https://doi.org/10.31925/farmacia.2026.1.11 **ORIGINAL ARTICLE**

ANTIHYPERGLYCAEMIC, ANTIDIABETIC, ANTI-ALFA-GLUCOSIDASE, AND ACUTE TOXICOLOGICAL STUDY OF *ODONTONEMA CALLISTACHYUM* (SCHTDL & CHAM) KUNTZE IN EXPERIMENTAL MOUSE MODELS

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Abstract

Diabetes, a chronic degenerative disease caused by insulin deficiency (Type 1) or resistance (Type 2), is among the most prevalent and deadly non-communicable diseases worldwide, posing a significant burden on global health systems. Despite the availability of several therapeutic options, there remains a pressing need for novel, safe, and effective antidiabetic agents, with medicinal plants representing a particularly valuable source of bioactive compounds. *Odontonema callistachyum*, a member of the *Acanthaceae* family, has demostrated various pharmacological properties, including antidiabetic activity. This present study evaluated the antidiabetic potential of *O. callistachyum in vivo*, *ex vivo* in the small intestine, as well as its acute toxicity in CD1 mice. The methanolic extract (ME) exhibited a significant antihyperglycaemic effect, as demonstrated by oral glucose and sucrose tolerance tests. Furthermore, the ME significantly inhibited glucose absorption in the mouse small intestine, an effect likely associated with α-glucosidase inhibition. Acute toxicological evaluation classified the ME of *O. callistachyum* as Category 5 according to OECD Test No. 423, indicating the absence of acute toxicity at a dose of 2,000 mg/kg.

Rezumat

Diabetul reprezintă una dintre cele mai prevalente și letale boli netransmisibile la nivel mondial, reprezentând o povară semnificativă pentru sistemele de sănătate globale. În ciuda disponibilității mai multor opțiuni terapeutice, există în continuare o nevoie stringentă de agenți antidiabetici noi, siguri și eficienți, plantele medicinale reprezentând o sursă deosebit de valoroasă de compuși bioactivi. *Odontonema callistachyum*, membru al familiei *Acanthaceae*, a demonstrat diverse proprietăți farmacologice, inclusiv activitate antidiabetică. Prezentul studiu a evaluat potențialul antidiabetic al *O. callistachyum in vivo*, *ex vivo* în intestinul subțire, precum și toxicitatea sa acută la șoarecii CD1. Extractul metanolic (ME) a prezentat un efect antihiperglicemic semnificativ, demonstrat prin testele de toleranță la glucoză și sucroză administrate oral. Mai mult, ME a inhibat semnificativ absorbția glucozei în intestinul subțire al șoarecilor, un efect probabil asociat cu inhibarea α-glucozidazei. Evaluarea toxicologică acută a clasificat ME al *O. callistachyum* în Categoria 5 conform OECD Test nr. 423, indicând absența toxicității acute la o doză de 2.000 mg/kg.

Keywords: *Odontonema callistachyum*, diabetes; antihyperglycaemic, α-glucosidase inhibition, acute oral toxicity

Introduction

Diabetes is one of the four non-communicable diseases with the highest prevalence and mortality worldwide. It is a chronic degenerative disorder that arises when the pancreas does not produce sufficient insulin or when biosynthesized insulin cannot be used effectively by the body (1).

Diabetes is classified into type 1 diabetes, which results from insufficient insulin production; type 2 diabetes, characterized by impaired insulin secretion or action; and gestational diabetes, a transient condition occurring during pregnancy (2). According to the International Diabetes Federation, 589 million adults aged 20 - 79 years were living with diabetes in 2024, corresponding to approximately one in nine individuals worldwide. This number is projected to increase to 853 million by 2050 (2). Diabetes is associated with severe complications, including stroke, myocardial infarction, kidney failure, limb amputations, and other long-term disabilities (3).

The use of oral antidiabetic drugs improves patients' quality of life; however, poor pharmacological adherence significantly reduces therapeutic effectiveness. Dose escalation, adverse drug

reactions, and drug - drug interactions are among the leading causes of non-adherence (4,5). Consequently, the continuous exploration of alternative therapeutic strategies, novel sources of bioactive molecules, and the scientific validation of traditional medicinal plant use remain highly relevant research areas. In this context, plants have historically constituted one of the most important sources of medicinal compounds (6).

Odontonema callistachyum, commonly known as "canutillo", is an herbaceous plant or shrub that can reach up to 3.5 m in height. Its young stems present glandular trichomes distributed evenly or arranged in two longitudinal lines and may also bear sessile glands. The leaves have petioles up to 2.7 cm in length, with ovate to ovate-elliptic or elliptical blades. The flowers display a pinkish-purple corolla, and the rachis is pubescent with eglandular trichomes arranged in an astral pattern, measuring 0.2 - 0.6 mm in length. The species is distributed from central Mexico to Guatemala and has been reported in several Mexican states, including San Luis Potosí, Querétaro, Hidalgo, Jalisco, Michoacán, Veracruz, Puebla, Guerrero, Oaxaca, Tabasco, and Chiapas. *O. callistachyum* is cultivated for ornamental and medicinal purposes; however, not all reported populations may be native (7). To date, pharmacological and toxicological studies on *O. callistachyum* remain scarce. Nevertheless, ethnomedical and phytochemical data are available for the closely related species *Odontonema strictum*.

Previous studies on *O. strictum* have identified the flavonoid kaempferol-3-β-D-(6″-O-p-coumaroyl)-glucopyranoside, which exhibited antidiabetic activity in murine models. In addition, antioxidant and antifungal activities have been reported, with flavonoids obtained using 70% (v/v) ethanol and supercritical carbon dioxide extraction methods (8,9). Flavonoids are widely recognized for their broad spectrum of biological activities, including antidiabetic effects (10).

Based on this background, *Odontonema callistachyum* may contain bioactive compounds with antidiabetic potential. Therefore, the present study aimed to evaluate the antidiabetic activity of its organic extracts in preclinical murine models, with secondary objectives including the investigation of the underlying mechanism of action and the assessment of acute toxicity in accordance with OECD guidelines.

Materials and Methods

Odontonema callistachyum was collected in Yajalón, Chiapas, Mexico (17°16′9.23″ N, 92°30′7.23″ W). Aerial parts (leaves, flowers, and stems) were harvested, and a voucher specimen was deposited in the Vascular Plants Collection under accession number UJAT-036514.

The plant material was cleaned and shade-dried at ambient temperature for two weeks. Subsequently, it was reduced in size using garden shears, ground in a manual mill (Molino Estrella®, Mexico), and sieved using a 1.4 mm mesh (ELE International®, USA), yielding 391.7 g of dried material.

Preparation of extracts

A total of 150 g of the dried plant material was subjected to sequential maceration in triplicate using organic solvents of increasing polarity: hexane, dichloromethane, and methanol. Each maceration was carried out for 72 h at room temperature. The resulting extracts (HE, DE, and ME) were filtered and concentrated to constant weight under reduced pressure using a rotary evaporator (BÜCHI®, Switzerland).

Phytochemical standardisation

The methanolic extract of *O. callistachyum* was chemically standardised based on its total phenolic content, determined by the Folin-Ciocîlteu method. Results were expressed as gallic acid equivalents (GAE) and used as a reproducible chemical marker to ensure batch-to-batch consistency of the extract employed in the *ex vivo* assays (10).

Animal experimentation considerations

The present study was designed as a pilot, exploratory investigation aimed at obtaining preliminary evidence of the antihyperglycaemic and antidiabetic potential of *O. callistachyum* extracts. Accordingly, a limited sample size (n = 5 animals *per* group) was employed, which is consistent with exploratory pharmacological studies. The results should therefore be interpreted with caution, as the small sample size may limit statistical power.

Male CD1 mice (25 - 30 g) were obtained from the biotherium of the Universidad Autónoma del Estado de Morelos and used for pharmacological evaluations. Animals were housed under standard light/dark cycles with ad libitum access to food (Purina®, Mexico) and water, in accordance with the Mexican Official Standard NOM-062-ZOO-1999. All experimental procedures were approved by the Research Ethics Committee of the Universidad de Quintana Roo (Project: ZS/PI-13/22; Ethical approval: CEI/DICT/005/2022).

Oral glucose tolerance test

Normoglycaemic mice (< 120 mg/dL) were divided into five groups (n = 5 *per* group). Following a 16-hour fasting period, treatments were administered as follows: Group 1 received glibenclamide (5 mg/kg), which was used as a positive control due to its well-established insulin secretagogue activity and its frequent use as a reference drug in OGTT models (Sigma-Aldrich®, Mexico). Groups 2, 3, and 4 were administered the plant extracts at a dose of 100 mg/kg (HE, DE, and ME, respectively). Group 5

received the vehicle (Vh; 10% Tween-80 in water) (Sigma-Aldrich®, Mexico).

Blood glucose concentrations were measured at 0.5, 1, 2, and 3 hours after glucose administration, and results were expressed as percentage glucose variation (%GV).

The %GV was used as a relative indicator of dynamic changes in blood glucose levels following treatment or glucose challenge and was calculated using the following formula: $\%GV = [(G_t - G_0) / G_0] \times 100$, where G_0 represents the baseline blood glucose concentration measured prior to treatment or glucose administration, and G_t corresponds to the glucose concentration at the specified time point after intervention (11).

Statistical comparisons between groups were performed using two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test, with a confidence level of 95%. Differences were considered statistically significant when $p < 0.05$. Comparisons were made between the extract-treated groups, the vehicle group, and the positive control group

Oral sucrose tolerance test

Animals were prepared under the same experimental conditions as those described for the oral glucose tolerance test. Group 1 received acarbose (50 mg/kg), which was used as a positive control (Sigma-Aldrich®, Mexico). Groups 2, 3, and 4 were administered the plant extracts at a dose of 100 mg/kg (HE, DE, and ME, respectively), while Group 5 received the vehicle (Vh; 10% Tween-80 in water). One and a half hours after extract administration, all animals received an oral sucrose load (2 g/kg). Blood glucose concentrations were measured at 0.5, 1, 2 and 3 hours following sucrose administration via tail vein puncture, and results were expressed as %GV (11).

Statistical comparisons between groups were performed using two-way ANOVA followed by a Bonferroni post hoc test, with a confidence level of 95%. Differences were considered statistically significant when $p < 0.05$. Comparisons were made between the extract-treated groups, the vehicle group, and the positive control group.

Acute antidiabetic assay in a non-insulin-dependent diabetes (NIDD) mouse model

To induce a non-insulin-dependent diabetes (NIDD) model, twenty male CD1 mice (20-30 g) were fasted for 13 hours prior to diabetes induction. Nicotinamide (20 mg/kg; Sigma-Aldrich®, Mexico) was administered intraperitoneally (i.p.) in sterile saline, followed by streptozotocin (STZ; 120 mg/kg; Sigma-Aldrich®, Mexico), also administered i.p. and freshly prepared in citrate buffer (pH 4.5), as described in the literature. This protocol was employed to induce partial pancreatic β-cell dysfunction while preserving residual insulin secretion. After two weeks, animals presenting fasting blood glucose levels above 200 mg/dL were considered diabetic and selected for the acute antidiabetic assay (11,12).

For the acute evaluation, NIDD mice were randomly assigned to five groups (n = 5) and fasted for 13 hours. Body weight and baseline blood glucose levels were recorded prior to treatment. Animals were then administered the respective treatments orally: the positive control group received glibenclamide (5 mg/kg), test groups received the plant extracts (HE, DE, or ME) at a dose of 100 mg/kg, and the vehicle group received 10% Tween-80. Blood glucose concentrations were measured at 1, 3, 5, and 7 hours after treatment *via* tail vein puncture, and results were expressed as %GV.

Statistical analysis

Statistical analysis was performed using two-way ANOVA followed by a Bonferroni post hoc test, with statistical significance defined as $p < 0.05$. Comparisons were made between extract-treated groups, the vehicle group, and the positive control group.

α-glucosidase ex vivo inhibition assay

CD1 mice were euthanised by manual cervical dislocation, and the small intestine was immediately excised and rinsed three times with sterile normal saline (NS) pre-warmed to 37°C. Duodenal segments (3 cm) were isolated, and both ends were ligated using polyglactin 910 sutures. All procedures were conducted under aseptic conditions, and tissues were maintained in NS at 37°C to preserve enzymatic activity.

Each intestinal segment was placed in a Petri dish containing NS on a temperature-controlled hotplate (37°C). A volume of 100 µL of sucrose (control), acarbose (10 mg/mL), or *O. callistachyum* methanolic extract (100 mg/mL), all prepared in NS containing sucrose (1 g/mL), was injected into the intestinal lumen. The concentrations employed exceeded physiological levels and were intentionally selected to maximise the detection of α-glucosidase modulation in an exploratory, mechanistic *ex vivo* model rather than to reproduce *in vivo* exposure conditions.

Samples were collected at 30, 60, 120, and 180 min, and glucose release was assessed using Fehling's reagent (13), followed by spectrophotometric measurement at 490 nm (Multiskan FC, Thermo Scientific®). Optical density values were used for the comparative analysis of reducing sugar release. Baseline measurements obtained at time 0 were used as operational blanks to normalise subsequent optical density readings.

Results were expressed as percentage glucose variation (%GV). Group comparisons were performed using an independent *t*-test with a 95% confidence level, and differences were considered statistically

significant at $p < 0.05$. Given the pilot and exploratory nature of the study, the analyses were intended to identify relative inhibitory trends rather than to quantify absolute enzymatic activity.

Calculation and interpretation of %GV

The %GV was calculated using relative optical density (OD) values obtained from the Fehling reaction measured at 490 nm, rather than absolute glucose concentrations. Baseline absorbance (OD_0) was defined at time 0, and subsequent absorbance values (OD_t) were recorded at each experimental time point. %GV was calculated according to the following equation:

$$\%GV = [(OD_t - OD_0) / OD_0] \times 100.$$

Increases in %GV reflect enhanced α-glucosidase-mediated sucrose hydrolysis and consequent glucose liberation, whereas lower or negative %GV values indicate reduced enzymatic activity. The use of OD-based %GV allows normalisation of inter-sample variability and is appropriate for exploratory, mechanistic *ex vivo* studies in which relative enzymatic modulation is the primary outcome.

Although extract-only interference controls were not included, all experimental conditions contained sucrose as a common substrate and were therefore interpreted comparatively across treatment groups. This relative analytical approach is suitable for preliminary *ex vivo* assessment of α-glucosidase modulation, while acknowledging potential limitations related to extract-associated optical interference.

Acute toxicity in mice

Acute toxicity was evaluated in accordance with OECD Guideline 423. Five female mice (8-12 weeks old, 25-32 g) were allocated into two groups. The treated group (n = 3) received a single oral dose of 2,000 mg/kg of the methanolic extract (ME) of *O. callistachyum*, selected based on its antihyperglycaemic and antidiabetic activity. The control group (n = 2) received no treatment.

Behavioural parameters were monitored at 20 min, 4 h, 24 h, and up to 14 days post-administration, following the modified Irwin test (14). Mortality was recorded over a 14-day observation period to allow classification of the extract according to OECD Guideline 423 (15).

Body weight was recorded prior to administration and on days 7 and 14. Mean body weight for each group was calculated, and percentage body weight variation (%WV) was determined. Statistical differences between treated and untreated groups were assessed using an independent t-test with a 95% confidence level, considering $p < 0.05$ as statistically significant.

At the end of the observation period, animals were euthanised by cervical dislocation, and blood samples were collected via cardiac puncture. Serum was obtained by centrifugation, and hepatic transaminases - aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - were quantified using an enzymatic assay. Liver enzyme levels were expressed as multiples of the upper limit of normal (ULN) relative to untreated controls. A gross necropsy was performed, and all macroscopic pathological findings were recorded. Data are presented as mean ± SEM, and statistical comparisons between groups were conducted using an independent *t*-test ($p < 0.05$).

Results and Discussion

Obtaining the extracts

Odontonema callistachyum was collected in Chiapas, Mexico, from a semi-humid environment with surrounding vegetation located near a stream. The plant was observed as a shrub and showed no visible signs of insect, animal, or microbial infestation.

The extraction yields of the hexane (HE), dichloromethane (DE), and methanolic (ME) extracts are presented in Table I, expressed both as percentage yield and absolute mass (g).

Table I

Extraction yields of *Odontonema callistachyum*

Extract	Yield (%)	Yield (g)
HE	0.62	0.94
DE	0.93	1.4
ME	3.17	4.76

HE, hexane extract; DE, dichloromethane extract; ME, methanol extract

The results indicate that the methanolic extract produced the highest yield (3.17%), which is consistent with the preferential extraction of polar secondary metabolites. Methanol is known to solubilise compounds such as phenolic acids, flavonoids, and other polar constituents. The dichloromethane extract yielded 0.93%, suggesting the presence of metabolites of intermediate polarity, including lipophilic flavonoids, terpenoids, or lignans. In contrast, the hexane extract showed the lowest yield (0.62%), which is consistent with the extraction of predominantly non-polar compounds, such as lipids and fatty acids.

Total phenolic content

The methanolic extract of *O. callistachyum* exhibited a total phenolic content of 5.67 ± 0.42 µg gallic acid equivalents (GAE)/mL at a test concentration of 1,000 µg/mL. This value was used as a chemical standardisation parameter to ensure batch-to-batch consistency of the extract employed in the biological assays.

The quantification of total phenolic content provides a reproducible and quantifiable phytochemical marker, supporting the reliability and comparability of the pharmacological evaluations conducted in this study.

Oral glucose tolerance test

The antihyperglycaemic effect of the extracts was evaluated in normoglycaemic mice at a dose of 100 mg/kg using an oral glucose tolerance test (OGTT). Figure 1 shows the time course (h) of the percentage glucose variation (%GV ± SEM). All three extracts significantly reduced the maximum %GV observed at 30 min following the glucose load (2 g/kg) when compared with the vehicle group ($p < 0.05$), indicating an antihyperglycaemic effect.

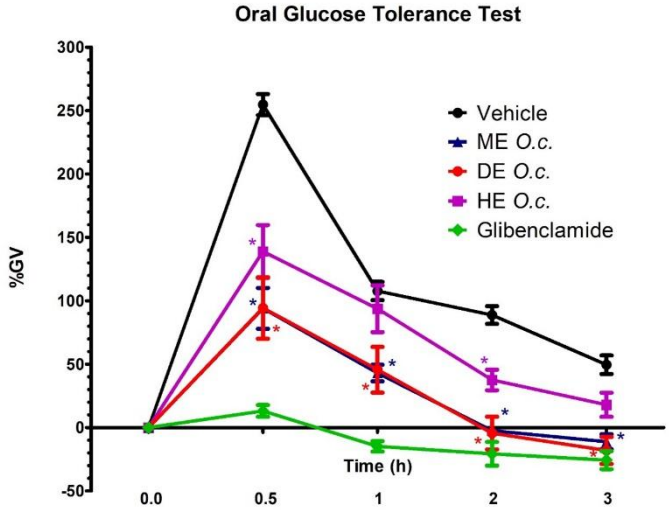

Figure 1.

Oral glucose tolerance test of extracts and controls. Data are presented as mean ± SEM (*$p < 0.05$ *vs.* vehicle)

The hexane extract (HE) produced a %GV of 138.91 ± 20.90% at 0.5 h, representing a significant reduction ($p < 0.05$) in the glycaemic peak compared with the vehicle group (254.80 ± 8.31% at 0.5 h). A further significant decrease ($p < 0.05$) was observed at 2 h, with %GV values of 37.58 ± 8.12% for HE versus 88.78 ± 7.02% for the vehicle. However, no statistically significant differences were detected between the extracts at later time points.

The dichloromethane extract (DE) significantly reduced the %GV at 0.5 h (94.31 ± 24.25%) compared with the vehicle group (254.80 ± 8.31%; $p < 0.05$). This antihyperglycaemic effect was sustained throughout the duration of the assay, with %GV values of 18.07 ± 10.63% at 3 h, compared with 49.53 ± 7.28% in the vehicle group.

Similarly, the ME significantly reduced the glycaemic peak at 0.5 h (%GV = 94.07 ± 16.13%) relative to the vehicle group (%GV = 254.80 ± 8.31%; $p < 0.05$), indicating inhibition of glucose absorption. At 1 h, the %GV observed for ME (43.00 ± 6.57%) was comparable to that of DE (45.62 ± 18.02%) and significantly lower than that of the vehicle group (107.70 ± 7.19%; $p < 0.05$). This significant difference was maintained throughout the 3 h experimental period.

Overall, OGTT results suggest that the extracts contain secondary metabolites contributing to antihyperglycaemic activity. While HE exhibited an early effect, this response was not sustained over time when compared with DE and ME. Previous reports indicate that moderately polar and polar extracts from species of the Acanthaceae family frequently display antihyperglycaemic activity (16,17), supporting the observed glucose-lowering effects of the methanolic and dichloromethane extracts.

3.1. Oral sucrose tolerance test

An oral sucrose tolerance test (OSTT) was conducted to further evaluate the ability of the extracts to attenuate the postprandial hyperglycaemic peak. The antihyperglycaemic effect of the extracts was assessed in normoglycaemic mice at a dose of 100 mg/kg. Figure 2 illustrates the time course (h) of percentage glucose variation (%GV ± SEM).

During the OSTT, the HE did not produce significant changes in %GV at any time point compared with the vehicle group ($p > 0.05$). The DE did not significantly reduce the maximum hyperglycaemic peak at 0.5 h; however, a significant reduction was observed at 2 h (%GV = 26.45 ± 6.12%) compared with the vehicle group (%GV = 77.17 ± 12.00%; $p < 0.05$).

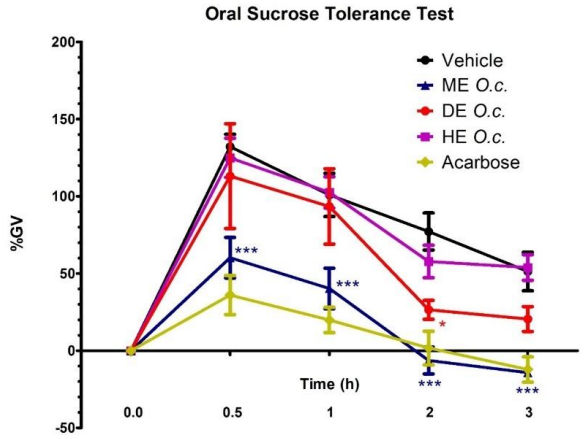

Figure 2.

Oral sucrose tolerance test of extracts and controls. Data are presented as mean ± SEM (*p < 0.05 *vs.* Vh, ***p < 0.001 *vs.* Vh)

In contrast, the ME demonstrated marked antihyperglycaemic activity throughout the assay. At 0.5 h, ME significantly reduced the hyperglycaemic peak (%GV = 60.20 ± 13.15%) relative to the vehicle group (%GV = 132.12 ± 8.00%; p < 0.001). This significant reduction was maintained at all subsequent time points. Moreover, direct comparison between extracts revealed that ME produced a significantly greater reduction in %GV than both DE (p < 0.05) and HE (*p* < 0.001).

The OSTT provides mechanistic information distinct from the OGTT, as sucrose - a disaccharide - was used as the carbohydrate load, whereas the OGTT employed glucose, a monosaccharide. The intestinal absorption of sucrose requires prior hydrolysis of the α-glycosidic bond between glucose and fructose, a process mediated by α-glucosidases. Inhibition of these enzymes prevents sucrose cleavage and consequently limits glucose absorption (18).

The pronounced and sustained activity observed with the ME in the OSTT suggests the presence of metabolites capable of reducing postprandial glycaemia, likely through inhibition of α-glucosidase activity. This mechanism plausibly explains the extract's ability to attenuate the hyperglycaemic peak following sucrose administration. To the best of our knowledge, this represents the first report of α-glucosidase-related antihyperglycaemic activity for the genus *Odontonema*, and specifically for *O. callistachyum.*

Although all extracts exhibited antihyperglycaemic effects in the OGTT, clear differences in efficacy emerged when sucrose was used as the carbohydrate challenge. The superior and selective performance of the ME in the OSTT supports a mechanism involving inhibition of carbohydrate hydrolysis rather than direct modulation of glucose uptake. Consequently, the methanolic extract was prioritised for further evaluation in the NIDD model and in the *ex vivo* α-glucosidase inhibition assay.

Acute antidiabetic assay in a non-insulin-dependent diabetes (NIDD) mouse model

To further investigate the antihyperglycaemic effects observed in normoglycaemic animals, the most active extracts were evaluated in a mouse model of non-insulin-dependent diabetes (NIDD). Based on their efficacy in the OGTT and OSTT, the dichloromethane extract (DE) and methanolic extract (ME) were selected, as both demonstrated an early glucose-lowering effect by attenuating the maximum postprandial peak.

In contrast to the OGTT and OSTT, the acute NIDD assay was performed in hyperglycaemic mice without administration of an exogenous carbohydrate load. Blood glucose levels were therefore intrinsically elevated at baseline. The antidiabetic effect was assessed at a dose of 100 mg/kg over a 7-h period. Figure 3 presents the percentage glucose variation (%GV ± SEM) over time.

The DE produced a significant reduction in %GV at 5 h (%GV = −66.99 ± 8.22%) and 7 h (%GV = −73.39 ± 9.01%) compared with the vehicle group (%GV = −9.50 ± 3.01% at 5 h; −22.00 ± 7.00% at 7 h; *p < 0.05). In contrast, the ME did not induce a significant reduction in %GV during the first hour post-administration (%GV = 0.05 ± 15.62% at 1 h). However, from 3 h onwards, ME produced a sustained and significant decrease in %GV relative to the vehicle group (*p < 0.05). No statistically significant differences were observed between the DE and ME groups at corresponding time points.

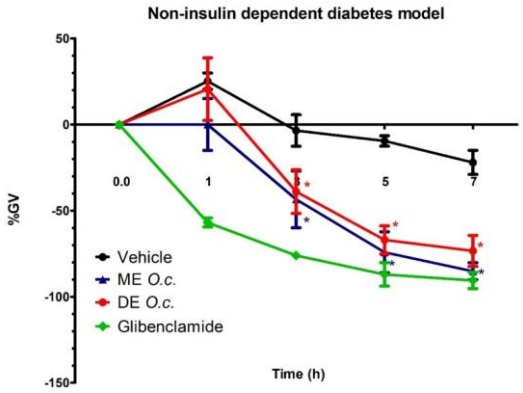

Figure 3.
Acute antidiabetic assay in NIDD mice treated with extracts and controls. Data are presented as mean ± SEM (*p < 0.05 *vs.* Vh)

The results obtained in the NIDD model suggest that metabolites of intermediate polarity present in the DE may contribute to the reduction of hyperglycaemia under diabetic conditions. The glucose-lowering effect observed for DE, together with its limited activity in the OSTT, indicates that its mechanism of action is unlikely to be related to α-glucosidase inhibition. Instead, an extra-pancreatic mechanism, such as improved peripheral insulin sensitivity or modulation of hepatic glucose output, may be involved.

In contrast, the ME exhibited antihyperglycaemic activity in the OGTT and a pronounced effect in the OSTT, supporting a mechanism that involves inhibition of carbohydrate hydrolysis through α-glucosidase modulation. The delayed yet sustained reduction in %GV observed in the NIDD model further suggests that ME may exert its antidiabetic effect through a combination of mechanisms, including α-glucosidase inhibition and additional extra-pancreatic actions.

Based on its consistent antihyperglycaemic profile across the OGTT, OSTT and NIDD models, the methanolic extract was selected for further mechanistic evaluation in the *ex vivo* α-glucosidase inhibition assay.

3.6. α-glucosidase *ex vivo* inhibition assay

In all experimental conditions, sucrose was present as the common substrate within the intestinal lumen. Variations in optical density over time therefore reflect relative changes in glucose release resulting from α-glucosidase-mediated sucrose hydrolysis. As the Fehling reaction selectively detects reducing sugars, changes in absorbance are attributable to glucose formation rather than to the presence of sucrose itself. Accordingly, %GV allows direct comparative assessment of enzymatic activity between control, extract-treated (*O. callistachyum*), and acarbose-treated groups.

The ME of *O. callistachyum* was evaluated using the mouse small intestine *ex vivo* α-glucosidase inhibition assay. The results are presented in Figure 4.

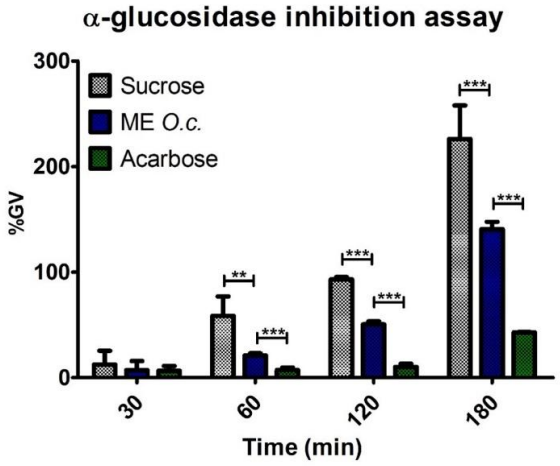

Figure 4.
Ex vivo intestinal α-glucosidase inhibition in mice treated with methanolic extract and controls. Data are presented as mean ± SEM (**p < 0.01, ***p < 0.001)

Sucrose hydrolysis increased progressively over time, with higher glucose levels detected in the medium as the experiment proceeded. Glucose concentrations remained low during the first 30 minutes, with no significant differences between groups. After 60 minutes, statistically significant differences were observed between the vehicle control and ME, as well as between ME and acarbose, indicating that the ME inhibits α-glucosidase activity, albeit to a lesser extent than acarbose. At 180 minutes, acarbose exhibited approximately 3.3-fold greater potency than the ME. The positive control behaved as expected, confirming the assay's sensitivity.

These results suggest that the polar methanolic extract of *O. callistachyum* contains metabolites capable of inhibiting α-glucosidase, consistent with the antihyperglycaemic effects observed in the OSTT and supporting the proposed mechanism of action through modulation of intestinal carbohydrate hydrolysis.

Acute toxicity in mice

Acute toxicity of the ME was evaluated in female mice following OECD guideline No. 423. After a single oral dose of 2,000 mg/kg, observations were recorded at baseline, 20 minutes, 4 hours, and 14 days post-administration. No significant differences were observed between the treated and untreated groups (data not shown due to lack of relevance).

None of the 22 evaluated parameters showed significant differences compared with the untreated group. These results suggest that ME at a dose of 2,000 mg/kg did not affect mouse behaviour. Quantitative analyses, including body weight variation (%WV), were performed in addition to qualitative observations. The mean %WV ± SEM values are presented in Table II.

Table II

Percentage of weight variation ± SEM

Time	Non-treated group	Treated group
First day of administration	0%	0%
1st week after administration	10.92% ± 4.47%	6.41% ± 1.28%
2nd week after administration	5.46% ± 2.23%	11.54% ± 0%

Analysis of %WV revealed no significant differences between the treated and untreated groups. The treated group exhibited a weight gain of 11.54% after two weeks, whereas the untreated group showed a weight loss of 5.46 ± 2.23% during the second week. Although the small sample size of the untreated group may have increased the standard error, no substantial differences were observed between groups.

Additionally, serum transaminase levels were compared between the untreated group and the ME-treated group (2,000 mg/kg) (Figure 5). The AST and ALT enzyme values in the acute toxicity model are shown in Figure 5. Each group is presented as the mean ± SEM, and statistical analysis was performed using Student's *t*-test

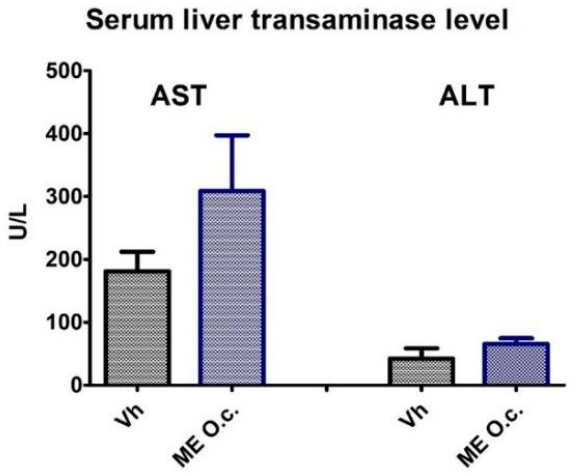

Figure 5.

Enzymatic AST and ALT values of the groups used in the acute toxicity model

Statistical analysis revealed no significant differences in AST and ALT levels between groups; however, a trend towards increased transaminase levels was observed. Reported reference values for AST and ALT in mice are comparable to those observed in rats (AST = 99.8 ± 21.51%, ALT = 48.3 ± 8.20%)

(19). Quantification of liver enzymes in mouse serum revealed increases of 1.7-fold ULN for AST and 1.6-fold ULN for ALT.

Gross necropsy examination (Figure 6) revealed fluid accumulation in the gallbladder, which has been described as cholestasis and can be classified as intrahepatic or extrahepatic depending on the site of the disorder (20, 21).

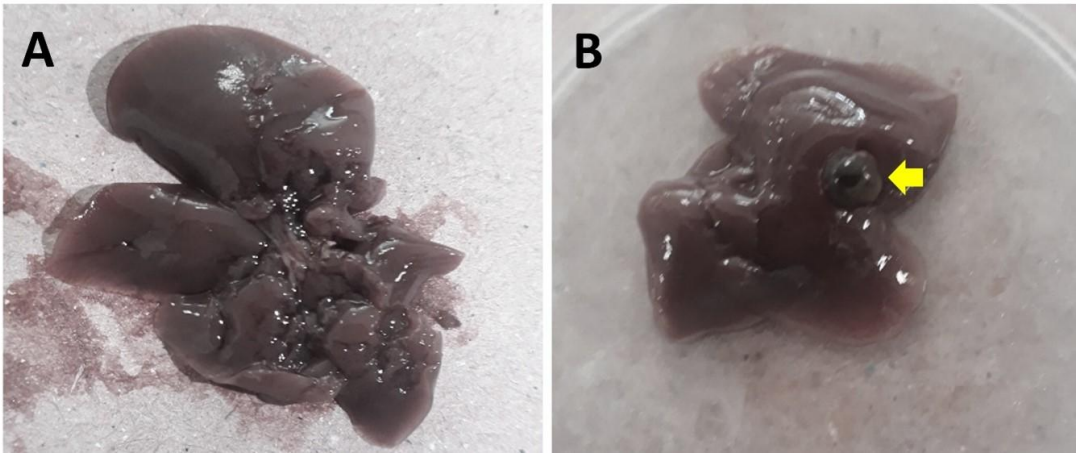

Figure 6.
Liver from the untreated group; B. Liver from the treated group (2,000 mg/kg, PO). The yellow arrow indicates potential cholestasis

Overall, toxicological evaluation indicated an elevation of serum transaminases to > 1× ULN but < 2× ULN, together with early signs of cholestasis. AST and ALT were assessed due to their clinical relevance, as increased serum levels may indicate tissue damage. AST is present in several tissues, including the liver, kidney, pancreas, brain, skeletal muscle, and cardiac muscle (22). ALT is more specific for liver injury, and elevated transaminase levels are commonly associated with toxic hepatitis (23). Drug-induced liver injury (DILI) can be identified during the drug research and development process (24). Although most drugs that cause severe DILI do so infrequently, many compounds may induce mild and transient liver injury, characterised by enzyme leakage and increases in serum aminotransferase activity, typically reaching three to five times, or occasionally more than five times, the ULN. ALT is generally considered more liver-specific than AST; however, a higher incidence of ALT elevation in treated subjects compared with controls is considered a sensitive, though not specific, indicator of potential severe DILI (25,26).

Cholestasis observed after treatment represents a clinical manifestation resulting from bile accumulation in the liver, blood, and other tissues, caused by impaired bile flow from the liver to the duodenum. Factors associated with cholestasis include drug toxicity, infections, tumour-related diseases, and viral hepatitis. Clinical manifestations may include jaundice, pruritus, diarrhoea, weight loss, dark urine, and pale stools (21,25). In the present study, cholestasis was observed in the treated group (2,000 mg/kg, PO); however, the mice did not exhibit clinical signs or symptoms associated with this condition. This may be attributable to the single acute dose administered. Further investigation using repeated dosing at the effective dose (100 mg/kg) is therefore recommended. The ME of *O. callistachyum* was not toxic following a single oral dose of 2,000 mg/kg and was classified as Category 5 according to OECD guideline No. 423 (15).

Conclusions

In the OGTT performed in normoglycaemic animals, all three extracts exhibited antihyperglycaemic effects. In the OSTT, the ME demonstrated a significant reduction in percentage glucose variation (%GV) from 30 minutes onwards compared with the vehicle, and this effect was maintained throughout the experimental period. In the acute test using the NIDD model, the ME produced a statistically significant decrease in %GV from the third hour relative to the vehicle, whereas the DE showed a significant reduction from the fifth hour. Although both extracts displayed antidiabetic activity, the ME exhibited greater efficacy. Furthermore, the *ex vivo* α-glucosidase inhibition assay revealed reduced glucose concentrations in the extraluminal medium, suggesting an inhibitory effect on intestinal carbohydrate-digesting enzymes.

In the acute toxicity assessment, the ME was not toxic following a single oral dose of 2,000 mg/kg and was classified as Category 5 according to OECD guideline No. 423, as no fatalities were observed. Behavioural observations conducted at baseline, 20

minutes, 4 hours, and 14 days post-administration revealed no significant alterations in the treated group compared with untreated controls, and no statistically significant changes in body weight were detected. Serum AST and ALT levels did not differ significantly between treated and untreated animals. However, gross necropsy examination revealed signs of cholestasis.

Overall, this preliminary study demonstrates the antidiabetic potential of *Odontonema callistachyum* extracts, particularly the ME. Nevertheless, further phytochemical investigations are required to identify the bioactive compounds responsible for the observed effects. In addition, comprehensive toxicity studies at therapeutically effective doses are warranted to confirm the safety profile and support the potential development of these extracts as antidiabetic agents.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article. The other data generated in the present study may be requested from the corresponding author.

Ethics approval and consent to participate

All experimental procedures were approved by the Research Ethics Committee of the Universidad de Quintana Roo (Project: ZS/PI-13/22; Ethical approval: CEI/DICT/005/2022)

AI use disclosure

During the preparation of this manuscript, the authors used ChatGPT for editing assistance and language improvement, specifically to improve spelling, grammar, word choice, readability, and clarity. The authors reviewed and revised all outputs from these tools and take full responsibility for the final content of the work.

Conflict of interest

The authors declare no conflict of interest.

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