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Original Article

HYPOGLYCEMIC ACTIVITY OF *COPERNICIACERIFERA* MART.L.EAF POWDER EXTRACT IN THE TREATMENT OF ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

Objective: This study characterize esters from carnauba crude powder and evaluates its hypoglycemic effect on the treatment of diabetic animals.

Methods: Groups of diabetic mice were induced by alloxan and treated with Carnauba crude powder. Blood samples were collected to determine the glucose serum level.

Results: The compound was identified and characterized as diester of the 4-methoxycinnamic acid (PCO-C) and presented the hypoglycemic effect in the concentrations of 100 and 150 mg/kgbody weight (b.w.) the concentration 150 mg/kg b.w..o.f PCO-C presented the best effect on controlling glucose levels ($p<0.05$),when compared to the reference drug.

Conclusion: The results indicate that the PCO-C is a promising therapeutic compound with hypoglycemic effect. This action can be justified by the presence of the diester of 4- methoxycinnamic acid.

Keywords: Carnauba wax, Cinnamic acid esters, Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone [1]. Complications from DM, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society [2].T.he total number of people with diabetes is projected to rise to366 million in 2030, for all age-groups worldwide. DM is a major public health problem in the 21st century [3].

Despite great efforts invested in diabetes research, its prevalence continues to grow, while current medications do not cover all of the symptoms and complications of the disease [4]. The use of medicinal plants is an important alternative in the treatment of diseases, especially in developing countries [5]. Several plant species have been pharmacologically or experimentally used to treat of DM [6, 7]. Nowadays, the main substances isolated from plants with hypoglycemic action are: terpenoids, alkaloids, coumarins, flavonoids and phenolic substances [8].

The *Copernicia cerifera* Mart, popularly known as Carnauba, Carnaubeira or Carandá, is a native palm tree of the semi-arid region of Brazilian northeast, mainly in the States of Ceará and Piauí.T.he most used product of the carnauba,the wax that covers the leaves, has valuable compounds useful for the pharmaceutical, cosmetic and food industries [9, 10, 11, 12]. Under the chemical point of view the carnauba wax is composed of a mixture of substances, mostly esters [13, 14]. Several studies have shown the hypoglicemic potential of esters [15, 16]. Thus, this study aimed to characterizeesters from the carnaúba leaf powder and evaluates its hypoglycemic activity potential. The Laboratory of Human Biochemistry, at the State University of Ceará has been evaluating the potential of extracts and substances derived from the Brazilian Northeastern flora, especially those with hypoglycemic, hypolipidemic, anti-inflammatory, antimicrobial and antioxidant activities. In this study, the hypoglycemic effect of PCO-C was evaluated due to its proximal structure to esters of gamma-oryzanol, which is reported to have hypoglycemic activity in the literature.

MATERIALS AND METHODS

Carnauba Leaf Powder Extract

The carnauba powder from unopened leaves of the bud of *Copernicia cerifera* Mart. was obtained from Pontes Indústria de Ceras Ltd a forty grams (40g) ofCarnauba powder were extracted by soaking in 500ml* of heptane: ethylene dichloride (90:10) at room temperature.T.he extract was filtered and evaporated in a rotary evaporator and the residue was stored at 2-8 °C until use.

The Carnauba powder extract obtained was placed on a column (46cm* x 3.8cm*) of Florisil, 400g (MERCK 60-100 meshes), and eluted with 2500ml* of the same solvent solution. The total elate was then run into a similar column of activated silica gel and after the column was washed with the addition 500ml* of the solvent. Mixture of the recovered waxy material showed absorption similar to that of aliphatic esters. The silica gel was stripped of the adsorbed residue with 2000ml* of 10% isopropanol in heptane. Upon concentration of the elute a yellowish solid was obtained [17, 18] and it was named PCO-C.

Characterization of the PCO-C

FTIR- measurements were performed with a PEKEIN ELMER FTIR instrument. NMR spectra were recorded with a Bruker DRX500 spectrometer at 500 MHz (^1H) using $CDCl_3$ as solvent. TLC analyses were made on Silica Gel 60 F_{254}, (Merck) plates using heptane: ethyl acetate (1:1) as mobile phase, and purity of the compounds was detected by spraying with 5% phosphomolybidic acid solution followed by heating at 120°C.

Acute Oral Toxicity Study

PCO-C at the dose range of 500-2000 mg/kg b.w. was orally administered to mice and mortality was observed after 14 days. Acute toxicity was determined in accordance with the method provided by the Organization for Economic Cooperation and Development (OECD) guidelines 425 [19].

Experimental animals

Healthy male Swiss albino mice (*Musmusculus*), aged approximately seven to eight weeks (weighing 20.0-25.0 g), were used in this study. The mice were housed in polypropylene cages, maintained under standard laboratory conditions, (i.e. 12:12 hour light and dark cycle; at an ambient temperature of 25 ± 3°C). Water and food were given *ad libitum* to the animals. The institutional ethics committee on the care and use of animals for experimentation approved the experimental protocols (nº.90/10).

Induction of experimental diabetes

Diabetes was induced in mice after fasting by a single intra-peritoneal injection of alloxan monohydrate, 150 mg/kg b.w. , freshly dissolved in cold sterile normal saline [7]. After 7 days, diabetes was confirmed by measuring the serum glucose level. Animals with blood glucose levels of more than 180 mg/dl were considered diabetic.

According with Jain and Arya [20] and Misra and Aiman [21], there are many inconsistencies and anomalies in alloxan-induced diabetic models. These observations have been reported in others studies, which indicate that alloxan-induced diabetic model is a doubtful model for antidiabetic studies. Hence, further studies are needed to evaluate an antidiabetic ideal model. This, however, remains contradictory to many other studies where alloxan has been successfully used, included the present study.

Experimental Protocol

Animals were divided into five groups of 12 mice each. The animals of all groups received the doses orally for 21 consecutive days, according to the adapted protocol [22].

Group 1: mice served as normal-control and received the vehicle (0,2 ml distilled water/day/mice);

Group 2: mice served as diabetic-control and received the vehicle (0,2 ml distilled water/day/mice);

Group 3: mice diabetic were administered PCO-C (100mg/kg b.w. /day) in distilled water as a fine aqueous suspensor orally;

Group 4: mice diabetic were administered PCO-C (150mg/kg b.w. /day) in distilled water as a fine aqueous suspensor orally;

Group 5: mice diabetic were administered Glibenclamide (10mg/kg b.w. /day) in distilled water as a fine aqueous suspensor orally.

Determination of blood glucose

Blood samples were collected from the retro-orbital plexus with the use of capillary tubes under mild ether anesthesia from overnight fasted mice. Blood glucose levels were analyzed on days 0, 10 and 21 of the study, which was performed by the machine METROLAB 2300 PLUS VERSION 1.7, which uses the kinetic method for the analysis of serum samples.

Statistical analysis

The data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) and Student-Newman-Keuls test was carried out for comparing results among treatments. The significant level was set at $p<0.05$

RESULTS

The PCO-C was obtained and characterized according to previously described methodology [17, 18], with 7% yield in relation to the carnauba powder. The PCO-C was identified as diester of the 4-methoxycinnamic acid by means of infrared spectroscopic analysis (FTIR), thin layer chromatography (TLC) melting point 94-95 °C, ^{1}HNMR and comparisons to the literature data [17, 18]. By the analysis of FTIR spectra of PCO-C, was possible to confirm the presence of the following characteristic functional groups: ester (1735 cm^{-1}, 1717 cm^{-1}, 1169 cm^{-1}), unsaturation (1630 cm^{-1}, 930 cm^{-1}), aromatic p-substituted (827 cm^{-1}) and the aromatic *p*-methoxy (1020 cm^{-1}).T.LC analysis of PCO-C and its hydrolysis products (saponifiable and unsaponifiable material) corroborate the results obtained with FTIR of PCO-C,and in the chromatogram obtained with saponifiable material was confirmed that this material contains 4-methoxycinnamic acid (RF 0.41) and its presence in thePCO-C structure was confirmed by ^{1}HNMR spectra analysis (Table 1 and Figure 1).

Table 1: Assignments of 1H NMR chemical shift (ppm) of PCO-C (diesters of 4-methoxycinnamic acid)

Chemical Shift (ppm)	Assignments
7.62	8 (d, 1 H, 16 Hz)
7.45	5, 3 (m, 2 H)
6.85	6, 2 (m, 2 H)
6.33	9 (d, 1H, 16Hz)
3.83, 3.90	7 (s, 3H)

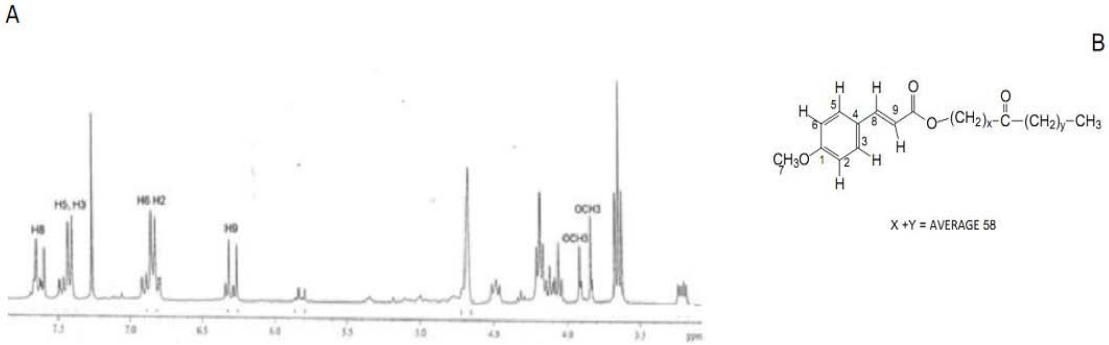

Fig. 1: (A) ^{1}HNMRSpectra of PCO-C (Expansion δ 3.32 - 8.08, CDCl3, 500.13 MHz) and (B) Structure of the diesterof4-methoxycinnamic acid.

A toxicology study of the PCO-C (diesters of 4-methoxycinnamic acid) was performed and, it was concluded that the maximum safe dose of PCO-C was 2000 mg/kg b.w. For the study of the PCO-C hypoglycemic effect, a range of 50-500 mg/kg b.w. Was used and the results showed that 100 and 150 mg/kg b.w. Doses had hypoglycemic action similar to the action of the reference drug (glibenclamide). The results of acute toxicity tests with mice showed that orally administration of PCO-C doses up to 2000mg/kg did not cause any adverse effects or mortality. The present study clearly indicates that the PCO-C (diesters of the 4-methoxycinnamic acid)has hypoglycemic effect on alloxan induced diabetic mice (Table 2). The administration of the PCO-C to diabetic mice resulted in a significant decrease ($p< 0.05$)in the blood glucose levels. It was found that 100 mg/Kg b.w. and 150 mg/kg b.w. Doses of PCO-C result in 30.5%and 41.5% blood glucose reduction, respectively. In the PCO-C treated mice, hypoglycemic effect was evident from the

10th day onwards; the decrease in blood glucose levels was highly pronounced on the 21st day. The hypoglycemic effect of PCO-C at 150 mg/kg b.w. dose was more prominent than glibenclamide.

DISCUSSION

According with the results of previously work [17, 18]and upon spectroscopic analysis including FTIR, ^{1}HNMR and melting point, the yellowish solid named PCO-C was characterized as esters of the 4-methoxycinnamic acid (fig. 1b). Ith as been reasonably well established that these substituted cinnamic acids occur predominantly as adiesters.

The PCO-C was effective in decrease blood glucose levels of diabetic mice. Previous studies have shown that substances with similar chemical structure to esters of cinnamic acid, such as the gamma-oryzanol and the policosanol, possess antioxidant, hypolipidemic and hypoglycemic properties [23-26]. The gamma-oryzanol, an important phytochemical of the rice brain, is a complex mixture of esters of the ferulic acid with triterpenic alcohols and sterols[27-29]. Study showed hypoglycemic effect of gamma-oryzanol on diabetic patients. This compound was able to reduce the concentration of fasting blood glucose in 29% and 33% of the patients with DM1 and DM2, respectively [30].

Policosanol is a complex mixture of long-chain primary alcohols and it isolated from the sugarcane wax [25]. Another study demonstrated that policosanol significantly reduced LDL - cholesterol (27.4% and 28.1%), total cholesterol (27.1% and 27.5%) and increased serum levels of HDL - cholesterol (17,6% and 17%) at doses of 20 and 40 mg / kg, respectively [26].

Table 2: Effect of PCO-C (diester of the 4-methoxycinnamic acid) on blood glucose levels in normal and diabetic mice

Groups	Dose (mg/Kg)	0 Day	10 Days	21 Days
I Normal control	-	98.6 ± 8.1	100.1 ± 9.7	100.1 ± 9.7
II Diabetic control	-	251.4 ± 26.3	241.2 ± 20.6	326.1 ± 31.0*
III Diabetic+PCO-C	100	210.6 ± 23.0	151.7 ± 57.3*	146.4 ± 31.8*
IV Diabetic+PCO-C	150	234.7 ± 23.7	174.2 ± 30.0*	137.2 ± 29.4*
V Diabetic+Glibenclamide	10	209.4 ± 33.9	110.8 ± 18.1*	147.7 ± 44.1*

Values expressed as means ± standard deviation (n=7), *P* values were analyzed using One-Way ANOVA followed by Newman-Keuls test. *p<0.05, when groups III, IV and V compared with diabetic control, i.e. group II.

CONCLUSION

In conclusion, our results showed that the use of PCO-C (diester of the 4-methoxycinnamic acid) has a wide margin of security risk and showed hypoglycemic effect in diabetic mice. These results are very promising and show that carnauba (*Coperniciacerifera* Mart), a native plant of the Caatinga biome, constitutes a rich source of active compounds for the treatment of Diabetes Mellitus.

CONFLICT OF INTERESTS

Declared None

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REFERENCES

1. Shrivastava RBL, Shrivastava PS, Ramasamy J. Role of self-care in management of diabetes mellitus. J Diabetes Metabolic Disorders 2013;12(14):1-5.
2. Tabish SA. Is diabetes becoming the biggest epidemic of the twenty-first century? Int J Health Sci 2007;1(2):5-8.
3. Wild S, Roglic G, Green A. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047-53.
4. Harley E, Mirsky N, Ofir R. Antidiabeticatributes of desert and steppicplantts: a review. Planta Med 2013;79(6):425-36.
5. OkpuzorJ, Adebesin O, Ogbunugafor H, Amadi I. The potential of medicinal plants in sickle cell disease control: a review. Int J Biom Health Sci 2008;4(2):47-55.
6. Leila Z, Eliandra DS, Luisa HC, Anildo CJ. Effect of crude extract and fractions from Vitexmegapotamica leaves on hyperglycemia in alloxan diabetic rats. J Ethnopharmacol 2007;109(1):151-55.
7. Katiyar D, Singh B, Lall AM, Hafiday of Ef chitooligosaccharides for the management of diabetes in alloxan induced mice: a correlative study with antihyperlipidemic and antioxidative activity. Eur J Pharm Sci 2011;44(4):534-43.
8. Negri G. Diabetes melito: plantas e princípios ativos naturais hipoglicemiantes. Rev Bras Ciênc Farm 2005;41(2):121-42.
9. Dang KH, Singh Z, Swinny EE. Edible coatings Influence fruit ripening, Quality, and aroma biosynthesis in mango fruit. J Agric Food Chem 2008;56(4):1361-70.
10. Arias MA, Loxley A, Eatmon C, Roey GV, Fairhurst D, Mitchnick M, *et al*. Carnauba wax nanoparticles enhance strong systemic and mucosal cellular and humoral immune responses to HIV-gp140 antigen. Vaccine 2011;29(6):1258-69.
11. Barman K, Asrey R, Pal RK. Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage. Sci Hortic 2011;130:795-800.
12. Jo WS, Song HY, Song NB, Lee JH, Min SC, Song KB. Quality and microbial safety of 'Fuji' apples coated with carnauba-shellac wax containing lemongrass oil. LWT-Food Sci Technol 2014;55(2):490-97.
13. Ullmann F, Gerhartz W, Yamamoto YS, Campbell FT, Pfefferkorn R, Rounsaville JF. Ullmann´s Encyclopedia of Industrial Chemistry. Frankfurt, Germany: VCH-Verlagsgesellschaft;1996.
14. Hwanga KT, Cuppett SL, Wellerc LC, Hannad AM. HPLC of grain sorghumwax classes highlighting separation of aldehydes fromwax esters and steryl esters. J Sep Sci 2002;25(9):619-23.
15. Weitzel G, Fretzdorff AM, Stock W. Syntheses and hypoglycemic activities of ethyl esters and various amides of omega-guanidino fatty acids with medium chain length. H Oppe Seylers Z Physiol Chem 1980;361(1):41-50.
16. Malaisse W, Lebrun P. Mechanisms of sulfonylurea-lnduced insulin release. Diabetes Care 1990;13(3):9-17.
17. Vandenburg LE, Wilder EA. Aromatic acids of carnauba wax. J Am Oil Chem Soc 1967;44(11):659-62.
18. Vandenburg LE, Wilder EA. The structural constituents of carnauba wax. J Am Oil Chem Soc 1970;47(12):514-18.
19. Organization for Economic Cooperation and Development (OECD) guidelines for acute toxicity of chemicals. No. 425 (Adopted: 3 October 2008).
20. Jain DK, Arya RK. Anomalies in alloxan-induced diabetic model: it is better to standardize it first. Indian J Pharmacol 2011;43(1):91.
21. Misra M, Aiman U. Alloxan: an unpredictable drug for diabetes induction? Indian J Pharmacol 2012;44(4):538-39.
22. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridaxprocumbens (Linn.). BMC Complement Altern Med 2009;9(48):1-7.
23. Kim JS, Godber JS, King J, Priyawiwatkul W. Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran in an aqueous model system. J Am Oil Chem Soc 2001;78(7):685-9.

24. Fang N, Yu S, Badger TM. Characterization of triterpene alcohol and sterol ferulates in rice bran using LC-MS/MS. J Agric Food Chem 2003;51(11):3260-67.
25. Berthold HK, Unverdorben S, Degenhardt R, Bulitta M, Gouni-Berthold I. Effect of policosanol on lipid levels among patients with hypercholesterolemia or combined hyperlipidemia: a randomized controlled trial. Jama 2006;295(19):2262-9.
26. Castaño G, Mas R, Fernandéz R, Ilinait J, Gámez R, Alvarez E. Effects of policosanol 20 versus 40 mg/day in the treatment of patients with type II hypercholesterolemia: a 6-month double-blind study. Int J Clin Pharmacol Res 2001;21(1):43-57.
27. Son MJ, Rico CW, Nam SH, Kang MY. Effect of oryzanol and ferulic acid on the glucose metabolism of mice fed with a high-fat diet. J Food Sci 2011;76(1):7-10.
28. Justo ML, Rodriguez-Rodriguez R, Claro CM, Alvarez de Sotomayor M, Parrado J, Herrera MD. Water-soluble rice bran enzymatic extract attenuates dyslipidemia, hypertension and insulin resistance in obese Zucker rats. Eur J Nutr 2013;52(2):789-97.
29. Spiazzi CC, Manfredini V, Barcellos da Silva FE, Flores EM, Izaguirry AP, Vargas LM, Soares MB, Santos FW. γ-Oryzanol protects against acute cadmium-induced oxidative damage in mice testes. Food Chem Toxicol 2013;55:526-32.
30. Marongiu B, Piras A, Porcedda S. Comparative analysis of the oil and supercritical CO2 extract of Elettariacardamomum (L.) Maton. J Agric Food Chem 2004;52(20):6278-82.