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Hepatoprotective and antioxidant effects of *Marjorana hortensis* plant in CCl_4-intoxicated rats

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ABSTRACT

The plant phenol compounds such as flavonoids and isoflavonids have an important role in the treatment of many diseases and some of them induce a potent hepatoprotective effect. The present study aimed to evaluate the hepatoprotective and antioxidant activity of methanolic extract of Marjorana hortensis (Marjoram) in a repeated (1 x 60 days) oral doses of 375 and 750 mg kg 1 then compared to control group and silymarin (standard) 100 mg kg 1, orally administered in albino rats for hepatoprotective action. Antioxidant enzymes as SOD, CAT and GSH were measured in liver homogenate. In vitro determination of the extract activity was compared to DPPH and measured spectrophotometrically. Oral administration of Marjorana hortensis significantly ($P < 0.05$) decreased liver enzyme levels when given in repeated doses. The small and large doses increased the antioxidant enzyme. It could be concluded that methanol extract of Marjorana hortensis have a significant hepatoprotective and antioxidant activity.

KEYWORDS

Marjorana hortensis;
Pharmacological effects;
Antioxidant;
Hepatoprotective.

INTRODUCTION

The liver disorders are a worldwide problem that causes high morbidity and mortality. There are no therapy can successfully control the progression of liver diseases even through newly developed drugs which have often side effects. Therefore, researches about herbal medicine that could replace the chemical drug were needed[1] Medicinal plants are promising source of hepatoprotective and antioxidants activity so has been used in the treatment of liver diseases. *Marjorana hortensis* (Family *Labaiatae*) used as a medicinal herb since ancient times and was valued by the Greeks as an antidote for poisons and for muscular cramps. The entire Marjoram herb is harvested and used. 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 and is imported from France. It has a warm & spicy aroma. Sweet marjoram was used medicinally by Romans and ancient Greek physicians. 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.

MATERIALS AND METHODS

Plant material

The selected plants were collected and taxonomic identifications were established by the staff members of

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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 ethanol extract were concentrated under reduced pressure at temperature not more than 50 ^{0}C and kept at –4^{0}C until used and the yield percentage was recorded in TABLE 1. The extract was freshly suspended in sterile distilled water with few drops of Tween 80 to a final concentration of 100 mg/ml.

TABLE 1 : Plant used for screening of the hepatoprotective and antioxidant effects and the yield of methyl alcohol extraction

Plant species	Plant family	Parts used	Yield / 200 g dry plant
Marjorana hortensis	Labaiatae	Aerial parts	71.32

Animals

A total of 25 mature albino rats (Sprague Dawley) from both sexes, weighting from140 to 170 g were used. Rats 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.wt.) 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 two months. The other four groups were given CCL4 (1 ml/kg b.wt. s.c) during the last five days of the experiment. One of these groups was used as a control positive (intoxicated non treated). Group III and IV were given the methanol extract of *Marjorana hortensis* orally at doses of 375 and 750 mg/kg b.wt. 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.wt. for two months. Blood samples were taken from the veins of orbital plexus of each animal with 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) and histopathological examination of the liver. 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[2]. 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 *Marjorana hortensis* 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[3]. 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(reactive reaction rate)=Abs. (DPPH solution) - Abs. (Sample) ÷Abs.(DPPH solution)× 100

Serum analysis

The enzymatic colometric determination of catalase activity according to the method of[4], superoxide dismutase activity according to method described by[5], glutathione peroxidase activity according to method described by[6], alkaline phosphates activity (ALP) according to the method of[7], AST or ALT activity in serum according to the method of[8] and Histopathological examination according to the method described by[9].

Statistical analysis

The data were expressed as mean± Standard deviation (S.D.). 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[10] using SPSS program version 15.

RESULTS

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

The antioxidant activity of ethanol extract of

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Marjorana hortensis was studied in liver homogenate of rats after prolonged oral administration (2 months) via determination of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) enzyme activity. The methanol extract significantly stimulated 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 the administration of methanol extract of *Marjorana hortensis* for 2 months on the levels of (SOD), (GSH-px), and (CAT) in liver homogenate of rats (n=5)

Group	Dose (mg/kg b.wt.)	SOD (U/g)	GSH-px (U/g)	CAT (U/g)
Control	0	188.00±0.577 [a]	0.117±0.000 [a]	0.14±0.006 [a]
Methanolic extract of Marjorana hortensis	375	303.23±1.862 [c]	0.156±0.011 [b]	0.87±0.088 [b]
	750	366.18±3.641 [e]	0.194±0.005 [c]	1.80±0.040 [d]

The effect of methanol extract of Marjorana hortensis at doses of 375and 750 mg/kg b.wt on liver enzymes (ALT, AST, and ALP) was reported in TABLE 3. CCL4 elevated the ALT level in the intoxicated group as compared to of the control (non treated) group. Rats pretreated with ethanol extract of Marjorana hortensis at doses 375 and 750 mg/kg b.wt. for 2 months significantly protected the liver and decreased the ALT as compared to CCL4 intoxicated group. Silymarin significantly decreased the enzyme activity. The CCL4 intoxicated group elevated the AST level compared to the control (non treated) group. The ethanol extract of Marjorana hortensis at doses of 375 and 750 mg/kg b.wt. for 2 months 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 ethanol extract of Marjorana hortensis at doses 375 and 750 mg/kg b.wt. for 2 months significantly decreased ALP enzyme levels compared to control (non treated) group. Silymarin was significantly decreased ALP enzyme level

TABLE 3 : Effect of the methanol extract of Marjorana hortensis, and Silymarin (standard) for 2 months on the serum activity of ALT, AST, and ALP in CCl4-intoxicated rats (n=5)

Groups	Dose mg/kg b.wt.	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]
CCL4control (intoxicated)	0	124.4±5.00[c]	263±7.68 [c]	229.4±1.91[c]
Ethanol extract of Marjorana hortensis	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]

In vitro evaluation of antioxidant activity using DPPH

Antioxidant activity of Marjorana hortensis methanol extract was evaluated in vitro as free radical scavenger activity. The reactive reaction rates (inhibition %) of Marjorana 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. The reactive reaction rates (inhibition %) of ascorbic acid as a standard antioxidant was 99.012±0.16 % in TABLE 4.

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

TABLE 3 : Showing reaction reactive rate (Inhibition %) of different concentrations of methanol extract of Marjorana hortensis 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]
Methanol extract of Marjorana hortensis	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]

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shown in Figure 1. Liver of CCL4- intoxicated rats showed loss of the normal liver architecture. There were a vacuolar degeneration of hepatocytes and individual hepatocellular necrosis Figure 2. 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 3. Concerning Marjorana hortensis at 375 mg/kg b.wt., 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 4. Liver sections of rats given Marjorana hortensis at large dose (750 mg/kg b.wt.) showed mild toxic effects of CCL4. 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 5.

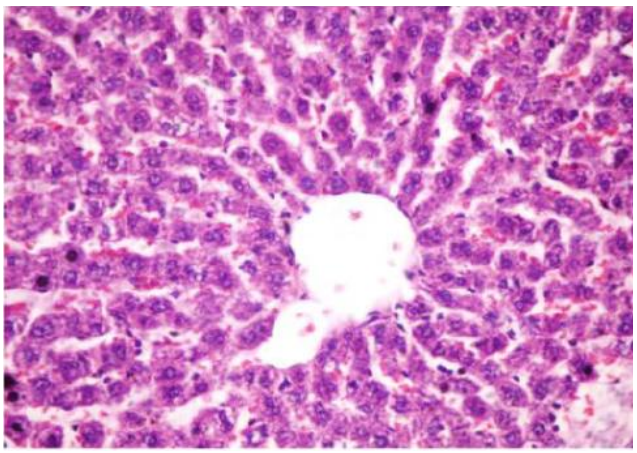

Figure 3 : Liver section of rat pretreated orally with silymarin 100 mg/kg b.wt. for 2 months then exposed to CCL4 showing normal size and shape of hepatocytes with large rounded vesicular nuclei and increase number of binucleated cells. (H and E X 400)

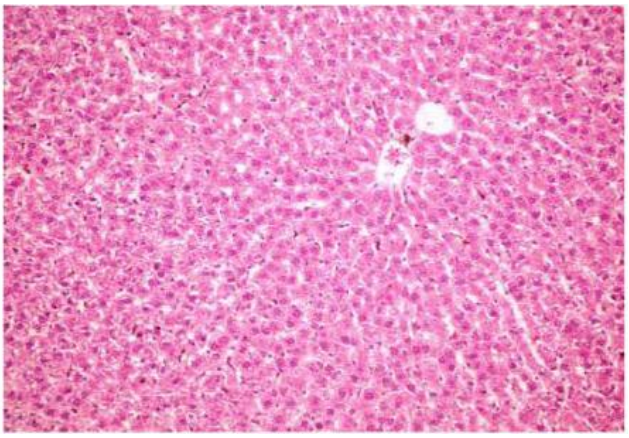

Figure 1 : Liver section of the control group showing normal architecture with normal central veins, portal tract, hepatocytes, and sinusoids. (H and E X 400)

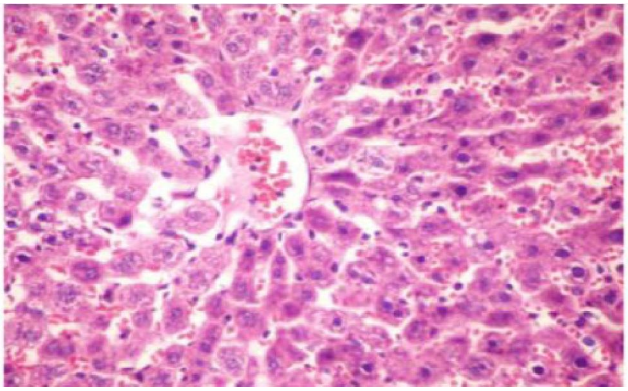

Figure 2 : Liver section of rat given CCL4 dose showing vacuolar degeneration of hepatocytes (v), sinusoidal congestion (c), individual hepatocellular necrosis (n) and increased the number of binucleated cells (b). (H and E X 400)

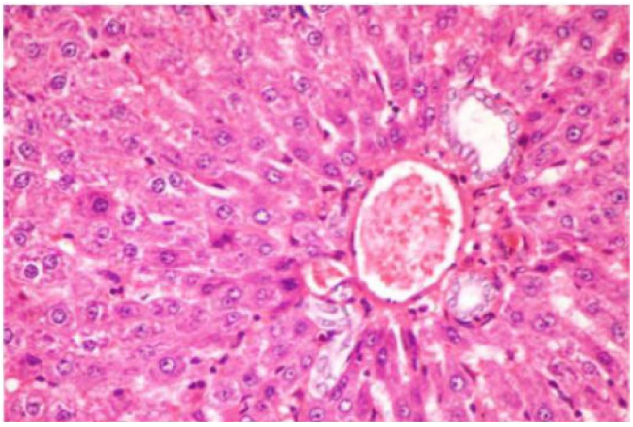

Figure 4 : Liver section of rat pretreated with methanolic extract of Marjorana hortensis at a dose of 375 mg/kg b.wt. for 2 months then treated with ccl4 showing regenerative activity with portal area demonstrating formation of newly formed bile ductules. (H and E X 400)

DISCUSSION

Oral administration of either Marjorana hortensis extract for 2 month before CCL4 intoxication offered protection to the rat liver. In addition, serum biochemical parameters as (AST, ALT, and ALP) were also increased compared to the control intoxicated group. The obtained results of the present study of Marjorana hortensis were supported by[11,12]. They evaluated the

protective effect of long-term dietary oregano on the alleviation of carbon tetrachloride-induced oxidative stress in rats and concluded that the dietary oregano may effectively improve the impaired antioxidant status in CCL4-induced toxicity in rats. In addition,[13] found that administration of carvacrol for 21 days prevented and improved liver enzymes toward normal after its elevation due to hepatotoxicity. The antioxidant activity of ethanol extract of the plant extract was proven by a significant increase in the levels of all the antioxidant enzymes (CAT, GSH and SOD) in liver homogenates in rats. In this concern, The obtained result showed that oral administration of ethanolic extract Marjorana hortensis significantly increased the activity of antioxidant enzymes in liver homogenate as catalase, glutathione peroxidase, superoxide dismutase (CAT, SOD, GSH-px) after prolonged oral administration up to 60 day. In this respect,[14] reported that co-administration of the extracts of Marjorana hortensis resulted in minimizing the hazard effects of ethanol toxicity on male fertility, liver, and brain tissues. They concluded that marjoram volatile oil and grape seed extract are useful herbal remedies, especially for controlling oxidative damages.

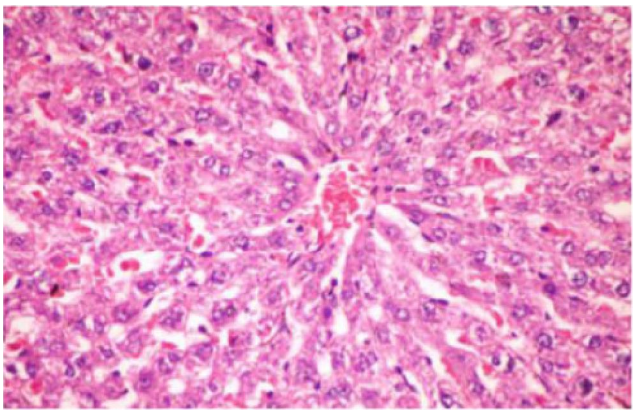

Figure 5 : Liver section of rat pretreated with methanolic extract of Marjorana hortensis at a dose of 750 mg/kgb.wt. for 2 months then treated with ccl4 showing mild vacuolar degeneration of hepatocytes (v), activation of kuffer cells (k), and increased the number of binucleated hepatocytes (b). (H and E X 400)

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[15,16,17]. From the obtained result, Marjorana hortensis methanolic extract was also proved to have a potent antioxidant activity. This finding is in agreement with that reported previously by (18). They mentioned that the antioxidant activities of the oil of Marjorana hortensis 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[19]. Additionally, Origanum vulgare had high free radical scavenger activity[20,21,22]. In this respect, the results of the present study are supported by[23], 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 blood and liver was observed in the Marjorana hortensis treated rats. This indicates that these plants 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[24], hence diminishing the toxic effects caused by their radical.

CONCLUSION

In conclusion, our results demonstrated that ethanol extract of Marjorana hortensis have a significant hepatoprotective, in vivo and in vitro antioxidant effect probably because of decreased liver enzyme levels with significant improvement to the histological liver sections. The antioxidant enzymes are significantly increased in pre treated extract rat liver homogenate. Inhibition% of Marjorana hortensis confirm its potent free radical scavenger activates.

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