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## Chronic hypoglycemic effect of *Malmea depressa* root on n5-streptozotocin diabetic rats

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### Abstract

The root of the “Elemuy” tree (*Malmea depressa*) is highly used in south east Mexico by the Mayan communities to treat type 2 diabetes. The long-term hypoglycemic effect (HbA1c) and the stimulation of insulin secretion resulting from the treatment with butanolic extract was studied using (n5-stz)-induced diabetic rats. We show that after 30 days of daily administration of 10 mg/kg of the butanolic extract, the glucose levels as well as the (HbA1c) levels were lower compared to the control group. This effect was also observed after 45 days of treatment, leading to the conclusion that the effects of chronic butanolic extract treatment are comparable to treatment with the standard drug Bieuglucon<sup>®</sup>. In an acute experiment, we found that a single administration of the extract at a dose of 50 mg/kg stimulates insulin release, which is similar to the result seen with Tolbutamide administration. The new compound, 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol, was isolated from the active fraction of the butanolic extract. The results presented here support the utility of the traditional use of the root and suggest that the active fraction of the plant extract could be developed as a phytomedicine.

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**Keywords:** *Malmea depressa*; Annonaceae; Neonatal-induced diabetic rats; Type 2 diabetes; Hypoglycemic plant

### 1. Introduction

Diabetes mellitus is a common metabolic disease characterized by increased circulating glucose concentrations associated with abnormalities in carbohydrate, fat, and protein metabolism, and a variety of microvascular, macrovascular, neurologic and infectious complications. The natural history of type 2 diabetes mellitus begins with a period of insulin resistance with augmented pancreatic insulin secretion. As the disease progresses, pancreatic function falters and is no longer able to meet peripheral demands. As a result, insulin levels fail to keep up with the body requirements (Inzucchi, 2003).

Normally, diabetes is detected by measuring glucose blood levels. However, due to wide variations in the circulating glucose concentrations, a randomized glucose measurement does not give clear data for overall glycemic control. A better approach to assess the level of control is the measurement of the glycated hemoglobin value (erythrocyte hemoglobin is non-enzymatically glycosylated at its amine residues = HbA1c). The percentage of hemoglobin molecules undergoing this reaction is proportional to the average glucose concentration during the preceding 60 days. HbA1c is therefore a commonly used laboratory test for assessing long-term diabetic control (Inzucchi, 2003). It has been reported that the animal model used here, streptozotocin (n5-stz) diabetic rats, exhibits high HbA1c values (Portha et al., 2002).

According to the World Health Organization (WHO, 2006), more than 176 million people are affected by this disease worldwide. In Mexico, about 10.6% of the population between 20 and 69 years old is affected by type 2 diabetes, which is the ninth highest rate in the world (FMD, 2007). The use of medicinal

*Abbreviations:* HbA1c, glycated hemoglobin; n5-stz, neonatal-induced diabetic rats at day 5 of birth.

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plants among the Mexican population is a tradition; the use of 306 species for the treatment of type 2 diabetes have already been reported (Andrade-Cetto and Heinrich, 2005a), and from these, *Malmea depressa* is widely used in southeast Mexico.

The aims of our study were to investigate the effects of an active extract of *Malmea depressa* on (n5-stz)-induced diabetic rats with two experiments: (a) chronic administration (HbA1c), and (b) insulin secretion after a single dose application.

## 2. Background

### 2.1. Plant taxonomy

*Malmea depressa* (Baill) R. E. Fries was recently renamed as *Mosannonna depressa* (Baill.) Chatrou, and it belongs to the Annonaceae family.

### 2.2. Plant ethnobotany

As mentioned before, the plant is traditionally used and is highly valued by the people in the Maya communities of the southeast region of Mexico, especially in the state of Yucatan, for the treatment of type 2 diabetes. The part of the plant used for the preparation of herbal teas is the root cortex. This herbal tea is reported to be prepared in two different ways: boiled or cold (macerated) in water (Andrade-Cetto et al., 2006).

### 2.3. Plant pharmacological activity

In a previous study, the acute hypoglycemic effect of water, BuOH extract, and EtOH was examined on stz diabetic rats. It was found that a BuOH (BE) extract showed a statistically significant hypoglycemic effect 60 min after oral administration (Andrade-Cetto et al., 2005b).

### 2.4. Plant phytochemical composition

From the pharmacologically active fraction (=BE), two phenylbutane derivatives (2-Hydroxy-3,4,5-trimethoxy-1-(2',4'-hydroxy-3'-dihydroxy)butyl-benzene and 2-hydroxy-3,4,5-trimethoxy-1-(2',3',4'-hydroxy)butyl-benzene) were identified (Andrade-Cetto et al., 2005b).

### 2.5. Animal model

The (n5-stz) rat model used here exhibits a clear basal hyperglycemia with glucose intolerance, high HbA1c values, a strong reduction of pancreatic insulin stores, a decreased (50%) basal plasma insulin level, and a lack of plasma insulin response to glucose (Portha et al., 2002).

## 3. Materials and methods

Using the methods from our previous studies (Andrade-Cetto and Wiedenfeld, 2001; Andrade-Cetto et al., 2005b) and others (Holmsted, 1991), we performed an ethnopharmacological

study using ethnobotanical, phytochemical and pharmacological methodologies.

### 3.1. Plant 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).

### 3.2. Preparation of the extract and isolation of the compound

The butanolic extract was prepared as previously described (Andrade-Cetto et al., 2000, 2005b) from the root (600 g), resulting in 12 g of extract (BE). The ratio of the herbal drug to the native herbal drug preparation (DER<sub>native</sub>) was 50:1 (Gaedcke and Steinhoff, 2003). The BE was subjected to phytochemical component identification by application onto a 100 cm × 2 cm Polyoprep 60–30 C<sub>18</sub> (Macherey & Nagel, Düren, Germany) flash-column and was eluted with H<sub>2</sub>O/MeOH/acetonitrile 70:15:15, 4 ml/min (10 ml fractions; monitored by UV-detection and controlled by HPLC). For the compound elucidation we use: GC–MS: Finnegan Mat GCQ/Polaris MS; injection 1 µl; column DB5, 50 m, 0.25 mm × 0.2 mm MS; source: 180 °C, interface: 280 °C; injector: 280 °C; full-scan mode.

### 3.3. Animals and induction of experimental diabetes

Newborn 5-day old Wistar rats; (weighing 10–12 g) were obtained from the Bioterium of the Science School, UNAM. They received 90 mg/kg i.p. of n5-stz (Sigma, no. 242-646-8) in acetate buffer 0.1 M, pH 4.5. The non-diabetic control group received only buffer i.p. At 4 weeks of age, rats were separated from their mothers and acclimatized with free access to food and water in an air-conditioned room (23 °C with 55% humidity) under a 12 h light:dark cycle. After 12 weeks, animals with fasting glucose values over 60 mg/dl were selected.

The animals were handled according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (OACU, 2007); all methods used in this study were approved by the Internal Council of the “Facultad de Ciencias”, Universidad Nacional Autónoma de México.

### 3.4. Chronic experiment (HbA1c, levels)

The diabetic animals were classified into four groups (1–4), each of which contained four rats. Group 1 (non-diabetic control) received 1.5 ml of physiological NaCl-solution (vehicle), group 2 (diabetic control) also received 1.5 ml of physiological NaCl-solution (vehicle), the rats of group 3 (diabetic) were given the standard oral hypoglycemic agent Bieglucon<sup>®</sup> (glibenclamide 3 mg/kg and metformin 500 mg/kg bodyweight (bw)) in the same vehicle, and group 4 (diabetic) received *Malmea depressa* (BE) (10 mg/kg bw). The treatments were administered twice a day, meaning that in each administration, we applied half

the dose, corresponding to the traditional use of the plant. The extract as well as the standard drug were redissolved in 1.5 ml of physiological NaCl-solution and administered by an esophagus canule, over a period of 45 days.

### 3.5. Acute experiment (insulin secretion)

The animals were classified into five groups (1–5), each of which contained five rats. Group 1 (non-diabetic control) received 1.5 ml of physiological NaCl-solution, group 2 (non-diabetic) received the standard hypoglycemic agent, tolbutamide (100 mg/kg), group 3 (diabetic control) received 1.5 ml of physiological NaCl-solution, group 4 (diabetic) received tolbutamide (100 mg/kg), and group 5 (diabetic) received *Malmea depressa* (BE) at a dose of 50 mg/kg. For the acute experiment, tolbutamide was selected as the control drug because Ohta et al. (1999) demonstrated in neonatal-induced streptozotocin diabetic rats that this drug leads to increases in insulin secretion, and that this effect is not glucose-dependent.

### 3.6. Collection of blood and determination of blood parameters

Blood samples were taken from the tail vein (according to the Guideline 9 IACUC, 99). Thirty-two microliters of blood were used for each assay; the glucose concentration was measured in plasma serum with a Reflotron equipment and confirmed by Accutrend GC and Accu-check compact meters (Roche). Cholesterol and triglycerides were measured with a Reflotron (Roche). Glycosylated haemoglobin was measured with a Micromat-BIORAD analyzer and confirmed with a DCA2000-Bayer analyzer.

For the chronic experiments, glucose, triglycerides, cholesterol, and glycosylated hemoglobin were measured in 8 h fasted rats at days 0, 30 and 45.

For the acute experiments, 52  $\mu$ l of blood were collected and 32  $\mu$ l were used for glucose determination, at 0 (before the oral administration of the drug or the plant extract), 15, and 75 min. The remaining 20  $\mu$ l were centrifuged and the plasma was stored ( $-20^{\circ}\text{C}$ ) for insulin determination. Insulin was measured by ELISA with an Ultra sensitive rat insulin kit (Chrysal Chemical, USA cat 90060) and Awareness Microelisa equipment.

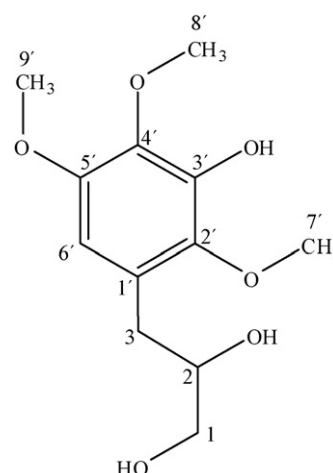


Fig. 1. New isolated compound from *Malmea depressa*: 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol.

### 3.7. Statistical analysis

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

## 4. Results

### 4.1. New compound

In addition to the previously described compounds, we isolated a new phenylpropane derivative, the structure of which was established by spectroscopic methods to be: 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol (Fig. 1). Data: MS:  $m/z$  (rel. int):  $[\text{M}]^+$   $\text{C}_{12}\text{H}_{18}\text{O}_6$  258 (8.0). NMR ( $\delta$  = ppm): C-6': 6.78 (1H, s)/108.92; C-5': 145.56; C-4': 141.35; C-3': 138.52; C-2': 144.38; C-1': 121.67; C-7': 3.84 (3H, s)/60.23; C-8': 3.84 (3H, s)/60.79; C-9': 3.84 (3H, s)/56.22; C-3: 2.72 (1H, d,  $J=7.0$  Hz) + 2.55 (1H, dd,  $J=7.0, 3.4$  Hz)/33.75; C-2: 3.43 (1H, dt,  $J=6.9, 6.8$  Hz); 72.26; C-1: 3.28 (2H, q,  $J=6.8$  Hz)/65.83.

### 4.2. Chronic activity in diabetic rats (HbA1c)

stz administration at a dosage of 90 mg/kg bw to newborn rats significantly increase the blood glucose levels compared to rats

Table 1  
Effect of daily oral administration of *Malmea depressa*, butanolic root extract on three different parameters in n5-stz diabetic rats after 45 days of treatment

Groups	Parameter	Glucose (mg/dl) T0	Glucose (mg/dl) T30	Glucose (mg/dl) T45	HbA1c (%) T0	HbA1c (%) T30	HbA1c (%) T45	Triglyceride (mg/dl) T0	Triglyceride (mg/dl) T30	Triglyceride (mg/dl) T45
1	Control (–) ND	53 $\pm$ 6	77 $\pm$ 7 b	76 $\pm$ 8	5 $\pm$ 0.1	4.9 $\pm$ 0.1	4.7 $\pm$ 0.2	78 $\pm$ 6	123 $\pm$ 15 b	123 $\pm$ 7 b
2	Control (+) D	54 $\pm$ 6	250 $\pm$ 7 a	233 $\pm$ 11 a	5.9 $\pm$ 0.2 a	6.1 $\pm$ 0.3 a	7.3 $\pm$ 1 ab	83 $\pm$ 20	89 $\pm$ 11	81 $\pm$ 16 a
3	Bieuglucon D	55 $\pm$ 6	184 $\pm$ 12 a	136 $\pm$ 6 abc	5.9 $\pm$ 0.4 a	5.6 $\pm$ 0.4 a	5.3 $\pm$ 0.3 ac	98 $\pm$ 10	103 $\pm$ 11	87 $\pm$ 9 a
4	<i>Malmea depressa</i> BuOH	56 $\pm$ 6	117 $\pm$ 6 abc	84 $\pm$ 8 bc	6.2 $\pm$ 0.2 a	5.5 $\pm$ 0.4 ac	5 $\pm$ 0.4 abc	97 $\pm$ 6 a	100 $\pm$ 3	64 $\pm$ 19 a

The values represent the mean  $\pm$  S.E.M. as compared with controls time intervals,  $n=4$ . Letter "a" shows significance versus control (–) group at each sample time (T0, T30, T45), letter "b" shows significance versus T0 of each treatment, letter "c" shows significance versus control (+) group at each sample time. All significance values at least at  $p < 0.05$ .

Table 2

Effect of a single oral administration of *Malmea depressa*, butanolic root extract on plasma glucose concentration and insulin secretion in n5-stz diabetic rats 75 min after treatment

Groups	Parameter	Glucose (mg/dl) T0	Glucose (mg/dl) T15	Glucose (mg/dl) T75	Insulin (ng/ml) T0	Insulin (ng/ml) T15	Insulin (ng/ml) T75
1	Control (–) ND	82 ± 2	102 ± 9	89 ± 5	2.015 ± 0.22	1.631 ± 0.20	1.902 ± 0.48
2	Control (–) ND tolbutamide (100 mg/kg)	82 ± 4	89 ± 5	50 ± 4 <sup>a,b</sup>	2.408 ± 0.52	3.01 ± 1.22 <sup>a</sup>	4.992 ± 0.82 <sup>a,b</sup>
3	Control (+) D	199 ± 14 <sup>a</sup>	265 ± 28 <sup>a</sup>	229 ± 25 <sup>a</sup>	2.118 ± 0.10	2.089 ± 0.10	2.361 ± 0.38
4	Control (+) D tolbutamide (100 mg/kg)	201 ± 18	224 ± 17	148 ± 15 <sup>a,b</sup>	2.540 ± 0.48	3.238 ± 0.92	5.036 ± 0.90 <sup>ab</sup>
5	<i>Malmea depressa</i> BuOH (50 mg/kg)	195 ± 6	201 ± 14 <sup>a</sup>	144 ± 8 <sup>a,b</sup>	1.879 ± 0.18	3.406 ± 0.42 <sup>a,b</sup>	2.198 ± 0.24

The values represent the mean ± S.E.M. as compared with controls time intervals,  $n = 5$ . All significance values at least at  $p < 0.01$ .

<sup>a</sup> Significance versus control (–) group at each sample time (T0, T15, T75).

<sup>b</sup> Significance versus T0 of each treatment.

injected with citrate buffer alone. The combination of metformin and glibenclamide (Bieuglucon<sup>®</sup>) lowered the plasma glucose levels at day 45 compared to the diabetic control, while the oral administration of the BE lead to a statistically significant hypoglycemic effect on the plasma glucose from day 30 to day 45 (Table 1).

The HbA1c data for the diabetic groups were significantly higher than in the control (non-diabetic) groups. The chronic administration of the control drug significantly lowered the HbA1c values after 45 days. The administration of the *Malmea depressa* BE produced a similar effect to the control drug, with the only difference being that, after 45 days, a significant difference was found against its own time 0 value as well (Table 1).

The values of cholesterol in the diabetic rats did not differ from the control group. Similarly, the values for triglycerides did not show any statistical significance; however it is remarkable that after 45 days, the *Malmea depressa* group showed decreased triglycerides compared to the control group and the group's own time 0 value (Table 1).

These results provide clear evidence that, for the glucose levels as well as for the Hb1Ac values, no significant differences can be found between the tested plant preparation and Bieuglucon<sup>®</sup> (standard hypoglycemic drug).

#### 4.3. Activity on the insulin secretion in diabetic rats

The oral administration of tolbutamide (100 mg/kg) to the non-diabetic control group significantly decreases plasma glucose levels after 75 min. A similar effect is observed with the diabetic animals. The oral administration of *Malmea depressa* BE at doses of 50 mg/kg also significantly lowered the plasma glucose levels 75 min after application. The intensity of this effect is similar to that seen with tolbutamide (Table 2).

The insulin level of the non-diabetic control group increased compared to control values 15 min after the administration of tolbutamide (100 mg/kg), and this effect is observed up to 75 min after administration. The insulin level of the diabetic group also increased within 15 min of tolbutamide administration, and was significant after 75 min compared to the diabetic control. Similarly, the oral administration of *Malmea depressa* BE increased the plasma insulin levels significantly

(also observed after 15 min) compared with the diabetic control animals (Table 2).

## 5. Discussion

The results of our investigations of the long-term (HbA1c) and immediate (insulin secretion) effects of *Malmea depressa* BE administration on a (n5-stz)-induced diabetic rat model confirmed that *Malmea depressa* exhibits a clear hypoglycemic activity, which is in accordance with our ethnopharmacological data as well as the effects reported with traditional use of the plant's root in the Yucatan state.

As the isolated compounds are the major constituents in the *Malmea depressa* BE, it is possible that they may play an important role in the hypoglycemic effect caused by this fraction.

Furthermore, we confirmed the previous observations (Portha et al., 2002) in which high levels of Hb1Ac were reported for (n5-stz)-induced diabetic rats together with corresponding increased plasma glucose values. Therefore, we can suggest that this model is suitable for studying the long-term effects (HbA1c) of drugs used for the treatment of type 2 diabetes.

The results of our chronic experiment confirmed that the long-term administration of the plant controls and reduces the values of the plasma glucose in (n5-stz)-induced diabetic rats as supported by the obtained HbA1c values.

In further agreement with Portha et al. (2002), we suggest that this animal model is also suitable for measuring insulin secretion in comparison with a (non-glucose-dependent) insulin-secreting drug like tolbutamide.

Our data concerning the acute effect of the BE provided clear evidence that an oral single dose of this plant fraction stimulates insulin secretion and this may partially explain the mechanism of the efficacy of *Malmea depressa*.

Thus, the results presented here confirm the traditional use of the root as hypoglycemic and suggest that this fraction could be developed as a phytomedicine.

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