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Effect of Purslane powder and Zinc supplementation on the performance, egg quality, antioxidant system and liver histopathology of lead-exposed laying Quails

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ABSTRACT: To determine effects of Purslane (*Portulaca oleracea*) and zinc supplementation in lead exposed quails, 180 adult female quails allocated into 9 groups. 1. Negative Control (NC, Fed with a corn-soy-based diet), 2. Corn-soy-based diet supplemented with 500 mg/kg lead acetate (Positive control), 3. Positive control supplemented with 0.5 % Purslane powder (PP), 4. Positive control supplemented with 1 % PP, 5. Positive control supplemented with 1.5 % PP, 6. Positive control supplemented with 140 mg/kg zinc, 7. Positive control supplemented with 0.5 % PP + 140 mg/kg zinc, 8. Positive control supplemented with 1 % PP + 140 mg/kg zinc, 9. Positive control supplemented with 1.5 % PP + 140 mg/kg zinc. Lead administration significantly decreased body weight, egg mass, egg production, liver weight, Haugh unit, serum concentrations of hematocrit, total protein, triglycerides and very low density lipoprotein concentration of quails ($P<0.05$). Serum alanine aminotransferase and lactate dehydrogenase activity significantly increased when compared with the NC ($P<0.05$). Superoxide dismutase and glutathione peroxidase activity in the liver and erythrocyte showed significant decrease ($P<0.05$). Lead administration resulted in a significant decrease ($P<0.05$) in total antioxidant capacity and increase in serum malondialdehyde. However, supplementation diet with 1.5% of PP reduced serum and liver malondialdehyde ($P <0.05$). Liver tissue of the birds in NC showed normal lobular architecture with central veins, radiating hepatic cords and portal triads, while this organ showed mild to severe tissue changes in lead exposed groups ($P<0.05$). It can be concluded that lead-exposure induced production of free radicals and weakened the antioxidant defenses of the quails. However, antioxidant status of quails partially improved when fed diets supplemented with 1.5 % PP and 140 ppm Zn.

Keywords: *Performance, Liver histopathology, Japanese quails.*

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INTRODUCTION

Lead is a toxic metal that is widely used in many industrial activities. It is well known that lead contamination occurs easily via contaminated food, water and food additives which ultimately enter the human body and threaten health and wellbeing (Yuan et al., 2013). Toxicity of lead and its compounds in animals and humans can be vary from soft tissues damage, mainly liver and kidney, to reduction of hematopoiesis, reproductive and nervous systems (Gupta, 2011).

Reactive oxygen species (ROS) production has been known as a main effect of heavy metals toxicity causing cellular oxidative stress. ROS are the by-products of degradation reactions in many tissues that affect normal metabolism of cells by damaging different cellular components for instance fatty acids, proteins and DNA (Xienia, 2000). Damron et al. (1969) reported that supplementation of 1000 ppm lead in the diet lead to a reduction in the growth rate in young broiler chickens. Morgan et al. (1975) reported growth rate reduction and anemia in Japanese quails fed diets containing 500 ppm lead. Saly et al. (2004) reported that dietary lead supplementation at 1000 ppm decreased egg weight, eggshell strength and eggshell thickness in laying hens. Naturally, different protective cellular mechanisms are developed to prevent peroxidation damage including enzymatic defense system (antioxidant enzymes) and free radicals scavengers (antioxidants). Antioxidants are chemical compounds that play an important role to protect human body against damage by ROS. Free radicals formed in the body due to normal physiological process can be scavenged by antioxidants (Usha and Pushpalatha, 2017).

Trace elements are interfered in the metabolic activities through metalloenzymes which are essential for the antioxidant conservation of cells in poultry (Petrovic et al., 2011(. Zinc (Zn) is one of the trace elements that play a role in the antioxidant system of the body. It is reported that Zn is an indispensible part of the SOD, which helps defend the broiler chickens against ROS production (Song et al., 2017). Cerklewski and Forbes (1976) showed that dietary Zn supplementation (200 ppm) could reduce lead concentration in the blood, liver and kidneys in rats exposed to 200 ppm of lead and alleviate lead toxicity. Rafique et al. (2010) demonstrated that the toxic effects of lead on male rats reproductive system decreased by Zn supplementation via activation antioxidant mechanisms.

During the last few decades, we have seen an increasing trend in the use of medicinal plants and extracts in poultry nutrition. Several studies showed that phenols, mostly flavonoids of some plants have antioxidant properties. One of the well known plants with effective antioxidant properties is Purslane (*Portulaca oleracea*). Purslane as a weed grows in the tropical and subtropical regions of the world (Sedaghati et al., 2019). It is a rich source of flavonoids and other antioxidant compounds such as α-tocopherol, ascorbic acid, and β-carotene as well as glutathione (Barbosa-Filho et al., 2008). Sadeghi et al. (2016) showed that the antioxidant status of broiler chickens was improved by dietary supplementation of Purslane powder. Ghorbani et al. (2013) reported that blood superoxide dismutase (SOD) activity and serum malondialdehyde (MDA) concentration were respectively increased and decreased in broiler chickens fed diets supplemented with 2% Purslane powder.

The effect of the Purslane powder in alleviation of lead toxicity has never been investigated; however previous studies reported the effect of some plants on lead toxicity in mice. Tangpong and Satarug (2010) found that Thunbergia laurifolia (Linn.) extract can ameliorate oxidative stress and reduce cell death in lead-exposed mice. Also, these researchers reported co-treatment of lead with Thunbergia laurifolia Linn. aqueous extract at 100 or 200 mg/kg led to increased plasma total antioxidant capacity (TAC). Khalaf et al. (2012) reported that green tea improves glutathione content and SOD activity in the brain of lead exposed rats. Although different natural herbs or their extracts has been studied to decrease toxic effects of lead, however, there is no previous study investigated the effect of Purslane in lead toxicity. Furthermore, it is demonstrated that each antioxidant has specificity for a particular ROS and supplementation of a single antioxidant might be not sufficient to prevent the oxidative stress caused by lead exposure. Therefore, in the present study, we investigated the effect of dietary Purslane powder, Zn and their combination on performance, egg quality, antioxidant status and liver histology in lead-exposed laying quails.

MATERIALS AND METHODS

Sampling and plant preparation

Purslane plant was purchased from a local field in Sanandaj (Kurdistan Province, Iran). After cleaning the whole plant including seeds, leaves, stems, and roots air drying, they were finely grounded to a size of 2 mm using a typical mill. Dried purslane plant powder stored in an air-tight containers at room temperature until use (Sadeghi et al., 2016). Proximate analysis of purslane plant powder was determined using methods described by AOAC (1994) with 6 replicates. The results of proximate analysis indicated

that Purslane powder contains 931.55 ± 4.11 Dry matter (g/kg), 241.90 ± 15.39 Ash (g/kg), 195.15 ± 6.12 Crude protein (g/kg), 16.83 ± 5.84 Crude fiber (g/kg), 85.47 ± 15.62 Ether extract (g/kg).

Antioxidant capacity of Purslane powder

The total phenolic compounds (TPC) in the Purslane powder were determined using the Folin-Ciocalteu reagent according to Halicia et al. (2005). 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH, IC50) of the Purslane was determined by the method reported by Gulcin et al. (2004). The ferric reducing property (FRAP) test was measured using the assay described by Yen and Chen (1994). The results of analysis antioxidant properties indicated that Purslane powder contains 326.96 ± 15.92 TPC (mg GAE/100 g), 1.27 ± 0.09 IC50 (mg/ml), 3.73 ± 0.23 FRAP (mg GAE/g).

Birds, Management and Treatments

All methods used in this study were approved by the guidelines of the Animal Ethics Committee in University of Kurdistan. A total of 180 14-week-old laying Japanese quails were randomly distributed between 36 cages. Feed (Table 1) and water were offered *ad libitum*. Light was provided for 16 h daily throughout the experiment. The animals were divided into 9 experimental treatments included into 9 groups. 1. Negative Control (NC, Fed with a corn-soy-based diet), 2. Corn-soy-based diet supplemented with 500 mg/kg lead acetate (Positive control), 3. Positive control supplemented with 0.5 % Purslane powder (PP), 4. Positive control supplemented with 1 % PP, 5. Positive control supplemented with 1.5 % PP, 6. Positive control supplemented with 140 mg/kg zinc, 7. Positive control supplemented with 0.5 % PP + 140 mg/kg zinc, 8. Positive control supplemented with 1 % PP + 140 mg/kg zinc, 9. Positive control supplemented with 1.5 % PP + 140 mg/kg zinc. All the experimental groups, except for the negative control group, received 500 ppm lead acetate. The composition of NC, LA and diets containing 0.5, 1 and 1.5 percent are shown in Table 1.

Table 1. Experimental diets ingredients and composition

	Diets								
Item (% or as noted)	NC	PC	0.5PP	1PP	1.5PP	140Zn	0.5PPZn	1PPZn	1.5PPZn
Ingredients									
Corn	53.82	53.71	53.00	52.29	53.82	53.82	53.00	52.29	53.82
Soybean Meal (44% CP)	34.88	34.90	34.81	34.72	34.88	34.88	34.81	34.72	34.88
Limestone	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Soybean Oil	3.57	3.61	3.90	4.19	3.57	3.57	3.90	4.19	3.57
Dicalcium phosphate	1.25	1.25	1.26	1.26	1.25	1.25	1.26	1.26	1.25
Common Salt	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Purslane	0	0	0.50	1	1.5	53.82	0.50	1	1.5
Zinc oxide (mg/kg)	0	0	0	0	0	87.24	87.24	87.24	87.24
Lead acetate (mg/kg)	0	500	500	500	500	500	500	500	500
Calculated composition (%)									
Metabolisable energy (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein	20	20	20	20	20	20	20	20	20
Calcium	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Available phosphorous	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Methionine	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Calculated zinc (mg/kg)	69.88	69.87	69.82	69.76	69.88	140.13	140.13	140.13	140.13
Analyzed zinc (mg/kg)	71.15	70.83	69.01	68.36	70.55	141.25	139.15	141.31	140.89
Calculated lead (mg/kg)	0	500	500	500	500	500	500	500	500
Analyzed lead (mg/kg)	2.75	498.36	491.98	505.12	506.47	492.60	495.30	511.44	500.36

NC, Negative control (Corn-soy-based diet); PC, Positive control (Corn-soy-based diet + 500 mg/kg Lead acetate); PP= purslane powder; Zn= Zinc oxide.

The vitamin premix contained (per kilogram of diet): vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 10 IU; vitamin K3, 2.5 mg; vitamin B12, 0.015 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; folic acid, 1 mg; choline, 1,000 mg; nicotinic acid, 30 mg and pantothenic acid, 10 mg. The mineral premix contained (per kilogram of diet): manganese, 60 mg; zinc, 80 mg; iron, 60 mg; copper, 8 mg; iodine, 0.35 mg; and selenium, 0.3 mg.

Performance measurements and egg quality

During the experiment daily egg number, egg weight and feed intake was recorded for each cage. The collected data (number of eggs and egg weight) were used to calculate egg production and egg mass per replicate. Feed intake was measured on a weekly basis. Data on feed intake and egg mass were used to calculate feed conversion ratio. Body weight change calculated for the whole period of the experiment. In the last 3 days of the experiment, 12 eggs from each treatment per day (36 eggs for 3 days) were randomly selected for the evaluation of egg quality parameters. Egg shape index was calculated as, egg shape index = width of egg/length of egg ×100. After weight the eggs, the albumen height of each egg was measured by a micrometer to calculate Haugh unit score [Hu = 100 log (H – 1.7 $W^{0.37}$ +7.6), Hu = Haugh unit, H = observed height of the albumen in mm, W = weight of egg (g)]. Furthermore, yolk weight, albumen weight, shell weight and eggshell thickness (from at least 4 places each egg with micrometer) were measured. Furthermore, egg yolk relative weight (EYRW), albumin relative weight (AlbRW) and eggshell relative weight (shellRW) were calculated.

Serum metabolites, relative organ weights and histopathology

At the end of the experiment, 2 birds from each pen were randomly selected and weighed and then blood samples were obtained from the wing vein using syringes collected from with no anticoagulant. Blood samples were centrifuged (3000 rpm, 15 min, 4 °C) and the serum was separated and stored in -20 °C for further analysis of glucose, total protein, uric acid, triglycerides, cholesterol and activity of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) using the commercial kits (Pars Azmun, Tehran, Iran). Blood for hematocrit measurement was drawn into EDTA tubes and hematocrit value was determined using microhematocrit capillary tubes by centrifuging for 5 min at 12,000 rpm (Campbell, 1995). Moreover, internal organs included liver, pancreas, heart, proventriculus, gizzard, caeca, spleen, oviduct, ovary, ileum, duodenum and jejunum were removed and weighed. Organ weights were expressed as a percentage of live body weight. For histopathological evaluation, appropriate tissue samples were collected from the livers then fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin-eosin for light microscopic examination.

Assay of antioxidants

The activity of glutathione peroxidase (GPx), SOD and Catalase (CAT) were measured in serum, erythrocytes and liver samples. Before measuring, liver samples were homogenized in a buffer solution (1.15% potassium chloride, pH 7.4) at refrigerated temperature. Homogenized samples were centrifuged for 15 min at 5000 r.p.m for a period of 15 minutes, and the supernatant was taken and used for the related measurements. The SOD in erythrocytes and liver was measured using a kit prepared by the company Randox. The principles of GPx measurement were based on the method described by Paglia and Valentine (1967). The activity of CAT was determined at room temperature by using the method explained by Aebi (1984). TAC was determined using Randox total antioxidant status kit (Randox Laboratories Ltd, Crumlin, UK). To measure serum malondialdehyde (MDA), 0.5 ml of serum was mixed with 2.5 ml trichloro acetic acid and after incubating for 15 min (room temperature), 1.5 ml TBA was added and mixed for 30 sec then, incubated at 95 °C for 30 min. Next, each sample incubated in ice bath for 3 hours and 4.0 ml n-butanol was added and mixed vigorously for 3 min. Finally MDA-TBA adduct was centrifuged at 950 × g for 10 min and absorbance was measured at 532 nm. 1,1,3,3-tetramethoxypropane was used to prepare MDA standard.

Statistical Analysis

The general linear model procedure of SAS software (SAS 2001) was used for analyzing the data in a completely randomized design model. The means of treatments were compared using Duncan's multiple range tests. Values of $P<0.05$ were considered statistically significant.

RESULTS

Growth performance

Egg weight, feed intake and feed/egg ratio of the laying quails were not affected by different experimental groups ($P>0.05$; Table 2). Although, lead exposed quails showed significant body weight, egg mass and egg production reduction when compared with the birds in NC group ($P<0.05$, Table 2), however, PP and Zn supplementation and their combination could not mimic it.

Egg quality

No significant difference ($P>0.05$) was observed for shell thickness, EYRW, AlbRW, shellRW and egg

shape index of quails after 5 weeks feeding experimental diets (Table 2). Feeding laying quails with diets containing lead decreased Haugh unit in comparison to the NC group (P<0.05), however dietary PP, Zn and their combination could not reduce these effect in birds exposed to lead toxicity (P<0.05).

Table 2. The effect of purslane and zinc on performance and egg quality of laying quails exposed to lead toxicity

	Experimental diets										
Items	NC	PC	T1	T2	T3	T4	T5	T6	T7	SEM	*P values*
BWC	29.3[a]	-44.8[b]	-31.7[b]	-39.9[b]	-25.1[b]	-35.3[b]	-33.9[b]	-32.8[b]	-27.3[b]	4.31	0.0001
EP (%)	94.2[a]	77.8[b]	82.6[b]	83.0[b]	82.7[b]	80.6[b]	84.5[b]	83.4[b]	81.2[b]	0.76	0.0001
EW (g)	11.5	11.4	11.2	11.3	11.4	11.4	11.3	11.2	11.5	0.04	0.57
EM(g/bird/day)	10.9[a]	8.8[b]	9.3[b]	9.4[b]	9.5[b]	9.2[b]	9.5[b]	9.3[b]	9.3[b]	0.09	0.0001
FI (g/bird/day)	28.5	28.8	28.1	29.2	29.7	30.0	30.0	29.1	28.6	0.49	0.98
FCR	2.6	3.0	3.0	3.1	2. 9	3.0	2.9	3.1	2.9	0.04	0.26
YRW (%)	34.9	32.4	34.2	35.8	31.4	34.1	33.4	32.7	34.5	0.62	0.85
ARW (%)	57.2	59.7	57.0	57.0	60.6	57.7	59.0	60.3	58.8	0.66	0.87
SRW (%)	7.8	7.8	8.6	7.1	7.9	8.1	7.5	6.8	6.6	0.18	0.18
STh (mm)	1.2	1.2	1.2	1.3	1.2	1.3	1.2	1.2	1.3	0.008	0.89
ShI	80.0	75.7	79.2	80.9	78.3	73.8	78.3	76.9	79.1	0.60	0.13
HaU	88.2[a]	67.6[b]	71.7[b]	68.8[b]	71.0[b]	73.2[b]	70.3[b]	71.7[b]	71.2[b]	1.50	0.02

Abbreviations: BWC. Body weight change; EP, Egg production; EW, Egg weight; EM, Egg mass; FI, Feed intake; FCR, Feed conversion ration; YRW, Yolk relative weight; ARW, Albumen relative weight; SRW, Shell reative weight; STh, Shell thickness; ShI, Shell index; HaU, Haugh unit; NC, Negative control (Corn-soy-based diet); PC, Positive control (Corn-soy-based diet + 500 mg/kg Lead acetate); T1 = PC + 0.5 % purslane powder (PP), T2 = PC + 1 % PP, T3 = PC + 1.5 % PP, T4 = PC +140 mg/kg Zinc oxide, T5 = PC + 0.5 % PP + 140 mg/kg Zinc oxide, T6 = PC + 1 % PP + 140 mg/kg Zinc oxide, T7 = PC + 1.5 % PP + 140 mg/kg Zinc oxide.
[a-c] Means with same superscript in each row are not significantly different.SEM= standard error of the means.

Serum Metabolites

Experimental groups did not alter serum levels of glucose, uric acid and cholesterol of laying quails (P>0.05; Table 3). In comparison to the NC group, concentrations of hematocrit, total protein, triglycerides and very low density protein (VLDL, estimated by Friedewald et al., 1972) showed a significant decrease in all other experimental groups (P<0.05). Moreover, serum activity of ALT and LDH of quails fed whit all experimental groups showed significant increase when compared with the NC group (P<0.05).

Relative internal organs weight

No significant change was observed in relative weights of pancreas, heart, proventriculus, gizzard, caeca, spleen, oviduct, ovary, ileum, duodenum and jejunum among the treatments (Table, 4). However, there was a significant decrease in relative weights of liver in quails fed other diets than the NC group (P<0.05).

Table 3. The effect of different experimental diets on serum parameters of laying quails exposed to lead

	Experimental diets										
Items	NC	PC	T1	T2	T3	T4	T5	T6	T7	SEM	*P values*
Hematocrit (%)	29.54[a]	21.47[b]	24.09[b]	23.39[b]	24.43[b]	23.71[b]	23.80[b]	23.87[b]	24.22[b]	0.51	0.02
Glucose (mg/dl)	276.4	272.1	237.0	344.0	253.2	272.5	297.6	286.1	352.7	17.71	0.89
Chol (mg/dl)	153.2	218.5	198.6	200.6	236.6	214.7	201.3	192.7	250.3	9.51	0.50
TG (mg/dl)	488.9[a]	366.0[b]	392.9[b]	375.3[b]	394.2[b]	377.2[b]	380.5[b]	383.2[b]	372.7[b]	8.67	0.01
TP (g/dl)	7.8[a]	4.9[b]	6.1[b]	6.1[b]	6.3[b]	5.4[b]	6.0[b]	6.1[b]	6.3[b]	0.18	0.03
UA (mg/dl)	5.5	5.6	5.3	5.1	5.7	4.2	5.5	5.6	6.0	0.17	0.54
VLDL (md/dl)	97.7[a]	73.2[b]	78.5[b]	75.0[b]	78.8[b]	75.4[b]	76.1[b]	76.6[b]	74.5[b]	1.73	0.01
ALT (U/L)	24.9[b]	51.0[a]	38.7[a]	44.8[a]	40.0[a]	41.4[a]	40.8[a]	38.6[a]	43.3[a]	1.70	0.03
LDH (U/L)	200.5[b]	395.0[a]	322.1[a]	330.1[a]	307.7[a]	314.4[a]	314.3[a]	329.1[a]	314.1[a]	12.02	0.02

Abbreviations: Chol, Cholesterol; TG, Triglycerides; TP, Total protein; UA, Uric acid; VLDL, Very low density lipoprotein; ALT, Alanine aminotransferase; LDH, Lactate dehydrogenase; NC, Negative control (Corn-soy-based diet); PC, Positive control (Corn-soy-based diet + 500 mg/kg Lead acetate); T1 = PC + 0.5 % purslane powder (PP), T2 = PC + 1 % PP, T3 = PC + 1.5 % PP, T4 = PC +140 mg/kg Zinc oxide, T5 = PC + 0.5 % PP + 140 mg/kg Zinc oxide, T6 = PC + 1 % PP + 140 mg/kg Zinc oxide, T7 = PC + 1.5 % PP + 140 mg/kg Zinc oxide.
[a-b] means with same superscript in each row are not significantly different. SEM= Standard error of the means.

Table 4. The effect of different experimental diets on relative weight of body organs of laying quails exposed to lead (%)

	Experimental diets										
Items (%)	NC	PC	T1	T2	T3	T4	T5	T6	T7	SEM	*P –values*
Liver	2.97[a]	1.20[b]	1.97[b]	2.05[b]	2.06[b]	2.05[b]	1.97[b]	2.07[b]	1.99[b]	0.10	0.02
Spleen	0.05	0.08	0.04	0.04	0.04	0.06	0.05	0.06	0.05	0.004	0.4
Heart	0.60	0.89	0.78	0.66	0.69	0.70	0.74	0.67	0.77	0.02	0.2
Jejunum	1.46	1.83	1.90	1.84	1.33	1.56	1.26	1.45	1.76	0.06	0.23
Duodenum	0.76	1.05	1.04	0.96	0.75	0.82	0.82	0.80	0.92	0.03	0.46
Ileum	0.2	0.19	0.22	0.28	0.20	0.23	0.22	0.23	0.23	0.007	0.08
Pancreas	0.17	0.25	0.22	0.29	0.22	0.20	0.23	0.26	0.25	0.01	0.42
Ovary	2.52	2.55	2.04	2.49	2.81	2.68	2.02	2.72	2.30	0.15	0.96
Oviduct	2.91	4.39	2.93	2.93	3.32	3.49	3.61	2.85	3.18	0.13	0.10
Gizzard	1.57	1.94	1.93	1.79	1.76	1.97	1.52	1.96	1.94	0.04	0.09
Cecum	0.54	0.68	0.63	0.63	0.69	0.59	0.52	0.59	0.51	0.03	0.91
Preventriculs	0.26	0.34	0.34	0.39	0.29	0.37	0.32	0.29	0.28	0.01	0.28

Abbreviations: NC, Negative control (Corn-soy-based diet); PC, Positive control (Corn-soy-based diet + 500 mg/kg Lead acetate); T1 = PC + 0.5 % purslane powder (PP), T2 = PC + 1 % PP, T3 = PC + 1.5 % PP, T4 = PC +140 mg/kg Zinc oxide, T5 = PC + 0.5 % PP + 140 mg/kg Zinc oxide, T6 = PC + 1 % PP + 140 mg/kg Zinc oxide, T7 = PC + 1.5 % PP + 140 mg/kg Zinc oxide. [a-b] means with same superscript in each row are not significantly different. SEM= Standard error of the means.

Antioxidant enzyme activities

The effects of treatments on liver, erythrocytes and serum antioxidants status are presented in Table 5. Dietary lead decreased SOD and GPx activities in the liver and erythrocyte than the NC group (P<0.05), however, had no effect on liver catalase activity (P<0.05). In addition, all dietary treatments decreased (P<0.05) TAC and increased serum and liver MDA levels compared to the NC group (P<0.05). The results showed that the negative effects of lead on serum activity of TAC, liver and serum MDA was ameliorated (P <0:05) by 1.5 % dietary PP supplementation, but PP supplementation could not restore the activity of TAC and MDA towards close the control levels (P <0.05).

Table 5. The effect of different experimental diets on liver, erythrocytes and serum antioxidants of laying quails exposed to lead (%)

	Experimental diets										
Items	NC	PC	T1	T2	T3	T4	T5	T6	T7	SEM	*P -values*
Liver											
MDA (nmol/mg of protein)	3.88[c]	6.45[a]	5.76[ab]	6.47[a]	4.40[bc]	5.22[abc]	5.43[abc]	5.19[abc]	4.46[bc]	0.22	0.04
CAT (unit/mg of protein)	10.83	13.71	12.21	12.11	11.51	12.88	13.41	12.54	11.27	0.29	0.28
GPx (unit/mg of protein)	25.19[a]	20.21[b]	22.02[b]	22.20[b]	22.28[b]	22.17[b]	22.23[b]	22.18[b]	21.36[b]	0.30	0.01
SOD (unit/mg of protein)	227.19[a]	174.85[b]	180.15[b]	175.16[b]	193.40[b]	188.98[b]	176.46[b]	187.64[b]	190.69[b]	4.05	0.04
Erythrocytes											
GPx (unit/mg Hb)	71.82[a]	42.80[b]	50.45[b]	51.06[b]	55.16[b]	47.71[b]	44.87[b]	45.32[b]	54.88[b]	2.16	0.04
SOD (unit/mg Hb)	1907.1[a]	1485.0[b]	1579.4[b]	1579.6[b]	1678.3[b]	1622.4[b]	1668.2[b]	1636.0[b]	1536.3[b]	28.99	0.02
Serum											
MDA (nmol/ml)	1.61[c]	3.04[a]	2.68[a]	2.59[ab]	2.00[c]	2.81[a]	2.65[a]	2.69[a]	2.05[bc]	0.09	0.0002
TAC (mmol/L)	1.31[a]	0.77[c]	0.88[bc]	0.99[bc]	1.09[ab]	0.84[bc]	0.96[bc]	0.98[bc]	0.99[bc]	0.03	0.02

Abbreviations: NC, Negative control (Corn-soy-based diet); PC, Positive control (Corn-soy-based diet + 500 mg/kg Lead acetate); T1 = PC + 0.5 % purslane powder (PP), T2 = PC + 1 % PP, T3 = PC + 1.5 % PP, T4 = PC +140 mg/kg Zinc oxide, T5 = PC + 0.5 % PP + 140 mg/kg Zinc oxide, T6 = PC + 1 % PP + 140 mg/kg Zinc oxide, T7 = PC + 1.5 % PP + 140 mg/kg Zinc oxide. CAT (Catalase), GPx (glutathione peroxidase) and SOD (Total superoxide dismutase) according to U/mg Protein, SOD (superoxide dismutase) according to mg/Hb, MDA (malondialdehyde) and TAC (Total antioxidant capacity) according to mmol/L. [a-c] means with same superscript in each row are not significantly different. SEM= standard error of the means.

Liver histopathology

Liver tissues from the quails in negative control group showed normal lobular architecture with central veins, radiating hepatic cords and portal triads, while this organ in the other experimental groups showed mild, moderate, and severe tissue changes (Figure 1A and 1B). The main histopathologic findings in the liver of quails, intoxicated with lead acetate alone, were hepatocyte degeneration, severe macrovesicular steatosis accompanied with marked hepatocellular ballooning, congestion and dilation of central veins and sinusoids, severe mononuclear cell infiltration in the liver parenchyma and around the portal area, proliferation of Kupffer cells, multifocal to diffuse necrosis and moderate fibrosis, particularly in the portal areas (Figure 1C–1F).

In comparison to lead acetate group, the quails treated with different Levels of PP powder and Zn showed improvement in its histological structure. However, mild vacuolar degeneration, a few hepatocytes with pyknotic nuclei, mild mononuclear cell infiltration and moderate venous congestion were observed in the treated groups. There was no evidence of fibrosis or coagulative necrosis in these groups. In comparison between all treated groups, the most therapeutic effect was seen with the dose of 1.5 % PP (Figure. 2A and 2D).

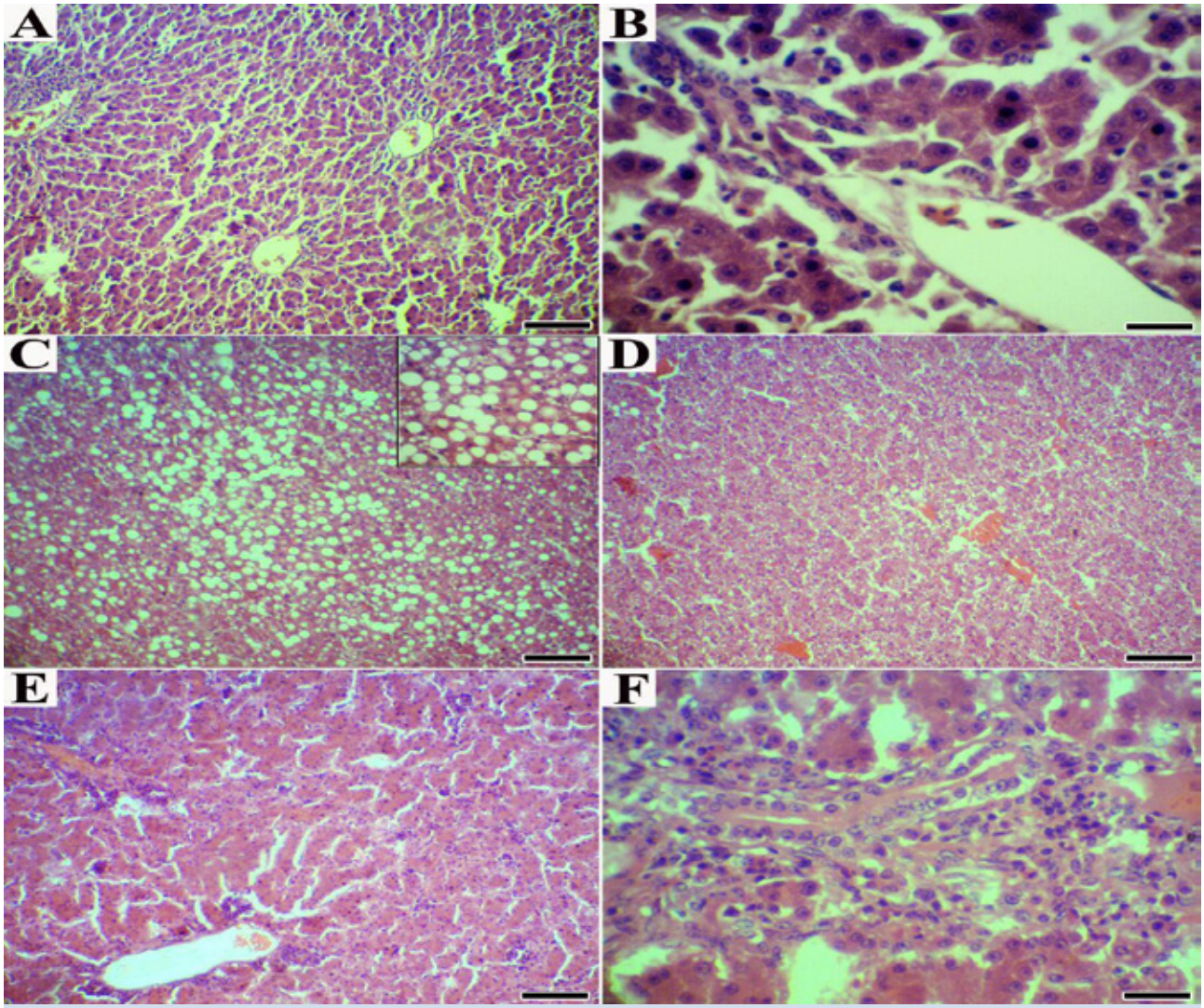

Figure 1. (A-B) Normal quails. (A) A normal lobular pattern with a centrilobular vein and radiating irregular anastomosing plates of hepatocytes with intervening sinusoids (H&E; Bar=150 µm); (B) Normal quails. Portal area with normal architecture (H&E; Bar=20 µm); (C-F) Quails received lead acetate. (C) Severe macrovesicular steatosis (H&E; Bar=150 µm); (D) Severe congestion and dilation of sinusoids (H&E; Bar=150 µm); (E) several foci of mononuclear cell infiltration in the liver parenchyma (H&E; Bar=150 µm); (F) Moderate infiltration of mononuclear cells and fibrous tissue around the portal area (H&E; Bar=20 µm).

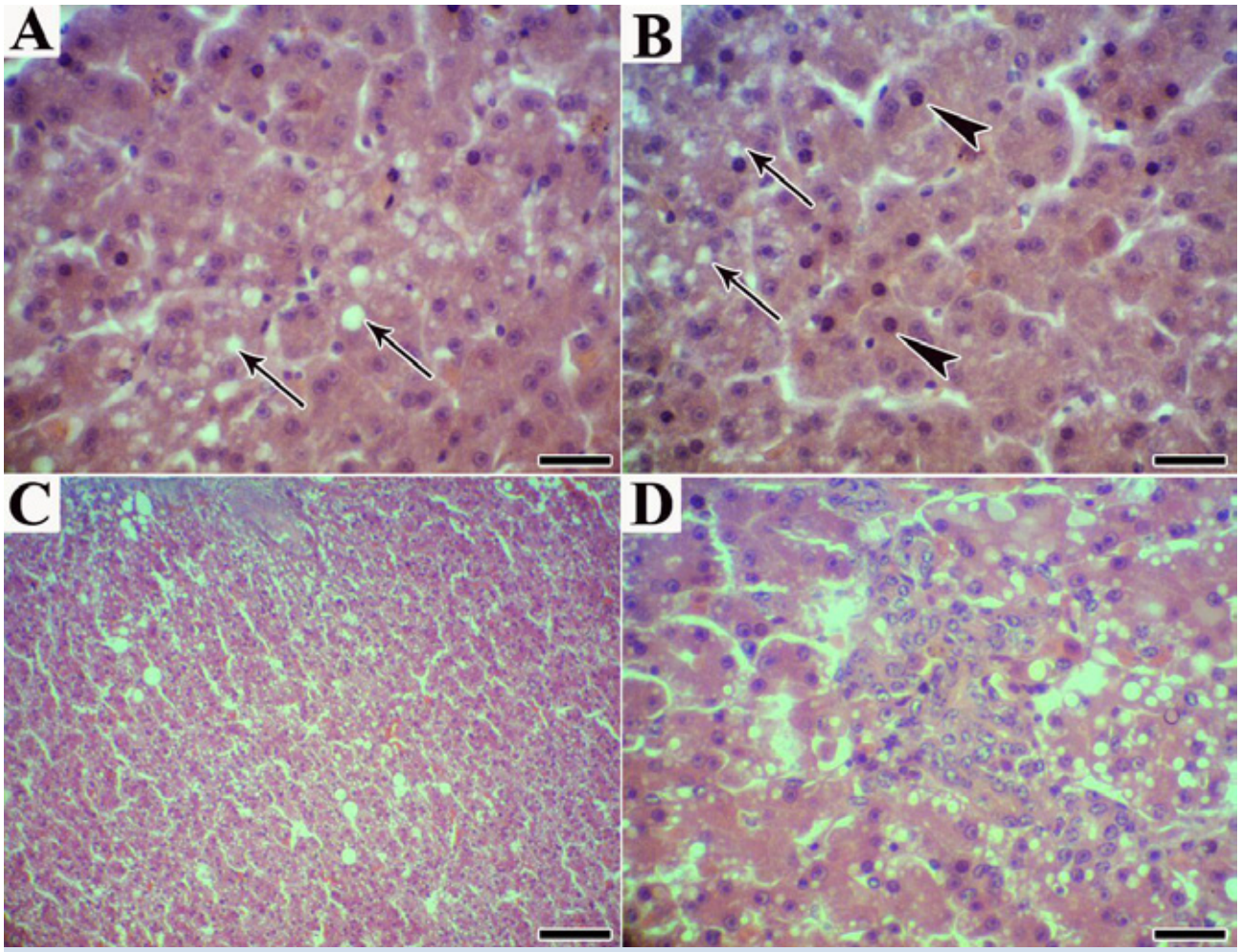

Figure 2. (A) Treated quails with zinc. Mild macrovesicular steatosis (arrows) (H&E; Bar=20 μm) (B) Treated quails with ***P. oleracea*** (1.5%). Presence of a few cytoplasmic vacuoles (arrows) with pyknotic nuclei (head arrows); (C) Mild congestion with disorganization of the hepatic cords (H&E; Bar=150 μm); (D) Treated quails with ***P. oleracea*** (1.5%). Mild infiltration of mononuclear cells in the in the liver parenchyma H&E; Bar=20 μm).

DISCUSSION

This study was designed to evaluate the protective effect of PP and Zn against lead-induced toxicity in laying quails. The present study showed that consumption of 500 ppm dietary lead for 5 weeks significantly decreased body weight, egg mass and egg production. In agreement with our results, Damron and Wilson (1975) reported that feeding 3000 ppm lead acetate to quails decreased body weight and increased mortality rate. Hossain et al. (2014) reported live weight reduction in lead-exposed (100 mg/kg of diet) broiler chickens after 42 days. Goldberg (1972) demonstrated that anemia is an early sign of lead toxicity. Morgan et al. (1975) reported inhibition of growth and anemia when lead acetate administered to Japanese quails diets at levels of 500 or 1000 ppm. In mammalian species it is demonstrated that lead administration resulted in a significant reduction in feed intake, erythrocytes count, haemoglobin and the concentration of blood iron (Saly et al., 2004). According to these reports, it is likely that body weight loss can be caused by toxic effects of lead on haemopoietic systems. This assumption is supported by lower haematocrit content in the blood of lead exposed laying quails. Oxidative stress has been known as the main mechanism of lead toxicity (Aykin-Burns et al., 2003). On the other hand, it has been demonstrated that free radical production, oxidative stress, could be strongly a possible reason for the body weight loss of quails in the present experiment (Hakim et al., 1997).

Yuan et al. (2013) reported that lead acetate reduced serum level of FSH, LH and progesterone in laying hens. Pillai et al. (2003) concluded that lead may attach to the steroid hormone receptors, for instance, estrogen and progesterone receptors; therefore, it finally prevents their secretion. Additionally, lead may interfere with calcium-dependent gonadotrophin-releasing

hormones through toxic effects on calcium homeostasis (Pillai et al., 2003). Calcium plays an important role in the regulation and secretion of gonadotropin releasing hormore and LH which lead to reduction of plasma estradiol level (Martinez De La Escalera et al., 1992). Estradiol induces the synthesis of vitellogenin in the avian liver (Gruber et al., 1976). Reduction synthesis of vitellogenesis could be a reason for the reduced yolk weight and egg weights (Faryadi and Sheikhahmadi 2017). Therefore, it seems probably that decrease in plasma LH level due to the addition of lead acetate to the diet of quails could be a possible reason to reduced egg production and egg mass.

Our results showed a significant decrease in Haugh unit in the lead exposed quails compared with the NC. Haugh unit has been accepted as an index of the quality of the albumen (Eisen et al., 1962(. Eggs with higher Haugh unit have better internal egg quality that could be due to lower protein damage in albumen (Begli et al., 2010). Therefore, it seems that Haugh unit reduction in the lead-exposed quails could be due to disorder in protein metabolism in the liver. It is strongly possible that toxicity effect of lead on liver cells and impairment of liver protein synthesis leading to reduced albumen protein production and especially the ovomucin which is a critical protein to creates viscosity character of egg albumen and increase Haugh unit (Omana et al., 2010).

In our present study, decrease in blood hematocrit, total protein, triglyceride, VLDL concentration and increase in the activity of ALT and LDH in the serum of the lead-exposed quails has been found when compared with the NC. These results are in accordance with Hamidipour et al. (2016), who reported higher ALT and LDH activity and decrease in triglyceride and total protein in quails exposed to lead acetate. The LDH is one of the most important liver glycolytic enzymes and can be found in the heart and other tissues. Hepatic impairment, heart failure, renal disorders, muscular dystrophy and hemolytic anemia can lead to increased levels of this enzyme in blood (Ebrahimi, 2011). In the present study, increased activity of LDH indicates damage in different tissues of the lead-exposed quails. However we measured ALT as it is well known that ALT is mainly in the cytoplasm of liver cells and any liver failures resulted in a release of this enzyme into the circulation system. Liver is one of the most important organs involved in the lead toxicity (Dzugan et al., 2012). Our findings showed that relative weight of the liver of quails decreased in lead-exposed groups. Previous work by Mahaffey et al. (1981) showed that organ/body weight ratio negatively affected by heavy metals.

Hamidipour et al. (2016) reported lower concentration of triglycerides in lead-exposed quails is due to small intestine villi damage which causes significant impairment in the absorption of fatty acids. Liver is the main organ of lipid biosynthesis and is particularly very active in laying birds (Aydin, 2005). Therefore, liver damage may decrease the synthesis of triglycerides. In addition, liver is responsible for synthesis of most of the plasma proteins. Therefore, total protein in the plasma is an important indicator of protein synthesis in the liver (Robin et al., 1987). It is reported that feeding birds with lead for 2 months could lead to degeneration of liver protein synthesis (Yuan et al., 2013). Totally, according to the present results, increased activity of ALT and LDH and decrease in serum triglyceride, VLDL and total protein concentration can indicate liver damage in lead-exposed laying quails.

It is reported that toxic metals act as catalysts in the production of reactive oxygen species (El-Maraghy et al., 2001). Free radicals can attack to lipid molecules leading to lipid peroxidation and change in antioxidant status of the cells (Stohs et al., 2001). Antioxidant enzymes of the cells play an important role in protection the homeostasis of free oxygen radicals (Qanungo et al., 1999). Alter of antioxidant enzyme activities such as SOD, CAT, and GPx and reduction in the concentrations of some antioxidant molecules, such as GSH has been reported in lead exposed animals. Previous studies suggested that oxidative damage is one of the important mechanisms of lead toxicity (Aykin-Burns et al., 2003). In the present study, a significant decrease in SOD and GPx activities in the liver and erythrocyte and serum TAC concentration along with increased liver and serum level of MDA are indicators of increased oxidative stress in lead-exposed birds (Pan et al., 2005). These results are in accordance with Erdogan et al. (2005), who showed that lead significantly increase plasma MDA in broilers. Several studies reported that MDA levels increased when lipid peroxidation develops (Tatli Seven et al., 2009). MDA is an index of lipid peroxidation that is associated with the oxidation of polyunsaturated fatty acids. Moreover, our present study demonstrates a significant decrease in the liver and erythrocyte SOD and GPx activity in all birds exposed to lead. Many studies have reported that lead bounds with

thiol groups and therefore reduce cellular glutathione levels (Fuhr and Rabenstein 1973). Under oxidative stress oxidized glutathione is reduced to GSH via glutathione reeducates which is an indirect combination of the antioxidant defense system. It has been shown that lead inhibits glutathione reductase by binding to sulfide groups at the active site of this enzyme leading to a reduction in the reduced glutathione which is a substrate for GPx (Sandhir and Gill 1995). This may explain the reduction of GPx activity in the present study. Strehlow et al. (2003) reported that estrogen up-regulates SOD expression. Previous studies reported that lead exposure resulted in a significant estrogen production (Paksy et al., 1992). Although we did not measure serum estrogen, however, it is strongly likely that reduced SOD activity in lead-exposed quails in our present study is due to lower estrogen production (Nampoothiri et al., 2007).

Antioxidants are free radical scavengers that suppress the formation of ROS. It is well known that Zn acts as an antioxidant to decrease oxidative damage of cell membrane. Zn is an important part of SOD, which protects body against free radicals by converting superoxide anions to hydrogen peroxide (Niles et al., 2008). It is suggested that increase dietary Zn can reduce toxic effects of lead in rats (Cerklewski and Forbes 1976). One of the antioxidant enzymes possess Zn involved in the active site is SOD (Nampoothiri et al., 2007). Hence in the present study we assumed that dietary Zn supplementation can increase SOD activity in lead-exposed laying quails. However, liver and erythrocyte SOD activity decreased in lead-exposed laying quails. It is strongly possible that ionic mechanism of action for lead resulted in substitution of Zn ions by lead in SOD and hence negatively affected its activity (Nampoothiri et al., 2007).

On the other hand, one natural source that could act as an antioxidant is Purslane (*Portulaca oleracea*), which has been using as an edible vegetable in many countries (Zhao et al., 2013). Purslane is a rich source of antioxidants (Simopoulos et al., 2005). In our present study, although, the negative effects of lead treatment on serum CAT activity ameliorated by dietary supplementation of PP at 1.5 %, however, it could not restore the increased activity of CAT towards the negative control levels. Moreover, TAC decreased in lead-exposed laying quails that could be due to higher production of ROS in these birds. Purslane is a rich source of glutathione that absorbed by gut and acts as a substrate for GSH-Px in animal cells and increase the antioxidant status of birds (Simopoulos, 2001). However, we did not see any significant increase in the activity of GSH-Px after exposing laying quails to lead for 5 weeks.

Histopathological examination of liver tissue in the groups that received lead acetate showed mild, moderate, and severe tissue changes. It has been reported that adding 400 ppm lead acetate to drinking water and diet leads to liver lesions in broiler chickens (Sipos et al., 2003). These liver lesions can be caused by stimulating the intercellular signals between kuffer cells and hepatocytes, which ultimately leads to increased proteolytic activity and damage to the liver tissue (Sipos et al., 2003). The low concentration of lead in the ration of birds can lead to low grade changes through the disruption of the normal biochemical processes of the liver system. The liver is the central organ for all metabolic processes, and because of its major role in the processing of foods and xenobiotics in the body, remarkable amounts of toxic lead are absorbed and stored in the liver. Therefore, the probable reason of damage in cells caused by its ability to replace with several metal ions, especially calcium and Zn in their binding sites (Garza et al., 2006). Lead causes oxidative damage to lipids and proteins, disruption of antioxidant mechanisms, and direct oxidative damage (Garza et al., 2006) that this effects whit addition of PP to the diet due to have antioxidant effects can slightly somewhat overcome on the toxic effects of lead. Furthermore, liver pathological results supported by the serum parameters findings.

CONCLUSIONS

From this study, it can be concluded that lead-exposure induced production of free radicals and weakened the antioxidant defenses of the quails. Supplementation of PP, Zn or their combination could not prevent the negative effect of lead on the performance of quails. However, antioxidant status of quails partially improved when fed diets supplemented with 1.5 % PP and 140 ppm Zn.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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