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Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats

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Abstract In the present study, the protective effect of peppermint (*Mentha piperita*) and parsley (*Petroselinum crispum*) leaves oils against hepatotoxicity is induced by carbon tetrachloride (CCl_4) in experimental rats. GC/MS results indicated that the main components in peppermint oil were menthol (35.9%) and menthone (25.6%), while in parsley oil were α-Pinene (26.6%) and Myristicin (20.3%). Hepatotoxicity by CCl_4 resulted in significant elevation of serum triglycerides, total cholesterol, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) and decreasing in serum high density lipoprotein (HDL-C). Moreover, kidney function tests for serum urea nitrogen, creatinine, and uric acid were found to be increased. Administration of 0.5 ml of each peppermint, parsley and their mixture oils attenuated the adverse effects and biochemical alterations caused by CCl_4 especially, at 0.5 ml of peppermint oil. CCl_4 caused significant increase in liver lipid peroxidation malondialdehyde (MDA) and significant decrease in liver antioxidant enzymes activity as superoxide dismutase (SOD) and glutathione (GSH). Peppermint, parsley and their mixture oils have strong radical scavenging activity and antioxidant activity specially, at 0.5 ml of peppermint oil that reversed these negative changes by significant increase in the activity of SOD, GSH and decreasing in MDA. Therefore, the results of this study show that peppermint, parsley and their mixture oils led to the protective effect against CCl_4 hepatotoxicity specially, peppermint oil. The results also revealed that the hepatoprotective effect of peppermint and parsley oil may be attributed to its antioxidant content and free radical scavenger effects.

Introduction

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury and death (Zheng and Storz, 2000). For example, reactive oxygen species (ROS) markedly alter the physical, chemical, and immunological properties of superoxide dismutase (SOD), which further exacerbates oxidative damage in cells. Although there are some

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synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), these compounds are associated with some side effects (Ito et al., 1983).

Many studies have been shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis (Reddy et al., 2003). Therefore, there has been a growing interest in research concerning alternative antioxidant active compounds, including plant extracts and essential oils that are relatively less damaging to the mammalian health and environment. Essential oils are known to possess multifunctional properties other than their classical roles as natural food additives and/or fragrances. Besides the antibacterial, antifungal, and anti-inflammatory activities (Bendimerad et al., 2005), many essential oils also have been confirmed to possess the antioxidant activity (Sokmen et al., 2005).

Peppermint (*Mentha piperita* L.), a perennial aromatic herb belonging to the Lamiaceae (Labiatae) family, is a natural hybrid between spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatica* L.). Dried peppermint leaves were found in the Egyptian pyramids, showing that the use of peppermint may date back to at least 1000 BC (Spirling and Daniels, 2001). The plant is widely used in folk remedies and alternative medical therapy for treatment of digestive disorders and nervous system actions because of its antitumor and antimicrobial activities, chemopreventive potential, its renal actions, antiallergenic effects, and also for lessening cramping, digestive complaints, anorexia, nausea and diarrhea (Keifer et al., 2007). The plant is cultivated mainly for its essential oil, which is obtained by steam distillation from the aerial parts (Yazdani et al., 2002). Furthermore, peppermint oil is used as a raw material in toothpowder, toothpaste, chewing tobacco, mouth fresheners, perfumes, analgesic balms, confectionary, cough drops, chewing gums, candies and the tobacco industry (Gupta, 1991). The essential oil is mostly made up of menthol (50%), menthone (10–30%), menthyl esters (up to 10%) and further monoterpene derivatives (pulegone, piperitone, menthofurane) (Saeidnia et al., 2005). Among the natural compounds, phenolic compounds, such as flavonoids, are one of the major groups of herbal compounds acting as antioxidants (Pietta, 1998). Studies revealed that peppermint had a good antioxidant activity (Atanassova et al., 2011).

Parsley, *Petroselinum crispum* (Mill.) Nym. syn. *P. sativum* Hoffm., family Apiaceae, a medicinal and food plant, is known for its aromatic leaves and roots. The essential oil is present in all parts of the plant. Leaf oil of the finest quality has a flavor that resembles the fresh herb and can only be obtained in low yield. Usually commercial essential oil is derived from mature seeds (fruits) and has a distinctly different flavor. It is used as a flavoring agent in food products or fragrance in perfumery and cosmetics, as stated in many patents. Antimicrobial, diuretic and weak antioxidant activities of parsley essential oil have been reported (Teissedre and Waterhouse, 2000). Myristicin from parsley oil is a potential cancer chemoprotective agent (Benevides et al., 1999). It was found that the leaf parsley seed essential oil was rich in Myristicin, while in root parsley seed oil apiol was a predominant compound (Lamarti and Bouriquet, 1991). The composition of the oil is influenced by genotype, environmental conditions and cultural systems. The antioxidative effect is mainly due to phenolic components, such as flavonoids. Antioxidants act as radical scavengers, inhibiting lipid peroxidation and oxidation processes and protect the human body from several diseases attributed to the reactions of radicals (Kurowska and Gałązka, 2006). Therefore, the present investigation was undertaken to evaluate the hepatoprotective effects of peppermint and parsley leaves oil on CCl_4-induced liver damage in rats.

Materials and methods

Materials

Plant materials and extraction of essential oils

Peppermint (*M. piperita*) and parsley (*P. crispum*) leaves were obtained from agricultural research center, Giza, Egypt. The dried samples were grounded and subjected to hydro distillation by Clevenger type apparatus for 3 h. (Mahboubi and Kazempour, 2014). The essential oils were separated and dried by sodium disulfate. The essential oils were kept in a dark vials at a cold place until analysis.

Carbon tetrachloride (CCL_4)

Carbon tetrachloride (CCl_4) was obtained from El-Gomhoryia Company for Chemical Industries, Cairo, Egypt. It was sold as a toxic chemical material for liver poisoning according to Passmore and Eastwood (1986). Paraffin oil was obtained from the pharmacy for dilution during the induction.

Methods

Gas Chromatography GC/GC–MS analysis of essential oils

Essential oils from peppermint and parsley leaves were analyzed using GC–FID and GC/MS. The GC apparatus was Agilent technology (HP) 6890 system with capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3 °C/min, and maintained for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230 °C, respectively. GC/MS analysis was conducted on a HP 6890 GC system coupled with a 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those stored in the Wiley 7n.1 mass computer library, NIST (National Institute of Standards and Technology) and with data published in the literature (Adams, 2001).

Experimental animals and diet

Thirty-six male rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 165 ± 5 g. The animals were kept under observation in a well-ventilated cage under day light and hygienic condition for five days before experiment and fed on standard diet and water ad libitum. The standard diet comprised of casein (200 g/kg), corn starch (497 g/kg), sucrose (100 g/kg), cellulose (30 g/kg), corn oil (50 g/kg), mineral mixture (100 g/kg), vitamin mixture (20 g/kg)

and DL-methionine (3 g/kg). The standard diet was performed according to Nelson (2000).

Experimental design

The experiment was performed in Animal House in the Institute of pathology, Giza. All rats were fed for one week on basal diet before starting the experiment, then divided into two main groups, the first group ($n = 6$ rats) was not injected with CCl_4 but fed on the basal diet only as a negative control (C −ve) normal rats. The rats of second main group ($n = 24$ rats) were injected by 0.5 ml/rat CCl_4 in paraffin oil 50% (3 ml/kg of body weight) administered by back subcutaneous injection twice a week for two weeks to induce hepatointoxication, according to Jayasekhar et al. (1997). After the injection of CCl_4, blood samples were obtained by hepatic portal vein method to ensure occurrence of liver injury and to estimate liver function then divided into 4 groups (each 6 rats) as follows:

Group (1): Normal rats feed on basal diet control (−ve) group.
Group (2): Hepatointoxicated rats kept without any treatment as a positive control (+ve) group and fed on basal diet.
Group (3): Injected rats with by CCl_4 were fed on basal diet containing 0.5 ml Parsley oil.
Group (4): Injected rats with by CCl_4 were fed on basal diet containing 0.5 ml peppermint oil.
Group (5): Injected rats with by CCl_4 were fed on basal diet containing 0.5 ml Parsley oil and 0.5 ml peppermint oil (mixture).

Blood sampling

At the end of the experiment period (28 days), rats were sacrificed after overnight fasting under ether anesthesia. Blood samples were collected from hepatic portal vein in a dry clean centrifuge tube. They were left to clot by standing at room temperature for 15 min, and then centrifuged at 3000 rpm for 10 min. Serum was carefully aspirated and transferred into clean quite fit plastic tubes and kept frozen at −20 °C until the time of analysis. Body weight gain was recorded through the experiment (28 day) by recorded initial and final body weight according to Chapman et al. (1959).

Biochemical analysis

Determination of liver functions

Serum alanine and aspartate aminotransferases (ALT & AST), alkaline phosphates (ALP) enzymes activity, total protein and albumin, were estimated according to Reitman and Frankel (1957), Kind and King (1954), Weichselbaum (1946) and Bartholomev and Delany (1966), respectively. Serum globulin (G) value was determined by subtracting the albumin from the total proteins according to Coles (1974). Serum gamma glutamyl transferase (GGT) was estimated according to Gowenlock et al. (1988). Serum total bilirubin was determined colorimetrically as described by Doumas et al. (1973).

Determination of kidney functions

Serum creatinine was determined according to the method described by (Bohmer, 1971). Serum uric acid was determined according to the method described by (Fossati and Prencipe, 1982). Serum urea in plasma was determined according to the enzymatic method of Patton and Crouch (1977).

Determination of liver antioxidant parameters

Livers of rats were rapidly removed and parts of them perfuse with 50–100 of ice cold 0.9% NaCL solution for estimation of superoxide dismutase (SOD) activity, glutathione (GSH), malondialdehyde (MDA) according to Nishikimi et al. (1972), Beuchamp and Fridovich (1971) and Habig et al. (1974) respectively.

Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test, and $p < 0.05$ was used to indicate significance between different groups (Snedecor and Cochran, 1980).

Results and discussion

Chemical composition of peppermint and parsley leaves oils

As shown in Table 1, the major components of peppermint essential oil were menthol (35.6%), and menthone (25.9%). In addition, we also found some other components of peppermint essential oil, including 1,8-Cineol, menthylacetate, caryophyllene. Many articles reported menthol as the main component of peppermint oil (Iscan et al., 2002; Behnam et al., 2005; Soković et al., 2009; Hussain et al., 2010) and menthone and limonene were the second component of peppermint oil from the Iscan et al. (2002) and Hussain et al. (2010), but one report showed β-terpinene, piperitone oxide as the main components of peppermint oil (Yadegarinia et al., 2006). Therefore menthol is not always the primary component of peppermint oil. The chemical composition of peppermint oil from this study is comparable to Iscan et al. (2002). Menthol and menthone are the main components of the oil. For the parsley essential oil, the major components were α-Pinene (26.6%), Myristicin (20.3 %), apiole (13.2%), as shown in Table 1. Although, in general, the major constituent in parsley essential oil was 1,3,8-p-menthatriene, followed by b-phellandrene, Myristicin, apiol and myrcene (Simon and Quinn, 1988), we did not find 1,3,8-pmenthatriene and myrcene in our analysis. This may be due to the plant's genetic base and development and environmental conditions.

Effect of peppermint and parsley leaves oils treatment on body weight and weight gain in CCL_4 induced hepatic damage in rats

The effect of feeding peppermint and parsley leave oils on body and weight gain in CCl_4 induced hepatic damage in rats

Table 1 Gas Chromatography (GC/GC–MS) analysis of the peppermint and parsley leaves oils.

Peppermint oil		Parsley oil	
Compound	Content (%)	Compound	Content (%)
1,8-Cineol	4.6	α-Pinene	26.6
Menthone	25.9	β-Pinene	10.5
Menthol	35.6	Myristicin	20.3
Menthylacetate	3.55	1-Allyl-2,3,4,5-tetramethoxybenzene	11.6
Caryophyllene	2.17	Apiole	13.2

is presented in Table 2. Obtained results showed that control (+ve) rat group showed a significant decrease in final body weight and body weight gain while the treatment with peppermint and parsley leaves oils, CCl_4 groups showed significant increase in these parameters compared with control (+ve) group, and non-significant difference compared with control (−ve) group.

Effect of peppermint and parsley leaves oils treatment on liver parameters in CCL_4 induced hepatic damage in rats

The serum biochemical parameters are considered the internal mirror that reflects the actual effect of both of the CCl_4 toxin and peppermint and parsley leaves oils as its counter. The measured biochemical parameters were as follows: ALT, AST, ALP, and GGT. The results indicated that the injection of rats with CCl_4 was able to induce hepatotoxicity as shown in Table 3. The activity levels of ALT, AST ALP, and GGT liver enzymes were significantly increased in CCl_4 group when compared with the control group ($p < 0.05$) group. The effect of CCl_4 mediated acute toxicity was increased permeability of the hepatocyte membrane and cellular leakage (Paduraru et al., 1996).

These results are in agreement with Leelaprakash et al. (2011), who reported statistically significant increase in plasma activities of ALT, AST, ALP, and GGT in CCl_4 induced animals. It was observed in this study that the feeding of rats with peppermint and parsley leaves oils was able to reduce and sometimes completely remove the toxic effect of CCl_4. Some parameters including ALT and AST were significantly decreased ($p < 0.05$) in the group injected and fed with CCl_4 and peppermint or parsley leaves oils compared with CCl_4 group. Frank et al. (2012) reported that CCl_4 produces oxidative damages and increased AST, ALT, ALP, total bilirubin levels and decrease in total protein. Therefore, the reduction in serum levels of AST, ALT, ALP by treatment of peppermint and parsley leaves oils is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This effect shows that serum levels of transaminases return to normal with the healing of hepatocytes (Ozturk et al., 2012).

Effect of peppermint and parsley leaves oils on Kidneys function in CCL_4 induced hepatic damage in rats

Table 4 showed that all kidneys function tests were elevated by CCl_4 administration. Urea level was found to be significantly lowered by peppermint and parsley leaves oils treatment. The best result was at mixture oils followed by parsley oil. Also, creatinine and uric acid levels were found to be significantly lowered by peppermint and parsley leaves oils supplementation with the same trend that recorded insignificant in these parameters. Table 4 showed also that, control (+ve) rats group showed a significant increase in serum bilirubin and significant decrease in serum total protein and albumin ($p > 0.05$) in comparison with control (−ve) group. Peppermint, parsley and their mixture leaves oils rat groups showed significant increase in serum bilirubin total protein, and albumin.

The protein and amino-acid metabolism of liver is characterized by production and breakdown of proteins, production and breakdown of amino acids as well as regulation of their concentrations in the blood, and detoxification of ammonium via synthesis of urea (excretory form) and glutamine (non-toxic transport or storage form) with simultaneous regulation of the acid–base balance (Gjøen et al., 1987). The hypoproteinemia is mostly associated with a reduction in albumin synthesis as found in liver disease or with excessive loss. The alteration in serum albumin is found more commonly in chronic liver disease (Yokogawa et al., 2006).

Parsley has been used for the treatment of inflammatory condition, liver diseases, constipation, flatulence, jaundice, colic pain, and rheumatism (David Hoffmann, 2010). Ethanolic extract of parsley leaves has been pharmacologically investigated for its hepatoprotective activity (Al-Howiriny et al., 2003). The extract dose dependently attenuated CCl_4 Induced increase in serum AST, ALT, ALP, and total bilirubin. The ethanolic extract of parsley leaves also showed significant anti-inflammatory (Al-Howiriny et al., 2003) and antioxidant (Wong and Kitts, 2006; Zhang et al., 2006) activities which may contribute to its hepatoprotective action. Phytochemical studies on peppermint and parsley leaves oil have showed the presence of menthol (35.9%) and menthone (25.6%), while in parsley oil were α-Pinene (26.6%) and Myristicin (20.3%).

Table 2 Effect of feeding peppermint and parsley leaves oils on Initial body weight, final body weight and body weight gain (BWG) on the experimental rats.

Groups	Initial body weight (g)	Final body weight (g)	BWG (g)
Control (−ve)	165.60 ± 18.34[a]	313.20 ± 37.62[a]	147.80 ± 11.66[a]
Control (+ve)	165.40 ± 34.90[a]	218.40 ± 9.03[c]	52.80 ± 9.76[c]
Peppermint Oils (PO)	165.00 ± 24.70[a]	304.60 ± 34.89[ab]	139.40 ± 18.01[ab]
Parsley Oils (MO)	165.60 ± 22.79[a]	310.40 ± 17.45[a]	145.40 ± 16.06[a]
MO + PO	165.60 ± 16.07[a]	293.20 ± 17.71[b]	127.60 ± 12.92[b]

Numbers followed by the same letter in the same column do not differ significantly by Duncan's multiple range test ($p > 0.05$).

Table 3 Effect of feeding peppermint and parsley leaves oils on liver parameters in CCl_4 induced hepatic damage on the experimental rats.

Groups	Alanine Transaminase ALT (IU/L)	Aspartate Transaminase AST (IU/L)	Alkaline Phosphotase ALP (IU/L)	Gamma Glutamyl Transferase GGT (IU/L)
Control (−ve)	75.10 ± 7.16^{d}	76.60 ± 11.48^{d}	103.10 ± 27.60^{d}	9.04 ± 1.13^{c}
Control (+ve)	125.85 ± 19.79^{a}	123.30 ± 17.89^{a}	155.20 ± 17.12^{a}	13.61 ± 2.19^{a}
Peppermint Oils (PO)	99.80 ± 10.50^{bc}	99.10 ± 9.92^{b}	120.0 ± 10.42^{c}	11.41 ± 1.35^{b}
Parsley Oils (MO)	102.20 ± 9.91^{b}	102.0 ± 10.98^{b}	132.60 ± 11.04^{b}	12.71 ± 1.59^{ab}
MO + PO	95.20 ± 7.90^{bc}	90.40 ± 6.50^{c}	126.20 ± 8.50^{bc}	11.42 ± 2.35^{b}

Numbers followed by the same letter in the same column do not differ significantly by Duncan's multiple range test ($p > 0.05$).

Table 4 Effect of feeding peppermint and parsley leaves oils on kidney function in CCl_4 induced hepatic damage on the experimental rats.

Groups	Urea (mmol/L)	Creatinine (mmol/L)	Uric acid (mmol/L)	Bilirubin (μmol/L)	T. protein (g/L)	Albumin (mmol/L)
Control (−ve)	4.60 ± 0.30^{d}	39.67 ± 3.67^{d}	0.23 ± 0.02^{e}	9.33 ± 0.85^{d}	81.33 ± 3.33^{a}	51.20 ± 2.14^{a}
Control (+ve)	7.87 ± 0.33^{a}	57.33 ± 4.33^{a}	1.12 ± 0.02a	21.67 ± 1.67^{a}	52.66 ± 2.11^{c}	46.41 ± 2.07^{b}
Peppermint Oils (PO)	6.27 ± 0.13^{b}	44.67 ± 2.03^{b}	0.49 ± 0.03^{c}	11.00 ± 1.00^{b}	67.00 ± 3.08^{b}	50.39 ± 3.08^{a}
Parsley Oils (MO)	6.33 ± 0.55^{b}	48.17 ± 3.53^{b}	0.76 ± 0.02^{b}	11.66 ± 1.53^{b}	65.67 ± 4.88^{b}	50.43 ± 1.07^{a}
MO + PO	5.89 ± 0.35^{c}	41.00 ± 2.08^{c}	0.33 ± 0.02^{d}	10.80 ± 0.58^{c}	69.67 ± 3.85^{b}	50.20 ± 3.17^{a}

Numbers followed by the same letter in the same column do not differ significantly by Duncan's multiple range test ($p > 0.05$).

Also flavones glycosides, apigenin-7-O-glucoside or cosmosiin, apigenin-7-O-apiosyl-O-glucoside/apiin, and the coumarin 2,3-dihydroxy furanocoumarin/oxypeucedaninhydrate (Al-Howiriny et al., 2003; Chaves et al., 2011).

The data in Table 5 demonstrated that, control (+ve) rat group showed significant decrease in the values of liver GSH and GPX were ($p < 0.05$) and significant increase in the values of liver MDA ($p < 0.05$) compared with control (−ve) group. The peppermint, parsley and their mixture leaves oils rats groups showed significant increase in the values of GSH and GPX, while was and significantly decrease in the values of liver MDA in comparison with control (+ve) group.

Results in Table 5 revealed that exposure of rats to CCl_4 resulted in depletion of antioxidant activities. In consonance with our results, Szymonik-Lesiuk et al. (2003) reported that CCl_4 intoxication leads to changes in antioxidant enzymes and reactive intermediates involved in the bioactivation of CCl_4 that may truss to those enzymes to prevent their inactivation. Furthermore, our results correspond with (Khan et al., 2009), and are in agreement with an investigation following CCl_4 intoxication (Manna et al., 2006).

Glutathione provides a first line of defense and scavenges free radical oxygen species (ROS). The decreased concentration of GSH in liver may be due to NADPH reduction or GSH utilization in the exclusion of peroxides (Yadav et al., 1997). GSH-dependent enzymes offer a second line of protection as they primarily detoxify noxious by-products generated by ROS and help to avert dissemination of free radicals (Gumieniczek, 2005). GSH-Px detoxifies peroxides by reacting with GSH and converting it into GSSG, which is reduced to GSH by GSR (Maritim et al., 2003). Our study revealed that CCl_4 treatment in rats markedly changed the activity of antioxidant enzymes, which was reverted by the administration of peppermint, parsley and their mixture leaves oils. The chemical components of peppermint and parsley are rich in phenolic compounds, such as mono- and dicaffeoylquinic acids and flavonoids, which have been extracted, isolated and identified as major chemical components (Wang et al., 2003). The observed

Table 5 Effect of feeding peppermint and parsley leaves oils on superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA), on the experimental rats.

Groups	SOD (U/mg)	GPX (μ/mg)	MDA (nmol/g)
Control (−ve)	142.60 ± 5.30^{a}	121.33 ± 17.13^{a}	6.23 ± 0.02^{d}
Control (+ve)	102.87 ± 5.33^{d}	78.14 ± 4.19^{d}	11.12 ± 0.02^{a}
Peppermint Oils (PO)	137.27 ± 8.13^{b}	114.38 ± 13.21^{b}	7.49 ± 0.03^{bc}
Parsley Oils (MO)	138.33 ± 7.55^{b}	89.59 ± 7.95^{c}	8.76 ± 0.0^{2b}
MO + PO	131.89 ± 8.35^{bc}	118.41 ± 11.18^{b}	7.33 ± 0.02^{bc}

Numbers followed by the same letter in the same column do not differ significantly by Duncan's multiple range test ($p > 0.05$).

improved endothelial reactivity could be due to the antioxidant contents of peppermint, parsley and their mixture leaves oils.

Conclusion

In the present study pretreatment with peppermint, parsley and their mixture leaves oils showed increased activity of antioxidant enzymes compared to CCl_4 treated animals indicating the potentiality of peppermint, parsley and their mixture leaves oils to act as an antioxidant by preventing the peroxidative damage caused by CCl_4.

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