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Hepatoprotective effect of *Opuntia dillenii* seed oil on CCl_4 induced acute liver damage in rat

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ABSTRACT

Objective: To investigate the hepatoprotective effect of *Opuntia dillenii* seed oil (ODSO) on CCl_4 provoked liver injury in rat. **Methods:** Animals were treated orally with ODSO at a concentration of 2 mL/kg, once daily for one week before the first intraperitoneal injection of CCl_4, and thereafter the administration of the oil was continued for 7 days until the introduction of the second injection of CCl_4. Fourteen hours after the last dose of CCl_4, rats were sacrificed, and the relative liver weight, weight gain, alkaline phosphatase, aspartate amino transferase, alanine aminotransferase, direct bilirubin, total bilirubin, triglycerides, total cholesterol, very low density lipoprotein, low density lipoprotein, high density lipoprotein, plasmatic glucose, urea, creatinine, acid uric and malondialdehyde were determined. **Results:** The significant increase was found in relative liver weight and plasma levels of alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, total bilirubin, direct bilirubin, triglycerides, very low-density lipoprotein, urea, uric acid and malondialdehyde. Likewise, the significant decrease was indicated in the weight gain and the level of glucose plasmatic, and high-density lipoprotein levels in CCl_4 produced liver injury in rats were re-established to normal levels when treated with ODSO. While, no change was observed in the total cholesterol, low-density lipoprotein and creatinine in all animals. **Conclusions:** We conclude that the ODSO has a protective effect on CCl_4-mediated liver injury. Hence, we suggest its inclusion as a preventive control of liver disorders.

1. Introduction

In the human body, the liver is considered important among the largest organs, it takes care of several functions, like the process of metabolism that is essential for life[1]. It is always vulnerable to different toxic molecules of foreign origin due to its location in the human body. These xenobiotic absorbed by the intestine pass first through the liver, which makes it threatened by diseases[2]. Currently, many people suffer from liver diseases induced by several hepatotoxic agents; among these agents are alcohol, infections and chemicals like carbon tetrachloride (CCl_4) and paracetamol[3,4]. Liver affections have become a global problem, and caused every year up to 20 000 deaths arise due to liver affections[5]. Treatment of liver affections with conventional medications may be inadequate

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or have adverse effects[6]. That is why it is very important to replace chemical drugs with herbal products.

CCl_4 is a toxin among the oldest toxins, and is most used for experimentally induced liver injury in laboratory animals[7]. A hepatotoxic agent that causes liver cell damage is comparable to that of the viral infection. When it enters the liver, CCl_4 is encountered as a xenobiotic, and is converted into two free radicals, which are trichloromethyl and trichloromethyl-peroxyl by the microsomal system dependent on monooxygenase P-450. These two radicals cause lipid peroxidation, which provokes serious liver injury[8]. Antioxidants have been reported to prevent oxidative injury to the liver, and they can prevent the risk of liver disease[9]. This is why we notice that there is a rising interest in natural products that have an antioxidant property, in order to use them to prevent liver pathologies related to oxidative stress[10].

Opuntia dillenii seed oil (ODSO) is an oil that is marked by a rise degree of unsaturated fatty acids, wherein linoleic acid is the prominent fatty acid, β - sitosterol is the sterol marker while γ -tocopherol is the only vitamin E in oil[11]. Antioxidant[12] and anti-inflammatory[13] activities are the only studies that have been done on this oil. The aim of this work is to evaluate the hepatoprotective effect of ODSO on Wistar rat with hepatotoxicity provoked by CCl_4.

2. Materials and methods

2.1. Chemicals

CCl_4 was purchased from Sigma chemicals, USA. Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total cholesterol, triglycerides, high density lipoprotein (HDL-c), bilirubin direct and total, glucose, total protein, urea, creatinine and acid uric kits were purchased from Biosystems, Spain. All other reagents used in this study were of high quality and analytical grade.

2.2. Collection of plant material

The fresh fruits of *Opuntia dillenii* used in this study were collected in February 2016 from regions in Essaouira, Morocco. The specimen was deposited at Mohammed First University, Oujda, Morocco, under the reference number HUMPOM 351 after its identification by the expert botanist Mohammed Fennan, from the scientific institute of the University Mohammed Ⅴ.

2.3. Preparation of Opuntia dillenii seeds powder

Fruits of *Opuntia dillenii* were peeled then the seeds were separated from the fruit, cleaned with the distilled water, dried in the oven at 37 ℃ for three days and then ground with a blender, until a fine and homogeneous powder was obtained and conserved at -20 ℃ until use.

2.4. Oil extraction

An amount of 100 g of seed powder was added in 500 mL of petroleum ether, and then the totality was shaken for 24 h under an ambient temperature. After filtration, the organic solvent was removed on a rotary evaporator under temperature at 40 ℃. Moreover, the oil was dried and stored at 4 ℃.

2.5. Animals

Twenty-four healthy adult Wistar rats [(200 ± 50) g, 10 weeks old] were employed in this research. Animals were taken from the animal house of the Faculty of Sciences, Mohammed First University, Oujda, Morocco. The rats were grouped in plastic cages in a well-ventilated room with soft bedding and accessibility to water and food *ad libitum* in an environmentally controlled room (22-26 ℃, with a 12/12 h light/dark cycle). The rats were adapted one week preceding treatment.

All rats were cared for in compliance with the internationally accepted Guide for the care and use of laboratory animals, published by the US National Institutes of Health (NIH Publication No. 85-23, Revised in 1985).

2.6. Experimental procedure

One week after the adaptation, the rats were arbitrarily grouped into four groups, each including six rats, and treated as follows: The normal control group and CCl_4 control group received distilled water (10 mL/kg). Moreover, the ODSO control group and ODSO + CCl_4 group received ODSO (2 mL/kg). The animals of groups CCl_4 control group, and ODSO+ CCl_4 group received CCl_4 intraperitoneally at a dose 1 mL/kg body weight (25% CCl_4, solubilized in olive oil; v/v) once a week for two weeks of treatment in order to induce liver injury. Body weights of the rats were measured before and after the treatment. All animals were treated and observed daily for two weeks.

2.7. Blood sampling

Twelve hours after the last intraperitoneal injection of CCl_4, blood samples from these treated rats were drawn from their carotid arteries after being anesthetized under a light ethyl ether. Then the blood was centrifuged at 3 000 rpm for 10 min and at 4 ℃ to obtain the plasma, thereafter the plasma was conserved at -20 ℃ until analysis. In addition, liver weights were measured and then used for making liver homogenate (10% w / v) in normal saline (pH 7.0) and conserved at -20 ℃ for biochemical analyzes.

2.8. Serum biochemical parameters determination

ALT and AST were estimated by IFCC method[14], ALP by *para*-nitrophenyl phosphate method[15], total cholesterol by enzymatic method[16], triglycerides by glycerol phosphate oxidase method[17], HDL-c by the ultra HDL assay, total bilirubin by diazonium saltmethod[18], direct bilirubin by diazoreaction method[19], plasma glucose by the oxidase-peroxidase method[20], urea by Urease-GLDH method[21], creatinine by kinetic alkaline picrate method[22], uric acid by uricase-peroxidase method[23] were analyzed following to the reported methods. All analyses were performed in triplicate

for every sample on the ARCHITECT c-Systems autoanalyzer (Hamburg, Germany) by using commercial reagent kits.

For low-density lipoprotein (LDL-c), its rate was estimated according to Friedewald *et al.*[24]. using the following formula:

LDL-c = total cholesterol – [HDL-c + very low-density lipoprotein (VLDL-c)]

In addition, VLDL-c was calculated according to the formula of [24], as follows:

VLDL-c = triglycerides/5

2.9. Determination of malondialdehyde (MDA)

In this study, hepatic lipid peroxidation was determined by the Buege & Aust method[25], and this method measures the level of TBARS production as described by Iqbal *et al.*[26]. After the preparation of the homogenate, 0. 5 mL of the homogenate was added in 0. 5 mL of trichloroacetic acid (30% w/v) and the whole was subject to a centrifugation during 10 min at 3 500 rpm and 4 ℃. Then 1 mL of thiobarbituric acid (0.67% w/v) was added to 1 mL of the supernatant obtained and the whole was placed in a boiling water bath during 10 min. Then to stop the reaction, the mixture was placed in an ice bath. At the end of this reaction, the spectrophotometer was used to measure the mixture of assays at 535 nm, and the calculation was made using the next molar extinction coefficient: 1.56×10^{5} M/cm^{-1}.

The results were expressed in nanomoles of MDA produced per gram of tissue.

2.10. Statistical analyses

The results were expressed as the mean ± SEM and were subjected to statistical analyses using Graph Pad Prism 5 Software, San Diego, CA, USA. Multiple-group comparisons were analyzed by one-way analysis of variance (ANOVA). $P < 0.05$ was considered as statistical significant difference.

3. Results

3.1. Effect of ODSO on variation in relative liver weight and body weight gain

The variation in body weight of rats at the end of the experiment was used to assess the growth performance of all the groups studied. The relative weight of the liver and the body weight gain for rats of the groups studied were shown in Table 1. CCl_4 administration at the end of the first and second week significantly attenuated weight gain and increased the relative weight of the liver in untreated rats (CCl_4 control group) compared to the healthy rats. Moreover, the quotidian treating of hepatotoxic rats by ODSO (ODSO + CCl_4 group) during the two weeks markedly improved growth performance. Indeed, the treatment of hepatotoxic rats (ODSO + CCl_4 group) with ODSO showed a significant reduction in the relative liver weights and an increase in body weight gain compared with the CCl_4 control group rats. Furthermore, the feeding of ODSO alone did not affect the growth performance in healthy rats.

Table 1

Effect of ODSO on the growth parameters in CCl_4-intoxicated rats.

Groups	Weight gain(g)	Relative liver weight (g/100 g BW)
Normal control group	38.16 ± 1.99	2.38 ± 0.04
CCl_4 control group	12.33 ± 0.77$^{###}$	4.00 ± 0.12$^{###}$
ODSO control group	39.83 ± 2.21	1.98 ± 0.03
ODSO + CCl_4 group	29.16 ± 2.16***	3.14 ± 0.03***

All values are represented as mean ± SEM, $n = 6$.

$^{###}P < 0.01$ compared with the normal control group. $^{***}P < 0.01$ compared with the CCl_4 control group.

3.2. Effect of ODSO on plasma hepatic markers (AST, ALT and ALP) in hepatotoxic rats.

The intoxication of the liver by CCl_4 was confirmed by measuring the activity of AST, ALT and ALP in the plasma (Figure 1). The CCl_4 injection of rats induced a significant rise in the activity of ALT, AST and ALP in comparison to the healthy rats. However, the feeding of rats with ODSO at a dose of 2 mL/kg significantly attenuated the elevation of these parameters compared to CCl_4 control group rats. Furthermore, the intake of ODSO alone did not affect the hepatic markers in healthy rats.

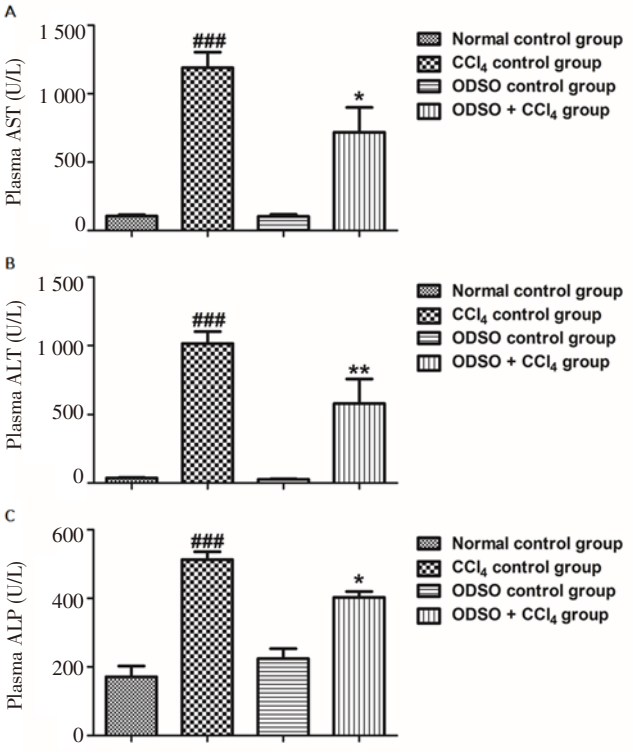

Figure 1. Effect of ODSO on CCl_4-induced alterations in plasma hepatic markers.

(A) AST, (B) ALT and (C) ALP. Data are mean ± SEM, n=6. $^{###}P$<0.001 versus normal control group. $^{*}P$<0.05, $^{**}P$<0.01 versus CCl_4 control group.

3.3. Effect of ODSO on plasma direct and total bilirubin in hepatotoxic rats

The activity of ODSO administration on direct and total bilirubin level in all rats of the groups studied was shown in the Figure 2. The

treatment of rats with CCl_4 induced a significant elevation in plasma bilirubin (direct and total). Moreover, the elevation in bilirubin concentration showed the dysfunction of the excretory function of hepatic cells. This abnormality was significantly corrected after the daily intake of ODSO at a concentration of 2 mL/kg. In addition, the administration of ODSO alone without induced CCl_4 injury did not influence the excretory function of the hepatic cells in the healthy rats.

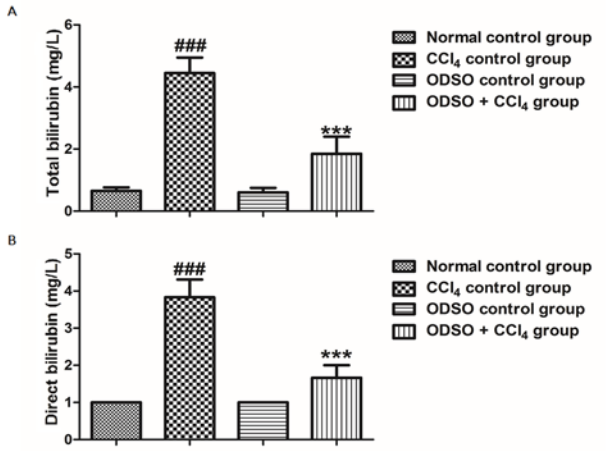

Figure 2. Effect of ODSO on plasma total bilirubin (A) and direct bilirubin (B) in CCl_4-intoxicated rats.
Values are mean ± SEM, $n = 6$. $^{###}P < 0.01$ compared with the normal control group. $^{***}P < 0.01$ compared with the CCl_4 control group.

3.4. Effect of ODSO on plasma total cholesterol, triglycerides and glucose in hepatotoxic rats

In this study, the levels of triglycerides, total cholesterol and glucose were investigated in order to evaluate the effect of ODSO on metabolic function of the liver (Figure 3). In our study, the intraperitoneal injection of CCl_4 to the rats induced a significant rise in the triglyceride amount, a significant decrease in glucose while the total cholesterol level was not impaired, in comparison with the normal control group rats. However, this alteration in triglyceride and glycemia level was recovered after the intake of ODSO at a concentration of 2 mL/kg. The administration of ODSO alone without CCl_4-induced injury did not influence the metabolic function of the cells hepatic in the healthy rats.

3.5. Effect of ODSO on plasma lipoproteins LDL-c and VLDL-c, HDL-c in hepatotoxic rats

The effect of ODSO on the plasma lipoprotein levels was shown in Figure 4. The administration of CCl_4 in rats induced a significant elevation in VLDL-c amount, a significant reduction in HDL content and did not affect the LDL-c content in comparison to the healthy rats. However, this change in VLDL-c and HDL-c level was slightly recovered after administration of ODSO at a concentration of 2 mL/kg. The administration of ODSO alone without CCl_4- induced injury did not influence the lipoprotein levels of the hepatic cells in the healthy rats.

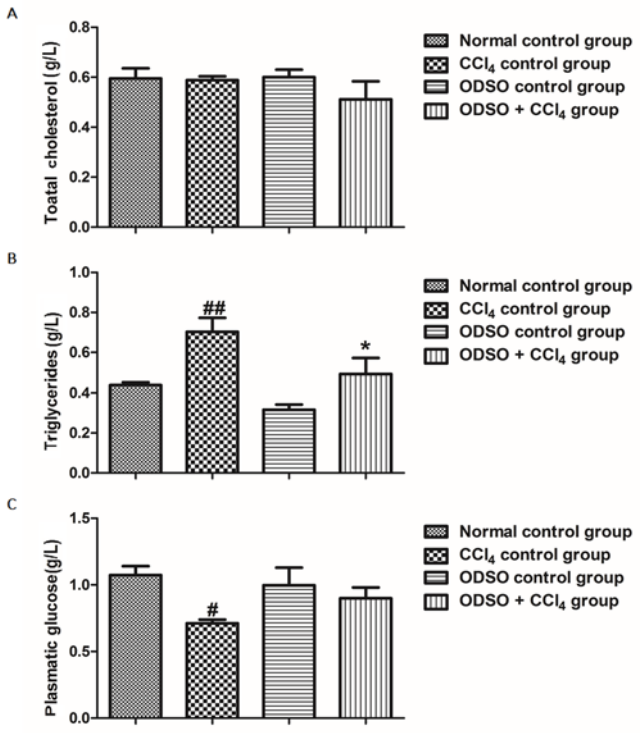

Figure 3. Effect of ODSO on plasma total cholesterol (A), triglycerides (B) and glucose (C) in CCl_4-intoxicated rats.
Data are mean ± SEM, n=6. $^{#}P$<0.05, $^{##}P$<0.01 as compared with the normal control group. $^{*}P$<0.05, as compared with the CCl_4 control group.

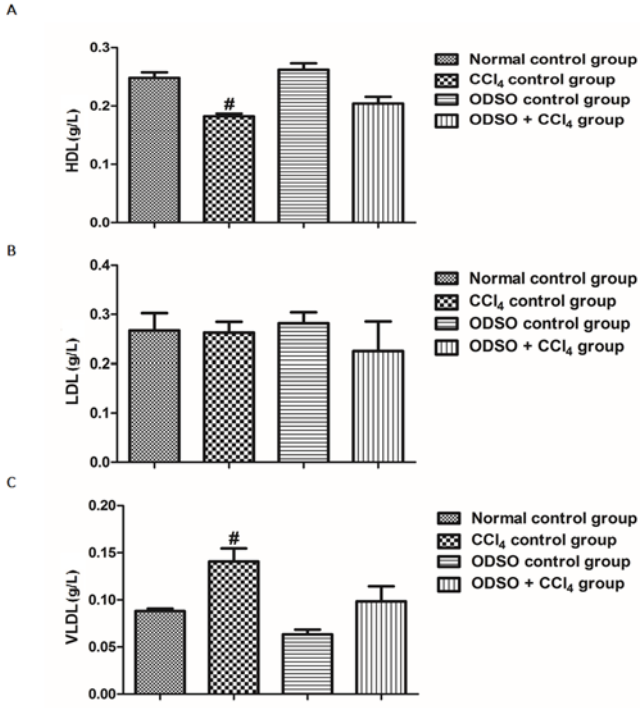

Figure 4. Effect of ODSO on plasma HDL-c (A), LDL-c (B) and VLDL-c (C) in CCl_4-intoxicated rats.
Data are presented as mean ± SEM, $n = 6$. $^{#}P$<0.05 as compared with the control group. Effect of ODSO on serum uric acid, urea and creatinine in hepatotoxic rats.

The plasma concentration of uric acid, creatinine and urea was

examined as biomarkers of renal function (Figure 5). The results of our study indicated that the intraperitoneal injection of CCl_4 in rats triggered a significant increase in uric acid, a slight increase of urea and does not affect creatinine level. In addition, the intake of ODSO at a concentration of 2 mL/kg slightly corrected the change of uric acid and urea that was induced by CCl_4.

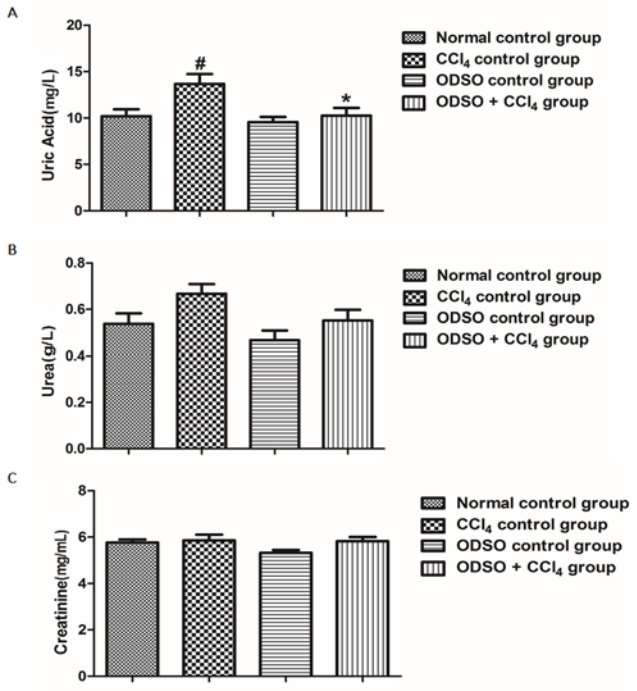

Figure 5. Effect of ODSO on plasma uric acid (A), urea (B) and creatinine (C) in CCl_4-intoxicated rats.

Data are presented as mean ± SEM, $n = 6$. $^{\#}P<0.05$ compared with the control group. $^{*}P<0.05$ compared with the CCL_4 control group.

3.6. Effect of ODSO on lipid peroxidation in hepatotoxic rats

The impact of ODSO on lipid peroxidation in rats for all groups studied was illustrated in Figure 6. The intake of CCl_4 provoked a substantial increase in the lipid peroxidation, as indicated by an elevated MDA level, compared to the healthy rats. The daily intake of rats (injected by CCl_4) with ODSO during two weeks induced a significant reduction in the content of MDA compared to CCl_4 control group. However, the MDA level in the rats treated only with ODSO was similar to that of the healthy rats.

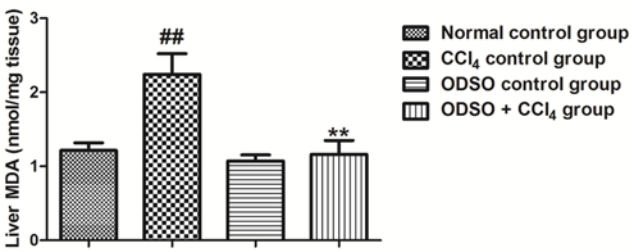

Figure 6. Effect of ODSO on lipid peroxidation in CCl_4-intoxicated rats.

Data are presented as mean ± SEM, $n = 6$. $^{\#\#}P < 0.01$ compared with the normal control group. $^{**}P < 0.01$ compared with the CCl_4 control group.

4. Discussion

The liver is considered important among the largest and most complex organs in the body. It is an organ that provides several physiological functions, such as protein synthesis, excretory and secretory function, storage of nutrients and maintenance of homeostasis. It also protects the body against the side effects induced by some drugs and xenobiotic[27]. The usual model used to study the hepatoprotective action of natural products is hepatotoxicity induced by CCl_4 solvent[28]. The latter is a chemical solvent that causes liver damage like to that produced by viral hepatitis[29]. The liver receives CCl_4 as a foreign toxin, and it turns it into two free radicals, which are trichloromethyl and trichloromethyl-peroxyl, by the microsomal system dependent on monooxygenase P-450. Moreover, these two free radicals cause the start of the oxidation of unsaturated lipids[8] and the oxidation of unsaturated lipids is recognized as the main causes of CCl_4-provoked hepatic affections[30]. In addition, numerous compounds are recognized by their beneficial effects against the liver impairment caused by CCl_4, by applying their protective action either by the attenuation of CCl_4-derived free radical production process, or by their antioxidant activities[31]. In our study, the hepatoprotective effect of ODSO against CCl_4 provoked liver injury in healthy adult Wistar rats was evaluated. Indeed, the hepatoprotective effect of ODSO was evaluated by assessing AST, ALT and ALP activities, as enzyme markers of liver injury[32]. In this work, we also studied the activity of ODSO on the growth performance (body weight gain and relative liver weight), metabolic function (triglycerides, total cholesterol, plasmatic glucose, HDL-c, LDL-c and VLDL-c) and excretory function (total and direct bilirubin) of the liver. Moreover, we evaluated the effect of ODSO on renal excretory function (serum uric acid, urea and creatinine), besides MDA, which represents the final product of the oxidation of unsaturated lipids in liver oxidative stress. The MDA level represents an important indicator of CCl_4-damaged liver[33]. In the current study, the intraperitoneal injection of CCl_4 to rats produced a significant rise in the plasma AST, ALT, ALP, direct bilirubin, total bilirubin, triglycerides, VLDL-c, uric acid, urea (slight increase), MDA and relative liver weight. In contrast, CCl_4 induced a significant reduction in plasma glucose, HDL-c and body weight gain compared to normal control group rats. In addition, CCl_4 did not affect the total cholesterol, LDL-c and creatinine. ODSO was found to exert a hepatoprotective effect by diminishing ALT, AST and ALP activities in plasma, in comparison with rats treated only by CCl_4. In addition, ODSO ameliorated the excretory function of the liver, and this activity was shown by decreasing of the total and direct bilirubin plasma levels. Moreover, ODSO also ameliorated the metabolic function of liver by restoration of the triglycerides, glucose and VLDL-c to normal value in comparison with CCl_4 control group. On the contrary, the oil did not restore the HDL-c to its normal value. In addition, ODSO ameliorated the renal excretory function by a slight correction of uric acid and urea levels in comparison with rats treated only by CCl_4. Finally, the oil was found also to improve the growth performance by diminishing relative liver index and raising the body weight gain. The effectiveness of a hepatoprotective

drug depends principally on its capacity to maintain standard physiological function or to reduce the damaging impact caused by hepatotoxic agents[34]. Therefore, based on these results obtained from our study, ODSO showed very high hepatoprotective effect in animals with CCL_4-provoked hepatotoxicity. Regarding the process by which CCL_4 acts in the liver, the generation of free radicals has a crucial role in the hepatotoxic effect[35]. Then, the hepatoprotective activity is related to the antioxidant activity, since it actions of damage are caused by the free radicals[36]. In a study regarding the chemical composition of ODSO, it has been reported that this oil was rich in phenolic compounds. In the same study, 11 phenolic compounds have been identified, namely, catechol, cinnamic acid, phenylpropionic acid, psoralen, syringic acid, sinapaldehyde, 3′-O-methylcatechin, (+)-gallocatechin, bisdemethoxycurcumin, 4′-O-methyl-(-)-epicatechin 3′-O-glucuronide, viscutin 1[37]. Other reports showed that this oil possesses a considerable antioxidant activity[12–37]. In that case, the protective effect of this oil against hepatotoxic CCl_4 can be due to its antioxidant effect, by the elimination of the free radicals resulting from the metabolism of CCl_4 in the liver, which are involved in the generation of liver damage. However, more studies on the active compounds of the ODSO and their biochemical mechanisms responsible for the hepatoprotective effect will be necessary.

In conclusion, based on results obtained, ODSO has a significant hepatoprotective effect on rats rendered hepatotoxic by CCl_4. Moreover, this effect has been represented by the improvement of the state of the enzyme marks related to hepatocellular damage, the metabolic and excretory function of the liver and lipid peroxidation in the liver. Therefore, it may be possible to use this oil as a hepatoprotective agent.

Conflict of interest statement

We declare that there is no conflict of interest.

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References

[1]Kandimalla R, Kalita S, Saikia B, Choudhury B, Singh YP, Kalita K, et al. Antioxidant and hepatoprotective potentiality of *Randia dumetorum* lam. leaf and bark via inhibition of oxidative stress and inflammatory cytokines. *Front Pharmacol* 2016; **7**: 205.

[2]Stickel F, Brinkhaus B, Krahmer N, Seitz HK, Hahn EG, Schuppan D. Antifibrotic properties of botanicals in chronic liver disease. *Hepato–gastroenterol* 2002; **49**: 1102-1108.

[3]Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-induced acute liver failure: Results of a United States multicenter prospective study. *Hepatology* 2005; **42**: 1364-1372.

[4]Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C, et al. A novel model of CCl_4-induced cirrhosis with ascites in the mouse. *J Hepatol* 2009; **51**(6): 991-999.

[5]Latha TB, Srikanth A, Kumar EK, Srinivasa MSK, Rao Y, Bhavani B. Comparative hepatoprotective efficacy of *Kumaryasava* and *livfit* against carbon tetrachloride induced hepatic damage in rats. *Pharmacol Online* 2009; **1**: 1127-1134.

[6]Takate S, Pokharkar R, Chopad V. Hepatoprotective activity of the aqueous extract of *Launaea intybacea* Beauv against carbon tetrachloride induced hepatic injury in Albino rats. *J Pharm Sci Tech* 2010; **2**(7): 247-251.

[7]Chiu HW, Hua KF. Hepatoprotective effect of wheat-based solid-state fermented antrodia cinnamomea in carbon tetrachloride-induced liver injury in rat. *PLoS One* 2016; **11**(4): e0153087.

[8]Zhao Q, Peng Y, Huang K, Lei Y, Liu HL, Tao YY, et al. Salvianolate protects hepatocytes from oxidative stress by attenuating mitochondrial injury. *Evid Based Complement Altern Med* 2016; **2016**: 5408705.

[9]Bertolami MC. Mechanisms of hepatotoxicity. *Arq Bras Cardiol* 2005; **85 Suppl 5**(1): 25-27.

[10]Dhanasekaran M, Ignacimuthu S, Agastian P. Potential hepatoprotective activity of ononitol monohydrate isolated from *Cassia tora* L. on carbon tetrachloride induced hepatotoxicity in wistar rats. *Phytomedicine* 2009; **16**(9): 891-895.

[11]Ghazi Z, Ramdani M, Fauconnier ML, El Mahi B, Cheikh R. Fatty acids sterols and vitamin E composition of seed oil of *Opuntia ficus indica* and *Opuntia dillenii* from Morocco. *J Mater Environ Sci* 2013; **4**(6): 967-972.

[12]Ghazi Z, Ramdani M, Tahri M, Rmili R, El msellem H, El Mahi B, et al. Chemical composition and antioxidant activity of seeds oils and fruit juice of *Opuntia ficus Indica* and *Opuntia dillenii* from Morocco. *J Mater Environ Sci* 2015; **6**(8): 2338-2345.

[13]El Hachimi F, Hajjaj G, Bendriss A, Cherrah Y, Alaoui K. Anti-inflammatory activity of seed oils of *Opuntia ficus–indica* L. and *Punica granatum* L. from Morocco. *World J Pharm Res* 2015; **4**(1): 284-294.

[14]Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. *J Clin Invest* 1955; **34**(1): 126-133.

[15]Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD. A reference method for measurement of alkaline phosphatase activity in human serum. *Clin Chem* 1983; **29**(5): 751-756.

[16]Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; **20**(4): 470-475.

[17]Fossati P, Prencipe L, Berti G. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-amino-phenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem* 1980; **26**: 227-231.

[18]Winsten S, Cehelyk B. A rapid micro diazo technique for measuring total bilirubin. *Clin Chim Acta* 1969; **25**(3): 441-446.

[19]Burtis CA, Ashwood ER. *Tietz textbook of clinical chemistry*. Third edition. Philadelphia: WB Saunders Co; 1999.

[20]Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; **6**(1): 24-27.

[21]Talke H, Schubert GE. Enzymatic determination of urea using the

coupled urease-GLDH enzyme system. *Mediat Inflamm*1965; **43**: 174-176.

[22]Jaffe M. About the precipitate which picric acid produces in normal urine and about a new reaction of creatinine. *J Physiol Chem* 1886; **10**(5): 391-400.

[23]Remaley AT, Rifai N, Warnick GR. Lipids, lipoproteins, apolipoproteins, and other cardiovascular risk factor. In: Burtis CA, Ashwood ER, Bruns DE. (eds). *Tietz textbook of clinical chemistry and molecular diagnostics*. St. Louis, Missouri: Elsevier; 2006, p. 903-968.

[24]Friedewald WT, Levy RI, Fredrickson DS. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of preparative centrifuge. *Clin Chem* 1972; **187**: 589.

[25]Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; **52**: 302-310.

[26]Iqbal M, Giri U, Giri DK, Alam MS, Athar M. Age-dependent renal accumulation of 4-Hydroxy-2 Nonenal (HNE)-modified proteins following parenteral administration of ferric nitrilotriacetate commensurate with its differential toxicity: Implications for the involvement of HNE-protein adducts in oxidative stress and carcinogenesis. *Arch Biochem Biophys* 1999; **365**: 101-112.

[27]Williamson EM, Okpako DT, Evans FJ. *The liver and biliary system: Selection, preparation and pharmacological evaluation of plant material*. London, UK: John Wiley and Sons; 1996.

[28]Lu Y, Hu D, Ma S, Zhao X, Wang S, Wei G, et al. Protective effect of wedelolactone against CCl_4-induced acute liver injury in mice. *Int Immunopharmacol* 2016; **34**: 44-52.

[29]Ponmari G, Annamalai A, Gopalakrishnan VK, Lakshmi PTV, Guruvayoorappan C. NF-kB activation and proinflammatory cytokines mediated protective effect of *Indigofera caerulea* Roxb. On CCl_4 induced liver damage in rats. *Int Immunopharmacol* 2014; **23**: 672-680.

[30]John Buege A, Steven Aust D. Microsomal lipid peroxidation. *Methods Enzymol* 1978; **52**: 302-310.

[31]Hewawasam RP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice. *Indian J Med Res* 2004; **120**(1): 30-34.

[32]Anand KK, Singh B, Chand D, Chandan BK. An evaluation of *Lawsonia alba* extract as hepatoprotective agent. *Planta Med* 1992; **58**: 22-25.

[33]Cheng N, Ren N, Gao H, Lei X, Zheng J, Cao W. Antioxidant and hepatoprotective effects of *Schisandra chinensis pollen* extract on CCl_4-induced acute liver damage in mice. *Food Chem Toxicol* 2013; **55**(3): 234-240.

[34]Kazeem MI, Bankole HA, Fatai AA. Protective effect of ginger in normal and carbon-tetrachloride induced hepatotoxic rats. *Annals Biol Res* 2011; **2**(1): 1-8.

[35]Zhang Y, He Y, Yu H, Ma F, Wu J, Zhang X. Liquiritigenin protects rats from carbon tetrachloride induced hepatic injury through PGC-1alpha pathway. *Evid Based Complement Altern Med* 2015; **649568**(10): 25.

[36]Muthy KNC, Jayaprakash GK, Singh RP. Studies on antioxidant activity of pomegranate (*Punica granatum*) peel extracts using *in vivo* models. *J Agric Food Chem* 2002; **50**(17): 4791-4795.

[37]Koubaa M, Mhemdi H, Barba FJ, Angelotti A, Bouaziz F, Chaabouni SE, et al. Seed oil extraction from red prickly pear using hexane and supercritical CO_2: Assessment of phenolic compound composition, antioxidant and antibacterial activities. *J Sci Food Agric* 2017; **97**(2): 613-620.