Protective effects of *Mentha piperita* L. leaf essential oil against CCl₄ induced hepatic oxidative damage and renal failure in rats

Khaled Bellassoued¹*, Anis Ben Hsouna², Khaled Athmouni³, Jos van Pelt⁴, Fatma Makni Ayadi⁵, Tarek Rebai⁶ and Abdelfattah Elfeki¹

Abstract

**Background:** *Mentha piperita* L. is a flowering plant belonging to the Lamiaceae family. *Mentha* plants constitute one of the main valuable sources of essential oil used in foods and for medicinal purposes.

**Methods:** The present study aimed to investigate the composition and in vitro antioxidant activity of *Mentha piperita* leaf essential oil (MpEO). A single dose of CCl₄ was used to induce oxidative stress in rats, which was demonstrated by a significant rise of serum enzyme markers. MpEO was administrated for 7 consecutive days (5, 15, 40 mg/kg body weight) to *Wistar* rats prior to CCl₄ treatment and the effects on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and γ-glutamyl transpeptidase (γ-GT) levels, as well as the liver and kidney superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activity and thiobarbituric acid reactive substances (TBARS) levels were evaluated. In addition, histopathological examinations of livers and kidneys was performed.

**Results:** The in vitro antioxidant activity of MpEO was lower than that of silymarin. Pretreatment of animals with MpEO at a dose of 5 mg/kg did not have a significant effect on ALT, AST, ALP, LDH, γ-GT, urea or creatinine levels in CCl₄-induced stress. Whereas pretreatment with MpEO at doses of 15 and 40 mg/kg prior to CCl₄, significantly reduced stress parameters (ALT, AST, ALP, LDH, γ-GT, urea and creatinine) compared to the CCl₄-only group. Moreover, a significant reduction in hepatic and kidney lipid peroxidation (TBARS) and an increase in antioxidant enzymes SOD, CAT and GPx was also observed after treatment with MpEO (40 mg/kg) compared to CCl₄-treated rats. Furthermore, pretreatment with MpEO at 40 mg/kg can also markedly ameliorate the histopathological hepatic and kidney lesions induced by administration of CCl₄.

**Conclusions:** We could demonstrate with this study that MpEO protects liver and kidney from CCl₄-induced oxidative stress and thus substantiate the beneficial effects attributed traditionally to this plant.

**Keywords:** *M. piperita*, Essential oil, Liver, Kidney, Oxidative stress, Histopathological

---

**Background**

Reactive oxygen species (ROSs) are various forms of activated oxygen. A disproportion of the reactive oxygen species and the absence of their scavenger systems in cells lead to oxidative stress and increases the risk of several human chronic diseases [1]. ROS contributes to the development of various diseases such as diabetes, atherosclerosis, cancer, neurodegenerative diseases, liver cirrhosis and the aging process [2]. The liver plays a central role in the maintenance of systemic lipid homeostasis and is especially susceptible to ROS damage. CCl₄ is now of greatest concern as an environmental contaminant [3]. It was reported that CCl₄ was one of the most commonly used toxins in the experimental study of liver diseases [4]. Abraham et al. [5] showed that the nephrotoxic effects of CCl₄ were also associated with free radical production.

To prevent the damage caused by ROS, living organisms have developed an antioxidant defense system that includes the presence of non-enzymatic antioxidant enzymes and the aging...
glutathione peroxidase (GPx) [6]. It has been anticipated that in addition to these natural antioxidants, other synthetic or natural ROS scavengers may reduce the incidence of free radical-mediated diseases. The use of antioxidants in the prevention and cure of various diseases is intensifying, and there is considerable interest in the study of the antioxidant activities of molecules such as plant polyphenolic and carotenoid components [6, 7]. Antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing lipid peroxidation [8].

A number of studies showed that various herbal extracts could protect liver and kidney against CCl₄-induced oxidative stress by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity [9]. Silymarin, a flavonolignan mixture of milk thistle (Silybum marianum), is one such important herbal hepatoprotective drug. Silymarin exhibits hepatoprotective effects by altering cytoplasmic membrane architecture and, in turn, preventing the penetration of hepatotoxic substances, such as carbon tetrachloride (CCl₄), thioacetamide and D-galactosamine [10].

The well-known and widely used peppermint (Mentha piperita L.) (Lamiaceae) is a cultivated natural hybrid of Mentha aquatica L. (water mint) and Mentha spicata L. (spearmint). Although a native genus of the Mediterranean region, it is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Peppermint oil is one of the most widely produced and consumed essential oils [11, 12]. Besides its uses in food, herbal tea preparations, and confectioneries, the medicinal uses of mint, which date back to ancient times, include carminative, anti-inflammatory, antispasmodic, antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anti-atharrhal application. It is also used against nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints. Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migraines [13, 14].

From the experimental and clinical studies performed on Mentha piperita leaf essential oil (MpEO), it seems that most of its pharmacological actions are due to its antioxidant activity which is mainly due to its ability to scavenge free radicals and/or inhibit lipid peroxidation [15, 16]. Antioxidants are substances that delay or prevent the oxidation of inter- or intra-cellular oxidizable substrates from oxidative stress. In this study, we report the chemical composition and antioxidant effects of MpEO in several in vitro systems (DPPH and superoxide scavenging activities). Besides, we are interested in determining the possible protective effects of MpEO against oxidative damage of the liver and kidney following an intraperitoneal administration of CCl₄, by assessing the oxidative stress profile and some serum biochemical parameters.

Methods

Plant material

Fresh leaves of M. piperita L. samples were harvested from the local market at Sfax (Tunisia) (N: 34.4426°, E: 10.4537°) during the vegetative stage in June 2013. The samples were identified and authenticated by a senior botanist, Pr. Ferjani Ben Abdallah, at the Faculty of Science of Sfax, University of Sfax (Tunisia). From 50 individual M. piperita L. plants each, a total of 80–100 leaves (= 12 cm² in size) were randomly collected from the base to the apex. The fresh leaves were mixed and immediately dried in the shade away from light at room temperature. After drying, the samples were grounded to a fine powder that was used for the extraction of essential oil.

Essential oil preparation

MpEO was extracted by the steam distillation method. A mass of 3 kg of dry plant material was hydrodistilled for 2 h in a Clevenger-type apparatus. The recovered (0.47%) essential oil was dried with anhydrous Na₂SO₄, and stored at 4 °C.

Mentha piperita essential oil composition

MpEO compositional analysis of the volatile constituents was performed on a Hewlett-Packard gas chromatograph GC: 5890 series II. The fused HP-Innowax capillary column (polyethylene glycol, 30 m, 0.25 μm, ID, 0.25 mm film thickness) was directly connected to the mass spectrometer. Nitrogen was used as a carrier gas at a flow rate of 1.2 ml/min. Oven temperature was initially set at 50 °C (1 min) and gradually raised to 250 °C (5 min) at 7 °C/ min. The temperatures of the injection port and detector were maintained at 250 and 280 °C, respectively. The mass spectrometer was operated (full scan-mode) in the EI-mode at 70 eV.

Component identification

The essential oil components were identified based on their mass spectra and computer matching with the data available in the Wiley 275 library (Wiley, New York).

In vitro antioxidant activities test

The antioxidant activity of the MpEO was determined by two methods and compared with the activity of silymarin, a standardized extract of the milk thistle seeds that contains a mixture of flavonolignans. Silymarin has a number of potential mechanisms including chemoprotective effects from environmental toxins and anti-inflammatory activity and is used as a drug.
Measurement of free radical-scavenging action

2,2-Diphenyl picrylhydrazyl (DPPH) free radicals scavenging activity was assessed according to Blois [17], with a slight modification. Different concentrations of the MpEO and silymarin (5–100 μg/ml) were mixed with 1 ml of 0.1 mM DPPH in ethanol solution and 450 μL of 50 mM Tris-HCl buffer (pH 7.4) was added. The solution was incubated at 37 °C for 30 min and the reduction of DPPH free radicals was measured by reading the absorbance at λ = 517 nm. Silymarin was used as reference standard. The activity is given as % DPPH scavenging and calculated according to the following equation:

\[
\% \text{DPPH scavenging} = \frac{(\text{control OD} - \text{sample OD})/\text{control OD}}{\times 100}
\]

The antioxidant activity of MpEO is expressed as IC50, defined as the concentration of MpEO required to cause a 50% decrease in initial DPPH concentration. Each sample was analyzed six times.

Scavenging of superoxide anion

The influence of MpEO on the generation of superoxide anion was measured according to the method described by Yen & Chen, 1995 [18]. Superoxide anion was generated in a non-enzymatic system and determined by spectrophotometric measurement for the reduction of nitroblue tetrazolium. The reaction mixture, which contained 100 μL of essential oil in ethanol, 800 μL of 1 M phosphate buffer (pH 7.4), 400 μL of distilled water, 100 μL of 0.1 M Na4EDTA, 100 μL of 1.5 mM NBT and 50 μL of 0.12 mM riboflavin was incubated at ambient temperature for 5 min, and the color was read at λ = 560 nm against blank samples.

\[
\% \text{superoxide anion scavenging} = \frac{(\text{blank OD} - \text{sample OD})/\text{OD blank}}{\text{OD blank}} \times 100
\]

Where blank OD is the absorbance of the control reaction and sample OD is the absorbance in the presence of MpEO. The IC50 was calculated from the plot of the inhibition percentage against the essential oil concentration. Each sample was analyzed six times.

In vivo antioxidant properties

Animal

Male Wistar rats, weighing about 200–220 g, were purchased from the Central Pharmacy of Tunisia (SIPHAT, Tunisia). They were housed at 22 ± 3 °C with light/dark periods of 12 h and a minimum relative humidity of 40%. The animals had free access to commercial pellet diet (SICO, Sfax, Tunisia) and water ad libitum. The general guidelines for the use and care of living animals in scientific investigations were followed [19]. The handling of the animals was approved by the Tunisian Ethical Committee for the Care and Use of laboratory animals.

Experimental design

After acclimatizing to the laboratory conditions for 1 week, 70 rats were divided into 7 groups of 10 animals and treated for 7 days as follows [20]:

The rats of group 1 served as normal control and received saline orally daily for 7 days and were injected with 1 ml/kg BW of just olive oil (the solvent of CCl4) on the 7th day. The rats of group 2 served as CCl4-hepato- and renotoxicity control and were received saline orally daily for 7 days and were injected with 1 ml/kg BW of CCl4 and olive oil mixture on the 7th day (a single intraperitoneal injection). The CCl4 dose was selected according to the reference dose for chronic oral exposure (RFD) as recommended for CCl4 (CASRN 56–23–5) [21].

The rats of group 3 were pretreated orally seven times with a dose of 50 mg/kg BW of reference drug silymarin with an interval of 24 h [22].

The rats of groups 4, 5, 6 and 7 were pretreated orally seven times with doses of 5, 15 and 40 mg MpEO/kg BW, respectively with an interval of 24 h [23].

After pretreatment with either silymarin or MpEO for 7 days, the rats of groups 3, 4, 5 and 6 received a single intraperitoneal injection of CCl4 (1 ml/kg BW) on the 7th day.

Rats were killed 24 h after vehicle or CCl4 single injection. The animals in the different groups were killed by cervical decapititation to avoid stress conditions.

Sample collection

Serum was prepared by centrifugation (1500×g, 15 min, 4 °C; Beckman-Coulter, Marseille, France) and stored at −80 °C for further biochemical assays. The liver and kidney tissues were immediately removed and dissected over ice-cold glass slides and a part was homogenized (10% w/v) with an Ultra Turrax homogenizer in iced cold, 1.15% KCl-0.01 M sodium, potassium phosphate buffer. Homogenates were centrifuged at 10000×g for 20 min at 4 °C. The resulting supernatants were used for immediate lipid peroxidation and protein oxidation determination. Homogenate aliquots were stored at −80 °C for further biochemical assays. Other parts of these livers and kidney tissues were fixed in 10% formaldehyde solution and processed for paraffin sectioning and histological studies.

Biochemical assays

Biochemical markers in plasma

Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), creatinine and urea rates were measured in plasma samples by standardized enzymatic procedures using commercial kits from (Biolabo, Maizy, France) on an
automatic biochemistry analyzer (Vitalab Flexor E, Diamond Diagnostics, Holliston, MA).

**Protein quantification**
Protein content in liver and kidney tissues were determined according to the method of Lowry et al. [24] using bovine serum albumin as a standard.

**Lipid peroxidation**
Malondialdehyde concentrations (marker for lipid peroxidation) in liver and kidney tissues were determined spectrophotometrically according to Draper & Hadley [25]. Briefly, an aliquot of liver and kidney extracts supernatant was mixed with 1 ml of 5% trichloroacetic acid and centrifuged at 2500×g for 10 min. One ml of thiobarbituric acid reagent (0.67%) was added to 500 μl of supernatant and heated at 90 °C for 15 min. The mixture was cooled and the absorbance measured at 532 nm using a spectrophotometer (Jenway UV-6305, Essex, England). The malondialdehyde values were calculated using 1,1,3,3-tetraethoxypropane as standard and expressed as nmol of malondialdehyde/mg of protein.

**Determination antioxidant enzyme activities in liver and kidney tissue**
Catalase (CAT) activity was measured according to Aebi [26]. A total of 20 μL tissue homogenate (about 1.5 mg proteins) was added to 1 ml phosphate buffer (0.1 M, pH 7) containing 100 mM H₂O₂. Rate of H₂O₂ decomposition was followed by measuring the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated using an extinction coefficient of 0.043 mM⁻¹ cm⁻¹ and expressed in international units (IU), i.e. in μmol H₂O₂ destroyed/min/ mg protein, at 25 °C.

Superoxide dismutase (SOD) activity was estimated according to Beyer and Fridovich [27]. The reaction mixture contained 50 mM of tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 mM riboflavin and 75 mM nitro blue tetrazolium (NBT). The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units/mg of protein, at 25 °C. Glutathione peroxidase (GPx) activity was measured by the procedure of Flohe and Gunzler [28]. One milliliter of reaction mixture containing 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of 2 mM glutathione (GSH), 0.1 ml of sodium azide (10 mM), 0.1 ml of H₂O₂ (1 mM) and 0.3 ml of liver and kidney supernatant were prepared. After incubation at 37 °C for 15 min, the reaction was terminated by adding 0.5 ml 5% TCA. Tubes were centrifuged at 1500×g for 10 min and the supernatant was collected. To 0.1 ml of this reaction supernatant, 0.2 ml of (0.1 M pH 7.4) and

### Table 1

| Peak | Compounds         | Retention Time (min) | Percentage (%) |
|------|-------------------|----------------------|---------------|
| 1    | α-pinene          | 4.42                 | 1.80          |
| 2    | β-pinene          | 6.07                 | 0.14          |
| 3    | Sabinene          | 6.42                 | 0.25          |
| 4    | Myrcene           | 7.81                 | 1.30          |
| 5    | Limonene          | 8.30                 | 8.0           |
| 6    | 1,8-cineole       | 8.48                 | 2.80          |
| 7    | 3-octanone        | 10.13                | 0.45          |
| 8    | 3-octanol         | 10.41                | 0.53          |
| 9    | Limonene oxide    | 13.44                | 0.59          |
| 10   | α-terpineol       | 18.62                | 0.37          |
| 11   | Linalool          | 15.73                | 2.64          |
| 12   | iso-menthone      | 15.14                | 33.00         |
| 13   | Menthy acetate    | 16.35                | 0.68          |
| 14   | Iso-pulegol       | 16.71                | 2.40          |
| 15   | Isomenthol        | 17.29                | 0.28          |
| 16   | Neo-iso-menthol   | 17.90                | 0.45          |
| 17   | Menthol           | 18.25                | 33.59         |
| 18   | Pulegone          | 18.39                | 1.6           |
| 19   | Neryl acetate     | 18.45                | 0.8           |
| 20   | Piperitone        | 19.09                | 3.2           |
| 21   | Myrtenol          | 19.56                | 0.55          |
| 22   | Carveol           | 19.99                | 0.31          |
| 23   | Caryophyllene     | 17.33                | 1.95          |
| 24   | Caryophyllene oxide | 20.10             | 0.11          |
| 25   | Germacrene D      | 20.80                | 0.11          |
| 26   | Δ-Cadinene        | 21.92                | 0.27          |

Monoterpene hydrocarbons (%) 16.23
Oxygenated monoterpenes (%) 79.5
Sesquiterpene hydrocarbons (%) 2.44
Total (%) 98.17

### Table 2

|     | MpEO | Silymarin |
|-----|------|-----------|
| 50% scavenging concentration (μg/ml) on DPPH radical | 61.28 ± 0.02* | 21.25 ± 0.13 |
| 50% scavenging concentration (μg/ml) on superoxide anion | 356.45 ± 0.35* | 39.04 ± 1.02 |

Values are represented as mean ± SEM of six different experiments

*p < 0.05 versus silymarin
Table 3 Effects of CCl4, MpEO and their combination MpEO/CCl4 on hepatic markers in serum of control and experimental rats

| Treatment and parameters | AST (U/L) | ALT (U/L) | ALP (U/L) | LDH (U/L) | γ-GT (U/L) |
|--------------------------|-----------|-----------|-----------|-----------|------------|
| Control                  | 164.82 ± 1.28 | 65.88 ± 1.44 | 149.61 ± 2.19 | 20.81 ± 0.52 | 3.17 ± 0.39 |
| CCl4                     | 524.12 ± 5.11*** | 179.23 ± 3.39*** | 217.47 ± 8.55*** | 40.66 ± 0.81*** | 5.43 ± 0.21*** |
| SL/CCl4                  | 191.15 ± 2.14### | 87.82 ± 7.61### | 157.50 ± 5.12### | 28.62 ± 0.62### | 3.47 ± 0.06### |
| MpEOa/CCl4               | 479.89 ± 36.41 | 162.02 ± 9.18 | 202.42 ± 7.62 | 37.75 ± 1.35 | 4.45 ± 0.25 |
| MpEOb/CCl4               | 356.82 ± 35.77### | 147.35 ± 5.67### | 186.74 ± 3.98### | 34.16 ± 1.35### | 4.06 ± 0.17### |
| MpEOc/CCl4               | 216.80 ± 7.26### | 97.82 ± 6.98### | 165.96 ± 5.12### | 31.64 ± 5.12### | 3.51 ± 0.20### |
| MpEOc                   | 160.80 ± 2.47 | 63.08 ± 1.16 | 139.77 ± 7.27 | 19.89 ± 0.18 | 2.81 ± 0.24 |

AST aspartate aminotransferase, ALT alanine aminotransferase, ALP alkaline phosphatase, LDH lactate dehydrogenase and γGT gamma glutamyl transferase.

Table 4 Effects of CCl4, MpEO and their combination MpEO/CCl4 on lipid profile in serum of control and experimental rats

| Treatment and parameters | T-Cholesterol (mmol/l) | T-Triglycerides (mmol/l) | HDL (mmol/l) | LDL (mmol/l) |
|--------------------------|------------------------|--------------------------|--------------|--------------|
| Control                  | 1.133 ± 0.033          | 0.736 ± 0.075            | 1.413 ± 0.023 | 0.123 ± 0.014 |
| CCl4                     | 1.750 ± 0.028***       | 1.743 ± 0.078***         | 0.446 ± 0.074*** | 0.726 ± 0.053*** |
| SL/CCl4                  | 0.966 ± 0.033**        | 1.043 ± 0.012***         | 1.240 ± 0.034*** | 0.176 ± 0.008** |
| MpEOa/CCl4               | 1.605 ± 0.063          | 1.610 ± 0.041            | 0.690 ± 0.135 | 0.640 ± 0.066 |
| MpEOb/CCl4               | 1.375 ± 0.076**        | 1.196 ± 0.039**          | 0.873 ± 0.053** | 0.253 ± 0.024** |
| MpEOc/CCl4               | 1.033 ± 0.033**        | 1.050 ± 0.010**          | 1.295 ± 0.039** | 0.186 ± 0.017** |
| MpEOc                   | 1.100 ± 0.115          | 0.763 ± 0.069            | 1.210 ± 0.106 | 0.113 ± 0.006 |

Chemical constitution of Mentha piperita L. leaf essential oil

Chemical composition of MpEO was determined by GC/MS analysis. The compounds, their percentages as well as the chemical structure of the oil are presented in Table 5.

Table 5 Effects of CCl4, MpEO and their combination MpEO/CCl4 on kidney markers in serum of control and experimental rats

| Treatment and parameters | Creatinine (µmol/l) | Urea (mmol/l) |
|--------------------------|---------------------|---------------|
| Control                  | 7.53 ± 0.59         | 2.50 ± 0.50   |
| CCl4                     | 15.41 ± 1.57***     | 12.79 ± 0.15**|
| SL/CCl4                  | 8.04 ± 0.32#        | 11.19 ± 0.27# |
| MpEOa/CCl4               | 12.52 ± 0.32        | 11.94 ± 0.47  |
| MpEOb/CCl4               | 10.50 ± 0.29#       | 11.45 ± 0.40# |
| MpEOc/CCl4               | 9.44 ± 0.67#        | 11.39 ± 0.30# |
| MpEOc                   | 7.34 ± 0.38         | 11.27 ± 0.35  |

Values are mean ± SE for eight rats in each group. CCl4, MpEO and MpEO/CCl4 treated groups vs control group; ** p < 0.01, *** p < 0.001, CCl4 group vs MpEO/CCl4 group; # p < 0.05, ## p < 0.01, ### p < 0.001.
Fig. 1  

**a.** Effects of CCl₄, MpEO and their combinations (MpEO/CCl₄) on hepatic TBARS of control (Con) and experimental rats. Con, control group; mod, CCl₄-model group; SL/CCl₄, silymarin 50 mg/kg + CCl₄; MpEO/CCl₄ 5 mg/kg + CCl₄ group; MpEO/CCl₄ 15 mg/kg + CCl₄ group; MpEO/CCl₄ 40 mg/kg + CCl₄ group; MpEO 40 mg/kg group. Values are mean ± SEM for ten rats in each group. CCl₄, MpEO, MpEO/CCl₄ treated groups vs control group; *p < 0.05, **p < 0.01, ***p < 0.001, CCl₄ group vs (MpEO/CCl₄) group; #p < 0.05, ##p < 0.01, ###p < 0.001.

**b.** Effects of CCl₄, MpEO and their combinations (MpEO/CCl₄) on kidney TBARS of control (Con) and experimental rats. Con, control group; mod, CCl₄-model group; SL/CCl₄, silymarin 50 mg/kg + CCl₄; MpEO/CCl₄ 5 mg/kg + CCl₄ group; MpEO/CCl₄ 15 mg/kg + CCl₄ group; MpEO/CCl₄ 40 mg/kg + CCl₄ group; MpEO 40 mg/kg. Values are mean ± SEM for ten rats in each group. CCl₄, MpEO, MpEO/CCl₄ treated groups vs control group; *p < 0.05, **p < 0.01, ***p < 0.001, CCl₄ group vs (MpEO/CCl₄) group; #p < 0.05, ##p < 0.01, ###p < 0.001.
their retention indices are listed in Table 1. MpEO is a mixture with 26 compounds representing 98.17% of the total oil composition. The most abundant chemicals categories for MpEO are oxygenated monoterpenes (79.50%), followed by monoterpene hydrocarbons (16.23%) and sesquiterpene hydrocarbons (2.44%). The major components of MpEO are menthol (33.59%) and iso-menthone (33.00%). In lower amounts we found a variety of compounds including limonene (8.00%), piperitone (3.20%), 1,8-cineole (2.80%), linalool (2.64%), iso-pulegol (2.40%), caryophyllene (1.95%) and pulegone (1.60%).

**Essential oil antioxidant activity**

The antioxidant activity of MpEO was compared to that of silymarin, a well-known antioxidant, using two different assays, namely DPPH and superoxide oxygen radicals inhibition, the results are reported in Table 2. DPPH showed for MpEO an IC50 value around 3 times higher than the one recorded for silymarin indicating that antioxidant activity of MpEO was lower than that of silymarin.

**Serum biochemical parameters**

The results of biochemical indicators of liver and kidney function are summarized in Tables 3, 4 and 5. The administration of CCl4 caused severe hepato and reno-toxicity in the treated rats, as evidenced by the significant elevations of serum ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL urea and creatinine levels, while HDL level was decreased compared to control animals.

Pretreatment with the MpEO at doses of 15 or 40 mg/kg significantly reduced levels of ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL urea and creatinine and increased the level of HDL compared to the CCl4 group. It is worth noting that the treatment with 5 mg/kg MpEO did not induce any significant changes in the biochemical parameters (ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL, urea, creatinine or HDL) when compared to the CCl4 group. Treatment of rats with only MpEO (40 mg/kg BW) did not result in significant alteration in biochemical parameters compared to control rats. Pretreatment with silymarin (50 mg/kg), used as positive control, significantly decreased the elevated levels of ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL, urea, creatinine or HDL when compared to the CCl4 group. Treatment of rats with only MpEO (40 mg/kg BW) did not result in significant alterations in biochemical parameters compared to control rats. Pretreatment with silymarin (50 mg/kg), used as positive control, significantly decreased the elevated levels of ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL urea and creatinine and increased of HDL level as compared to CCl4 group. Its effect was comparable in reducing of liver and kidney damage induced by CCl4 with that observed for the highest dose of MpEO (40 mg/kg).

**Effects on lipid peroxidation**

TBARS level is widely used as a marker for free radical mediated lipid peroxidation injury. We determined TBARS levels in the liver and kidney tissues of the investigated animals and our results are shown in Fig. 1a, b. The levels of TBARS were significantly increased in both liver and kidney tissues of CCl4-treated animals when compared to control untreated rats.

Pre-treatment with the MpEO at doses of 15 and 40 mg/kg BW significantly reduced levels of TBARS in liver and kidney tissues as compared to CCl4 group. There was a dose effect; treatment with MpEO at 5 mg/kg BW did not induce any significant decrease in the levels of TBARS in liver and kidney as compared to CCl4 group. When rats were treated with only MpEO (40 mg/kg BW), no significant differences in the TBARS values was observed compared to control rat. Pretreatment with silymarin (50 mg/kg) significantly decreased the elevated levels of TBARS in both liver and kidney compared to CCl4 control. Moreover, the effect of silymarin (50 mg/kg) in attenuation of TBARS levels in liver and kidney was comparable with highest dose of MpEO (40 mg/kg).

**Effects on antioxidant enzymes**

Results presented in Tables 6 and 7 showed a significant decrease in the levels of CAT, SOD, GPx in liver and kidney tissues of rats treated with MpEO and CCl4 compared to control rats. These results indicated a significant increase in the activities of antioxidant enzymes in the liver and kidney tissues of CCl4-treated rats, as evidenced by the significant elevations of CAT, SOD, GPx compared to the CCl4 group. Treatment with MpEO at doses of 15 or 40 mg/kg BW significantly reduced levels of TBARS in liver and kidney tissues of CCl4-treated animals when compared to control rats.

**Table 6** Effects of CCl4, MpEO and their combination MpEO/CCl4 on the activities of enzymatic antioxidants in liver of control and experimental rats

| Treatment       | SOD (Units/mg protein) | CAT (μmol H2O2/min/mg protein) | GPx (μmol GSH/min/mg protein) |
|-----------------|------------------------|--------------------------------|-------------------------------|
| Control         | 16.04 ± 0.11           | 14.03 ± 0.29                   | 7.66 ± 0.51                   |
| CCl4            | 13.60 ± 0.50***        | 11.13 ± 0.37***                | 5.25 ± 0.25**                 |
| SL/CCl4         | 15.80 ± 0.50**         | 13.48 ± 0.29***                | 7.41 ± 0.16***                |
| MpEO/CCl4       | 13.92 ± 0.40           | 11.34 ± 0.41                   | 5.41 ± 0.31                   |
| MpEO/CCl4       | 14.98 ± 0.19**         | 12.32 ± 0.32**                 | 6.04 ± 0.17**                 |
| MpEO/CCl4       | 15.65 ± 0.44**         | 13.20 ± 0.19***                | 7.26 ± 0.22***                |
| MpEOc          | 15.83 ± 0.31           | 13.85 ± 0.33                   | 7.45 ± 0.33                   |

*Values are mean ± SEM for ten rats in each group. CCl4, MpEO and MpEO/CCl4 treated groups vs control group; ** p < 0.01, *** p < 0.001, CCl4 group vs MpEO/CCl4 group; * p < 0.05, ** p < 0.01, *** p < 0.001

**Table 7** Effects of CCl4, MpEO and their combination MpEO/CCl4 on the activities of enzymatic antioxidants in kidney of control and experimental rats

| Treatment       | SOD (Units/mg protein) | CAT (μmol H2O2/min/mg protein) | GPx (μmol GSH/min/mg protein) |
|-----------------|------------------------|--------------------------------|-------------------------------|
| Control         | 15.13 ± 0.22           | 12.90 ± 0.15                   | 5.68 ± 0.27                   |
| CCl4            | 12.12 ± 0.50***        | 10.05 ± 0.34***                | 4.06 ± 0.28**                 |
| SL/CCl4         | 14.52 ± 0.35**         | 12.23 ± 0.24***                | 5.56 ± 0.14**                 |
| MpEO/CCl4       | 12.92 ± 0.20           | 10.80 ± 0.22                   | 4.46 ± 0.33                   |
| MpEO/CCl4       | 13.69 ± 0.10*          | 11.02 ± 0.22*                  | 5.10 ± 0.28*                  |
| MpEO/CCl4       | 14.20 ± 0.35**         | 12.04 ± 0.19***                | 5.32 ± 0.06**                 |
| MpEOc          | 14.91 ± 0.21           | 12.62 ± 0.33                   | 5.59 ± 0.27                   |

*Values are mean ± SEM for ten rats in each group. CCl4, MpEO and MpEO/CCl4 treated groups vs control group; ** p < 0.01, *** p < 0.001, CCl4 group vs MpEO/CCl4 group; * p < 0.05, ** p < 0.01, *** p < 0.001
Fig. 2 (See legend on next page.)
kidney in CCl₄-treated group when compared to control group. The decrease in hepatic and kidney CAT, SOD and GPx levels induced by CCl₄ injection were significantly restored (elevated) in the MpEO and silymarin groups, and this effect was more pronounced with the increase of essential oil concentration. Pretreatment with MpEO at doses of 15 and 40 mg/kg significantly increased levels of hepatic and kidney CAT, SOD and GPx as compared to CCl₄ group. It is worth noting that the treatment with MpEO at a dose 5 mg/kg did not induce any significant increase in the levels of hepatic and kidney CAT, SOD and GPx as compared to CCl₄ group. No significant differences in the values were observed in rats treated with MpEO only (40 mg/kg) compared to control rat values. Moreover, the effect of silymarin (50 mg/kg) was comparable in attenuation of levels of hepatic and kidney CAT, SOD and GPx with highest dose of MpEO (40 mg/kg).

**Histopathological findings**

The normal liver architecture was observed in liver histology of control group (Fig. 2a). Large numbers of inflammatory cells such as lymphocytes together with hepatic sinusoidal inflammation, hepatocyte necrosis and devastating liver architecture were observed in the CCl₄ group (Fig. 2b). However, pretreatment with MpEO (40 mg/kg, Fig. 2f) can remarkably ameliorate the histopathological hepatic lesions induced by administration of CCl₄. MpEO 15 mg/kg showed very few inflammatory cells along with prominent nucleolus (Fig. 2e). The highest dose of MpEO (40 mg/kg) and silymarin (50 mg/kg) significantly attenuated the damaged liver depicting marked focal regenerative changes which are illustrated by presence of actively dividing cells with a prominent nucleolus (Fig. 2f). In addition, silymarin at the dose of 50 mg/kg has shown to produce hepatoprotection evidenced by area of regeneration and dark nucleus (Fig. 2c). The histological pattern was almost normal in rats treated with MpEO oil alone. By analyzing the histopathological scoring attributed to the liver tissues it is possible to note that the highest dose of MpEO (40 mg/kg) or silymarin (50 mg/kg) pretreatment just conferred good protection on CCl₄-induced liver damage (Table 8).

Kidney sections of normal histological appearance (Fig. 3a) and the CCl₄ control group showed some nephrotoxic lesions, as evidenced by the glomerular and tubular necrosis (Fig. 3b). However, pretreatment with MpEO (40 mg/kg, Fig. 3f) can remarkably ameliorate the histopathological kidney lesions induced by administration of CCl₄ (Fig. 3f). In addition, silymarin at the dose of 50 mg/kg has shown to produce renoprotection evidenced by amelioration the histopathological kidney lesions induced by injection of CCl₄ (Fig. 3c). The histological pattern in kidney was almost normal in rats treated with MpEO alone. By analyzing the histopathological scoring attributed to the kidney tissues it is possible to note that the highest dose of MpEO (40 mg/kg) or silymarin (50 mg/kg) pretreatment just conferred good protection on CCl₄-induced kidney damage (Table 9).

**Discussion**

Chemical composition of the essential oil obtained from MpEO was determined by GC-MS analysis. The compounds, their percentages as well as the retention indices are listed in Table 1. The essential oil is a complex mixture with 26 compounds representing 98.17% of the total oil composition. The major component of the essential oil is menthol (33.59%) followed by iso-menthone (33.00%). In lower amounts we found a variety of compounds including limonene (8.00%), pipertitone (3.20%), 1,8-cineole (2.80%), linalool (2.64%), iso-pulegol (2.40%), caryophyllene (1.95%) and pulegone (1.60%). The obtained results are in accordance with previous studies of *M. piperita* oils from Turkey, Spain (Barcelona), Norway and Poland that also had menthone and menthol as their most important components [30–32]. On the other hand, the composition of the essential oil from Iran is totally different, with α-terpinene (19.70%), isomenthone (10.30%), trans-carveol (14.50%), pipertitinin oxide (19.30%) and β-caryophyllene (7.60%) as the major compounds, and also the oil from the Girona region (Spain) is different, where limonene and 1,8-

| Table 8 | Grades of inflammatory cells and cellular necrosis in rat liver |
|---------|---------------------------------------------------------------|
| Groups  | Zero | I | II | III | IV | N | P value vs Control | P value vs CCl₄ |
| Control | 10 0 0 0 0 | 10 | - |
| CCl₄   | 0 1 3 3 3 | 10 | P < 0.001 | - |
| SL/CCl₄| 8 2 0 0 0 | 10 | ns | P < 0.001 |
| MpEO/CCl₄ | 0 2 6 1 1 | 10 | P < 0.05 | ns |
| MpEO/SL/CCl₄ | 6 4 0 0 0 | 10 | ns | P < 0.05 |
| MpEO/SL | 7 3 0 0 0 | 10 | ns | P < 0.001 |
| MpEO   | 9 1 0 0 0 | 10 | P < 0.001 |

* MpEO (5 mg/kg BW), ** MpEO (15 mg/kg BW), *** MpEO (40 mg/kg BW), SL: Silymarin (50 mg/kg BW)
cineole, eucalyptol are the main compounds (33.37% and 30.75%, respectively) [33, 34].

These studies showed variable chemotypes of M. piperita L. extracts with various major oil components. Differences in chemical composition observed for essential oils is likely related to abiotic factors such as soil type and climate specific regions of provenance samples and geographical factors [35]. Furthermore, menthol and iso-mentholene, found at relatively high concentrations in the MpEO used in the present study, have been reported to exhibit anti-inflammatory activity [14], making MpEO a promising candidate against oxidative damage of the liver and kidney following an intraperitoneal administration of CCl4. To be noted: working with natural the liver and kidney following an intraperitoneal administration of CCl4. To be noted: working with natural

The liver plays a fundamental role in the metabolism of lipids. Injection of CCl4 caused a significant increase in the triglyceride, total cholesterol, and LDL levels and decrease in HDL level. Increase in the cholesterol levels might be due to the increased esterification of fatty acids, inhibition of fatty acid β-oxidation, and decreased excretion of cellular lipids [41]. CCl4 stimulates the transfer of acetate into liver cells (probably by increasing access to acetate) and leads to an increase in cholesterol synthesis. It also increases the synthesis of fatty acids and triglyceride from acetate and enhances lipid esterification [42]. The accumulation of triglyceride in liver might occur due to the inhibition of lysosomal lipase activity and VLDL secretion [43].

The administration of CCl4 induced also renal toxicity evidenced by an elevation of serum creatinine and urea [44, 45]. These pathological changes can also be attributed to damages touching the structural integrity of nephrons [46], which is consistent with reports confirming that the level of serum creatinine increases only if at least half of the kidney nephrons are already damaged [47].

Treatment of rats with MpEO prior to CCl4 exposure resulted in a dramatically protective effect against acute hepatotoxicity and oxidative stress, which was further also confirmed by the hepatic histopathological examinations. The stimulation of hepatic regeneration makes the liver more resistant to damage by the toxin [48]. Treatment with silymarin (50 mg/kg) or MpEO (40 mg/kg) significantly decreased the elevated levels of ALT, AST, ALP, LDH, γGT, total cholesterol, triglycerides, LDL, urea and creatinine and increased of HDL level as compared to CCl4 group. Pharmacological studies have shown that essential oil derived from various plant materials possesses anti-inflammatory activities [49, 50] knowing that sesquiterpenes have excellent anti-inflammatory activities [51], the anti-inflammatory activity of M. piperita L. leaf essential oil could be partly explained by the presence of sesquiterpenes, such as spathulenol, cadinene,
caryophyllene oxide. The ethanolic extract of parsley leaves also showed significant anti-inflammatory [52] and antioxidant activities [53, 54] which may contribute to its hepatoprotective action. Furthermore, menthol and iso-menthone, found at relatively high concentrations in the MpEO used in the present study, have been reported to exhibit anti-inflammatory activity [14].

In the present study, the two-fold increase in the TBARS levels and reduce activity of SOD, CAT and GPx observed in liver and kidney homogenate of CCl 4-intoxicated rats. Silymarin significantly reversed CCl 4-induced TBARS levels elevation but values obtained with MpEO at the highest dose was comparable in attenuation of TBARS levels in liver and kidney. Silymarin reversed CCl 4-induced SOD, CAT and GPx activities decrease but values obtained with MpEO at the highest dose (40 mg/kg) was comparable in attenuation of SOD, CAT and GPx activities in liver and kidney.

These results suggested that MpEO could exert its antioxidant and/or radical scavenging activities thus preventing the formation of the carbon free radicals originated from CCl 4 metabolism as well as ROS and peroxidation products. This hypothesis is supported by the recent findings on the in vitro antiradical and antioxidative activities of MpEO [16]. Previous studies showed that menthol and its derivatives were the major compounds responsible for antioxidant activity of MpEO [15].

In the present study, the rats of group 2 served as CCl 4-hepato and renotoxicity control and rats of groups 3, 4, 5 and 6 were injected with 1 ml/kg BW of CCl 4 and olive oil mixture on day seven (a single intraperitoneal injection). It has already been shown that a single dose of CCl 4 initiates lipid peroxidation [55–58] that results in the disruption of cellular and organelle membrane integrity and subsequent leakage of cellular contents into the blood [59, 60]. CCl 4 is further well known to induce fibrosis of the hepatic tissue that may further progress to cirrhosis if the stimuli persists [61–65]. Thus, a single CCl 4 injection in mice can be used as an attractive and highly reproducible model of liver regeneration after toxic injury. The first appearance of histological fibrosis and scarring fibers is usually observed after repeated CCl 4 treatment for 2 to 3 weeks, depending on the dosage and mouse strains used [66]. In the present work, the hepatic histoarchitecture of the CCl 4-treated rats resulted large numbers of inflammatory cells such as lymphocytes along with hepatic sinusoidal inflammation, hepatocyte necrosis and devastating liver architecture. The highest dose of MpEO (40 mg/kg) or silymarin (50 mg/kg) significantly attenuated the damaged liver depicting marked focal regenerative changes which are illustrated by presence of actively dividing cells with a prominent nucleolus. The administration of MpEO reducing the histological alterations in liver provoked by CCl 4 was quite noticeable. In fact, the histological changes seen in the kidney of rats treated with CCl 4 were characterized by some nephrotoxic lesions, as evidenced by the glomerular and tubular necrosis. Our results confirmed previous findings of Ozturk et al. [67] who had found degenerative changes in kidney of rats exposed to CCl 4. The results suggest that MpEO treatment prior to CCl 4 intoxication could prevent the CCl 4-induced alterations in kidney tissues of treated animals.

Conclusions

The contents of MpEO not only protect the integrity of plasma membrane but, at the same time, increased the regenerative and reparative capacity of the liver and kidney. These results suggest that the compound present in MpEO has hepatorenal protective effects against CCl 4 induced oxidative stress in rats. Further investigations are essential to elucidate the precise mechanism of active agents of MpEO protection against CCl 4-induced hepatotoxicity and nephrotoxicity and it has to be tested against other biological parameters.

Abbreviations

BHT: Butylated hydroxytoluene; CAT: Catalase; DPPH: 1-Diphenyl-2-picrylhydrazyl; DTNB: 5,5′-Dithio-bis(2-nitrobenzoic acid); EDTA: Ethylenediaminetetraacetic acid; GPX: Glutathione peroxidase; GSH: Glutathione; M. pipreta: Mentha piperita; MpEO: Mentha piperita essential oil; NBT: Nitroblue Tetrazolium; ROS: Reactive oxygen species; SD: Standard deviation; SOD: Superoxide dismutase; TBA: Thiobarbituric Acid; TBARS: Thiobarbituric acid reactive substances; TBS: Tris Buffered Saline; TCA: Trichloroacetic Acid; TRIS: Trishydroxymethyl aminomethane

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included within the article.

Authors' contributions

KB, ABR, KA, JvP, FMA, TR and AE designed and wrote the paper. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Department of Life Sciences, Animal Ecophysiology Laboratory, Faculty of sciences, University of Sfax Tunisia, Road of Soukra Km 3.5, BP 1171, PC 3000 Sfax, Tunisia. 2Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax, PO Box 1177, Road Sidi Mansour km 6, 3018 Sfax, Tunisia. 3Department of life sciences, Laboratory of Biodiversity and Aquatic Ecosystems, Faculty of sciences, University of Sfax Tunisia, Road of Soukra Km 3.5, BP 1171, PC 3000 Sfax, Tunisia. 4Laboratory of Clinical Digestive Oncology, Department of Oncology, KU, Leuven, Belgium. 5Biochemistry Department of Life Sciences, Animal Ecophysiology Laboratory, Faculty of sciences, University of Sfax Tunisia, Road of Soukra Km 3.5, BP 1171, PC 3000 Sfax, Tunisia.
References
1. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr. 2005;135:969–72.
2. Basaga HS. Biochemical aspects of free radicals. Biochem Cell Biol. 1990;68:89–98.
3. ATSDR. "Agency for Toxic Substance and Diseases Registry". Toxicological profile for carbon tetrachloride, U.S. Department of Health and Human Services, Public Health Service, TP-39/02. Atlanta, CA, 1994.
4. Wang CY, Ma FL, Liu JT, Tian JW, Fu FH. Protective effect of salvianic acid A on acute liver injury induced by carbon tetrachloride in rats. Biol Pharm Bull. 2007;30:44–7.
5. Abraham P, Wilfred G, Cathrine SP. Oxidative damage to the lipids and proteins of the lung, testis and kidney of rats during carbon tetrachloride intoxication. Clin Chim Acta. 1999;289:177–90.
6. Liang M, Leibfried D, Morcol J, Cronin MT, Mazur M, Telsner J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
7. Fang YZ, Yang S, Wu G. Free radicals antioxidants and nutrition. Nutrition. 2002;18:872–9.
8. Bansal AK, Bansal M, Soni G, Bhatnagar D. N-nitrosodiethylamine induced oxidative stress in rat liver. Chem Biol Interact. 2005;156:101–11.
9. Shahjahan M, Sabitha KE, Jainu M, Shyamala Devi CS. Effect of Mentha piperita L. extracts against carbon tetrachloride induced liver fibrosis in mice by their antimutagenicity. J Agric Food Chem. 1995;43:27–81.
10. Vinh VM, Uskoković S, Matić S, Vuković V, Lukić M, Matić V, Uskoković S. Hepatoprotective effects of Hedyotis Corymbosa and Alternative Medicine. 2013; https://doi.org/10.1155/2013/569037.
11. Laboratory of Histology and Embryology, Faculty of Medicine of Sfax, Sfax Tunisia, Road Menzel Chaker km 0.5, CP 3029 Sfax, Tunisia.
12. Brown D. Encyclopaedia of herbs and their uses. London, U.K: Dorling Kindersley; 1995.
13. Foster S. Peppermint, Mentha piperita, In Botanical Series, American Botanical Council: Austin, Texas, no 306, 1990.
14. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev.
15. Lee CP, Shih PH, Hsu CL, Yen GC. Hepatoprotection of tea seed oil (Camellia oleifera Abel). Food Chem Toxicol. 2004;42:665–71.
16. Brown D. Encyclopedia of herbs and their uses. London, U.K: Dorling Kindersley; 1995.
17. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem. 1995;43:27–52.
18. Council of European Communities. Council instructions about the protection of living animals used in scientific investigations. Off J Euro Comm. 2010; 276:1-33.
19. Hlavatý M, Viklický P, Albritton R, Kim J, Macean J, Eck J, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr. 2005;135:969–72.
20. Bruckner JV, Mackenzie WF, Muralidhara S, Luthra R, Kyle M, Acosta D. Oral toxicity of carbon tetrachloride: acute, subacute and subchronic studies in rats. Fundam Appl Toxicol. 1993;21:225–30.
21. Pardini P, Madsen C. Subchronic toxicity study of peppermint oil in rats. Toxicol letter. 1992;62:215–20.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem. 1951;193:265–75.
23. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;241:2–31.
24. Aebi H. Catalase in vitro. Methods Enzymol. 1984;21:121–6.
25. Beyer JW, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem. 1987;135:59–66.
26. Schuegerl K, Gündüz WA. Assays for glutathione peroxidase. Methods Enzymol. 1984;105:114–21.
27. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol. 1991;13:372–4.
28. Kim NS, Lee DS. Comparison of different extraction methods for the analysis of fragrances from Lavandula species by gas chromatography-mass spectrometry. J Chromatogr. 2002;22:31–47.
29. Rohloff J, Dragland S, Mordal R, Iversen TH. Effect of harvest time and drying method on biomass production, essential oil yield, and quality of peppermint (Mentha piperita L.). J Agric Food Chem. 2005;53:4143–8.
30. Skalikova-Wozniak K, Walasek M. Preparative separation of menthol and pulegone from peppermint (Mentha piperita L) by high-performance counter-current chromatography. Phytochem Lett. 2014;22:97; https://doi.org/10.1016/j.phytol.2014.06.007.
31. Yadegariatina G, Gachkar L, Rezaei MB, Taghizadeh M, Alipoor-Astaneh S, Rasouli J. Biochemical activities of Iranian Mentha piperita L. and Myrtus communis L. essential oils. Phytochemistry. 2006;60:1249–55.
32. Ruiz Del Castillo ML, Blanch GP, Herrera M. Natural variability of the enantiomeric composition of bioactive chiral terpenes in Mentha piperita. J Chromatogr A. 2004;1054:87–93.
33. Orav A, Raal A, Aarak E. Comparative chemical composition of the essential oil of Mentha piperita L. from various geographical sources. Proc Estonian Acad Sci. 2004;53:174–81.
34. Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem. 2001;75:197–202.
35. Singh G, Mirmuthu P, De Heluanis CS, Catalanzan AN. Antioxidant and biocidal activities of Curcum nigrum (seed) essential oil, oleocin, and their selected components. J Agric Food Chem. 2006;54:74–81.
36. Pradeep K, Victor Raj Mohan C, Gobianand K, Karthikeyan S. Protective effect of Cissus fistula Linn. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biol Res. 2010;43:113–25.
37. Botsoglou NA, Tzitzoglou 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 AgricFood Chem. 2008;56:2687–93.
38. Zargar RC, Benson JM, Burnett VL, Springer DL. Cytochrome P4502E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes. Chem Biol Interact. 2000;125:23–43.
39. Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. J Nutr. 2005;135:2075–8.
40. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of halothalanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol. 2003;33: https://doi.org/10.1081/JTR-120017963.
41. Marimuthu S, Adluri RS, Rajagopalan R, Menon VP. Protective role of ferulic acid on carbon tetrachloride-induced hyperlipidemia and histological alterations in experimental rats. J Basic Clin Physiol Pharmacol. 2013;24:59–66. https://doi.org/10.1515/bcpp-2012-0053.
42. Abdul Moniem AE, El-Deib KM. The possible protective effects of Physalis peruviana on carbon tetrachloride-induced nephrotoxicity in male albino rats. Life Sci J. 2012;9:1038–52.
43. Al-Ja'yiha M, Mothana R, Al-Said M, Al-Doobi M, Al-Muaybdeh M, Al-Sohabani M, Parvez MK, Rafaullah S. Attenuation of CCl4-Induced Oxidative Stress and Hepatotoxicity by Saudi Sdr Honey in Rats, Evidence-Based Complementary and Alternative Medicine. 2013; https://doi.org/10.1155/2013/569037.
44. Khan RA, Khan NR, Saheen S, Bokhari J. Prevention of CCl4-induced nephrotoxicity with Sonchus Asper in rat. Food Chem Toxicol. 2010;48:2469–76.
45. Bhattacharya H, Lun L, Gomez R. Biochemical Effects to Toxicity of CCl4 on Rosy Barbs (Puntius conchonius). Our Nat. 2005;23:20–25.
46. Sadasivan S, Latha PG, Sasikumar JM, Rajasekaran S, Shyamal S, Shine VJ. Hepatoprotective studies on Hedysites Cymbrosia (L.) Lern. J Ethnopharmacol. 2006;105:25–49.
47. Martins FT, Dongietto AC, De Souza TC, De Souza KR, Moreira MB, Barbosa LC. Composition, and Anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of Garcinia brasiliensis. Chem Biodivers. 2008;5:251–8.
48. Kim JY, Oh TH, Kim BJ, Kim KS, Lee NH, Hyun CG. Chemical composition and anti-inflammatory effects of essential oil from Farfugium japonicum flower. J Oleo Sci. 2008;56:223–8.
51. Liu X, Sun Z, Zhang M, Meng X, Xia X, Yue F, Liu C. Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber Apostichopus japonicas. Carbohydr Polym. 2012;90:1664–70.

52. Al-Howiriny T, Al-Sohaibani M, El-Tahir K, Rafatullah S. Prevention of experimentally-induced gastric ulcers in rats by an ethanolic extract of "parsley" Petroselinum crispum. Am J Chin Med. 2003;31:699–711.

53. Wong PYY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. Food Chem. 2006;97:505–15.

54. Zhang H, Chen F, Wang X, Yao HY. Evaluation of antioxidant activity of parsley (Petroselinum crispum) essential oil and identification of its antioxidant constituents. Food Res Int. 2006;39:833–9.

55. Haefeman DG, Hoekstra WG. Protection against carbon tetrachloride-induced lipid peroxidation in the rat by dietary vitamin E, selenium, and methionine as measured by ethane evolution. J Nutr. 1977;107:656–65.

56. Lee PY, Mccay PB, Hombrook KR. Evidence for carbon tetrachloride-induced lipid peroxidation in mouse liver. Biochem Pharmacol. 1982;31:405–9.

57. Burk RF, Lane JM, Patel K. Relationship of oxygen and glutathione in protection against carbon tetrachloride-induced hepatic microsomal lipid peroxidation and covalent binding in the rat. Rationale for the use of hyperbaric oxygen to treat carbon tetrachloride ingestion. J Clin Invest. 1984;74:1996–2001.

58. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther. 1989;43:139–54.

59. Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 1983;33:401–8.

60. Brattin WJ, Glende EA, Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. J Free Radic Biol Med. 1985;127–38.

61. Post J, Earle DP, Patek AJ, Victor J. Effects of yeast and food intake on experimental carbon tetrachloride cirrhosis of the liver in the rat. Am J Pathol. 1942;18:661–73.

62. Constandinou C, Henderson N, Iredale JP. Modeling liver fibrosis in rodents. Methods Mol Med. 2005;117:237–50.

63. Smyth R, Munday MR, York MJ, Clarke CJ, Dare T, Turton JA. Comprehensive characterization of serum clinical chemistry parameters and the identification of urinary superoxide dismutase in a carbon tetrachloride-induced model of hepatic fibrosis in the female Hanover Wistar rat. Int J Exp Pathol. 2007;88:361–76.

64. Smyth R, Lane CS, Ashiq R, Turton JA, Clarke CJ, Dare TO, York MJ, Griffiths W, Munday MR. Proteomic investigation of urinary markers of carbon-tetrachloride-induced hepatic fibrosis in the Hanover Wistar rat. Cell Biol Toxicol. 2009;25:499–512.

65. Fuji T, Fuchs BC, Yamada S, Lauwers GY, Kulu Y, Goodwin JM, Lanuti M, TANABE KK. Mouse model of carbon tetrachloride induced liver fibrosis. Histopathological changes and expression of CD133 and epidermal growth factor. BMC Gastroenterol. 2010;10:79.

66. Liedtke C, Luedde T, Sauerbruch T, Scholten D, Streetz K, Tacke F, Tolba R, Trautwein C, Trebicka J, Weiskirchen R. Experimental liver fibrosis research: update on animal models, legal issues and translational aspects. Fibrogenesis Tissue Repair. 2013:6:19.

67. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology. 2003;62:353–6.