Effects of *Origanum majorana L.* on cadmium induced hepatotoxicity and nephrotoxicity in albino rats

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ABSTRACT

الأهداف: دراسة التأثير الوقائي لمستخلص نبات البردقوش على كبد وكلى الجرذان البيضاء بعد تعرضها للتسمم بالكادميوم.

الطريقة: أجريت هذه الدراسة بجامعة الملك خالد، أبها، المملكة العربية السعودية خلال الفترة من سبتمبر حتى ديسمبر 2010م. تم تقسيم 36 جرذاً إلى 6 مجموعات كالتالي: المجموعة الضابطة، مجموعة الكادميوم، مجموعة البردقوش، مجموعة الكادميوم مع البردقوش، مجموعة ما قبل المعالجة بالبردقوش (جرعة بمستخلص البردقوش لمدة 14 يوماً ثم الكادميوم لمدة 14 يوماً)، مجموعة ما بعد المعالجة بالبردقوش (جرعة بالكادميوم لمدة 14 يوماً ثم بمستخلص البردقوش لمدة 14 يوماً). تمت عملية الحقن والمعالجة يومياً بجرعة مقدارها ٥ ملغ/كلغ من الكادميوم، و١ جرام/كلغ من البردقوش لمدة 28 يوماً. تم قياس معدل أنزيمات الكبد ومستويات البروتين واليوريا والكرياتين في مصل جميع المجموعات. كما تم قياس مستويات أنزيمات الجلوتاتيون المختزل وسوبرأوكسيد الديزميوتاز والكاتاليز ومعدل أكسدة الدهون في كبد وكلى جميع المجموعات. كما تم عمل مسح للتغيرات الجينية في الكبد لجميع المجموعات باستخدام 3 بادئات جينية (chi 25, 5.8S and NS3).

النتائج: أن استخدام الكادميوم يرفع من مستويات أنزيمات الكبد، واليوريا، والكرياتين ويقلل من مستويات البروتين. انخفضت أنشطة التأكسد لدى الكبد والكلى وارتفعت معدل أكسدة الدهون في مجموعة الكادميوم مقارنة بالمجموعة الضابطة. كما لوحظ تحسن في وظائف الكبد والكلى لمجموعتي الكادميوم مع البردقوش وما بعد المعالجة بالبردقوش. لوحظ أن البادئ الجيني NS3 قادرا على إظهار التغيرات الجينية في الكبد لجميع مجموعات التجربة.

خاتمة: أن استخدام مستخلص البردقوش له أثر فعال ويقلل من التأثيرات الفسيولوجية والجزيئية الضارة للكادميوم على كبد وكلى الجرذان البيضاء.

Objectives: To evaluate the effect of *Origanum majorana L.* (OM) against cadmium (Cd)-induced hepatotoxicity and nephrotoxicity.

Methods: This study was performed at King Khalid University, Abha, Kingdom of Saudi Arabia from September to December 2010. Thirty-six male albino rats were divided into 6 groups: control, Cd, OM, OM+Cd, OM pre-treated (received OM extract for 14 days followed by Cd for 14 days), OM post-treated (received Cd for 14 days followed by OM extract for 14 days). All treatments were orally administered once a day (Cd: 5 mg/kg by weight [b.w] and OM: 1 g/kg b.w) for 28 days. Changes in liver biochemical markers namely, alanine transaminase, aspartate aminotransferase, alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), and levels of total protein, urea, and creatinine in the serum were determined. Levels of liver and kidney antioxidants namely, glutathione, superoxide dismutase and catalase, and lipid peroxidation (LPO) were estimated. Differential display using 3 primers: chi 25, 5.8S and NS3 was performed for liver homogenates.

Results: Exposure to Cd increased the levels of liver biochemical markers, urea, and creatinine, and lowered total protein levels. Exposure to Cd lowered activities of liver and kidney antioxidants, while it increased LPO levels. Levels of all disrupted parameters were alleviated by co-administration and post-administration of OM extract. The NS3 primer was able to show genetic variability in livers of all experimental groups.

Conclusion: The OM showed apparent protective and curative effect on Cd-induced hepatotoxicity and nephrotoxicity.

Saudi Med J 2011; Vol. 32 (8): 797-805

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Received 2nd February 2011. Accepted 2nd June 2011.

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Cadmium (Cd) is an environmental contaminant that is present in air, soil, water, food, and cigarette smoke.[1] The Cd has been considered as a risk factor for humans, as it accumulates in the body tissues, such as the liver, lungs, kidneys, bones, and reproductive organs.[2,3] It has been reported that Cd generates reactive oxygen species (ROS) causing oxidative damage in various tissues.[4] It has been shown that exposure to Cd via different routes causes increased lipid peroxidation (LPO) in membranes of erythrocytes and tissues, such as kidney, liver, brain, and testes where thiobarbituric acid reactive substances (TBARS) and hydroperoxides are used as indicators of oxidative damage.[5-7] Intake of Cd results in utilization of glutathione (GSH) and protein binding sulfhydryl groups, and consequently enhance the levels of free radicals, such as hydrogen peroxide, hydroxide, and superoxide anions.[8] The liver and kidney are considered the most vulnerable organs to Cd toxicity.[9-12] An increase on the levels of serum hepatic marker enzymes, such as aspartate aminotransferase (AST) and alanine transaminase (ALT) following Cd exposure, which indicates the damaging effect of Cd on the liver has been reported in various studies.[13,14] It has also been reported that Cd nephrotoxicity resulted from generating frees radicals, and by inducing cell necrosis and apoptosis.[15,16] Moreover, a variety of changes in liver non-enzymatic antioxidants, such as GSH and liver enzymatic antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) associated with Cd exposure have been reported.[17] Herbal and natural products represent one of the most common forms of complementary and alternative medicines.[18] They are readily available, and can be obtained from supermarkets and pharmacies. As these products are usually used without medical prescription, they must be safe for human health.[19] Numerous studies have reported the antioxidant properties of many natural products against many toxic materials.[20-22] Some natural product extracts, such as honeybee solution,[9] green tea,[23] ginger[24] and black cumin[25] have been found to have a variety of pharmacological and antioxidant effects on Cd toxicity. The *O. majorana (OM)*, a member of the mint family, *Lamiaceae*, is a widely used plant in folk Saudi Arabian medicine. It contains flavonoids, phenolic terpenoids, phenolic glycosides, tannins, and sitosterol.[26] The antiviral, bactericidal, antiseptic, and antifungal effects of *OM* are attributed to ursolic acid and essential oil, and particularly to thymol and carvacrol.[26,27] The antioxidant and antitumor activities of *OM* have been reported in various studies.[28-31] As there has been no scientific report on the protective, or therapeutic effect of *OM* against Cd induced toxicity on liver and kidney of intoxicated animals, this study aimed to evaluate the protective, therapeutic, and/or chelating effects of the aqueous extract of *OM* against hepato- and nephrotoxicity induced by Cd in male rats.

Methods. ***Natural product preparation.*** Whole plant of *OM* was purchased from the local market in KSA. The plant was identified by Department of Biology staff at King Khalid University, Abha, KSA from September to December 2010. The plant was washed with distilled water and then dried. The dried plant was ground to a powder before being extracted by maceration in distilled water (200 g/ 1000 ml) for 2 days at 37°C. The extract was then filtered using a filter funnel, and the excess water was evaporated under reduced pressure in a rotary evaporator. Once evaporated, the extract produces a dry extract (18 g of solid residue). The extract was dissolved in deionized distilled water to a final stock concentration of one g/ml for further use. The procedure was repeated weekly to get fresh extract in this study

Experimental design. Thirty-six male albino rats, 6-8 weeks old, and weighing 150-200 g were obtained from the Experimental Animal Unit, College of Science, King Khalid University, Abha, KSA and followed the international guidelines for the care and use of laboratory animals. Ethical approval was obtained from the College of Science Ethical Committee, King Khalid University, KSA. The rats were maintained in standard conditions, in well-ventilated cages with raised floors of wide wire mesh. The temperatures were maintained at 22°C with a 12-h light/dark cycle, and allowed to consume standard rat pellet chow and water *ad libitum* before treatments. The experiment started after 10 days of rat adaptation, and procedures were performed in sterilized conditions.

Acute toxicity tests for OM. This test was carried out with a modified method of Seth et al.[32] Rats were divided into 7 groups. The control group received deionized water, and the other groups received: Group 2 - 250 mg, Group 3 - 500 mg, Group 4 - 1000 mg, Group 5 - 2000 mg, Group 6 - 4000 mg, and Group 7 - 8000 mg of the aqueous extract dissolved in deionized distilled water per kg b. w. Immediately after administration, the animals were observed for the first 4 hours, and twice daily for 7 days for signs of any behavioral changes and mortality.

Sub-acute dose and animal grouping. Rats were divided into 6 groups of 6 each. These groups were: Group 1 (control group), rats received isotonic saline solution; Group 2 (Cd group), rats were administered daily with 5 mg/kg b.w. cadmium chloride for 28 days by gavage; Group 3 (OM group), rats were orally administered with the aqueous extract of *OM* in a daily dose of 1000 mg/kg b.w. for 28 days; Group 4 (OM+Cd group), rats were daily co-administered with 5 mg/kg $CdCl_2$ and 1000 mg/kg of the aqueous extract of *OM*

for the experiment period; Group 5 (OM pre-treated group), rats were daily pre-treated with 1000 mg/kg b.w. of *OM* extract for the first 14 days and followed by daily administration of 5 mg/kg b. w. $CdCl_2$ for the remaining 14 days; Group 6 (OM post-treated group) rats were daily pretreated with $CdCl_2$ (5 mg/kg b.w.) for the first 14 days and followed by daily administration of *OM* (1000 mg/kg b.w.) for the other 14 days of the experiment period.

Serology. At the end of the experimental period rats were fasted overnight, however distilled water was made available ad libitum. Blood samples were collected from the rat tails into plain tubes under ether anesthesia, and animals were then sacrificed by decapitation. Blood samples were centrifuged at 3000 revolutions per minute (rpm) for 5 min to obtain serum. Sera were kept frozen at -80°C until used for later biochemical analysis.

Biochemical analysis. Activities of serum ALT and AST were spectrophotometrically assayed according to the method of Reitman and Frankel.[33] The alkaline phosphatase (ALP) activity in the serum was spectrophotometrically measured at 405 nm by the formation of para-nitrophenol from para-nitrophenol - phosphate as a substrate using the method of Belfield and Goldberg.[34] activity of gamma-glutamyltransferase (GGT) in the serum was spectrophotometrically measured using diagnostic kits (Sigma Chemical Co., St. Louis, MO, USA) according to Szasz.[35] Serum total protein levels, documenting the extent of liver dysfunction, were determined spectrophotometrically according to the method of Lowry et al.[36] using a Sigma Diagnostic kit (Sigma Chemical Co., St. Louis, MO, USA). Serum urea was estimated by using the diagnostic kit based on the method of Fawcett and Scott.[37] Creatinine in the serum was estimated using the diagnostic kit based on the methods of Teitz.[38]

Preparation of tissue homogenate. Liver and kidney tissues were quickly removed, washed in ice-cold, isotonic saline, and blotted individually on ash-free filter paper. The tissues were then homogenized separately in 0.1M 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride buffer, pH 7.4 using a Potter-Elvehjem homogenizer at 4°C, the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 min in cold centrifuge, and the supernatant was kept at -20°C for estimation of GSH, SOD and CAT activities.

Estimation of reduced GSH, CAT and SOD activities. Activity of reduced GSH in the liver and kidney was assayed using GSH Assay Kit (BioVision, Inc., USA) following the manufacture's instruction based on the method of Akerboom and Sies.[39] The CAT activity in the liver and kidney was assayed using a commercially available CAT activity assay Kit (BioVision, Inc., USA) following manufacture's instruction based on the method of Aebi.[40] The activity of SOD in the liver and kidney was assayed using a commercially available SOD activity assay kit (Randox, Crumlin, UK) following the manufacture's instruction based on the method of Misra and Fridovich.[41]

Measurement of LPO. The LPO in the liver and kidney of control and all treated groups was measured by the quantification of TBARS and determined by the method of Buege and Aust.[42]

Isolation of total ribonucleic acid (RNA) from liver homogenates, complementary DNA synthesis and differential display. 100 mcgl of liver homogenate was subjected to RNA extraction using QIAGEN-QIAamp RNA blood mini kit according to the manufacturer's instructions (QIAGEN, Hilden, Germany). Reverse transcription reactions were performed using an oligo dT primer. The 25 mcgl reaction mixture was prepared with 2.5 mcgl of 5x buffer with magnesium chloride ($MgCl_2$), 2.5 mcgl of 2.5 mM deoxynucleotide triphosphates (dNTPs), 1mcgl of 10 pmol primer, 2.5 mcl RNA and 0.2 mcl reverse transcriptase enzyme (100U/mcgl). polymerase chain reaction (PCR) amplification was performed in a thermocycler (Eppendorf) programmed at 95°C for 5 min, 42°C for 1 h, 72°C for 10 min, and a soak at 4°C.[43]

Differential display was performed using 3 different arbitrary primers (**Table 1**). The PCR reaction was performed following the method of Abd El Fattah et al[44] briefly, a 25 mcgl reaction mixture was prepared with 2.5 mcl 10x Taq DNA polymerase buffer (10 mM Tris HCl (pH 8.3), 25 mM potassium chloride (KCI), 2.5 mcl 50mM $MgCl_2$, 2 mcl primer (40 pmol/mcl), and 0.25 mcl of Taq polymerase (AmpliTaq, Perkin- Elmer, 5 u/mcgl), 2.5 mcgl from the cDNA, 2.5 mcgl dNTPs 4mM, and 12.75 mcl of distilled water. The PCR

Table 1 - The DNA nucleotide sequences and annealing temperature of the primers used in the differential display examination.

Annealing (°C)	Primer sequence 5→3'	Primers
56	GAY TTR GAT TGG GAA TAY CC	Chi 25
55	CGC TGC GTT CTT CAT CG	5.8S
53	GCA AGT CTG GTG CCA GCC	18S rRNA (NS3)

reaction was performed in 9700 thermal cycler (Perkin-Elmer), and the PCR conditions were performed as follows: initial denaturation at 95°C for 5 min; followed by 40-cycles (94°C for 1 min); 55, 53, 56°C for 1 min; 72°C for 2 min; and final extension, 72°C for 10 min. Electrophoresis was performed at 80 volts with 0.5x tris-borate buffer (TBE) buffer in 1.5% agarose gel was stained in 0.5 mcg/ml (w/v) ethidium bromide solution and destained with deionized water. Finally, the gel was visualized and photographed using a gel documentation system.

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 10.0 (SPSS, Chicago, IL, USA). Data are presented as means ±standard deviations. Statistical evaluation of the data was performed using one-way ANOVA followed by a post-hoc least significant difference test. Values were considered statistically significant at $p \leq 0.05$.

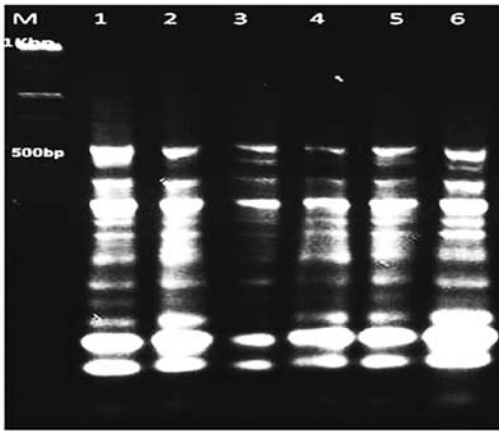

Figure 1 - Differential display for the 6 rat groups using Chi 25 as an arbitrary primer. Lanes; M - 1kbp DNA marker, 1 - control group, 2 - Cd group, 3 - OM group, 4 - OM+Cd group, 5 - OM post-treated group, 6 - OM pre-treated group. OM - *Origanum majorana,* Cd - cadmium

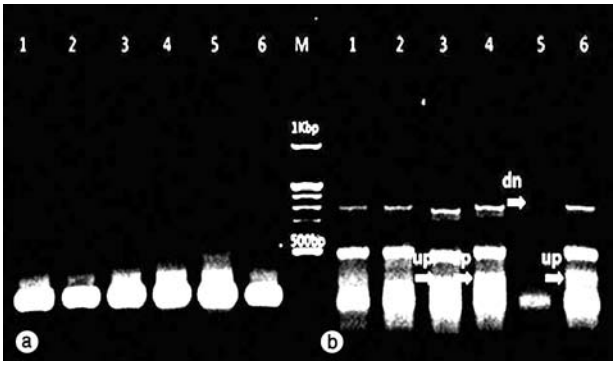

Figure 2 - Differential display for the 6 rat groups using the primers: **a**) 5.8S, and **b**) NS3 as arbitrary primers. Lanes, M - 1kbp DNA marker, 1 - control group, 2 - Cd group, 3 - OM group, 4 - OM+Cd group, 5 - OM post-treated group, 6 - OM pre-treated group. Up - up-regulated gene, dn - down-regulated gene, OM - *Origanum majorana*, Cd - cadmium, PCR - polymerase chain reaction

Results. Liver enzymes in the serum represents the levels of the hepatic enzymes ALT, AST, ALP, and GGT in the serum of control and all experimental rat groups (**Table 2**). The results showed that Cd induced a significant increase in the activities of those enzymes in the Cd group compared to the control group. Oral administration of *OM* alone to rats did not affect the levels of those enzymes indicating the safe use of this natural product. Compared to the Cd group, the activities of ALT, AST, ALP, and GGT enzymes were significantly decreased in those rats co-treated with Cd and *OM,* and those post-treated with *OM* after exposure to Cd. However, the ameliorative effect of post-treatment of *OM* was more pronounced than that of co-treatment with Cd and *OM.* The disrupted liver enzymes, however, were not ameliorated by pre-treatment of *OM* before exposure to Cd, as their levels in OM pre-treated group did not show significant difference when compared with the Cd group.

Total protein, urea, and creatinine in the serum. There was a significant decrease in the levels of serum total protein in the Cd group when compared with the control group (**Table 2**). However, levels of this compound in the serum were significantly increased in OM + Cd group and OM post-treated group when compared with the Cd group. Levels of urea and creatinine in the serum of the Cd group were significantly increased when compared with the control group (**Table 2**). However, levels of serum urea and creatinine were significantly decreased in OM+ Cd group and OM post-treated group when compared with the Cd group. The ameliorative effect of post-treatment of *OM* on the levels of serum total protein, urea and creatinine was more prominent than that of co-treatment with Cd and *OM* (**Table 2**). The administration of *OM* alone to rats did not affect the levels of all these compounds indicating the safe use of this extract. However, post treatment with *OM* after exposure to Cd did not improve the disrupted levels of serum total protein, urea and creatinine as there was no significant difference between their levels in OM pre-treated group and Cd group.

The GSH, SOD, and CAT in the liver and kidney. In the Cd group, the levels of liver and kidney GSH and the activities of SOD, and CAT decreased as a result of Cd administration when compared with the control group (**Table 3**). Compared to the Cd group, activities of these enzymes were significantly increased in OM+

Table 2 - Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), g-glutamyl transpeptidase (g-GT), total protein, urea and creatinine in the serum of control and experimental groups of rats.

Group parameter	Control	OM	Cd	OM+Cd	OM post-treated	OM pre-treated
ALT (U/L)	67.01 ± 2.30	65.25 ± 3.30	80.75 ± 6.50*	68.25 ± 0.56†	66.25 ± 0.96†	73.0 ± 3.55
AST (U/L)	99.5 ± 5.32	105.3 ± 3.30	177 ± 0.50*	120.5 ± 3.32†	105 ± 1.63	183.3 ± 4.57
ALP (U/L)	53.75 ± 1.89	51.5 ± 2.38	73 ± 2.45*	53.5 ± 2.38†	55.5 ± 4.23†	73 ± 5.41
γ-GT (U/L)	4.05 ± 0.05	3.97 ± 0.46	8.1 ± 0.23*	6.62 ± 0.27†	4.48 ± 0.37†	7.8 ± 0.11
Protein (g/dl)	6.8 ± 0.59	6.22 ± 0.47	5.57 ± 0.35*	5.95 ± 0.31	6.87 ± 0.20†	5.55 ± 0.65
Urea (mg/dl)	48.1 ± 1.28	46.0 ± 1.63	77.5 ± 3.71*	45.75 ± 3.40†	49.5 ± 6.50†	74.25 ± 3.86
Creatinine (mg/dl)	0.43 ± 0.01	0.42 ± 0.02	0.54 ± 0.02*	0.44 ± 0.02†	0.44 ± 0.02†	0.55 ± 0.01

Origanum majorana (*OM)* and cadmium (Cd) values are expressed as mean ± standard deviation for groups of 6 animals each. Control group in comparison with Cd groups *$p \leq 0.0001$, Cd groups in comparison with OM+Cd, OM post-treated and OM pre-treated groups †$p \leq 0.0001$

Table 3 - Levels of reduced GSH and activities of SOD and CAT and TBARS in the liver and kidney of the control and all experimental groups of rats.

Group (parameter)	Control	OM	Cd	OM + Cd	OM post-treated	OM pre-treated
Liver						
GSH (mg/100g tissue)	62.93 ± 3.72	92 .0 ± 5.230‡	35.15 ± 2.52*	58.25 ± 2.63†	62.25 ± 5.62†	41.0 ± 3.65
SOD (U/mg)	8.35 ± 1.18	8.75 ± 1.10	4.77 ± 0.27*	8.11 ± 0.356†	8.42 ± 0.70†	5.55 ± 0.52
CAT (U/mg)	75.75 ± 4.22	77.6 ± 6.24	52.25 ± 5.37*	71.75 ± 4.03†	74.0 ± 5.48†	59.25 ± 4.3
TBARS (nmol/mg tissue)	27.41 ± 2.31	26.91 ± 3.11	43.1 ± 3.62*	31.41 ± 2.03†	28.21 ± 2.45†	40.1 ± 2.72
Kidney						
GSH (mg/100g tissue)	42.25 ± 3.20	64.4 ± 2.08‡	29.25 ± 1.25*	37.25 ± 3.86†	46.25 ± 2.5†	30.25 ± 1.89
SOD (U/mg)	6.82 ± 1.18	6.55 ± 0.574	4.35 ± 0.39*	6.3 ± 0.357†	6.95 ± 0.34†	4.55 ± 0.9
CAT (U/mg)	80.25 ± 5.51	78.0 ± 5.34	65.0 ± 3.57*	74.5 ± 1.29†	77.0 ± 1.14†	67.75 ± 1.89
TBARS (nmol/mg tissue)	20.53 ± 1.81	21.03 ± 1.32	33.63 ± 1.75*	28.22 ± 1.67†	22.93 ± 1.76†	32.11 ± 2.21

Origanum majorana (*OM)* and cadmium (Cd) values are expressed as mean ± standard deviation for groups of 6 animals each. Control group in comparison with Cd groups *$p \leq 0.0001$, Cd groups in comparison with OM+Cd groups, OM post-treated and OM pre-treated groups †$p \leq 0.0001$, control group in comparison with OM groups ‡$p \leq 0.0001$. GSH - glutathione, SOD - superoxide dismutase, CAT - catalase, TBARS - thiobarbituric acid reactive substances

Cd group and OM post-treated group. However, the ameliorative effect of post-treatment of *OM* was more pronounced than that of co-treatment with Cd and *OM*. However, the disruption in these enzymes was not ameliorated by pre-treatment of *OM* before exposure to Cd as there was no significant difference between their levels in OM pre-treated group and Cd group. The level of liver and kidney GSH was significantly increased in rats administered with *OM* alone when compared with the control group (**Table 3**).

Lipid peroxidation. An increase in LPO in the livers and kidney homogenates of the Cd group was indicated by the significant increase in TBARS (**Table 3**). The levels of TBARS in the liver and kidney of OM+Cd and OM post-treated groups were significantly less than that of the Cd group. However, the effect of OM post-treated group on the levels of TBARS was more pronounced than that of OM + Cd group (**Table 3**). However, levels of TBARS were not affected by pre-treatment of *OM* before exposure to Cd as there was no significant difference between their levels in OM pre-treated group and Cd group.

Differential display results. Approximately 78 bands were obtained when the primer Chi 25 was used (**Figure 1**). The molecular weights of the obtained bands ranged from 500 bp to 50 bp. There was fluctuation in the gene expression in all the amplified genes with the chi 25 primer (**Figure 1**). Moreover, Chi 25 and 5.8S primers failed to differentiate between the examined samples. When 5.8S primer used, approximately 30 bands were obtained (**Figure 2a**). The molecular weights of these bands ranged from 800-200 bp. Primer NS3 succeeded to differentiate between the examined animals where approximately 24 bands with molecular weight ranged from 900-100 bp were obtained, and all those bands were monomorphic except the band at molecular weight 300 bp, this gene was up-regulated in OM group, OM+Cd group, and OM pre-treated group where it was down-regulated in the OM post-treated group (**Figure 2b**). The gene at 800 bp was down-regulated only in the OM post-treated group (**Figure 2b**).

Discussion. The Cd is known to be one of the most dangerous occupational and environmental toxins.[1] It

can be found in water, atmospheric air, and even in food. Products of vegetable origin are the main carrier of Cd compounds in food.[1] Many studies have reported that the concentration of Cd in different organs of different fish species collected from different areas in KSA was high but still within the safety permissible level for human use.[45,46] However, as smoking is one of the main sources of CD in the general population.[47] Bassiony[48] reported that a prevalence range of 2.4-52.3% for current smoking in Saudi Arabia with a higher rate for males (13-38%) than females (1-16%). This gives alarm that exposure of Saudi people to CD is increasing. In the acute toxicity test in this study, animals treated with the water extract of *OM* (up to 8 g/kg body weight) did not manifest any significant clinical, or macroscopic signs of toxicity or mortality indicting that this plant does not have toxic effect. This is in agreement with previous studies.[29,49]

It has been shown in many studies that Cd induces oxidative damage by producing ROS,[4,50] and decreasing the biological activities of some antioxidant enzymes, such as SOD and CAT,[51,52] which play an important role in antioxidant profile, and in scavenging of free radicals. The Cd has also been reported to cause damage to lipids, and by that to generate LPO.[16,53] This study also showed that exposure to Cd has led to an increase of LPO indicated by the elevation of TBARS levels, which associated with a distinct decrease in the activity of the antioxidants SOD, CAT, and GSH in the liver and kidney of the animals exposed to Cd. The SOD, CAT, and GSH are essential parts of cellular antioxidant defense system, and they play an essential role in protection against oxidative stress.[54] It has been proposed that Cd induces oxidative stress and LPO by depleting GSH, or by inhibition of antioxidant enzymes.[54] Moreover, Cd has been shown to exert a direct inhibitory effect on SOD and CAT activities via Cd-enzyme interaction with a resultant perturbation of enzyme topography critical for catalytic activity[54,55] Our results showed that there was a marked increase of hepatic and nephric LPO, which is indicted by the elevation of the levels of Cd administration. This was consist with other reports on Cd-intoxicated rats.[12,55] The significant decrease in the activities of SOD and CAT in the livers and kidneys of the Cd groups compared to control groups may be attributed, in part, to an overwhelming oxidative modification of enzymatic proteins and biomembrane lipids by ROS, as evident by heightened level of LPO.[55] The ability of *OM* to prevent liver dysfunction and alterations of antioxidative parameters induced by Cd in this study may be due to the fact that *OM* possess scavenging free radicals properties as it has been reported to have antioxidant properties.[29,30] Therefore, it is possible that the *OM* aqueous extracts could have spared the consumption of GSH, SOD, and CAT occasioned by Cd-induced oxidative stress. In addition, the protective effects of this extract may be related to their ability to chelate/sequester Cd via formation of Cd-flavonoid complexes[29,56] as a result of many conditions including cell necrosis, improved or increased synthesis, and alterations in the permeability of enclosing cell membrane.[57]

As a result of the imbalance among antioxidants/oxidants ratio in the cells, the levels of hepatic enzymes (AST, ALT, ALP and GGT) elevate in liver damage due to tissue necrosis or membrane damage.[57] In this study, a severe hepatic damage noticed by the significant increase of the hepatic enzymes ALT, AST, ALP and GGT in the serum of the Cd group when compared with the control group. These characteristic features of Cd-induced liver toxicity are similar to those previously reported by other investigators.[53,58] Serum levels of the measured hepatic enzymes were not affected by oral administration of the aqueous extract of *OM* in rats suggesting a safe use of this plant on liver. In addition, the disruption in the levels of the hepatic enzymes in Cd intoxicated rats was ameliorated by both post- and co-administration with the *OM* aqueous extract, as there was a significant decrease in the levels of these enzymes in rats' sera when compared to Cd group. However, this effect was more profound and more effective in the OM post-treated group, in which the levels of these enzymes came back to approximately the normal levels. The decreased levels of these hepatic enzymes in the OM post-treated group suggests that the *OM* aqueous extract possessed a curative and therapeutic effect, and it is able to cure the hepatocytes from Cd-induced liver injury and damage and subsequent leakage of enzymes into the serum. Furthermore, the significant decrease in the levels of these enzymes in the OM+ Cd group suggests a chelating effect of this plant against Cd ions. Moreover, levels of the measured hepatic enzymes in the Cd group were not ameliorated by pre-treatment with *OM,* which gives a very poor evidence that the plant may have a protective effect against Cd induced hepatic damage.

Whereas, no studies have examined the effect of *OM* on Cd hepatotoxicity, some studies have shown its ameliorative effects on disrupted liver enzymes of mice exposed to other toxic elements such as lead.[29] Serum total protein represents a complex mixture containing a number of components, which differ in properties, and function. Hypoproteinemia is the deficiency of protein in the plasma, partly because of dietary insufficiency, subsequent impairment in protein synthetic machinery, or excessive excretion.[59] Total protein levels are one element used to monitor the liver function.[60] In the present study, there was a significant decrease in serum total protein levels in the Cd groups compared with control. This is in agreement with similar studies that reported

hepatotoxicity in Cd intoxicated rats.[61,62] The decreased total protein maybe attributed to hepatocellular injury or hepatic inflammation induced by Cd intoxication and subsequently disturbed the protein biosynthesis.[59] In the current study, a significant increase in serum total protein levels was observed in OM+Cd group and OM post-treated Cd group. However, this effect was more profound and more effective in the post-treated group indicating that the therapeutic ability of this plant to stimulate the regeneration of hepatic tissue, and thus increased protein synthesis in damaged liver cells was more effective than its chelating effect as proposed by other studies.[63]

It has been postulated that increased levels of serum urea and creatinine is linked to kidney disease.[59] Urea is the main nitrogenous end product of protein catabolism. It represents 90% of the total urinary nitrogen excretion. In this study, the Cd group showed a significant increase in serum urea and creatinine that might suggest the inability of the kidney to excrete these products, indicating an impairment of kidney functions. These effects could be attributed to the changes in the threshold of tubular re-absorption, renal blood flow, and glomerular filtration rate.[64] This damaging effect of Cd on kidneys has been described by other authors.[55,65] Some studies have shown increased urea concentrations in the serum indicating reduced glomerular filtration rate in Cd-exposed rats.[66,67] It has been suggested that CD-induced tubular damage leads to a certain degree of interstitial nephritis, which in turn results in a decreased glomerular filtration rate.[68] In addition, it has also been proposed that Cd exerts a direct toxic effect on the glomerulus, and this leads to decrease in urea and creatinine clearance.[67] The obtained results in the current study showed that the water extract of *OM* had a therapeutic and chelating effect against Cd induced kidney damage. This was obvious as there was a significant decrease in urea and creatinine levels in the OM post-treated group and OM+Cd group when compared with the Cd group. This suggests a potent therapeutic and mild chelating effect of this plant against Cd nephrotoxicity. However, the oral administration of the aqueous extract of *OM* alone to rats did not change the levels of these parameters suggesting a safe use of this plant on kidneys.

Many studies have reported that Cd can modulate gene expression and signal transduction, reduce activities of proteins involved in antioxidant defenses and interfered with DNA repair processes.[69,70] The obtained molecular results in this study showed that most of the amplified liver genes with the 3 used arbitrary primers were monomorphic, and few bands were polymorphic. Polymorphic bands were observed only with the primers NS3. There was fluctuation in the gene expression in all the amplified genes with chi 25 primers, but a uniform expression was shown with the amplified bands with the other 2 primers. With the primers NS3, there were 3 up-regulated genes and a down- regulated gene. This would lead to the fact that NS3 primer could be used as molecular markers for genetic variability between liver tissues subjected to different treatments with *OM*.

There are some limitations to this study. For example, no histopathological assessment of the liver and kidney tissues in treated and non-treated rat groups was performed. In addition, no cloning or sequencing analysis of the up and down regulated DNA bands was carried out. This was because this research was self-funded, and there was no fund obtained from any source. However, these limitations should be considered in future research. In addition to the fact that this study points out the harmful effects of Cd on the liver and kidney at the physiological and molecular levels, it should motivate researchers to investigate the positive roles of natural products other than *OM* that may alleviate hepato and nephrotoxicity induced by Cd.

In conclusion, the aqueous extracts of *OM* exhibited therapeutic and chelating effects against Cd induced hepato and nephrotoxicity. However, the therapeutic effect (post-treatment with the plant extract after Cd exposure) was more potent than its chelating effect (co-treatment of the plant extract with Cd). Therefore, it could be recommended that populations exposed to Cd can use the aqueous extract of *OM*.

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