# Could Alpha-Lipoic Acid Protect Against Sub-chronic Toxicity of Heavy Metals Mixture in Japanese Quails?

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**Abstract**: **Context:** The toxicity of heavy metals considered a serious event in human and animals practice. Objective: The sub-chronic toxicity of heavy metals mixture was investigated in Japanese quail, and whether the administration of alpha lipoic acid (ALA) able to reverse or enhance the toxicity. **Materials and methods**: For this purpose 50 seven days old Japanese quail were randomly and equally divided into five groups (n=10). Group I; was kept on normal diet, birds in other four experimental groups were provided with drinking water containing a mixture of lead acetate, cadmium chloride and mercuric chloride at dose of 100 ppm for each metal for 30 days. Experimental diets for metals-treated groups were prepared to contain 0.5, 1.0, 3.0 g/kg ALA in basal diet (groups III, IV, and V respectively). **Results**: revealed that the administration of toxic metals mixture in drinking water for 30 days induced a significant decrease in the final body weight, increased tissues lipid peroxidation, and induced hepatic, renal and cardiac damage. Also, caused perturbation of lipid profile with increased concentration of heavy metals and copper in liver, kidneys, brain, and muscle tissues. Furthermore, the results showed that alpha-lipoic acid fails to reduce tissue concentration of heavy metals, lipid peroxidation and exert a pro-oxidant activity especially at low concentration. Only at high doses alpha-lipoic acid improved biliary and cardiac functions. **Discussion and Conclusion**: We concluded that alpha-lipoic acid has no metal-chelating activity and cannot protect Japanese quails from heavy metals toxicity.
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## 1. Introduction

Metals are probably well-known to the humans and animals as the oldest toxic substances. Among existing toxic metals, the most important are cadmium, lead, and mercury, which are harmful to the animal's health (Suttle, 2010). Treatment of heavy metals poisoning is sometimes warranted to prevent or reverse the toxicity particularly for those metals that are known to be cumulative and persistent (Goyer and Clarkson, 2001). Alpha-Lipoic acid (ALA), also known as thioctic acid is a naturally occurring compound widely distributed among microorganisms, plants, and animals (Packer *et al.,* 1995). Endogenous ALA is bound to proteins, but exogenous ALA supplementation found to be unbound in the circulation. Lipoic acid is lipophilic, readily absorbed from an oral dose and able to penetrate the cell membranes reaching high intracellular concentrations within 30 seconds of its administration (Handelman *et al.,* 1994).

ALA had been used as an antioxidant and for treatment of diabetic polyneuropathy. Inside the body, ALA is reduced to dihydrolipoic acid (DHLA); a potent antioxidant in both fat- and water-soluble medium. DHLA is capable of regenerating ascorbic acid from dehydro-ascorbic acid, glutathione, and vitamin E. Additionally, ALA and DHLA had been shown to form complexes with manganese, zinc, cadmium, lead, cobalt, nickel, and iron ions preventing the free radical induced tissue damage or enzyme inactivation (Lyn Patrick, 2002). ALA as a sulfur-containing antioxidant might be a beneficial component in the treatment of heavy metals toxicity particularly cadmium, arsenic, lead, and mercury due to its ability to decrease the oxidative stress that cause cellular damage (Bludovska *et al.,* 1999; Caylak *et al.,* 2008; Liu *et al.,* 2010).

Theoretically, ALA as a dithiol compound appears capable of chelating some toxic metals, and improves their clearance from the body. *In vitro*, ALA chelate mercury from renal slices (Keith *et al.,* 1997). But certain studies suggested that the toxicity of cadmium or mercury was decreased mainly by the antioxidant activity of ALA rather than by removal of metals from tissues (Bludovska *et al.,* 1999; Aposhian *et al.,* 2003). On the other hand, previous studies had shown that ALA reduce the food intake and cause profound weight loss in rats in a dose-dependent manner (Kim *et al.,* 2004; Prieto-Hontoria *et al.,* 2009; Seo *et al.,* 2012). ALA may, also, exert oxidant activity and triggers the apoptosis in a variety of cells through different mechanisms. The signaling involved in the triggering of ALA induced apoptosis includes the tensin homologue deleted on

chromosome 10 (PTEN)/Akt pathway, up-regulation of poly (ADP-ribose) polymerase, activation of caspases, increase of intracellular $Ca^{2+}$ concentration, p53 activation, increase expression of Bax, down-regulation of Bcl-2, release of cytochrome-c from mitochondria, down regulation of survivin, as well as induction of pro-apoptotic JNK signaling (Moungjaroen *et al.,* 2006; Simbula *et al.,* 2007; Shi *et al.,* 2008; Choi *et al.,* 2009). Another study explored that, ALA triggers the eryptosis through the increase of the cytosolic $Ca^{2+}$ concentration, ceramide formation, and enhanced the effect of glucose depletion on the erythrocytes (Bhavsar *et al.,* 2010).

In the light of the above, it is clear that many unanswered questions remain regarding the benefits of the long-term use of ALA against the toxicity of heavy metals in veterinary practice. Therefore, the aim of this study is to investigate the toxic effect of a mixture between lead, cadmium and mercury on Japanese quails, and whether the supplementation of ALA can reverse or enhance the toxicity and tissue concentrations of the tested metals.

## 2. Material and Methods

### Chemicals and diagnostic kits

Lead acetate was obtained from pure laboratory Chemicals, EL Nasr pharmaceutical chemicals Co, Egypt. Mercuric chloride was provided from lobachemie PVT, LTD, India and cadmium chloride from chemajet Co, Egypt. Alpha-lipoic acid was obtained from Sigma Co., USA. Biochemical diagnostic kits were obtained from Vitro Scient Co., Germany for measurement of serum biochemical parameters.

### Birds and experimental design

Our 30-day study was carried out on a total 50 seven-day-old Japanese quails (*Coturnix coturnix japonica*) of both sexes which were obtained from the department of animal breeding, Faculty of Veterinary Medicine, Alexandria University. The animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local authorities (Faculty of Veterinary Medicine, Alexandria University, Egypt). After 7 days acclimatization period, birds were randomly assigned into 5 groups (n =10), and maintained in individual cages in room where the temperature was approximately 28±2 °C, 12 hour light/dark cycle and supplied with commercial diet with free access to food and water. Each cage contained a random selection of 5 female and 5 male birds. Group I; the birds received normal water and feeds and considered the negative control group, birds in groups II, III, IV and V were supplied with toxic water contained a mixture of cadmium chloride, mercuric chloride and lead acetate) in a concentration of 100 ppm for each metal and were fed ALA containing diet at dose of 0.0, 0.5, 1.0 and 3.0 g/kg mixed with food, respectively.

### Biochemical analysis

At the end of experiment, birds were bled by slaughtering for blood sample and then dissected for the collection of internal organs. Blood was centrifuged at 1300 rpm for 10 min for separation of the serum. Liver, kidney, brain, breast muscle (pectoralis major) and serum were collected and stored at -20 °C till analysis. Serum ALT, AST, ALP, LDH, GGT, and CPK activities, and protein, albumin, cholesterol, triacylglycerol, HDL-c, urea, uric acid, and calcium levels were measured spectrophotometrically using available commercial diagnostic kits (Vitro Scient Co., Germany) according to manufacturer's instructions. ELISA procedure was used for quantitative determination of serum troponin I according to manufacturer's instructions.

### Assessment of lipid peroxidation

The extent of lipid peroxidation was determined by the method of (Ohkawa *et al.,* 1997) by measuring the malondialdehyde (MDA) concentration in liver, kidney, breast muscle and brain. MDA is known as a product of lipid peroxidation that reacts with thiobarbituric acid under high temperature giving a red color absorbing at 535 nm.

### Analysis of heavy metals

Samples of liver, kidney, breast muscles and brain were collected and digested with a mixture of 5 ml $HNO_3$ and 1 ml HCL per 1 g of sample. Digests were diluted to a constant volume with de-ionized water. Samples were analyzed for the presence of lead, cadmium, mercury and copper using 4100 MP-AES Spectrometer (Microwave Plasma-Atomic emission Spectrometry), Agilent Tech., Germany, at wave lengths 405.78, 228.80, 253.7 and 324.75 nm, respectively. Metals concentrations are expressed on a wet tissue weight basis.

### Statistical analysis

The obtained data were presented as mean ± SE. The significance of the difference between control and treated groups parameters was analyzed using one way ANOVA test by computerized Costat program.

## 3. Results

### Effect of toxic metals mixture with or without ALA on clinical status, post-mortem picture, and body weight

All birds in metals-treated groups with or without ALA showed depression, ruffled feathers, generalized weakness and a significant decrease in the final body weight compared with the control

group. Our daily observation revealed a decrease in the food intake of lipoic acid treated groups in a dose dependent manner. Final body weight was significantly decreased in birds co-exposed to 3 g/kg ALA and metals compared with birds exposed only to toxic metals mixture as shown in table (1). Gross pathological examination revealed paleness and enlargement of the liver and kidneys in heavy metals-exposed birds including those administered ALA.

**Effect of toxic metals mixture with or without ALA on the biochemical parameters of the serum**

Effect of toxic metal mixture with or without ALA on hepatobiliary and cardiac biomarkers

As shown in table (2), birds sub-chronically exposed to toxic metals mixture without ALA exhibited a significant increase in the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK) and troponin I level, and significant decrease of alanine aminotransferase (ALT) with non-significant changes in serum total bilirubin level as compared to control birds. Co-exposure of lipoic acid with toxic metals has been shown a modification in the activities of some enzymes in a dose dependant manner. Dietary supplementation of 0.5 g/kg ALA significantly decrease the serum activities of ALP and GGT, while the activities of LDH, ALT, and CK were elevated with non-significant changes in troponin I level in comparison with metals-treated group. Birds co-administered 1.0 g/kg ALA with toxic metals showed a significant elevation in the activities of ALT, AST, LDH, and CK while the activities of ALP and GGT and troponin I level were decreased in comparison with metals received group. ALA 3g/kg caused an elevation in the activities of ALT and AST, while the activities of ALP, GGT, LDH, and CK and troponin I level were declined as compared to metals treated group.

Effect of toxic metals mixture with or without ALA on renal functions, serum proteins and calcium levels

Table (3) showed that the birds sub-chronically intoxicated with metal mixture exhibited a significant increase in the levels of uric acid, and blood urea nitrogen (BUN) and significant decrease in serum total protein, albumin, globulins and calcium levels as compared to control group. Birds received ALA at a dose of 0.5 g/kg showed a significant decrease in serum total protein and albumin levels with non-significant changes in serum globulins, uric acid and urea levels when compared to metal intoxicated birds. Feeding of ALA at dose of 1 g/kg had no significant changes on serum protein, albumin, globulins, uric acid and urea levels, while ALA at dose of 3g/kg significantly increased serum total protein, albumin, globulins, and calcium levels, decreased uric acid with non-significant change in serum urea level as compared to metals-intoxicated birds.

Effect of toxic metal mixture with or without ALA on serum lipid profile

The data represented in table (4) revealed that administration of toxic metal mixture significantly decreased serum triacylglycerol (TAG), cholesterol and VLDL-c levels, increased HDL-c level with non-significant change in serum LDL-c level when compared to control group. Moreover, the co-administration of lipoic acid at different doses with toxic metals significantly increased serum TAG, and VLDL-c levels and decreased serum HDL-c and LDL-c levels with non-significant changes on serum cholesterol level as compared to intoxicated group.

**Effect of toxic metals mixture with or without ALA on lipid peroxidation products (MDA)**

The data represented in table (5) revealed that intoxication of birds with metals combination significantly increased malondialdehyde (MDA) level in liver, kidney, brain and muscle as compared to control group. Treatment with ALA at different doses had no significant effects on MDA level in different organs as compared to intoxicated birds.

**Effect of toxic metals with or without ALA on the concentrations of toxic metals and some elements in selected organs**

Our results as represented in table (6) clearly revealed that there is a significant increase in lead, cadmium and mercury concentrations in liver, kidney, brain and muscles in metals exposed groups with or without ALA in comparing with control group. In liver, the dietary supplementation of ALA especially at high dose significantly increased lead content and decreased cadmium concentration as compared to intoxicated group. Cadmium concentration was below the detectable level in brain and muscles of birds in all experimental groups. Lead and cadmium contents in kidneys don't showed any significant alteration between metals exposed birds with or without ALA. Considering analysis of copper, it was showed that there is an elevation of the content of copper in the liver, kidneys and brain of toxic metals-treated group. The most significant changes in the copper concentration evident in liver in all experimental groups, while only slight elevation in the copper content was pronounced in kidneys especially in groups received high doses of ALA. Moreover, the co-exposure of ALA with toxic metals decreased the brain content of copper towards the level of negative control group in a dose dependent manner when compared with metals-exposed birds, while copper concentration in breast muscle don't showed any significant alteration between experimental groups and control one.

**Table 1. Effect of toxic metals mixture with or without ALA on the final body weight**

| Groups | I | II | III | IV | V |
|---|---|---|---|---|---|
| Initial body weight (g) (7 days old) | 42.0±0.8 | 41.1±0.4 | 43.0±0.4 | 42.1±0.7 | 40.5±0.3 |
| Final body weight (g) (30 days old) | 188.0±5.5 | 134.4±12.2[a] | 133.3±18.0[a] | 111.1±12.0[a] | 82.5±9.4[a,b] |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA.

[a] ***p* <0.01** compared with control group (**I**), [b] ***p* <0.01** compared with only metals-exposed group (**III**)

**Table 2. Effect of toxic metals mixture with or without ALA on hepatobiliary and cardiac biomarkers in quails**

| | ALT (U/l) | AST (U/l) | ALP (U/l) | GGT (U/l) | LDH (U/l) | CPK (U/l) | Troponin I (ng/ml) |
|---|---|---|---|---|---|---|---|
| I | 20.6±2.7 | 175.1±5.4 | 1193.5±49.0 | 4.9±0.4 | 262.9±8.1 | 80.4±17.4 | 0.35±0.02 |
| II | 14.6±0.3[a] | 348.9±11.7[c] | 2048.9±77.1[c] | 17.6±0.7[c] | 916.8±21.9[c] | 186.4±5.5[c] | 0.47±0.03[a] |
| III | 22.4±0.4[b] | 350.3±20.3[c] | 1351.7±119.2[d] | 11.0±0.8[c,d] | 1179.5±139.0[c,b] | 322.4±7.0[c,d] | 0.49±0.02[a] |
| IV | 22.7±1.8[b] | 608.7±88.2[b,c] | 1712.8±48.3[c,b] | 9.6±0.3[c,d] | 1039.8±37.8[c,b] | 236.9±2.2[c,d] | 0.37±0.01[b] |
| V | 20.4±1.9[b] | 646.1±70.0[c,d] | 1560.7±163.2[b] | 4.0±0.2[d] | 823.2±34.2[c,b] | 116.7±4.3[d] | 0.24±0.01[c,d] |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA.

[a] ***p* <0.05** compared with control group (**I**) [c] ***p* <0.01** compared with control group (**I**)

[b] ***p* <0.05** compared with only metals-exposed group (**II**) [d] ***p* <0.01** compared with only metals-exposed group (**II**)

**Table 3. Effect of toxic metals mixture with or without ALA on serum proteins, urea and uric acid levels in quails**

| | Protein (g/dl) | Albumin (g/dl) | Globulins (g/dl) | Uric acid (mg/dl) | Urea (mg/dl) | Ca (mg/dl) |
|---|---|---|---|---|---|---|
| I | 3.9±0.02 | 1.67±0.00 | 2.22±0.01 | 7.0±0.1 | 6.4±0.3 | 5.1±0.1 |
| II | 3.0±0.02[c] | 1.32±0.01[c] | 1.78±0.01[c] | 15.8±0.5[c] | 19.2±1.7[c] | 3.7±0.1[c] |
| III | 2.7±0.07[c,d] | 1.29±0.00[c] | 1.46±0.07[c,b] | 14.9±0.4[c] | 17.3±0.4[c] | 5.9±0.2[a,d] |
| IV | 2.9±0.06[c] | 1.33±0.01[c] | 1.66±0.03[c] | 15.0±0.3[c] | 20.8±0.6[c] | 7.2±0.3[c,d] |
| V | 3.3±0.11[a] | 1.58±0.03[d] | 1.98±0.10[a] | 8.9±0.8[d] | 15.9±1.2[c] | 4.9±0.2[b] |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA.

[a] ***p* <0.05** compared with control group (**I**) [c] ***p* <0.01** compared with control group (**I**)

[b] ***p* <0.05** compared with only metals-exposed group (**II**) [d] ***p* <0.01** compared with only metals-exposed group (**II**)

**Table 4. Effect of toxic metals mixture with or without ALA on serum lipid profile in quails**

| | cholesterol (mg/dl) | TAG (mg/dl) | HDL-c (mg/dl) | LDL-c (mg/dl) | VLDL-c (mg/dl) |
|---|---|---|---|---|---|
| I | 227.7±2.5 | 280.6±6.6 | 99.3±5.7 | 71.5±6.1 | 55.6±1.6 |
| II | 207.7±3.6[c] | 136.1±5.6[c] | 116.8±1.4[a] | 63.6±5.5 | 22.5±0.5[c] |
| III | 200.7±5.8[c] | 212.4±9.8[c,d] | 91.1±4.4[d] | 47.6±8.1[a] | 45.8±1.9[a,d] |
| IV | 209.6±3.0[c] | 282.2±6.1[d] | 91.8±2.2[d] | 65.0±2.2 | 57.0±1.5[d] |
| V | 206.9±7.9[c] | 276.3±6.0[d] | 94.9±10.5[d] | 49.0±4.3[a] | 53.3±1.2[d] |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA.

[a] ***p* <0.05** compared with control group (**I**) [c] ***p* <0.01** compared with control group (**I**)

[b] ***p* <0.05** compared with only metals-exposed group (**II**) [d] ***p* <0.01** compared with only metals-exposed group (**II**)

**Table 5. Concentration of malondialdehyde (nmole/g tissue) in selected organs after the exposure to toxic metals with or without ALA**

| Group | Liver | Kidney | Brain | Muscle |
|---|---|---|---|---|
| I | 9.81±0.1 | 22.1±1.8 | 26.1±1.3 | 14.1±1.3 |
| II | 14.23±0.7[a] | 46.6±1.8[a] | 35.7±2.1[a] | 24.5±1.3[a] |
| III | 14.97±0.6[a] | 53.1±2.2[a] | 39.1±2.4[a] | 28.3±1.1[a] |
| IV | 14.76±0.8[a] | 51.3±1.2[a] | 39.0±2.0[a] | 25.5±2.2[a] |
| V | 16.20±0.6[a] | 49.6±1.0[a] | 39.7±1.7[a] | 25.1±1.4[a] |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA**.**

[a] ***p* <0.01** compared with control group (**I**)

**Table 6. Concentration of metals in selected organs after the exposure to toxic metals with or without ALA**

| | Lead (ppm) | | | | Cadmium (ppm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver | Kidney | Brain | Muscle | Liver | Kidney | Brain | Muscle |
| I | 9.6±3.5 | 1.4±0.2 | 5.2±0.5 | 0.5±0.2 | 1.2±0.2 | 0.3±0.0 | ND | ND |
| II | 62.6±4.3[c] | 102.1±3.1[c] | 16.3±0.5[c] | 16.3±1.3[c] | 37.2±7.6[c] | 24.4±2.6[c] | ND | ND |
| III | 77.4±4.4[c] | 100.8±0.6[c] | 15.1±0.5[c] | 14.9±3.2[c] | 28.6±2.4[c] | 23.6±3.1[c] | ND | ND |
| IV | 80.3±4.2[c,b] | 109.1±1.7[c] | 14.9±0.7[c] | 15.0±1.4[c] | 25.4±1.2[c,b] | 25.4±0.8[c] | ND | ND |
| V | 79.7±2.0[c,b] | 103.1±1.0[c] | 15.1±0.3[c] | 11.8±0.8[c,b] | 27.8±2.1[c,b] | 27.8±2.0[c] | ND | ND |
| | Mercury (ppm) | | | | Copper (ppm) | | | |
| | Liver | Kidney | Brain | Muscle | Liver | Kidney | Brain | Muscle |
| I | 0.4±0.0 | 0.6±0.1 | 0.3±0.4 | 0.1±0.0 | 8.1±1.4 | 6.8±0.6 | 4.1±0.8 | 1.4±0.7 |
| II | 26.1±2.1[c] | 30.1±3.1[c] | 15.5±5.1[c] | 9.8±3.1[c] | 14.6±1.8[a] | 7.0±0.2 | 5.4±1.6 | 1.3±0.5 |
| III | 23.2±3.2[c] | 31.2±2.2[c] | 16.3±4.0[c] | 8.6±2.6[c] | 13.0±0.5[a] | 7.5±0.9 | 4.4±1.3 | 1.4±0.8 |
| IV | 22.3±2.2[c] | 29.5±2.3[c] | 14.8±3.7[c] | 8.8±1.5[c] | 14.6±0.4[a] | 8.8±0.1 | 4.6±1.1 | 1.8±0.5 |
| V | 22.2±1.0[c] | 31.5±0.5[c] | 14.4±2.3[c] | 8.7±1.2[c] | 14.6±2.0[a] | 8.8±0.4 | 3.1±0.2 | 1.3±0.6 |

All data represented as mean ± SE

**I**; Control group, **II**; Birds treated with toxic metals only, **III**; Birds treated with toxic metals and 0.5 g/kg ALA, **IV**; Birds treated with toxic metals and 1 g/kg ALA, **V**; Birds treated with toxic metals and 3 g/kg ALA

[a] ***p* <0.05** compared with control group (**I**) [c] ***p* <0.01** compared with control group (**I**)

[b] ***p* <0.05** compared with only metals-exposed group (**II**) [d] ***p* <0.01** compared with only metals-exposed group (**II**)

## 4. Discussion

The present study was undertaken to investigate the sub-chronic toxicity of lead, cadmium, and mercury combination in Japanese quails, and whether lipoic acid able to counteract or increase the adverse effect of these metals.

Our data showed that the sub-chronic exposure of Japanese quails to a mixture of toxic metals induced a significant lowering in the final body weight compared with control birds. The same observation was previously recorded by (Sant'Ana *et al.,* 2005; Rahman and Joshi, 2009; Abdul jaleel and shuhaimi-Othman, 2013), they found that lead and cadmium reduced body weight gain in chicken and Japanese quails. Lowered body weights in the metals-treated birds could be due to the reduction in the feed utilization or due to the metabolic disarray resulting in loss of the cellular functions and tissue damage (Erdogan *et al.,* 2005). Their results also explored that the dietary supplementation of lipoic acid together with metals aggravate the negative effect of metals on the body weight gain in a dose dependent manner. This could be explained by the decrease in the food intake which observed during the experiment in the birds treated with high dose of lipoic acid together with metals. In this context, several authors had also observed this anorexic effect of ALA in rats and the mechanism was attributed to the suppression of hypothalamic AMP-activated protein kinase activity with no adverse pathology, blood cell counts, and blood chemistry were unchanged indicating that anti-obesity effect o ALA is not due to toxicity (Kim *et al.,* 2004; Seo *et al.,* 2012). However, it was suggested that the inhibition of the feed intake is not the only mechanism underlying the body weight lowering action of ALA, but also the inhibitory action of ALA on the intestinal sugar absorption could also contribute to the lower feed efficiency observed in the ALA-treated animals (Prieto-Hontoria *et al.,* 2009). Moreover, ALA increased the lean mass loss possibly by suppressing the protein synthesis in the skeletal muscle by down-regulating the mTOR signaling pathway (Wang *et al.,* 2010).

The liver is the first organ to encounter the ingested nutrients, drugs, and environmental toxicants that enter the hepatic portal vein from the digestive system, and liver function can be detrimentally altered by injury resulting from acute or chronic exposure to the toxicants. The present study showed that the mixture of heavy metals; lead (Pb), mercury (Hg), and cadmium (Cd) induced significant increases of serum AST, ALP, LDH, and GGT activities with reduction in serum ALT activity. Similar observations were reported in many experimental investigations on the animals exposed to Pb (Bersényi *et al.,* 2003; Shalan *et al.,* 2005; *et al.,* 2007; Liu *et al.,* 2010), Hg (Bersényi *et al.,* 2003; Agarwal *et al.,* 2010; Bashandy *et al.,* 2011), Cd (Fouad *et al.,* 2009; Renugadevi and Prabu, 2010; Swapna and Reddy, 2011) which may be due to the induction of free radical, and lipid peroxidation that can damage the biomembrane leads to leakage of enzymes into blood. The decreased ALT activity after the heavy metal intoxication may be correlated with Karatas and Kalay (2002) and Roy (2002) which similarly reported decreased activity of ALT in *Tilapia zilli*, and *Boleopthalmus dussumier*, respectively, received water contaminated with lead and toxicants, indicating severe or chronic damage to liver cells estimated by the high level of AST and

decreased ALT activity. Administration of lipoic acid at different concentrations restore the activity of ALT toward the control level without lowering AST activity indicating that lipoic acid counteract the chronicity induced by the toxic metals. Regarding biliary biomarkers, lipoic acid produced a significant decrease in the activity of ALP and GGT without significant difference in serum bilirubin level (data not shown) between the metals groups with or without ALA. Our findings were in agreement with recently previous work in rats which reporting that lipoic acid able to lowering of ALT, AST and GGT activities, without significant difference in bilirubin level between bile duct ligating group (BDL) and BDL+ALA group (Somi *et al.,* 2013).

Analysis of cardiac biomarkers suggested that sub-chronic toxicity of the heavy metals induced a significant elevation in AST, LDH, and CK activities, and troponin I level. Ahmed and Hassanien (2013) reported that the chronic exposure of Pb significantly increased the cardiac high sensitivity C-reactive protein (HS-CRP), IL-6, E-selectin, troponin I levels, and serum CK-MB activity compared to the control. These cytokines and markers were assayed as indicators of inflammation and tissue damage in the heart degenerative cells and serum of rats treated with lead. Various mechanisms were suggested to explain these effects: inhibition of the calcium-pump or a transport protein, disturbances in mineral metabolism, inactivation of several enzymes (Patrick, 2006). Also, Oriquat *et al.* (2012) reported that the sub-chronic exposure to mercuric chloride in rats significantly increased serum AST, LDH, and CK activities. The significant elevation of CK activity was indicating multi-organ damage; cardiac, skeletal muscles, and brain tissues. These findings were in agreement with those of Kuliczkowski *et al.* (2004) and Lim *et al.* (2010) who found marked elevation of serum CK after mercury poisoning. Moreover, Prabu *et al.* (2013) found that the rats intoxicated with cadmium for four weeks had significant higher activities of AST, LDH, CK-MB, ALT, and ALP enzymes. Co-administration of low doses of ALA together with the toxic metals significantly increased serum CK activity and troponin I level, while at higher concentration of ALA, these parameters were declined when compared with intoxicated group indicating an ameliorating effect of ALA only at higher doses on the toxic metals-induced cardiac toxicity. Our results were in accordance with El-Awady *et al.* (2011) who reported that administration of lipoic acid attenuated the elevated activities of serum LDH, CK, and CK-MB, and serum cardiac troponin I level induced by cisplatin in rats. Also, Hussein *et al.* (2012) demonstrated that prior administration of ALA significantly attenuated the cisplatin-evoked elevation in serum LDH and CK activities, urea, and creatinine levels and protected both kidney and heart tissues. Another explanation of CK attenuation at high dose of ALA may be attributed to the pro-oxidant and damaging effect of ALA on the protein as confirmed by Scott *et al.* (1994) who reported that DHLA, the reduced metabolite of ALA, may have pro-oxidant effects, via its ability to reduce iron and generate reactive sulfur-containing radicals that can damage the proteins such as alpha 1-antiproteinase and creatine kinase. In another study, supplementation of ALA failed to prevent cardiac complications in diabetic rats and led to cardiac toxicity as indicated by the pathological changes and the elevation of serum cardiac biomarkers; so that the pro-oxidant effects of ALA suggest that the careful selection of appropriate doses of ALA in reactive oxygen species-related diseases are critical (Al-Rasheed *et al.,* 2013).

The obtained results also revealed a significant decrease in the serum calcium level in birds sub-chronically exposed to metals without ALA. Many reports correlated between the exposure to toxic metals, particularly lead and cadmium and the disturbance of calcium metabolism. Cadmium toxicity affects the calcium metabolism, and individuals with severe cadmium nephropathy may have renal calculi and excess excretion of calcium, probably related to increase urinary loss and associated skeletal changes are probably related to calcium loss and include bone pain, osteomalacia, and/or osteoporosis (Goyer and Clarkson, 2001). Also, lead able to inhibit the synthesis of calcitriol resulting in decrease of calcium and phosphorous absorption at the intestinal and renal tubular levels which leads to hypocalcemia and hypophosphotemia (Dongre *et al.,* 2013). Co-administration of lipoic acid with toxic metals resulted in a significant increase in the serum calcium level when compared with the birds treated with the metals only. Interestingly, serum calcium level of the birds exposed to 0.5 and 1 g/kg LA significantly exceeded control group. To our knowledge, there is no information available on the effects of lipoic acid on serum calcium level. ALA induced-hypercalcemia may be explained in the light of the results obtained by Schweizer and Richter (1996) who strongly suggested that alpha-lipoic acid stimulates the $Ca^{2+}$-specific release pathway from intact rat's liver mitochondria. Prolonged stimulation of $Ca^{2+}$ release by lipoic acid may contribute to the detrimental, prooxidant-like effects seen with higher concentrations of lipoic acid.

Our results regarding lipid profile revealed that heavy metal toxicity significantly decreased serum cholesterol, triacylglycerol, and VLDL-c levels while

increased serum HDL-c level. This result came in accordance with Sunita *et al.* (2009) who reported that body weight and serum cholesterol were significantly decreased after cadmium chloride administration in domestic fowls. Also, an increase in HDL cholesterol accompanied by reduced total cholesterol after lead exposure was reported by Cocco *et al.* (1995). Co-administration of ALA with the heavy metals significantly decreased serum HDL-c, and LDL-c levels and increased triacylglycerol, and VLDL-c levels toward control level without any changes in serum total cholesterol as compared to heavy metal intoxicated birds. Effect of ALA on serum TAG also investigated by Hamano (2006) who reported that the addition of lipoic acid to the broiler ration decreased glucose and increased the TAG level. Our finding revealed that dietary administration of ALA able to counteract the adverse effect of metals mixture on the serum lipid profile in quails. In the same context, Seo *et al.* (2012) also reported that intake of ALA inhibited weight gain and improved blood and liver lipid profiles in diet-induced obese rats.

Oxidative stress constitutes an important mechanism of several toxicants that causes several pathological lesions that affect poultry growth. MDA is formed as end product of lipid peroxidation and therefore the extent of lipid peroxidation by ROS can be monitored by MDA level (Sumida *et al.,* 1989). The observed elevation in MDA level of birds exposed to lead in this study may be due to either overproduction or accumulation of ROS resulting from lead exposure. Lead altered lipid metabolism and enhanced lipid peroxidation directly through increasing fluxes of superoxide, hydrogen peroxide and hydroxyl radicals (Gurer *et al.,* 1999) and indirectly through damage the protective antioxidant barrier (Patra *et al.,* 2011). An inhibition of δ-ALAD activity leads to accumulation of δ-ALA, which undergoes auto-oxidation inducing free radicals and so lipid peroxidation (Flora *et al.,* 2008). Previous studies had reported that levels of lead and MDA were increased as a result of lead supplementation (Hamed *et al.,* 2010). Also, it had been reported that cadmium may induce oxidative damage in a variety of tissues by enhancing peroxidation of membrane lipids due to inhibition of antioxidant enzymes (Shukla *et al.,* 2000). Furthermore, MDA level was significantly increased in brain and liver of rats after the exposure to mercuric chloride (El-Demerdash, 2001). The present study also showed that ALA didn't reduce the elevated MDA level in tested tissues indicating that ALA fails to protect against metals-induced oxidative stress. The obtained data were in disagreement with other works confirmed the antioxidant activity of lipoic acid (Chen *et al.,* 2011; Abdou and Abdel-Daim, 2014; Li *et al.,* 2014). This may be explained in that ALA may exhibit pro-oxidant effect depending on the alteration of the trace element homeostasis as reported by (Kayali *et al.,* 2007). Another explanation of the pro-oxidant effect of ALA stated by Moini *et al.* (2002) who reported that alpha-Lipoic acid and dihydrolipoic acid promoted the mitochondrial permeability transition in permeabilized hepatocytes and isolated rat liver mitochondria. Dihydrolipoic acid also stimulated superoxide anion production in rat liver mitochondria and submitochondrial particles. Furthermore, Cakatay *et al.* (2005) found increasing levels of protein oxidation markers such as protein carbonyl (PCO), nitrotyrosine (NT), and advanced oxidation protein products (AOPP) in ALA-supplemented aged rats which may be due to the prooxidant effects of ALA. Consistent with these findings, Bhatti *et al.* (2005) observed that alpha-lipoic acid increases NADPH-induced $O^{\cdot -2}$ and expression of p47phox in the nondiabetic kidney and thus by acting as a pro-oxidant, causes deleterious effects in the healthy kidney.

As expected, tissue levels of lead, cadmium and mercury were increased significantly in birds sub-chronically exposed to metals mixture. However, elevated tissue levels of all metals remained non-significantly affected by ALA treatment. The obtained data suggested that lipoic acid doesn't have clearly ameliorating effect on tissue residues of tested metals. Our results were in disagreement with the hypothesis that considered lipoic acid as a one of heavy-metal chelating agent (Biewenga *et al.,* 1997; Flora *et al.,* 2013). This might be explained that chelating activity of lipoic acid doesn't enhance or facilitate metals excretion, as showed by results of many previous studies which suggested that lipoic acid cannot decrease tissue content of cadmium, mercury or lead (Aposhian *et al.,* 2003; Wang *et al.,* 2008). Administration of LA was not effective in decreasing blood or tissue lead levels compared to a well-known chelator, succimer that was able to reduce them to control levels (Gurer *et al.,* 1999). As estimated by AAS, Cd content in the liver, the kidneys, the brain and the testes remained unaffected by alpha-LA treatment (Bludovska *et al.,* 1999). Recently, another study explored that lipoic acid has mostly been used in combination with other chelating agent like DMSA, due to the fact that lipoic acid itself does not have metal chelating ability but it can consistently tackle the generated oxidative stress (Flora *et al.,* 2012). In this work, measurement of tissue copper concentration showed that the copper content in the liver, kidney, brain and muscle tissues increased significantly in all metals treated groups with and without ALA when compared to control.

This may be attributed to the administration of cadmium chloride and mercuric chloride. In former studies in rats, it was demonstrated that the administration of mercuric chloride or cadmium chloride induced a marked elevation in the copper content of liver and kidney (Huang and Lin, 1997; Noel *et al.,* 2004). Also, administration of lead acetate (70 mg Pb/kg body wt twice a week) and cadmium chlorate (20 mg Cd/kg body wt once a week) intragastrically for 7 weeks singly or in combination significantly increased copper level in rat liver, kidneys, brain and heart tissues (Skoczynska *et al.,* 1994). An association between increased blood lead levels and Fe deficiency was postulated by Muwakkit *et al.* (2008) and Hegazy *et al.* (2010). This impairment in iron metabolism may be incriminated in elevated level of copper since it play important role in iron metabolism; absorption and utilization of iron (Jones *et al.,* 1997). Regarding the effect of lipoic acid on tissue copper level, El-Nabarawy *et al.* (2010) mentioned that treatment with ALA did not reduce the elevated serum copper level in diabetic patients. In conclusion, the sub-chronic exposure to a mixture of lead, cadmium and mercury in Japanese quail resulted in a significant decrease of final body weight, deterioration of many biochemical parameters, and elevation of oxidative stress in selected organs along with increase the body burden of tested metals.

**Conclusion**

Dietary administration of lipoic acid with metals exhibited advantage and disadvantage, as it improve biliary system, lipid profile and the specific cardiac biomarkers. On the other hand lipoic acid fails to decrease tissue levels of tested metals or protect against oxidative damage induced by metals mixture. The observed undesirable effect of lipoic acid on feed intake and body weight gain make the use of it in veterinary practice questioned. Also, our results emphasize the importance of monitoring the dose of LA supplementation, duration of treatment and its potential harmful effects.

**Declaration of interest**

The authors have no declaration of interest

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