Original Article *Spatula DD. 2014; 4(1):33-40*

Evaluation of the antioxidant activity of Marjoram plant (*Origanium majorana* L.) in CCl_4- intoxicated rats

Marjoram bitkisinin (*Origanium majorana L.*) CCl_4 toksikasyonlu sıçanlardaki antioksidan aktivitesinin değerlendirilmesi

Nehal Aly Afifi, Mostafa Abass Shalaby, Hosney Awad El-banna, Shimaa Emam Ramadan

Pharmacology Department, Faculty of Veterinary medicine, Cairo University, Giza, Egypt.

SUMMARY

Aim: This work was performed to evaluate the antioxidant activity and hepatoprotective effect of methanolic extract of Marjoram plant (*Origanium majorana* L.) in CCl_4-intoxicated rats.

Methods: A total of 25 mature albino rats (Sprague Dawley) from both sexes, weighting from140 to 170 g were used. The animals were randomly divided into 5 groups each of 5 animals. These groups were used to investigate the *in vivo* hepatoprotective and antioxidant effects of the tested plant extract when given orally in two doses (375 and 750 mg/kg b.w.). The first group was kept as normal healthy control. The other four groups were given CCl_4 (1 ml/kg b.w. subcutaneously) for five days. One of these was used as a CCl_4-intoxicated non treated control. Group III and IV were given the methanol extract of *Origanium majorana* plant orally at doses of 375 and 750 mg/kg b.w. per day, for two months. Group V was used as a standard group and received silymarin orally at a dose of 100 mg/kg b.w. for two months. The antioxidant activity of methanol extract of *Origanium majorana* plant was studied in rats liver homogenate after the prolonged oral administration (2 months) via determination of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) enzyme activity. The *in vitro* antioxidant activity was compared to DPPH and measured spectrophotometrically. The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in the different groups was evaluated as well as the histopathological examination of the liver.

Results: Oral administration of *Origanium majorana* plant increased the antioxidant enzymes and significantly ($P < 0.05$) decreased liver enzyme levels when given in repeated doses.

Conclusion: Thus methanolic extract of *Origanium majorana* possesses a significant antioxidant activity.

Key words: *Origanium majorana,* antioxidant activity, hepatoprotective, CCl_4-intoxication, rat.

ÖZET

Amaç: Bu çalışma CCl_4 intoksikasyonlu sıçanlarda Marjoram bitkisinin metanolik ekstresinin antioksidan ve karaciğer koruyucu etkisini değerlendirmek için gerçekleştirildi.

Yöntem: Her iki cinsiyetten 140 ila 170 gram arasında toplam 25 erişkin sıçan (Sprague Dawley) kullanıldı. Hayvanlar rastgele olarak her birinde 5 sıçan bulunan 5 gruba ayrıldı. Bu gruplar, oral iki dozda (375 ve 750 mg/kg vücut ağırlığına) verildiğinde, test edilen bitki ekstrelerinin *in vivo* hepatoprotektif ve antioksidan etkilerini araştırmak için kullanıldı. İlk grup normal sağlıklı grup olarak korundu. Diğer dört gruba CCl_4 (1 ml/kg vücut ağırlığına, derialtı) dört gün boyunca verildi. Bunlardan birisi CCl_4 toksike tedavisiz kontrol grubu olarak kullanıldı. Grup III ve IV'e 2 ay süreyle, günde 375 ve 750 mg/kg vücut ağırlığına olacak şekilde, oral olarak *Origanium majorana* bitkisi methanol ekstresi verildi. Grup V standart grup olarak kullanıldı ve oral olarak 100 mg/kg vucüt ağırlığına olacak şekilde 2 ay boyunca silimarin tedavisi aldı. *Origanium majorana* metanol ekstresinin antioksidan aktivitesi, oral uygulamaların sonunda (2 ay) sıçanların karaciğer homojenatlarında, katalaz (CAT), süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) enzim aktivitelerinin saptanması ile çalışıldı. İn vitro antioksidan aktivite DPPH ile karşılaştırıldı ve spektrofotometrik olarak ölçüldü. Karaiciğer histopatolojik çalışması yanında, her bir gruptaki aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve alkalen fosfataz (ALP) enzimleri değerlendirildi.

Bulgular: *Origanium majorana* bitkisinin oral uygulaması tekrarlayan dozlarda verildiğinde antioksidan enzimleri arttırdı ve anlamlı olarak ($P < 0.05$) karaciğer enzim seviyelerini azalttı.

Sonuç: *Origanium majorana* metanolik ekstraktı belirgin bir antioksidan aktiviteye sahiptir.

Anahtar kelimeler: *Origanium majorana*, antioksidan aktivite, hepatoprotektif, CCl_4 intoksikasyonu, sıçan.

Corresponding Author:
Dr. Nehal Aly Afifi,
Pharmacology Department, Faculty of Veterinary medicine, Cairo University, Giza, Egypt.
E-mail: drnehal_affifi@ cu.edu.eg

Received November 28, 2013; accepted January 31, 2014
DOI 10.5455/spatula.20140131074641
Published online in ScopeMed (www.scopemed.org).
Spatula DD. 2014; 4(1):33-40.

INTRODUCTION

From ancient times the medicinal properties of plants were discovered. Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs [1, 2]. Even today this area holds much more hidden treasure as almost 80% of the human population in developing countries is dependent on plant resources for healthcare [3].

The liver disorders are a worldwide problem that causes high morbidity and mortality. There is no therapy can successfully control the progression of liver diseases therefore, researches about herbal medicine that could replace the chemical drug were needed [4]. Medicinal plants are promising source of hepatoprotective and antioxidants activity so has been used in the treatment of liver diseases. *Origanium majorana* is the most important member of the Lamiaceae family [5]. *Origanium majorana* is used worldwide as a spice and crude drug and possesses high antioxidant and anticancer properties [6]. The entire Marjoram herb is harvested and used in Egypt.

The herb is a proximally ten inches tall and has small white, or sometimes pink, flowers. Sweet Marjoram oil is distilled from the leaves and flowering tops of the plant. Some traditional uses include tense muscles relaxation and in relieve spasms, calming and promoting restful sleep, relieving migraine headache, comforting the heart, lowering high blood pressure, assist breathing and disinfecting.

Phytochemical constituents of *Origanium majorana* such as flavonoids, tannins, sterols, triterpens and volatile oils had been previously isolated and identified [7, 8]. Moreover, the chemical composition of *Origanium majorana* leaves extract was identified by [9-11]. About 26 volatile components were identified. The major components were found to be terpinen-4-ol, γ-terpinene, trans-sabinene hydrate, linalool, trans-sabinene hydrate acetate, thujanol, terpinolene, and thymol. In addition to higher concentrations of light oxygenated compounds, which give the oil a superior aroma. Recently the composition of the essential oils of fresh aerial parts of *Origanium majorana* herb is determined by Gc-Ms. The main identified oils constituents was γ-terpinene (19.77%), sabinene hydrate (17.56%), terpinen-4-ol (14.96%), ∂-terpinene (13.25%) and sabinene (12.35%) in *Origanium majorana* [5].

Our study was performed to evaluate the antioxidant and hepatoprotective activities of marjoram herb in healthy and CCl_4-intoxicated rats.

MATERIAL AND METHODS

Plant material

The selected plants were collected and taxonomic identifications were established by the staff members of the Department of Flora, Ministry of Agriculture. A voucher sample was kept in the Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt. The air-dried plant material (250 g) was pulverized, and stored for further use.

Preparation of the methanolic extract

Two hundred grams of the dried parts of the plant was extracted with methanol 70% for at least 24 h, followed by percolation for 5 to 7 times till complete exhaustion. The methanolic extract were concentrated under reduced pressure at temperature not more than 50 ˚C and kept at –4˚C until used .The extract was freshly suspended in sterile distilled water with few drops of Tween 80 to a final concentration of 100 mg/ml.

Animals and Husbandry

A total of 25 mature albino rats (Sprague Dawley) from both sexes, weighting from140 to 170 g were used. Rats were kept under a 12 h light/dark cycle and fed good quality concentrates, with free access to water. The experiments were carried out according to the National regulations on animal welfare and institutional animal Ethical Committee (IAEC).

Experimental design

Animals were allocated randomly into 5 equal groups. These groups were used to test the hepatoprotective and antioxidant effect *in vivo* of oral dose (375 and 750 mg/kg b.w.) of the tested plant. Each group was placed into a separate cage. The animals were randomly divided into 5 groups each of 5 animals. Group I (control healthy) received distilled water orally (1 ml per day) for 60 days. The other four groups were given CCl_4 (1 ml/kg b.wt. s.c) for five days One of these groups was used as a control positive (intoxicated non treated). Group III and IV were given the methanol extract of *Origanium majorana* plant orally at doses of 375 and 750 mg/kg b.w. per day, for 60 days. Group V was used as a standard group and received silymarin orally at a dose of 100 mg/kg b.w. for 60 days.

Blood samples were taken from the veins of orbital plexus of each animal without anticoagulant at the end of the experimental period. Serum samples were separated by centrifugation at 3000 rpm for 10 min. These samples were used for estimating the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Histopathological examination of the liver was also studied. The activity of antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase were determined in liver homogenate.

Evaluation of the *in vitro* antioxidant activity

The scavenging activity of (1, 1-Diphnyl, 2-picryl hydrazyl) DPPH radical was investigated according to the method described by [12]. A methanol solution of DPPH (2.95 ml) was added to 50 µl extract sample (the tested extracts were dissolved in methanol at different concentration, 10.000-25 µg/ml for the methanol extract of *Origanium majorana* plant in a disposable cuvette. Ascorbic acid was used as a standard at 0.1 M concentration which equal to 17613 µg/ml as described by [13]. The absorbance of the standard and samples were measured at 517 nm at regular interval of 15 sec for 5 min. The inhibition percent for each sample was calculated using the following formula:

% inhibition = [Abs. (DPPH sol) - Abs. (Sample) % Abs.(DPPH sol)] x 100

Determination of hepatic antioxidant enzymes (CAT, SOD, GSH-px)

The antioxidant activity of methanol extract of *Origanium majorana* was studied in liver homogenate of rats after oral administration for 60 days. The enzymatic colometric determination of catalase (CAT), activity according to the method of[14], superoxide dismutase (SOD) activity according to method described by [15], and glutathione peroxidase (GSH-px) activity according to method described by[16].

Serum analysis

The activity of serum aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured calorimetrically according to [17], Alkaline phosphates activity (ALP) according to the method of [18].

Histopathological analysys

The Histopathological was examination according to the method described previously [19].

Statistical analysis

The data were expressed as mean±standard deviation (SD). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at level $P < 0.05$ according to [20] using SPSS program version 15.

RESULTS

***In vitro* shoot multiplication**

Antioxidant activity of *Origanium majorana* methanol extract was evaluated *in vitro* as free radical scavenger activity. The reactive reaction rates (inhibition %) with a mean value±standard error of Majorana hortensis were 93.99±1.29, 55.73±0.18 and 12.96±0.59 % at concentrations of 20000, 5000 and 25 µg/ml methanolic solution of the plant extract, respectively. As shown in Table 1 and Figure 1. The reactive reaction rates (inhibition %) of ascorbic acid as a standard antioxidant was 99.012±0.16 %.

Table (1): Showing reaction reactive rate (Inhibition %) of different concentrations of methanolic extract of *Origanium majorana* at different time intervals as compared with ascorbic acid (standard).

Tested material	Concentration (µg/ml)	Reaction reactive rate (Inhibition %)
Ascorbic acid (Standard)	17613	99.12 ± 0.16^{a}
Methanolic extract of *Origanium majorana*	20000	93.99 ± 1.29^{b}
	10000	72.63 ± 1.03^{c}
	5000	55.73 ± 0.18^{d}
	100	44.48 ± 0.15^{e}
	50	25.35 ± 0.309^{f}
	25	12.96 ± 0.59^{g}

Values represent the mean± S.E. of five samples for each group. Values in the column with different superscript letters are significantly different at $P< 0.05$

The antioxidant activity of methanol extract of *Origanium majorana* was determined in rats liver homogenate after prolonged oral administration for 60days by measuring levels of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) enzyme activity. The Marjoram methanol extract significantly stimulate the antioxidant activity in liver homogenate of treated rats as evident by the increased levels of the tested antioxidant enzymes (Table 2).

Table 2: Effect of oral administration of methanolic extract of Origanium majorana for 2 months on the levels of catalase (CAT) , superoxide dismutase (SOD),and glutathione peroxidase (GSH-px), in liver homogenate of rats (n=5).

Group	Dose (mg/kg b.w.)	CAT (U/g)	SOD (U/g)	GSH-px (U/g)
Control (non treated)	0	0.14 ± 0.006^{a}	188.00 ± 0.577^{a}	0.117 ± 0.000^{a}
Methanolic extract of *Origanium majorana*	375	0.87 ± 0.088^{b}	303.23 ± 1.862^{c}	0.156 ± 0.011^{b}
	750	1.80 ± 0.040^{d}	366.18 ± 3.641^{e}	0.194 ± 0.005^{c}

Values represent the mean± S.E. of five samples for each group.
Values in the column with different superscript letters are significantly different at P< 0.05

Table 3: Effect of oral administration of methanolic extract of Origanium majorana and silymarin (standard) for 2months on the serum activity of ALT, ASTand ALP in CCl4- intoxicated rats (n=5).

Groups	Dose (mg/kg b.w.)	ALT (U/ml)	AST (U/ml)	ALP (U/ml)
Control (non treated)	0	82.8 ± 2.24^{b}	133.4 ± 1.96^{a}	135.8 ± 2.63^{a}
CCL_4-control (intoxicated)	0	124.4 ± 5.00^{c}	263 ± 7.68^{c}	229.4 ± 1.91^{c}
Methanolic extract of *Origanium majorana* plant	375	75.6 ± 3.12^{ab}	145.2 ± 4.07^{ab}	148.4 ± 3.65^{b}
	750	72.0 ± 4.42^{ab}	136 ± 2.21^{ab}	135.4 ± 3.2^{a}
Silymarin (standard)	100	68.8 ± 3.26^{a}	136.6 ± 1.89^{ab}	135.4 ± 1.86^{a}

Values represent the mean± S.E. of five samples for each group.
Values in the column with different superscript letters are significantly different at P< 0.05

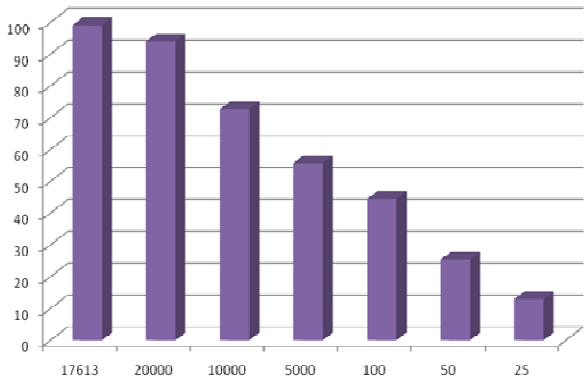

Figure 1: Reaction reactive rate (Inhibition %) of different concentrations of methanol extract of *Origanium majorana* at different time intervals as compared with ascorbic acid (standard).

The effect of methanol extract of Marjorana at doses of 375and 750 mg/kg b.w. on liver enzymes (ALT, AST, and ALP) was reported in Table 3. CCl_4 elevated the ALT level in the intoxicated group as compared to of the control (non treated) group. Rats pretreated with methanol extract of Marjoram plant at doses 375 and 750 mg/kg b.w. for 60 days significantly protected the liver and decreased the ALT as compared to CCl_4 intoxicated group. Silymarin significantly decreased the enzyme activity. The CCl_4 intoxicated group elevated the AST level compared to the control (non treated) group. The methanol extract of *Origanium majorana* plant at doses of 375 and 750 mg/kg b.w. significantly decreased AST level. Silymarin significantly decreased AST enzyme level. The level of ALP enzyme was elevated in the intoxicated rats compared to the control (non treated) group. The methanol extract of *Origanium majorana* plant at doses 375 and 750 mg/kg b.w. for 60 days significantly decreased ALP enzyme levels compared to control (non treated) group. Silymarin was significantly decreased ALP enzyme level.

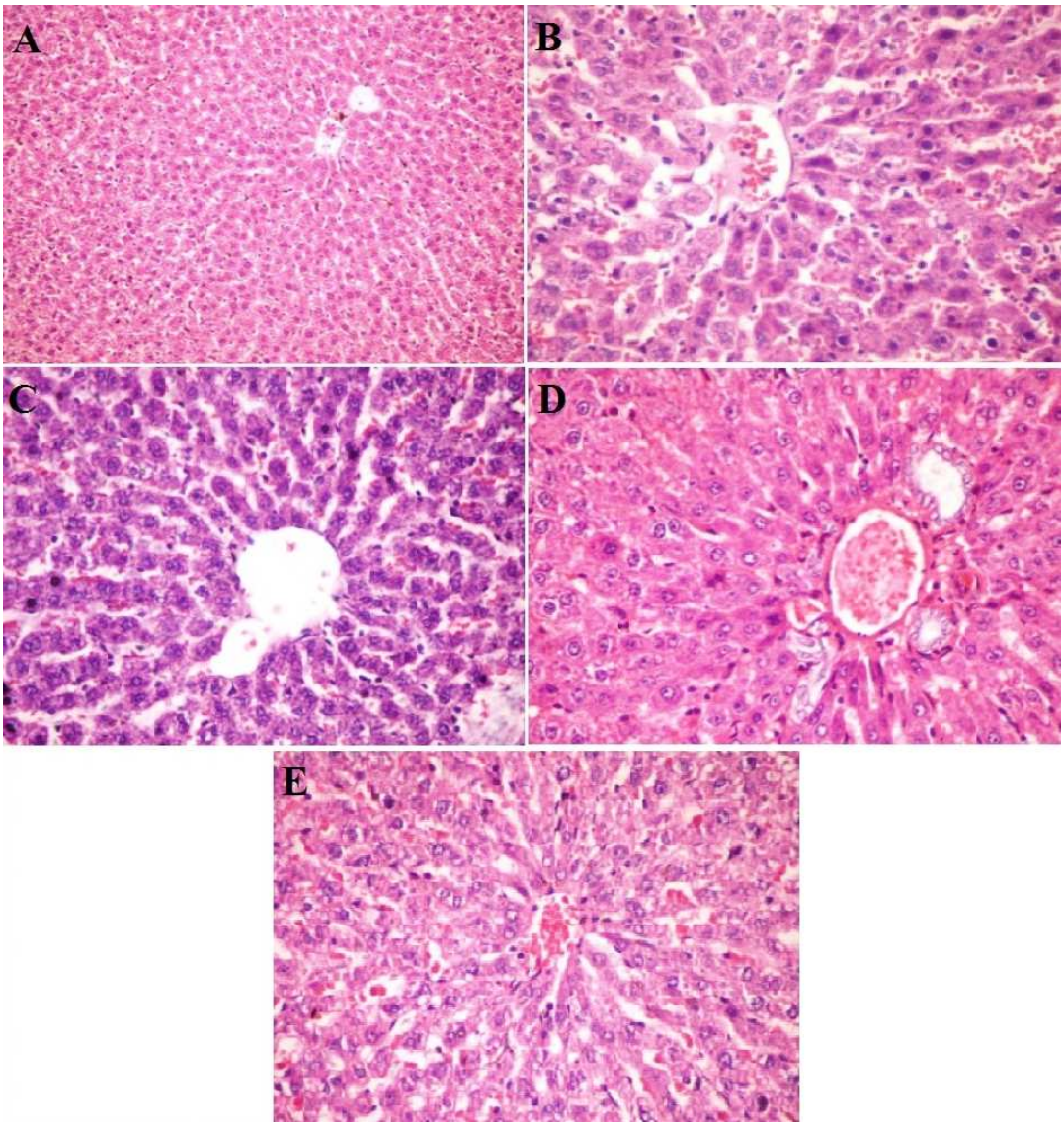

Figure 2: (A) Liver section of the control group showing normal architecture with normal central veins, portal tract, hepatocytes, and sinusoids. (H&E, 20X); **(B)** Liver section of rat given CCl_4 dose showing vacuolar degeneration of hepatocytes, sinusoidal congestion, individual hepatocellular necrosis and increased the number of binucleated cells. (H&E, 40X); **(C)** Liver section of rat pretreated orally with silymarin 100 mg/kg b.w. for 2 months then exposed to CCl_4 showing normal size and shape of hepatocytes with large rounded vesicular nuclei and increase number of binucleated cells. (H&E, 40X); **(D)** Liver section of rat pretreated with methanolic extract of *Origanium majorana* plant at a dose of 375 mg/kg b.w. for 2 months then treated with CCl_4 showing regenerative activity with portal area demonstrating formation of newly formed bile ductules. (H&E, 40X); **(E)** Liver section of rat pretreated with methanolic extract of *Origanium majorana* plant at a dose of 750 mg/kg b.w. for 2 months then treated with CCl_4 showing mild vacuolar degeneration of hepatocytes, activation of kuffer cells (k), and increased the number of binucleated hepatocytes. (H&E, 40X)

.

Histopathological examination

Microscopically, the liver of control non treated rats revealed normal architecture of hepatic lobules. The central veins, portal tract, hepatocytes, and sinusoids appear normal. The lobular unit was well identified as shown in Figure 2A. Liver of CCl_4^- intoxicated rats showed loss of the normal liver architecture. There were a vacuolar degeneration of hepatocytes and individual hepatocellular necrosis (Figure 2B). Histopathological examination of silymarin treated rats' revealed normal size and shape of hepatocytes with large rounded vesicular nuclei and increase number of binucleated cells as shown in Figure 2C. Concerning *Origanium majorana* plant at

375 mg/kg b.w., the liver section showed a marked improvement compared to the intoxicated group. Liver showed normal hepatocytes, hepatic cord, and sinusoids. Some of hepatocytes showed regenerative activity and the portal area showed formation of newly formed bile ductules as illustrated in Figure 2D. Liver sections of rats given Marjoram plant at large dose (750 mg/kg b.w.) showed mild toxic effects of CCl_4. No necrosis were noticed and only mild vascular degeneration of hepatocytes, activation of kuffer cells with increased number of binucleated hepatocytes were observed as demonstrated in Figure 2E.

DISCUSSION

CCL_4 induced hepatic injury is a commonly used model for studying the hepatoprotective effects of drugs or medicinal plant extracts, and the extent of hepatic damage is assessed by the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase [21]. Further, the extent of hepatic damage is assessed by histopathological examination [22]. The results of the present study undertaken to evaluate the hepatoprotective activity of methanolic extract of Origanum majorana in CCL_4 induced liver injury of rats showed that the oral administration of methanolic extract of *Origanium majorana* plant (375 and 750 mg/kg b.w.) for 60 days before CCl_4 intoxication significantly reduced the toxic effect of CCL_4, similar to the standard silymarin in the levels of liver function serum markers, viz AST, ALT and ALP and offered protection to rats liver. This hepatoprotective effect is greater in the methanolic extract of *Origanum majorana* at 750 mg/kg b.w, which is comparable to the reference drug silymarin (100 mg/kg b.w.). The results showed that pretreatment with the methanolic extract restored the biochemical parameters, thereby indicating their protection against the injurious effects of CCL_4, which may be due to the inhibitory effects on cytochrome P450 resulting in the inhibition of formation of hepatotoxic free radicals[21, 23]. The obtained results were supported by [24-26] who evaluated the protective effect of long-term dietary *Origanium* on the alleviation of CCl_4-induced oxidative stress in rats and concluded that the dietary oregano may effectively improve the impaired antioxidant status in CCl_4-induced toxicity in rats. In addition, the administration of carvacrol for 21 days prevented and improved liver enzymes toward normal after its elevation due to hepatotoxicity. The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, or DNA and can lead to degenerative disease. Antioxidant compounds like phenolic acids, polyphones, terpenoids and flavonoids scavenge free radicals such as peroxide, hydro peroxide and thus inhibit the oxidative mechanisms that lead to degenerative disease [27]. In the present investigation, different antioxidant assays have been used to evaluate the antioxidant activity of the methanolic extract of *Origanum majorana*.

In the present study, the antioxidant activity of methanolic extract of the plant was proven by a significant increase in the levels of all the antioxidant enzymes (CAT, GSH and SOD) in liver homogenates after prolonged oral administration up to 60 day as conducted by [21, 28]. In this concern, Kumar et.al, concluded that the methanolic extract of *Origanum majorana* possess free radical scavenging activity under *in vitro* conditions and could protect the liver tissue against CCL_4 induced oxidative stress probably by increasing antioxidant defense activities.

There is a growing interest in the antioxidant properties of many herbs and spices that were reported to be effective in retarding the process of lipid peroxidation in oils and fatty acids [29, 30]. From the obtained result, Marjorana methanolic extract was also proved to have a potent antioxidant activity. This finding is in agreement with that reported previously by [31]. They mentioned that the antioxidant activities of the oil of Marjorana were slightly lower than those of ascorbic acid leaves of Syrian oreganum [*Origanum syriacum* L. (Lauraceae). Similar findings were previously recorded for other herbs and spices as Lamiaceae (Labiatae) family possess a significant antioxidant activity [32]. Additionally, *Origanum vulgare* had high free radical scavenger activity, [33]. In this respect, the results of the present study are supported by Kintzios et al. [34], since they found that *Origanum majorana* L. essential oil exhibited concentration-dependent inhibitory effects on 2, 2'-diphenylpicrylhydrazyl (DPPH).

In the present study, the elevation of GSH levels in liver was observed in the Marjorana treated rats. This indicates that this plant can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen [35], hence diminishing the toxic effects caused by their radical.

The outcome of this study clearly demonstrated the antioxidant potential of *Origanium majorana* extract. The results show that Marjorana plant possessed high levels of antioxidants, could scavenge neutralize oxidants and free radicals.

CONCLUSION

We concluded that methanol extract of *Origanium majorana* plant have a significant antioxidant activity and hepatoprotective effects that can protect CCl_4-intoxicated rats.

Acknowledgements

The authors acknowledge Pharmacology Department, Faculty of Veterinary Medicine, Cairo University for the great help in rearing and handling of rats. This work has been performed by the authors of their own interest.

REFERENCES

1. Abd El-Razek MH. A new ester isolated from *Ferula assafoetida* L. Bioscience, biotechnology and biochemistry. 2007; 71:2300-3.
2. Mahendra P, Bisht S. *Ferula assafoetida*: Traditional uses and pharmacological activity. Pharmacognosy Reviews. 2012; 6:141-6.
3. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bulletin of the world health organization. 1985; 63:965-81.
4. Bruck R, Hershkoviz R, Lider O, Aeed H, Zaidel L, Matas Z, et al. Inhibition of experimentally induced liver cirrhosis in rats by a non peptic mimetic of extracellular matrix of associated Arg-Gly-Asp epitope. J Hepatol. 1996; 24:731-8.
5. Gharib FA, Teixeira daSilva JA. Composition, total phenolic content and antioxidant activity of the essential oil of four Lamiaceae herbs. Medicinal and aromatic plant science and biotechnology. 2013; 7(1):19-27.
6. Rameilah RM. Anticancer and anti oxidant activities of *Matricaria chamomilla* L. and *Majorona hortensis* essential oils. Research Journal of medicine and medical sciences. 2009; 4(2):332-9.
7. Vagi E, Rapavi E, Hadolin M, Vasarhelvine PK, Balazs A, Blázovics A, et al. Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. J Agric Food Chem. 2005; 53(1):17-21.
8. Al–Howiriny T, Alsheikh A, Algasoumi S, Al-Yahya M, ElTahir K, Rafatullah S. Protective effect of *Origanum majorana* L. 'Marjoram' on various models of gastric mucosal injury in rats. Am J Chin Med. 2009; 37(3):531-45.
9. EL-Ghorab AH, Mansour AF, EL-Massry KF. Effect of extraction methods on the chemical composition and antioxidant activity of Egyptian marjoram (*Majorana hortensis* Moench). Flavour and Fragrance Journal. 2004; 19(1):54-61.
10. Verma RS, Sashidhora KV, Yadav A, Naqvi AA. Essential oil composition of *Majorana hortensis* (Moench) from subtropical India. Acta pharmaceutica sciencia. 2010; 52:19-22.
11. Verma R, Verma R, Chauhan A, Yadav A. Changes in the essential oil composition of *Majorana hortensis* Moench. Cultivated in India during plant ontogeny. Journal of the Serbian chemical Society. 2010; 75: 441-7.
12. Miliauskas G, Venskutonis PR, Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004; 2:231-7.
13. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AKS, Mehrota S, et al. Studies on the antioxidant activity of *Desmodium gangeticum*. Biolo Pharmaceut Bulletin. 2003; 26(10):1424-7.
14. Aebi H. Colorimetric determination of catalase in tissue. Methods Enzymol. 1984; 105:121-6.
15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Bioch Biophys. 1984; 21:130-2.
16. Paglia DE, Valentine WN. Determination of glutathione peroxidase in tissue UV method. J Lab Clin Med. 1967; 70:158-69.
17. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am J Clini Patholo. 1957; 28:56-63.
18. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. J Clin Pathol. 1954; 7:322-6.
19. Carleton H. Carleton's. Histological technique. 4th ed., London, Oxford University press New York, Toronto; 1976. pp. 91-95.
20. Snedecor GW, Cocharn WG. Statistical methods, 8th Ed, Iowa State University Press, Ames, Iowa, USA; 1990. p.507.
21. Vishnu Kumar K S, Palaksha M N, Venkatesh K, Sandip Kumar Y, Naik R R. Antioxidant and hepatoprotective effects of methanolic extract of *Origanum majorana* in CCl_4 induced liver injury in rats. World Journal of Pharmacy and Pharmaceutical Sciences. 2013; 2(6): 5898-912.
22. Manjunatha BK, Mankani KL, Vidya SM, Krishna V, Manohara YN. Hepatoprotective activity of *Butea superba* against carbon tetrachloride induced hepatic damage in rodents. Pharmacog Mag. 2008; 4:41-5.
23. Surendra V, Prakash T, Uday RS, Divakar G, Snehal DF, Kotresha D. Hepatoprotective activity of aerial parts of *Cynodon dactylon* against CCL_4 induced in rats. Pharmacog Mag. 2008; 4:195-201.
24. Baser KH. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr Pharm Dis. 2008; 14(29):3106-19.
25. Botsoglou NA, Taitzoglou IA, Botsoglou E, Lavrentiadou SN, Kokoli AN, Roubies N. Effect of long-term dietary administration of oregano on the alleviation of carbon tetrachloride-induced oxidative stress in rats. J Agric Food Chem. 2008; 56(15):6287-93.
26. Aristatile B, Al-Numair K.S, Veeramani C, Pugalendi KV. Antihyperlipidemic effect of carvacrol on D-galactosamine-induced hepatotoxic rats. J Basic Clin Physiol Pharmacol. 2009; 20(1):15-27.
27. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995; 28:25-30.
28. Palaniswamy R, Padma PR. Antioxidant activity of *Majorana hortensis* leaves subjected to oxidative stress in an *in vitro* system. IRJP. 2011; 2:153-7.
29. Namiki M. Antioxidants/antimutagenes in Food. Critical reviews in food science and nutrition. 1990; 39:273-300.

30. Pokorny J. Natural antioxidants for food use. Trends in Food Science Technology. 1991; 9:223-7.
31. Alma MH, Mavi A, Yildirim A , Digrak M, Hirata T. Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. Biol Pharm Bull. 2003; 26(12):1725-9.
32. Tsimidou M., Boskou, D. Antioxidant activity of essential oil from the plants of Lamiaceae family. I: Charalamboru G. Spices, Herbs and Edible Crops. Elsevier, Amsterdam, Holland. 1994.
33. Miron TL, Plaza M, Bahrim G, Ibáñez E, Herrero M. Chemical composition of bioactive pressurized extracts of Romanian aromatic plants. J Chromatogr A. 2010; 1218(30):4918-27.
34. Kintzios S, Papageorgiou K, Yiakoumettis I, Baricevic D, Kusar A. Evaluation of the antioxidants activities of four Slovene medicinal plant species by traditional and novel biosensory assays. J Pharm Biomed Anal. 2010; 53(3):773-6.
35. Mossa, AT, Nawwar, GA. Free radical scavenging and antiacetylcholinesterase activities of Origanum majorana L. essential oil. Hum Exp Toxicol. 2011; 30(10):1501-13.