, safety, affordability and acceptability (Valiathan, 1998). Also, World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes (WHO, 1980). In Mexico, medicinal plants and their products have been used in the traditional medicine and have shown experimental or clinical anti-diabetic activity (Grover et al., 2002; Dineshkumar et al., 2009). In Mexico, the decoction of *Marrubium vulgare* is used in the traditional medicine for treatment of diabetes (Castillo-España and Monroy-Ortiz, 2000). However, there are conflicting reports about the hypoglycemic effect of *M. vulgare* since on the one hand it is reported that the aqueous extract (plant collected in Mexico) did not significantly reduce blood glucose levels (Herrera-Arellano et al., 2004) while moreover been shown that administration of the aqueous extract (plant collected in Algeria) at a dose of 300 mg / kg induces significant decrease glucose levels in diabetic rats (Boudjelal et al., 2012) and that the aqueous extract (500 mg / kg daily for 28 days) of leaves of *M. vulgare* (collected in Saudi Arabia) reduced blood glucose levels in diabetic rats (Ahmed et al., 2011). We believe that the variation of these results is due to using the same plant but collected in different countries which surely make the metabolic content in quantity and variety is distinct, together they employ different plant parts and different ways extraction (Deluc et al., 2009). Therefore, this study was carried out in order to investigate and corroborate the antidiabetic effect of *M. vulgare* (collected in Morelos, Mexico) it is part of a group of plants subjected to pharmacological and phytochemical study with the purpose of offering it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential antidiabetic effects, in this sense, further experiments are in progress in order to isolate and characterize secondary metabolites responsible for the hypoglycemic activity and elucidate the mechanism of action.

Materials and methods

Chemicals

Glucose was purchased from Sigma-Aldrich® Co. (St. Louis, MO, USA), glibenclamide was obtained from MP Biomedicals® Co. (Lllkirch, France). Solvents and other reagents were analytical grade from local sources.

Plant material and extraction

Marrubium vulgare was collected and identified by Dr. Patricia Castillo-España in August 2009 in Yautepec, Morelos, Mexico. A voucher specimen was deposited at the HUMO-Herbarium of the Centro de Estudios Ambientales e Investigación "Sierra de Huautla" (CEAMISH) of Morelos State University. Briefly, the plant material was separated [roots (100 g), leaves (100 g) and stems (100 g)] and subjected to successive maceration with ethanol (3 times for 72 h at room temperature). After filtration, extracts were concentrated *in vacuo* at 40 °C and finally crude ethanolic extracts were obtained.

Animals

Adult male Wistar rats (200–300 g) were obtained from the FES Iztacala-UNAM vivarium. Animals were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2 °C and humidity 45–65%) and were fed with standard rodent diet and water *ad libitum*. All animal procedures were approved by the ethical committee in accordance with our Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture, NOM-062-ZOO-1999, Mexico).

Hypoglycemic activity

Administration of test material

A protocol previously described was used. Rats were deprived of food for 16 h before experimentation but allowed free access to tap water throughout the experiment. All experiments were carried out using five animals per group. The extracts were suspended in 0.05% of Tween 80 in isotonic saline solution (vehicle) and were administered orally by intragastric route at 100 mg/kg (in a volume of 2 mL of saline solution) using an intragastric tube.

Acute experimental model

Animals were classified in to five groups. Group 1 was treated with 100 mg/kg of roots extract, group 2 was treated with 100 mg/kg of leaves extract, group 3 was treated with 100 mg/kg of stems extract, group 4 was treated with 5 mg/kg of glibenclamide (hypoglycemic reference drug). Control rats received a single dose of 2 mL of the vehicle (group 5). Blood samples were collected from the tail tip at 0 (before oral administration), 1, 3, 5, and 8 h after vehicle, extract and drug administration. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using a commercial glucometer and test-strips (Accutrend GCT, Roche).

Measurement of extract effect on blood glucose levels after glucose loading

Thirty minutes after administration of 100 mg/kg of roots extract (most active), a dose of 2 g/kg of glucose solution was administered to each rat. Roots extract and vehicle were administered to rats in the same volume of solution. Blood samples were collected from the tail tip at 0 (before oral administration), 1, 1.5, 2 and 3 h after substrate administration. Blood glucose concentration was estimated as described.

Statistical analysis

All data are presented as mean ± standard error of mean (S.E.M.). Statistical analysis was performed by analysis of variance (ANOVA). P-values less than 0.05 were considered to be significant.

Results

The roots ethanolic extract (100 mg/kg) induced a significant decrease ($p < 0.05$) of blood glucose levels (~20%) after 5 h (Figure 1a). This effect was more effectiveness than glibenclamide, a therapeutic drug used as positive control (5 mg/kg). Leaves extract and

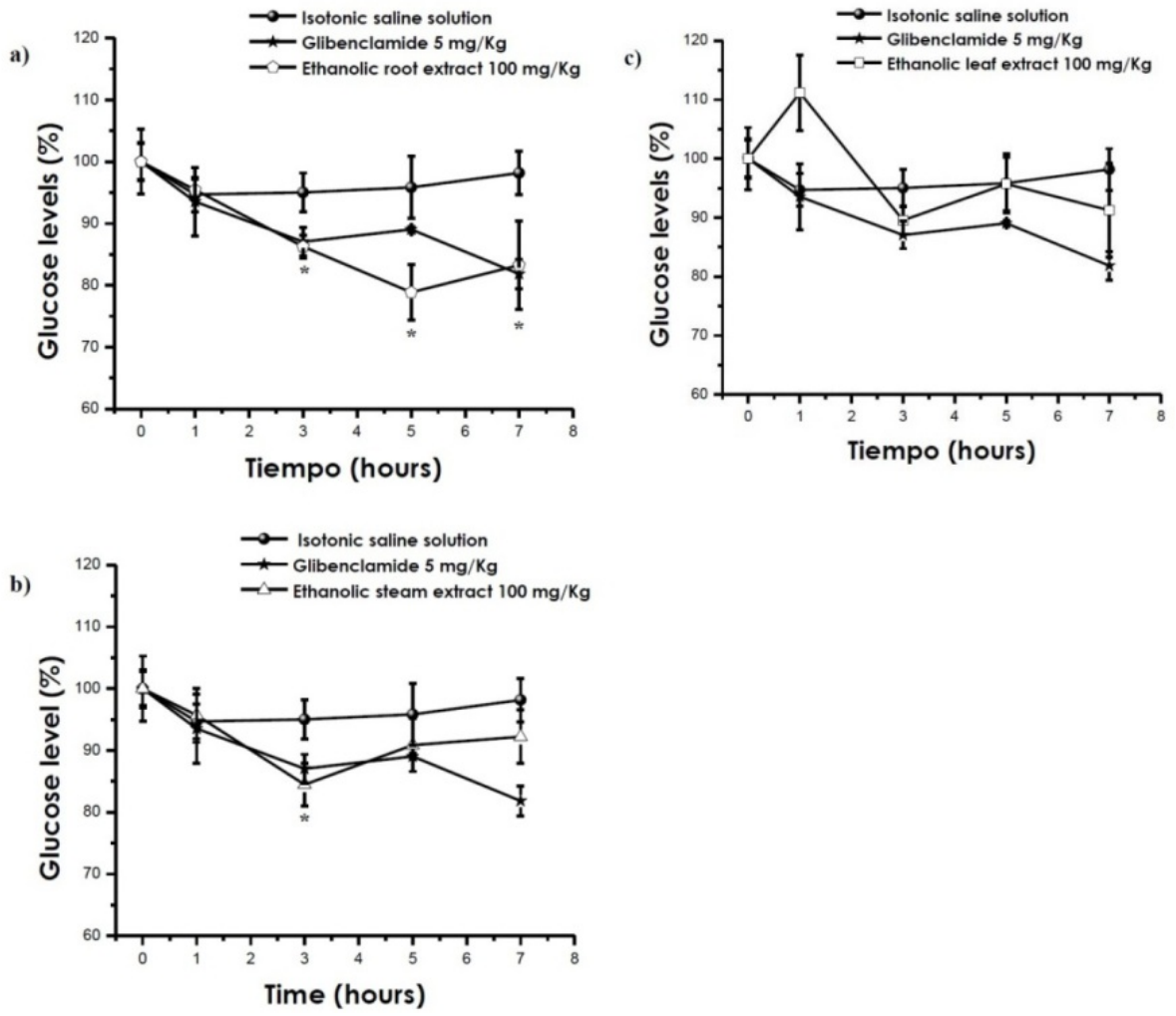

Figure 1 Acute hypoglycemic effect a) roots ethanolic extract, b) stems extract and c) leaves extract from *Marrubium vulgare* on normoglycemic rats. Values are expressed as the mean ± S.E.M., from five animals, $*p < 0.05$ vs. control group (vehicle treated rats) and positive control (glibenclamide treated rats).

stems extract also showed hypoglycemic effect but it was not statically significant (Figure 1b and Figure 1c). Also, the extract controlling postprandial glucose levels in a short time (Figure 2).

Discussion

Roots ethanolic extract motivated hypoglycemia could be linked to more than one mechanism. One might involve modulation of insulin secretion and/or insulin action probably related with extrapancreatic and pancreatic effects. In addition, recent studies suggest that postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications (Ortiz-Andrade et al., 2007; Sánchez-Salgado et al., 2007; Sunil et al., 2011). Therefore, control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications (Ortiz-Andrade et al., 2007; Sánchez-Salgado et al., 2007; Sunil

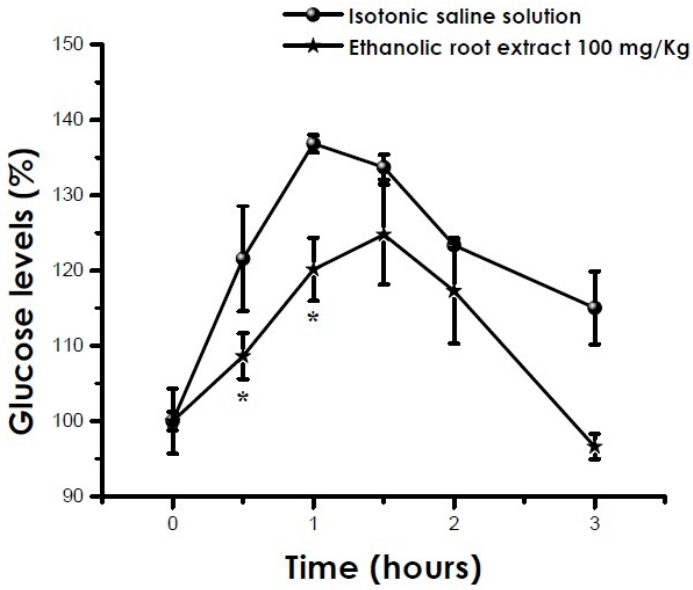

Figure 2 Effect of roots ethanolic extract from *Marrubium vulgare* on blood glucose levels after a single oral administration of 2 g/kg of glucose in male Wistar rats. Each plot represents the means ± S.E.M., from five animals, *$p < 0.05$ vs. control group (vehicle treated rats).

et al., 2011). In this context, we elected roots ethanolic extract to elucidate the mechanism of action as hypoglycemic agent by postprandial blood glucose test. The glycemic response after single oral glucose ingestion was examined. As observed in Figure 2, extract induced a significant decrease in the glycemic response, at a dose of 100 mg/kg compared to control. The postprandial blood glucose was lower at 30 min and this activity was similar during 60 and 90 min post-ingestion of carbohydrate. Results indicate that the control of postprandial glucose level showed by roots ethanolic extract might involve an anti-hyperglycemic effect, mediated by the regulation of glucose uptake from the intestinal lumen, through the inhibition of carbohydrate digestion or absorption. This could be possible by retarding the postprandial glucose levels by inhibition of intestinal α-glucosidase complex or possible blockade of glucose co-transporter as Glut-2 and Glut-4, main carrier of glucose from intestine to circulation (Ortiz-Andrade et al., 2007; Sánchez-Salgado et al., 2007; Sunil et al., 2011). Further experiments are in progress to elucidate this mechanism of action. Phytochemical studies have shown that flavonoids, terpenes and phenylpropanoid esters are the main constituents of *M. vulgare* (Sahpaz et al., 2002; Nawwar et al., 1989; 15. Meyreilva et al., 2005). However, these constituents did not show hypoglycemic effect. Therefore, it was necessary to direct the attention to compounds present in organic extracts. In conclusion, the present results provide pharmacological support for the use of *M. vulgare* in ethnomedical practices as antidiabetic in Morelos, Mexico. Moreover, present efforts are directed to isolate the active constituents from extracts of this species to allow us understanding its mechanism(s) of action and to design new therapeutic agents with potential antidiabetic effects.

Acknowledgement

This study was financed by grants from “Fomento a la generación o aplicación innovadora del conocimiento o fomento a la investigación aplicada o desarrollo tecnológico” (PROMEP-SEP) and “Programa para el fortalecimiento de la Investigación” (PROFI-UQRO-

O). The authors are thankful to Dr. Patricia Castillo-España for the authentication of the plant and Dr. Rafael Villalobos-Molina for providing necessary experimental animals.

Conflict of interests

The authors declared no conflict of interest.

References

Ahmed A. Elberry, Fathalla M. Harraz, Salah A. Ghareib, Salah A. Gabr, Ayman A. Nagy, Essam Abdel-Sattar. (2011). Methanolic extract of *Marrubium vulgare* ameliorates hyperglycemia and dyslipidemia in streptozotocin-induced diabetic rats. *International Journal of Diabetes Mellitus*, http://dx.doi.org/10.1016/j.ijdm.2011.01.004 (corrected proofs).

Boudjelal A, Henchiri C, Siracusa L, Sari M, Ruberto G. (2012). Compositional analysis and *in vivo* anti-diabetic activity of wild Algerian *Marrubium vulgare* L. infusion. *Fitoterapia*, 83 (2): 286-292.

Castillo-España P, Monroy-Ortiz C. (2000). Plantas Medicinales Utilizadas en el Estado de Morelos. Second ed. Cuernavaca: Universidad Autónoma del Estado de Morelos.

Deluc LG, Quilici DR, Decendit A, Grimplet J, Wheatley MD, Schlauch KA, Mérillon JM, Cushman JC, Cramer GR. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics, 10: 212.

Dineshkumar B, Mitra A, Manjunatha M. (2009). In vitro and in vivo studies of anti-diabetic Indian medicinal plants: a review. *Journal of Herbal Medicine and Toxicology,* 3 (2): 9-1.

Gilman G, Goodman LS. (2007). The pharmacological basis of therapeutics. 10th ed., New York: Macmillan.

Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. (2002). *Journal of Ethnopharmacology*, 81: 81-100.

Herrera-Arellano A, Aguilar-Santamaría L, García-Hernández B, Nicasio-Torres P, Tortoriello J. (2004). Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Phytomedicine*, 11 (7-8): 561- 566.

Meyre-Silva C, Yunes RA, Schlemper V, Campos-Buzzi F, Cechinel-Filho V. (2005) Analgesic potential of marrubiin derivatives, a bioactive diterpene present in *Marrubium vulgare* (Lamiaceae). *Il Farmaco*, 60: 321–26.

Momin A. (1987). Role of indigenous medicine in primary health care. New Delhi: First International Seminar on Unani Medicine, pp. 54.

Nawwar M, El-mousallamy A, Barakat H, Buddrus J, Linscheid M. (1989). Flavonoid lactates from leaves of Marrubium vulgare. *Phytochemistry*, 28: 3201-06.

Ortiz-Andrade R, García-Jiménez S, Castillo-España P, Ramírez-Ávila G, Villalobos-Molina R, Estrada-Soto S. (2007). Alpha-Glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an anti-hyperglycemic agent. *Journal of Ethnopharmacology*, 109: 48-53.

Sahpaz S, Garbacki N, Tits M, Bailleul F. (2002). Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. *Journal of Ethnopharmacology*, 389-92.

Sánchez-Salgado JJ, Ortiz-Andrade RR, Aguirre-Crespo F, Vergara-Galicia J, León-Rivera I, Montes S, Villalobos-Molina R, Estrada-Soto S. (2007). Hypoglycemic, vasorelaxant and hepatoprotective effects of *Cochlospermum vitifolium* (Willd.) Sprengel: A potential agent for the treatment of metabolic syndrome. *Journal of Ethnopharmacology*, 109: 400-5.

Spiller HA, Sawyer TS. (2006). Toxicology of oral antidiabetic medications. *American Journal of Health-System Pharmacy*, 63: 929-38.

Stenman PHS, Groop K, Laakkonen E, Wahlin-Boll E, Melander A. (1990). Relationship between sulfonylurea dose and metabolic effect. *Diabetes*, 39: 108A.

Sunil K, Vipin K, Om P. (2011). Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, 347-52.

Valiathan MS. (1998). Healing plants. *Current Sciences*, 75 (10-11): 122-126.

WHO. Expert Committee on Diabetes mellitus - technical report series 646. 2nd report. (1980) Geneva: World Health Organization, pp. 1-8.

Wild S, Rolglic G, Green A, Sicress R, King H. (2004). Global prevalence of diabetes. *Diabetes Care*, 27: 1047-53.

