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Modulatory effect of *Origanum majorana* extract against cisplatin-induced dyslipidemia in rats

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ABSTRACT

Cisplatin (CDDP) drug is one of the platinum compounds that used for the treatment of a variety of human neoplasms. However, its high doses produce undesirable toxic side effects. *Origanum majorana* is a natural herbal product used in the management of many diseases. Therefore, the present study investigated the curative effect of *Origanum majorana* ethanolic extract (OMEE) on the lipid profile abnormalities induced by cisplatin. Male Wistar rats were randomly separated into four groups (n=6). Rats in group 1 act as controls and received distilled water orally for 14 days. Rats of groups 2, 3 and 4 injected a single dose of CDDP (3 mg/kg body weight, i.p); then after 3 days of CDDP injection, rats were challenged with distilled water, OMEE (500 mg/kg body weight), and silymarin (150 mg/kg/ body weight); respectively orally for 14 consecutive days. The present study revealed that CDDP caused body, liver and kidney weights loss within the experimental period. Moreover, CDDP increase the serum total lipid, triglycerides and LDL-cholesterol levels significantly. Conversely, it causes a significant decrease in the serum HDL-cholesterol level. OMEE treatment elevates the body, liver and kidney weights significantly. Additionally, it declines the levels of total lipid, triglycerides, and LDL-cholesterol and increase the HDL-cholesterol level significantly. In conclusion, these findings may exhibit a positive hypolipidemic potential of OMEE against dyslipidemia induced by CDDP and suggest that OMEE might serve as novel adjuvant therapy that can be used with CDDP.

Key words:

Cisplatin, *Origanum majorana* extract, Body weight, Cholesterol, Lipoproteins.

INTRODUCTION

Cisplatin (cis-diaminedichloroplatinum) (CDDP) is an eminent member of the most efficient anti-neoplastic drugs (Reck *et al.,* 2010). In spite of its anticancer activity, its use is primarily restricted due to severe toxic side effects against normal tissues that interfere with its therapeutic efficacy (Park *et al.,* 2009). Dyslipidemia is considered as adverse effects resulted from CDDP chemotherapy (Boyer *et al.,* 1990). Owing to the continuous aggressive high-dose cisplatin chemotherapy, it is necessary to investigate agents to overcome the cisplatin side effects without affecting its antitumor efficacy. Recently, several researchers suggested that cotherapy of cisplatin with plant extracts can minimize the cisplatin adverse effects beside the enhancement of its antitumor efficacy (Hadjzadeh *et al.,* 2012; Chen *et al.,* 2014). *Origanum majorana* L. is a member of the mint family Lamiaceae. It is mainly distinguished by its common names, such as oregano and sweet marjoram (Kumar *et al.,* 2011).
The Food and Drug Administration regards *O. majorana* to be generally safe (El-Ashmawy *et al.,* 2005). It is generally used as a spice and it is well-liked home remedies for asthma, depression, rheumatism, nervous headaches, cardiovascular diseases, epilepsy, diuretic and stomach disorders (Al-Harbi, 2011). *Origanum majorana* has high antioxidant and anticancer activities (Vági *et al.,* 2005; El-Ghany and Nanees, 2010). Additionally, the alcoholic and aqueous extracts of *O. majorana* possess antihyperglycemic and antilipidemic effect (Pimple *et al.,* 2012). Furthermore, clinical research has confirmed the efficacy and safety of silymarin against toxicity induced by different drugs (Eminzade *et al.,* 2008). As it is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation and its associated cell damage in some experimental models. Thereby, the present investigation aims to evaluate the therapeutic effect of ethanolic extract of *Origanum majorana* against cisplatin-induced dyslipidemia in male albino rats in comparison with silymarin as a classic drug used in same conditions.

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MATERIALS AND METHODS

Preparation of *Origanum majoranum* ethanolic extract (OMEE)

Marjoram (*Origanum majorana*) was purchased from local market in Egypt and authenticated by the herbarium of Botany Department, Faculty of Science, Cairo University. Marjoram ethanolic extract was prepared from marjoram powder

according to Ryszard *et al.* (2008). Briefly, twenty five grams of marjoram powder mixed with 150 ml ethanol for 24 h and repeated this process again (2x extraction). After filtration, the total filtrate obtained was then concentrated and dried using a lyophilizer (LABCONCO lyophilizer, shell freeze system, USA) and stored in desiccator until use.

Phytochemical screening of OMEE

The phytochemical screening was analyzed for *Origanium majorana* extract as described by Verma *et al*. (2010).

***In vitro* antioxidant assay using DPPH (2, 2-diphenyl-1-picrylhydrazyl)**

The DPPH free radical scavenging assay was carried out according to the method adopted by Brand *et al*. (1995). The OMEE and ascorbic acid (standard antioxidant) were prepared with various concentrations (10-70 mg/ml) and added to freshly prepared DPPH (0.1 mM in methanol). The solution was then shaken vigorously and incubated at room temperature in the dark for 30 min. The control solution (DPPH only) underwent the same processes. The absorbance (Abs) was measured at 517 nm against methanol. The radical scavenging activity was calculated from the following equation:

$$\% \text{ DPPH inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Animals

Adult male Wistar albino rats (*Rattus norvegicus*), weighing 150-170 g were obtained from the animal house of the National Research Center (NRC), Egypt. All animals had access to a standard diet and clean drinking water. The rats were left for one week prior the commencement of the experiment for acclimatization. The experimental animal facility and protocol were approved by the Institutional Animal Care and Use Committee (IACUC) (CUFS/F/PHY/05/13) of the Faculty of Science, Cairo University, Egypt.

Acute toxicity test

The acute oral toxicity test was applied according to the organization for economic cooperation and development (OECD) based on acute oral toxicity up and down procedure 425 guideline (OECD, 2008). In this study, rats (n=10) were divided into two equal groups. The 1st one serves as a control and received distilled water, and the 2nd group administered the OMEE orally only once at a limit dose of 5000 mg/kg body weight. The LD_{50} was predicted to exceed 5000 mg/kg, if three of five rats were survived. Weights were estimated periodically at 0, 7 and 14 days of administration, and the body weight change was calculated at the end of the study. Furthermore, general macroscopic observations were noticed such as changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system and motor activity and behavior pattern. Additionally, signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were observed at 0 min., 30 min., 1, 2, 4, 6 h and thereafter every day for 14 days to check abnormal clinical manifestation and mortality. On day 15, all survived rats were euthanized. Blood was collected and serum was prepared for determination of liver and kidney function markers. Moreover, the liver and both kidneys were excised, weighed and fixed in 10% formalin for 24 h. Biopsy of liver and kidneys were processed and stained with hematoxylin and eosin stain for histopathological examination (Suvarna *et al.,* 2013).

Determination of hepatic markers

Serum aspartate aminotransferase (ASAT) and serum alanine aminotransferase (ALAT) were determined, by colorimetric method, using Biodiagnostic kit according to the method described by Reitman and Frankel (1957). The gamma glutamyl transferase (ɣGT) activity was determined using Spectrum kit, according to the kinetic calorimetric method described by Szasz *et al.* (1974).

Determination of renal markers

For determination of kidney function, serum creatinine, urea and uric acid were estimated according to the methods of Bartles *et al*. (1972), Fawcett *et al*. (1960), Barham and Trender (1972); respectively, using Biodiagnostic kit. The blood urea nitrogen (BUN) was calculated from urea concentration by multiplying the urea result by 0.467.

Experimental design

Twenty four rats were divided randomly into four groups of 6 animals each; the first group was used as a control and received distilled water orally for 14 days. Rats of groups 2, 3 and 4 injected with a single dose of CDDP (3 mg/kg body weight, i.p), then after 3 days of CDDP injection, rats were challenged with distilled water (group 2), group 3 received OMEE (500 mg/kg/day), and group 4 received silymarin (150 mg/kg/day) orally for 14 consecutive days. At the end of the study period, animals were fasted overnight. Following sodium pentobarbital anaesthesia (50 mg/kg body weight), blood samples were withdrawn by the heart puncture in centrifuge tubes, left 20 minutes to clot, then centrifuged at 3000 rpm for 15 minutes. The obtained sera were separated and stored at -20°C for biochemical measurements. Liver and kidneys were excised and washed with saline for histological examination.

Body and organs weights of the rats

The body weight of the rats was recorded and the change in body weight was calculated as follows: Body weight change = body weight at autopsy – starting body weight. Liver and kidneys were removed and weighted. Their ratios were calculated as follows: Organ ratio (%) = Organ weight (g) ×100/Body weight (g).

Determination of lipid profile

The Biodiagnostic appropriate kits were used for the determination of biochemical lipid profiles indicators. Serum total lipid was detected according to the method of Zollner and kirsch (1962), triglycerides (Fassati and Prencipe, 1982), low density cholesterol (LDL) (Wieland and Seidel, 1983) and high density cholesterol (HDL) (Lopez-Virella *et al.,* 1977).

Statistical analysis of data

Statistical analysis was performed using the SPSS computer program (SPSS Inc., Chicago, IL, USA) for Windows Version 15.0. Data are expressed as mean ± standard error of mean (SEM). Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Student's t-test for group comparisons. Data were considered statistically significant at a P value < 0.05.

RESULTS

Phytochemical constituents of OMEE

Phytochemical constituents of OMEE showed the presence of different active components such as flavonoids, tannins, sterols, triterpens, glycosides, alkaloids and saponins.

Antioxidant activity of OMEE

The ethanolic extract of *O. majorana* exhibited potent antioxidant activity, since the lowest inhibition value for the extract to scavenge DPPH free radical was 50% and the highest inhibition value was above 70% (Figure 1). These results were comparable to vitamin C as a standard antioxidant.

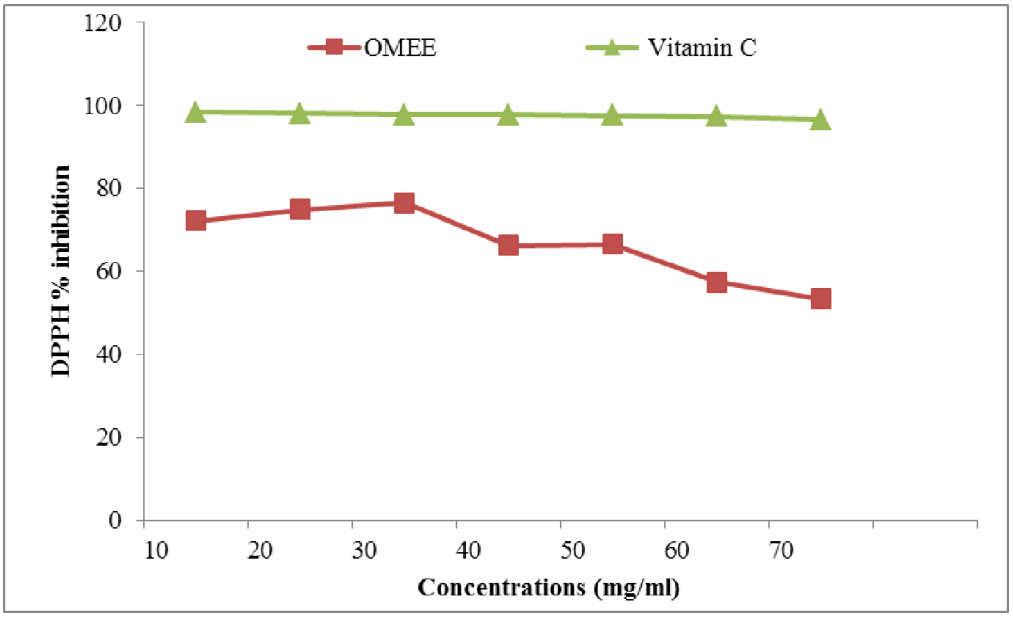

Fig. 1. DPPH radical scavenging activity of the OMEE

Acute oral toxicity study

No mortality was observed at 5000 mg OMEE/kg body weight. Further, no significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, behavior, salivation, sleep of the OMEE treated group as well as the control animals were found to be normal. Moreover, there is no significant difference in histological examination of liver (Fig. 2) and kidneys (Fig. 3) or biochemical markers (Tables 1 and 2), of both control and treated groups. Thereby, the results of the acute oral toxicity (Guideline 425) calculations concluded that, the LD_{50} value of OMEE was more than 5000 mg/kg body weight.

The supposed median effective dose (ED_{50}) of OMEE was considered to be one tenth of the LD_{50}, i.e. 500 mg/kg body weight.

Effect of OMEE treatment on body and organs weights of CDDP

There was a significant loss ($p<0.05$) in the body weight of the CDDP rats when compared with the control rats. Moreover, a significant reduction ($p<0.05$) in the liver and kidneys weight ratios noticed in CDDP rats when compared with the control ones (Table 3). While, the CDDP groups treated with OMEE or silymarin showed a significant increase ($p<0.05$) in their body weight change, liver and kidneys ratios as compared to the untreated CDDP group.

Effect of OMEE on lipid profile of CDDP

Administration of cisplatin resulted in a significant increase ($p<0.05$) in the levels of serum total lipids, triglycerides, and LDL-cholesterol and a significant decrease ($p<0.05$) in serum HDL-cholesterol, as compared to control group. Conversely, the groups treated with OMEE or silymarin showed a significant decrease ($p<0.05$) in serum total lipids, triglycerides, and LDL-cholesterol and a significant increase ($p<0.05$) in serum HDL-cholesterol, as compared to CDDP group (Table 4).

Despite the anticancer effectiveness of cisplatin (CDDP), its high-dose therapy is limited due to its cumulative toxicity (Naqshbandi *et al.,* 2013). Recently, several studies suggested that using of the plant-derived agents in combination with the chemotherapeutic agents lower the toxicity of the later to the normal tissues; besides the ability of these natural agents to enhance the efficacy of the chemotherapeutic agents (Silici *et al.,* 2011; Ma *et al.,* 2014). Therefore, the current study aims to investigate the effectiveness of *Origanum majorana* ethanolic extract (OMEE) to reduce CDDP toxicity.

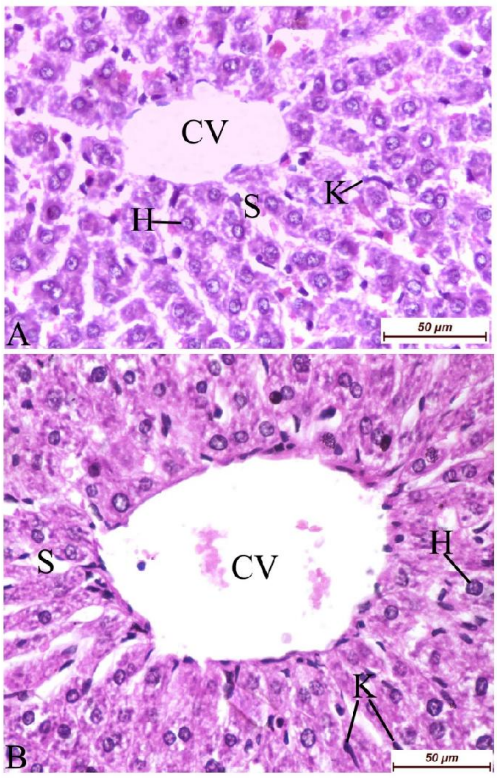

Fig. 2. Photomicrographs of liver sections stained by hematoxylin and eosin showing the effect of OMEE at an acute limit dose (5000 mg/kg b.wt) on liver architecture. (A) Control group, showing the normal liver architecture with distinct hepatocytes (H), sinusoidal spaces (S), central vein (CV), kupffer cells (K), well preserved cytoplasm and prominent nuclei. (B) OMEE group, showing more or less normal liver architecture. Scale bar = 50µm

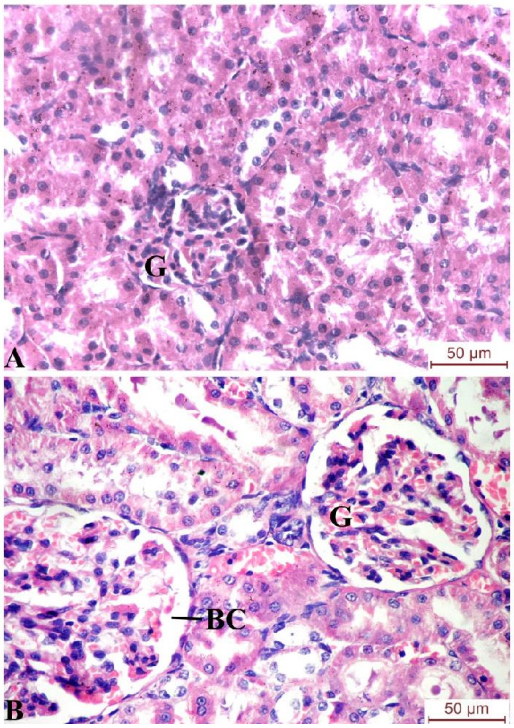

Fig. 3. Photomicrographs of kidney sections stained by hematoxylin and eosin showing the effect of OMEE at an acute limit dose (5000 mg/kg b.wt) on kidney architecture. (A) Control group, showing the normal kidney architecture with glomerulus (G) and Bowman's capsule (BC). (B) OMEE group, showing more or less normal kidney architecture. Scale bar = 50µm

Again, it is an attempt to discover a new nutraceutical extract used together with the CDDP to enhance its role. The present preliminary phytochemical analysis showed that OMEE contains alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic compounds, glycosides and hydroquinone. This is in accordance with the study of Zheng and Wang (2001); Raina and Negi (2012) and Benchikha *et al*. (2013). Additionally, the current study revealed the antioxidant capacity of the OMEE; as it can scavenge the DPPH. Since, Molyneux (2004) clarified that DPPH has been used extensively to predict the antioxidant activities of natural products. The antioxidant potency of the OMEE may be due to its various antioxidant components (flavonoids, phenols and alkaloids). Safety is the overriding criterion in the selection of natural supplements for use in health care systems. The idea that the herbal drugs are safe and free from side effect is a false idea (Calixto, 2000). The results of the present study demonstrated that the OMEE did not cause any apparent toxicity to healthy rats, since, no death or signs of toxicity were observed in rats treated with OMEE at a dosage of 5000 mg/kg body weight.

The present study revealed that OMEE did not have any toxic effect on the vital organs (liver and kidney) either in their functions or histological architcture. These results are in agreement with the results of Aristatile *et al*. (2009). Therefore, OMEE can be considered one of the medicinal safe agent. Body and organs weights measured to evaluate biochemical functions and tissue injury assessment (Mossa *et al.,* 2013). Raju *et al*. (2011) reported that a general reduction in body weight and internal organs weight are simple indices of toxicity after exposure to tested substances. Sellers *et al*. (2007) added that organs weight changes are an important endpoint for detecting harmful effects of chemicals. The present study evidenced that cisplatin injection had a toxic effects on the liver and kidney of the rats. Since, it was noticed that administration of CDDP (3 mg/kg body weight) resulted in a significant decrease in the body, liver and kidney weights of the animals; as compared to control rats and these results are in consonance with the results of Arhoghro *et al*. (2012).

This weight loss observed in the CDDP group may be due to reduced appetite, disturbance in gastrointestinal tract physiology, food absorption and assimilation and enhanced catabolic rate, which are considered as the obvious side effects of chemotherapeutic drugs (Abdel-Wahhab *et al.,* 2013). It could be also due to the sever or prolonged emesis induced by CDDP as suggested by Ballatori and Roila (2003). Regarding lipid profiles, the present study revealed that a single dose of cisplatin significantly elevated serum level of triglycerides and LDL and reduced HDL in comparison with the control group and these are in agreement with the previous study of Saleh *et al.* (2014). The elevated serum LDL-cholesterol and teriglycerides levels herein may be attributed to centrilobular necrosis, which results in translocation and accumulation of fats from peripheral adipose tissue in the liver, increased hepatic synthesis of fatty acids and reduce cholesterol catabolism (Reddy and Rao, 2006). Furthermore, the elevated LDL level in CDDP rats may be due to hepatic secretion of apoprotein B-100 which increased by CDDP and responsible for the secretion of LDL that eventually elevate its level (Chan, 1992). On the other side, the reduction in HDL may be related to the reduction in Apo-A1 which is a principle protein

Table 1. Serum ALAT, ASAT and γGT activities of control and treated groups

Groups	ASAT (U/ml)	ALAT (U/ml)	γGT (U/L)
Control	76.675 ± 2.632	48.725 ± 2.045	4.053 ± 0.614
OMEE	76.825 ± 5.367	45.075 ± 3.914	5.067 ± 0.204

Table 2.Serum creatinine, urea, BUN and uric acid concentrations of control and treated groups

Groups	Creatinine (mg/dl)	Urea (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)
Control	0.579 ± 0.024	38.145 ± 2.406	81.679 ± 5.152	2.472 ± 0.262
OMEE	0.567 ± 0.054	40.205 ± 2.339	86.093 ± 5.010	2.391 ± 0.314

All values are means ± SEM (n= 5)

Table 3. Effect of OMEE treatment on the body weight and relative weights of liver and kidney of CDDP

Groups / Parameters	Control	CDDP	CDDP+OMEE	CDDP+Silymarin
Body weight change (g)	40.383±1.657	-21.083±3.729 [a]	40.167±1.939 [b]	31.833±1.574 [b]
Liver ratio (%)	3.143±0.073	2.745±0.101 [a]	3.233±0.123 [b]	3.417±0.108 [b]
Kidney ratio (%)	0.682±0.019	0.617±0.027 [a]	0.650±0.013 [b]	0.722±0.049 [b]

All values are means ± SEM (n= 6). [a]: significant at $p<0.05$ as compared to control group.[b]: significant at $p<0.05$ as compared to CDDP group

Table 4. Effect of OMEE treatment on serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of CDDP

Groups / Parameters	Control	CDDP	CDDP+OMEE	CDDP+Silymarin
Total lipids (mg/dl)	18.949±1.045	26.204±0.353 [a]	18.901±0.930 [b]	17.550±2.007 [b]
HDL-Cholesterol (mg/dl)	56.752±3.561	36.533±1.201[a]	52.624±5.052 [b]	54.521±4.426 [b]
LDL-Cholesterol (mg/dl)	58.106±3.521	85.165±7.720 [a]	57.379±5.450 [b]	57.144±1.356 [b]
Triglycerides (mg/dl)	43.543±3.377	67.518±3.161 [a]	37.510±2.970 [b]	39.804±4.094 [b]

All values are means ± SEM (n= 6). [a]: significant at $p<0.05$ as compared to control group. [b]: significant at $p<0.05$ as compared to CDDP group

of HDL synthesis (i.e., impaired synthesis of HDL) that can be induced by cisplatin intoxication as clarified by Mohammadi *et al*. (1998). The elevation of triglycerides level in CDDP group may be due to the impaired removal and destruction of triglycerides rich in lipoproteins such as, LDL, IDL, VLDL and remnants or to the increased hepatic synthesis of fatty acids (Karakus *et al.,* 2011). A marked recovery was observed in OMEE treated group. OMEE increase the body weight and liver and kidneys relative weights significantly, as compared to CDDP group. It seems that its phytochemical constituent could act as gastrointestinal tract stimulant that relief lack of appetite and indigestion (Leeja and Thoppil, 2007). Moreover, ethanolic extract of *Origanum majorana* in the present study was effective in reducing the values of the biochemical parameters of lipid profiles. OMEE and silymarin significantly decrease serum total lipid, triglycerides, and LDL-cholesterol and increase serum HDL-cholesterol, as compared to CDDP group. These results are in the same line with those of Bushuty and Shanshan (2012). The antihyperlipidemic and hypocholesterolemic effects of OMEE could be attributed to the presence of flavonoids, saponins, glycosides, tannins and phenolics as reported by Nagm (2002). Their actions may be due to increased inhibition of intestinal absorption of cholesterol, interfere with lipoprotein production, increased expression of hepatic LDL receptors; leading to an increased removal of LDL-cholesterol from the blood and its increased degradation and catabolism of cholesterol from the body (Brown and Goldstein, 1986).

Conclusion

From the above mentioned data, it could be concluded that *Origanum majorana* ethanolic extract supplementation ameliorated the change in lipid profiles induced by cisplatin. This antihyperlipidemic effect of OMEE may be attributed to the presence of flavonoids, saponins, glycosides, tannins and phenolics and their free radical scavenger activity. These results evidenced that OMEE has a high curative role against the cisplatin toxicity; and can be used to enhance cisplatin efficiency as anticancer drug, i.e. the present work would provide a promising strategy for prevention of toxicity in cisplatin-based chemotherapy.

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