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Effects of Silymarin on Growth Performance, Internal Organs and Some Blood Parameters in Japanese Quail Subjected to Oxidative Stress Induced by Carbon Tetrachloride

Behboodi HR[1], Samadi F[1], Shams Shargh M[2], Ganji F[3] & Samadi S[4]

[1]Department of Animal and Poultry Physiology, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[3]Department of Biology, Faculty of Science, Golestan University, Gorgan, Iran
[4]Department of Horticulture, College of Agriculture, Shiraz University, Shiraz, Iran

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Corresponding author
Firooz Samadi
F.samadi@gau.ac.ir

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Abstract

The effects of Silymarin on growth performance, internal organs, and some blood parameters were investigated in Japanese quail that were subjected to oxidative stress induced by carbon tetrachloride (CCl_4). An experiment was conducted as a completely randomized design in a factorial arrangement (2 × 2) with four replicates of 30 birds each. Factors included two levels of Silymarin (0 and 1 mL/kg of body weight (BW)) and two levels of CCl_4 (0 and 1 mL/kg of BW). Results showed that Silymarin did not affect productive parameters, whilst CCl_4 significantly ($P < 0.05$) reduced feed intake and body weight gain. Silymarin did not affect the relative weights of breast, gizzard and heart, whereas CCl_4 reduced relative weights of breast and heart. Both Silymarin and CCl_4 administration resulted in higher pancreases relative weight. Birds treated with Silymarin had greater blood serum concentration of total protein and lower concentrations of glucose, triglyceride and total cholesterol ($P < 0.05$). In contrast, birds that received CCl_4 showed decreased total protein and increased glucose concentrations of blood serum ($P < 0.05$). The interaction effect between Silymarin and CCL_4 showed that Silymarin ameliorated the adverse effects of CCl_4 on blood albumin. Treatment of CCl_4 increased blood concentration of alkaline phosphatase compared with Silymarin ($P < 0.05$). This study showed that Silymarin may be a useful antioxidant source to ameliorate the adverse effects of oxidative stressors in Japanese quail.

Introduction

Stress results in reactive oxygen species (ROS) production. Excess generation of ROS can cause oxidative damage to macromolecules resulting in lipid peroxidation, mutagenesis, and carcinogenesis (Khan and Sultana, 2009). Tetrachloride carbon (CCl_4) has long been known as a model toxicant and has been shown to induce oxidative stress both *in vitro* and *in vivo* (Manibusan *et al*., 2007). ROS produced by CCl_4 cause serious damage to some tissues by

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stimulating lipid peroxidation (Janbaz and Gilani, 2002).

In poultry farms, stress can reduce productive performance. Researchers have reported positive effects of medicinal plants on broiler's performance when challenged with stress (Ponte and Rosado, 2008). Medicinal plants possess beneficial properties including anticoccidial, antifungal, and antioxidant capabilities (Knekt *et al.,* 2002) and they have potential to prevent side effects of oxidative stress (Sonkusale *et al.,* 2011) by removing hydrogen peroxide (Schaffer *et al.,* 2004). Milk thistle (*Silybum marianum*) is a mixture of flavonolignans, and is known as Silymarin. The main active ingredients of its seeds are silybin, isosilybin, silycristin, and silydianin (Ding *et al,* 2001). Some features of *S. marianum* include anti-oxidation and immune modulators (Katiyar, 2004). *S. marianum,* is also used in hepatoprotection against experimentally induced oxidative stress by various chemicals including CCl_4 (Gadgoli and Mishra, 1999).

In the present study, the effects of *in vivo* Silymarin administration was assessed on growth performance, internal organs, and some blood parameters in Japanese quail subjected to oxidative stress induced by CCl_4.

Materials and Methods

Plant preparation and extraction

S. marianum used in this experiment was collected from the heights of Ravansar (34°00' – 52" north latitude and 46°00' - 27" east longitude; altitude: 1650 m), Kermanshah province, Iran. Its total values of phenolic compounds, flavonoids, and antioxidants were measured colorimetrically, using Folin-Ciocalteu method (Guo *et al*., 2000; Chang *et al*., 2002). To prepare Silymarin, seeds were powdered with an electrical mill. Then powder was defatted by petroleum ether, dried, and mixed with ethanol 80% (2:10). Thereafter, it was shaken and passed through filter paper, and the remaining alcohol was removed by distillation under vacuum (Harborne, 1998; Bahraminejad *et al*., 2008).

Birds, diets and experimental design

A total of 480 day-old Japanese quail chicks were reared for 42 days at a research farm of Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Golestan, Iran). The ambient temperature on d 1 was 38±1°C and then decreased by 1°C every two days until a constant temperature of 24°C was reached. The lighting schedule provided 23 hrs of light per day. The experiment was performed as a completely randomized design with 4 replicates of 30 birds in each, using a 2 × 2 factorial arrangement with Silymarin and CCl_4 (olive oil solution at the volume ratio of 1:1) as main effects. Factors included 2 levels of Silymarin (0 and 1 mL/kg per body weight) and CCl_4 (0 and 1 mL/kg per body weight).

Silymarin was fed directly into crop using a syringe equipped with a plastic nozzle and a feeding tube (Nova Cath®, No. 10). Intraperitoneal injection of CCl_4 was performed on day 22 and again every three days thereafter. Chickens in the CCl_4 control group received an intraperitoneal injection of 0.9% sodium chloride solution instead (Sharma *et al*, 2006) and those in the Silymarin control group were fed distilled water (1 mL/kg body weight) by an oral gavage. Birds were provided with free access to feed and water throughout the experimental period. The composition of the basal diet is shown in Table 1. All experimental protocols were approved by the Animal Care and Use Committee of the College of Animal Science of the Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Golestan, Iran).

Traits measured

Body weight gain (BWG) and feed intake (FI) were measured weekly and used to calculate feed conversion ratio (FCR) which is the quotient of FI divided by BWG. At the end of experiment, two birds 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. The serum samples were analyzed for triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-c), total protein, albumin, glucose, and various liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), using enzyme kits (Pars-Azmoon Co., Tehran, Iran). The relative weight (percentage of body weight) of carcass, trunk, and chest as well as internal organs (stomach, gizzard, pancreas, proventriculus, liver and heart) were evaluated to assess carcass performance at the end of the period.

Table 1. Composition and nutrients of basal diet[1]

Ingredients	(%)
Corn	49.16
Soybean meal (44% protein)	45.05
Soybean oil	2.76
Calcium carbonate	1.30
Dicalcium phosphate	0.75
Salt	0.35
Vitamin premix[2]	0.25
Mineral premix[3]	0.25
DL-Methionine	0.13
Calculated analysis:	
ME (Kcal/kg)	2900
CP (%)	24.00
Calcium (%)	0.80
Available phosphorus (%)	0.30
Sodium (%)	0.15
Lysine (%)	1.30
Methionine (%)	0.50
Methionine + Cystine (%)	0.88

[1] Calculated composition according to NRC (1994).
[2] Vitamin premix (each kg contained): Vitamin A, 3600000 IU; Vitamin D3, 800000 IU; Vitamin E, 9000 IU; Vitamin K3, 1600 mg; Vitamin B1, 720 mg; Vitamin B2, 3300 mg; Vitamin B3, 4000 mg; Vitamin B5, 15000 mg; Vitamin B6, 150 mg; Vitamin B9, 500 mg; Vitamin B12, 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 in a completely randomized design with four treatments and four replicates, each with 30 broilers, using a 2 × 2 factorial arrangements with Silymarin and CCl_4 as main effects. Data were analyzed using GLM procedure of SAS software (SAS, 2003). The main effect means and the interactions are reported and Duncan's multiple range tests were used to compare the treatment effects. Differences were considered statistically significant at $P < 0.05$.

Results

Total phenol, flavonoids and antioxidant of Silymarin

The amounts of total phenol, flavonoids and antioxidants in Silymarin are shown in Table 2.

Table 2. Total phenol, flavonoids and antioxidants of Silymarin

Compounds	Dry weight
Total phenol	2.60 mg/g
Flavonoids	1.96 mg/g
Antioxidants	74.23%

Growth performance

The main and interaction effects of Silymarin and CCl_4 on growth performance of Japanese quails at 42 d of experiment are shown in Table 3. Silymarin did not affect productive parameters (FI, BWG and FCR) while CCl_4 significantly ($P < 0.05$) reduced BWG and FI. Experimental treatments did not affect FCR.

The relative weight of internal organs

There were significant main and interaction effects between Silymarin and CCl_4 on the relative weights of some internal organs ($P < 0.05$) including breast, gizzard, pancreas, and heart (Table 4). Silymarin did not affect the relative weights of breast, gizzard and heart, but CCl_4 reduced relative weights of breast and heart. Both Silymarin and CCl_4 resulted in higher relative pancreas weight.

Table 3. Effect of Silymarin and carbon tetrachloride (CCl_4) on weekly growth performance of Japanese quails at 42 d (d 1 to 42)

	BWG[1] (g)	FI[2] (g)	FCR[3]
Silymarin			
0 mL	36.26	143.60	3.96
1 mL	34.60	141.10	4.08
SEM	0.79	1.33	0.09
CCl_4			
0 mL	36.71[a]	144.80[a]	3.94
1 mL	34.15[b]	140.00[b]	4.10
SEM	0.79	1.33	0.09
Interactions			
0 mL Silymarin × 0 mL CCl_4	37.72	145.07	3.85
1 mL Silymarin × 0 mL CCl_4	35.70	144.52	4.05
0 mL Silymarin × 1 mL CCl_4	34.80	142.17	4.07
1 mL Silymarin × 1 mL CCl_4	33.50	137.85	4.10
SEM	1.11	1.88	0.13
Significance			
Silymarin	0.16	0.22	0.41
CCl_4	0.04	0.02	0.34
Silymarin × CCl_4	0.75	0.33	0.52

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

Table 4. Effect of Silymarin and carbon tetrachloride (CCL_4) on internal organs of Japanese quails at 42 d (% of live body weight)

	Carcass	Breast	Corpus	Gizzard	Proventriculus	Pancreas	Liver	Heart
Silymarin								
0 mL	0.38	0.26	0.12	0.02	0.004	0.004[a]	0.03	0.01[a]
1 mL	0.39	0.27	0.12	0.02	0.005	0.003[b]	0.02	0.008[b]
SEM	0.006	0.006	0.004	0.001	0.0002	0.0003	0.002	0.0003
CCl_4								
0 mL	0.38	0.28[a]	0.12	0.01[b]	0.004	0.004[a]	0.02	0.01[a]
1 mL	0.38	0.25[b]	0.13	0.02[a]	0.005	0.002[b]	0.03	0.008[b]
SEM	0.006	0.006	0.004	0.001	0.0003	0.0002	0.002	0.0003
Interactions								
0 mL Silymarin × 0 mL CCl_4	0.37	0.27	0.11	0.02	0.004	0.005	0.03	0.01
1 mL Silymarin × 0 mL CCl_4	0.40	0.27	0.12	0.01	0.004	0.003	0.02	0.01
0 mL Silymarin × 1 mL CCl_4	0.39	0.24	0.13	0.02	0.004	0.002	0.03	0.01
1 mL Silymarin × 1 mL CCl_4	0.37	0.26	0.13	0.02	0.005	0.003	0.03	0.01
SEM	0.007	0.008	0.005	0.002	0.0002	0.0002	0.0023	0.0004
Significance								
Silymarin	0.53	0.22	0.63	1.000	0.14	0.005	0.14	0.01
CCl_4	0.75	0.018	0.07	0.18	0.61	0.0002	0.14	0.01
Silymarin × CCl_4	0.014	0.22	0.17	0.18	0.14	0.033	0.61	0.07

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

Blood biochemical parameters

Birds administered Silymarin showed greater blood serum concentrations of total protein and albumin but lower concentrations of glucose, triglyceride, and total cholesterol ($P < 0.05$). CCl_4 decreased total protein concentrations but increased glucose concentrations of blood serum ($P < 0.05$). The interaction effect between Silymarin and CCl_4 showed that Silymarin ameliorated the adverse effect of CCl_4 on albumin (Table 5).

Table 5. Effect of Silymarin and carbon tetrachloride (CCL_4) on blood parameters of Japanese quails at 42 d

	Total protein (g/dL)	Albumin (g/dL)	Glucose (mg/dL)	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	HDL-C[1] (mg/dL)
Silymarin						
0 mL	2.80[b]	1.08[b]	306.25[a]	192.63[a]	170.12[a]	39.25
1 mL	3.20[a]	1.37[a]	295.25[b]	157.38[b]	152.62[b]	36.37
SEM	0.093	0.016	1.77	8.99	2.86	1.83
CCl_4						
0 mL	3.21[a]	1.22	297.50[b]	161.88	162.00	36.87
1 mL	2.75[b]	1.23	304.00[a]	188.13	160.75	38.75
SEM	0.093	0.016	1.77	8.99	2.86	1.83
Interactions						
0 mL Silymarin × 0 mL CCl_4	3.07	1.15[c]	303.25	171.25	174.25	37.50
1 mL Silymarin × 0 mL CCl_4	3.35	1.45[a]	291.75	152.50	149.75	36.25
0 mL Silymarin × 1 mL CCl_4	2.52	1.02[d]	309.25	214.00	166.00	41.00
1 mL Silymarin × 1 mL CCl_4	3.05	1.30[b]	298.75	162.25	155.50	36.50
SEM	0.131	0.023	2.51	12.72	4.05	2.59
Significance						
Silymarin	0.01	0.0001	0.001	0.016	0.001	0.28
CCl_4	0.007	0.611	0.023	0.061	0.76	0.48
Silymarin × CCl_4	0.362	0.0001	0.841	0.219	0.11	0.54

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

Blood liver enzymes

Data on hepatic enzyme activities in blood serum (AST, ALT, and ALP) are presented in Table 6. Alkaline phosphatase (ALP) was the only hepatic enzyme that was influenced by experimental treatments, as CCl_4 significantly increased its activity compared to Silymarin treatment ($P < 0.05$).

Table 6. Effect of Silymarin and carbon tetrachloride (CCl_4) on blood liver enzymes of Japanese quails at 42 d

	AST[1] (U/L)	ALT[2] (U/L)	ALP[3] (U/L)
Silymarin			
0 mL	274.50	7.12	179.50
1 mL	280.25	6.00	167.00
SEM	7.84	0.61	14.27
CCl_4			
0 mL	279.00	6.00	146.00[b]
1 mL	275.75	7.12	200.50[a]
SEM	7.84	0.61	14.27
Interactions			
0 mL Silymarin × 0 mL CCl_4	271.50	6.50	154.00
1 mL Silymarin × 0 mL CCl_4	286.50	5.50	138.00
0 mL Silymarin × 1 mL CCl_4	277.50	7.75	205.00
1 mL Silymarin × 1 mL CCl_4	274.00	6.50	196.00
SEM	11.09	0.86	20.18
Significance			
Silymarin	0.61	0.21	0.54
CCl_4	0.77	0.21	0.01
Silymarin × CCl_4	0.42	0.88	0.86

[1] Aspartate aminotransferase; [2] Alanine aminotransferase; [3] Alkaline phosphatase.
[a-c] Means within a column without a common superscript differ significantly ($P < 0.05$).

Discussion

Oxidative stress caused by excessive reactive oxygen species is one of the main factors that negatively affect organismal performance (Dalloul *et al.,* 2006; Lin *et al.,* 2006) and underlies pathogenesis of several important diseases (Kris-Etherton *et al.,* 2004). Oxidative

stress induced by carbon tetrachloride is involved with the cytochrome P_{450}-NADPH enzyme system through the metabolism of chloromethyl and proxy chloromethyl reactive radicals (Ha *et al.,* 2005). These radicals attack unsaturated fatty acids, alkalizing proteins, and other cellular macromolecules that lead to lipid peroxidation of the cell membrane, changes in enzyme function, and ultimately cell damage (Kodai *et al.,* 2007). In the current study, CCl_4 significantly reduced feed intake and body weight gain, which is consistent with previous studies (Khorramshahi *et al.,* 2014; Khodadust *et al.,* 2015). Reduced body weight gain could be due to reduced nutrient digestion and absorption as a result of low bile secretion (Panovska *et al.* 2007). Silymarin did not improve growth performance in birds treated with CCl_4 which contrasts the results of Ebrahimi *et al* (2013), who used powdered Silymarin at various concentrations (0, 100, and 200 mg/kg) in broilers. This difference may be due to species differences and experimental doses. In this regard, susceptibility of broilers due to genetic selection should be taken into consideration. The relative increase in gizzard weight in birds treated with CCl_4 may be due to fat accumulation in gizzard. It was documented that CCl_4 can increase blood lipid concentrations which can lead to the fat accumulation in visceral organs (Devarshi *et al.*, 1986). However, although changes in the relative weight of pancreas and heart require further investigation, the relative weight changes may be due in part to changes in cardiac blood circulation and basal metabolism. The loss of relative carcass and breast weight in birds treated with CCl_4 can be due reduced secretion of bile due to liver oxidative damage and thereby reduced digestion and absorption of nutrients (Panovska *et al.,* 2007). In addition, it was reported that toxins produced through the destruction of intestinal epithelial cells or released during changes in the intestinal ecosystem have an adverse impact on performance parameters (Applegate *et al.,* 2009).

Hepatocytes are involved in protein metabolism; therefore, any damage to hepatocytes can lead to reduced circulation of protein concentrations (Chlopčiková *et al.,* 2004). In congruence with our results, there are some studies reporting adverse effects of CCl_4 on blood serum protein concentration (Kumar *et al.,* 2009; Sonkusale *et al.,* 2011; Jothi *et al.,* 2012). CCl_4 reduces blood serum albumin concentration, possibly because of ribosome breakdown (Redman, 1969). In agreement with our findings, positive effects of some medical plants including Silymarin (Neshatgharamaleki and Mohajeri, 2014) and peppermint (Mehri *et al.,* 2015) on hepatocyte protein synthesis have been reported. Additionally, Huseini *et al.* (2006) stated that using medicinal plants enhances concentrations of total protein, albumin and globulin; hence albumin: globulin ratio in blood serum of broiler chickens.

The mechanism by which the *Silybum marianum* reduces blood sugar is not well understood, but it may work by inducing beta cells of the pancreas to produce insulin (Soto *et al.,* 2004). Silymarin repairs and renovates the pancreatic tissue, which plays an important role in the regulation of blood sugar (Soto *et al.,* 2004). Soto *et al* (2003) studied the effect of Silymarin on pancreatic function in diabetic animals and noted that Silymarin plays a protective role in pancreatic tissue against damaging elements, thereby exerting its hypoglycemic effect. Indeed, we observed a reduction in blood glucose levels with Silymarin treatment. In addition, flavonoids such as Silymarin could regulate liver enzymes involved in the metabolism of carbohydrates, and therefore reduce blood sugar and restore weight. This occurs as a result of reduced liver phosphorylase enzyme activity and increased activity of glucokinase and glycogen synthase (Abascal and Yarnell, 2003a-b).

It has been reported that beta-oxidation of fatty acids and hydrolysis of triglyceride lessens under the influence of CCl_4. This leads to enhanced availability of fatty acids for esterification, and consequently, CCl_4 facilitates the synthesis of fatty acids and triglycerides through acetate. This process can be the result of acetate transfer to liver cells, followed by cholesterol upsurge (Boll *et al.,* 2001). Hasani-Ranjbar *et al.,* (2010) reported that there is a significant decrease in levels of total cholesterol and LDL-c, and improvement in total antioxidant power after treatment with *S. marianum*, garlic, and wheat germ. Banaee *et al.,* (2011) showed that oral administration of Silymarin to fish significantly reduced plasma glucose and total cholesterol levels. Tumova *et al.,* (2010) reported that administration of Silymarin resulted in lower serum cholesterol levels and mild increase in HDL-c levels. These

results may be due to fat-mediated improved bioavailability and/or by inhibition of resorption of dietary cholesterol. *S. marianum* seed extract (Silymarin) caused a significant decrease in LDL-c (Huseini *et al.,* 2006).

Liver cells contain high concentrations of ALT, AST and ALP enzymes. ALT and AST are enzymes present in hepatocytes and liver parenchymal cells, respectively. Increased levels of these transaminases in hepatocytes are indicators of poor hepatic integrity. Destruction of 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). CCl_4 is a toxic agent that can destroy hepatocyte membrane integrity and raise blood levels of AST, ALT, and ALP enzymes (Tsukamoto *et al.,* 1990; Soni and Nishant, 2008). It has been reported that the most important causes of increased serum AST in birds are liver diseases (Campbell and Coles, 1986). The impact of Silymarin on liver transaminases has been reported. Silymarin can treat damaged hepatocytes and restore normal liver function (Muriel & Mourelle, 1990; Wang *et al*., 2013). Neshatgharamaleki and Mohajeri, (2014) showed that administration of Silymarin in rats resulted in a significant reduction in ALP. Similar to our observations, Silymarin also lowered alanine aminotransferase levels in broiler chickens under stress induced by aflatoxin B_1 (Fani Makki *et al.,* 2014).

In general, this study showed that Silymarin did not affect productive parameters, while CCl_4 significantly reduced feed intake and body weight gain. CCl_4 reduced relative weights of breast and heart. Birds treated with Silymarin showed greater blood serum concentration of total protein and lower concentrations of glucose, triglyceride and total cholesterol, while CCl_4 decreased total protein and glucose concentrations in blood serum. The interaction effect between Silymarin and CCl_4 showed that Silymarin ameliorated the adverse effects of CCl_4 on albumin. CCl_4 significantly increased blood levels of ALP compared to Silymarin treatment. Therefore, this study suggests that Silymarin may be a useful antioxidant source to ameliorate the adverse effects of oxidative stressors in poultry farms.

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References

Abascal K & Yarnell E. 2003a. The many faces of *silybum marianum* (Milk Thistle): Part 1 - Treating cancer and hyperlipidemia and restoring kidney function. Alternative and Complementary Therapies, 9: 170-175. DOI: 10.1089/107628003322256878

Abascal K & Yarnell E. 2003b. The Many Faces of *Silybum marianum* (Milk Thistle): Part 2 - Clinical uses, safety, and types of preparations. Alternative and Complementary Therapies, 9: 251-256. DOI: 10.1089/107628003322490698

Applegate TJ, Schatzmayr G, Pricket K, Troche C & Jiang Z. 2009. Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poultry Science, 88: 1235-1241. DOI: 10.3382/ps.2008-00494

Bahraminejad S, Asenstorfer RE, Riley IT, & Schultz CJ. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). Journal of Phytopathology, 156: 1-7. DOI: 10.1111/j.1439-0434.2007.01309.x

Banaee M, Sureda A, Mirvaghefi, AR & Rafei GR. 2011. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 37:885-896. DOI: 10.1007/s10695-011-9486-z

Boll M, Lutz WD, Weber E & Stampfl A. 2001. Pathogenesis of carbon tetrachloride-induced hepathocyte injury bioactivation of CCL_4 by cytochrome P450 and effects on lipid homeostasis. Zeitschrift für Naturforschung C, 56: 111-121. DOI: 10.1515/znc-2001-1-218

Campbell TW & Coles EH. 1986. Avian clinical pathology. In: Coles EH. (Eds). Veterinary Clinical Pathology. Saunders Company. Philadelfia. Pages. 203-209.

Chang CC, Yang MH, Wen HM & Chern JC. 2002. Estimation of total flavonoid content in proplis by two complementary colorimetric

methods. Journal of Food and Drug Analysis, 10: 178-182.

Chlopčiková S, Psotová J, Miketová P & Šimánek V. 2004. Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Part I. Silymarin and its flavonolignans. Phytotherapy Research, 18: 107-110. DOI: 10.1002/ptr.1415

Dalloul RA, Lillehoj HS, Lee JS, Lee SH & Chung KS. 2006. Immunopotentiating effect of a *Fomitella fraxinea*-derived lectin on chicken immunity and resistance to coccidiosis. Poultry Science, 85: 446–451. DOI: 10.1093/ps/85.3.446

Devarshi P, Kanase A, Kanase R, Mane S, Patil S & Varute AT. 1986. Effect of mandur bhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCL_4 induced hepatic injury. Journal of Biosciences, 10: 227-234. DOI: 10.1007/BF02703480

Ding TM, Tian SJ, Zhang ZX, Gu DZ, Chen YF, Shi YH & Sun ZP. 2001. Determination of active component in Silymarin by PR-LC and LC/MS. Journal of Pharmaceutical and Biomedical Analysis, 26: 155-161. DOI: 10.1016/S0731-7085(01)00364-8

Ebrahimi R, Mahmmadabadi T, Sari M, Sallari S, Zamiri MJ & Beygi Nasiri MT. 2013. Effect of silymarin on lead-induced oxidative stress in broilers. Iranian Journal of Animal Science Research, 5: 302-312.

Fani Makki O, Omidi A, Afzali N, Sarir H, Frouzanmehr M & Shibak A. 2014. Efficacy of *Silybum Marianum* Seeds in Ameliorating the Toxic Effects of Aflatoxin B1 in Broilers. Iranian Journal of Toxicology, 8: 977-982.

Gadgoli C & Mishra SH. 1999. Antihepatotoxic activity of *p*-methoxy benzoic acid from *Capparis spinosa*. Journal of Ethnopharmacology, 66: 187–192. DOI: 10.1016/S0378-8741(98)00229-3

Guo FC, Savelkoul HFJ, Kwakkel RP, Williams BA & Verstegen MWA. 2000. Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. World's Poultry Science Journal, 59: 427- 440. DOI: 10.1079/WPS20030026

Ha KT, Yoon SJ, Choi DY, Kim DW, Kim JK & Kim CH. 2005. Protective effect of *lycium chinense* fruit on carbon tetrachloride-induced hepatotoxicity. Journal of Ethnopharmacology, 96: 529-535. DOI: 10.1016/j.jep.2004.09.054

Harborne AJ. 1998. Phytochemical methods, a guide to modern techniques of plant analysis. Springer Nether;ands, Pages, 270.

Hasani-Ranjbar S, Nayebi N, Moradi L, Mehri A, Larijani B & Abdollahi M. 2010. The efficacy and safety of herbal medicines used in the treatment of hyperlipidemia; a systematic review. Current Pharmaceutical Design, 16: 2935-2947. DOI: 10.2174/138161210793176464

Hetrog MGL & Hollmann PCH. 1998. Potential health effects of the dietary flavonol quercetin: review. European Journal of Clinical Nutrition. 50: 63-71.

Huseini HF, Larijani B, Heshmat R, Fakhrzadeh H, Radjabipour B, Toliat T & Raza M. 2006. The efficacy of *Silybum marianum* (L.) Gaertn (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. Phytotherapy Research, 20: 1036-1039. DOI: 10.1002/ptr.1988

Janbaz KH & Gilani AH. 2002. Menthol prevents liver damage induced by paracetamol and CCl_4. Pakistan Journal of Biological Science, 5: 1101-1103.

Jothi G, Bhuvaneswari S & Radhika J. 2012. Antihepatotoxic activity of *Samanea saman* (Jacq.) Merr. against carbon tetrachloride induced hepatic injury in rats. Research Journal of Pharmacy and Technology, 5: 393-397.

Katiyar SK. 2004. Silymarin and skin cancer prevention: Anti-inflammatory, antioxidant and immunomodulatory effects (Review). International Journal of Oncology, 18: 101-112.

Khan TH & Sultana S. 2009. Antioxidant and hepatoprotective potential of *Aegle marmelos Correa*. against CCl_4-induced oxidative stress and early tumor events. Journal of Enzyme Inhibition and Medicinal Chemistry, 24: 320-327 DOI: 10.1080/14756360802167754

Khodadust MR, Samadi F, Ganji F, Jafari Ahangari Y & Asadi GH. 2015. Effects of peppermint (*Mentha piperita* L.) alcoholic extract on carbon tetrachloride-induced hepatotoxicity in broiler chickens under heat stress condition. Poultry Science Journal, 3: 1-16. DOI: 10.22069/psj.2015.2323

Khorramshahi M, Samadi F & Ganji F. 2014. The effects of *Cynara scolymus* L. on carbon tetracholoride induced liver toxicity in Japanese quail. International Journal of AgriScience 4: 362-369.

Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T & Aromaa A. 2002. Flavonoid intake and risk of chronic diseases. American Journal of Clinical Nutrition, 76: 560–568.

Kodai S, Takemura S, Minamiyama Y, Hai S, Yamamoto S, Kubo S, Yoshida Y, Niki E, Okada S, Hirohashi K & Suehiro S. 2007. *S*-allyl cysteine prevents CCl_4-induced acute liver injury in rats. Free radical Research, 41: 489-497. DOI: 10.1080/10715760601118361

Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CL & Etherton TD. 2004. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. Annual Reviews of Nutrition, 24: 511–538. DOI: 10.1146/annurev.nutr. 23.011702.073237

Kumar SS, Kumar BR & Mohan GK. 2009. Hepatoprotective effect of *Trichosanthes cucumerina* Var *cucumerina* L. on carbon tetrachloride induced liver damage in rats. Journal of Ethnopharmacology, 123: 347-350. DOI: 10.1016/j.jep.2009.02.023

Lin H, Decuypere E & Buyse J. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144: 11–17. DOI: 10.1016/j.cbpa.2006.01.032

Manibusan MK, Odin M & Eastmond DA. 2007. Postulated carbon tetrachloride mode of action: A review, Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews, 25: 185-209. DOI: 10.1080/10590500701569398

Mehri M, Sabaghi V & Bagherzadeh-Kasmani F. 2015. *Mentha piperita (peppermint)* in growing Japanese quails' diet: Serum biochemistry, meat quality, humoral immunity. Animal Feed Science and Technology, 206: 57-66. DOI: 10.1016/j.anifeedsci.2015.05.022

Muriel P & Mourelle M. 1990. Prevention by silymarin of membrane alterations in acute CCL_4 liver damage. Journal of Applied Toxicology, 10: 275-279. DOI: 10.1002/jat.2550100408

Neshat Gharamaleki M & Mohajeri D. 2014. Study the protective effects of Black Cumin (*Nigella sativa linn*.) ethanolic extract against Rifampin-induced hepatotoxicity in rats. Qom University of Medical Sciences Journal, 8: 73-84.

NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press. Washington, DC. 176 Pages.

Panovska TK, Kulevanova S, Gjorgoski I, Bogdanova M & Petrushevska G. 2007. Hepatoprotective effect of the ethyl acetate extract of *Teucrium polium L.* against carbontetrachloride-induced hepatic injury in rats. Acta Pharmaceutica, 57: 241–248. DOI: 10.2478/v10007-007-0020-x

Parmar MY, Shah PA, Thakkar VT, Al-Rejaie SS & Gandhi TR. 2012. Hepatoprotective effect of *amomum subulatum* roxb seeds on carbon tetrachloride-induced liver damage in rats. Journal of Pharamcy, 2: 38-43.

Ponte PIP and Rosado CMC. 2008. Pasture intake improves the performance and meat sensory attributes of free-range in broilers. Poultry Science, 87: 71-79. DOI: 10.3382/ps. 2007-00147

Redman CM. 1969. Biosynthesis of serum proteins and ferritin by free and attached ribosomes of rat liver. Journal of Biological Chemistry, 244: 4308-4315.

SAS Institute. 2003. SAS/STAT® 9. User's Guide. SAS Institute Inc. Cary, NC. USA.

Schaffer S, Eckert GP, Müller WE, Llorach R, Rivera D, Grande S, Galli C & Visioli F. 2004. Hypochlorous acid scavenging properties of local mediterranean plant foods. Lipids, 39: 1239-1247. DOI: 10.1007/s11745-004-1353-9

Sharma A, Sharma MK & Kumar M. 2006. Protective effect of *Mentha piperita* against arsenic-induced toxicity in liver of swiss albino mice. Basic and Clinical Pharmacology and Toxicology, 100: 249–257. DOI: 10.1111/j.1742-7843.2006.00030.x

Soni B & Nishant P. 2008. Ameliorative action of cyanobacterial phycoerythrin on CCl_4-induced toxicity in rats. Toxicology 248: 59-65. DOI: 10.1016/j.tox.2008.03.008

Sonkusale P, Bhandarker AG, Kurkare NV, Ravikanth K, Maini S & Sood D. 2011. Hepatoprotective activity of superliv liquid and repchol in CCl_4 induced FLKS syndrome in broilers. International Journal of Poultry Science, 10: 49-55.

Soto C, Mena R, Luna J, Cerbón M, Larrieta E, Vital P, Uria E, Sánchez M, Recoba R, Barrón H, Favari L & Lara A. 2004. Silymarin induces recovery of pancreatic function after

alloxan damage in rats. Life Sciences, 75: 2167-2180. DOI: 10.1016/j.lfs.2004.04.019

Soto C, Recoba R, Barrón H, Alvarez C & Favari L. 2003. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 136: 205-212. DOI: 10.1016/S1532-0456(03)00214-X

Tsukamoto H, Matsuoka M & French SW. 1990. Experimental models of hepatic fibrosis: a review. Seminars in Liver Disease, 10: 56-65.

Tumova L, Tuma J, Megusar K & Dolezal M. 2010. *Substituted pyrazinecarboxamides as* abiotic elicitors of flavolignan production in *Silybum marianum* (L.) gaertn cultures in vitro. Molecules, 15: 331-340. DOI: 10.3390/molecules15010331

Wang CH, Zhang T, Cul X, Li SH, Zhao X & Zhang X. 2013. Hepatoprotective effects of a Chinese herbal formula, Longyin Decoction, on carbon-tetrachloride-induced liver injury in chickens. Evidence-based Complementary and Alternative Medicine, 39: 27-43. DOI: 10.1155/2013/392743