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Evaluation of protective effect of cactus pear seed oil (*Opuntia ficus-indica* L. MILL.) against alloxan-induced diabetes in mice

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ABSTRACT

Objective: To evaluate the *in vitro* antioxidant power of cactus pear seed oil [*Opuntia ficus-indica* L. MILL. (CPSO)] and its protective effect against chemically induced diabetes mellitus in mice.

Methods: The *in vitro* antioxidant effect of CPSO was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The preventive effect was conducted on Swiss albino mice treated with CPSO (2 mL/kg, *per os*), before and after a single intraperitoneal alloxan administration (100 mg/kg). Survival rate, body weight and fasting blood glucose were measured and histopathological analysis of pancreas was performed to evaluate alloxan-induced tissue injuries.

Results: CPSO exhibited an antioxidant effect in DPPH scavenging assay. Moreover, the administration of CPSO (2 mL/kg) significantly attenuated alloxan-induced death and hyperglycemia ($P < 0.001$) in treated mice. Morphometric study of pancreas revealed that CPSO significantly protected islets of langerhans against alloxan induced-tissue alterations.

Conclusions: Based on theses results, CPSO can prevente alloxan-induced-diabetes by quenching free radicals produced by alloxan and inhibiting tissue injuries in pancreatic β-cells.

1. Introduction

According to International Diabetes Federation, 382 million people worldwide have diabetes mellitus (DM) [1]. This disease results from the interaction of genetic predisposition, behavioral and environmental risk factors. Then, the growing DM prevalence requires efficient preventive interventions [2].

It has been shown that intolerable amounts of cellular free radicals, known as redox imbalance, are harmful and could be fatal for the host cells [3]. Although the genetic basis of DM, there is solid evidence that redox imbalance is a determinant factor leading to the development of this chronic metabolic disease [4,5]. Therefor, it is well known that healthy diet and regular physical activity are beneficial to subjects at high risk of diabetes.

Nutritional status characterized by good supply of antioxidants, might be helpful to prevent DM induced by oxidative stress. In this context, many vegetable oils, containing polyunsaturated fatty acids (PUFAs) and antioxidants (tocopherols, polyphenols and carotenoids, *etc.*), are reported to be antidiabetic by preventing diabetes complications [6,7], and even more, by inhibiting the disease development [8–11].

Cactus pear seed oil (CPSO), extracted from (*Opuntia ficus-indica* L. Mill.) seeds, contains high amounts of PUFAs and antioxidant compounds that can be useful in DM management [12–16]. In a previous work, we demonstrated that CPSO improves postprandial hyperglycemia in normal and streptozotocin-induced DM in rats by a partial inhibition of the intestinal glucose absorption [17]. Though, there is no study reporting preventive effect of CPSO against alloxan (Allx) induced-diabetes in mice.

The aim of this work is to evaluate the antioxidant activity and the preventive effect of CPSO against DM by its oral intake before and during Allx diabetogenic phase. The effect of CPSO was compared to D-α-tocopherol acetate-enriched cooking oil (TCO).

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2. Materials and methods

2.1. Plant materiel

CPSO was freely provided by Argan Oil Company, Casablanca, Morocco. *Opuntia ficus-indica* L. Mill. seeds have been separated from fresh fruit collected in summer period (June–August). Separated seeds have been dried at room temperature and then cold-pressed by use of oil extraction machine. The extraction was conducted without use of solvent system. Extracted CPSO was bottled in 40 mL glass bottle and stored in dark at 6 °C until use.

2.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH scavenging assay was performed according to the method described by Liu *et al.* [18] with some modifications. One milliliter of ethanolic solution of DPPH (0.001%; w/v) (Sigma Aldrich) was added to 1.5 mL of final concentrations of CPSO in ethanol (6.00 mg/mL, 4.00 mg/mL, 1.00 mg/mL, 0.40 mg/mL and 0.10 mg/mL) prepared from a stock solution of 10 mg/mL. The mixture was shaken by vortex and the absorbance was immediately determined by spectrophotometer (Spectronic® 20 GENESYS®, Spectronic Instruments USA) at 517 nm. The absorbance was monitored at 30 min intervals during 90 min of incubation at room temperature in dark. Ascorbic acid, a stable synthetic antioxidant, was used as standard reference. All assays were done in triplicates. The scavenging activity of the samples was calculated according to the following formula:

$$\text{DPPH Scavenging percentage } (\%S) = 100 \times [(A_B - A_S)/A_B]$$

where A_B and A_S are the absorbance of control and tested samples, respectively, at the measurement time.

2.3. Effect of cactus pear seed oil on Allx-induced DM

2.3.1. Animals

Swiss albino mice (19 weeks old) were used for this experiment. The animals were supplied by the animal house of the Faculty of Sciences, Mohammed First University, Oujda, Morocco. The animals were housed in polycarbonate cages in environmental conditions and fed standard diet and water *ad libitum* under 12 h light/12 h dark (light period 7:00 AM–7:00 PM). All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health [19].

2.3.2. Diabetes induction

Diabetes was induced in mice by a single intraperitoneal (*ip.*) dose (100 mg/kg of body weight) of Allx monohydrate (Allx monohydrate 98%, ACROS Organics). The diabetogenic dose of Allx was freshly prepared in phosphate-citrate buffer (pH = 4.5) and injected to overnight fasted mice.

2.3.3. Experimental design

To evaluate the effect of CPSO treatment on the incidence of Allx-induced DM, mice were randomly divided into four groups: control group, positive control group, CPSO treated group and TCO (20% D-α-tocopherol acetate in cooking oil, v/v) treated group.

On the beginning day (day 0), treated animals received oral administration of CPSO (2 mL/kg of body weight and TCO (2 mL/kg of body weight) to their respective groups, followed by a single dose injection of Allx at 1 h of time interval. The treatment was sustained for a week after Allx administration (Figure 1). Control group and positive control group received oral administration of distilled water (10 mL/kg of body weight) followed by an intraperitoneal injection of phosphate-citrate buffer and Allx respectively (Figure 1).

On the termination day (day 7), animals were euthanized and the splenic part of pancreas was quickly removed and immediately deposed in 10% buffered formalin to perform histopathological study.

Blood was withdrawn from the mice tail vein, from overnight fasting mice, and glycaemia was measured at the start and at the end of study by electronic glucometer (*Optium xceed*, Abbott Diabetes Care Ltd., United Kingdom).

2.3.4. Histopathological study of pancreas

The splenic part of pancreas from each mouse was fixed in 10% buffered formalin for 17 h and processed via the paraffin wax embedding method (dehydration, clearing and embedding). The paraffin embedded-sections were cut at 7-micron thickness using microtome (Leitz 1512, Germany), and then stained by hematoxylin and eosin standard method. Stained sections of pancreas were qualitatively (morphological) analyzed on Olympus microscope (Olympus CH Microscope, Japan).

The quantitative (morphometric) analysis was carried out on photomicrographs by use of ImageJ Software [20]. Islets density was determined at 100× magnification; though, the islet diameter, islet area and islet's cell number were determined at 400× magnification.

2.4. Statistical analysis

The collected data were analyzed by GraphPad Prism 6 for Mac (version 6.0f, Trial) and expressed as means ± standard error of mean (SEM). Glycaemia data were analyzed using Two-way ANOVA analysis of variance, followed by Bonferroni *post-hoc* test. The other data were analyzed by one-way ANOVA, followed by Bonferroni *post-hoc* test. The difference was considered statically significant when $P < 0.05$.

3. Results

3.1. DPPH scavenging assay

CPSO antioxidant activity was evaluated over a range of concentrations and the results of DPPH scavenging effect were plotted in Figure 2. CPSO exhibited a significant antioxidant activity with an IC_{50} value of 0.96 mg/mL. However, the maximal efficiency (at 90 min) of CPSO on DPPH scavenging [(86.20 ± 0.13)%, 6 mg/mL] was slower than that of ascorbic acid [(97.12 ± 0.57)%, 1.6 mg/mL]. Additionally, the antioxidant ability of CPSO increased proportionally to its concentration in the milieu.

3.2. Effect of CPSO administration on Allx diabetes induction

3.2.1. Effect on survival rate

Treatment with CPSO enhanced the survival rate after Allx (100 mg/kg) injection (77.77%) compared to the group treated