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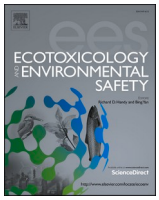

Potential ameliorative effect of dietary quercetin against lead-induced oxidative stress, biochemical changes, and apoptosis in laying Japanese quails

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ABSTRACT

Lead (Pb) is a widespread environmental pollutant which is a toxic threat to human and animal health. The present study was designed to evaluate the ameliorative role of quercetin in laying quails exposed to Pb. A total of 112 birds were randomly divided into four groups. The control group was fed with basal diet, the Pb group was fed with ration supplemented with Pb at the dose of 100 mg/kg (as Pb (II) acetate trihydrate), the Quercetin group was fed with ration supplemented with quercetin at the dose of 400 mg/kg, and the Pb+ Quercetin group was fed with ration supplemented with Pb at the dose of 100 mg/kg and quercetin at dose of 400 mg/kg. Results showed that serum total protein, glucose, albumin, and blood urea nitrogen (BUN) values of the Pb + Quercetin group partially improved with quercetin supplementation. Meanwhile, serum creatinine values of the Pb + Quercetin group was found to be significantly lower than that of the Pb group. Aspartate aminotransferase (AST) and alanine transaminase (ALT) enzyme activities in the Quercetin and Pb + Quercetin groups were similar to those of the Control group, unlike the Pb group. Moreover, alkaline phosphatase (ALP) enzyme activity of the Pb + Quercetin group significantly improved with the addition of quercetin. We also found that malondialdehyde (MDA) levels of the kidney, liver, and heart were significantly reduced by quercetin supplementation. The glutathione, catalase, and glutathione peroxidase activities of the kidney, liver, and heart tissue were increased by quercetin supplementation. These results were in line with the observed apoptotic markers. The expression of caspase-3 and caspase-9 were significantly decreased by quercetin supplementation. It may be concluded that dietary supplementation with quercetin ameliorates the toxic effects of Pb exposure by alleviating oxidative stress, biochemical changes, and apoptosis in quails.

1. Introduction

Lead (Pb) is a chemical element with the symbol Pb in the carbon group. It is the first element characterized by its toxicity. A wide range of toxic effects of Pb in animal systems has been observed and it is known as one of the permanent heavy metals found everywhere. Pb poisoning is a real threat to public health, especially in developing countries. Therefore, great efforts are being made to reduce the dangers of this metal in terms of occupational and public health. Hematopoietic, kidney, reproductive, and central nervous systems are part of the human body that are vulnerable to high levels of Pb exposure (Assi et al., 2016). High doses of Pb result in reproductive disorders in animals (Wrzecinska et al., 2021). As Pb is a global environmental pollutant, primarily in industrial regions, animals may be easily exposed to Pb. Pb poisoning in animals is usually from many environmental sources, and this may be monitored from the industrial pollution and agricultural applications by the contamination of soil and feed. In addition, exposure to high doses of Pb may lead to decreased performance, poisoning, or even death in animal (Seven et al., 2021). Pb is an environmental pollutant which threatens human and animal life in many ways, especially during the

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development age Pb may cause oxidative damage to the brain, heart, kidney, and reproductive organs. The mechanism of Pb-induced oxidative stress involves the effects of Pb on the membranes, DNA, and antioxidant defense systems of cells (Seven et al., 2021). Moreover, it is reported that the toxic effects of Pb may cause conditions such as disruption of calcium homeostasis, stimulation of calcium release from mitochondria, production of reactive oxygen species, and disruption of oxidant/antioxidant balance in tissues (Dabrowska et al., 2015).

Plant polyphenols, especially flavonoids, draw attention due to their various biological effects (Bessa et al., 2021). Flavonoids are natural compounds found in vegetables, fruits, and medicinal plants. Flavonoids have been reported to have antiinflammatory, antioxidant, growth promoter, antiviral, liver protective, antibacterial, antiallergic, anticarcinogenic, antithrombotic, and immune system activating effects in various animals and different poultry species (Saeed et al., 2017). Quercetin, 2- (3,4-dihydroxyphenyl) -3, 5, 7- trihydroxychromen-4-one is a flavonoid commonly found in fruits and vegetable foods (Batiha et al., 2020). It is known that quercetin, which is abundant in vegetables, tea, fruit, cider, and onions, is used in poultry production and health due to its positive effects. Supplementation of quercetin may cause a lower incidence of diseases and infections, since quercetin improves the immune system by stimulating lymphocytes, macrophages, and IgY antibody production, and by increasing the activity of natural killer cells and the weight of lymphoid organs like the spleen, thymus and bursa (Saeed et al., 2017). It is also reported that quercetin has antioxidant and antibacterial effects (Batiha et al., 2020). It shows antioxidant effect by chelating with metal ions and removing free radicals (Durukan, 2020). Quercetin cleans reactive oxygen species produced by a photosynthetic electron transport system in plant cells and protects body tissues from free radicals (Kicinska and Jarmuszkiewicz, 2020). The aim of this study was to investigate the effects of quercetin in laying Japanese quails exposed to Pb toxicity. For this, the effects of quercetin on antioxidant parameters and the expression levels of apoptotic proteins were investigated in laying quails exposed to Pb toxicity.

2. Materials and methods

2.1. Supplements

Quercetin was purchased from Pure Bulk Inc (Roseburg, OR, USA). Pb (II) acetate trihydrate [(CH3COO)2 Pb.3H2O] was purchased from Merck (CAS #: 114 6080-56-4).

2.2. Animal, diet, and experimental design

A total of 112 laying quails (Coturnix Coturnix japonica) were obtained from a commercial company in Gaziantep region. Each group comprised 28 female animals at the age of five weeks (each with four replicates consisting of seven birds). The quails were randomly divided into four groups, which had four replicates including seven quails in each group. This research was carried out using the standard cages designed for laying quails with the approval of the Local Ethics Committee for Animal Experiments of the Ministry of Food, Agriculture, and Livestock (29.05.2018/2018-1). The water and feed were provided adlibitum. The photo period was 16 h/8 h (light/dark). Birds were kept in quail layer cages in a temperature-controlled room. The basal diet prepared according to the National Research Council (1994) was supplied by a commercial company (Table 1). The experimental groups were arranged as follows: fed a corn-soy bean basal ration and no supplement (Group I; Control) supplement of 100 mg/kg Pb (as Pb (II) acetate trihydrate) to the ration (Group II; Pb), supplement of 400 mg/kg quercetin to basal ration (Group III; Quercetin), and supplement of 100 mg/kg Pb and 400 mg/kg quercetin to basal diet (Group IV; Pb + Quercetin). At the end of the experiment (56 days), six quails from each group were slaughtered by decapitation for analyzes. Serum glucose, total protein, albumin, globulin, creatinine, and BUN, as well as enzyme activities of ALT, AST, and ALP, were measured using an autoanalyzer (Olympus AU-600, Olympus Corporation, Tokyo, Japan).

Table 1
Ingredients and nutrient composition of diet[a].

Ingredients	%	Nutritional Composition	%
Maize	66.27	Dry matter	89.9
Soybean meal (44% CP)	24.44	Crude protein	17.0
Wheat bran	1.31	Crude cellulose	3.31
Salt	0.25	Ether extract	1.89
L-Lysine hydrochloride	0.21	Crude ash	9.78
L-Threonine	0.13	Calcium[c]	2.50
Sodium bicarbonate	0.10	Phosphorus[c]	0.35
DL-Methionine	0.12	Sodium[c]	0.16
Vitamin-Mineral premix[b]	0.32	Lysine[c]	1.00
Limestone	5.46	Threonine[c]	0.75
Calcium phosphate	1.39	ME, kcal/kg[c]	2800
Total	**100**		

[a] Quercetin (400 mg quercetin per kg diet) was added to the basal diet.
[b] Vitamin-mineral premix (per 1 kg): vitamin A, 8000 IU; vitamin D3, 3000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg; copper (copper sulphate), 10 mg; iodine (ethylenediamine dihydriodide), 1.0 mg; iron (ferrous sulphate monohydrate), 50 mg; manganese (manganese sulphate monohydrate), 60 mg; zinc (zinc sulphate monohydrate), 60 mg; selenium (sodium selenite), 0.42 mg.
[c] Calculated.

2.3. Oxidative stress

2.3.1. Sample collection and homogenate preparation

After the quails were slaughtered, the tissues taken were washed with phosphate buffer (PBS) and each of them were wrapped in aluminum foil, placed in polyethylene bags, and labeled. The tissues were kept under − 20 °C until the analysis. For the analysis, the tissues were weighed and transferred to glass tubes by maintaining the coldness. Tris buffer (pH 7.4) was added on the tissues in a 1/10 ratio. By keeping coldness of the tissue, it was homogenized in the homogenizer. The tissue total protein was determined by Lowry et al. (1951) method.

2.3.2. Determination of malondialdehyde (MDA) level

The amount of MDA produced in the tissue was used as an indicator of the lipid peroxidation level. The MDA levels were determined to the spectrophotometric method defined by Placer et al. (1966). The pink colored complex of MDA formed by thiobarbituric acid (TBA) was measured at 532 nm.

2.3.3. Tissue glutathione (GSH) level

The GSH level was determined by the spectrophotometric method of Sedlak and Lindsay (1968). Since the color intensity of the yellow-colored complex formed by the example 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) was directly proportional to the concentration of GSH in the environment, the samples were measured at 412 nm.

2.3.4. Measurement of glutathione peroxidase (GSH-Px) levels in the tissue

The level of GSH-Px activity in the tissue was determined by the spectrophotometric method described by Lawrence and Burk (1976). The yellow-color complex which was formed as a result of mixing the samples with DTNB solution was measured at 412 nm.

2.3.5. Measurement of catalase (CAT) enzyme level in tissue

The CAT activity in tissue was determined by the spectrophotometric method described by Goth (1991). When the tissue is incubated with the substrate containing hydrogen peroxide (H_2O_2), the H_2O_2 is cleaved to H_2O and O_2 by catalase activity. The ammonium molybdate added to the medium combines with H_2O_2 to terminate the reaction. During this period, the color change was measured at 405 nm.

2.4. Western blotting

Tissues were homogenized with cold RIPA lysis buffer (Santa Cruz Biotechnology, Sc24948A), then centrifuged at 14,000 rpm at 4 °C for 45 min; then, the supernatant was separated. Total protein content was determined spectrophotometrically according to the BCA (Thermo Fisher, 23227) method (Smith et al., 1985). Samples were electrophoresed by loading 60 μg of protein in each well, and the first well with protein marker to the polyacrylamide gel (stacking gel 4%; separating gel 12%) (Laemmli, 1970). After the Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, specific proteins were transferred to the polyvinylidene difluoride (PVDF) membrane by western blotting (Towbin et al., 1979). The membranes of PVDF were blocked by 5% Bovine Serum Albumin, and then washed three times with the Tris-Buffered Saline-Tween 20 (TBST) to prevent nonspecific binding for 5 min. After blocking, the membranes were incubated with primary antibodies caspase-9 (ab69514; 1/500), caspase-3 (ab90437; 1/500), and beta-actin (ab8226; 1/5000) during the night. After incubation time, the membranes were washed three times with TBST for 5 min. Subsequently, the bands which were obtained by chemiluminescent conjugate (ECL; Bio-Rad, 1705060) on membranes treated with the appropriate secondary antibody (ab97240; 1/10000, ab6721; 1/10000) were visualized in the chemiluminescence imaging system (Bio-Rad ChemiDoc ™ XRS +) (Kielkopf et al., 2012; Bass et al., 2017). The band intensities in images were measured with the appropriate analysis system (Bio-Rad Image Lab ™ Software version 5.2.1, Bio-Rad Laboratories, Inc., USA). Protein expression levels were normalized to beta-actin, which was used as an internal control.

2.5. Statistical analyses

All values were presented as the mean ± SE. Whether the values obtained as a result of the study showed a normal distribution was determined by Shapiro–Wilk test. As a result of the Shapiro–Wilk normality analysis, it was determined that the data showed normal distribution. The differences between the mean of the groups were detected using the One-Way ANOVA analysis of variance and the post-hoc Duncan test in IBM SPSS Statistics 22 computer program (SPSS. IBM, 2013). The results were considered significant at $P < 0.05$.

3. Results

3.1. Serum biochemical parameters

When the effects of quercetin on serum biochemical parameters of the experimental groups were examined, it was seen that glucose, total protein, albumin, BUN ($P < 0.05$), and creatinine ($P < 0.01$) were positively affected by quercetin. The effects of quercetin on globulin and albumin/globulin (A/G) ratio were not found to be significant (Table 2). Serum ALT, ALP ($P < 0.001$), and AST ($P < 0.05$) activities of Quercetin and Pb + Quercetin groups were like the Control group, which is different from the Pb group.

3.2. Oxidative stress parameters

The MDA, GSH, GSH-Px, and CAT values of the kidney, liver, and heart tissues are presented in Table 3. The highest MDA levels in the kidney ($P < 0.01$), liver, and heart ($P < 0.001$) tissues were in the Pb group. The MDA levels of the heart, kidney, and liver tissues of Quercetin group were like the control, and the supplementation of quercetin in the Pb + Quercetin group significantly reduced the MDA level of the kidney ($P < 0.01$), liver, and heart ($P < 0.001$). The GSH, GSH-Px and CAT values of the kidney ($P < 0.01$), heart, and liver tissues ($P < 0.001$) in the Pb group were significantly lower than the other groups.

Table 2
Effect of lead and/or quercetin serum biochemical parameters of experimental groups.

Parameters	Control	Pb	Quercetin	Pb+Quercetin	P
Glucose (mg/dL)	141.50 ± 14.60^{b}	211.17 ± 7.04^{a}	173.67 ± 17.69^{ab}	203.67 ± 23.73^{a}	*
Total Protein (g/dL)	2.90 ± 0.12^{a}	2.43 ± 0.07^{b}	2.85 ± 0.12^{a}	2.62 ± 0.16^{ab}	*
Albumin (g/dL)	1.37 ± 0.15^{a}	1.00 ± 0.06^{b}	1.30 ± 0.07^{a}	1.18 ± 0.03^{ab}	*
Globulin (g/dL)	1.53 ± 0.22	1.47 ± 0.04	1.55 ± 0.06	1.44 ± 0.14	NS
A/G ratio	1.19 ± 0.47	0.78 ± 0.05	0.84 ± 0.03	0.85 ± 0.06	NS
Creatinine (mg/dL)	0.03 ± 0.02^{b}	0.09 ± 0.01^{a}	0.03 ± 0.01^{b}	0.06 ± 0.01^{ab}	**
BUN (mg/dL)	11.17 ± 1.58^{b}	17.67 ± 1.93^{a}	9.50 ± 1.34^{b}	12.17 ± 1.70^{b}	*
Enzymes					
AST (U/L)	308.50 ± 17.49^{b}	367.33 ± 22.82^{a}	290.50 ± 9.54^{b}	315.33 ± 16.59^{b}	*
ALT (U/L)	3.00 ± 0.63^{b}	7.33 ± 0.67^{a}	2.33 ± 0.21^{b}	2.83 ± 0.31^{b}	***
ALP (U/L)	418.83 ± 13.35^{b}	650.67 ± 21.97^{a}	474.17 ± 26.62^{b}	627.67 ± 23.03^{a}	***

Pb: lead; A/G: Albumin/Globulin ratio; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase; The data are presented as mean ± SE. a, b: Mean values with different superscripts within a row differ significantly; *: P < 0.05; **: P < 0.01; ***: P < 0.001; NS: non-significant.

Table 3
Effect of lead and/or quercetin on oxidant/antioxidant status in kidney, heart and liver tissues of experimental groups.

Parameters		Control	Pb	Quercetin	Pb+Quercetin	P
Kidney	MDA	29.52 ± 2.50^{b}	38.94 ± 0.88^{a}	27.77 ± 2.09^{b}	30.19 ± 1.76^{b}	**
	GSH	3.48 ± 0.27^{a}	2.55 ± 0.05^{b}	3.92 ± 0.38^{a}	3.31 ± 0.14^{a}	**
	GSH-Px	24.45 ± 0.72^{a}	17.14 ± 2.39^{b}	28.77 ± 1.43^{a}	24.06 ± 2.33a	**
	CAT	6.90 ± 0.94^{a}	3.08 ± 0.39^{b}	6.57 ± 0.55^{a}	6.12 ± 0.47^{a}	**
Heart	MDA	14.97 ± 1.16^{c}	27.78 ± 0.87^{a}	15.27 ± 0.75^{c}	20.28 ± 1.34^{b}	***
	GSH	3.52 ± 0.06^{ab}	2.66 ± 0.10^{c}	3.70 ± 0.15^{a}	3.23 ± 0.04^{b}	***
	GSH-Px	21.54 ± 0.96^{a}	15.84 ± 0.73^{b}	21.91 ± 1.81^{a}	20.25 ± 0.80^{a}	***
	CAT	11.44 ± 0.40^{a}	4.15 ± 0.47^{c}	11.30 ± 0.66^{a}	8.34 ± 1.33^{b}	***
Liver	MDA	29.03 ± 3.09b	50.12 ± 1.03^{a}	28.44 ± 2.94^{b}	32.17 ± 0.55^{b}	***
	GSH	4.83 ± 0.26^{a}	2.57 ± 0.05^{c}	4.93 ± 0.30^{a}	3.37 ± 0.12^{b}	***
	GSH-Px	30.40 ± 0.51^{a}	24.09 ± 0.60^{b}	31.47 ± 1.25^{a}	31.65 ± 1.39^{a}	***
	CAT	12.69 ± 1.16^{a}	4.92 ± 0.23^{b}	12.98 ± 0.78^{a}	9.88 ± 1.48^{a}	***

Pb: lead; MDA: malondialdehyde; GSH: glutathione; GPx: glutathione peroxidase; CAT: catalase. The data are presented as mean ± SE. a, b, c: Mean values with different superscripts within a row differ significantly. **: P < 0.01; ***: P < 0.001; NS: non-significant.

3.3. Caspase-3 and caspase 9 protein expression levels

The caspase-3 and caspase-9 protein expression levels are presented in the Figs. 1, 2 and 3, respectively. The caspase-3 ($P < 0.001$) and caspase-9 ($P < 0.01$) protein expression levels of liver tissue in the Pb group were significantly higher than the others. Caspase-3 and caspase-9 protein expression levels of the Control and Quercetin groups were similar, and the supplementation of quercetin in the Pb + Quercetin

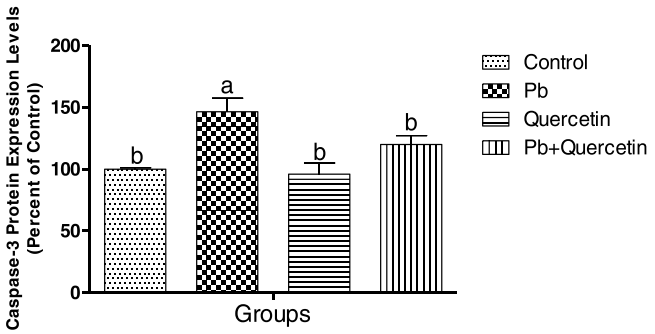

Fig. 1. Effects of lead and/or quercetin on caspase-3 protein expression levels in the liver tissue.
Protein expression is normalised to β-actin. Data are shown as percent of control and expressed as mean ± SE. a,b: mean values with different superscripts within the same column show statistically significant differences between the groups. Significance is $P < 0.001$. Pb: Lead.

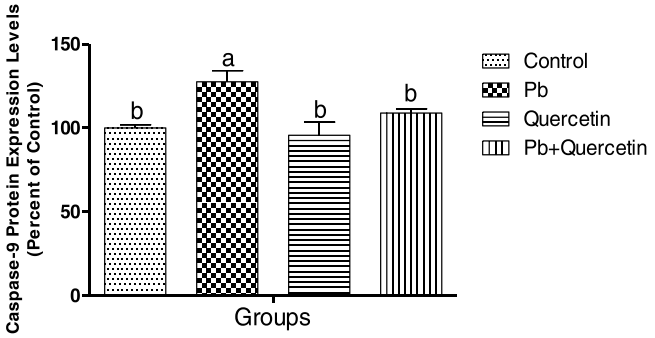

Fig. 2. Effects of lead and/or quercetin on caspase-9 protein expression levels in the liver tissue.
Protein expression is normalised to β-actin. Data are shown as percent of control and expressed as mean ± SE. a,b: mean values with different superscripts within the same column show statistically significant differences between the groups. Significance is $P < 0.001$. Pb: Lead.

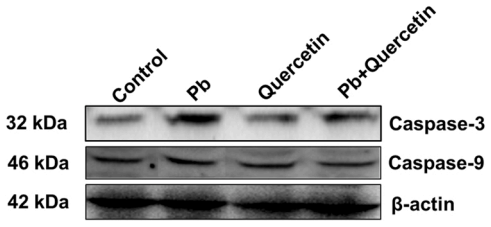

Fig. 3. Effects of lead and/or quercetin on Western Blot Band Image of Caspase-3 and Caspase-9 in the liver tissue. Pb: Lead.

group according to the Pb group significantly decreased caspase-3 ($P < 0.001$) and caspase-9 levels ($P < 0.01$).

4. Discussion

Free radicals are produced excessively in conditions like stress and aging which cause structural anomalies and dysfunctions of the cell and mitochondrial membranes (Liu et al., 2014). Free radicals can originate from oxygen and nitrogen. Oxygen-originated ones are called reactive oxygen species (ROS) (Moreira and Lyon, 2020). Pb is an environmentally toxic substance that causes oxidative stress through the formation of a ROS, which has been reported as an important mechanism underlying Pb toxicity (Qu et al., 2019). Oxidative stress occurs when ROS production exceeds the antioxidant system's ability to defend cells against oxidized molecules (Sharifi-Rad et al., 2020). Pb toxicity occurs entirely at the molecular and cellular levels. Oxidative damage mechanism caused by Pb consists of lipid peroxidation and weakening of antioxidant defense systems that carry the thiol (−SH) group in cells due to the increase in ROS (Balali-Mood et al., 2021).

Serum ALT and ALP levels of the groups exposed to Pb toxicity were found to be significantly higher than those of other groups ($P < 0.001$). Additionally, AST levels of Pb groups were found to be higher ($P < 0.05$). Recent studies show that the differences between enzyme activity related to the result of hepatotoxicity (Ezedom and Asagba, 2016; Alijagic et al., 2018; Suljevic et al., 2020). Consistent with the result of this study Suljevic et al. (2021) reported that Pb exposure influences serum biomarkers in Japanese quails. It has been reported that these changes in serum analytes may be the result of liver damage due to metallothionein, which is synthesized in the liver and has a high affinity for heavy metals. Thus, Pb is rapidly distributed in the liver and causes hepatotoxicity (Wong et al., 2017; Suljevic et al., 2021). In this study, Pb increased the levels of serum glucose, total protein, albumin, BUN ($P < 0.05$), and creatinine ($P < 0.01$). Hamidipour et al. (2016) reported that Pb showed a significant increase in blood glucose, creatinine, and uric acid, and showed a significant decrease in total protein, albumin, and globulin levels in Japanese quails exposed to a 0.4 mg/kg diet of a lead acetate for 21 days. These findings are consistent with the findings of our study. High creatinine level in the Pb group may be a sign of glomerular filtration and kidney dysfunction (Hamidipour et al., 2016), and changes in albumin and globulins and total proteins in the plasma may be used as a clinical symptom for monitoring the health of the immune system, liver, and kidneys of animals (Salighezadeh et al., 2014; Hamidipour et al., 2016). In addition, increased glucose levels in the Pb groups may be due to reasons such as Pb poisoning, impaired carbohydrate metabolism, increase energy demand of cells, cellular ATP decrease, or even decreased levels of acetylcholinesterase (Hamidipour et al., 2016).

Pb is known to have indirect oxidative effects on biological systems and cells. Lipid peroxidation caused by Pb results in the formation of harmful aldehyde by products like MDA (Toz and Deger, 2018). It has been reported in previous studies that Pb toxicity increases the MDA level (Alagawany et al., 2018; Toz and Deger, 2018; Kou et al., 2020). In this study, increased MDA levels in laying quails exposed to the Pb group compared to the Control and Quercetin groups were found to be statistically significant. MDA levels were found to be higher in the kidney ($P < 0.01$), liver, and heart tissue ($P < 0.001$) of laying quails exposed to Pb toxicity compared to the other groups. In a previous study, Kou et al. (2020) reported that the MDA level was found to be significantly increased in the liver tissue of the Pb-exposed group in female quails. These results are consistent with those of our study.

It has been reported that Pb toxicity causes oxidative stress and increases the production of ROS as the main mechanisms of its toxic effect, thus causing cell structure damage, lipid peroxidation (Ma et al., 2017; Alagawany et al., 2018). The potential effect of Pb in the induction of oxidative stress suggests that some medicinal plants and herbs with antioxidants and possible chelating activities may help to modulate Pb-induced toxicity (Alagawany et al., 2018). It has been reported in the literature that quercetin is a powerful antioxidant (Salehi et al., 2020).

Free radicals in the organism are removed by an endogenous (enzymatic or nonenzymatic) and exogenous antioxidant system. CAT and GSH-Px are enzymatic antioxidants while glutathione (GSH) is one a nonenzymatic antioxidant (Moussa et al., 2020). In this study, CAT, GSH-Px, and GSH activities of the kidney ($P < 0.01$), liver, and heart ($P < 0.001$) were found to be lower in the Pb group compared to the Control and Quercetin groups. Like this study, Kou et al. (2020) reported that CAT and GPx activity was significantly reduced in the liver tissue of female quails exposed to Pb toxicity. As in previous study (Alagawany et al., 2018) this might have been due to the disruption of the antioxidant defense system when exposed to Pb and the increase in cell vulnerability to the attack of free radicals that cause oxidative damage.

Like the findings of this study, Almasmoum et al. (2019) reported that 1000 mg/L lead acetate in drinking water significantly increased the MDA concentration in the liver tissue and decreased antioxidative enzyme activities (CAT, GSH-Px, and GSH) in rats. Quercetin shows its antioxidant activity mainly by its effect on GSH, enzymatic activity, signal transduction pathways and ROS arising from environmental and toxicological factors. Quercetin shows a powerful antioxidant property by protecting the oxidative balance (Dong et al., 2019). In this study decreased MDA levels and increased antioxidant enzyme activities in the Quercetin groups than in those of the Pb group may be attributed to the reported antioxidant properties. Small amounts of ROS that act as signal molecules to support normal physiological activities in physiological conditions are found in the animal body. Excessive amounts of ROS that may cause apoptosis may be produced when oxidative damage occurs (Chen et al., 2018).

Pb is known to cause wide spectrum pathogenesis, including the hemopoietic system, liver, kidney, brain, heart, and the reproductive system. After Pb is absorbed, it enters the bloodstream and accumulates in the erythrocytes. It then distributes preferably to many organs, such as the liver and kidney. After accumulation in organs, Pb causes the production of ROS and binds with functional -SH groups. Excessive ROS production may oxidatively damage the cellular macromolecules and trigger apoptotic events (Dewanjee, 2015). Apoptosis has an effective role in protecting and maintaining body health by eliminating unhealthy and dead cells. Apoptosis causes some changes in cell morphology by causing shrinkage in the cells, fragmentation of DNA, and degradation of messenger RNA. Cell death is caused by the activation of caspase-3 by caspase-9, which causes cell death by breaking down proteins. Cell death occurs when caspase-3, which causes cell death by breaking down proteins, is activated by caspase-9 (Safhi, 2018). Caspase-3 and caspase-9 have been reported to play an important role in the process of apoptosis induced by different stimuli (Gul Baykalir et al., 2020).

In this study, oxidative damage caused by Pb increased the expression level of caspase-3 ($P < 0.001$) and caspase-9 ($P < 0.01$) in the liver tissue of the Pb group compared to the Control and Quercetin groups (Figs. 1, 2 and 3), which is consistent with the findings of Iflazoglu Mutlu et al. (2021). Dewanjee et al. (2015) reported that caspase-3 and -9 expressions increased significantly ($P < 0.01$) in hepatocytes exposed to lead acetate. It has been reported that the protective effect of quercetin in an oxidative injury of some cells is through modulation of mitochondrial dysfunction and inhibition of caspase activity (Akan and Garip, 2013; Alshammari et al., 2021). The low caspase levels in the Quercetin groups may be due to the antioxidative properties of Quercetin. In a previous study by Al-Omair and Elsawy (2017), quercetin was reported to reduce the negative effect of lead acetate toxicity on blood and testicles in rats. Additionally, Akan and Garip (2013) reported that quercetin has a protective effect on apoptosis in *K562* erythroleukemia cell line exposed to H_2O_2.

5. Conclusion

In conclusion, it may be declared that oxidative stress and the expression levels of apoptotic proteins (caspase-3 and-9) increased in the laying quails exposed to Pb toxicity and the supplementation of quercetin reduced oxidative stress and apoptotic protein expression levels. The protective effects of quercetin may be linked to its antioxidant activity. For these reasons, the use of quercetin may be recommended to reduce the negative effects of Pb, which is an important environmental pollutant in poultry.

CRediT authorship contribution statement

Aslihan Sur Arslan: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Ismail Seven**: Investigation, Writing – review & editing. **Seda Iflazoglu Mutlu**: Investigation, Formal analysis, Writing – review & editing. **Gozde Arkali**: Conceptualization, Methodology, Data curation, Formal analysis. **Nurgul Birben**: Investigation, Writing – review & editing. **Pinar Tatli Seven**: Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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