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A jojoba (*Simmondsia chinensis*) seed cake extracts express hepatoprotective activity against paracetamol-induced toxicity in rats

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ABSTRACT

This study aimed to investigate the hepatoprotective activity of jojoba seed cake extracts against an acute paracetamol (PC) intoxication. Two aqueous extracts from jojoba (*Simmondsia chinensis*) seed cake, a simmondsin-rich extract (WE), and a simmondsin-hydrolyzed extract (NE) using Viscozyme L enzyme have been prepared and characterized. After enzyme treatment, simmondsin content decreased from 33.0 % to 3.0 % and glucose content increased from 16.2 % to 27.3 % reflecting simmondsin hydrolysis. Both extracts were administered to different rat groups via gavage (0.6 g/kg b.w.) before PC treatment (2 g/kg b.w.) three times a week for 3 weeks. The PC intoxication altered the serum biomarkers, the oxidative status, and the Tumor necrosis factor alpha (TNF-α), Bax and Bcl-2 protein expressions of tested animals. In addition, the histological analysis of liver tissues proved significant injury and hepatocellular necrosis. WE and NE extract showed a relatively high in vitro radical scavenging (ORAC) and averting activities (HORAC) with a polyphenol content of 3.6 % and 2.9 %, respectively. Both extracts showed a powerful in vivo hepatoprotective activity against PC-induced toxicity by improving the hepatocellular antioxidant status and blocking proteins expression (TNF-α, Bax and Bcl-2), involved in inflammation and liver damage. However, the enzymatic treatment improved the hepatoprotective activity of NE despite its lower simmondsin content and lower in vitro antioxidant capacity. This enhancement could be linked to the synergetic effect between the antioxidant components and the new hydrolytic products as glucose, uronic acids, arabinose and simmondsin-aglycons. These results suggest that jojoba waste could be potentially valorized in developing hepatoprotective drugs.

1. Introduction

N-acetyl-p-aminophenol (acetaminophen), also known as paracetamol (PC) is an analgesic and antipyretic drug largely used in the world. However, it is considered among the main drugs causing hepatotoxicity when consumed at an overdose by humans or with experimental animals, leading to a fatal hepatic damage [1]. PC is transformed into N-acetyl-*p*-benzoquinone (NAPQI) through the cytochrome P450 enzymatic system in the liver. In humans, NAPQI is excreted by the urinary tract when conjugated with hepatic glutathione (GSH). However, at a PC

Abbreviations: ALT, Alanine aminotransferase; ALP, Alkaline phosphatase https://www.webmd.com/digestive-disorders/alkaline_phosphatase_test; Novozyme® NE, Aqueous simmondsins-hydrolyzed extract using Viscozyme L; WE, Aqueous simmondsins-rich extract; AST, Aspartate aminotransferase; AN, *Aspergillus niger*; b. w., Body weight; CAT, Catalase; Didemethylsimmondsin5DMS, (DDMS), Dry weight (DW)5-demethylsimmondsin; 4DMS, 4-demethylsimmondsin; GAE, Gallic acid equivalents; GGT, Gamma-glutamyl transferase; GSH, Glutathione; GSSG, Oxidized glutathione; HE, Hematoxylin-Eosin; HPLC, High performance liquid chromatography; OH·, Hydroxyl radicals; HORAC, Hydroxyl radical averting capacity; LDH, Lactate dehydrogenase; MDA, Malondialdehyde; NAPQI, N-acetyl-*p*-benzoquinone; ORAC, Oxygen radical absorbance capacity; PC, Paracetamol; ROS, Reactive oxygen species; S, Simmondsin; Cis-FS, Simmondsin-2-*cis*-ferulate; Trans-FS, Simmondsin-2-*trans*-ferulate; $\cdot O_2$-, Superoxide anions; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid-reactive substance; 3TE, -(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)Trolox equivalents; TEAC, Trolox equivalent antioxidant capacity; TNF-α, Tumor necrosis factor alpha.

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overdose, the NAPQI accumulation exhausts the hepatic GSH stores, which promotes the tumor necrosis factor alpha (TNFα) synthesis. This leads to reactive oxygen species (ROS) production, such as hydroxyl radicals (OH·), H_2O_2, and superoxide anions (·O_2-), damaging lipids and macromolecules, such as proteins and nucleic acids in hepatocytes in the liver. As a result, cells undergoing inflammation and apoptosis through pathological oxidative stress lead to liver cell death [2,3]. In the case of PC overdose, the main clinical treatment is the administration of N-acetylcysteine, which is a potent ROS scavenger suppressing oxidative stress. For that reason, many plant-derived compounds have been evaluated for their antioxidant activities and protective effects against PC-induced hepatotoxicity [4–7].

Jojoba (*Simmondsia chinensis*), a dioecious plant growing in desert and semi-desert areas, has been used by Native Americans for medicinal purposes, such as a remedy for cancer, obesity, and throat warts [8,9]. Jojoba seeds are used for producing approximately 50 % of oil which has many cosmetic and medicinal applications, such as skin disorder curing, wound healing, lubricating, anti-inflammatory and antioxidant [8,10,11]. The resulting seed cake is a rich source of proteins (25–30 %) and carbohydrates (50 %) which could be valorized as a feed for livestock. However, the residue could contain a substantial amount of simmondsin reaching 21 % which is considered an anti-nutritional factor due to its cyano group [12,13]. The removal or detoxification of simmondsins from jojoba seed cake is required prior to its use as a feed ingredient. Different chemical, microbiological and enzymatic methods have been used for simmondsins neutralization [14–17]. In addition, a simple water/ethanol simmondsins extraction or using a closed microwave vessel technique with high extraction yield have been investigated [13,18]. It was demonstrated that simmondsin degrade to glucose and aglycon when *β*-glucosidase enzyme is used [19]. Furthermore, the endogenous enzymes of jojoba cake could replace the cyano group into the aglycon moiety by amide group, thus eliminating the simmondsin toxicity [14].

When mice are fed on a high simmondsin dose of 750 mg/kg body weight (b.w.), a food intake reduction has been shown with hepatotoxicity and an intestinal hemorrhage signs resulting in mice death [17]. However, when the doses are lowered to 250 mg/kg b.w, the food intake and the body weight have been decreased without observable harmful impacts on liver, spleen and kidney [20]. In addition, at non-toxic range, simmondsin administration has been linked with several interesting biological properties, such as antifeedant, antifungal, insecticidal, and angiostatic activities [20–23]. Recent studies have demonstrated the antioxidant properties of simmondsin against a hyperglycemia-induced oxidative stress and pancreatic beta-cell damage [24]. Moreover, jojoba seed extract, has expressed beneficial effects against the oxidative stress status of liver in rats [25,26]. The improvement of antioxidant liver capacity has been attributed to the simmondsin and polyphenol molecules and to the possible synergistic effect between different bioactive molecules present in the jojoba seeds extracts. However, it was not clear enough whether namely simmondsins are the major contributor to the observed hepatoprotective effects. To the best of our knowledge, no previous studies on the protective effects of jojoba seed cake extracts against PC-induced toxicity in rats has been reported. Therefore, the aim of the present study was to investigate the hepatoprotective activity of jojoba seed cake extracts in a model of paracetamol-induced toxicity in rats. In order to better understand the simmondsins contribution against the acute paracetamol intoxication, simmondsin-rich and simmondsin-hydrolyzed aqueous extracts were prepared and characterized, and their hepatoprotective activities were investigated and compared. The information obtained from this study could facilitate the development of new functional foods and hepatoprotective drugs from jojoba seed cake.

2. Materials and methods

2.1. Extracts preparation from jojoba seed cake

Jojoba cake was prepared as previously described [13]. Aqueous simmondsin-rich extract (WE) and simmondsin-hydrolyzed extract (NE) have been prepared and characterized in order to investigate and compare their hepatoprotective activity against PC-induced toxicity in rats. Both extracts were prepared in Erlenmeyer flasks, using a ratio of water to jojoba cake 10:1 (w/w), total volume of 200 mL and agitation of 150 rpm at 25 °C on a Brunswick Innova® incubator (Connecticut, USA). The WE was obtained by extracting jojoba cake with water for 2 h. The NE was prepared using a commercially available AN enzyme complex (Viscozyme® L, Novozymes, Canada) with an enzyme/water ratio of 1:200 (v/v) and maceration time of 24 h. The different enzymatic activities of the commercial enzyme preparation are summarized in supplementary Table A.1. Different extracts were centrifuged at 2250 ×*g* for 30 min, using a Hettich Rotina 420 R centrifuge (Tuttlingen, Germany). The collected supernatants were freeze-dried using a Christ®Alpha 1–4 LSClyophilizer (Osterode am Harz, Germany) and stored at −80 °C for further investigations.

2.2. Total polyphenol and flavonoid content analyses

The total polyphenol content was determined according to the method of Singleton and Rossi [27] with Folin–Ciocalteu's reagent, using gallic acid as a calibration standard. The results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (DW). The total flavonoid content was determined according to Chang, Yang, Wen and Chern [28] method, using quercetin dihydrate as a calibration curve standard. The results were presented as mg quercetin equivalents (QE) per 100 g DW.

2.3. Total uronic acid content analysis

The total uronic acid content was evaluated according to the *m*-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen [29]. The results were expressed as w/w% DW using galacturonic acid as a standard.

2.4. High performance liquid chromatography (HPLC) determination of simmondsin and carbohydrate composition

Simmondsins (Didemethylsimmondsin - DDMS, 5-demethylsimmondsin - 5DMS, 4-demethylsimmondsin - 4DMS, simmondsin - S, simmondsin-2-*cis*-ferulate - Cis-FS, and simmondsin-2-*trans*-ferulate-Trans-FS) were analyzed quantitatively with standard curves for each molecule, as described by Feki, Klisurova, Ali Masmoudi, Choura, Denev, Trendafilova, Chamkha and Sayadi [13]. A ZORBAX C18 column and a DAD detector with two mobile phases of 0.1 % acetic acid in water (mobile phase A) and of 100 % acetonitrile (mobile phase B) were used. The results were expressed as percent (%) of DW.

HPLC determination of sugars was performed on a Nexera-*i*LC2040C Plus UHPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a Shimadzu RID 20 A detector, and a Carbohydrate column (5 μm, 4.6 × 150 mm, ZORBAX) with a precolumn Reliance Cartridge (ZORBAX, Agilent Technologies). In an isocratic regime, an eluent of water and acetonitrile mixture (20:80, v/v) was used, with a temperature of 30 °C and flow rate of 1.0 mL/min [30]. The results were expressed as percent of DW. All sugars were identified by comparing the retention times of unknown analytes with analytical grade standards (Glc, Fru, Gal, Rha, Xyl, Fuc, Ara, sucrose, and maltose) purchased from Sigma-Aldrich.

2.5. In vitro antioxidant activity

Oxygen radical absorbance capacity (ORAC) was measured according to the method of [31] with a modification of [32]. The method measures the antioxidant scavenging activity against peroxyl radical induced by 2,2-azobis-(2-amidino-propane) dihydrochloride at 37 °C. ORAC results were expressed as micromole Trolox equivalents per gram (μmol TE/g) of dry extract.

Hydroxyl radical averting capacity (HORAC) was measured as described previously [33]. The method measures the metal-chelating activity of antioxidants under the conditions of Fenton-like reactions, employing a Co (II) complex and hence protecting ability against the formation of hydroxyl radical. HORAC results were expressed as μmol gallic acid equivalents (μmol GAE/g) per gram of dry extract.

2.6. Animals and study design

In the present study, all procedures with animals were conducted in strict conformity with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe N° 123, Strasbourg, 1985). The male Swiss rats, aged five weeks and reached a body weight of 250 g, were purchased from SIPHAT-Tunisia. The rats were adapted to laboratory conditions with dark/light periods of 12 h, at room temperature of 22 ± 3 °C, at a minimum relative humidity of 40 %. They were provided with free access to water and diet and were purchased from the company of animal Nutrition-Sfax-Tunisia. After adaptation of one week, the rats were divided into five groups of eight rats per each.

- Group I (C): served as a control group of rats supplied only with a commercial diet.
- Group II (PC): treated group with PC at a dose of 2 g/kg b.w.
- Group III (WE+PC): treated group with 0.6 mg/kg b.w. of jojoba WE and PC (2 g/kg b.w.)
- Group IV (NE+PC): treated group with 0.6 mg/kg b.w. of NE and PC (2 g/kg b.w.).

Prior to the experiment the animals were fasted for around 24 h and allowed access to distilled water only. After that, the respective dose of tested extract solutions was received by each group through oral gavage 3 times per week for 3 weeks. Except for group I, the oral administration of PC was performed 3 h after extract administration until the 19th day.

2.7. Serum and liver sample preparation

After 48 h of the last PC administration, corresponding to the 21th day, the animals were lightly anesthetized by inhalation and the blood from the brachial artery was collected in heparinized tubes. After centrifugation at 450g for15 min at 4 °C, the supernatant was recovered as serum. Then, the liver was carefully dissected out and weighed. Both liver and serum samples were stored for subsequent analyses at −80 °C.

2.8. Serum biochemical analysis

Quantitative estimations of plasma levels of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), https://labtestsonline.org/tests/gamma-glutamyl-transferase-ggt -:~:text=GGT%20is%20elevated%20in%20the,first%20liver%20enzyme%20to.lactate dehydrogenase (LDH), and alkaline phosphatasehttps://www.webmd.com/digestive-disorders/alkaline_phosphatase_test (ALP) were determined using an automatic biochemical analyzer (VitalabFlexorE, USA) at the clinical and Biochemical laboratory of the HediChaker University Hospital, Sfax, Tunisia.

2.9. Cytosol extraction from liver

Liver cytosol extraction was performed using a T25ULTRA-TURRAX® homogenizer (IKA, China). The liver was mixed with saline phosphate buffer and protease inhibitor cocktail (Sigma-Aldrich). After homogenization, the mixture was centrifuged at 14,000 × *g* and 4 °C for 30 min. The obtained supernatant was used for the different kinds of analyses.

2.10. Liver oxidative stress marker determinations

The thiobarbituric acid-reactive substance (TBARS) concentration, as a marker of lipid peroxidation, was measured in liver cytosol samples using the method of [34]. Malondialdehyde (MDA), produced by 1,1,3,3-tetramethoxypropane hydrolysis, was used for a calibration curve construction. TBARS was measured as a total MDA content expressed as nmol/g tissue. The Trolox equivalent antioxidant capacity (TEAC) was carried out according to [35], applying a Trolox standard curve. The results were expressed as μM of TE. Catalase (CAT) activity was determined according to [36] and [37]. Twenty μl of liver cytosolic extract were added to 200 μl of 500 mM H_2O_2 and 780 μl of 0.1 M phosphate buffer (pH 7.5). The H_2O_2 decomposition rate was followed by measuring the decrease in absorbance at 240 nm for 1 min. The CAT activity was definite as the micromoles of H_2O_2 decreased per milligram of protein for a minute and the results were expressed in international units (IU): U/mg protein. Superoxide dismutase (SOD) activity was assayed based on the inhibition of the photoreduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to [38]. One unit of SOD activity was determined as the amount of enzyme that inhibited the oxidation of MTT by 50 % and expressed as U/mg protein. The protein content in liver cytosolic extracts, used for CAT and SOD activity calculations, was determined based on the method of [39]. Hepatic contents of total, reduced and oxidized glutathione were determined according to the method of Chatuphonprasert et al. [40]. The sample was mixed with the reaction mixture, which consisted of EDTA, NADPH, DTNB, and glutathione reductase in PBS buffer (pH 7.0). The total GSH content was determined at 412 nm after 5 min. To determine the GSSG content, the homogenate was treated with 4-vinylpyridine before the addition of the reaction mixture and then incubated at room temperature for 1 h. The GSH levels (total GSH − [2 × GSSG]) and the redox index (defined as the GSH/GSSG ratio) were then calculated.

2.11. Histopathological and immunohistochemical analysis

The histopathological analysis using Hematoxylin-Eosin (HE) staining and the immunohistochemistry analysis using the primary rabbit polyclonal antibodies against Bax, Bcl-2, and TNF-α were realized as previously described [35,41]. Microscopic images of liver tissue (400 × magnification) were evaluated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA) and scored according to [42,43].

2.12. Statistical analysis

All samples were analyzed for at least three times and all tests were repeated at least twice. All quantitative results were expressed as means ± SD. Differences between treatments were analyzed by one-way ANOVA, followed by Tukey's Post-Hoc test for multiple comparisons with statistical significance.

3. Results and discussion

3.1. Chemical characterization of jojoba seed cake extracts

The aqueous extracts of Jojoba cake WE and NE were prepared, after a kinetic study of simmondsin contents, identified with HPLC in the bulk

Table 1
Chemical characterization of aqueous simmondsin-rich extract (WE) and simmondsin-hydrolyzed extract (NE), obtained from jojoba seed cake.

	WE	NE
Glucose, %	16.2 ± 1.0	27.3 ± 1.3**
Arabinose, %	n.f.[a]	1.5 ± 0.1
Total uronic acid content, %	0.7 ± 0.1	1.9 ± 0.0*
Simmondsins, %	33.0 ± 3.3	3.0 ± 0.9*
Didemethylsimmondsin	10.6 ± 2.0	0.6 ± 0.6*
5-demethylsimmondsin	3.7 ± 1.1	0.1 ± 0.1*
4-demethylsimmondsin	3.0 ± 0.7	0.0 ± 0.0*
Simmondsin	10.8 ± 0.6	0.2 ± 0.2**
Simmondsin-2-*trans*-ferulate	4.9 ± 1.0	2.2 ± 0.0*
Total phenolics, %	3.6 ± 0.1	2.9 ± 0.1*
Total flavonoid content, %	0.2 ± 0.0	0.1 ± 0.0*
ORAC[b], (μmol TE[c]/g)	730.7 ± 68.5	533.1 ± 49.2**
HORAC[d], (μmol GAE[e]/g).	217.5 ± 9.9	146.9 ± 27.4*

[a] not found,
[b] Oxygen radical absorbance capacity;
[c] Trolox equivalents;
[d] Hydroxyl radical averting capacity;
[e] Gallic acid equivalents. The different parameter significance for NE vs. WE: **$p \leq 0.01$, *$p \leq 0.05$.

solutions, during different extraction conditions (results not shown). The selected experimental conditions at the bench scale, based on higher and lower simmondsin content, were used in a scaled-up experiment. The aim was to produce a higher quantity of two extracts for further characterization and investigations with experimental animals in order to identify the main bioactive compound (s) possibly associated with their hepatoprotective properties.

3.1.1. Simmondsin and carbohydrate content of extracts

The analysis of the different extracts showed that after enzyme treatment the simmondsin content decreased from 33.0 % to 3.0 % (Table 1). The decrease in simmondsin content could be explained by the β-glucosidase activity of AN enzyme preparation, able to degrade simmondsin into sugar (glucose) and aglycon [19]. In order to confirm simmondsin degradation, glucose content of both extracts was measured using HPLC and presented in Table 1. After enzyme treatment, glucose content increased from 16.2 % to 27.3 %. Logically, the decreasing of simmondsin content contributed to the increase of glucose content, which was a clear sign that simmondsins degraded. This degradation would evidently result in the increase of the aglycon content in the extract [19]. More specifically, individual simmondsin molecules of different extracts were identified and quantified using HPLC analysis. Table 1 showed that DDMS, 5DMS, 4DMS, S and *trans*-FS content decreased from 10.6 %, 3.7 %, 3.0 %, 10.8 % 4.9–0.6 %, 0.1 %, 0.0 %, 0.2 %, 2.2 %, respectively. Overall, most of the individual simmondsin molecules decreased drastically reflecting β-glucosidase effectiveness on degrading simmondsin into glucose and aglycon. However, the *trans*-FS content was less affected by the enzymatic treatment, probably due to the steric hindrance of the ferulic acid moiety of FS to the β-glucosidase, thus protecting partially *trans*-FS from degradation.

The analysis of the different extracts showed that the enzymatic treatment affected the quality of jojoba cake carbohydrates. Accordingly, uronic acids and arabinose were found as products of enzyme hydrolysis (Table 1). From the results it was evident that the total uronic acid concentration increased from 0.7 % to 1.9 %, which was due to the ability of the AN enzyme preparation to hydrolyse homogalacturonan fragments of pectin in jojoba seed cake into mono- and oligomeric α-D-galacturonic acid [44,45]. Additionally, arabinose a new monosaccharide that was not detectable in the initial WE, appeared in NE (1.5 %) after the enzymatic treatment. As reported before, arabinose could be found in jojoba carbohydrate hydrolysate [46]. Moreover, it is possible that *Aspergillus* enzyme treatment of pectin and hemicellulose, could accumulate monosaccharides like arabinose, rhamnose, etc. [47]. The noticeable increase in the amount of free sugar fraction could be explained by the synergistic hydrolytic effects of *Aspergillus* enzymes and endogenous enzymes on jojoba seed polysaccharides (pectin, cellulose, hemicellulose, starch) leading to the liberation of monomeric, dimeric, and other oligomeric sugars, such as galactose, xylose, glucose, isoprimeverose, fucose, etc. [48]. In fact, the enzyme complex from *Aspergillus sp.* contains many cell wall degrading enzymes with synergistic effects, especially *endo*-pectinase, CMC-ase, and xylanase (Table A1). These enzymes can cleave the long-chain cellulosic fibers and a part of pectic polymers and hemicelluloses into smaller molecules and release them to the bulk solution (saccharification). Other monosaccharides, oligosaccharides and smaller polysaccharide fractions, which were not analyzed, could exist in the treated extract resulting from jojoba cake saccharification. However, rhamnose, fructose, xylose and galactose were not detected.

3.1.2. Polyphenol, and flavonoid content of extracts

The analysis of WE showed that phenolic content in the sample was 3.6 % (Table 1). After enzyme treatment, the content decreased to 2.9 %. As mentioned previously, the carbohydrases could release other compounds such as aglycons and saccharides which could decrease the purity and the quantity of polyphenol in NE. Furthermore, the flavonoid content decreased by 50 %, from 0.2 % to 0.1 % after enzyme treatment, which could reflect the flavonoid bioconversion under the β-glucosidases activity by leading to aglycon liberation [49].

From these results it could be concluded that the enzyme treatment enhanced not only the simmondsin removal from the extract but also increased the content of glucose, arabinose and uronic acids. Glucose was the most important new component reflecting the high simmondsin degradation and aglycon liberation in the extract. On the other hand, the total polyphenol content slightly decreased, however, the total flavonoid content showed a considerable reduction in the extract.

3.2. ORAC and HORAC antioxidant activities

In vitro free radical scavenging activity of simmondsin-rich and hydrolyzed extracts were tested by measuring their ORAC activity against peroxyl radicals and HORAC activity against the formation of hydroxyl radicals. The ORAC values of WE and NE were 730.7 ± 68.5 and 533.1 ± 49.2 μmol TE/g, respectively. Similar to ORAC, HORAC values decreased from 217.5 ± 9.9–146.9 ± 27.4 μmol GAE/g, for WE and NE, respectively (Table 1). To the best of our knowledge, such data for jojoba cake extracts for the HORAC capacity are lacking in the available literature. These findings show that both extracts exhibited a metal chelating antioxidant capacity, protecting against hydroxyl radical ($HO^{\cdot}$) generation via the Fenton reaction. Compared to our recent study [13], both extracts exhibited quite high ORAC values. It has been demonstrated that this ORAC antioxidant activity was highly correlated with the polyphenol and simmondsin contents [13]. However, the enzyme treatment highly decreased the simmondsin content by 90.9 %, which did not highly affect the ORAC antioxidant activity (decrease by 27.0 %). Thus, after simmondsin hydrolysis to glucose and aglycon, and/or possible aglycon cyano group transformation to amino group [14], the jojoba cake extract maintained its high radical scavenging and radical averting capacity. Probably, simmondsin products had maintained good correlation with those antioxidant activity which could explain the relatively high antioxidant activities of NE.

3.3. Effect of extracts against paracetamol-induced toxicity in rats

The simmondsins administered doses to rat groups from WE and NE were about 198 and 18 mg/kg of rat's b.w., respectively, which is in the range of non-liver-toxic doses for rats as demonstrated by Boozer and Herron [20]. Both extracts were used as potential food supplements for different groups of rats in order to evaluate their in vivo hepatoprotective activities, using a PC-induced hepatic injury model. The

Table 2
Body and organ weight parameters of male rats.

Groups	C[a]	PC[b]	WE+PC[c]	NE+PC[d]
Initial body weight (g)	269 ± 5	272 ± 7	267 ± 3	268 ± 8
Final body weight (g)	280 ± 13	281 ± 10	274 ± 12	278 ± 9
Absolute liver weight (g)	7.6 ± 1.5	11.1 ± 0.8****	8.8 ± 0.6+++	8.4 ± 0.4+++
liver weight/ body weight (%)	3.1 ± 0.2	5.3 ± 0.4****	3.7 ± 0.5+++	3.3 ± 0.1+++

[a] Control;
[b] Paracetamol;
[c] Group treated with aqueous simmondsin-rich extract (WE) and PC;
[d] Group treated with aqueous simmondsin-hydrolyzed extract (PE) and PC. Treated (PC) vs. Control (C): ****$p \leq 0.0001$. Treated (WE+PC, NE+PC) vs. Treated (PC): $^{+++}p \leq 0.001$, $^{++++}p \leq 0.0001$.

different physical and biochemical parameters were monitored and discussed below.

3.3.1. Body and liver weight

The results of the experimental tests performed with the extracts on rats' body and liver weights are summarized in Table 2. The obtained data showed that there was no significant difference in the rats' mean b. w. between different groups. However, the PC treatment induced an increase in the absolute and the mean relative liver weight, as compared to the control group. This indicated that PC induced vital signs change in rats. The increase in liver weight might be due to edema in the tissue, caused by PC overdose, as reported by [50]. Interestingly, the pretreatment of PC intoxicated rats with different jojoba extracts blocked the weight of liver gain and maintained the weight near to those of control group. This result suggested that both extracts had a positive effect in improving metabolic function in the rat liver.

3.3.2. Serum biochemical parameters

Measurements of blood biochemical parameters, such as AST, ALT, GGT, ALP, and LDH, usually used for early detection of hepatic damage, were done at the end of the experiment. Fig. 1 show a significant ($p < 0.001$) increase in the plasma levels of these different enzymes in the PC-treated group when compared to the control group. An increase of AST by 47.2 % and ALT by 113.9 % was observed. As a classical biochemical index, this enhancement indicated liver dysfunction and cell damage. The observed increase in GGT level by 337.5 %, also indicated liver injury. In fact, at normal hepatic function, GGT enzyme is mostly found in the canalicular area of the hepatocyte plasma membrane. Thus, the increases in GGT activity in serum proved liver damage. Moreover, the increase in ALP by 196.4 % activity reflects the pathological alteration of biliary flow under PC overdose intoxication. Accordingly, the observed increase in LDH activity by 165.6 % reflected an alteration in the plasma membrane permeability and/or integrity. Thus, the marked release of these biochemical markers into rat's blood circulation (which normally exist in small contents in the serum) indicated severe damage in the hepatic tissue membranes during PC intoxication. These findings were in agreement with different previous studies reporting similar results for AST, ALT, GGT, ALP, and LDH activity increase under PC-intoxication [4,7].

The pretreatment of PC intoxicated rats with WE or NE jojoba extracts induced a reduction in plasma levels of AST, ALT, GGT, ALP and

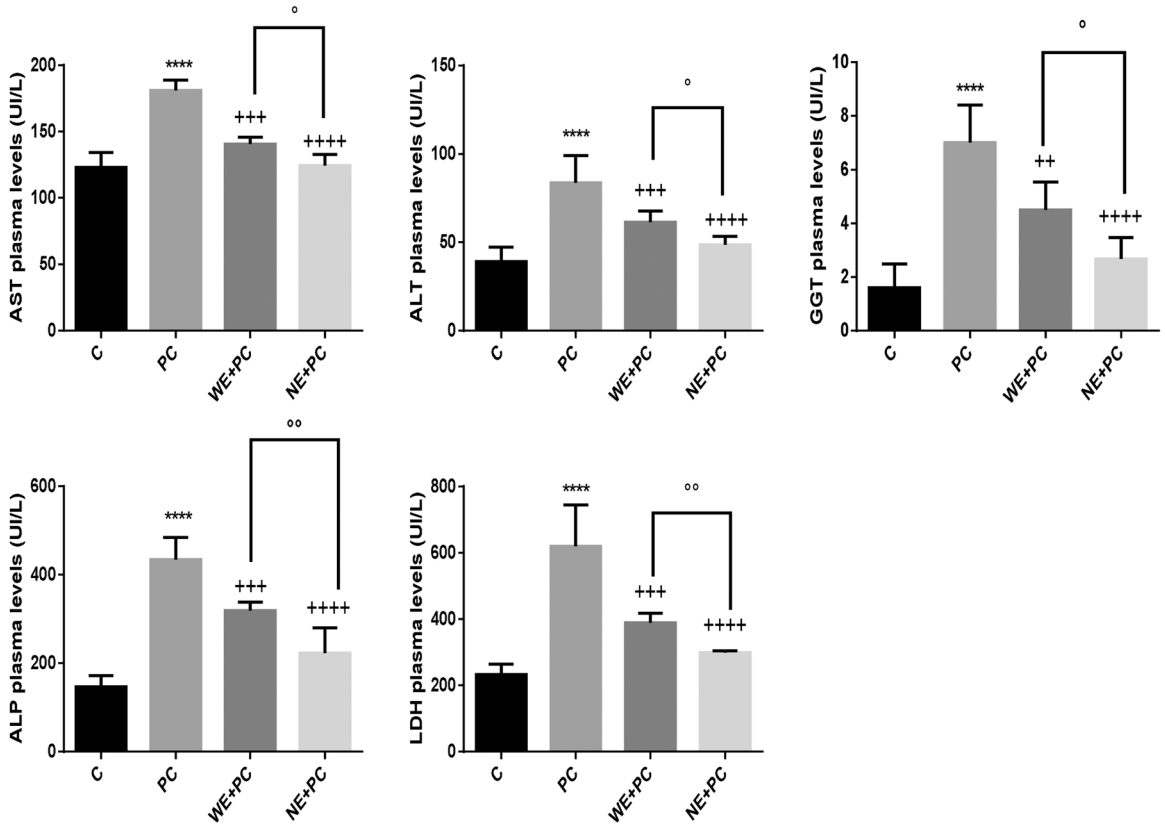


Fig. 1. Effect of jojoba-seed extracts on plasma levels' changes (UI/L) of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase https://www.webmd.com/digestive-disorders/alkaline_phosphatase_test(ALP), and lactate dehydrogenase (LDH). Control group (C), treated group with paracetamol (PC), treated group with aqueous simmondsin-rich extract and PC (WE+PC), and treated group with aqueous simmondsin-hydrolyzed extract and PC (NE+PC). Treated (PC) vs. Control (C): ****$p \leq 0.0001$. Treated (WE+PC, NE+PC) vs. Treated (PC): $^{++++}p \leq 0.0001$, $^{+++}p \leq 0.001$, $^{++}p \leq 0.01$. Treated WE+PC vs. $^{\circ\circ}p \leq 0.01$, $^{\circ}p \leq 0.05$.

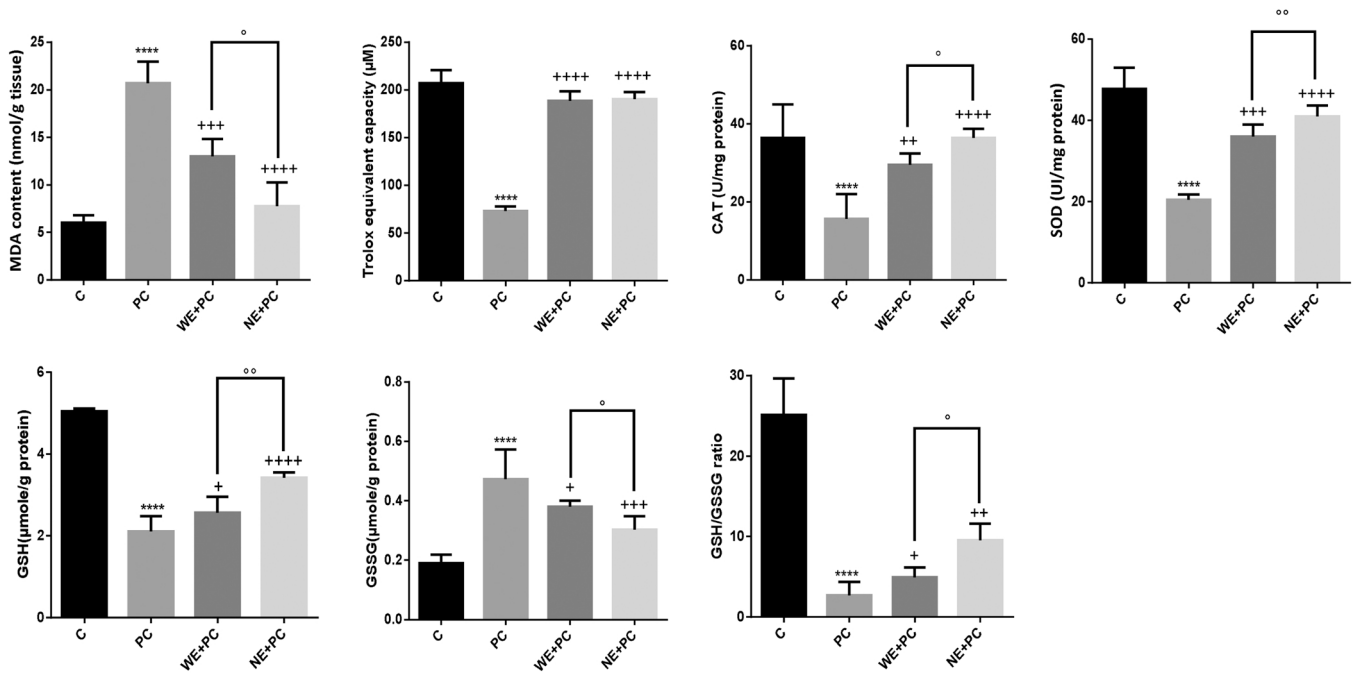


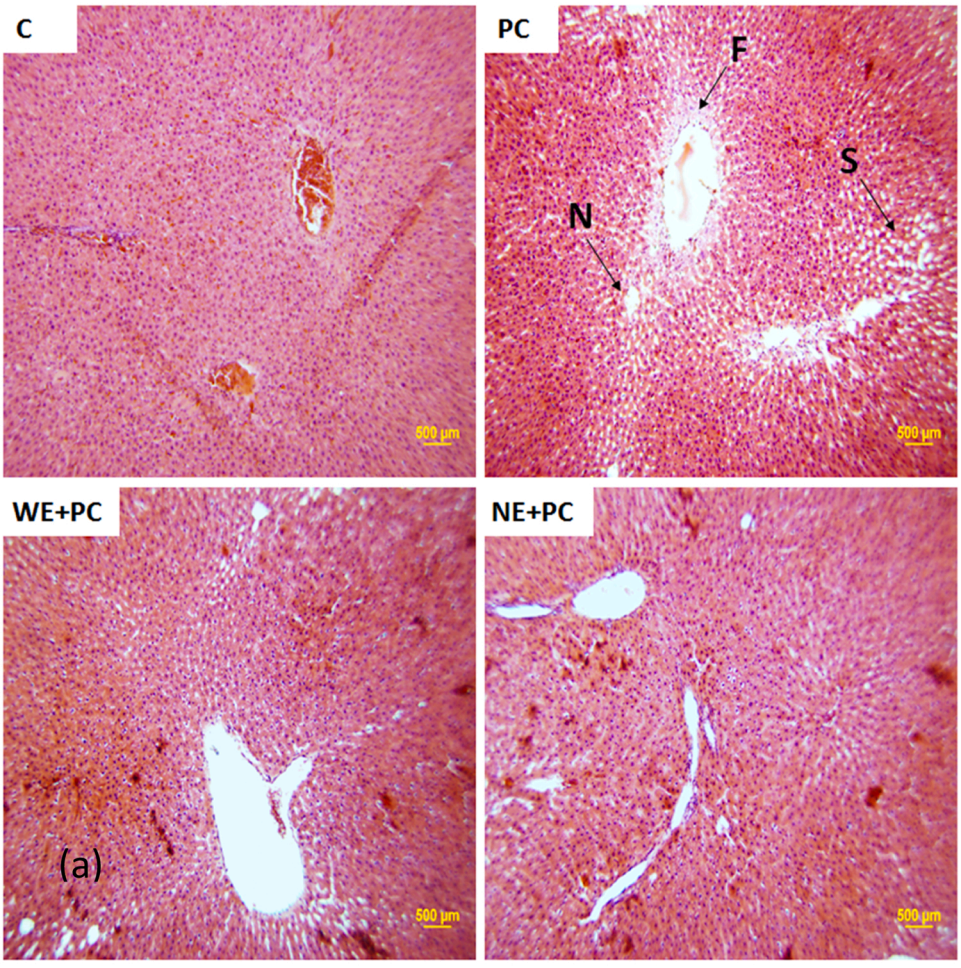
Fig. 2. Effects of paracetamol (PC), aqueous simmondsin-rich extract (WE) and simmondsin-hydrolyzed extract (NE) on Trolox equivalent antioxidant capacity (TEAC), lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), oxidized glutathione (GSSG) and the redox index (GSH/GSSG ratio). Control group (C), treated group with paracetamol (PC), treated group with PC and WE (WE+PC), and treated group with PC and NE (NE+PC). Treated (PC) vs. Control (C): ****p ≤ 0.0001, *p ≤ 0.05. Treated (WE+PC, NE+PC) vs. Treated (PC): + ++ +p ≤ 0.0001, + ++p ≤ 0.001, + +p ≤ 0.01, +p ≤ 0.05. Treated WE+PC vs. Treated NE+PC: °°p ≤ 0.01, °p ≤ 0.05.

LDH (Fig. 3). When compared to the PC-treated group, a significant ($p \leq 0.01$) reduction was observed for the WE group by 22.4 %, 26.9 %, 35.7 %, 26.4 % and 37.2 %, respectively. Higher significant ($p \leq 0.0001$) decrease by 31.2 %, 42.0 %, 61.9 %, 48.6 % and 51.9 %, respectively, was observed for the NE group. Both jojoba cake extracts were able to promote liver protection against PC induced hepatotoxicity. The protective effects of both jojoba cake extracts against PC-induced biochemical index deterioration can be related to their antioxidant constituents, as polyphenols (e.g. flavonoids), simmondsins leading to their high antioxidant free radical scavenging capacity (ORAC and HORAC) as described in the previous 3.2. section. Many authors have reported that antioxidants including food-derived molecules and plant extracts can relieve the liver PC-induced toxicity [4,6,7,51,52]. However, according to the different plasma levels of the liver injury biomarkers, and despite its relatively lower in vitro antioxidant activities by 27 % for ORAC and 32.5 % for HORAC, and its lower polyphenol and flavonoid contents, the NE had a higher prevention effect than WE. This hepatoprotective activity enhancement could be induced by the new carbohydrate hydrolytic bioactive compounds produced after jojoba enzymes treatment, such as glucose, uronic acids and arabinose, which contents increased to 27.3 %, 1.9 % and 1.5 %, respectively. Indeed, the antioxidant compounds, such as polyphenols (e.g flavonoid, etc.) of NE extract could be associated with the synergetic effect of these new produced bioactive compounds and thus enhanced the liver protection against PC-induced toxicity in rats. An in-depth analysis is required to get a comprehensive understanding of the hepatoprotective effect of both extracts against PC-induced toxicity, which will be developed in the next sections.

3.3.3. *Liver oxidative stress status*

In this study, the oxidative stress status that plays an important role in the development of PC-induced hepatotoxicity [4], was evaluated in the liver by measuring the levels of TEAC, MDA, CAT, SOD and GSH. As it is shown in Fig. 2, a high dose of PC caused a pathological higher level of MDA by 245.2 %, compared to the control group. This observation could result from membrane lipid peroxidation through the ROS accumulation. This remarkable increase in MDA level, reflecting indirectly the hepatic cell injury level, was associated with a significant decrease in the total antioxidant capacity of liver tissue. It was expressed as TEAC by 64.7 % and with a significant decrease in the liver antioxidant enzymes activities of SOD (by 57.1 %) and CAT (by 56.9 %), suggesting that the antioxidant system was weakened. Both enzymes (SOD and CAT) catalyze the conversion of $\cdot O_2^-$ into H_2O_2, which is then transformed into oxygen and water. Their decreases contributed to the ROS increases and liver oxidative status deterioration as reported by [4,5,53]. These results were followed by a significant GSH decrease by 58.2 % and GSSH elevation by 149.1 %, indicating the depletion of the GSH stores and leading to the reduction of GSH/GSSG ratio by 89.3 %. In fact, in healthy liver tissues, the majority of glutathione is found in its reduced form. After hepatic tissue intoxication, GSH-Peroxidase detoxifies peroxides by converting reduced GSH to oxidized form (GSSG), which could be in turn reduced through its specific reductase to restore the GSH level [54]. As predicted, the GSH and the redox index decreased due to the GSH oxidation to GSSG and probably due to the NAPQI accumulation. This exhausted the hepatic GSH stores, resulting in different biochemical indicators and oxidative status perturbations. These results were in agreement with those reported before [4,6,33,53].

The intake of the WE or NE extracts before rats' intoxication with PC improved the hepatocellular antioxidant capacity and reduced the lipid peroxidation of liver tissue towards the control values. As it is shown in Fig. 2, a significant decrease in MDA level for WE and NE treated groups, compared to PC group, by 37.2 % and 62.6 % was observed, respectively. Considering MDA as a vital indicator of lipid peroxidation, the significant decrease of its level suggested that WE and NE had a potent preventive effect against lipid peroxidation. Fig. 2 also shows a significant increase in TEAC, CAT, SOD, GSH levels and GSH/GSSG ratio for WE and NE treated groups, compared to PC group, by 157.8 %, 88.3 %, 75.6 %, 21.7 %, 83.4% and 160.6 %, 100 %, 131.8 %, 62.4 %, 253.9 %, respectively. However, the GSSG significantly decreased by 19.7 % with WE and by 36.0 % with NE. The results demonstrated that both extracts



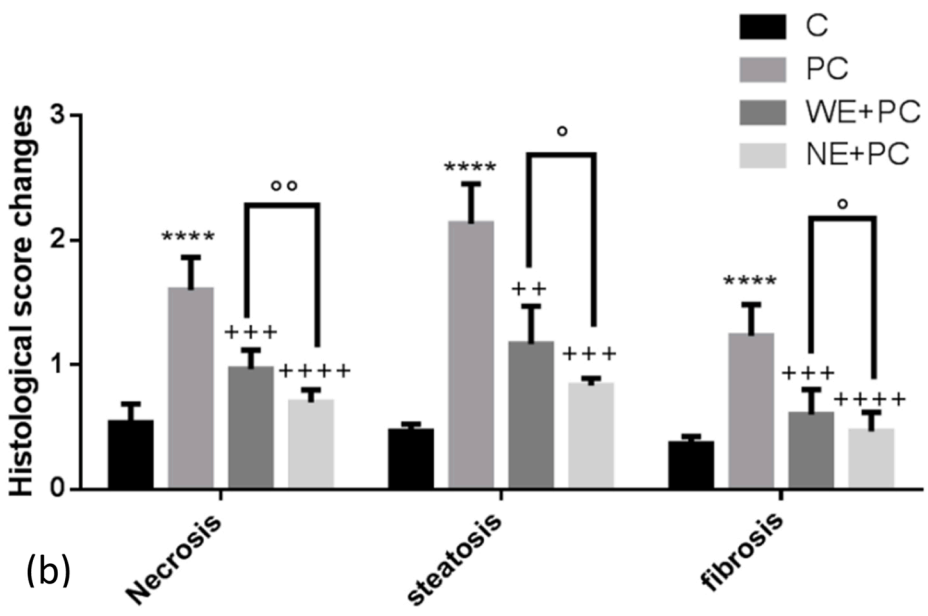


Fig. 3. Histological aspect (100 x) (a) and Histological score changes of steatosis, necrosis and fibrosis (b) of livers of different groups. Control group (C), treated group with paracetamol (PC), treated group with aqueous simmondsin-rich extract and PC (WE+PC), and treated group with aqueous simmondsin-hydrolyzed extract and PC (NE+PC). Treated (PC) vs. Control (C): $^{****}p \leq 0.0001$. Treated (WE+PC, NE+PC) vs. Treated (PC): $^{++++}p \leq 0.0001$, $^{+++}p \leq 0.001$, $^{++}p \leq 0.01$. Treated WE+PC vs. Treated NE+PC: $^{\circ\circ}p \leq 0.01$, $^{\circ}p \leq 0.05$. Notes: Semi-quantitative assessment of liver injury was performed using scores ranging from 0 to 4 as follows: representing a 25 % loss (1 +), representing a 50 % loss (2 +), representing a 75 % loss (3 +) and representing more than 75 % loss (4 +).

reverted the CAT and SOD levels which represent the first line of the antioxidative defense system against ROS. The GSH, GSSG levels and GSH/GSSG ratio followed the improvement and reflected consequently the reduction of PC toxicity effect by possible decrease in NAPQI accumulation. This improvement mediated by the inhibition of the oxidative stress and the enhancement of the anti-oxidative system maintained the level of liver parameters close to the normal level. These results prove that the WE, and the NE improved the enzymatic and non-enzymatic oxidative status in the rat liver and simultaneously reduced the PC-induced lipid peroxidation. However, the different performance of

hepatic oxidative and antioxidant markers (MDA, SOD, CAT, TEAC and GSH) of WE and NE was depending on several metabolic pathways and biochemical factors, which in turn are affected by the quality and the quantity of different active molecules in both extracts.

The present results were in accordance with previous findings showing that incorporation of jojoba seed extract in the diet of rats might restore the liver oxidative status deterioration induced by high-fat diet and high-fructose diet, as well [26]. In a previous study, the same authors demonstrated that simmondsin-containing jojoba seed extract and purified simmondsin are sufficient to reduce pancreatic beta-cell damage [24]. These findings may confirm the liver protective antioxidant properties of WE characterized by a rich-simmondsin content of 33.0 %. In fact, the nitrile group-attached to the sugar or to the cyclohexane ring of simmondsin may induce the expression of hepatic detoxification enzymes, as it has already been observed for glucosinolate aglycons [55]. Furthermore, the relatively high antioxidant potential and free radical scavenging capacity of both extracts, which was previously correlated with simmondsin and polyphenol content, could reflect their high capacity to prevent the oxidative stress [7,51,56]. In fact, the hydroxylated active compounds in WE and NE, having the ability to donate hydrogen, could play a vital role in protection against ROS. Many studies reported that a large variety of phenolic and flavonoid constituent have well contributed on the protective liver herbal extracts such as betacyanins [4], resveratrol [52], luteolin and quercetin 3-β-D-glucoside [6], as well as phenolic-rich oil [57] etc. In fact, the phenolic compounds enhance the hydroxyl groups availability and the ability to donate hydrogen reacting with free radicals liberated after liver intoxication. Therefore, bioactive phytochemicals with high antioxidant potential, superior free radical-scavenging ability, and inhibition of oxidation are contributed to the hepatoprotective traits in animal models [4,52,56,57].

However, the NE with a lower in vitro antioxidant capacity revealed a greater improvement of hepatic antioxidative status in PC-treated rats, when compared to WE. As mentioned before, this observation proved that the hepatoprotective activity improvement is not only attributed to the in vitro antioxidant capacity and common active components between both extracts as polyphenols, simmondsins, etc. It could be related, on the other hand, to the new compounds released by enzymatic treatment, such as aglycons, glucose, arabinose, uronic acids, etc. The aglycon simmondsin product accumulated in NE after enzymes treatment could be more effective in releasing the PC liver intoxication than untreated simmondsins in WE. Probably, aglycon simmondsin products intestinal absorption is better and faster, which could enhance the improvement of the oxidative status. In addition, the glucose released at high content after AN enzyme treatment can be further metabolized in the pentose phosphate pathway in hepatocytes. This might generate NADPH, which is important for the antioxidant defensive systems in the liver, xenobiotic (PC) detoxification by cytochrome P450 and different anabolic processes. In addition, the multiplicated content of uronic acid by 2.7 times and the arabinose appearance under the effect of AN enzyme jojoba cake saccharification, could enhance synergistically the synthesis of the antioxidant enzyme in the liver, leading to improved liver antioxidant status, as reported by Liu, Wu, Wang, Wu, Li, Gao, Liu, Zhang, Cai and Su [58].

Compared to PC group, the significant beneficial effect of WE ($p \leq 0.01$) and NE ($p \leq 0.0001$) groups, indicated that both jojoba cake extracts were able to promote liver protection against PC-induced antioxidant capacity deterioration. After enzyme treatment, the produced simmondsin aglycons, glucose, uronic acids and arabinose significantly and synergistically improved the hepatoprotective activities of NE with the antioxidant and bioactive compounds as polyphenols and simmondsins.

3.3.4. Histopathological study

In order to confirm the biochemical index analysis and oxidative stress status results, histopathological analysis was performed. As shown in Fig. 3a, the liver tissues of the control group exhibited a normal cellular structure with a well-preserved nucleolus, prominent nucleus and cytoplasm with visible thin sinusoids and central veins. In contrast, significant evidence of injury with many leukocytes and severe hepatic architecture loss were observed in the PC treated group liver sections, proving hepatocellular necrosis. The liver section of PC treated group confirmed that besides focal necrosis, there were areas of steatosis and fibrosis with severe fatty changes in the hepatocytes vacuolations, necrotic cells and bundles of confluent stained fibroblasts laying down fresh collagen fibers (Fig. 3a). These histological changes induced by PC within liver tissue were evaluated using semi-quantitative scores ranging from 0 to 4 [43]. Results depicted in Fig. 3b recorded very high levels on the overall histological scores changes as steatosis, fibrosis and necrosis in PC group liver section. These histological changes induced by PC within liver tissue were significantly ($p \leq 0.05$) ameliorated by the supplementation of WE and NE jojoba cake extracts and the hepatic lesions were reversed. The correspondent sections of liver tissue illustrated minor histopathological changes characterized by the reduction of hepatic necrosis, fibrosis and steatosis, which did not return to 100 % of normal level. However, some biochemical hepatic TEAC, serum AST and ALT almost returned to normal. This observation could be explained by the fact that the hepatic histological structure is not dependent only on those parameters but also on oxidative stress status, apoptotic and inflammatory parameters, which were not restabled to 100 % of the normal level.

Furthermore, the histological score calculations confirmed that the NE jojoba extract exhibited a significant ($p \leq 0.5$) improvement in the anatomical structure of liver tissue better than the WE extract. Thus, the observation of HE stained liver sections clearly showed that both jojoba cake extracts noticeably restored PC-induced histological changes. In addition, it confirmed that the new hydrolytic compounds after AN

Table 3
Scoring criteria of immunohistochemistry assay with specific antibodies used in this study.

TNF-α						
Positive stained cells			Staining intensity		Final score product	
Groups	Percent %	Score 1	Intensity	Score 2	Score 1 X Score 2	Score 3
C	<5 %	0	absent	0	0–1	0(-)
PC	51–75 %	3	strong	3	9–12	3+ (+++)
WC+PC	26–50 %	2	moderate	2	5–8	2+(++)
NE+PC	6–25 %	1	Weak	1	2–4	1+(+)
Bcl-2						
C	51–75 %	3	strong	3	9–12	3+ (+++)
PC	6–25 %	1	Weak	1	2–4	1+(+)
WC+PC	26–50 %	2	moderate	2	5–8	2+(++)
NE+PC	26–50 %	2	moderate	2	5–8	2+(++)
Bax						
C	<5 %	0	absent	0	0–1	0(-)
PC	51–75 %	3	strong	3	9–12	3+ (+++)
WC+PC	6–25 %	1	Weak	1	2–4	1+(+)
NE+PC	<5 %	0	absent	0	0–1	0(-)

Control group (C), treated group with paracetamol (PC), treated group with aqueous simmondsin-rich extract (WE) and PC, (WE+PC), and treated group with aqueous simmondsin-hydrolyzed extract (NE) and PC, (NE +PC).
Note: Scoring results are based on screening 12 consecutive microscopic fields. Percent positive cells (score 1) multiplied by the staining intensity (score 2) equals to final product score (score 3). Either 0 or (-) depicts negative staining. For example, an individual slide having <5 % of stained cells (= 0) with a staining intensity of 1 (= weak) will generate a final product score of $0 \times 1 = 0$; another slide showing 80 % of stained cells (= 4) with a staining intensity of 3 (= strong) will give a final product score of $4 \times 3 = 12$ (3 +).

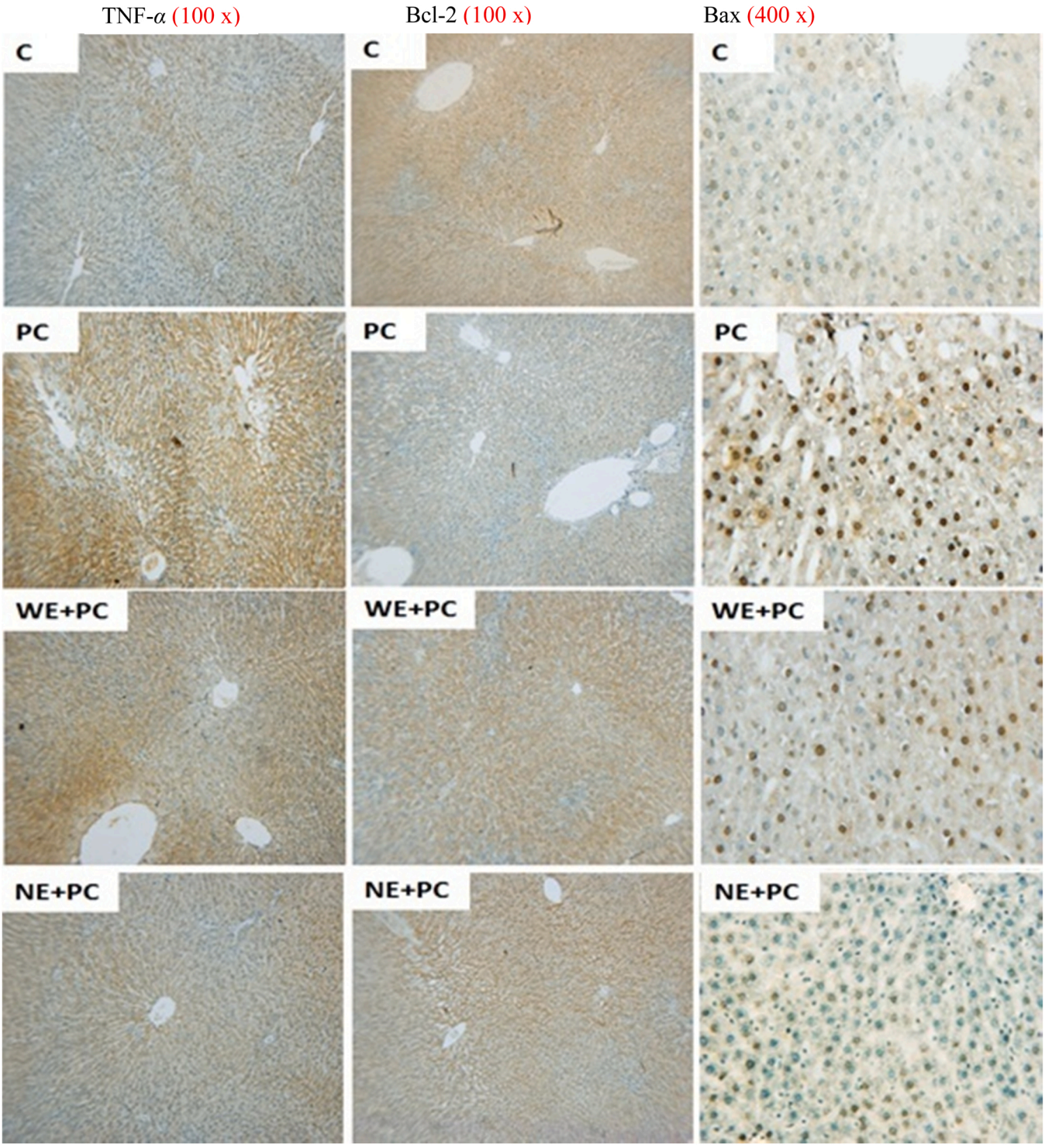


Fig. 4. Immunohistochemical staining with anti-TNF-α, Bcl-2 (100 ×), and Bax (400 ×) in the liver tissues of rats treated with paracetamol (PC), aqueous simmondsin-rich extract (WE) and simmondsin-hydrolyzed extract (NE). (C) Control group, (PC) treated group with PC, (WE+PC) group treated with WE and PC, and (NE+PC) group treated with NE and PC.

treatment improved this hepatoprotective activity better.

3.3.5. *Liver immunohistochemical study*

The hepatotoxicity mechanisms of PC may include antioxidant depletion, reactive metabolite formation, and protein alkylation. More in-depth, the intrinsic toxicity chiefly depends on the genetic variants expression, and the death of hepatocyte follows typically a necrotic or apoptotic pathway depending on many transcription factors [51,59]. To understand the potential mechanisms of the observed effects, the immunostaining was investigated to analyze the TNF-α, Bcl-2 and Bax key proteins implicated in proinflammatory and hepatic apoptosis. Bcl-2, as an anti-apoptotic protein, controls the apoptosis by regulating the caspases cascade. However, the Bax protein, as a pro-apoptotic protein, can form a heterodimer with Bcl-2 inducing the liver cell apoptosis activation by releasing the apoptogenic factors (cytochrome c, Smac/DIABLO, etc.) from the mitochondria to the cytoplasm [51,60]. Table 3 and Fig. 4

show the results of immunohistochemical intensity staining for differently labeled cells. The observation of PC treated rats showed a marked staining with TNF-α reflecting its up-regulating, this result was confirmed with the high scoring criteria of immunohistochemistry (Table 3). In fact, as a pro-inflammatory cytokine, TNF-α could initiate two pathways of nuclear factor kappa B (NF-*k*B) activation and death signaling induction involved in fever induction, apoptotic cell death and inflammation [61]. In addition, a marked staining with Bax protein was observed reflecting its up-regulating, which was associated with a reduction of Bcl-2 immunostaining protein reflecting its down-regulation. These results demonstrated that PC-caused cellular apoptosis. A similar finding was reported by [59] who showed that PC overdose elicited hepatotoxicity in rats with a similar impact on concerned inflammatory and apoptotic markers (TNF-α Bcl-2, and Bax).

Fig. 4 shows that the administration of WE and NE decreased the TNF-α and Bax labeled cells, and increased the Bcl-2 labeled cells which were confirmed by the score criteria calculation of immunohistochemistry presented in Table 3. These results were consistent with the previous improvement in HE staining cytoarchitecture, antioxidant status, and biochemical markers demonstrating that anti-inflammatory and anti-apoptotic biological activity of jojoba cake extracts can be one of the important molecular mechanisms protecting the liver against PC-induced toxicity. These findings are in accordance with the earlier study of Abdel-Wahhab, Joubert, El-Nekeety, Sharaf, Abu-Salem and Rihn [25], who indicated the protective effect of ethanolic jojoba seed extract on livers of rats fed with a fumonisin-contaminated diet. They demonstrated that the incorporation of this polyphenol-simmondsin-rich extract in rats' diet attenuated the rat liver inflammation and significantly decreased the TNF-α, IL-1α, and NO levels in serum.

The NE revealed a higher preventive effect against disturbance of inflammatory and apoptotic markers (TNF-α, Bcl-2, and Bax) in comparison to WE. This inflammatory and apoptotic protective activity enhancement was consistent with previous vital parameters improvement obtained with NE-treated rats. The accumulated components after enzymes treatment such as aglycons, glucose, and uronic acids could enhance these activities against PC induced liver toxicity. Accordingly, Zanobbio, Palazzo, Gariboldi, Dusio, Cardani, Mauro, Marcucci, Balsari and Rumio [62] have reported that glucose oral administration prevented PC-induced liver toxicity and repressed the rat liver inflammation by inducing the secretion of IL-10. [Wu, Fan, Huang, Wu and Guo [59]] reported that *Poria cocos* polysaccharide fraction has pharmacological effects against PC-induced liver injury in mice, characterized by underlying molecular mechanisms of suppressing the NF-kB pathway-based apoptosis and inflammatory stress in liver cells. However, more in-depth investigations are required to elucidate specific mechanisms and pathways for activation modes involved in the hepatoprotective activity of jojoba cake extract against PC-induced toxicity. Besides, the safety of the developed jojoba cake extract should be checked before its use as a food supplement with a hepatoprotective activity.

4. Conclusion

The results from the current study indicated that despite of their simmondsin content, jojoba extracts had powerful protective effects against PC-induced hepatotoxicity and expressed antioxidant, anti-inflammatory and antiapoptotic activities. Both extracts increased the production of antioxidant enzymes, decreased the oxidative biomarkers production inside the liver and blocked the expression of the proteins involved in inflammation and liver damage (TNF-α, Bax and Bcl-2). When WE (simmondsin content 33 %) and NE (simmondsin content 3 %) were administered, the GGT and MDA serum levels were decreased by 35.7 % (WE), 61.9 % (NE), and 37.2 % (WE), 62.6 % (NE), respectively. Furthermore, a significant beneficial effect of WE ($p \leq 0.05$) and NE ($p \leq 0.0001$) were observed on all serum biochemical parameters (AST, ALT, GGT and LDH), and antioxidative defense system parameters (GSH, TEAC, catalase CAT and SOD). Despite the lower ORAC (533.1 μmol TE/g) and HORAC (146.9 μmol GAE/g) antioxidant capacities of NE, its hepatoprotective activity was higher than WE. These findings revealed that the hepatoprotective activity of both extracts could be associated not only with the in vitro antioxidant activity and common bioactive compound content but also with hydrolytic products released after simmondsin and polysaccharide enzyme hydrolysis. Although the positive effect found in this study attributed to such as simmondsin, polyphenol and polysaccharide fractions contained in jojoba seed extracts, it would be interesting to investigate the contribution of purified polysaccharide fractions on the hepatoprotective activity.

CRediT authorship contribution statement

Firas Feki: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Asma Mahmoudi:** Investigation, Methodology, Writing – review & editing. **Petko Denev:** Resources, Writing – review & editing. **Ines Fki:** Conceptualization, Writing – review & editing. **Manol Ognyanov** and **Yordan Georgiev:** Investigation, Writing – review & editing. **Sirine Choura:** Investigation. **Mohamed Chamkha** and **Antoaneta Trendafilova:** Resources. **Sami Sayadi:** Conceptualization, Writing – review & editing, Supervision.

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Conflict of interest statement

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113371.

References

[1] G.W. Przybyła, K.A. Szychowski, J. Gmiński, Paracetamol–an old drug with new mechanisms of action, Clin. Exp. Pharmacol. Physiol. 48 (1) (2021) 3–19.
[2] H. Jaeschke, A. Ramachandran, Mechanisms and pathophysiological significance of sterile inflammation during acetaminophen hepatotoxicity, Food Chem. Toxicol. 138 (2020), 111240.
[3] J.A. Hinson, D.W. Roberts, L.P. James, Mechanisms of acetaminophen-induced liver necrosis, Advers. Drug React. (2010) 369–405.
[4] H.A. González-Ponce, M.C. Martínez-Saldaña, P.G. Tepper, W.J. Quax, M. Buist-Homan, K.N. Faber, H. Moshage, Betacyanins, major components in Opuntia red-purple fruits, protect against acetaminophen-induced acute liver failure, Food Res. Int. 137 (2020), 109461.
[5] T. Lakshmi, B. Sri Renukadevi, S. Senthilkumar, P. Haribalan, R. Parameshwari, R. Vijayaraghavan, S. Rajeshkumar, Seed and bark extracts of Acacia catechu

protects liver from acetaminophen induced hepatotoxicity by modulating oxidative stress, antioxidant enzymes and liver function enzymes in Wistar rat model, Biomed. Pharmacother. 108 (2018) 838–844.
[6] S. Shanmugam, P. Thangaraj, Bd.S. Lima, R. Chandran, A.A. de Souza Araújo, N. Narain, M.R. Serafini, L.J.Q. Júnior, Effects of luteolin and quercetin 3-β-d-glucoside identified from Passiflora subpeltata leaves against acetaminophen induced hepatotoxicity in rats, Biomed. Pharmacother. 83 (2016) 1278–1285.
[7] M.T. Olaleye, A.C. Akinmoladun, A.A. Ogunboye, A.A. Akindahunsi, Antioxidant activity and hepatoprotective property of leaf extracts of Boerhaavia diffusa Linn against acetaminophen-induced liver damage in rats, Food Chem. Toxicol. 48 (8) (2010) 2200–2205.
[8] J.R. Al-Obaidi, M.F. Halabi, N.S. AlKhalifah, S. Asanar, A.A. Al-Soqeer, M. Attia, A review on plant importance, biotechnological aspects, and cultivation challenges of jojoba plant, Biol. Res. 50 (2017).
[9] J. Wisniak, The chemistry and technology of jojoba oil, Am. Oil Chem. Soc. (1987).
[10] E. Ranzato, S. Martinotti, B. Burlando, Wound healing properties of jojoba liquid wax: an in vitro study, J. Ethnopharmacol. 134 (2) (2011) 443–449.
[11] R.R. Habashy, A.B. Abdel-Naim, A.E. Khalifa, M.M. Al-Azizi, Anti-inflammatory effects of jojoba liquid wax in experimental models, Pharmacol. Res. 51 (2) (2005) 95–105.
[12] S. Agarwal, S. Khan, Comparative molecular and chemical fingerprinting found accession "Clone-64" as the best genetic material for jojoba industry, Meta Gene 17 (2018) 115–123.
[13] F. Feki, D. Klisurova, M. Ali Masmoudi, S. Choura, P. Denev, A. Trendafilova, M. Chamkha, S. Sayadi, Optimization of microwave assisted extraction of simmondsins and polyphenols from Jojoba (Simmondsia chinensis) seed cake using Box-Behnken statistical design, Food Chem. (2021), 129670.
[14] A. Bouali, A. Bellirou, N. Boukhatem, A. Hamal, B. Bouammali, Enzymatic detoxification of jojoba meal and effect of the resulting meal on food intake in rats, Nat. Prod. Res. 22 (7) (2008) 638–647.
[15] R.M. Elsanhoty, A. Al-Soqeer, M.F. Ramadan, Effect of detoxification methods on the quality and safety of jojoba (Simmondsia chinensis) meal, J. Food Biochem. 41 (5) (2017), e12400.
[16] A.J. Verbiscar, T.F. Banigan, C.W. Weber, B. Reid, R.S. Swingle, J.E. Trei, E. A. Nelson, Detoxification of jojoba meal by lactobacilli, J. Agric. Food Chem. 29 (2) (1981) 296–302.
[17] A.J. Verbiscar, T.F. Banigan, C.W. Weber, B. Reid, J.E. Trei, E.A. Nelson, R. F. Raffauf, D. Kosersky, Detoxification of jojoba meal, J. Agric. Food Chem. 28 (3) (1980) 571–578.
[18] R.A. Holser, T.P. Abbott, Extraction of simmondsins from defatted jojoba meal using aqueous ethanol, Ind. Crops Prod. 10 (1) (1999) 41–46.
[19] C.A. Elliger, A.C. Waiss, R.E. Lundin, Simmondsin, an unusual 2-cyanomethyle-necyclohexyl glucoside from Simmondsia californica, J. Chem. Soc. Perkin Trans. 1 (1973) 2209–2212.
[20] C. Boozer, A. Herron, Simmondsin for weight loss in rats, Int. J. Obes. 30 (7) (2006) 1143–1148.
[21] M.A. Abbassy, S.A. Abdelgaleil, A.-S.H. Belal, M.A.A. Rasoul, Insecticidal, antifeedant and antifungal activities of two glucosides isolated from the seeds of Simmondsia chinensis, Ind. Crops Prod. 26 (3) (2007) 345–350.
[22] A. D'Oosterlynck, S. Raes, Simmondsin for Use as Angiogenesis Inhibitor US7387999B2 (2008).
[23] B. De Paepe, Anti-angiogenic agents and cancer: current insights and future perspectives, Recent Pat. Anti Cancer Drug Discov. 4 (2) (2009) 180–185.
[24] S. Belhadj, O. Hentati, G. Hamdaoui, K. Fakhreddine, E. Maillard, S. Dal, S. Sigrist, Beneficial effect of Jojoba seed extracts on hyperglycemia-induced oxidative stress in RINm5f beta cells, Nutrients 10 (3) (2018).
[25] M.A. Abdel-Wahhab, O. Joubert, A.A. El-Nekeety, H.A. Sharaf, F.M. Abu-Salem, B. H. Rihn, Dietary incorporation of jojoba extract eliminates oxidative damage in livers of rats fed fumonisin-contaminated diet, Hepatoma Res. 2 (2016) 78–86.
[26] S. Belhadj, S. Dal, F. Khaskhoussi, E. Maillard-Pedracini, O. Hentati, S. Sigrist, Anorexic and metabolic effect of jojoba: potential treatment against metabolic syndrome and hepatic complications, Nutr. Metab. 17 (1) (2020) 1–10.
[27] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (3) (1965) 144–158.
[28] C.-C. Chang, M.-H. Yang, H.-M. Wen, J.-C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Anal. 10 (3) (2002).
[29] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal. Biochem. 54 (2) (1973) 484–489.
[30] Y.N. Georgiev, T.G. Batsalova, B.M. Dzhambazov, M.H. Ognyanov, P.N. Denev, D. V. Antonova, C.W. Wold, I.Z. Yanakieva, I.I. Teneva, B.S. Paulsen, S.D. Simova, Immunomodulating polysaccharide complexes and antioxidant metabolites from Anabaena laxa, Oscillatoria limosa and Phormidesmis molle, Algal Res. 60 (2021), 102538.
[31] B. Ou, M. Hampsch-Woodill, R.L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, J. Agric. Food Chem. 49 (10) (2001) 4619–4626.
[32] P. Denev, M. Ciz, G. Ambrozova, A. Lojek, I. Yanakieva, M. Kratchanova, Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties, Food Chem. 123 (4) (2010) 1055–1061.
[33] B. Ou, M. Hampsch-Woodill, J. Flanagan, E.K. Deemer, R.L. Prior, D. Huang, Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe, J. Agric. Food Chem. 50 (10) (2002) 2772–2777.
[34] S.-Y. Park, S.-H. Bok, S.-M. Jeon, Y.B. Park, S.-J. Lee, T.-S. Jeong, M.-S. Choi, Effect of rutin and tannic acid supplements on cholesterol metabolism in rats, Nutr. Res. 22 (3) (2002) 283–295.
[35] I. Fki, S. Sayadi, A. Mahmoudi, I. Daoued, R. Marrekchi, H. Ghorbel, Comparative study on beneficial effects of hydroxytyrosol- and oleuropein-rich olive leaf extracts on high-fat diet-induced lipid metabolism disturbance and liver injury in rats, BioMed. Res. Int. 2020 (2020), 1315202.
[36] H. Aebi, [13] Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.
[37] H. Jemai, I. Fki, M. Bouaziz, Z. Bouallagui, A. El Feki, H. Isoda, S. Sayadi, Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats, J. Agric. Food Chem. 56 (8) (2008) 2630–2636.
[38] W.F. Beyer Jr., I. Fridovich, Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions, Anal. Biochem. 161 (2) (1987) 559–566.
[39] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1–2) (1976) 248–254.
[40] W. Chatuphonprasert, L. Udomsuk, O. Monthakantirat, Y. Churikhit, W. Putalun, K. Jarukamjorn, Effects of Pueraria mirifica and miroestrol on the antioxidation-related enzymes in ovariectomized mice, J. Pharm. Pharmacol. 65 (3) (2013) 447–456.
[41] A. Mahmoudi, H. Ghorbel, R. Marrekchi, H. Isoda, S. Sayadi, Oleuropein and hydroxytyrosol protect from bisphenol A effects in livers and kidneys of lactating mother rats and their pups', Exp. Toxicol. Pathol. 67 (7–8) (2015) 413–425.
[42] Y.X. Li, L. Zhang, D. Simayi, N. Zhang, L. Tao, L. Yang, J. Zhao, Y.Z. Chen, F. Li, W. J. Zhang, Human papillomavirus infection correlates with inflammatory Stat3 signaling activity and IL-17 level in patients with colorectal cancer, PLoS One 10 (2) (2015), e0118391.
[43] T.N. Slaughter, A. Paige, D. Spires, N. Kojima, P.B. Kyle, M.R. Garrett, R.J. Roman, J.M. Williams, Characterization of the development of renal injury in Type-1 diabetic Dahl salt-sensitive rats, Am. J. Physiol. Regul., Integr. Comp. Physiol. 305 (7) (2013) R727–R734.
[44] H. Kuroyama, N. Tsutsui, Y. Hashimoto, Y. Tsumuraya, Purification and characterization of a β-glucuronidase from Aspergillus niger, Carbohydr. Res. 333 (1) (2001) 27–39.
[45] S. Samanta, Microbial pectinases: a review on molecular and biotechnological perspectives, J. Microbiol. Biotechnol. Food Sci. 2021 (2021) 248–266.
[46] T. Watanabe, K. Takahashi, K. Matsuda, Carbohydrates of jojoba seed: isolation and characterization of xyloglucan, in: D.M. Yermanos (Ed.), Proceedings of the Third International Conference on Jojoba and Its Uses, Riverside, Calif, 1978, 83–105.
[47] S.A.A. Saleh, W.A. Abdel Wahab, A.N. El-Dein, W.A. Abdelwahab, A.A.M. Ahmed, W.A. Helmy, F.A. Mostafa, Characterization of Aspergillus niger MK981235 xylanase with extraction of anti-hepatotoxic, antioxidant, hypocholesterolemic and prebiotic Corchorus olitorius stems xylooligosaccharides, Int. J. Biol. Macromol. 166 (2021) 677–686.
[48] T. Watanabe, K. Takahashi, K. Matsuda, Isolation and characterization of oligosaccharides from purified cellulase digest of Jojoba (Simmondsia chinensis) seed xyloglucan, Agric. Biol. Chem. 44 (4) (1980) 791–797.
[49] R.R. Singhania, A.K. Patel, R.K. Sukumaran, C. Larroche, A. Pandey, Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production, Bioresour. Technol. 127 (2013) 500–507.
[50] A.B. Baptista, M.M. Sarandy, R.V. Gonçalves, R.D. Novaes, C. Gonçalves da Costa, J.P.V. Leite, Md.C.G. Peluzio, Antioxidant and anti-inflammatory effects of Anacardium occidentale L. and Anacardium microcarpum D. Extracts on the liver of IL-10 knockout mice, Evid. Based Complement. Altern. Med. 2020 (2020).
[51] Y.-t Liu, B.-n Lu, J.-y Peng, Hepatoprotective activity of the total flavonoids from Rosa laevigata Michx fruit in mice treated by paracetamol, Food Chem. 125 (2) (2011) 719–725.
[52] A.C.N. de Moraes, C.B.V. de Andrade, I.P.R. Ramos, M.L. Dias, C.M.P. Batista, C. F. Pimentel, J.J. de Carvalho, R.Cd.S. Goldenberg, Resveratrol promotes liver regeneration in drug-induced liver disease in mice, Food Res. Int. 142 (2021), 110185.
[53] D.-D. Luo, J.-F. Chen, J.-J. Liu, J.-H. Xie, Z.-B. Zhang, J.-Y. Gu, J.-Y. Zhuo, S. Huang, Z.-R. Su, Z.-H. Sun, Tetrahydrocurcumin and octahydrocurcumin, the primary and final hydrogenated metabolites of curcumin, possess superior hepatic-protective effect against acetaminophen-induced liver injury: Role of CYP2E1 and Keap1-Nrf2 pathway, Food Chem. Toxicol. 123 (2019) 349–362.
[54] J. Zhao, H. Hu, Y. Wan, Y. Zhang, L. Zheng, Z. Hong, Pien Tze Huang Gan Bao ameliorates carbon tetrachloride-induced hepatic injury, oxidative stress and inflammation in rats, Exp. Ther. Med. 13 (5) (2017) 1820–1826.
[55] C.W. Nho, E. Jeffery, The synergistic upregulation of phase II detoxification enzymes by glucosinolate breakdown products in cruciferous vegetables, Toxicol. Appl. Pharmacol. 174 (2) (2001) 146–152.
[56] A.E. El-Hadary, M.F. Ramadan, Antioxidant traits and protective impact of Moringa oleifera leaf extract against diclofenac sodium-induced liver toxicity in rats, J. Food Biochem. 43 (2) (2019), e12704.
[57] A.E. El-Hadary, R.M. Elsanhoty, M.F. Ramadan, In vivo protective effect of Rosmarinus officinalis oil against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats, PharmaNutrition 9 (2019), 100151.
[58] J. Liu, Y. Wu, Y. Wang, X. Wu, Y. Li, C. Gao, Y. Liu, Q. Zhang, J. Cai, Z. Su, Hepatoprotective effect of polysaccharide isolated from Sonneratia apetala fruits on acetaminophen-induced liver injury mice, J. Funct. Foods 86 (2021), 104685.
[59] K. Wu, J. Fan, X. Huang, X. Wu, C. Guo, Hepatoprotective effects exerted by Poria Cocos polysaccharides against acetaminophen-induced liver injury in mice, Int. J. Biol. Macromol. 114 (2018) 137–142.

[60] A. Peña-Blanco, A.J. García-Sáez, Bax, Bak and beyond—mitochondrial performance in apoptosis, FEBS J. 285 (3) (2018) 416–431.
[61] A. Annibaldi, P. Meier, Checkpoints in TNF-induced cell death: implications in inflammation and cancer, Trends Mol. Med. 24 (1) (2018) 49–65.
[62] L. Zanobbio, M. Palazzo, S. Gariboldi, G.F. Dusio, D. Cardani, V. Mauro, F. Marcucci, A. Balsari, C. Rumio, Intestinal glucose uptake protects liver from lipopolysaccharide and D-galactosamine, acetaminophen, and alpha-amanitin in mice, Am. J. Pathol. 175 (3) (2009) 1066–1076.