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journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2015.06.013>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 these results, CPSO can prevent 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]. Therefore, 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, Casa-blanca, 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 deposited 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 \times magnification; though, the islet diameter, islet area and islet's cell number were determined at 400 \times magnification.

2.4. Statistical analysis

The collected data were analyzed by GraphPad Prism 6 for Mac (version 6.0f, Trial) and expressed as means \pm 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 IC₅₀ value of 0.96 mg/mL. However, the maximal efficiency (at 90 min) of CPSO on DPPH scavenging [(86.20 \pm 0.13)%, 6 mg/mL] was slower than that of ascorbic acid [(97.12 \pm 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

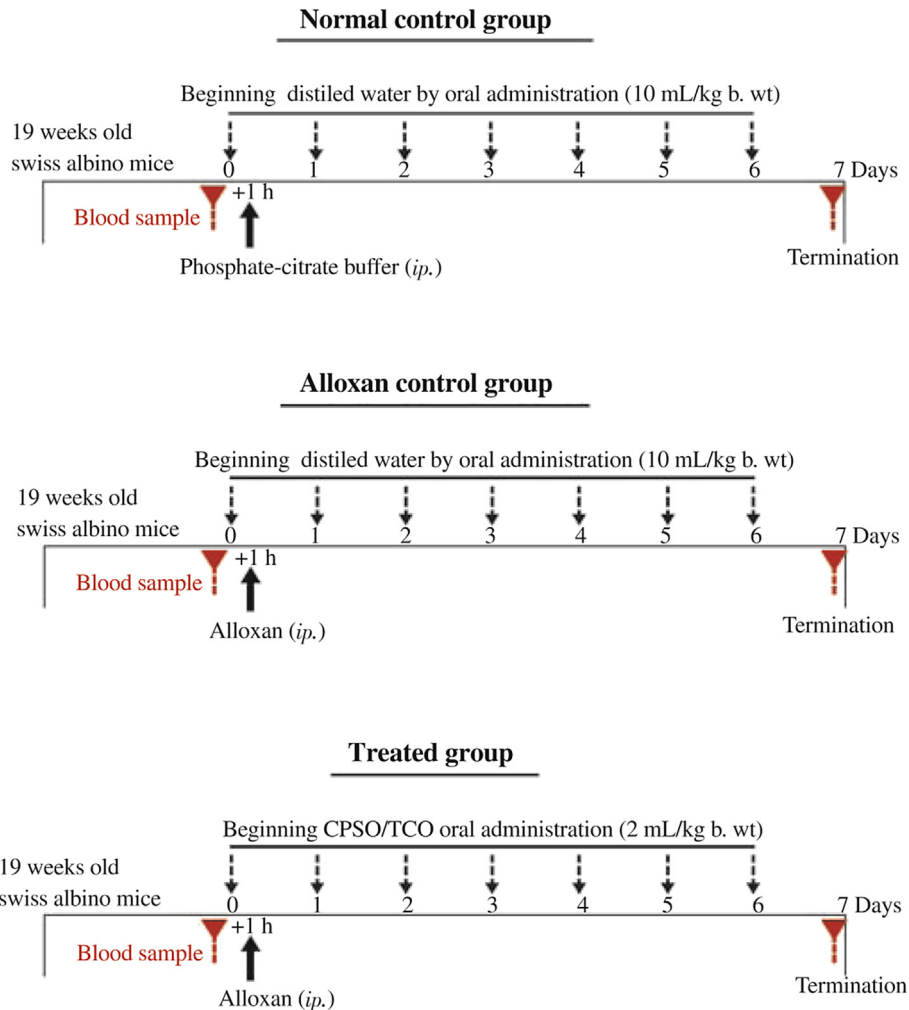


Figure 1. Preventive effect of CPSO and TCO against Allx-induced diabetes in mice.

with Allx only (40.00%). In the same way, TCO administration prevented mortality due to Allx administration compared to Allx group (Figure 3).

3.2.2. Effect on blood glucose level

The effect of CPSO administration on blood glucose level of Allx-treated mice is presented in Figure 4. Allx injection

induced a significant ($P < 0.001$) increase in fasting blood glucose of Allx group compared to normal control group [(4.41 ± 0.19) mmol/L]. The administration of CPSO (2 mL/kg) with Allx in mice decreased significantly the incidence Allx-induced hyperglycemia in CPSO-treated group [(4.37 ± 0.38) mmol/L, $P < 0.001$] compared to the group treated with only Allx [(13.13 ± 0.95) mmol/L]. Treatment with TCO (2 mL/kg) attenuated significantly (68%, $P < 0.001$) the Allx-induced hyperglycemia compared to Allx group.

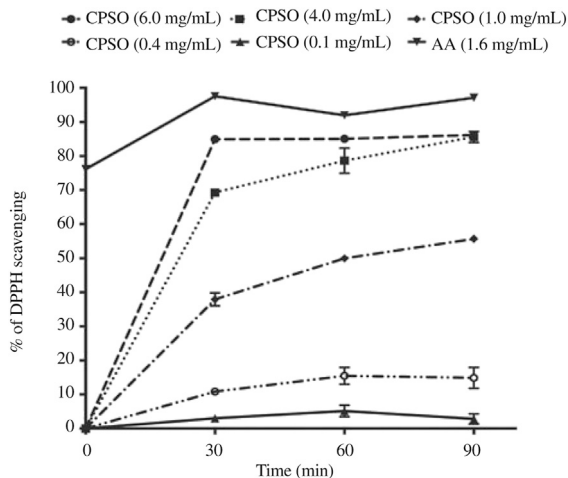


Figure 2. Effect on DPPH scavenging by CPSO various concentration during 90 min incubation at room temperature in dark. Ascorbic acid (AA) was used as standard antioxidant ($n = 3$).

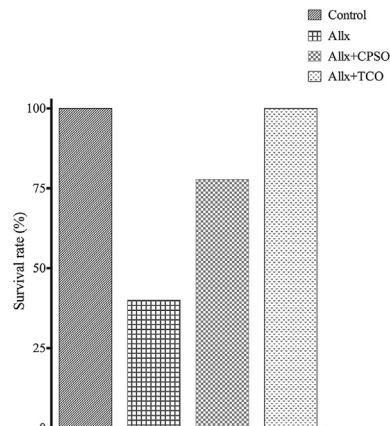


Figure 3. Effects of CPSO and TCO on survival rate of swiss albino mice after Allx administration.

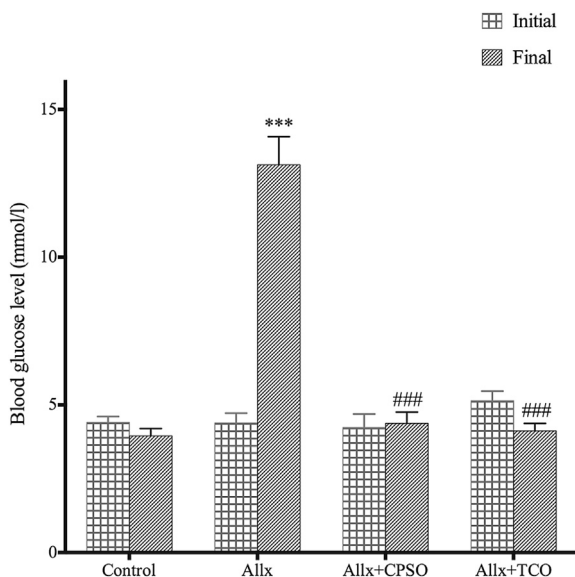


Figure 4. Effect of oral administration of CPSO and TCO on blood glucose level before (Day 1) and after (Day 8) Allx intraperitoneal administration in mice.

$n = 6-8$, *** $P < 0.001$ compared to normal control group at the corresponding time. ### $P < 0.001$ compared to Allx group at the corresponding time.

These results suggest that CPSO treatment significantly attenuated the development of Allx-induced DM in swiss albino mice.

3.2.3. Effect on body weight

The body weight variation is shown in Table 1. Allx induced a significant decrease in body weight in Allx group $[-(6.84 \pm 3.20)\%$, $P < 0.001$] compared to control group $[(+8.27 \pm 2.17)\%$. Though, CPSO intake with Allx inhibited significantly the body weight loss in treated group $[(+8.780 \pm 2.88)\%$, $P < 0.001$] compared to the group treated with only Allx.

3.2.4. Histopathological study of pancreas

Light micrographs of hematoxylin and eosin stained sections of pancreas are grouped in Figure 5. It is notable that Allx induced a degeneration of islets of langerhans in animals treated with only Allx. Administration CPSO and TCO with Allx attenuated the destructive effect of Allx on pancreatic islets.

The results of the morphometric analysis of pancreas are presented in Table 2. Allx administration induced a significant

Table 1

Changes in body weight before and after Allx intraperitoneal administration in CPSO and TCO treated mice groups.

Groups	Body weight (g)		Changes in body weight (%)
	Initial	Final	
Control	24.71 \pm 1.07	26.92 \pm 0.93	+(8.27 \pm 2.17)
Allx (100 mg/kg)	25.13 \pm 1.39	23.54 \pm 1.16	-(6.84 \pm 3.20)***
Allx + CPSO (2 mL/kg)	25.23 \pm 0.78	27.79 \pm 1.02	+(8.780 \pm 2.88)###
Allx + TCO (2 mL/kg)	22.51 \pm 0.44	23.25 \pm 0.42	+(3.18 \pm 0.55)#

Values are means \pm SEM ($n = 6-8$), *** $P < 0.001$ compared to control group, # $P < 0.05$; ### $P < 0.001$ compared to Allx group.

reduction in the numerical density of islets (number of islets/ mm^2 of pancreas) ($P < 0.001$), islet diameter ($P < 0.01$), islet area ($P < 0.01$) and numerical number of cells per islet ($P < 0.01$) compared to normal control group. Whereas, the oral administration of CPSO prevented significantly the Allx-induced pancreas injuries by preserving islet diameter $[(267.10 \pm 23.47) \mu\text{m}]$, islet area $[(0.0416 \pm 0.0091) \text{mm}^2]$ and insular number of cells $[(380.80 \pm 54.23) \text{cell/islet}]$ in normal state (no significant difference) compared to normal mice. However, it was a significant reduction in islet density $[(1.79 \pm 0.20) \text{islet}/\text{mm}^2]$, $P < 0.01$ compared to normal control group.

4. Discussion

This study was undertaken, mainly, to assess the protective effect of cactus pear seed oil against Allx-induced DM in mice.

Allx is an unstable compound, which presents a molecular shape analogy with glucose. Though, Allx by itself is not toxic, but once it is infiltrated to the pancreatic β -cells through the GLUT2 transporter [21], Allx is reduced to dialuric acid in the presence of different cellular reducing agents. The presence of both Allx and its reduction product leads to the establishment of redox cycle for generation of superoxide radicals (O^{\bullet}). Thereafter, a dismutation of superoxide radicals induces a production of hydrogen peroxide (H_2O_2), which reacts with ferrous (Fe^{2+}) to produce hydroxyl radical (OH^{\bullet}), a high oxidative agent [22]. The intolerable rise in oxidative agents provokes necrosis of pancreatic β -cells known to be vulnerable to redox imbalance [23]. This suggests that Allx diabetogenicity is a free radical mediated process. Furthermore, Allx toxicity is related to animal death due to hypoglycemic fatal convulsions [24].

This study shows that oral administration of CPSO prevented the diabetogenic effect of Allx; this is possibly due to the presence of antioxidant compounds, which act by inhibiting Allx-induced free radicals production or/and more probably by quenching them if they are produced. This hypothesis is supported by DPPH scavenging ability of CPSO confirmed in this paper.

A chemical composition of cactus pear seed oil is characterized by the presence of fat-soluble vitamins (tocopherols, β -Carotene and vitamin K_1) [13]. Recent study demonstrated that $\text{D-}\alpha$ -tocopherol-enriched diet prevents against Allx-induced diabetes in mice [25]. Though, γ -tocopherol is a major vitamin E in CPSO. It has been reported that γ -tocopherol have an antioxidant and anti-inflammatory activities [26]. These findings were supported by Tomasch *et al.* which demonstrated that γ -tocopherol rich oil intake improves plasmatic antioxidant capacity and decreases low-density lipoproteins in healthy male volunteers [27]. While γ -tocopherol is less powerful antioxidant than α -tocopherol, it is more efficient in macromolecules protecting against oxidation [28].

Moreover, phenolic compounds could also be involved in the protecting action of CPSO against Allx cytotoxicity. It has been shown that there are more than 20 phenolic compounds in cactus pear seeds and this content is highly correlated to their antioxidant activity [16]. It is well known that phenolic compounds have high antioxidant efficiency and they are effective in care of degenerative diseases [29,30].

Furthermore, reactive oxygen species are involved in free calcium (Ca^{2+}) rise in β -cells cytosol [31]. This abnormal state induces supraphysiological insulin exocytosis, which leads to

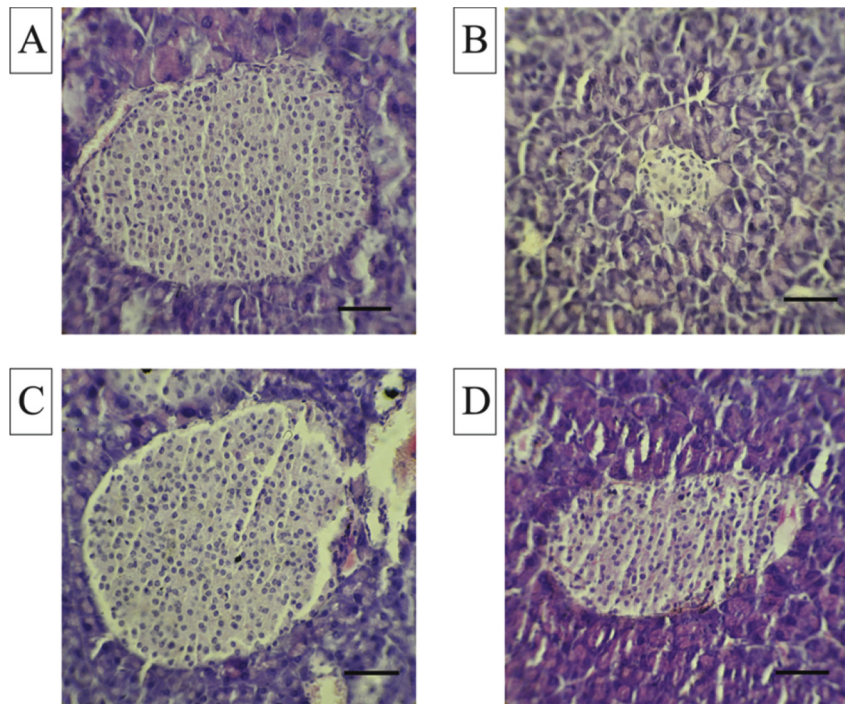


Figure 5. Photomicrographs (400× magnification) of pancreatic islets of langerhans of normal control (A), Allx (100 mg/kg) group (B), alloxan and cactus pear seed oil group (2 mL/kg) (C) and Allx and TCO (2 mL/kg) (D).

Note a conserved islet diameter, islet area, and islet cell number in cactus pear seeds oil treated group versus alloxan control group (scale bar = 50 μm).

Table 2

Effect of CPSO and TCO administration on histomorphometric parameters of hematoxylin and eosin stained pancreas of Allx treated mice.

Groups	Islets density (islets/mm ²)	Islet diameter (μm)	Islet area (mm ²)	Number of cells/islet
Control	2.99 ± 0.20	269.50 ± 26.28	0.0421 ± 0.0063	375.60 ± 56.64
Allx (100 mg/kg)	1.10 ± 0.24***	133.90 ± 14.13**	0.0101 ± 0.0015**	103.80 ± 16.96**
Allx + CPSO (2 mL/kg)	1.79 ± 0.20**	267.10 ± 23.47###	0.0416 ± 0.0091###	380.80 ± 54.23###
Allx + TCO (2 mL/kg)	2.09 ± 0.22#	253.00 ± 16.15##	0.0345 ± 0.0033#	315.80 ± 30.65#

Values are means ± SEM ($n = 6$, for each animal), * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to normal control group, # $P < 0.05$; ## $P < 0.01$ compared to Allx group.

hypoglycemic fatal convulsions in mice. Free radicals quenching ability of CPSO could be involved in the improvement of survival rate in mice treated by CPSO with Allx.

On the other hand, the lipophilic fraction of CPSO contains 83% of unsaturated fatty acids (UFAs) represented by 56% of linoleic acid (ω -6) and 20% of oleic acid (ω -9) [32]. It was confirmed that ω -6 rich oil prevented Allx-induced diabetes in rats by preserving redox homeostasis in β -cells and by increasing cytoprotective antioxidant compounds such as vitamin E, superoxide dismutase and glutathione reductase [10]. In another study, it has been demonstrated that pretreatment with γ -linolenic acid and arachidonic acid, a metabolites of linoleic acid, prevented the incidence of Allx induced diabetes in rats by 100% [33]. However, some studies reported that ω -6 fatty acids are pro-inflammatory components, but it seems that there are many contradictions between findings.

Lifestyle and diet could be effective in diabetes prevention [34]. Also, healthy diet as source of antioxidants and good fats, is more recommended to manage the metabolic equilibrium in both healthy and diabetic people [7].

In spite of its actual expensive price, CPSO valorization might be helpful in the oil's price lowering and then more consumers could use it.

In conclusion, CPSO prevented Allx-induced diabetes in mice; this effect might be due to the synergism of their antioxidant compounds in quenching free radicals and the capacity of their UFAs to enhance the antioxidant status in pancreatic β -cells. Further studies could be accomplished to assess the anti-diabetic effect in other DM models.

Conflict of interest statement

We declare that we have no conflict of interest.

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