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The curative effects of dietary yeast beta-1,3/1,6-glucan on oxidative stress and apoptosis in laying quails (*Coturnix coturnix japonica*) exposed to lead

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Abstract: The objective of this study was to investigate the effects of supplementation of beta-glucan to the basal diet of laying quails exposed to lead (Pb) toxicity. A total of 112 birds were randomly divided into 4 groups. The first group was fed a corn-soybean based basal diet without any supplementation (control). The second (Pb) and third (beta-glucan) groups were supplemented with 100 mg/kg of Pb (as Pb acetate) and 100 mg/kg of beta-glucan, respectively. The fourth group (Pb + beta-glucan) was fed a basal diet supplemented with 100 mg/kg of Pb and 100 mg/kg of beta-glucan. It was determined that serum total protein, albumin, and creatinine values of the Pb + beta-glucan group partially improved ($P < 0.05$) with the addition of beta-glucan, but globulin and blood urea nitrogen (BUN) values were similar to the Pb group ($P < 0.001$). Globulin and albumin/globulin ratio were not affected by the supplements. Aspartate aminotransferase (AST) ($P < 0.01$) and alanine transaminase (ALT) ($P < 0.001$) enzyme activities in the beta-glucan and Pb + beta-glucan groups were similar to the control group, unlike the Pb group. However, ALP enzyme activity was similar in all supplemented groups, unlike the control group ($P < 0.01$). Malondialdehyde ($P < 0.001$) was reduced in the liver, heart, and kidney tissues of beta-glucan supplemented groups compared to the Pb group. Glutathione levels of liver and heart tissues in the Pb + beta-glucan group were significantly higher than in the Pb group ($P < 0.001$). Catalase and glutathione peroxidase activities were also significantly increased in all tissues by supplementation of beta-glucan as compared to the Pb group. The caspase-3 and caspase-9 protein expression levels in the liver tissue of the beta-glucan group decreased ($P < 0.001$) compared to the Pb group. As a result, we conclude that beta-glucan helps reduce the harmful effects of Pb toxicity in laying quails.

Key words: Beta-glucan, lead toxicity, oxidative damage, apoptosis, quail

1. Introduction

Lead (Pb), which is found naturally in the earth, is the most important toxic metal and also one of the most prevalent heavy metal contaminants in the world [1,2]. Pb threatens human and animal life in many ways, especially during the development stages [3]. Hematopoietic, kidney, reproductive, and central nervous systems are parts of the human body that are vulnerable to exposure to high levels of Pb [4]. Pb poisoning is a real threat to public health, especially in developing countries. Therefore, great efforts are being made to reduce the dangers of the metal in terms of occupational and public health. Animals can easily be exposed to Pb because it is a global environmental pollutant, primarily in the industrial regions [4].

Beta-glucans are carbohydrates consisting of glucose chains in the cell wall of yeast, fungi, bacteria, and cereals [5]. Beta-1,3/1,6-glucan is formed as a result of the formation of glucose monomers by chaining with beta-1,3 glycosidic bonds and by the binding of glucose monomers to these chains by beta-1,6 linkages. Beta-glucans can be found in many organisms, from bacteria to trees and from seeds to fungi. Beta-1,3/1,6-glucan from polysaccharides, which has different functions, helps the mechanical wall hardness and integrity of the cell wall [6–8]. Beta-glucan has several beneficial health outcomes such as antibacterial, antitumor, antiviral, enhancing immunity, and wound healing activities [9]. Some features of beta-glucans, such as molecular weight, degree of branching, solubility, primary structure, and polymer charge can

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change its effect on the immune system and biological activity [10].

Pb poisoning in animals can usually occur from a large number of environmental sources and can be monitored via contamination of soil and feed because of industrial pollution and agricultural applications. A wide range of toxic effects of Pb in animal systems is observed. Poisoning of wild birds following ingestion of Pb from ammunition in relation to hunting activities has been recognized. Pb taken in this way endangers the growth and survival of nestlings, causes adverse effects on reproduction by reducing egg production and plasma calcium level, causes hemolytic anemia and behavioral impairments [11,12]. Exposure to high doses of Pb can result in reproductive disorders, poor performance, oxidative stress, apoptosis, poisoning, or even death in animals [10,13–19]. Pb can cause oxidative damage to the reproductive organs, kidney, heart, and liver. The mechanism of oxidative stress induced by Pb involves the effects of Pb on the antioxidant defense systems, membranes, and DNA of cells [3,20]. While new cells are formed in living organisms, some of the existing cells are eliminated by cell death, thereby maintaining a constant balance. These existing cells are destroyed by various types of cell death, such as physiological, programmed cell death (apoptosis), and pathological cell death (necrosis). Reactive oxygen species (ROS) can react with macromolecules due to oxidation and cause them to undergo apoptosis or necrosis [21,22]. Beta-glucans show their antioxidant effects as a strong intracellular free radical scavenger [23]. Free oxygen radicals cause lipid peroxidation and consequently membrane damage and destruction [24]. Beta-1,3-glucan has a protective effect against oxidative damage by suppressing lipid peroxidation. Also, it controls lipid peroxidation due to inhibiting the increased levels of malondialdehyde (MDA), which is an indication of lipid peroxidation [25]. It directs the immune system to recognize and destroy macrophages in mutated cells. Beta-glucans show their effects in tissue regeneration and repair by accelerating the repair of damaged tissue. They show their adjuvant effects by increasing antibacterial, antifungal, antiviral, and antiparasitic effects [6].

Nowadays, many researchers focus on the effects of natural dietary supplements on oxidative stress and apoptosis, and the relationship between oxidative stress and apoptosis. The aim of this study was to examine the effects of beta-glucan, which has various natural biological functions, on laying quails exposed to Pb toxicity, which has become increasingly widespread during today's industrialization period.

2. Materials and methods

2.1. Supplements

Beta-1,3/1,6-glucan originating from *Saccharomyces cerevisiae* containing at least 70% beta-1,3/1,6-glucan was purchased from Pure Bulk Inc. (Roseburg, OR, USA). Pb (II) acetate trihydrate [$(CH_3COO)_2Pb.3H_2O$] was purchased from Merck (CAS #: 6080-56-4).

2.2. Animal, diet, and experimental design

A total of 112 laying quails (*Coturnix coturnix japonica*) were obtained from a local commercial company. Each group consisted of 28 female animals aged 5 weeks (each with four replicates consisting of 7 birds). The quails were randomly divided into 4 groups, which had 4 replicates including 7 quails in each group. The research was carried out using standard cages (34 × 62.5 × 23 cm from the CIMUKA company) designed for laying quails with the approval of the Local Ethics Committee for Animal Experiments of the Ministry of Food, Agriculture, and Livestock (05.29.2018/2018-1). Water and feed were provided ad libitum. The photoperiod was 16/8 h (light/dark). The birds were kept in quail layer cages in a temperature-controlled room. A basal diet prepared according to National Research Center [26] was supplied by a commercial company (Table 1). The experimental groups were arranged as follows: fed a corn-soybean basal diet and no supplementation (group I; control), supplementation with 100 mg/kg of Pb (as Pb (II) acetate trihydrate) to the diet (group II; Pb), supplementation with 100 mg/kg of beta-glucan to basal diet (group III; beta-glucan), and supplementation with 100 mg/kg of Pb and 100 mg/kg of beta-glucan to basal diet (group IV; Pb + beta-glucan). At the end of the experiment (after 56 days), 6 quails from each group were slaughtered for analysis by decapitation.

Research studies on Pb toxicity in quail were used for determining the dosage of Pb [27,28]. The dose of beta-glucan was determined according to Moon et al. [29] and Zhu and Wu [30].

2.3. Oxidative stress

2.3.1. Sample collection and homogenate preparation

After the quails were slaughtered, liver, heart, and kidney tissues were washed with phosphate buffer (PBS) and each was wrapped in aluminum foil, placed in polyethylene bags, and labeled. The tissues were kept at –20 °C until analysis. For the analysis, the tissues were weighed and transferred to glass tubes while maintaining the coldness. Tris buffer (pH 7.4) was added to the tissues in a 1/10 ratio. While maintaining coldness, the tissue was homogenized in the homogenizer. The tissue total protein was determined by Lowry et al. [31] method, which used bovine serum albumin as the standard.

2.3.2. Determination of malondialdehyde (MDA) level

The amount of MDA produced in the liver, heart, and kidney tissues was used as an indicator of lipid peroxidation level. The colored complex of MDA formed by thiobarbituric acid (TBA) was measured at 532 nm. The

Table 1. Ingredients and nutrient composition of basal ration.

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[b]	2.50
Sodium bicarbonate	0.10	Available phosphorus[b]	0.35
DL-Methionine	0.12	Sodium[b]	0.16
Vitamin-Mineral premix [a]	0.32	Lysine[b]	1.00
Limestone	5.46	Threonine[b]	0.75
Calcium phosphate	1.39	ME, kcal/kg [b, c]	2800
Total	100		

[a]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.
[b] Calculated.
[c]Metabolizable energy (ME) = 53 + 38 [(Crude protein, %) + (2.25 x Ether extract, %) + (1.1 x Starch, %) + (Sugar, %)]

MDA levels were determined by the spectrophotometric method defined by Placer et al. [32].

2.3.3. Tissue glutathione (GSH) level

The GSH level was determined by the spectrophotometric method of Sedlak and Lindsay [33]. Since the color intensity of the colored complex formed by the 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 tissue

The level of GSH-Px activity in the liver, heart, and kidney tissues was determined by the spectrophotometric method described by Lawrence and Burk [34]. The yellow color complex that 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 [35]. When the tissue is incubated with the substrate containing hydrogen peroxide (H_2O_2), 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), centrifuged at 14,000 rpm at 4 °C for 45 min, and the supernatants were separated. Total protein content was determined spectrophotometrically according to the BCA (Thermo Fisher, 23227) method [36]. Samples were electrophoresed by loading 60 µg of protein in each well, and the first well with a protein marker, to the polyacrylamide gel (stacking gel 4%; separating gel 12%) [37]. After sodium dodecyl sulphate-polyacrylamide gel electrophoresis, specific proteins were transferred to polyvinylidene difluoride (PVDF) membrane by western blotting [38]. The membranes of PVDF were blocked by 5% bovine serum albumin (BSA), and then washed 3 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) overnight. After incubation, the membranes were washed 3 times with TBST for 5 min. Subsequently, the bands that were obtained by chemiluminescent conjugate (ECL; Bio-Rad, 1705060) on membranes treated with the appropriate

secondary antibody (ab97240; 1/10,000, ab6721; 1/10,000) were visualized in the chemiluminescence imaging system (Bio-Rad ChemiDoc™ XRS +) [39,40]. 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). Relative expression levels of protein were normalized to beta-actin, which was used as an internal control.

2.5. Statistical analyses

All values were presented as mean ± SE. Whether the values obtained as a result of the study show a normal distribution was determined by the Shapiro–Wilk test. As a result of Shapiro–Wilk normality analysis, it was determined that the data showed normal distribution. The differences between the mean of groups were detected using one-way ANOVA and the posthoc Duncan test in IBM SPSS Statistics 22 computer program [41]. The results were noted as significant at $P < 0.05$.

3. Results

When the effects of beta-glucans on the serum biochemical parameters of the experimental groups were examined, it was seen that serum total protein, albumin, and creatinine values of Pb + beta-glucan group were partially improved ($P < 0.05$) by the addition of beta-glucan, but globulin and BUN values were similar to Pb group ($P < 0.001$). Globulin and albumin/globulin ratio were not affected by supplements (Table 2). Serum AST ($P < 0.01$) and ALT ($P < 0.001$) activities of beta-glucan and Pb + beta-glucan groups were similar to the control and the group different than the Pb group, but ALP activities of the control group were higher than in the other groups ($P < 0.01$; Table 2).

The MDA, GSH, GSH-Px, and CAT values of kidney, liver, and heart tissues are presented in Table 3. The highest MDA levels in all tissues were in the Pb group ($P < 0.001$). The MDA levels of heart, kidney, and liver tissues of the beta-glucan group were similar to the control, and also the supplementation of beta-glucan in the Pb + beta-glucan group significantly reduced the MDA level ($P < 0.001$). The GSH, GSH-Px, and CAT values of kidney, heart, and liver tissues in the Pb group were significantly lower than in the other groups ($P < 0.05$). Similar to the control group, caspase-3 (Figure 1) and caspase-9 (Figure 2) protein expression levels of the beta-glucan group decreased according to the Pb group in liver tissue ($P < 0.001$).

4. Discussion

Birds can reduce the amount of metal residue in their eggs by reducing the accumulation of the mineral. This type of protection may be enough to prevent the accumulation of some metals such as Mn and Cr but is inadequate for Pb [42]. Therefore, birds are very sensitive to Pb exposure. Oxidative stress has been reported to be the main mechanism of toxicity induced by Pb [16,17,27,43]. Oxidative stress formed by the effect of Pb occurs due to two different mechanisms working at the same time. The first one is the formation of ROS, like singlet oxygen, hydroperoxides, and H_2O_2, and the second one is the depletion of the antioxidant reserves [44,45]. Apoptosis is a type of programmed cell death regulated by Bcl-2 and caspase protein families [46].

Recent studies show that Pb can cause oxidative stress by increasing oxidation production, suppressing the activities of antioxidant enzymes, and eventually activating

Table 2. Effects of beta-glucan on serum biochemical parameters of experimental groups.

Parameters	Control	Pb	Beta-glucan	Pb + Beta-glucan	P
Glu (mg/dL)	139.50 ± 5.90^{b}	190.50 ± 12.89^{a}	149.00 ± 10.96^{b}	177.83 ± 5.96^{a}	**
TP (g/dL)	3.08 ± 0.10^{a}	2.42 ± 0.09^{b}	2.82 ± 0.18^{ab}	2.87 ± 0.20^{ab}	*
Albumin (g/dL)	1.52 ± 0.09^{a}	1.15 ± 0.06^{b}	1.30 ± 0.10^{ab}	1.33 ± 0.08^{ab}	*
Globulin (g/dL)	1.57 ± 0.09	1.27 ± 0.04	1.52 ± 0.09	1.53 ± 0.14	NS
A/G ratio	1.00 ± 0.12	0.90 ± 0.04	0.86 ± 0.02	0.90 ± 0.06	NS
Cre (mg/dL)	0.02 ± 0.00^{b}	0.04 ± 0.01^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{ab}	*
BUN (mg/dL)	11.67 ± 1.09^{b}	19.70 ± 1.58^{a}	12.20 ± 1.24^{b}	16.25 ± 1.08^{a}	**
Enzymes					
AST (U/L)	294.17 ± 10.27^{b}	382.60 ± 14.98^{a}	296.33 ± 18.89^{b}	300.40 ± 20.24^{b}	**
ALT (U/L)	4.25 ± 0.16^{b}	7.75 ± 0.70^{a}	3.40 ± 0.24^{b}	3.20 ± 0.48^{b}	***
ALP (U/L)	562.40 ± 19.95^{a}	400.17 ± 25.48^{b}	465.75 ± 28.92^{b}	445.17 ± 17.30^{b}	**

Pb: lead; Glu: glucose; TP: total protein; Cre: creatinine; A/G: albumin/globulin ratio; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase; $^{a, b}$: mean±SE values with different superscripts within a row differ significantly; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: nonsignificant

Table 3. Malondialdehyde (MDA, nmol/g), glutathione (GSH, nmol/g), glutathione peroxidase (GSH-Px, IU/g protein) and catalase (CAT, kU/g protein) values of kidney, heart, and liver tissues of experimental groups.

Parameters		Control	Pb	Beta-glucan	Pb + Beta-glucan	P
Kidney	MDA	30.90 ± 1.33^{c}	39.40 ± 1.77^{a}	29.71 ± 1.22^{c}	35.25 ± 1.12^{b}	***
	GSH	2.47 ± 0.11^{a}	2.12 ± 0.29^{b}	2.54 ± 0.26^{a}	2.33 ± 0.12^{ab}	*
	GSH-Px	22.32 ± 1.83^{a}	16.80 ± 1.74^{b}	21.95 ± 0.96^{a}	20.19 ± 1.18^{a}	*
	CAT	5.32 ± 0.18^{a}	3.41 ± 0.55^{b}	5.94 ± 0.50^{a}	5.24 ± 0.61^{a}	*
Heart	MDA	15.67 ± 1.47^{c}	24.31 ± 0.80^{a}	15.18 ± 0.92^{c}	20.88 ± 1.30^{b}	***
	GSH	3.69 ± 0.02^{a}	2.78 ± 0.12^{c}	3.78 ± 0.15^{a}	3.08 ± 0.03^{b}	***
	GSH-Px	24.90 ± 1.63^{a}	18.91 ± 1.24^{b}	25.02 ± 2.19^{a}	23.81 ± 1.15^{a}	*
	CAT	10.53 ± 0.88^{a}	4.98 ± 0.38^{c}	10.50 ± 0.46^{a}	7.62 ± 0.17^{b}	***
Liver	MDA	28.04 ± 1.22^{c}	48.82 ± 1.69^{a}	27.60 ± 1.75^{c}	37.59 ± 1.71^{b}	***
	GSH	5.06 ± 0.26^{a}	3.02 ± 0.20^{c}	5.68 ± 0.38^{a}	3.98 ± 0.24^{b}	***
	GSH-Px	33.00 ± 2.83^{a}	22.77 ± 0.78^{b}	33.20 ± 1.03^{a}	30.38 ± 2.01^{a}	**
	CAT	9.22 ± 0.53^{a}	2.35 ± 0.06^{c}	9.81 ± 0.50^{a}	4.79 ± 0.59^{b}	***

Pb: lead; ^{a,b,c}: mean ± SE values with different superscripts within a row differ significantly; * P < 0.05; ** P < 0.01; *** P < 0.001

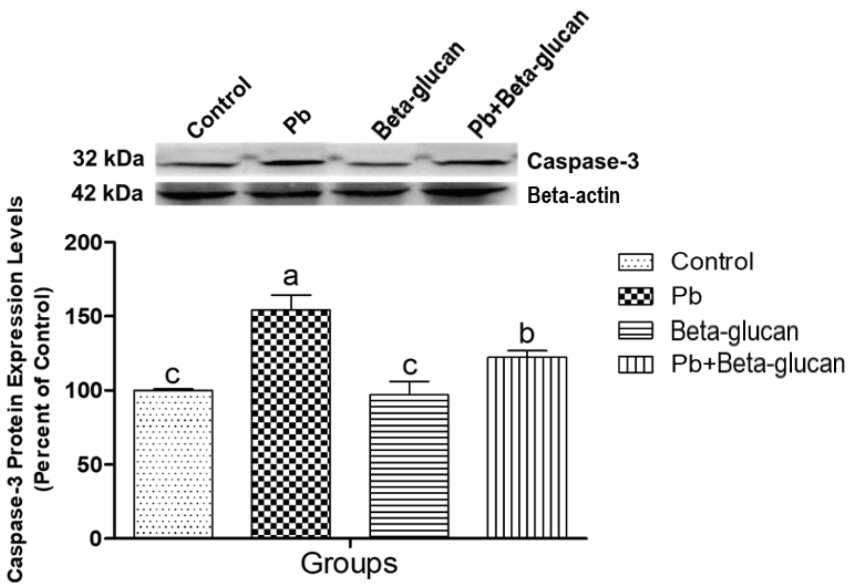

Figure 1. Liver tissue caspase-3 protein expression levels (P < 0.001; mean ± SE).

apoptosis [18,47]. Kofuji et al. [48] reported that beta-glucan from barley has higher antioxidant activity than gelatin, pectin from apple and citrus, curdlan, dextrin, chitosan, pullulan, gellan gum, and sodium alginate.

Bahakaim et al. [49] used Glycomoss (foreign product) or Mox (local product) as a source of mannan oligosaccharides (MOS) and beta-glucans (28% MOS and 34% beta-glucans) 0.5 and 0.75 g/kg in quail diet from 7 to 13 weeks of age. In MOS and beta-glucans supplemented groups, when compared with the control, they reported that the plasma total protein and globulin were significantly increased, but the A/G ratio was significantly decreased (P < 0.05). Also, the plasma concentration of albumin and the activities of AST and ALT enzymes were not affected in this research. Similarly, Mousa et al. [50] reported that dietary MOS plus beta-glucans supplementation (0.5 and 0.75 g/kg) increased the plasma total protein, globulin, and glucose, and decreased the A/G ratio (P < 0.05). However,

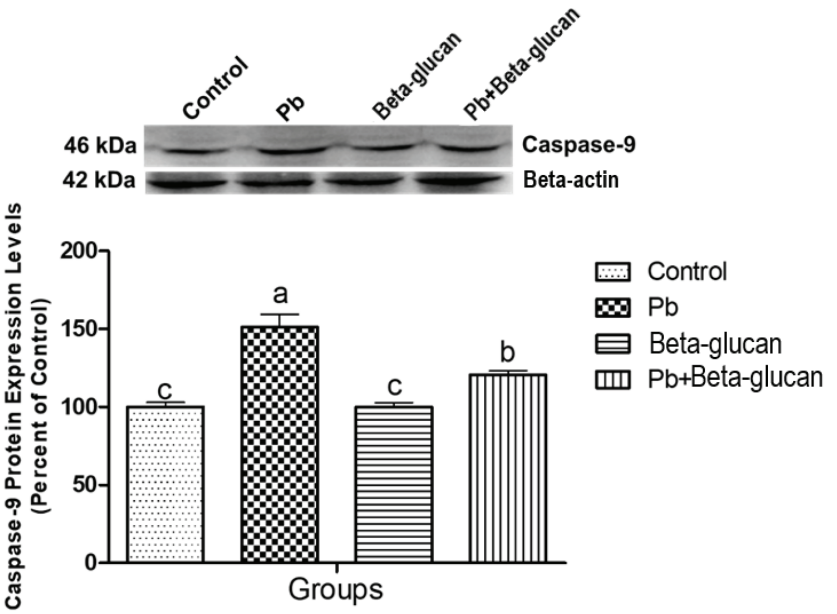

Figure 2. Liver tissue caspase-9 protein expression levels ($P < 0.001$; mean ± SE).

plasma albumin, AST, and ALT values were not affected by dietary beta-glucan. Unlike our study, Bahakaim et al. [49] and Mousa et al. [50] reported that supplementation with beta-glucan and MOS did not change the plasma albumin, AST, and ALT values, increased the globulin and total protein levels and decreased the A/G ratio. This may be attributed to exposure to Pb toxicity or to the effect of MOS and/or MOS and glucan interaction. The organism naturally exhibits defense behavior against Pb-induced oxidative stress and apoptosis [18,47]. Since one of the organs most affected by this condition is the liver, there is an increase in liver enzymes AST and ALT activities. Similar to our study, Alagawany et al. [27] reported that Pb (100 mg/kg) supplementation to the diet of quails for 8 weeks has significantly decreased total protein, albumin, and globulin, and increased ALT and AST enzyme activities ($P < 0.001$). Also, Seven et al. [16] reported that the addition of 200 mg/kg Pb to broiler diet for 42 days as compared to the control group has significantly increased AST activity ($P < 0.05$), but there was no effect on glucose, ALP, and albumin. Similarly, Yuan et al. [19], in their study, in which the basal diet (corn-soybean meal) of Hy-Line Brown hens was supplemented with 15, 30, and 60 mg/kg of Pb, reported that the supplementation with Pb at 60 mg/kg resulted in increased levels of BUN, urea acid, and AST activity ($P < 0.05$).

Seven et al. [16] reported that the 200 mg/kg of Pb supplementation to broiler diet significantly increased the MDA level in plasma ($P < 0.01$) and liver tissue ($P < 0.05$), and the GSH values in plasma and heart tissue ($P < 0.01$). In addition to the above effects, they reported that Pb supplementation increased the superoxide dismutase (SOD) activity of plasma and CAT activities of hemolysate, liver, and heart tissues. The water-soluble yeast beta-1,3-glucan, which can participate in the body's natural defense mechanisms, has a perfect radical scavenging effect [10]. By means of exhibiting an action positively correlated with its dose, water-soluble yeast beta-1,3-glucan may increase SOD activity in serum [10,51]. Yeast beta-glucan has a powerful antioxidative effect that can increase antioxidant activity by removing free radicals, preventing the activity of ROS and antioxidants, and increasing the body's antioxidant capacity [10,52,53]. In a study investigating the protective role of beta-glucan against oxidative damage caused by sepsis in rats, Şener et al. [25] gave 50 mg/kg beta-glucan to rats once a day by intragastric gavage for 10 days. They reported that the application of beta-glucan according to the saline-treated group significantly decreased the MDA levels in the brain ($P < 0.05$), liver, heart, lung, diaphragm ($P < 0.01$), and kidney tissues ($P < 0.001$). Moreover, beta-glucan use prevented the decline of GSH levels in the brain, heart, lung, diaphragm, kidney ($P < 0.05$), and liver tissues ($P < 0.001$). In a study that examined the effects of 0, 50, 250, 500, and 1000 mg/kg Pb solution on liver histology and oxidative stress for 49 days, Kou et al. [43] reported that the activities of GSH-Px, SOD (only 500 mg/kg), CAT enzymes, and total antioxidant capacity significantly decreased in liver tissues of Pb groups, but MDA levels increased. They also noted that Pb exposure caused damages to bird health by inducing ultrastructural and microstructural injury, lipid metabolism disorder, and oxidative damage in liver tissue.

Alagawany et al. [27] reported that supplementation of Pb (100 mg/kg) to the diet of quails for 8 weeks increased serum MDA level compared to the control ($P < 0.001$), decreased the SOD and CAT ($P < 0.001$). However, they reported that the GSH level of Pb and control groups was similar ($P < 0.001$). In a study that explored whether or not the mannan/beta-glucans from the yeast cell wall (BYCW) (0, 0.2, 1, and 5 mg/mL) have a protective role in suppressing the cellular ROS in intestinal porcine epithelial cells from the jejunum (IPEC-J2) cells damaged by deoxynivalenol (DON; 1 μM), Guo et al. [54] reported that treatment with BYCW in the attack of DON, which was a Fusarium mycotoxin, caused a drop in MDA and ROS and a rise of GSH in IPEC-J2 cells. Moreover, they reported that there were similar improvements in MDA, ROS, and GSH levels in the cells after treatment with CPDT (25 μM) and BYCW (0.2, 1, 5 mg/mL) for 12 and 24 h in the IPEC-J2 cells under DON attack. All these research reports are compatible with our study. Kofuji et al. [48] reported that beta-glucan from barley has higher antioxidant activity than oat and black yeast-derived beta-glucan and they also reported that this effect is directly proportional to beta-glucan density. In addition, they reported that the hydroxyl radical scavenging activity of beta-glucans extracted under alkali or acid conditions was slightly higher than extracted in neutral conditions with warm water, and this activity was reduced with a drop in their molecular size.

Caspase-3 and caspase-9 have been noted to play a significant role in the formation phase of apoptosis caused by different stimuli [18,55]. Xu et al. [18] reported that Pb could induce an increase in caspase-3 activity, and the apoptosis induced by Pb occurs via the activation of apoptosis practitioners such as caspase-3. In a study that investigated the cytotoxic effect of Pb in human leukemia (HL-60) cells, which were cultured with lead nitrate for 24 h, Yedjou et al. [47] reported that Pb at very low concentration has a similar effect with control on the viability of HL-60 cells, but significantly decreased between 25 and 50 μg/mL of lead nitrate ($P < 0.05$). Also, they noted that the apoptosis of HL-60 cells induced by Pb was associated with the activation of caspase-3, and the numbers of caspase-3 positive cells (apoptotic cells) were significantly increased in all doses (10, 20, 30, and 40 μg/mL) of lead nitrate ($P < 0.05$). These research results are in line with ours.

5. Conclusion

Research on beta-glucan added to poultry diets focuses on its effects on the immune system, but there is no research on its effects on oxidative stress and/or apoptosis in the literature. Here, we demonstrated that the addition of Pb, which is known as the inducer of oxidative stress and apoptosis, to the diet of laying quails had a negative effect on some blood parameters (glucose, total protein, albumin, creatinine, and BUN) and liver enzymes (AST, ALT, and ALP). In addition, Pb caused oxidative stress in the kidney, heart, and liver tissues. Beta-glucan was able to ameliorate these adverse effects. Moreover, Pb sensitized liver cells to apoptosis through the activation of caspase-3 and caspase-9, but this effect was suppressed by beta-glucan. To increase the profitability in poultry farming, the effects of beta-glucan, a natural product, on oxidative stress and apoptosis need to be investigated in future research.

Acknowledgments/discaimers/ conflict of interest

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The authors of this research declare that there is no conflict of interest in the publication of this article.

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