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Effects of Peppermint (*Mentha piperita* L.) Alcoholic Extract on Carbon Tetrachloride-induced Hepatotoxicity in Broiler Chickens Under Heat Stress Condition

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Abstract

In order to investigate the effects of peppermint (*Mentha piperita* L.) alcoholic extract on liver injury caused by the oxidant carbon tetrachloride (CCl_4), an experiment was performed as a completely randomized design in a factorial arrangement (2 × 2) with 4 replications of 10 broilers each. Factors included two levels of peppermint leaf alcoholic extract (0 and 2 mL/Kg body weight) and CCl_4 (0 and 1 mL/Kg body weight). Results showed significant ($P<0.05$) interactions for body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) on d 42 of the experiment. The alcoholic extract of peppermint leaf did not improve growth performance, whereas CCl_4 worsened BWG and FCR ($P<0.05$). The interaction between peppermint extract and CCl_4 indicated an ameliorative effect of peppermint extract on BWG and FCR ($P<0.05$). The interaction effects between peppermint extract and CCl_4 significantly differed for blood serum concentrations of total protein, albumin, albumin:globulin ratio, glucose, triglyceride, total cholesterol, HDL_C, LDL_C, LDL_C:HDL_C ratio, and $VLDL_C$ as well as the amount of blood liver enzymes ($P<0.05$). Peppermint extract significantly increased blood serum concentrations of total protein, albumin, triglyceride and HDL_C, whilst CCl_4 decreased those concentrations ($P<0.05$). Blood serum concentrations of total cholesterol, LDL_C, LDL_C:HDL_C ratio, $VLDL_C$ and glucose were decreased by peppermint extract, whereas those concentrations were increased by CCl_4 ($P<0.05$). A significantly higher level of liver enzymes was found in blood serum of birds treated by CCl_4 than those by peppermint extract ($P<0.05$). A moderate effect on blood serum liver enzymes was observed by the interaction between 2 mL of peppermint extract and 1 mL of CCl_4 ($P<0.05$). Generally, this study indicated that *in vivo* administration of peppermint alcoholic extract ameliorated the adverse effects of CCl_4 on growth performance and liver function, therefore it might be useful for the prevention of oxidative stress-induced hepatotoxicity in broilers.

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Introduction

Liver is involved in many metabolic functions. Meanwhile, it is the target for a number of toxicants (Meyer and Kulkarni, 2001) and chemically induced injury (Khan and Sultana, 2009), which consequently result in disturbance of hepatic metabolic functions (Wolf, 1999). It is well documented that oxidative stress is the key point results in hepatic damage. Oxidative stress occurs when production of reactive oxygen species (ROS) exceeds from its elimination by antioxidants. At this condition, ROS damages biomolecules (Khan and Sultana, 2009).

Nowadays, poultry industry is challenging with a number of stressors that lead to a lower performance. Heat stress is one of the most challenging environmental conditions affecting commercial poultry, particularly in broiler chickens which are more sensitive than other domestic animal species (Geraert *et al*., 1993; Mashaly *et al*., 2004; Yu *et al*., 2008). Heat stress leads to creation of an imbalance in favor of prooxidants and consequently an increase in ROS generation and inactivity of cellular antioxidant defenses. As a result, heat stress is likely to induce oxidative changes in cells, in particular, hepatic cells (Altan *et al*., 2003; Mahmoud and Edens, 2003).

Unfortunately, hepatic damage due to the oxidative stress is increasingly growing and synthetic drugs used in the prevention and treatment of liver damages and disorders are sometimes with serious side effects (Rao *et al*., 2006). Thus, there is growing interest to the traditional herbal medicines that are claimed to possess hepatoprotective activity with the least side effects. Medicinal plants may serve as a vital source of potentially useful compounds for the development of effective therapy to combat a variety of liver problems. Peppermint (*Mentha piperita* L.), one of these medical plants, belong to the Lamiaceae family. Its essential oils are mainly made up of menthone, menthol and methyl acetate (Murray, 1995). This plant is usually used as an antiseptic, antispasmodic, carminative, mild tonic, antimicrobial and the treatment for irritable bowel syndrome, inflammatory bowel disease, disorders of the biliary system and liver problems (Taylor, 1984; Foster, 1996; Bouchra *et al*. 2003). Taylor (1984) reported that most of the effects of peppermint are related to its effect on bile flow and liver function. Studies have demonstrated that the antioxidant function of peppermint contributes to the prevention and treatment of diseases associated with oxidative stress through scavenging free radicals and neutralizing ferryl ion-induced peroxidation (Sharma *et al*., 2006; Singh and Gupta, 2011). Peppermint has also been documented for compounds like eugenol, caffeic acid, rosmarinic acid, flavonoids and α-tocopherol shaping its antioxidant and anti-peroxidant traits (Rastogi and Mehrotra, 1993). It has been documented that flavonoid compounds exhibit antioxidant and antitumor properties (Knekt *et al*., 2002). These biological functions are attributed to the radical-scavenging properties of flavonoids (Wang and Huang, 2004). Vokovic-Gacic and Simic (1993) reported that peppermint extract can increase the number of hepatic two nuclear cells and intracellular RNA concentration and rate

of restoration of liver cells. These properties of the peppermint are thought to provide many beneficial effects against chronic liver damage and liver fibrosis induced by oxidative stressors like carbon tetrachloride (CCl_4) in chickens. CCl_4 has long been known as a model toxicant and has been the focus of many *in vitro* and *in vivo* toxicological studies (Manibusan *et al*., 2007). Meanwhile, the liver is the major target organ of CCl_4 toxicity owing to its high content of cytochrome P-450 (Södergren *et al*., 2001). It is well established that exposure to CCl_4 results in necrosis and cirrhosis of the liver tissue, which can be shown by hepatic metabolic disturbance and leakage of the liver enzymes into the blood stream (Mujumddar *et al*., 1998). It has been reported that medical plants have a role in the prevention of food poisoning (Sonkusale *et al*., 2011), through removing of hydrogen peroxide (Schaffer *et al*., 2004). One recent study reported that methanol extract of some medical plants has a good level of hepatoprotection function against CCl_4 (Ahsan *et al*., 2009). Due to the antioxidant properties, peppermint has been shown as possessing various antioxidant properties, but there are no enough documents that peppermint inhibits tissue injury due to oxidative stress. In the present study, *in vivo* antioxidant activities of peppermint were assessed against hepatotoxicity induced by CCl_4 in the broiler chickens.

Materials and Methods

Plant preparation and extraction

Peppermint plant used in this experiment was collected in summer when the plant was in vegetative stage, from the research farm (36°00' – 16" north latitude and 59°00' - 36" east longitude; altitude: 985 m) of Ferdowsi University of Mashhad, Mashhad, Razavi Khorasan province, Iran. Collected leaves were shadow dried and ground with a laboratory hammer mill (Iran Khodsaz Gristmill, ELS 300C, Iran). The total values of phenolic compounds, flavonoids and antioxidants were measured colorimetrically, using Folin- Ciocalteu method (Table 1) (Guo *et al*., 2000). In order to prepare peppermint extract, 200 g of dried leaf powder was mixed with 1 L ethanol 80% with the ratio of 2:10. Then it was shaken for 24 hrs to be completely mixed, thereafter passed through a filter paper and the resulting alcohol was removed by distillation under vacuum (Harbone, 1998).

Table 1. Total phenol, flavonoids and antioxidants of peppermint leaf

Compounds	Dry weight
Total phenol	2.719 (mg/g)
Flavonoids	1.835 (mg/g)
Antioxidants	72.531 %

Birds, diets and experimental design

A total of 160 day-old broiler chicks (Ross, 308) were purchased from a local commercial hatchery and raised over a 42-d experimental period. The chickens were housed in an environmentally controlled poultry house with wood shavings as litter at the research farm of Animal Science Faculty, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan province, Iran. The temperature was set at 32°C at 1 d of age and then decreased by 1°C every 2 days until a permanent temperature of 24°C was reached at 35 d of experiment. The heat stress was applied once daily (from 08:00 to 16:00 hr = 8 hrs/d) during the last experimental week by increasing room temperature to reach 34°C. From 18:00 to 08:00 hr, the environmental temperature was reduced to 21°C. The lighting schedule provided 23 hrs of light per day.

A two-phase feeding program was used, with a starter diet until 21 d and a finisher diet from 22 to 42 d of age. The composition of the basal diet is shown in Table 2. Diets were formulated to meet or exceed NRC (1994) recommendations. The experiment was performed as a completely randomized design in a factorial arrangement (2 × 2) with 4 replications of 10 broilers each. Factors included 2 levels of peppermint alcoholic extract (0 and 2 mL/Kg body weight) and CCl_4 (0 and 1 mL/Kg body weight). Treatments were applied from 22 d of experiment. Peppermint extract was fed directly into the chicken crop via syringe equipped with a plastic nozzle and feeding tube (Nova Cath®, No. 10). Intraperitoneal injection of CCl_4 was performed from day 22nd, every three days (Sonkusale *et al*., 2011). In order to homogenize the stress for the control group, 1 mL/Kg body weight sodium chloride 0.9% solution was injected intraperitoneally (Sharma *et al*., 2006) and also 2 mL/Kg body weight of distilled water was fed by oral gavage. Birds had free access to feed and water throughout the experiment. All experimental protocols were approved by the Animal Care and Use Committee of the Faculty of Animal Science of Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Golestan province, Iran).

Serum biochemistry

On d 42, two male chickens from each replicate were selected and blood samples were collected in nonheparinized tubes from the brachial vein. Serum was obtained by centrifuging at 1500 × g for 7 min at 4°C, and stored at -20°C until biochemical analysis. Serum samples were analyzed for total protein and albumin as indicators of liver damages (Sathesh Kumar *et al*., 2007), globulin, glucose, triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDL_C), using enzymatic related kits (Pars-Azmoon Co., Tehran, Iran). Meanwhile, serum liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured, using a spectrophotometer.

Table 2. Composition and calculated analyses of the basal diet[1]

Ingredients (%)	0 to 21 d	22 to 42 d
Ingredients:		
Corn	56.55	60.61
Soybean meal	37.27	32.33
Soybean oil	2.38	3.69
Dicalcium phosphate	1.44	1.09
Calcium carbonate	1.28	1.38
Vitamin premix[2]	0.25	0.25
Mineral premix[3]	0.25	0.25
DL-Methionine	0.15	0.07
Salt	0.43	0.33
Calculated analysis:		
Metabolisable energy (Kcal/Kg)	2950	3100
Crude Protein (%)	21.2	19.38
Calcium (%)	0.92	0.87
P (available) (%)	0.41	0.34
Sodium (%)	0.18	0.15
Lysine (%)	1.15	0.03
Methionine (%)	0.48	0.37
Methionine + Cystine (%)	0.83	0.69
Threonine (%)	0.81	0.73

[1]Claculated composition was according to NRC (1994).
[2]Vitamin premix (each Kg contained): Vitamin A, 3600000 IU; Vitamin D_3, 800000 IU; Vitamin E, 9000 IU; Vitamin K_3, 1600 mg; Vitamin B_1, 720 mg; Vitamin B_2, 3300 mg; Vitamin B_3, 4000 mg; Vitamin B_5, 15000 mg; Vitamin B_6, 150 mg; Vitamin B_9, 500 mg; Vitamin B_{12}, 600 mg; Biotin, 2000 mg.
[3]Mineral premix (each Kg contained): Mn, 50000 mg; Fe, 25000 mg; Zn, 50000 mg; Cu, 5000 mg; Iodine, 500 mg; Choline chloride 134000 mg.

Statistical analysis

This study was performed as a completely randomized design with 4 replications of 10 broilers each, using a 2 × 2 factorial arrangement with peppermint extract and CCl_4 as main effects. Data was analyzed, using GLM procedure of SAS software (SAS, 2003). Main effect means and the interactions are reported. Duncan's multiple test was used to compare the treatment effects. Differences were considered statistically significant at $P<0.05$.

Results

Growth performance

The main and interaction effects of alcoholic extract of peppermint leaf and CCl_4 on growth performance of broilers are shown in Table 3. There were significant ($P<0.05$) differences among the interaction effects for body weight gain

(BWG), feed intake (FI) and feed conversion ratio (FCR) on d 42 of experiment. The alcoholic extract of peppermint leaf did not improve growth performance, whereas CCl_4 worsened BWG and FCR ($P<0.05$). Interestingly, peppermint extract ameliorated the adverse effects of CCl_4 on BWG and FCR ($P< 0.05$).

Table 3. Effect of peppermint leaf extract and carbon tetrachloride (CCl_4) on growth performance of broiler chickens

	0-21 d			0-42 d		
	BWG[1] (g)	FI[2] (g)	FCR[3]	BWG[1] (g)	FI[2] (g)	FCR[3]
Extract:						
0 mL	671.56	965.97	1.43	2037.82	4016.62[a]	1.98[a]
2 mL	677.07	977.05	1.42	2084.79	3941.65[b]	1.89[b]
SEM	9.63	9.15	0.011	20.43	22.78	0.018
CCl_4:						
0 mL	679.79	976.10	1.44	2149.28[a]	3957.83	1.84[b]
1 mL	668.84	967.38	1.44	1973.33[b]	4000.44	2.03[a]
SEM	9.63	9.15	0.011	20.43	22.78	0.018
Interactions:						
0 mL extract × 0 mL CCl_4	668.86	958.19	1.43	2151.04[a]	4003.41[a]	1.86[c]
2 mL extract × 0 mL CCl_4	690.71	994.01	1.44	2147.51[a]	3912.25[b]	1.82[c]
0 mL extract × 1 mL CCl_4	674.25	973.75	1.43	1924.60[c]	4029.83[a]	2.09[a]
2 mL extract × 1 mL CCl_4	663.43	961.19	1.44	2022.07[b]	3971.25[a]	1.96[b]
SEM	13.61	12.95	0.015	28.91	32.23	0.026
Significance:						
Extract	0.6926	0.3911	0.5794	0.1302	0.0384	0.0006
CCl_4	0.4368	0.5137	0.8112	0.0001	0.2110	0.0001
Extract × CCl_4	0.5427	0.2445	0.9290	0.0003	0.1120	0.0001

[1]Body weight gain; [2]Feed intake; [3]Feed conversion ratio.
[a-c]Means within a column without a common superscript differ significantly ($P<0.05$).

Blood biochemical parameters

The main effect means of peppermint alcoholic extract and CCl_4 and their interactions on the blood serum biochemical parameters in broilers are shown in Tables 4 and 5. Significant interactions were observed between peppermint extract and CCl_4 on total protein, albumin, albumin:globulin ratio, glucose, triglyceride, total cholesterol, HDL_C, LDL_C, LDL_C:HDL_C ratio and $VLDL_C$ ($P<0.05$). The interaction effects showed that the use of 2 mL peppermint extract and 0 mL of CCl_4 significantly increased and decreased blood serum concentrations of total protein and glucose, respectively. Meanwhile, interaction effect between 0 mL

peppermint extract and 1 mL of CCl_4 resulted in significant lower serum concentrations of total protein and albumin (*P<0.05*).

The interaction between peppermint extract and CCl_4 revealed that 2 mL peppermint extract and 0 mL of CCl_4 significantly decreased blood serum concentrations of triglyceride and $VLDL_C$ (*P<0.05*). Meanwhile, interaction effect between 0 mL peppermint extract and 1 mL of CCl_4 resulted in a significant increase of blood serum concentrations of triglyceride, total cholesterol, LDL_C, LDL_C:HDL_C ratio and $VLDL_C$, whereas HDL_C serum concentration decreased.

Table 4. Effect of peppermint leaf extract and carbon tetrachloride (CCl_4) on blood biochemical parameters of broiler chickens at 42 d

	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Alb:Glo[1] (g/dL)	Glucose (mg/dL)
Extract:					
0 mL	4.17[b]	2.55[b]	1.62	1.57	205.79[a]
2 mL	4.77[a]	3.08[a]	1.69	1.93	166.05[b]
SEM	0.11	0.09	0.10	0.14	4.57
CCl_4:					
0 mL	4.79[a]	3.12[a]	1.67	1.92	171.61[b]
1 mL	4.14[b]	2.50[b]	1.64	1.58	200.18[a]
SEM	0.11	0.09	0.10	0.14	4.57
Interactions:					
0 mL extract × 0 mL CCl_4	4.47[b]	2.95[ab]	1.52	1.89[ab]	198.31[ab]
2 mL extract × 0 mL CCl_4	5.11[a]	3.29[a]	1.82	1.97[a]	144.91[c]
0 mL extract × 1 mL CCl_4	3.87[c]	2.14[c]	1.72	1.27[b]	213.26[a]
2 mL extract × 1 mL CCl_4	4.42[b]	2.86[b]	1.56	1.88[ab]	201.09[b]
SEM	0.15	0.13	0.14	0.19	6.47
Significance:					
Extract	0.0014	0.0018	0.6743	0.096	0.0001
CCl_4	0.0008	0.0006	0.8649	0.0981	0.0008
Extract × CCl_4	0.0006	0.0004	0.4455	0.0848	0.0001

[1]Albumin to globulin ratio.

[a-c]Means within a column without a common superscript differ significantly (*P<0.05*).

Blood liver enzymes

Data on the levels of blood serum liver enzymes (AST, ALT, and ALP) are presented in Table 6. Alcoholic extract of peppermint, CCl_4 and their interaction significantly affected concentrations of blood serum liver enzymes (*P<0.05*). In terms of the interaction effects between peppermint extract and CCl_4, blood serum concentrations of liver enzymes were significantly lower in 2 mL peppermint extract and 0 mL of CCl_4. In contrast, the interaction between 0 mL peppermint extract and 1 mL of CCl_4 showed an increase function of blood liver enzymes compared to others (*P<0.05*). A moderate effect on blood serum concentrations of liver enzymes was observed by the interaction between 2 mL peppermint extract and 1 mL of CCl_4 (*P<0.05*).

Table 5. Effect of peppermint leaf extract and carbon tetrachloride (CCL_4) on blood lipids of broiler chickens at 42 d

	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL_C[1] (mg/dL)	LDL_C[2] (mg/dL)	LDL_C:HDL_C (mg/dL)	$VLDL_C$[3] (mg/dL)
Extract:						
0 mL	99.67[a]	183.30[a]	64.81[b]	98.17[a]	1.59[a]	19.94[a]
2 mL	79.21[b]	163.75[b]	79.39[a]	73.45[b]	1.04[b]	15.91[b]
SEM	2.93	3.82	2.70	5.04	0.103	0.59
CCl_4:						
0 mL	77.87[b]	159.34[b]	76.84[a]	66.93[b]	0.89[b]	15.57[b]
1 mL	101.01[a]	187.71[a]	62.37[b]	104.69[a]	1.73[a]	20.28[a]
SEM	2.93	3.82	2.70	5.04	0.103	0.59
Interactions:						
0 mL extract × 0 mL CCl_4	87.68[b]	165.56[bc]	72.60[ab]	75.43[bc]	1.05[bc]	17.54[b]
2 mL extract × 0 mL CCl_4	68.06[c]	153.12[c]	81.08[a]	58.43[c]	0.74[c]	13.61[c]
0 mL extract × 1 mL CCl_4	111.66[a]	201.04[a]	57.03[c]	120.92[a]	2.17[a]	22.35[a]
2 mL extract × 1 mL CCl_4	90.36[b]	174.39[b]	67.70[bc]	88.47[b]	1.33[b]	18.22[b]
SEM	4.14	5.40	3.61	7.13	0.146	0.83
Significance:						
Extract	0.0003	0.0035	0.0196	0.0046	0.0026	0.0004
CCl_4	0.0001	0.0002	0.0016	0.0002	0.0001	0.0001
Extract × CCl_4	0.0001	0.0003	0.0034	0.0003	0.0001	0.0001

[1]High-density lipoprotein cholesterol; [2]Low-density lipoprotein cholesterol; [3]Very low density lipoprotein cholesterol.

[a-c]Means within a column without a common superscript differ significantly ($P<0.05$).

Table 6. Effect of peppermint leaf extract and carbon tetrachloride (CCl_4) on blood serum liver enzymes of broiler chickens at 42 d

	AST[1] (U/L)	ALT[2] (U/L)	ALP[3] (U/L)
Extract:			
0 mL	302.00[a]	19.79[a]	1851.13[a]
2 mL	270.86[b]	15.98[b]	1679.75[b]
SEM	4.54	0.436	12.29
CCl_4:			
0 mL	267.00[b]	13.31[b]	1614.25[b]
1 mL	305.88[a]	21.45[a]	1916.88[a]
SEM	4.54	0.436	12.29
Interactions:			
0 mL extract × 0 mL CCl_4	283.50[b]	16.22[c]	1642.25[c]
2 mL extract × 0 mL CCl_4	250.50[c]	13.40[d]	1556.75[d]
0 mL extract × 1 mL CCl_4	320.50[a]	23.35[a]	1930.05[a]
2 mL extract × 1 mL CCl_4	291.25[b]	19.55[b]	1772.75[b]
SEM	6.42	0.617	17.39
Significance:			
Extract	0.0004	0.0001	0.0001
CCl_4	0.0001	0.0001	0.0001
Extract × CCl_4	0.0001	0.0001	0.0001

[1]Aspartate aminotransferase; [2]Alanine aminotransferase; [3]Alkaline phosphatase.

[a-d]Means within a column without a common superscript differ significantly ($P<0.05$).

Discussion

Stressor conditions are unavoidable parts of poultry production systems. There is an increasing interest in the use of herbs and medicinal plants in poultry feeding to overcome these problems. Medicinal plants, due to having secondary metabolites, have positive effects on immune function and growth performance of broilers. Most of these plants normally function as ergogenic, antiparasitic, antibacterial, anti-flatus, antifungal and antiseptic (Huang *et al*., 1992). Madrid *et al*. (2003) reported that herbal extracts improve gastrointestinal function by improving hepatic and pancreatic secretory function. These positive effects have been attributed to the existence of active substances such as carvacrol, flavonoids and menthol which ultimately enhance the digestibility of nutrients and thereby improve growth performance. In the current study, peppermint leaf extract did not improve growth performance in terms of BWG and FCR, which is inconsistent with the others (Al-Kassi and Witwit, 2010; El Iraqi *et al*., 2013; Akbari and Torki, 2014). Nevertheless, our results showed that peppermint leaf alcoholic extract ameliorated the adverse effects of CCl_4 on BWG and FCR. Previously, an antimicrobial function of peppermint has been reported (Helander, 1998). It seems that peppermint, by restricting the growth and function of the intestinal harmful microorganisms, might improve growth performance (Lovkova *et al*., 2001). Consistent with our findings, Sonkusale *et al*. (2011) also reported that CCl_4 has negative effect on broilers performance. CCl_4, like many other toxins, impairs liver and digestive system function (Lieber, 2000). A study by Ahsan *et al*. (2009) showed that methanol extract of some medical plants had a good level of hepatoprotection function against CCl_4. Nateghi *et al*. (2013) and Mehmetçik *et al*. (2008) also reported the positive effect of Artichoke extract on broiler and rat liver damage induced by CCl_4, respectively. Shimizu *et al*. (2001) reported the destructive effect of CCl_4 on liver damage, as the bubble-like structures around the central veins and bubbles of fat in the midsection of some liver cells were observed. Sonkusale *et al*. (2011) also demonstrated that the severe granular and vacuolar degenerative changes associated with focal necrotic areas in the liver of broilers poisoned with CCl_4. Of course, previous studies have shown that chickens unlike other laboratory animals against necrosis (tissue death) are resistant to CCl_4-induced liver damage (Slater, 1966; Diaz Gomez *et al*., 1975). It has been demonstrated in pigeons, as well (Diaz Gomez *et al*., 1983). Researchers documented that the lack of sensitivity in chickens is due to the lower ability of the liver to activate CCl_4 to active metabolites including free radicals of CCl_3-. The low capacity of CCl_4 activation can be due to a lower content of p-450 in the liver of chickens and pigeons compared with susceptible species such as mice and rats (Diaz Gomez *et al*., 1975). P-450 is a complex which plays a role in activation of CCl_4 and other toxins to metabolites through the liver. These metabolites, in turn, react with lipids and proteins and eventually cause liver damage (Castro and Diaz Gomez, 1972; Uehleke *et al*., 1973; D'Acosta *et al*., 1973; Villarruel *et al*., 1975).

The results of this study suggest the important role of peppermint extract to control of liver function. Consistent with our results, Akbari and Torki (2014) reported that peppermint extract has increased total protein and HDL-cholesterol and decreased blood serum concentrations of total cholesterol, triglycerides, LDL-cholesterol and glucose of broilers subjected to heat stress. Thus, these authors suggested the protective role of peppermint extract against heat stress condition. Falah *et al*. (2013) also reported that peppermint extract has increased albumin, total protein, albumin:globulin ratio and HDL-cholesterol and significantly reduced total cholesterol, triglycerides, LDL-cholesterol and glucose in broilers. It seems as some components of peppermint including menthol and menthone have a potential to decrease blood lipids in broilers (Escop, 2003), also in this case, increased albumin:globulin ratio is believed to be linked with the improved liver function because of the administration of peppermint extract. Mansoub (2011) also reported that peppermint extract increases albumin, total protein and HDL-cholesterol and also significantly decreases glucose, triglycerides, total cholesterol, LDL-cholesterol and LDL:HDL ratio in serum of broilers. This author suggested that the main reason for the reduction of serum lipids in broilers is due to the active ingredients of peppermint leaves such as tocopherol and menthol.

Sonkusale *et al*. (2011) showed that intraperitoneal injection of CCl_4 to broilers significantly increases blood concentrations of triglycerides, total cholesterol, LDL-cholesterol and LDL:HDL ratio and decreases serum concentrations of albumin, total protein, albumin:globulin ratio and HDL-cholesterol, indicating the adverse effect of CCl_4 on the liver function. Samudram *et al*. (2008) in an experiment about the destructive effects of CCl_4 on the rat liver function reported a lower blood serum protein level. The decrease in total protein and albumin levels in liver toxicity could be due to its reduced biosynthesis. By the impairment of ribosomal protein biosynthesis of endoplasmic reticulum, CCl_4 reduces protein biosynthesis (Clawson, 1989). Gad *et al*. (2011) also showed that CCl_4 decreases serum protein and albumin levels, but increases serum lipid levels, showing the adverse effect of CCl_4 on the liver function. Moawad (2007) also in a similar research showed that CCl_4 increases serum cholesterol and triglycerides via liver damage. Owen (1990) noted an increase in serum cholesterol under liver diseases which is related to undermining the role of the liver to remove cholesterol from the blood circulation. Administration of CCl_4 is followed by increased cholesterol, triglycerides and free fatty acids levels in plasma and tissues. The CCl_4 enhances the synthesis of fatty acids and triglycerides by the use of acetate as a substrate. This process can be resulted from the transfer of acetate into liver cells followed by a rise in blood lipids (Boll *et al*., 2001).

Liver cells contain high concentrations of ALT, AST and ALP enzymes. ALT is present in the cytoplasm, while AST is present in the mitochondria of liver cells (Drotman and Lawhorn, 1978; Adzet *et al*. 1987). Destruction of the liver cells leads to the leakage of these enzymes into the blood stream (Parmar *et al*., 2012).

Therefore, an increased concentration of liver enzymes in the blood circulation is one of the main indicators of liver damage due to the toxins (Hetrog and Hollmann, 1998). Sonkusale *et al*. (2011) showed that injection of CCl_4 to broilers increases blood liver enzymes levels. It has been reported that the most important causes of increased serum AST in birds are liver diseases (Campbell and Coles, 1986; Dein, 1986). CCl_4 is converted to trichloromethyl (CCl_3·) in the liver, which is a highly reactive free radical. Therefore, this free radical is converted to proxy trichloromethyl (CCl3OO) by reacting with oxygen. The latter combination attacks hepatic cells and leads to lipid peroxidation and consequently increased blood liver enzymes concentrations. Patil and Mall (2012) as well as Jain *et al*. (2012) reported a protective role for peppermint extract against liver damage by CCl_4. These authors documented that peppermint extract lowers serum liver enzymes of ALT, AST and ALP, whilst CCl_4 does the contrary in rats. Sharma *et al*. (2006) reported that extracts of peppermint significantly decreases the amount of liver enzymes including ALP, AST and ALT, and also increases HDL-cholesterol levels in the blood of mice. These researchers also expressed that peppermint extract due to the active ingredients of α-tocopherol, caffeic acid and menthol has a protective role against arsenic-induced toxicity in mice. Meanwhile, the hepatoprotective function for other medical plants is well documented. Nateghi *et al*. (2013) reported that artichoke leaf extract reduces ALT level in broilers poisoned with CCl_4. Mehmetçik *et al*. (2008) and Fallah *et al*. (2013) also reported that administration of artichoke leaf extract to rats caused a significant decrease in blood levels of liver enzymes compared with the group poisoned with CCl_4. Yildiz *et al*. (2008) also reported that 5% of the diet artichoke powder in egg laying hens, reduces blood levels of ALP. In contrast, Abdo *et al*. (2007) reported that the use of different levels of artichoke leaves in broiler diets had no significant effect on liver enzymes. Radwan *et al*. (2007) also reported that the use of different levels of artichoke leaves in egg laying hens had no significant effect on the levels of serum ALT and AST. It seems that sensitivity of different animals and also the used form of medical plant (powder or extract) are the main reasons for the discrepancy of different investigations.

In conclusion, these findings suggest that *in vivo* administration of peppermint leaf extract ameliorates the adverse effect of CCl_4 on liver function and growth performance and consequently might be useful for the prevention of oxidative stress-induced hepatotoxicity in broilers.

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