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Cassia grandis fruit extract reduces the blood glucose level in alloxan-induced diabetic rats

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ABSTRACT

Introduction: *Cassia grandis* Lf fruits are ethnobotanically used for digestive disorders, anemia, and for reducing blood glucose. However, there are no studies about the antidiabetic activity nor the oral toxicity of the plant fruit-extracts. This paper aims to evaluate the hypoglycemic effect of *C. grandis* fruits extract *in vivo,* and assess the acute oral toxicity, and sub-acute oral toxicity. The antioxidant activity and the α-glycosidase inhibitor effect were also evaluated.

Methods: The extract was obtained by maceration of the fruit pulp with 70% hydroalcoholic solution (1:2, m:v). The extractive solution was concentrated in a vacuum rotary evaporator, up to a drug: solvent ratio of 2:1 (g/ml). Soluble solids, relative density, refractive index, pH, total phenolics, and flavonoids were determined. A preliminary phytochemical screening was made, followed by the quantitation of volatiles by GC/MS. The acute and sub-acute oral toxicity was evaluated in Sprague Dawley rats, by using biochemical and hematological parameters. The radical scavenging activity (DPPH·, ABTS·) and α-glycosidase inhibitory effect were tested. The hypoglycemic effect was assessed in alloxan-induced diabetic rats.

Results: The extract of *C. grandis* contains alkaloids, coumarins, flavonoids, free amino acids, amines, phenols, tannins, reduced sugars, resins, saponins, steroids, and triterpenes, plus 38 volatile compounds, being linalool the most abundant (1,66%). The extract exhibited an LD_{50} > 2000 mg/kg, and after a continuous administration (1000 mg/kg, 28-days), the hematological and biochemical parameters were normal. The extract showed hypoglycemic effect, being the dose 200 mg/kg no statistically different from glibenclamide at 25 mg/kg. Good antioxidant activity and a potent α-glycosidase inhibitory effect were also observed.

Conclusion: *C. grandis* extract is an excellent hypoglycemic and non-toxic plant product. The hypoglycemic mechanism could be associated with the antioxidant effect and with the α-glycosidase inhibition. Up to the best of our knowledge, this is the first report on the hypoglycemic effect *in vivo* of *C. grandis* fruits extract.

1. Introduction

Diabetes mellitus is a metabolic disease widespread all over the world, characterized by excess in blood glucose and changes in the carbohydrate metabolism. The global prevalence of diabetes among adults over 18 years in 2014 was 4%, and it was estimated at 5.22% (366 million of people) for 2030 [1]. Despite the progress of the diabetes management, it is still not possible to avoid its lethal consequences. However, antidiabetic drugs treatments are reduced [1,2]. For this reason, many people use medicinal plants for lowering the

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blood glucose levels.

Various species of the genus Cassia are used by diverse populations to treat diabetes [3,4], the hypoglycemic effect of some species has been confirmed *in vivo* [5–8]. Inhabitants of Central America, the Caribbean, and South America use the fruit pulp of *Cassia grandis* Lf for digestive disorders, anemia [9], and diabetes [10,11]. The species *Cassia grandis* (Cañandonga, Cassia Rosa, Cassiagrande), belongs to Fabaceae family, subfamily Cesalpinaceae. The stem bark extract of *Cassia grandis* reduces the blood glucose in normal rats and diabetic rats [12]; however, removing the stem bark of plants can cause severe damages. Contrarily, the fruits are harvested twice a year, producing a considerable amount of pulp. The fruit pulp has a characteristic smell and strong sweet flavor. Additionally, the ripe fruit contains phenols, flavonoids, coumarins, terpenes, reducing sugars, amino acid, amines, saponins, and glycosides [13]. Some of these metabolites could explain the ethnobotanical use of this plant fruit.

The hydroalcoholic extract of *C. grandis* fruits is non-genotoxic in mice [14]. Nonetheless, there is neither acute oral toxicity nor subacute oral toxicity studies of the *C. grandis* fruit extract (CgE). In the same way, the hypoglycemic effect was never assayed *in vivo*. Thus, this paper aims to evaluate the hypoglycemic potential of the CgE in alloxan-induced diabetic rats and assess the acute and 28-day sub-acute oral toxicity in Sprague-Dawley rats. The antioxidant activity and the α-glycosidase inhibitory activity were also evaluated. Additionally, the extract was characterized, using spectrophotometric techniques, and GC/MS, to obtain a vegetal product that could be used in pharmaceutical preparations.

2. Materials and methods

2.1. Plant material

Fruits of *C. grandis* L. f were collected in El Caney (20.0569 N; −75.7719 W), Cuba, in April 2016. A sample of the species was identified by Félix Acosta Cantillo and was deposited in the Eastern Center of Ecosystems and Biodiversity herbarium (BIOECO), in Santiago de Cuba, Cuba, with voucher No.1965.

2.2. Extract preparation

The ripe fruit pulp (1 kg) was macerated with 2 l of 70% hydroalcoholic solution for 72 h, at room temperature [15]. After that, the extract was filtered at vacuum and concentrated using a rotary evaporator at 40 °C (KIKA WERKE GMBH & Co. Germany). The final drug/solvent ratio was 2:1 (w/v) [16]. CgE was poured into an amber flask and stored protected from light, at 25 ± 2 °C.

2.3. Phytochemical evaluation

A preliminary phytochemical screening was made, to detect the presence of metabolites such as mucilage (polysaccharide), saponins, alkaloids, triterpenes, steroids, quinones, coumarins, resins, essential oils, reducing sugars, phenols and tannins, flavonoids, free amino acids, amines, and glycosides [16].

Total solids, relative density, and the refractive index of the CgE were determined [17]. The pH was directly measured using a pH-meter (Tecnopon, Brazil) previously calibrated with buffer solutions (pH 4 and 7, Alphatec, Brazil). Total phenolics as pyrogallic acid and Total flavonoids as rutin were spectrophotometrically determined [18]. All the measures were made in triplicate.

2.4. Gas chromatography/mass spectrometry analysis

Three successive extractions from 100 ml of CgE were performed, using a separatory funnel, and ethyl ether as solvent (15 mL). The extracts were pooled and dried for 12 h over anhydrous sodium sulfate. After that, the ethereal extract was concentrated to 10 ml under a nitrogen stream. The volatile composition was performed by GC/MS (SHIMADZU GCMS-QP500, Japan). A DB-5MS capillary column (Agilent Technologies, USA) of 30 m × 0.32 m and 0.25 mm thick film was used. The temperature was programmed at 40 °C for 3 min, with an increase of 5 °C/min up to 250 °C. In the end, the temperature was kept constant at 250 °C. The mass spectra were recorded over 60–260 AMU range, with ionization energy of 70 eV. The injection volume was 10 μL with a split ratio of 100:1. Helium was used as the carrier gas at a flow rate of 0.5 mL/min. Both, the injector and detector temperatures were kept at 280 °C. The constituents were identified by comparing the retention Kovac index and the mass spectrum with the indexes and reference recorded in the FFNSC 1.3 library and NIST database. The percentage composition was calculated using the peak normalization method. Results were expressed in g/100 ml of extract.

2.5. Evaluation of acute oral toxicity

Female Sprague-Dawley rats, nulliparous of 10 weeks old, with body weight between 150–200 g, were used. Animals were supplied by the National Center for Production of Laboratories Animal (CENPALAB), Havana City. They were randomly grouped (Two groups, 5 animals each) and marked to allow the individual identification. Before the administration, animals were acclimatized for seven days at a controlled temperature of 23 ± 2 °C, relative humidity of 60 ± 10% and a 12/12 h light/dark cycle [19]. Animals had free access to food (standard chow CMO-1000) and distilled water. All the tests were conducted agreeing to the Good Laboratory Practice. The ethical considerations established by the Ethics Committee of the Toxicology and Biomedicine Center (TOXIMED), Medical University of Santiago de Cuba, Cuba, were taken into account.

The first day, after a fasted period of 12 h, the animals were weighed. After that, the Group I (Control) received distilled water (2 mL) and the Group II received 2000 mg/kg [20] of CgE in one dose, dissolved in distilled water (up to 2 mL). An intragastric cannula (Vygón, France) was used for administration. Three hours after, the food and water access were restored.

2.5.1. Gross observation, food and water intake, and relative organ weight

The animal's behavior was recorded the first 4 h after the administration, and occasionally along the rest of the day. The record was made twice a day for the next 14 days. Clinical observations were made to evaluate the physical condition and the nasal-ocular mucosa. Particular attention was paid to possible changes in the skin and coat, the respiratory rhythm, nervousness and possible death occurrence. Daily, the food and water intake were recorded [19,20]. The day 14, animals were euthanized using a ketamine overdose (ip) and subjected to a gross necropsy for detecting possible pathological changes. The kidney, lung, liver, pancreas, vessel, spleen, stomach, intestines, and heart were removed, rinsed with distilled water, dried with filter paper, and weighed. Macroscopic observations were made for detecting morphological changes/and or anatomical alterations. The relative organ weight (ROW) in percent, was calculated (Organ weight ×100/ Animal body weight).

2.6. Evaluation of 28-dayssub-acute oral toxicity

Female Sprague-Dawley rats, 14 weeks old, body weight 225–265 g were used. The Pharmaceutical Research Laboratory, Federal University of Amapá, Brazil, supplied the animals. They were acclimatized for a week at controlled temperature (22 ± 3 °C), relative humidity (60 ± 10%), and a 12/12 h light-dark cycle. The feed was standard chow (NUVILAB MCP 689) and distilled water, *ad-libitum*. Ethical considerations established by the Ethics Committee of the Federal University of Amapá (Protocol Number 012/2017), were taken into account.