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Topic: Natural Products and Hepatocellular Carcinoma

Dietary incorporation of jojoba extract eliminates oxidative damage in livers of rats fed fumonisin-contaminated diet

Mosaad A. Abdel-Wahhab[1], Olivier Joubert[2], Aziza A. El-Nekeety[1], Hafiza A. Sharaf[3], Ferial M. Abu-Salem[4], Bertrand H. Rihn[2]

[1]Food Toxicology and Contaminants Department, National Research Center, Dokki, Cairo 12311, Egypt.
[2]Faculty of Pharmacy, EA 3452 CITHEFOR, Lorraine University, 54001 Nancy Cedex, France.
[3]Department of Pathology, National Research Center, Dokki, Cairo 12311, Egypt.
[4]Department of Food Science and Technology, National Research Center, Dokki, Cairo 12311, Egypt.

ABSTRACT

Aim: This study aimed to determine the composition of ethanol extract of jojoba seeds, and to evaluate its hepatoprotective effects in rats fed fumonisin B_1 (FB_1)-contaminated diet. **Methods:** Jojoba seeds were extracted in 95% ethanol, and the chemical composition was determined. Male rats were divided into six groups and treated for 8 weeks as follows: (1) Untreated control; (2) FB_1-contaminated diet (80 mg/kg diet); (3) low dose (0.5 mg/kg b.w.) jojoba extract; (4) high dose (1.0 mg/kg b.w.) jojoba extract; (5) low dose jojoba extract plus FB_1; and (6) high dose jojoba extract plus FB_1. Blood and liver samples were collected for different biochemical analyses and histological examinations. **Results:** The results indicated that the ethanolic extract of jojoba is rich in protein, phenolic compounds, phytic acid, and considerable amounts of simmondsin. Animals fed FB_1-contaminated diet showed severe biochemical and histological changes typical to those reported in literature. Treatment with jojoba seed extract alone at the two tested doses did not induce significant alterations in all parameters tested. Combined treatment of jojoba seed extract with FB_1 eliminated hepatotoxicity induced by FB_1, especially at low dose of jojoba seed extract. **Conclusion:** The authors concluded that jojoba seed extract can be incorporated in FB_1-contaminated feed to eliminate FB_1-induced hepatotoxicity.

Key words: Fumonisin B_1; health hazards; jojoba seed; liver; mycotoxins; oxidative stress

Address for correspondence:
Prof. Mosaad A. Abdel-Wahhab, Food Toxicology and Contaminants Department, National Research Center, Dokki, Cairo 12311, Egypt.
E-mail: mosaad_abdelwahhab@yahoo.com

Received: 19-07-2015, **Accepted:** 07-10-2015

INTRODUCTION

Fumonisins B (FBs) are mycotoxins produced by the fungal species *Fusarium*, including *Fusarium verticillioides* and *Fusarium proliferatum*.[1,2] This mycotoxin is mainly produced on corn and possibly sorghum, which remain the primary sources of human exposure.[3,4] At least 28 FBs have been isolated and characterized.[5] Fumonisin B_1 (FB_1) is the most common toxin, which has been classified by the International Agency for Research on Cancer as a Group 2B carcinogen (possibly carcinogenic in humans).[6] Long-term studies indicated that FB_1 was hepatocarcinogenic in rats[7-9] while another study reported on the nephrocarcinogenicity and cancer promoting activity in rats.[10,11] Epidemiological

Access this article online	
Website: http://www.hrjournal.net/	Quick Response Code
DOI: 10.4103/2394-5079.168078	

How to cite this article: Abdel-Wahhab MA, Joubert O, El-Nekeety AA, Sharaf HA, Abu-Salem FM, Rihn BH. Dietary incorporation of jojoba extract eliminates oxidative damage in livers of rats fed fumonisin-contaminated diet. Hepatoma Res 2016;2:78-86.

evidence suggests that it may be an etiological agent in human esophageal cancer.[12,13] Several studies in rodents have shown that FB_1 promotes pre-neoplastic lesions in the liver, suggesting a role for FB_1-induced genotoxicity.[14] Recently, Chuturgoon *et al*.[15] reported that FB_1 induces global DNA hypomethylation and histone demethylation in human hepatoma cells that causes chromatin instability and may lead to liver tumourigenesis. FB_1 is resistant to conditions normally used in food processing and, therefore, poses a significant hazard to human and animal health.[16] The cytotoxic mechanism of FB_1 is attributed to its disruption of sphingolipid metabolism; the underlying mechanisms of its cancer initiating/promoting properties are unknown. This disturbance of sphingolipid metabolism plays a role in membrane and lipoprotein structure and in cell regulation as secondary messengers for growth factors, differentiation factors, and cytokines.[8]

Jojoba (*Simmondsia chinensis* L) is a perennial woody shrub native to semi-arid regions all over the world.[17] Currently, it is cultivated in the Ismailia Desert in Egypt.[18] The jojoba plant produces seeds that contain up to 50% liquid wax used as a lubricant additive and in cosmetics.[19] It has been reported that jojoba seeds possess anti-inflammatory activity.[20] Moreover, jojoba liquid wax was used in folk remedies for renal colic, sunburn, chaffed skin, hair loss, headache, wounds, and sore throat.[21] Jojoba meal is the protein residue remaining after oil extraction, and it has potential as dietary supplements for animal feeds, as well as for the treatment of overweight animals and humans.[22] This protein meal consists mainly of 79% albumins and 21% globulins.[23] Previous reports indicated that jojoba meal contained anti-nutritional compounds known as simmondsins (5-demethylsimmondsin, 4,5-didemethylsimmondsin, simmondsin, and simmondsin 2'-ferulate),[24] which have been identified as the component in jojoba that is most responsible for the inhibition of food intake and for appetite suppression in rodents, rats, dogs, and chickens.[25] However, the meal also contains several beneficial compounds, such as phytic acid and polyphenols, which shows antioxidant and anti-cancer activity.[26] The aim of this study was to evaluate the effect of ethanol extract of jojoba seed in rats fed FB_1-contaminated diet.

METHODS

Chemicals and kits

FB_1 standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits for analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides, and cholesterol were obtained from Quimica Clinica Aplicada (SA, Spain). Interleukin-1α (IL-1α), procollagen III, and tumor necrosis factor-alpha (TNF-α) kits were purchased from Orgenium (Helsinki, Finland). Kits for measuring nitric oxide (NO), malondialdehyde (MDA), total antioxidant capacity (TAC), carcinoembryonic antigen (CEA), and superoxide dismutase (SOD) were obtained from Biodiagnostic (Giza, Egypt).

Preparation of jojoba seed extract

Jojoba (*S. chinensis*) seeds were obtained from the Crops Department, National Research Center, Dokki, Cairo, Egypt. The seeds (200 g) were ground to a powder and immersed in 95% ethanol overnight. The extract was filtered and evaporated under reduced pressure of nitrogen to obtain a semisolid residue.

Determination of chemical composition in jojoba seed extract

Crude protein (N X 6.25) was determined according to AOAC[27] and phytic acid content in jojoba seed extract was determined according to the method described by Mohamed *et al*.[28] Total phenolics were determined according to the modified method described by Chandler and Dodds[29] and simmondsin content was determined according to Abbott *et al*.[30]

FB_1 production

FB_1 was produced through the fermentation of corn by *Fusarium moniliforme* (obtained from Plant Pathology Department, National Research Center, Dokki, Cairo, Egypt) as described by Voss *et al*.[31] The fermented corn was autoclaved; ground to a powder and the FB_1 content was measured by high-performance liquid chromatography (HPLC) according to Shaphard *et al*.[32] The corn powder was incorporated into the basal diet to provide the desired level of 80 mg FB_1/kg diet. The diet containing FB_1 was analyzed, and the presence of FB_1 was confirmed by HPLC.

Experimental animals

Three months old male Sprague-Dawley rats (100-120 g) were purchased from the Animal House Colony, Giza, Egypt and were maintained on standard laboratory diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy: 12.08 MJ) in artificial illuminated and temperature controlled room free from any other sources of chemical contamination at the Animal House Lab., National Research Center, Dokki, Cairo, Egypt. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt.

Experimental design

After an acclimatization period of 1 week, the animals were divided into six groups (10 rats/group) and housed in filter-top polycarbonate cages. The rats were maintained on their respective diet for 8 weeks as follows: (1) Untreated control; (2) FB_1-contaminated diet (80 mg/kg diet); (3) low dose of jojoba seed extract (JELD) (0.5 mg/kg b.w.); (4) high dose of jojoba seed extract (JEHD) (1.0 mg/kg b.w.); (5) FB_1-contaminated diet and treated with JELD; and (6) FB_1-contaminated diet and treated with JEHD. Body weight and food intake were recorded daily throughout the treatment period. At the end of the treatment period, blood samples were collected from the retro-orbital venous plexus of all animals after fasting for 12 h. The blood sample from each animal was left to clot and centrifuged at 5,000 *g* under cooling for 10 min to separate the serum for the determination of ALT, AST, ALP, triglycerides, cholesterol, NO, IL-1α, TNF-α, and CEA according to the respective kit instructions. After collection of blood samples, all animals were sacrificed and liver samples of each animal were dissected, weighed, and homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate. This homogenate was centrifuged at 1,700 *g* and 4 °C for 10 min and the supernatant was stored at -70 °C for the determination of lipid peroxidation by measuring the formed MDA using thiobarbituric acid reactive substances. The level of lipid peroxidation was expressed as nmol MDA per gram tissue. The liver homogenate was further diluted to give 5% homogenate (w/v), centrifuged at 3,000 *g* for 5 min at 0 °C and used for the determination of SOD and TAC. Another liver sample of each animal was dissected, excised, and fixed in 10% neutral formalin; dehydrated in ascending grades of ethanol; cleaned in xylene; and embedded in paraffin. Five micrometer thick sections were prepared and stained with hematoxylin and eosin according to Drury *et al*.[33]

Statistical analysis

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System.[34] The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio.[35] All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

Composition of ethanol extract of jojoba seeds

The results of this study revealed that ethanol extract of jojoba seeds is rich in crude protein (27.32 g/100 g seeds) and total phenolic content (65.53 mg/100 g). Phytic acid content was 6.83 mg/g, and simmondisin content reached 12.43 mg/g [Figure 1].

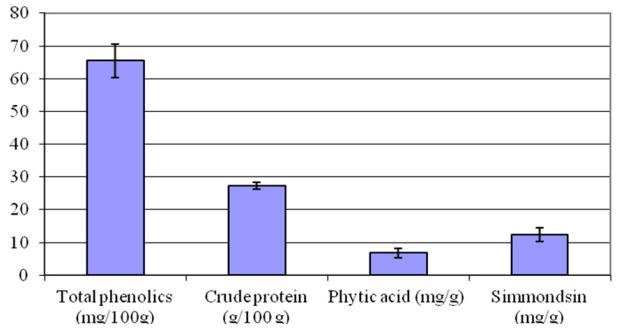

Figure 1: Chemical composition of the ethanol extract of jojoba seeds

Effect of jojoba seed extract on food intake and body weight

The effect of different treatments on food intake indicated that the acute toxicity of FB_1 first appeared as a significant decrease in food intake [Figure 2a]. Animals fed FB_1-contaminated diet showed a significant decrease in food intake throughout the treatment period compared to the control group. Animals treated with jojoba seed extract at both the low and highdoses also showed a gradual decrease in food intake which became severe by the 7th week of treatment and was pronounced in the JEHD group. The combined treatment of FB_1 and jojoba seed extract induced a significant improvement in food intake, although the food consumption was still lower than in the control group. It is of interest to mention that the improvement in food intake was pronounced in the group fed FB_1-contaminated diet and treated with JELD [Figure 2a].

The effect of different treatments on body weight gain of rats is depicted in Figure 2b. Animals fed FB_1-contaminated diet failed to gain weight; however, animals treated with jojoba seed extract showed slight weight gain, although there was a significant difference between these groups and the control. Moreover, animals in the groups treated with the FB_1 and jojoba seed extract did not show any significant increase in body weight, and they were below the normal weight of the control group. Animals receiving combined treatment of FB_1 and jojoba seed extract showed slightly higher weight gain than those receiving FB_1 alone.

Biochemical effects of treatment with FB_1 and jojoba seed extract

The biochemical results [Table 1] revealed that FB_1 alone induced a significant increase in all biochemical parameters tested. The jojoba seed extract alone at both low and high doses did not induce any significant changes in ALT, AST, and triglycerides. However, ALP

showed a significant increase accompanied by a significant decrease in cholesterol level, especially in the high dose group. Animals fed FB_1-contaminated diet and treated with jojoba seed extract showed a significant improvement in all biochemical parameters; although all levels tested were still higher than in the control group. The observed improvement in all biochemical parameters was more pronounced in the group fed FB_1 and treated with JELD.

The data presented in Table 2 showed that treatment with FB_1 resulted in a significant increase in serum CEA, TNF-α, IL-1α, and NO. Animals treated with JELD or JEHD alone were comparable to the control group in terms of the levels of CEA, TNF-α, and NO, however, the level of IL-1α showed a significant increase. Treatment with FB_1 plus JELD or JEHD resulted in a significant improvement in all the tested parameters toward the control values; in the JEHD group, CEA and TNF-α levels were normalized [Table 2].

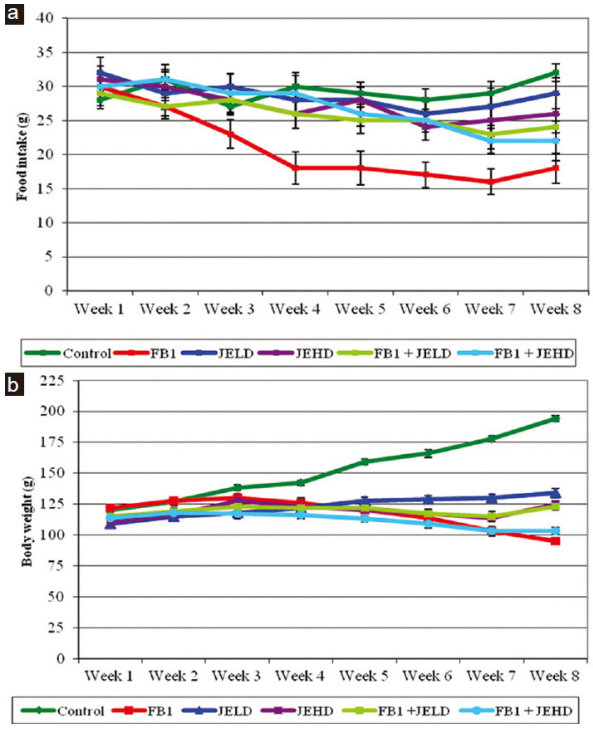

Figure 2: Effect of jojoba extract on (a) food intake and (b) body weight in rat fed FB_1-contaminated diet. FB_1: fumonisin B_1; JELD: low dose of jojoba seed extract; JEHD: high dose of jojoba seed extract

The effect of different treatments on MDA level, glutathione (GSH), and TAC in liver tissue [Table 3] revealed that animal fed FB_1-contaminated diet showed a significant increase in MDA accompanied by a significant decrease in GSH and TAC. Treatment with JELD or JEHD did not affect MDA significantly; however, it resulted in a significant increase in GSH and TAC levels. The combined treatment of FB_1 with jojoba seed extract resulted in a significant improvement in the activity of antioxidant enzymes and decreased lipid peroxidation in the liver tissues although they were still significantly different from the control. Of note, treatment with JEHD showed the best results at improving antioxidant enzymes activity and at decreasing lipid peroxidation.

Histological changes induced by treatment with FB_1 and jojoba seed extract

The above biochemical findings were further confirmed by histological examinations in the liver tissues. The microscopic examination of the liver section of the control rats showed the normal histological structure of liver lobule and hepatocytes which form cords radiating from the central vein [Figure 3a]. The liver sections of rats fed FB_1-contaminated diet showed vacuolar degeneration, hepatocellular necrosis, and congestion of blood sinusoids which were surrounded by an aggregation of inflammatory cells, proliferation and dilation of bile duct, as well as signs of fibrosis [Figure 3b]. The liver sections of rats treated with JELD showed normal hepatocytes architecture, and dilation of sinusoid [Figure 3c]; however, liver sections of rats treated with JEHD did not show any pathological changes [Figure 3d].

The liver of rat fed FB_1-contaminated diet and treated with JELD showed marked improvement in the histological features of the hepatic tissue although minimal vacuolar degeneration was still present [Figure 3e]. However, the liver sections of rats fed FB_1-contaminated diet and treated with JEHD showed histological features resembling normal hepatocytes [Figure 3f].

DISCUSSION

Previous reports indicated that jojoba meal contained 25-30% crude protein, was high in dietary fiber, and could

Table 1: Effect of jojoba extract on biochemical parameters in rats fed FB_1-contaminated diet

Groups parameter	Control	FB_1	JELD	JEHD	JELD + FB_1	JEHD + FB_1
ALT (IU/L)	25.43 ± 2.73^a	76.21 ± 5.18^b	26.72 ± 1.33^a	27.44 ± 2.28^a	34.93 ± 2.28^c	38.21 ± 2.93^c
AST (IU/L)	85.16 ± 3.27^a	112.73 ± 4.22^b	88.25 ± 2.53^a	88.76 ± 4.73^a	96.23 ± 4.92^c	102.32 ± 2.83^c
ALP (IU/L)	82.25 ± 4.28^a	123.32 ± 6.43^b	92.28 ± 4.34^c	97.28 ± 6.22^c	95.28 ± 7.22^c	115.24 ± 2.93^d
Triglycerides (mg/dL)	122.21 ± 3.74^a	243.24 ± 6.43^b	122.34 ± 3.37^a	125.74 ± 2.56^a	142.32 ± 4.89^c	142.73 ± 3.82^c
Cholesterol (mg/dL)	87.23 ± 3.26^a	287.82 ± 7.78^b	79.83 ± 5.38^c	72.34 ± 5.64^c	111.96 ± 3.88^d	117.26 ± 3.95^d

Within each row means superscript with different letters are significantly different at $P \leq 0.05$. FB_1: fumonisin B_1; JELD: low dose of jojoba seed extract; JEHD: high dose of jojoba seed extract; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase

serve as a feed supplement.[19] It represents one of the non-traditional plant protein sources. However, several trials have demonstrated growth retardation in animals consuming diets supplemented with jojoba meal[36] due to the presence of simmondsin and simmondsin-2-ferulate.[37] These compounds were considered toxic probably after metabolism by gut microorganisms.[38] However, elimination of jojoba seed meal anti-nutritional factors could be done by different methods, including solvent extraction, heat, chemical treatment, and microbial fermentation.[39]

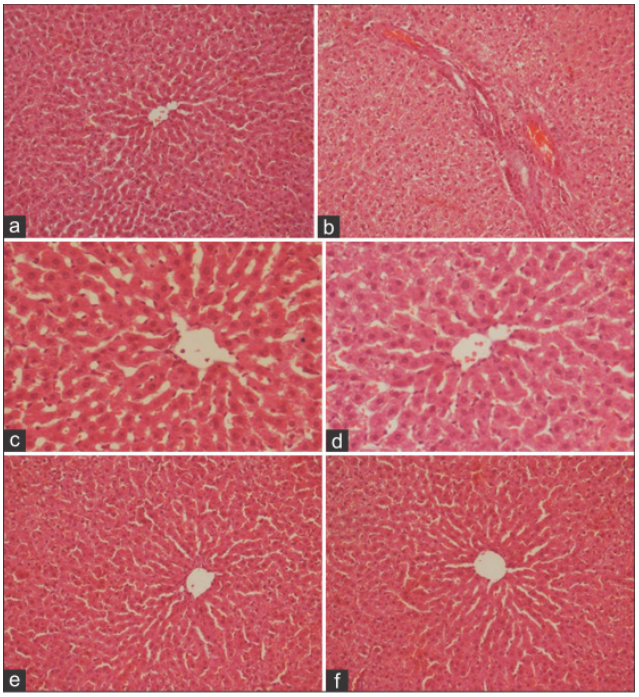

Figure 3: A photomicrograph of liver section of (a) control rat showing normal structure of hepatic lobule, central vein, and blood sinusoid(s); (b) rat fed FB_1-contaminated diet showing vacuolar degeneration, hepatocellular necrosis, and congestion of blood sinusoids which are surrounded by aggregation of inflammatory cells, proliferation and dilation of bile duct, and signs of fibrosis; (c) rat treated with JELD showing normal hepatocytes architecture and dilation of sinusoid; (d) rat treated with JEHD showing no pathological changes; (e) rat fed FB_1-contaminated diet and treated with JELD showing marked improvement in histological features of hepatocyte tissue with minimal vacuolar degeneration still present; and (f) rat fed FB_1-contaminated diet and treated with JEHD showing histological features resembling that of normal hepatocytes (a, e, f: HE, ×200; b, c, d: HE, ×400). FB_1: fumonisin B_1; JELD: low dose of jojoba seed extract; JEHD: high dose of jojoba seed extract

Table 2: Effect of jojoba extract on serum cytokines and NO in rats fed FB_1-contaminated diet

Groups parameter	CEA (ng/mL)	TNF-α (ng/L)	IL-1α (ng/mL)	NO (μmol/L)
Control	1.99 ± 0.42^a	43.2 ± 3.53^a	0.68 ± 0.02^a	23.72 ± 2.11^a
FB_1	8.66 ± 1.43^b	87.32 ± 3.21^b	5.12 ± 0.87^b	57.28 ± 3.21^b
JELD	1.92 ± 0.72^a	43.32 ± 1.98^a	0.81 ± 0.04^c	26.72 ± 1.73^c
JEHD	1.98 ± 0.62^a	45.37 ± 3.25^a	0.81 ± 0.06^c	29.83 ± 1.83^d
FB_1 + JELD	3.53 ± 0.43^c	62.11 ± 3.47^c	1.55 ± 0.12^d	28.94 ± 1.29^d
FB_1 + JEHD	2.14 ± 0.22^a	42.18 ± 2.33^a	1.22 ± 0.07^d	32.93 ± 2.94^e

Within each row means superscript with different letters are significantly different at $P \leq 0.05$. FB_1: fumonisin B_1; JELD: low dose of jojoba seed extract; JEHD: high dose of jojoba seed extract; CEA: carcinoembryonic antigen; TNF-α: tumor necrosis factor-alpha; IL-1α: interleukin 1 alpha; NO: nitric oxide

In this study, we evaluated the ability of ethanol extract of jojoba seeds to protect the liver of laboratory animals from the toxic effects of FB_1. The tested animals were given an extreme FB_1 challenge to ensure induction of severe response. The selected doses of FB_1 and jojoba seed extract were based on our previous work and others,[8,40] respectively. The current results indicated that the ethanol extract of jojoba seeds is rich in total phenolics, crude protein, phytic acid, and simmondsin. These results were in accordance with those reported previously.[19,41-43] Moreover, Shrestha *et al*.[23] reported that jojoba protein consisted mainly of albumins and globulins. The decrease in body weight gain and food intake reported in this study in the group fed FB_1-contaminated diet indicated the presence of adverse effects and toxicity in rats caused by ingestion of FB_1. This decrease may indicate protein catabolism, thereby contributing to the observed kidney injury.[8,9,44,45] Similar decrease in body weight gain and food intake had been reported in rats,[9,44] swine,[45] horses,[46] broiler,[47] and Turkey poults[48,49] fed fumonisin. Previously, Abdel-Wahhab *et al*.[8] and El-Nekeety *et al*.[9] stated that administration of FB_1 to rats enhanced lipid peroxidation which presumably resulted from free-radical-mediated toxicity. Stockmann-Juvala *et al*.[50] found that FB_1 evoked oxidative stress, which may contribute at least in part to FB_1-induced toxicity and carcinogenicity.

The elevation of ALT, AST, ALP, triglycerides, and cholesterol in the group fed FB_1-contaminated diet indicated necrosis or hepatocellular injury.[9] The results of this study also revealed that treatment with FB_1 resulted in a significant increase in serum CEA, TNF-α, IL-1α, and NO suggesting that FB_1 can induce hepatotoxicity in rats. Similar results suggested earlier indicated that TNF-α, IL-1α, and NO were produced by macrophages, and they played a vital role in tumor conditions.[51] Moreover, TNF-α is an essential factor in tumor promotion[52] and is a key factor that regulates the production of other

Table 3: Effect of jojoba extract on antioxidants and lipid peroxidation in liver of rats fed FB_1-contaminated diet

Groups parameter	Control	FB_1	JELD	JEHD	JELD + FB_1	JEHD + FB_1
MDA (mol/mg protein)	66.85 ± 2.37^a	115.36 ± 3.44^b	67.26 ± 2.73^a	68.23 ± 3.16^a	87.74 ± 3.19^c	85.91 ± 3.02^c
SOD (u/mg protein)	331.43 ± 8.65^a	166.74 ± 7.34^b	352.33 ± 3.46^c	348.93 ± 5.88^c	258.33 ± 6.72^d	277.76 ± 4.27^e
TAC (mol/g protein)	82.25 ± 4.28^a	123.32 ± 6.43^b	92.28 ± 4.34^c	97.28 ± 6.22^c	95.28 ± 7.22^c	104.33 ± 2.75^d

Within each row means superscript with different letters are significantly different at $P \leq 0.05$. FB_1: fumonisin B_1; JELD: low dose of jojoba seed extract; JEHD: high dose of jojoba seed extract; MDA: malondialdehyde; SOD: super oxide dismutase; TAC: total antioxidant capacity

cytokines involved in chronic inflammation and tumor development via the nuclear factor kappa B pathway.[53] Moreover, the increase of NO and MDA and the decreased level of SOD and TAC in rats fed with FB_1 suggested that FB_1 administration enhanced the generation of free radicals which directly led to free radical-mediated toxicity.[8,9,54,55] The generation of free radicals is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenesis induced by many carcinogens.[56,57] In this respect, Hassan *et al*.[58] reported that liver damage was directly related to free radical mediated toxicity which was known to attack the highly unsaturated fatty acids of the cell membrane and considered a key process in many pathological events induced by oxidative stress[59] Another mechanism of FB_1-induced injury was suggested by Pinelli *et al*.[60] who stated that FB_1-induced a down-regulation of cytoplasmic phospholipase A2 activity and arachidonic acid metabolism by a mechanism involving prostaglandin production, cyclic adenosine monophosphate synthesis, and protein kinase activation, as well as global DNA hypomethylation and histone demethylation that causes chromatin instability and may lead to liver tumorigenesis.[61]

The histological findings of the liver strongly confirmed the biochemical results. We demonstrated that jojoba seed extract had a protective role against FB_1-induced liver damage, as indicated by improvements in the histological structure of the liver tissues. Similar histological changes in the liver tissues were reported previously.[9] Moreover, Abdel-Wahhab *et al*.[8] and Voss *et al*.[61] stated that FB_1 specifically disrupt cellular sphingolipid metabolism causing, among other things, increased levels of the sphingoid base sphinganine and an increased sphinganine/sphingosine ratio. Such disruption was associated with a diversity of animal diseases. These include liver and kidney lesions in rats,[8] liver and brain lesions in horses,[62] liver and lung lesions in pigs,[63] and liver lesions in chickens.[64] FB_1 was reported to induce liver lesions in rats which consisted of one or more of the following features: single cell necrosis, hepatocellular cytoplasmic vacuolation, variation in nuclear size and staining properties, pyknosis, fibrosis and bile duct proliferation, mild to marked hepatocellular hyperplasia, mitotic figures and foci of cellular alteration were found in the more severely affected livers.[9,54]

In this study, animals treated with the ethanol extract of jojoba seeds at both the low and high doses did not show an acute decrease in body weight and food intake which may be due to the low levels of simmondsin due to the ethanol extraction.[65,66] The slight decrease in food intake and body weight gain in these groups may be due to the presence of simmondsin residue which was reported to induce food restriction and growth retardation.[36,67,68] Treatment with jojoba seed extract to rats fed FB_1-contaminated diet improved food consumption and body weight gain which may be due to the withdrawal of the effect of simmondsin.[69] Similar growth retardation was observed in male rats fed defatted jojoba meal which, therefore, concluded that the growth retardation seen with defatted jojoba meal was due to its simmondsin activity through its role in food intake reduction.[70]

The results of this study also revealed that treatment with jojoba seed extract at both low and high doses did not affect the activity of ALS, AST, triglycerides level, or serum cytokines suggesting that the treatment did not cause liver toxicity. However, jojoba seed extract induced a slight increase in NO. According to Kampf *et al*.,[70] jojoba contains a natural antioxidant postulated to be an allylic derivative of hydroxytoluene. Van Boven *et al*.[71] isolated eight glucoside compounds from jojoba seeds and Bouali *et al*.[40] reported that jojoba is rich in phytic acid and omega-3 fatty acid. Phytic acid is well known to have anti-radical effects by chelating iron required for the MPP-enhanced •OH generation *via* the Fenton-type reaction.[72,73] Phytic acid was also shown to have anti-cancer property,[74] and to improve serum and hepatic lipid levels in aged mice fed a high-cholesterol diet by increasing their fecal lipid content. Moreover, Pacheco *et al*.[75] reported that phytic acid protected the membranes of the Intestinal Porcine Epithelial cell line (IPEC-1) against cell damage induced by the mycotoxin deoxynivalenol.

The antioxidant activity of glucoside was reported by Mehta *et al*.[76] Abdel-Wahhab *et al*.[77] concluded that glucoside decreased DNA damage and hepatocarcinogenesis induced by aflatoxin B_1 by activating the phase II enzymes GSH S-transferase and GSH peroxidase. These results suggest that glucoside is capable of counteracting FB_1 toxicity by suppressing cytochrome P450 mediated bioactivation of FB_1. Jojobenoic acid in jojoba seed extract also has antioxidant activity and has the ability to bind metal ions, representing an additional mechanism underlying their pharmacological effects.[40] More importantly, jojoba seed extract itself was not toxic and did not exert any significant changes in the biochemical parameters tested or the histological structure of the liver.

Previous reports showed that jojoba extract did not show any toxic manifestation on the general body metabolism and the blood serum parameters were within the normal range.[20,21] Moreover, jojoba oil supplement resulted in a 40% reduction of blood cholesterol and altered

lipoprotein pattern which may be attributed to the higher omega-3 fatty acid content.[78] Moreover, Vermauti *et al*.[79] reported that jojoba was rich in saponin which was well known to stimulate the cell-mediated immune system, as well as to enhance antibody production.[80] It was reported to inhibit the growth of cancer cells *in vitro*,[81,82] to exert an anti-cancer effect at the intestinal level, to reduce the formation of carcinogenic substances in the colon, and to have antioxidant properties.[83] The higher total phenolic content in the extract reported in this study suggested another mechanism for its antioxidant activity.[84] In this respect, Zheng and Wang[85] reported that active polyphenol components such as flavonoids and phenolic acids possess antioxidant activities. Consequently, the protective effects of jojoba seed extract against FB_1-induced biochemical and histological changes in the liver reported herein may be due to the direct mechanism as free radical scavenger, and the indirect mechanism by which the extract may induce its protective effect through the enhancement of the synthesis of antioxidant enzymes in the liver.[86]

It could be concluded that the ethanolic extract of jojoba seeds is rich in protein, phenolic compounds, and phytic acid. The extract has antioxidant effects and can protect against FB_1-induced hepatotoxicity. This action may be due to its content of several antioxidant compounds that have the ability to scavenge free radicals generated by FB_1 and consequently prevent lipid peroxidation, and/or the enhancement of antioxidant enzyme activities in the cell.

Financial support and sponsorship

This work was supported by the National Research Centre, Dokki, Cairo, Egypt (Project No. 10070112).

Conflicts of interest

There are no conflicts of interest.

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