Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2017.11432.1194

The Effects of Silymarin on Oxidative Status and Bone Characteristics in Japanese Quail Subjected to Oxidative Stress Induced by Carbon Tetrachloride

Moradi F[1], Samadi F[1], Dastar B[2] & Samadi S[3]

[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]PhD Student of Horticulture Department of Shiraz University, Iran

Poultry Science Journal 2017, 5 (2): 97-104

Keywords
Bone index
Japanese quail
Oxidative stress
Blood biochemical parameters

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

Article history
Received: August 4, 2016
Revised: June 6, 2017
Accepted: June 23, 2017

Abstract

This experiment was conducted to assess the effects of Silymarin on oxidative status, bone characteristics, and some blood parameters in Japanese quail subjected to oxidative stress induced by carbon tetrachloride (CCl_4). The experiment was performed as a completely randomized design with four replicates, each with 30 birds, using a 2 × 2 factorial arrangement with two doses of Silymarin (0 and 1 mL/kg BW) and CCl_4 (0 and 1 mL/kg BW). Results revealed that the interaction between Silymarin and CCl_4 on concentrations of total cholesterol, triglycerides, glucose, albumin, calcium, and alkaline phosphatase were significant ($P < 0.05$). In contrast, concentrations of phosphorus, total protein, and high density lipoprotein-cholesterol in blood serum did not differ between experimental treatments. Experimental treatments had a significant effect on superoxide dismutase activity in blood serum ($P < 0.05$), but not on glutathione peroxide activity and malondialdehyde concentration. Experimental treatments significantly affected the weight, thickness, and external and internal diameters of tibia bone ($P < 0.05$), but not its length, ash, volume, and density. This study shows that Silymarin has potential to attenuate adverse effects of oxidative stress induced by CCl_4 in Japanese quail.

Introduction

Poultry experience various stressors which can result in oxidative stress. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds their elimination by different antioxidant agents. ROS damage biomolecules by disrupting intracellular reduction-oxidation balance (Khan and Sultana, 2009).

Carbon tetrachloride (CCl_4) is a model toxicant that has been assessed in both *in vitro* and *in vivo* toxicological studies (Manibusan *et al*., 2007). CCl_4 causes serious damage to tissues by stimulating lipid peroxidation (Janbaz *et al*, 2002). Bone tissue is sensitive to free radical damage (Basu *et al*., 2001) as ROS influences bone mineralization by modulating bone cell function (Wauquier *et al*., 2009).

It was reported that medical plants can remove hydrogen peroxide and have a role in the preventing the side effects of oxidative stress (Schaffer *et al*., 2004; Sonkusale *et al*., 2011). Milk

Please cite this article as: Moradi F, Samadi F, Dastar B & Samadi S. 2017. The Effects of Silymarin on Oxidative Status and Bone Characteristics in Japanese Quail Subjected to Oxidative Stress Induced by Carbon Tetrachloride. Poult. Sci. J. 5 (2): 97-104.

thistle (*Silybum marianum*), known as Silymarin, is a herb with antioxidant properties and contains a mixture of flavonolignans with the active ingredients. The main substances in seed extract of this plant are silybin, isosilybin, silycristin, and silydianin (Ding *et al*, 2001). *Silybum marianum* has numerous functions including antioxidant (Katiyar, 2005), anti-inflammatory (Horváth *et al*, 2001) and immune modulation (Katiyar, 2005), and protection against non-melanoma skin cancer (Vayalil *et al,* 2003). As an antioxidant polyphenol flavonoid, Silymarin has the natural ability to clean up free radicals (Skottova & krecman 1998). In this study, *in vivo* antioxidant function of Silymarin was assessed against oxidative stress induced by CCl_4 in Japanese quail.

Materials and Methods

Plant preparation and extraction

Silybum marianum used in this experiment was collected in the summer from the heights of Ravansar (34° 52′ N 46° 27′ E; altitude: 1650 m), Kermanshah, Iran. The total values of phenolic compounds, flavonoids, and antioxidants were measured calorimetrically according to the Folin-Ciocalteu method (Chang *et al*., 2002). To prepare Silymarin, *S. marianum* seeds were powdered by a full electric mill (DM-WP120), then defatted by petroleum ether. Samples were dried and mixed (2:10 v/w) with 80% ethanol, then shacked for 24 hrs and passed through paper filter. The alcohol fraction was removed under vacuum (Harbone, 1998).

Birds, diets and experimental design

A total of 480 day-old Japanese quail chicks (BW±SE, 7±1 g) were obtained from a local commercial hatchery and raised over a 42-d experimental period. The birds were housed in an environmentally controlled poultry house with paper roll as litter at the research farm of Animal Science Faculty, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan, Iran. Ambient temperature was initially set at 38±1°C and then decreased by 1°C every 2 days until a constant temperature of 24°C was reached at day 35 of experiment. The lighting schedule provided 23 hrs of light per day.

The experiment was performed as a completely randomized design with four replicates of 30 birds each, using a 2 × 2 factorial arrangement with two doses of Silymarin (0 and 1 mL/kg BW) and CCl_4 (0 and 1 mL/kg BW). Experimental treatments were applied beginning on day 22 of the study. Silymarin was fed directly into crop *via* a syringe equipped with a plastic nozzle and feeding tube (Nova Cath®, No. 10). Intraperitoneal injection of CCl_4 was performed beginning on day 22, and again every three days (Sonkusale *et al*., 2011). In order to homogenize the stress, the control group received 1 mL/kg BW 0.9% sodium chloride solution *via* intraperitoneal injection (Sharma *et al*., 2006) and also 1 mL/kg BW of distilled water by oral gavage. Birds had free access to feed and water throughout the experiment. The composition of the basal diet is shown in Table 1.

Table 1. Ingredients and chemical compositions of the 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] Claculated composition was 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.

Traits measured

On day 42, two birds from each replicate were selected, and blood samples were collected in nonheparinized tubes from the brachial vein. Sera were obtained by centrifuging at 1500 × *g* for 7 min at 4°C and stored at -20°C until biochemical analysis (Khodadust *et al*., 2015). The serum samples were analyzed for total

protein, albumin, glucose, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-c), calcium, phosphorous, and alkaline phosphatase (ALP) activity using enzymatic related kits (Pars-Azmoon Co., Tehran, Iran).

The activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured in red blood cells. Blood serum SOD activity was measured according to the Woolliams *et al.,* (1983). Briefly, the inhibition of nitroblu tetrazolium (NBT) reduction with xanthine-xanthine oxidase was used as a superoxide generator. One unit of SOD function was defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Activity of GPX was determined according to methods described by Paglia and Valentine (1967).

Following the slaughtering on day 42, the right tibia from two birds of each replicate was collected. Tibia length was measured as distance between the two ends of the bone using a digital caliper (ACCUD SERIES 111). Tibia volume was measured under the assumption that the specific gravity of water is 1 g/cm^3 at room temperature (Kim *et al*, 2004). External and internal diameters and tibia thickness were determined using a digital caliper. To measure tibia density, bones were placed for 48 hrs in an oven at 60°C to dry. Thereafter, they were cooled in desiccators and weighed. Bone density was calculated by dividing the dry weight of each bone to its volume (Zhang & Coon, 1997). Tibia ash was determined following its defatting by chloroform (AOAC, 1980).

Statistical analysis

The data was analyzed using GLM procedures of SAS software (SAS, 2003). Duncan's multiple tests were used to compare the effects of treatment. Differences were considered statistically significant at $P < 0.05$.

Results

Total phenol, flavonoids, and antioxidant of Silymarin

In vitro examinations showed that the prepared Silymarin contained 2.606 mg/g total phenol, 1.964 mg/g flavonoids, and 74.231% antioxidants.

Blood biochemistary

The main effects of Silymarin, CCl_4, and their interactions on blood serum biochemistry in Japanese quail chicks are depicted in Table 2. In birds treated with Silymarin, albumin concentration was greater, whereas ALP hepatic enzyme activity and cholesterol and glucose concentrations were lower ($P < 0.05$). Administration of CCl_4 resulted in lower concentrations of calcium and triglyceride and a higher concentration of glucose ($P < 0.05$). There were significant interactions between Silymarin and CCl_4 on albumin, calcium, ALP, triglycerides, cholesterol, and glucose in blood sera ($P < 0.05$).

Table 2. Effect of Silymarin and carbon tetrachloride (CCl_4) on blood biochemical parameters of Japanese quail at 42 d

	Total protein (g/dL)	Albumin (g/dL)	Calcium (mg/dL)	Phosphor (mg/dL)	ALP[1] (U/L)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL[2] (mg/dL)	Glucose (mg/dL)
Silymarin (mL/kg BW)									
0	2.97^{b}	1.1^{b}	9.9	8.5	218.8^{a}	156.4	165.9^{a}	76.0	304.7^{a}
1	3.25^{a}	1.3^{a}	10.1	8.5	188.7^{b}	156.9	151.4^{b}	70.6	294.3^{b}
SEM	0.05	0.01	0.3	1.5	13.48	5.10	1.88	3.32	0.73
CCl_4 (mL/kg BW)									
0	3.15	1.2	10.4^{a}	8.3	206.5	178.3^{a}	162.7	74.2	297.5^{b}
1	3.07	1.1	9.5^{b}	8.7	201.0	135.0^{b}	154.6	72.4	304.5^{a}
SEM	0.07	0.01	0.12	1.4	13.97	3.57	2.13	3.45	0.74
Silymarin × CCl_4									
0 × 0	3.0	1.1^{bc}	10.3^{a}	8.1	206.7^{a}	179.3^{a}	170.6^{a}	81.2	301.4^{a}
1 × 0	3.3	1.3^{a}	10.3^{a}	8.5	206.4^{a}	177.4^{a}	154.8^{ab}	67.2	287.6^{b}
0 × 1	2.9	1.1^{c}	9.3^{b}	8.8	231.0^{a}	133.6^{b}	161.2^{ab}	70.8	308.0^{a}
1 × 1	3.2	1.2^{ab}	9.7^{b}	8.5	171.0^{b}	136.4^{b}	148.0^{b}	74.0	301.0^{a}
SEM	0.05	0.01	0.1	1.6	13.05	3.78	1.86	82.9	53.0
P-value									
Silymarin	0.01	0.00	0.36	0.95	0.02	0.97	0.01	0.23	0.01
CCl_4	0.50	0.41	0.00	0.50	0.70	0.00	0.19	0.69	0.02

[1]Alkaline phosphatase. [2] High-density lipoprotein cholesterol.

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

Antioxidant status

The main effects of Silymarin, CCl_4, and their interactions on the activities of antioxidant enzymes and MDA content in sera of Japanese quails are shown in Table 3. The interaction between Silymarin and CCl_4 was significant on SOD activity ($P < 0.05$), but not GPX activity and MDA content. Silymarin decreased activity of SOD hepatic enzyme in blood serum, while CCl_4 increased SOD activity ($P < 0.05$).

Table 3. Effect of Silymarin and carbon tetrachloride (CCl_4) on antioxidant enzymes and MDA of Japanese quail at 42 d

Treatments	SOD[1] (U/gHb)	GPX[2] (U/gHb)	MDA[3](nmol/L)
Silymarin (mL/kg BW)			
0	1271.4[a]	50.26	2.08
1	1007.1[b]	47.08	2.22
SEM	3.89	2.36	0.11
CCl_4 (mL/kg BW)			
0	1098.5	49.02	2.27
1	1180.0	48.32	2.03
SEM	4.86	2.51	0.10
Silymarin × CCl_4			
0 × 0	1362.0[a]	51.84	2.34
1 × 0	835.0[c]	46.20	2.20
0 × 1	1180.8[b]	48.68	1.82
1 × 1	1179.2[b]	47.96	2.24
SEM	2.38	2.4	0.08
P-value			
Silymarin	0.00	0.14	0.36
CCl_4	0.42	0.75	0.11
Silymarin × CCl_4	0.04	0.31	0.06

[1]Superoxide dismutase. [2] Glutathione peroxidase. [3] Malondialdehyde.

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

Table 4. Effect of Silymarin and carbon tetrachloride (CCl_4) on Index of Tibia Bone of Japanese quail at 42 d

Treatments	Weight (g)	Length (mm)	Volume (cm^3)	Density (g/ cm^3)	Thickness (mm)	External diameter (mm)	Internal diameter (mm)	Ash (%)
Silymarin (mL/kg BW)								
0	0.78[a]	51.55	0.78	0.96	0.59	3.64[a]	2.89	0.55
1	0.72[b]	51.23	0.74	0.96	0.53	3.44[b]	3.11	0.53
SEM	0.002	0.44	0.002	0.000	0.004	0.02	0.08	0.002
CCl_4 (mL/kg BW)								
0	0.76	51.60	0.78	0.97[a]	0.60[a]	3.63[a]	2.81[b]	0.56[a]
1	0.74	51.20	0.75	0.96[b]	0.52[b]	3.45[b]	3.19[a]	0.52[b]
SEM	0.003	0.43	0.002	0.001	0.004	0.02	0.06	0.001
Silymarin × CCl_4								
0 × 0	0.80[a]	51.54	0.81	0.97	0.56[b]	3.44[b]	2.87[b]	0.56
1 × 0	0.71[b]	50.84	0.74	0.97	0.63[a]	3.46[b]	2.76[b]	0.57
0 × 1	0.71[b]	51.56	0.75	0.96	0.50[b]	3.45[b]	2.92[b]	0.51
1 × 1	0.72[b]	51.61	0.74	0.96	0.54[b]	3.81[a]	3.46[a]	0.53
SEM	0.002	0.44	0.002	0.000	0.03	0.01	0.01	0.001
P-value								
Silymarin	0.01	0.44	0.12	0.4	0.07	0.01	0.11	0.44
CCl_4	0.47	0.34	0.14	0.02	0.01	0.03	0.00	0.02
Silymarin × CCl_4	0.04	0.52	0.08	0.14	0.01	0.01	0.01	0.12

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

Bone indexes

The main effect of Silymarin, CCl_4, and their interactions on bone indexes of Japanese quail at day 42 are shown in Table 4. Silymarin and CCl_4 had significant interactions on weight, thickness, and external and internal diameters of tibia. Both Silymarin and CCl_4 decreased tibia weight ($P < 0.05$). Tibia thickness increased in quails treated with Silymarin, but CCl_4 did not have an effect ($P < 0.05$). The external and internal diameters of tibia bone increased in birds that received both treatments ($P < 0.05$). CCl_4 resulted in lower density and ash percentage of tibia bone ($P < 0.05$).

Discussion

Stressful conditions are unavoidable problems in poultry production systems. There is an increasing interest in the use of herbs and medicinal plants in poultry feeding to help overcome the stress. Medicinal plants have positive effects on performance of broilers due to their secondary metabolites, which can be ergogenic, antiphrastic, antibacterial, anti-flatus, antifungal, and antiseptic (Huang *et al*., 1992). It has been shown that Silymarin reduces the concentrations of glucose, cholesterol, and triglyceride in blood of broilers challenged with lead (Ebrahimi *et al*, 2013). Consistent with the current study, Case *et al.* (1995) reported that plant secondary metabolites (for example, thymol, carvacrol, and silybin) may inhibit the function of hepatic enzymes involved in the synthesis of cholesterol and fatty acids. Tollba *et al.* (2010) demonstrated that using various herbal ingredients can reduce blood lipids in broilers, hens and quails. Silymarin can also impact the kinetics of glucose 6–phosphatase and inhibit gluconeogenesis to decrease blood glucose (Guigas *et al*, 2007). Fanimakki *et al.,* (2014) reported that Silymarin has no effects on total protein and albumin in blood serum, which contrasts our findings in albumin. It seems that this difference is due to the sensitivity of different species, as well as the form of Silymarin (powder or extract) used. It was reported that Silymarin has no effect on blood serum calcium and phosphorus (Amiridumari *et al.*, 2013), which is consistent with the current study. Sonkusale *et al* (2011) reported that intraperitoneal injection of CCl_4 to broilers significantly increased blood concentrations of total cholesterol and decreased serum concentrations of albumin, total protein and HDL-c. Samudram *et al*. (2008) reported a lower total protein levels in blood serum following the destructive effects of CCl_4 on the function of rat liver. The decrease in blood serum levels of total protein and albumin could be due to its reduced biosynthesis, which could occur by impairing ribosomal protein biosynthesis of endoplasmic reticulum (Clawson, 1989). Similarly, Gad *et al.* (2011) showed that CCl_4 decreases serum protein and albumin levels, but increases serum lipid levels, showing the adverse effect of CCl_4 on liver function. Owen (1990) noted an increase in serum cholesterol under liver diseases related to undermining role of the liver to remove cholesterol from the blood stream.

CCl_4 can lead to liver damage and necrosis by inducing production of free radicals. Hepatic necrosis elevates blood serum enzymes such as ALP and decreases activities of antioxidant enzymes such as SOD and catalase (Eidi, 2010) which is consistent with our findings of SOD. Studies have shown that the antioxidant properties of Silymarin can increase cellular glutathione, superoxide dismutase, and glutathione peroxidase function in brain tissue of rat (Nencini *et al*, 2007) which contrasts the current experiment. These differences could be due to differences in the duration of the experiment, the amount of Silymarin used, experimental materials, and the studied tissues (brain or blood stream). The administration of Silymarin in rats with diabetes mellitus leads to appropriate changes in MDA (an index of lipid peroxidation) and antioxidant enzymes such as SOD **(**Baluchnejadmojarad *et al*, 2010). However, in our study, CCl_4 did not increase lipid peroxidation, which could be due to the sensitivity of experimental species and usage dose.

Studies in humans and animals have shown that secondary metabolites of plants have modulatory effects on bone mineralization (Sunita & Pattanayak, 2011). The current study shows that Silymarin has more positive effects on tibia thickness than CCl_4, perhaps due to the similarity between Silybin and estrogen in the mineralization of bone tissue (Gebhardt, 2002). Estrogen has positive effects on bone mineralization by increasing the function of the vitamin D receptor in intestinal mucosa and osteoblast cells, which leads to synthesis of bone

matrix protein and mineral storage (Liel *et al*, 1999). Similarly, Kim *et al*., (2012) documented osteoblastic function of Silymarin in tibia-fractured mice. The positive effects of medicinal plants in the chemical composition and physical properties of tibia in broiler chickens were reported (Pisarski & Kwiecien, 2003). Tahmasebi *et al*. (2012) showed that herbal phytoestrogens such as *Withania somnifera* enhances bone calcification. Few studies have been conducted on the effects of toxins, especially CCl_4, on tissues and bone characteristics. It has been documented that CCl_4 can disrupt the balance between oxidants and antioxidants, which can cause oxidative damage to biological molecules (Soni *et al*, 2008), such lipid peroxidation in bone tissue. For example, teratogenic effects of the pesticide diazinon were reported on the growth of cartilage and bone (Meneely & Wyttenbach, 1989) in chicken and quail embryos. Field studies have correlated exposure to organochlorines with loss of alveolar bone in gray seals (Bergman *et al*, 1992), reduced bone density in deer mice (Johnson *et al*., 2009), and altered mineralization in clapper rails (Rodriguez-Navarro *et al*., 2006). In general, it can be concluded that Silymarin has potential to attenuate adverse effects of oxidative stressors in Japanese quail by stabilizing lipid peroxidation.

Acknowledgements
The authors would like to thank Gorgan University of Agricultural Sciences and Natural Resources for the financial and facilities support.

References

Amiridumari H, Sarir H, Afzali N & Fani Makki O. 2013. Effects of milk thistle seed against aflatoxin B_1 in broiler model. Journal of Research in Medical Sciences, 18: 786-790.

Association of Official Analytical Chemists. 1980. Method of Analysis 13th Edition. Washington, DC.

Baluchnejadmojarad T, Roghani M & Khastehkhodaie Z. 2010. Chronic treatment of silymarin improves hyperalgesia and motor nerve conduction velocity in diabetic neuropathic rat. Phytotherapy Research, 24: 1120-1125. DOI: 10.1002/ptr.3078

Basu S, Michaëlsson K, Olofsson H, Johansson S & Melhus H. 2001. Association between oxidative stress and bone mineral density. Biochemical and Biophysical Research Communications, 288:275–279. DOI: 10.1006/bbrc.2001.5747

Bergman A, Olsson M & Reiland S. 1992. Skull-bone lesions in the Baltic grey seal (*Halichoerus grypus*). AMBIO, 21: 517–519.

Case GL, He L, Mo H & Elson CE. 1995. Induction of geranyl pyrophosphate pyrophosphatase activity by cholesterol-suppressive isoprenoids. Lipids, 30: 357-359. DOI: 10.1007/BF02536045

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.

Clawson GA. 1989. Mechanisms of carbon tetrachloride hepatotoxicity. Journal of Pathology and Immunopathology Research, 8: 104-112. DOI: 10.1159/000157141

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, Mohamadabadi T, Sari M, Salari S, Zamiri MJ & Beige Nasiri MT. 2013. Effect of Silymarin against lead induced in broiler chickens. Iranian Journal of Animal Science Research, 5: 302-312.

Eidi A, Eidi M, Al-Ebrahim M, Haeri Rohani A, Mortazavi P. 2010. Protective effects of sodium molybdate on carbon tetrachloride-induced hepatotoxicity in rats. Journal of Trace Elements in Medicine and Biology, 14: 67-71. DOI: 10.1016/j.jtemb.2010.12.003

Fani Makki O, Omidi A, Afzali N, Sarir H, Frouzanmehr M & Shibak A. 2014. Effecacy of *Silybum Marianum* seeds in ameliorating the toxic effects of aflatoxin B_1 in Broilers. Iranian Journal of Toxicology, 8:977-982.

Gad AS, Khadrawy YA, El-Nekeety AA, Mohamed SR, Hassan NS & Abdel-Wahhab MA. 2011. Antioxidant activity and hepatoprotective effects of whey protein and *Spirulina* in rats. Nutrition, 27: 582-589. DOI: 10.1016/j.nut.2010.04.002

Gebhardt R. 2002. Oxidative stress, plant-derived antioxidants and liver fibrosis. Planta Medica, 68: 289-296. DOI: 10.1055/s-2002-26761.

Guigas B, Naboulsi R, Villanueva GR, Taleux N, Lopez-Novoa JM, Leverve XM & El-Mir MY. 2007. The flavonoid silibinin decreases glucose-6-phosphate hydrolysis in perifused rat hepatocytes by an inhibitory effect on glucose-6-phosphatase. Cellular Physiology and Biochemistry, 20:925-934. DOI: 10.1159/000110453

Harborne AJ. 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer. London, 302 Pages.

Horváth ME, González-Cabello R, Blázovics A, Looij MVD, Barta I, Müzes G, Gergely P & Fehér J. 2001. Effect of silibinin and vitamin E on restoration of cellular immune response after partial hepatectomy. Journal of Ethnopharmacology, 77: 227- 232. DOI: 10.1016/S0378-8741(01)00298-7

Huang YF, Ma HL, Wu DF, Zhou JI, Zhou KS & Qi ZY. 1992. Effect of chinese medicinal herbs additives on the growth of broilers. Journal of Fujian Agriculture College, 21: 93-96.

Janbaz KH, Saeed SA & Gilani AH. 2002. Protective effect of rutin on paracetamol-and CCl_4-induced hepatotoxicity in rodents. FITOTERAPIA, 73: 557-563. DOI: 10.1016/S0367-326X(02)00217-4

Johnson KE, Knopper LD, Schneider DC, Ollson CA & Reimer KJ. 2009. Effects of local point source polychlorinated biphenyl (PCB) contamination on bone mineral density in deer mice (*Peromyscus maniculatus*). Science of the Total Environment, 407: 5050–5055. DOI:10.1016/j.scitotenv.2009.06.004

Katiyar SK. 2005. Silymarin and skin cancer prevention: Anti-inflammatory, antioxidant and immunomodulatory effects (Review). International Journal of Oncology, 26: 169-176. DOI: 10.3892/ijo.26.1.169

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

Kim JL, Park SH, Jeong D, Nam JS & Kang YH. 2012. Osteogenic activity of silymarin through enhancement of alkaline phosphatase and osteocalcin in osteoblasts and tibia fractured mice. Experimental Biology and Medicine, 237: 417-428. DOI: 10.1258/ebm.2011.011376

Kim WK, Donalson LM, Herrera CL, Woodward LF, Kbena DJ, Nisbet & Ricke. 2004. Effects of different Bone preparation methods (fresh, dry, and fat-free dry) on bone parameters and the correlations between bone breaking strength and the other bone parameters. Poultry Science, 83: 1663-1666. DOI: 10.1093/ps/83.10.1663

Liel Y, Shany S, Smirnoff P, & Schwartz B. 1999. Estrogen increases 1, 25-dihydroxyvitamin D receptors expression and bioresponse in the rat duodenal mucosa. Endocrinology, 140: 280-285. DOI: 10.1210/endo.140.1.6408

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

Meneely G & Wyttenbach CR. 1989. Effects of the organophosphate insecticides diazinon and parathion on bobwhite quail embryos: Skeletal defects and acetylcholinesterase activity. Journal of Experimental Zoology, 252: 60-70. DOI: 10.1002/jez.1402520109

Nencini C, Giorgi G & Micheli L. 2007. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine, 14: 129-135. DOI: 10.1016/j.phymed.2006.02.005

Owen JS. 1990. Extrahepatic cell membrane lipid abnormalities and cellular dysfunction in liver disease. Drugs, 40: 73-83. DOI: 10.2165/00003495-199000403-00008

Paglia DE & Valentina WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70: 158-169.

Pisarski RK & Kwiecien M. 2003. The effect of hulless oats and Barley upon the chemical composition and durability of broiler chickens tibia bones. Annales Universitatis Mariae Curie-Sklodowska, 21: 215- 221.

Rodriguez-Navarro AB, Romanek CS, Alvarez-lloret P & Gaines KF. 2006. Effect of *in ovo* exposure to PCBs and Hg on clapper rail bone mineral chemistry from a contaminated

salt marsh in coastal georgia. Environmental Science and Technology, 40: 4936–4942. DOI: 10.1021/es060769x

Samudram P, Rajeshwari H, Vasuki R, Geetha A & Sathiya P. 2008. Hepatoprotective activity of Bi-herbal ethanolic extract on CCl_4 induced hepatic damage in rats. African Journal of Biochemistry Research, 2: 61-65.

SAS (Statistical Analysis System). 2003. SAS/STAT 9. User's Guide. SAS Intitute Inc. Cary, North Carolina.

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 & Clinical Pharmacology &Toxicology, 100: 249–257. DOI: 10.1111/j.1742-7843.2006.00030.x

Skottova N & Krecman V. 1998. Silymarin as a potential hypocholesterolaemic drug. Physiological Research, 47: 1-7.

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.

Soni B, Visavadiya NP, Madamwar D. 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

Sunita P & Pattanayak SP. 2011. Phytoestrogens in postmenopausal indications: A theoretical perspective. Pharmacognosy Review, 5: 41-47. DOI: 10.4103/0973-7847.79098

Tahmasbi AM, Mirakzehi MT, Hosseini SJ, Agah MJ & Kazemi Fard M. 2012. The effects of phytase and root hydroalcoholic extract of *Withania somnifera* on productive performance and bone mineralisation of laying hens in the late phase of production. British Poultry Science, 53: 204-214 DOI: 10.1080/00071668.2012.662628

Tollba AAH, Shabaan SAM & Abdel-Mageed MAA. 2010. Effects of using aromatic herbal extracts and blended with organic acids on productive and physiological performance of poultry. 2- The growth during cold winter stress. Egyptian Poultry Science Journal, 30: 229-248.

Vayalil PK, Elmets CA & Katiyar SK. 2003. Treatment of green tea polyphenols in hydrophilic cream prevents UVB-induced oxidation of lipids and proteins, depletion of antioxidant enzymes and phosphorylation of MAPK proteins in SKH-1 hairless mouse skin. Carcinogenesis, 2: 927-936. DOI: 10.1093/carcin/bgg025

Wauquier F, Leotoing L, Coxam V, Guicheux J & Wittrant Y. 2009. Oxidative stress in bone remodeling and disease. Trends in Molecular Medicine, 15: 468-477. DOI: 10.1016/j.molmed.2009.08.004

Woolliams JA, Wiener G, Anderson PH & Mcmurray CH. 1983. Variation in the activities of glutathione-peroxidase and superoxide-dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Research in Veterinary Science, 34: 253-256.

Zhang B & Coon CN. 1997. The relationship of various tibia bone measurements in hens. Poultry Science, 76: 1698-1701. DOI: 10.1093/ps/76.12.1698