PharmacologyOnLine December 30, 2012
Archives • 2012 • vol.3 • 1 - 5

Hypoglycemic effect of the Rhizophora Mangle Cortex on STZ-NA-Induced Diabetic Rats

Adolfo Andrade-Cetto[1]* and María Luisa Rubalcaba Mares[1] *
[1] *Laboratorio de Etnofarmacología, Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, D.F. México.*
*Dr. Adolfo Andrade-Cetto Address: Apartado Postal 70-359, Coyoacan México DF, aac@ciencias.unam.mx

Summary

This study sought to confirm the hypoglycemic effects of two extracts of the plant Rhizophora mangle (RM) in nicotinamide-streptozotocin induced diabetic rats (STZ-NA). RM is traditionally used in Mexico to treat type 2 diabetes, (T2D). Two different RM extracts were prepared and tested. The first extract was a water extract (WE), similar to the traditionally used tea, and the second extract was an ethanol:water extract (EWE). The extracts (WE 5.9 and 59 mg/kg, and EWE 9 and 90 mg/kg) were tested on STZ-NA rats to determine whether hypoglycemia occurred after administration of the extracts. Phytochemistry- Two different extracts were prepared, n-hexane and buthanol, to test for the presence of alkaloids, terpenes and phenolic compounds.

The extracts that were administered to the STZ-NA-induced diabetic rats produced a significant hypoglycemic effect compared with the control group, similar to that achieved with glibenclamide. We also determined that phenolic compounds were the main components of the RM cortex.

Diabetic people living near the Mexican coastal zones use the cortex of the Rhizophora mangle plant to treat type 2 diabetes. The results presented here support that extracts of this plant have hypoglycemic effects, which is in agreement with the traditional use.

Keywords: Type 2 diabetes, hypoglycemic agent, medicinal plant, Rhizophora mangle.

Abbreviations: RM, Rhizophora mangle; STZ-Na, Nicotinamide-streptozotocin; EWE, Ethanolic extract; WE, Water extract; T2D; Type 2 diabetes., DER, Drug Extraction Ratio.

Introduction

Diabetes mellitus is defined as hyperglycemia that is associated with inadequate insulin secretion, with or without impairment of insulin action.

Type 2 diabetes, is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. An individual may present primarily with insulin resistance or beta cell deficiencies, and these abnormalities can range from mild to severe [1]

T2D is a public health problem. According to the World Health Organization [2], more than 176 million patients are affected by this disease worldwide. In 2010, the WHO acknowledged that this disease is a major cause of mortality in Mexico. The 2008 Mexican health services report, found that diabetes is the second-highest cause of mortality in Mexico [3].

In the rural areas of Mexico, medicinal plants are traditionally used to treat diabetes [4].

Rhizophora Mangle L. (Rhizophoraceae), traditionally known as 'mangrove' or 'red mangrove', is a 25 m-tall tree that is grown in associations known as mangroves and is distributed along the Pacific and Gulf Coasts of Mexico. The main characteristics of the tree are a tall, straight trunk with abundant roots, a round treetop with simpodic ramifications, and a bitter, red internal cortex [5].

Traditionally, the cortex is used to treat a variety of ailments such as diarrhea, leprosy, menstrual regulation and diabetes [6]. This plant is thought to have hypoglycemic activity and may be useful for treating type 2 diabetes [4]. The anti-hyperglycemic effect was demonstrated by [7], who administered a 4 ml/kg aqueous decoction of the stem to healthy rabbits; after which, a glucose tolerance test was performed. The authors concluded that the plant caused a reduction in blood glucose, as indicated by a 16.3% reduction in the area under the curve.

The aim of the current study was to examine the acute hypoglycemic effects of Rhizophora Mangle bark cortex extracts that were made with either water or a mixture of ethanol and water in nicotinamide-streptozotocin (STZ-NA)-induced diabetic rats. We also sought to characterize the main components of the plant.

Methods

Ethnobotany

Direct interviews about the plant use were conducted with the inhabitants of the coastal lagoons of the following regions in Mexico: Coyuca de Benitez Guerrero, Catemaco Veracruz and Manialtepec Oaxaca. Samples were collected with the help of the informants in Manialtepec. The identity of the samples was confirmed, and voucher specimens were deposited at the "Instituto Mexicano del Seguro Social" Herbarium in Mexico City. Five kg of the Rhizophora mangle cortex were collected from different trees, the plant material was dried under constant conditions at 40° C, ground in an IKA Mf10 mill, and stored at room temperature.

Plant extracts

Plant extracts were prepared to study their hypoglycemic effects and to investigate the plant's basic phytochemical composition. The water extract (WE), 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 ethanolic extract (EWE) was prepared by adding 50 g of the plant material to 500 ml an ethanol and water mixture (50:50); the extract was then heated at 40° C for four hours and filtered three times, followed by evaporation in a Buchi rotary evaporator. Phytochemical analysis of the plant was performed by extracting 200 g by the soxhlet method, first with n-hexane followed by methanol. The dry methanol extract was partitioned with butanol and water in a 1:1 ratio, and the butanolic phase was then dried, followed by evaporation of all of the extracts in a rotary Buchi evaporator. All of the extracts were kept at -4 o C until they were used.

TLC Analysis

The n-hexane and the methanolic extracts were analyzed by standard TLC methods. Briefly, the samples were applied to a Merck 20 x 20 mm 60 F_{254} Silica Gel plate with tree solvent systems. For alkaloids, a mixture of dichloromethane, 85: MeOH, 14: NH_4OH (25%), 1, was used and the plate was revealed with Dragendorff's reagent. For phenolics, a mixture of n-butanol, 48: isopropanol, 17: acetic acid, 17: water, 18, and revealed with diphenylborinic acid. For terpenes, a mixture of dichloromethane, 50: n-hexane 50, was used, revealed with vanillin [8].

Animals and Induction of experimental diabetes

Eight-week-old male Wistar rats weighing 200-220 g were obtained from the Bioterium of the Science School, UNAM, and were acclimatized with free access to food and water for at least one week in an air conditioned room (25° C with 55% humidity) with a 12 h light-dark cycle prior to performing the experiments. The animals were handled according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals [9]. All methods used in this study were approved by the Internal Council of the "Facultad de Ciencias" of the Universidad Nacional Autónoma de México. Experimental diabetes was induced as described by Masiello [10]. Overnight fasted rats were injected intraperitoneally with 230 mg/kg nicotinamide (NA) (Sigma, N3376) 15 min, before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130).

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 WE (5.9 mg/kg and 59 mg/kg), and groups 6 and 7 received EWE (9 mg/kg and 90 mg/kg), respectively. The extracts were dissolved in 1.5 ml of physiological NaCl solution.

Collection of blood and extract administration

Plasma glucose concentrations were measured with a Reflotron instrument and were confirmed with an Accutrend GC (Roche). In total, 32 µl of blood was used for each assay. All the extracts were orally administered with 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 consumed an average of 20 g dry plant.

Statistical analysis

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

Results

Ethnobotany

The plant was deposited at the IMSS, Herbarium in Mexico City with the voucher number IMMSM15816. We confirmed, as a result of direct interviews, that the cortex of this plant is used to treat type 2 diabetes. For this purpose, approximately 20 g of dry plant material are boiled in 1000 ml water, which is consumed during the day. We also confirmed that the use of the plant extends throughout the Mexican territory because the plant is also used in the same manner along the Pacific, Gulf and Caribbean coasts.

Yield and Phytochemical composition

The drug, according to DER native, was 4.8:1 for the WE and 3:1 for the EWE [11]. We confirmed by TLC that the main compounds of the plant cortex are phenolic, which were present in both extracts,

whereas terpenes were detected in the n-hexane extract. We could not detect the presence of alkaloids in the extracts (data not shown).

3.3 Acute hypoglycemic effect

We confirmed that the administration of Na followed by an injection of STZ to normal rats significantly ($p < 0.001$) elevated blood glucose levels compared with rats that were injected with citrate buffer alone as reported by Masiello et al. in 1998 (Table 1).

In the diabetic rats, the extracts showed significant hypoglycemic effects (Table 1). Rats treated with the 5.9 mg/kg WE dose demonstrated hypoglycemia after 60 min, which was significant at 120 and 180 min ($p < 0.001$), compared with time 0. At the 59 mg/kg dose, the rats treated with the WE demonstrated hypoglycemia from 60 min ($p < 0.01$) to 180 min ($p < 0.001$) compared with time 0, at this dose the WE treated rats also had significant hypoglycemia when compared with the control group ($p < 0.01$). The maximal effects of the water extracts were observed after 180 min of treatment.

Treatment of the diabetic rats with 9 mg/kg EWE led to a significant decrease in plasma glucose levels, compared with time 0, at the 60 min ($p < 0.005$) through the 180 min ($p < 0.001$) time points, and at 120, 180 min against the control group. At doses of 90 mg/kg, there was a significant effect after 60 min through 180 min compared to time 0 and control group. The maximum activity was observed after 180 min using the ethanol-water extract.

These results support acceptance of the null hypothesis that there are no significant differences between treatment with the tested plant extracts and glibenclamide, a standard hypoglycemic drug.

Discussion

In the present manuscript, we confirm that the STZ-NA-induced diabetic rat is a suitable model to test the effects of plants that induce hypoglycemia. The extracts studied here as well as glibenclamide, a standard diabetic drug, were able to significantly decrease glucose levels in the diabetic rats; thus, we may conclude that RM causes hypoglycemia in STZ-NA-induced diabetic rats. Diabetic people living near the Mexican coastal zones use the cortex of the red mangrove, Rhizophora mangle, to treat type 2 diabetes. For this propose, they drink an extract of the boiled cortex in water. The results presented here suggest that the plant extract has a hypoglycemic effect, which supports its traditional use. Furthermore, we detect the presence of phenolic compounds in the active extracts. More detailed studies are needed to understand the hypoglycemic effects of the plant.

Acknowledgements

The authors wish to thank M.V. Z. Mario Soriano-Bautista, Biol. Dora Salazar and Biol. Isabel Antúnez for housing the animals and Anamarel Medina Hernández for her collaboration. This work was partially supported by the DGAPA, PAPIIT project IN228510 and CONACyT CB-0151264.

References

1) Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2003;.26: S5.
2) Word Health Organization, 2012, web page; http://www.who.org, (accessed: May, 2012).
3) SINAIS, 2012, National Health Information System. Mexican Government, web page; http://sinais.salud.gob.mx (accessed: May, 2012) In spanish.
4) Andrade-Cetto A. Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 2005; 99: 325–248.
5) Pennington, T.D., Sarukhán, J., Tropical Trees of Mexico. UNAM-FCE, Méxco, 1998: 404-405.
6) Argueta A. ed. Atlas of the traditional Mexican medicinal plants, National Indigenous Institute, Mexico, 1994: 81.
7) Alarcón-Aguilara F. J., Roman-Ramos R, Pérez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J. of Ethnopharmacol 1998; .61: 101-110.
8) Andrade-Cetto, A.. Estudio Etnofarmacologico de Equisetum myriochaetum Schlechtendal & Cham y Cecropia obtusifolia Bertol. Tesis Doctoral. Facultad de Ciencias, UNAM, México 1999; 97.
9) Institutional Animal Care and Use Committee, Guideline of

Selected Techniques for Rat and Mouse Blood Collection, USA 1999; 9: 6.
10) Masiello, P., Broca, C., Gross, R., Roye, M., Mantheghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G. Development of a new model in adult rats administered streptozotocin and nicotinamida 1998; Diabetes 47; 224-229
11) Gaedcke F. Steinhoff B. Herbal Medicinal Products. Medpharm CRC, Germany, 2003: 177.

Groups	*Gl (mg/dl) T0*	*Gl (mg/dl) T60*	*Gl (mg/dl) T120*	*Gl (mg/dl) T180*
1 ND Control	104 ± 11	112 ± 8	110 ± 6	119 ± 3
2 D Control	174 ± 3^{3}	184 ± 15^{3}	174 ± 11^{3}	179 ± 12^{3}
3 D + G *5 mg/kg*	172 ± 3	138 ± $8^{2,c}$	118 ± $5^{3,c}$	123 ± $5^{3,c}$
4 D + EE *9 mg/kg*	173 ± 3	160 ± 4^{a}	153 ± $3^{1,c}$	150 ± $5^{1,c}$
5 D + EE *90 mg/kg*	172 ± 5	152 ± $4^{1,a}$	145 ± $4^{2,c}$	148 ± $3^{2,c}$
6 D + WE *5.9 mg/kg*	173 ± 2	157 ± 7	155 ± 3^{c}	140 ± $3^{c,2}$
6 D +WE *59 mg/kg*	176 ± 5	146 ± $5^{1,a}$	138 ± $4^{1,b}$	134 ± $4^{1,c}$

Table 1. Acute hypoglycemic effects of the *Rhizophora mangle* cortex on NA-STZ-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 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; EE, ethanol-water extract; WE, water extract.

ISSN: 1827-8620