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Hypoglycemic effect of *Malmea depressa* root on streptozotocin diabetic rats

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Abstract

The hypoglycemic effects of water, ethanolic and butanolic extracts prepared from the root of *Malmea depressa* (Baill) R.E. Fries. (Annonaceae) were studied in diabetic rats (streptozotocin induced). Oral application of water extracts at doses of 40 and 80 mg/kg, ethanolic (112 mg/kg) and butanolic (80 mg/kg) extracts significantly lowered the plasma glucose levels in diabetic rats within three hours. Glibenclamide and metformin were used as references and showed similar hypoglycemic effects like the extracts. The three extracts have a similar chemical composition (HPLC analysis).

Keywords: *Malmea depressa*; Hypoglycemic effect; Streptozotocin-induced diabetic rats; Mexican plants

1. Introduction

Malmea depressa (Baill) R.E. Fries. (Annonaceae), traditional names: 'Elemuy', 'Sufricaya' and 'Nazareno Prieto', is a tree up to 10 m high growing associated to disturbed areas in the tropical rainforest of Southeast Mexico and Guatemala. Its main physical features are a tall straight trunk; flowers with fleshy oval-shaped yellowish petals and its external cortex which is plain and light gray (Standley and Steyermark, 1946).

In Mexico, the plant is traditionally used by the people of the Maya communities of the southeast region, specifically in the state of Yucatan, for the treatment of diabetes type 2. The part used for the preparation of herbal teas is the root cortex (Heinrich et al., 2002). This herbal tea is reported to be prepared in two different ways: boiled or cold (macerated) in water. Beside this, in the market of Merida ethanolic extracts are sold for the same treatment.

Phytochemical studies in the aerial part cortex have revealed the presence of phenolic compounds (alfa-asaron), propenylbenzenes and some alkaloids. Those compounds were told to decrease cholesterol and triglycerides in rats (Argueta, 1994). Also antiseptic properties of the plant have been demonstrated (Gutiérrez-Lugo et al., 1996.)

Although there exist ethnobotanical reports as a hypoglycemic plant there is no scientific report about its efficacy.

The aim of our study was to investigate the hypoglycemic effect of water, ethanolic and butanolic extracts in streptozotocin (STZ)-induced diabetic rats and to identify the main chemical constituents occurring in the applicated extracts.

2. Materials and methods

According to our previous studies (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001; Andrade-Cetto and Wiedenfeld, 2004) and further definitions (Holmsted, 1991), we perform an ethnopharmacological study with re-

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spect to ethnobotanical, phytochemical and pharmacological methodologies.

2.1. Ethnobotany

Traditional use of the plant was recorded at Merida market in 1993 and 1997 by ourselves, Argueta (1994) reported the use of the root and bark for the treatment of kidney problems and diabetes in the Mexican states of Yucatan and Quintana Roo. Similar findings were reported by Ankli (2000) and Heinrich et al. (2002). Our own ethnopharmacological studies were performed in the community of Chikindzonot in Yucatan in 2003.

Diabetic people were identified by the local health services and local healers. All informations were obtained about the plant and its special usage based on structured and unstructured interviews with the traditional healers and the diabetic people, respectively. The data were referred to plant samples (mini-herbarium) collected at its natural habitats, and stored as herbarium vouchers for exact identification.

2.2. Materials

With the help of traditional healers, samples of *Malmea depressa* were collected in Chikindzonot, Yucatan, Mexico. Their identity was confirmed and voucher specimens were deposited at the IMSS Herbarium in Mexico City (IMSS 14702 and IMSS 14706).

2.3. Preparation of the extracts and isolation of compounds

Three different sorts of plant extracts were prepared from root samples. (1) boiled in the traditional way filtrated and liophilizated (18 g), resulting in 2.4 g of dry extract (WE), DER native 7.5:1; (2) the ethanolic extract obtained from Merida market was liophilized to dryness (ET); (3) the butanolic extract was prepared as already described (Andrade-Cetto et al., 2000) from root (250 g) resulting in 2.5 g of extract (BE), DER native 100:1. The BE was used for the phytochemical identification of the main components by application on a 100 cm × 2 cm Polygoprep 60-30 C_{18} (Macherey & Nagel, Düren, Germany) flash-column and eluted with H_2O/MeOH/AcCN 70:15:15, 4 ml/min (10 ml fractions; monitored by UV-detection and controlled by HPLC).

2.4. Animals

Male Wistar rats were used; eight weeks old (weighing 200–250 g) obtained from the Bioterium of the Science School, UNAM, and acclimatized with free access to food and water for at least 1 week in an air conditioned room (25 °C with 55% humidity) under a 12 h light: 12 h dark cycle prior to the experiments. All the animal handling was in accordance with the Federal Government legislation on animal care.

2.5. Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (Sigma, no. 242-646-8) (65 mg/kg in acetate buffer 0.1 M, pH 4.5) to overnight-fasted rats. Control rats received only the buffer.

Diabetes was identified by polydipsia, polyuria and by measuring non-fasting plasma glucose levels 48 h after injection of STZ. Animals which did not develop more than 250 mg/dl glucose levels were rejected.

2.6. Experimental groups

The diabetic animals were classified into eight groups (1–8) each of them with eleven rats. Group 1 as non diabetic control received 1.5 ml of physiological NaCl-solution (vehicle), group 2 as the diabetic control received also 1.5 ml of physiological NaCl-solution (vehicle), the rats of group 3 were given the standard oral hypoglycemic agent glibenclamide (3 mg/kg bodyweight (bw)) in the same vehicle, group 4 received the hypoglycemic agent metformin (14 mg/kg bw), groups 5 and 6 received WE (40 mg/kg bw) and WE (80 mg/kg bw), respectively, group 7 received ET (112 mg/kg bw) and group 8 received BE (80 mg/kg bw). The extracts were redissolved in 1.5 ml of physiological NaCl-solution and administered orally by a canule.

2.7. Collection of blood and determination of blood glucose

Blood samples were taken from the tail vein (according to the Guideline 9 (3/10/99) IACUC) before oral administration of the extracts or the vehicle and at times 0, 60, 120 and 180 min thereafter. Thirty-two microliters of blood were used for each assay; the glucose concentration was measured in plasma serum with Reflotron equipment and confirmed by Accutrend GC and Accu-check compact equipments (Roche).

2.8. Statistical analysis

The data were statistically analyzed by ANOVA tukey test. The plasma glucose levels were expressed as the mean (S.E.M.).

3. Results

3.1. Ethnobotany

The results of our field study in Chikinzonot Yucatan confirmed that *Malmea depressa* is used as a hypoglycemic agent against diabetes type 2, it is also used against kidney problems. The tree is locally named by its Maya name “Elemuy”. In general, the people drink the infusion of the root after boiling 30–36 g in 1 l water; also the same amount

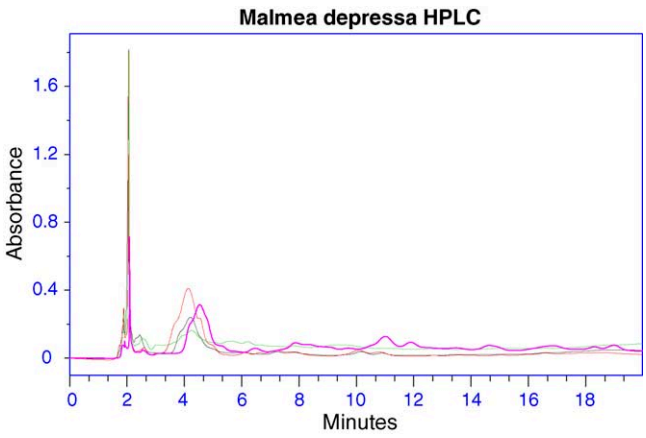

Fig. 1. HPLC, comparison of the extracts of *Malmea depressa*, on a Beckman System gold HPLC. The same main compounds (**2**) are observed on the four extracts; on the right side of the graphic from top to bottom the lines are ET, BU, WH, WC.

of plant is overnight macerated on cold water. The tea or the macerated is drunk during the day as "agua de uso". The dry root is sold at the main market place in Merida, Yucatan, with the same preparation way and a similar recommended dose, furthermore an ethanolic preparation was found, this preparation is recommended for the treatment of type 2 diabetes, some drops (about 1.5 ml) are added to 250 ml water, and drunk several times per day. Those results confirm the previously reported use of *Malmea depressa* for the treatment of diabetes type 2.

3.2. Compounds

A similar composition of ingredients in all three extracts was confirmed by HPLC analysis (Fig. 1). Because both water preparations (hot decoction and cold maceration) show the same compounds only the first preparation was tested in the animal model.

The structures of the isolated compounds (Fig. 2) were established by spectroscopical methods as phenylbutane-derivatives (2-hydroxy-3,4,5-trimethoxy-l-(2′,4′-hydroxy-3′-dihydroxy)butyl-benzene 1; 2-hydroxy-3,4,5-trimethoxy-l-(2′,3′,4′-hydroxy)butyl-benzene 2).

3.3. Activity in diabetic rats

STZ administration at a dosage of 65 mg/kg bw to normal rats significantly elevated the blood glucose levels compared with rats injected citrate buffer alone (Table 1) as in previous reports (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001; Andrade-Cetto and Wiedenfeld, 2004). In our diabetic rats, the extracts showed significant hypoglycemic effects (Table 1). The water extract at doses of 40 and 80 mg/kg bw showed activity at 120 and 180 min, with a significant reduction ($p < 0.01$) of plasma glucose levels. The water extract at a dosage of 40 mg/kg bw did not show significant activity at 60 min while at dose of 80 mg/kg the hypoglycemic effect show statistical significance ($P < 0.01$).

The ethanolic (112 mg/kg) and the butanolic (80 mg/kg) extracts led to a significant decrease in plasma glucose level compared with the control. The effect was significant from 60 min ongoing until 180 min, the ET presented a higher effect at 60 min. The maximum activity of both extracts was observed after 180 min, too.

Glibenclamide (3 mg/kg) and the metformin (14 mg/kg) produced a significant decrease in plasma glucose from 60 min until 180.

These results indicate that there is no significant difference between the tested plant preparations in compari-

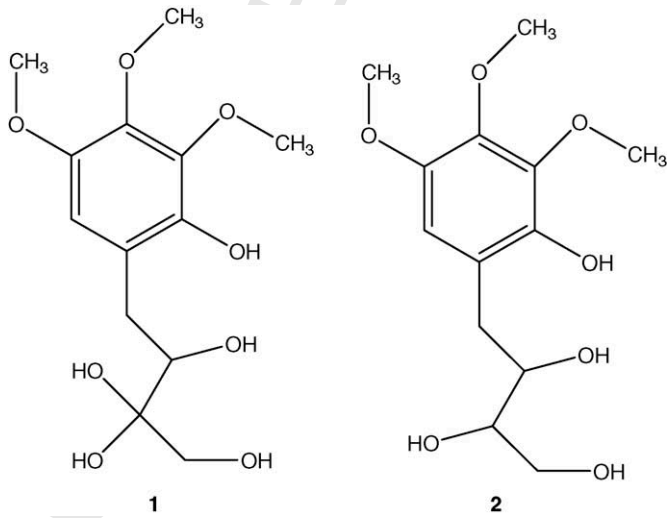

Fig. 2. Isolated compounds from *Malmea depressa*. (**1**) 2-Hydroxy-3,4,5-trimethoxy-l-(2′,4′-hydroxy-3′-dihydroxy)butyl-benzene; (**2**) 2-hydroxy-3,4,5-trimethoxy-l-(2′,3′,4′-hydroxy)butyl-benzene.

Table 1
Effect of oral administration of extracts of *Malmea depressa* root on plasma glucose concentration in diabetic rats

Treatments	Blood glucose levels (mg/dl) ± standard error			
Time (min)	T0	T60	T120	T180
Control (+) no diabetic (vehicle)	131 ± 6a	143 ± 2a	137 ± 3a	132 ± 5a
Control (−) (vehicle)	410 ± 10a[1]	408 ± 25a[1]	413 ± 19a[1]	442 ± 12a[2]
Glibenclamide (3 mg/Kg bw)	418 ± 11a[1]	349 ± 26a[1]	317 ± 28b[2]	318 ± 19b[2]
Metformin (14.16 mg/Kg bw)	390 ± 20a[1]	308 ± 19a[1]	260 ± 16b[2]	239 ± 23b[2]
Malmea depressa aqueous extract (40 mg/Kg bw)	411 ± 11a[1]	344 ± 16a[1]	268 ± 21b[2]	296 ± 28b[2]
Malmea depressa aqueous extract (80 mg/Kg bw)	408 ± 17a[1]	328 ± 7a[1]	329 ± 14a[1]	306 ± 17b[2]
Malmea depressa aqueous extract (113 mg/Kg bw)	407 ± 12a[1]	324 ± 14a[1]	277 ± 14b[2]	248 ± 11b[2]
Malmea depressa aqueous extract (80 mg/Kg bw)	397 ± 17a[1]	347 ± 17b[2]	313 ± 20b[2]	316 ± 12b[2]

The values represent the mean ± S.E.M. as compared with control time intervals.

son to glibenclamide and metformin (standard hypoglycemic drugs)

4. Discussion

Our ethnopharmacological studies as well as our experimental pharmacological data confirm that *Malmea depressa* shows clear hypoglycemic activity which is in accordance to the traditional use in Yucatan state as an infusion of the root for the treatment of diabetes type 2.

The diabetes induction by STZ and the use of glibenclamide and metformin in this animal model were discussed previously (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001; Andrade-Cetto and Wiedenfeld, 2004).

All three extracts (aquous, ethanolic, butanolic) of *Malmea depressa* root produce a hypoglycemic effect in rats. The water extracts show a significant activity after 120 min; the higher dosage shows a higher activity compared with the lower one at 60 min. The maximum effect of both water extracts was observed after 180 min of treatment.

Our preparations given in the animal model is equivalent to the usage given daily traditionally to man (dosage of 80 mg/kg WE in rat = 36 g/1 WE in man). Besides the already discussed administration as an "agua de uso" we tested the acute effect of Malmea-extracts, too.

Therefore we included as a single-dose the application of 40 mg/kg (in rat = 18 g/l in man) in our study. Similarily, the ethanolic extract was administered. The results of our testings are shown in Table 1.

As we could determine in all extracts the same compounds contained we may assume that those components are connected with the pharmacological activity.

Further studies will be done to determine the mechanisms of the activity of the extracts as well as for isolated compound.

Uncited reference

IACUC (1999).

Acknowledgments

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References

Argueta, V.A. (Coordinador) 1994. Atlas de las Plantas de la Medicina Tradicional Mexicana. II. Institute Nacional Indigenista, Mexico, p. 611.

Ankli, A. 2000. Yucatec Mayan Medicinal Plants: Ethnobotany, Biological Evaluation and Phytochemical Study of Crossopetalum gaumeri. Ph. D. Dissertation ETH Ziirich, Nr. 13555, p. 289.

Andrade-Cetto, A., Wiedenfeld, H., Revilla-Monsaive, M.C., Islas, S., 2000. Hypoglycemic effect of Equisetum myriochaetum aerial parts on streptozotocin diabetic rats. Journal of Ethnopharmacology 72, 129–133.

Andrade-Cetto, A., Wiedenfeld, H., 2001. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. Journal of Ethnopharmacology 78, 145–149.

Andrade-Cetto, A., Wiedenfeld, H., 2004. Hypoglycemic effect of *Acosmium panamense* bark on streptozotocin diabetic rats. Journal of Ethnopharmacology 90, 217–220.

Gutiérrez-Lugo, M.T., Barrientos-Benitez, T., Luna, B., Ramirez-Gama, R.M., Bye, R., Linares, E., Mata, R., 1996. Antimicrobial and cytotoxic activities of some crude drug extracts from Mexican Medicinal Plants. Phytomedicine 2, 341–347.

Heinrich, M., Ankli, A., Bork, P., Wolfram, L., Bauerfeind, P., Bran, R., Schmid, C., Weiss, C., Brugisser, C., Gertsch, J., Wasescha, M., Stcher, O., 2002. Yucatec Mayan medicinal plants: evaluation based on indigenous uses. Journal of Ethnopharmacology 79, 43–52.

Holmsted, B., 1991. Historical perspective and future of Ethnopharmacology. Journal of Ethnopharmacology 1, 7–24.

Institutional Animal Care and Use Committee, 1999. Guideline of Selected Techniques for Rat and Mouse Blood Collection, Guideline 9 IACUC, USA, p. 6.

Standley, P., Steyermark, J., 1946. Flora of Guatemala and the adjacent regions. IV. Fieldiana Botany 24, 270–294.