



The protective role of Coenzyme Q10 in metallothionein-3 expression in liver and kidney upon rats' exposure to lead acetate

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Abstract

Metallothionein-3 (MT3) is an antioxidant protein that alters after exposure to heavy metals. In this study, we investigated the hepatic and renal expression of MT3 gene following exposure to lead acetate (PbAc) alone and PbAc plus CoQ10 as an adjuvant antioxidant. Twenty-four rats were allocated into three groups, including control, PbAc (free access to drinking water contaminated with PbAc at 1 g/100 ml), and PbAc plus CoQ10 (10 mg/kg/day Oral). After 28 consecutive days of treatment, the mRNA expression of MT3 and Cyt-c genes and MT3 protein levels were assessed using real-time PCR and immunosorbent assay. The serum lipid profile was also monitored in the three groups. PbAc exposure significantly reduced the hepatic and renal MT3 mRNA and protein expression compared to the control group. This reduction was significantly increased with addition of CoQ10 to levels near those of the control group. The hepatic and renal expression of Cyt-c mRNA increased after treatment with PbAc, while such effect was reversed after addition of CoQ10. Alteration in lipid profile including increased cholesterol and low-density lipoprotein levels were observed after PbAc exposure which were counteracted by CoQ10. Our results confirm the cytotoxic effects of acute lead exposure manifested as changes in the serum lipid profile and cellular levels of Cyt-c mRNA. These cytotoxic effects may have been caused by decreased MT3 gene expression and be reduced by the protective role of CoQ10.

Keywords Lipid profile · Pb · CoQ10 · Cyt c · MT3

Introduction

Lead (Pb) is a heavy metal and environmental contaminant that induces toxicity in various body organs, including the liver, kidney, and brain [1]. Pb-induced intoxication is mainly caused by contaminated food, occupational conditions, inhalation of contaminated dust and activity in industries [2]. Pb is known as an oxidative stress factor and Pb exposure increases Reactive Oxygen Species (ROS) levels, decreases cellular antioxidant levels and consequently damages the proteins, DNA, mitochondria and biological membranes [3]. Some studies have investigated the lipid profile after lead exposure. The long-term effects of exposure to low-dose lead acetate on the liver and kidney of rats show

a significant increase in maleic dialdehyde levels, which is a characteristic of increased lipid peroxidation [4]. Furthermore, previous studies have reported a significant increase in liver enzyme activities and serum cholesterol and triglyceride and a reduction in serum HDL in rats following lead poisoning [5].

Metallothioneins (MTs) are a family of cysteine-rich, low-molecular-weight proteins that bind to Pb in the liver and kidney [6]. MTs are four isoforms of the murine MT (MT1 to MT4) and a metallothionein-like gene (MTL5) [7]. Among the four isoforms of MTs, MT1 and MT2 are largely expressed in mammalian cell types, with the highest expression being in the liver. MT3 was mainly expressed in the brain, and also in small amounts in the liver, kidney, heart, pancreas, and intestine [8–10]. Also, MT3 was expressed in the liver and kidney [11–13]. MT4 is expressed exclusively in the skin and tongue [8, 9]. Nonetheless, the expression of MT3 in the liver and kidney is not well unclear.

Several studies have investigated the protective role of MTs against heavy metal toxicity [6]. MT1 and MT2 have a role in the detoxification of heavy metals [14]. They are mainly

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expressed in the liver and kidney of mice exposed to cadmium and lead [4, 14]. The high cysteine content of MT1 and MT2 makes them capable of binding to heavy metal ions (cadmium, silver, and lead) *in vivo* and *in vitro* [14]. Unlike MT1 and MT2, MT3 isoform has been very little studied. MT3 has been shown to play a role in the development and cell apoptosis in mammalian cells, protection from DNA damage, diagnosis of tumorigenesis and cancer, and pathogenesis of some neurodegenerative diseases [8–10]. Previous studies indicated that Pb can bind to the metallothionein isoform MT3 [15, 16]. The reduction of MT3 expression in Alzheimer's disease shows its decreased protective effect [17]. Furthermore, lithium chloride-treated mice reported a reduced expression of MT3 in the brain [18]. However, none of the other MT isoforms show growth inhibitory activities, which reveals that this activity is a specific functional characteristic of MT3 [17].

Lead causes oxidative disturbances through the production of oxidative radicals and reduces cellular antioxidant defense [19]. Antioxidant enzymes are one of the targets of lead, which Pb is capable of electron transport form covalent bonds to the SH-group of antioxidant enzymes [20]. Furthermore, lead prevents the cellular balance of some important scarce elements that are necessary for the catalytic activity of endogenous enzyme antioxidants [21]; as a result, it causes the accumulation of ROS within the cells. ROS participates in direct cellular oxidative damage and causes cellular apoptosis [22]. The induction of ROS along with impairments in the cellular redox defense leads to a reduction in antioxidant defense effects against lead-induced toxicity [23].

Coenzyme Q10 (CoQ10) acts as a fat-soluble antioxidant to fully protect the lipid membranes and lipoproteins against oxidative stress [24]. CoQ10 inhibits adipocyte differentiation and cholesterol, reduces white adipose tissue content, and regulates fat metabolism [25]. CoQ10 can reduce oxidative stress and increase antioxidant enzyme activity in the coronary arteries and hemodialysis patients [26]. Other studies have shown that CoQ10 protects against cadmium and lead-induced toxicity by increasing the antioxidant capacity and decreasing cellular oxidative stress [27]. Due to the association of lead exposure with cellular oxidative damage, the antioxidant function of CoQ10 and its protective effects against lead-induced damage have become the focus of much attention. This study was therefore conducted to determine the effect of CoQ10 on serum lipid concentrations, MT3 mRNA and protein expression and the Cyt-c gene mRNA expression in the liver and kidney of rats after lead exposure.

Materials and Methods

Animals

Twenty-four male adult Wistar rats weighing 200–250 g were procured from Pasteur Institute of Iran. All the rats were housed in a room with a 12/12-h dark–light cycle with controlled temperature (22 ± 3 °C). The rats had access to fresh water and food and were acclimatized to laboratory conditions for one week prior to the experiments. All protocols for animal experiments were approved by the institutional animal Ethical Committee, Parand branch, Islamic Azad University, Parand, Iran (IR. IAU.PIAU.REC.1399.003).

Drugs and experimental groups

Lead acetate (acetate trihydrate, Sigma-Aldrich, Merck, Germany) and CoQ10 (Sigma, St. Louis, MO, USA) were purchased from Kimia Pars Inc., Tehran, Iran. Lead acetate (PbAc) was dissolved in water and CoQ10 was dissolved in corn oil.

The rats were randomly divided into three equal groups as follows: Group 1 or control group was fed a normal diet for 28 consecutive days. Group 2 received (PbAc) (1 g/100 ml) by drinking water for 28 consecutive days. Group 3 received PbAc (1 g/100 ml) by drinking water + CoQ10 (10 mg/kg/day) by gavage for 28 consecutive days. All the groups received oral corn oil as a vehicle of CoQ10. The PbAc solution was prepared daily. The dose of PbAc and CoQ10 was chosen according to previous research, respectively [28, 29]. On the twenty-ninth day, the rats were rapidly euthanized with a combination of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg), their thoracic was opened, and blood samples were drawn from their left ventricle. Then, their liver and kidney were collected and washed using saline solution, and sections of the liver and kidney tissues were used for the enzyme-linked immunosorbent assay (Elisa) assays, and the remainder of the liver and kidney tissues was stored at -80 °C for mRNA isolation [30].

Blood biochemistry

To determine the serum concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), the blood samples obtained from the heart were stored in test tubes. Serum TC, TG, LDL and HDL levels were measured using enzymatic kits (Wako, Osaka, Japan) according to the manufacturer's instructions.

Sample preparation for protein expression by Elisa

The level of MT3 protein was measured by a sandwich Elisa kit according to the manufacturer's protocol (BioSource, Inc.). In brief, one ml PBS was added to 0.5 g of the liver and kidney tissues of the rats and was homogenized and centrifuged at 4 °C, 600×g, for 10 min. Then, their supernatant was centrifuged at 1000×g, 4 °C, for 20 min. The resultant sediment was removed and diluted by 1 ml PBS, and then, 1 mmol Ethylenediaminetetraacetic acid (EDTA), 10 mmol tris at PH=7.4, 200 ml PBS, and 66 ml Sodium Dodecyle Sulfate (SDS) %16 were also added. This homogenate was centrifuged at 1100×g for 20 min at room temperature. The supernatant was removed and stored at – 80 °C and then used for the Elisa assays to determine the derived protein.

RNA extraction and complementary DNA (cDNA) synthesis

Total RNA extraction was performed using the RNX plus™ kit according to the manufacturer's instructions (Cinnagen Inc, Tehran, Iran). The quantity of the extracted RNA was evaluated by the optical observation of sample electrophoresis on agarose gel 2%. The 18S and 28S ribosomal RNA bands were visible in the intact RNA sample. The quality of the extracted RNA was evaluated by a spectrophotometer (Nanodrop ND-2000, NanoDrop Technologies, Wilmington, Delaware, US), and only a pure RNA sample with an A260/A280 ratio, ranging from 1.8 to 2, was used for the cDNA synthesis. The cDNA was synthesized by 100 ng of RNA, using a first strand cDNA synthesis kit according to the manufacturer's instruction (Fermentas, Thermo Scientific, Waltham, MA, USA). The synthesized cDNA was stored at – 80 °C to perform further investigations on it.

Real-time PCR using SYBR Green

The real-time quantitative PCR was performed using an Applied Biosystems 7500 Real-time PCR system (Applied Biosystems, USA). The relative quantification was performed by measuring the increase in fluorescence light as a result of SYBR Green bonding. The quantitative real-time PCR reactions were run in 20 mL, including 1 µl of cDNA, 10 µl of SYBR Green Master Mix, 5 pmol of each complementary primer at volumes of 0.5 µl and 8 µl of deionized water. The BLAST tool (NCBI, www.ncbi.nlm.nih.gov/blast) was used

to design the primers of MT3, Cyt-c and GAPDH as an internal reference. Table 1 presents the primer sequences used for real-time PCR.

The amplification conditions for MT, Cyt-c and GAPDH were as follows: Initial denaturation at 94 °C for 20 s, followed by 40 cycles at 60 °C for 30 s and a final extension at 72 °C for 30 s. All the experiments were repeated at least three times in order to ensure reproducibility. The relative expression of mRNA was calculated using the $\Delta\Delta CT$ method. The Relative Fold Increase (RFI) was calculated using the following equation: $RFI = 2^{-\Delta\Delta CT}$ [31, 32].

Statistical analysis

Statistical analysis was performed with SPSS 22 for Windows. One-way analysis of variance was used to compare the difference between groups (Tukey's test). The results are presented as the mean \pm SEM. $P < 0.05$ was considered a significant differences association.

Results

Effect of PbAc and PbAc + CoQ10 on the lipid profile of rats' blood serum

Total cholesterol and LDL levels increased in the blood serum of the rats treated with PbAc (1 g/100 mL) compared to the control group (1.136 times, $P = 0.007$ and 1.235 times, $P = 0.037$, respectively). This increase was countered by CoQ10 (10 mg/kg/day) (0.91 times, $P = 0.05$ and 0.738 times, $P = 0.004$, respectively). HDL level also increased in the blood serum of the rats treated with CoQ10 + PbAc compared to the control and PbAc groups (1.092 times, $P = 0.05$ and 1.099 times, $P = 0.037$, respectively). No significant differences in the rat's blood serum TG and HDL composition between PbAc and control group were found ($P = 0.54$ and $p = 0.98$, respectively; Fig. 1).

The effect of PbAc and PbAc + CoQ10 on MT3 gene mRNA expression in rats' liver and kidney using real-time PCR

MT3 gene mRNA expression decreased in the liver and kidney of the rats treated with PbAc (1 g/100 ml) compared to the control group, ($P < 0.01$ and $P < 0.001$, respectively). MT3 gene mRNA expression increased in the liver and kidney

Table 1 Gene-special primers used for Real-time PCR

Gene	Forward Primer	Reverse Primer
MT3	5'AGGGCTGTGTCTGGAAAGGTG 3'	5'AGGAAACTGGGTGGAGGTG 3'
GAPDH	5'AAGTTCAACGGCACAGTCAAGG 3'	5'CATACTCAGCACCAGCATCACC 3'
Cyt-c	5'GTCTGATCCTTTGTGGTGTGAC 3'	5'GGTCCAGTCTTATGCTTGCCT 3'

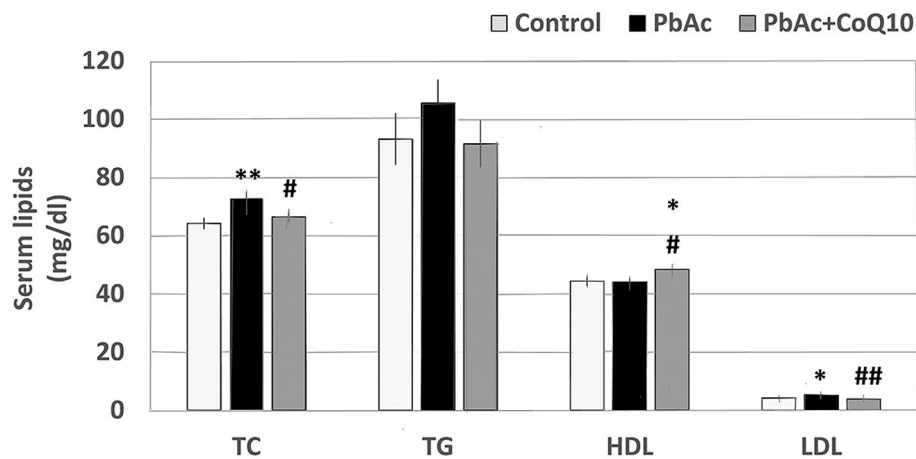


Fig. 1 The effect of PbAc and PbAc+CoQ10 on the serum lipid profile of rats. TC and LDL increased significantly in the PbAc-treated group compared to the control group but decreased significantly in the PbAc+CoQ10-treated group compared to the PbAc-treated group. HDL increased significantly in the PbAc+CoQ10-treated group compared to the PbAc-treated and control groups. Data were

expressed as mean \pm SEM ($n=8$ per group). *Compared to the control group, $P<0.05$; **Compared to the control group, $P<0.01$; #Compared to the PbAc group, $P<0.05$; ##Compared to the PbAc group, $P<0.01$. TC: Total Cholesterol; TG: Triglycerides; LDL: Low-Density Lipoprotein cholesterol; HDL: High-Density Lipoprotein cholesterol

of rats treated with PbAc+CoQ10 compared to the PbAc-treated group ($P<0.05$ and $P<0.001$, respectively). This level decreased in the PbAc+CoQ10-treated group compared to the control group ($P<0.05$; Fig. 2).

The effect of PbAc and PbAc+CoQ10 on level of MT3 protein in rats' liver and kidney using Elisa assays

The level of MT3 protein decreased in the liver and kidney of the rats treated with PbAc compared to the control group ($P<0.001$). On the contrary, the level of MT3 protein increased in the rats treated with PbAc+CoQ10 compared to the rats treated with PbAc ($P<0.01$; Fig. 3).

The effect of PbAc and PbAc+CoQ10 on Cyt c gene mRNA expression in rats' liver and kidney using real-time PCR

Cyt c gene mRNA expression increased in the liver and kidney of the rats treated with PbAc compared to the control group ($P<0.001$). Cyt c gene mRNA expression decreased in the liver and kidney of rats treated with PbAc+CoQ10 compared to the PbAc-treated group ($P<0.01$ and $P<0.001$, respectively; Fig. 4).

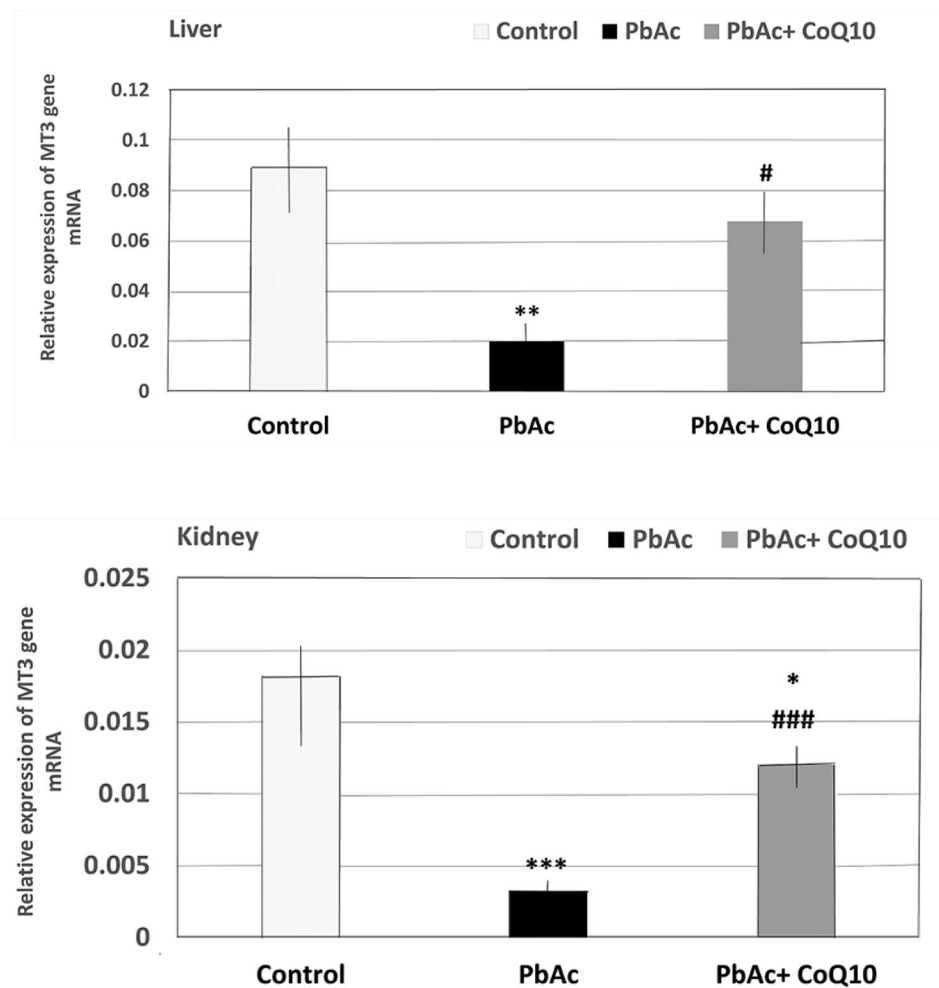
Discussion

Several investigators reported that lead-induced hepatotoxicity decreases HDL significantly and increases their serum cholesterol, triglyceride levels and also serum liver enzyme

activities in rats. The increased liver enzyme is a biomarker of liver damage, the destruction of the liver cell membrane, and the induction of lipid peroxidation [5, 33]. The present findings showed that PbAc-induced toxicity elevates serum cholesterol and LDL levels compared to the controls, but no change was observed in triglycerides and HDL levels in this treatment group, which may be due to the concentration of PbAc (100 mg/ml) used. Lead exposure changes cell surface receptors for lipoprotein or inhibits lipoprotein lipase function in liver, which then reduces removal of lipoproteins [34–36]. Moreover, lead alters lipid metabolism enzymes activity in liver, this can limit the bile acids biosynthesis, which is the only route for elimination of body cholesterol [34, 36]. So, the balance between biosynthesis and catabolism of cholesterol by the liver is a critical determinant of its concentration in the blood serum. Other studies have also shown that the main mechanism of lead-induced toxicity is the enhancement of cellular oxidative stress due to increased free radical production or antioxidant enzyme reduction [37], which leads to metabolic abnormalities such as abnormal lipid profile [38].

In this study, to determine if CoQ10 affects PbAc-induced toxicity, CoQ10 (10 mg/kg/day by gavage) was added to the PbAc (1 g/100 ml) regimen of the rats as well. The results demonstrated for the first time that CoQ10 administration in rats fed with PbAc causes a down-regulation of the cholesterol and LDL in the rats' blood serum and increases HDL compared to the PbAc-treated and control rats. These results are consistent with previous findings on the antioxidant activity and protective effects of CoQ10 in different tissues [3, 39, 40] and the reduction

Fig. 2 The effect of PbAc and PbAc + CoQ10 on MT3 mRNA expression in the rats' liver and kidney. MT3 mRNA decreased significantly in the PbAc-treated group compared to the control group, but increased significantly in the PbAc + CoQ10-treated group compared to the PbAc-treated group in the rats' liver and kidney. MT3 mRNA decreased significantly in the PbAc + CoQ10-treated group compared to the PbAc-treated group in the rats' kidney. The results were reported as mean \pm SEM (n = 8 per group). *Compared to the control group, $P < 0.05$; **Compared to the control group, $P < 0.01$; ***Compared to the control group, $P < 0.001$; #Compared to the PbAc group, $P < 0.05$; ###Compared to the PbAc group, $P < 0.001$



in blood cholesterol following CoQ10 treatment [25]. The suppression of lipid peroxidation as a result of CoQ10 treatment may be caused by the ability of CoQ10 to inhibit ROS [39]. Also, Yousef et al. (2019) showed that CoQ10 causes a balance between the oxidants and antioxidants and has beneficial effects against lead-induced neuronal damage. Thus, the present study showed that CoQ10 may be reduced hyperlipidemia induced by PbAc due to its ability to neutralize oxidative damage and inhibiting ROS generation.

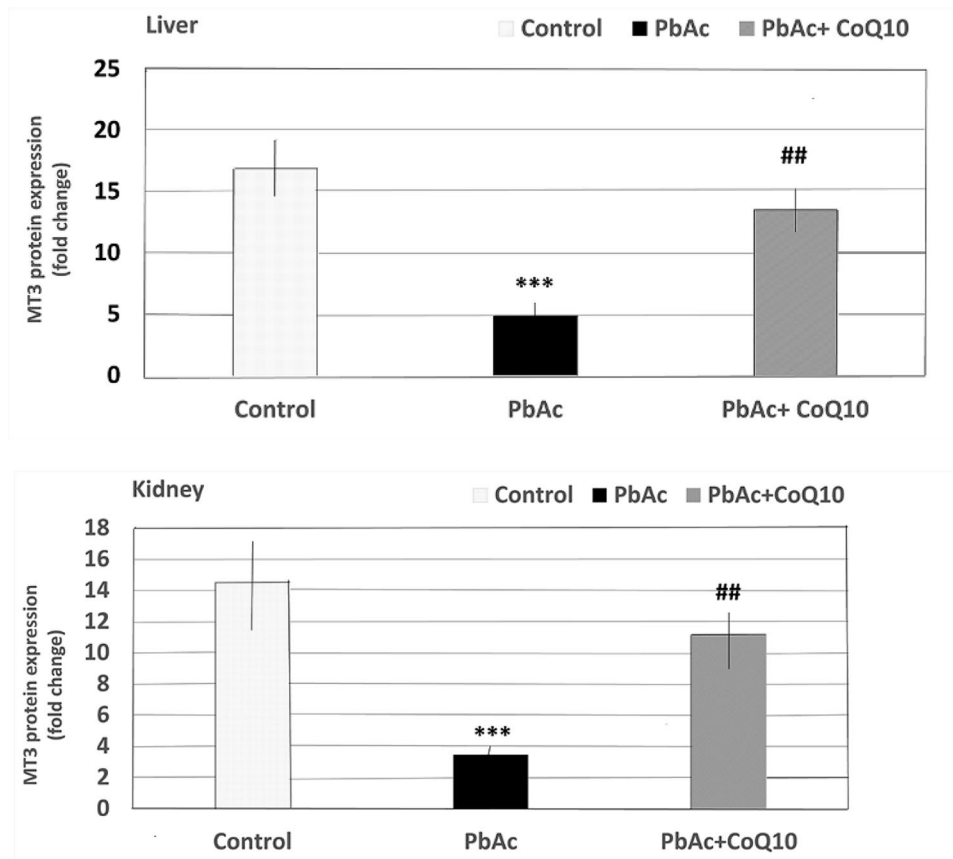
Previous studies have demonstrated MT3 protein and mRNA expression in various organs of rats, mainly in the brain, and in a low amount in the liver, kidney pancreas, prostate, testis, and tongue [8–10, 41]. Moreover, MT3 mRNA and protein overexpression has been detected in many cases of human bladder, prostate and breast cancers [42]. MT3 mRNA and protein are also expressed in normal human kidneys, renal carcinoma and the nuclei isolated from rat nephrons [9, 11, 12]. Also, MT3 expression was also identified in Broiler's liver [13]. In present study, MT3 mRNA and protein were expressed in rats' liver and kidney. These localizations are also confirmed by the studies

that showed MT3 protein expression in different peripheral organs of rats and humans [12].

MT3 expression was highly increased in the liver after exposure to cadmium [11], which shows that MT3 mediates the mechanism of cytotoxicity in different mammalian organs. Tsui et al. (2019) have shown that MT3 is a tumorigenesis factor and increasing invasiveness and cell growth of bladder carcinoma, which is upregulated by hypoxia and arsenic in vivo [43]. Also, the overexpression of the mRNA or protein of MT3 has been observed in many human bladder, prostate and breast cancers [42]. Moreover, the MT3 gene is involved in cellular growth and heavy metals' metabolism during oxidative stress situations in the human brain [44], and the MT3 gene expression also decreases with oxidative stress [45]. Thus, the present findings suggest that MT3 expression can decrease after acute exposure to PbAc during oxidative stress conditions [44].

The reduction of MT3 levels is indicated in metal-associated neurodegenerative diseases, and this function is due to the diminution of cellular capacity to neutralize ROS [17, 41]. Many studies demonstrated that cadmium exposure increased the cells' resistance against cell apoptosis with

Fig. 3 The effect of PbAc and PbAc + CoQ10 on level of MT3 protein in the rats' liver and kidney. An Elisa assay was performed and the results were reported as mean \pm SEM (n = 8 per group). The level of MT3 protein decreased significantly in the PbAc-treated group compared to the control group in the rats' liver and kidney but increased significantly in the PbAc + CoQ10-treated groups compared to the PbAc-treated group. ***Compared to the control group, $P < 0.001$; ##Compared to the PbAc group, $P < 0.01$



elevated MT3 levels [13]. On the other hand, Somji et al. (2004) showed that when the cells are in proliferation/regeneration, MT3 expression decreases and occurs cell destruction through cell apoptosis. In the present study, the expression of MT3 mRNA and protein decreased in the liver and kidney of the PbAc-treated rats. Thus, the present findings suggest that PbAc exposure may decrease the cellular capacity for the neutralization of ROS and cause cellular apoptosis by inducing oxidative stress [17, 46]. The reduction of MT3 levels may be indicative of PbAc-induced apoptosis due to toxicity in the liver and kidney of rats.

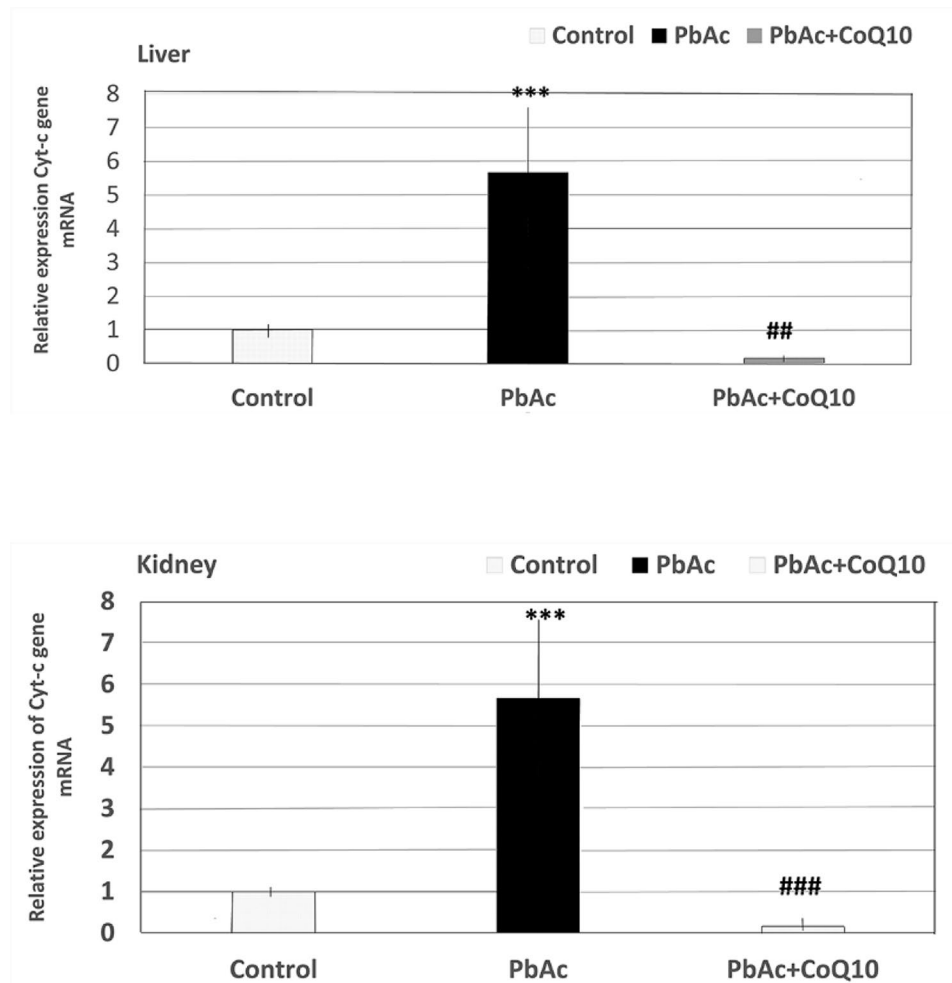
The mechanism of reduction of MT3 expression by Pb toxicity in the liver and kidney is not known. Mammalian MT3 is an unusual protein with a puzzling role [47]. However, the proposed mechanism may be operative here. The binding Affinity of Pb to MT3 is more compared to its binding with Zn [15, 48] and thus during Pb-induced toxicity, Zn may be replaced by Pb and affecting liver and kidney function. Furthermore, Bonaventura et al. (2018) has shown that expression level of MT3 gene is dependent on toxic conditions. However, the mechanisms that show in the PbAc-induced reduction of MT-3 in the liver and kidney remain to be investigated.

Several studies have shown that the mechanism of toxicity of Pb occurs through the production of free radicals and

generation of ROS, which result in cellular apoptosis generation [49]. During apoptotic stimuli, Cyt c is released into the cytosol, and mediates the activation of the adaptor molecule apoptosis-protease activating factor 1 (Apaf-1), which is required for the activation of effector caspases (caspases 3, 6, and 7) and induces apoptosis [23]. Also, El-Tantawy et al. (2016) showed a markedly increase of hepatic caspase-3 levels in PbAc-treated rats [50]. In the present study, Cyt-c mRNA expression in the liver and kidney of the PbAc-treated rats was increased.

Several researches showed that CoQ10 has been implicated in the metabolic pathways that are associated with protecting organisms against oxidative damage [26, 39, 40]. CoQ10 is a potent antioxidant that protects against the production of mitochondrial ROS generated by oxidative stress [49]. One study has shown the ability of CoQ10 to protect against PbAc-induced neurotoxicity in rats by restoring the balance between the antioxidants and oxidants through its antioxidant and anti-apoptotic activities [3]. The present study also showed that CoQ10 can increase MT3 protein and mRNA levels in rats' liver and kidney after PbAc exposure. CoQ10 also reduced the upregulation of Cyt c mRNA levels. This effect of CoQ10 has modulated the upregulation of anti-apoptotic mitochondrial-related proteins through its antioxidant

Fig. 4 The effect of PbAc and PbAc + CoQ10 on Cyt-c mRNA expression in the rats' liver and kidney. Cyt-c mRNA increased significantly in the PbAc-treated group compared to the control group. Cyt-c mRNA decreased significantly in the PbAc + CoQ10-treated group compared to the PbAc-treated group. The results were reported as mean \pm SEM (n=8 per group). ***Compared to the control group, $P < 0.001$; ##Compared to the PbAc group, $P < 0.01$; ###Compared to the PbAc group, $P < 0.001$



activity and the inhibition of ROS generation by CoQ10 by neutralizing ROS through protective effects against free radicals [3].

Conclusion

It seems that PbAc exposure can generate oxidative stress along with an increase in cholesterol, LDL, and Cyt c mRNA levels and a reduction in the expression of MT3 in the liver and kidney of rats. The decrement of MT3 expression can be used as an indicator of PbAc-induced cytotoxicity. CoQ10 has protective effects against PbAc toxicity by reducing cholesterol, LDL and Cyt c mRNA levels and an increase in HDL and MT3. These results suggested that CoQ10 plays an important role in PbAc-induced detoxification by reducing oxidative stress.

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Authors' contribution PK designed the research. PK and AAM conducted the studies. PK analyzed the data. PK interpreted the results. PK and AAM wrote the manuscript. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors report no conflicts of interest.

Ethical approval The Ethics Committee of the Islamic Azad University-Parand Branch approved and oversaw this study (IR.IAU.PIAU.REC.1399.003).

Research involving human participants No human sample was used.

References

1. Togao M, Nakayama SM, Ikenaka Y, Mizukawa H, Makino Y, Kubota A, Matsukawa T, Yokoyama K, Hirata T, Ishizuka M (2020) Bioimaging of Pb and STIM1 in mice liver, kidney and brain using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and immunohistochemistry.

- Chemosphere 238:124581. <https://doi.org/10.1016/j.chemosphere.2019.124581>
2. Ericson B, Landrigan P, Taylor MP, Frostad J, Caravanos J, Keith J, Fuller R (2016) The global burden of lead toxicity attributable to informal used lead-acid battery sites. *Ann Glob Health* 82(5):686–699. <https://doi.org/10.1016/j.aogh.2016.10.015>
 3. S Yousef AO, A Fahad A, Abdel Moneim AE, Metwally DM, El-Khadragy MF, Kassab RB (2019) The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. *Int J Environ Res Public Health* 16(16):2895. <https://doi.org/https://doi.org/10.3390/ijerph16162895>
 4. Dai S, Yin Z, Yuan G, Lu H, Jia R, Xu J, Song X, Li L, Shu Y, Liang X (2013) Quantification of metallothionein on the liver and kidney of rats by subchronic lead and cadmium in combination. *Environ Toxicol Pharmacol* 36(3):1207–1216. <https://doi.org/10.1016/j.etap.2013.10.003>
 5. Abdelhamid FM, Mahgoub HA, Ateya AI (2020) Ameliorative effect of curcumin against lead acetate-induced hematobiochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environ Sci Pollut Res*. <https://doi.org/10.1007/s11356-020-07718-3>
 6. Thirumoorthy N, Kumar KM, Sundar AS, Panayappan L, Chatterjee M (2007) Metallothionein: an overview. *World J Gastroenterol WJG* 13(7):993. <https://doi.org/10.3748/wjg.v13.i7.993>
 7. Rahman A, Khan KM, Rao MS (2018) Exposure to low level of lead during preweaning period increases metallothionein-3 expression and dysregulates divalent cation levels in the brain of young rats. *Neurotoxicology* 65:135–143. <https://doi.org/10.1016/j.neuro.2018.02.008>
 8. Sabolić I, Breljak D, Škarica M, Herak-Kramberger CM (2010) Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. *Biometals* 23(5):897–926. <https://doi.org/10.1007/s10534-010-9351-z>
 9. Thirumoorthy N, Sunder AS, Kumar KM, Ganesh G, Chatterjee M (2011) A review of metallothionein isoforms and their role in pathophysiology. *World J Surg Oncol* 9(1):1–7. <https://doi.org/10.1186/1477-7819-9-54>
 10. Juárez-Rebollar D, Rios C, Nava-Ruiz C, Méndez-Armenta M (2017) Metallothionein in brain disorders. *Oxid Med Cell Longev*. <https://doi.org/10.1155/2017/5828056>
 11. Al-Waeli A, Pappas A, Zoidis E, Georgiou C, Fegeros K, Zervas G (2012) The role of selenium in cadmium toxicity: interactions with essential and toxic elements. *Br Poult Sci* 53(6):817–827. <https://doi.org/10.1080/00071668.2012.751523>
 12. Sabolić I, Škarica M, Ljubojević M, Breljak D, Herak-Kramberger CM, Crljen V, Ljubešić N (2018) Expression and immunolocalization of metallothioneins MT1, MT2 and MT3 in rat nephron. *J Trace Elem Med Biol* 46:62–75. <https://doi.org/10.1016/j.jtemb.2017.11.011>
 13. Zoidis E, Papadomichelakis G, Pappas AC, Theodorou G, Fegeros K (2019) Effects of selenium and cadmium on breast muscle fatty acid composition and gene expression of liver antioxidant proteins in broilers. *Antioxidants* 8(5):147. <https://doi.org/10.3390/antiox8050147>
 14. Shen X, Liu W, Chen Y, Guo Y, Gao M, Chen W, Liu Y, Liu S (2019) Diagnostic significance of metallothionein members in recognizing cadmium exposure in various organs under low-dose exposure. *Chemosphere* 229:32–40. <https://doi.org/10.1016/j.chemosphere.2019.04.192>
 15. Carpenter M, Shami Shah A, DeSilva S, Gleaton A, Su A, Goundie B, Croteau M, Stevenson M, Wilcox D, Austin R (2016) Thermodynamics of Pb (II) and Zn (II) binding to MT-3, a neurologically important metallothionein. *Metallomics* 8(6):605–617. <https://doi.org/10.1039/c5mt00209e>
 16. Pérez-Zúñiga C, Leiva-Presa À, Austin RN, Capdevila M, Palacios Ó (2019) Pb (ii) binding to the brain specific mammalian metallothionein isoform MT3 and its isolated α MT3 and β MT3 domains. *Metallomics* 11(2):349–361. <https://doi.org/10.1039/c8mt00294k>
 17. Yu WH, Lukiw WJ, Bergeron C, Niznik HB, Fraser PE (2001) Metallothionein III is reduced in Alzheimer's disease. *Brain Res* 894(1):37–45. [https://doi.org/10.1016/s0006-8993\(00\)03196-6](https://doi.org/10.1016/s0006-8993(00)03196-6)
 18. Chetcuti A, Adams LJ, Mitchell PB, Schofield PR (2008) Microarray gene expression profiling of mouse brain mRNA in a model of lithium treatment. *Psychiatr Genet* 18(2):64–72. <https://doi.org/10.1097/YPG.0b013e3282fb0051>
 19. Chander K, Vaibhav K, Ahmed ME, Javed H, Tabassum R, Khan A, Kumar M, Katyal A, Islam F, Siddiqui MS (2014) Quercetin mitigates lead acetate-induced behavioral and histological alterations via suppression of oxidative stress, Hsp-70, Bak and upregulation of Bcl-2. *Food Chem Toxicol* 68:297–306. <https://doi.org/10.1016/j.fct.2014.02.012>
 20. Dewanjee S, Dua TK, Khanra R, Das S, Barma S, Joardar S, Bhattacharjee N, Zia-Ul-Haq M, Jaafar HZ (2015) Water spinach, *Ipomoea aquatica* (Convolvulaceae), ameliorates lead toxicity by inhibiting oxidative stress and apoptosis. *PLoS ONE* 10(10):e0139831. <https://doi.org/10.1371/journal.pone.0139831>
 21. Flora G, Gupta D, Tiwari A (2012) Toxicity of lead: a review with recent updates. *Interdiscip Toxicol* 5(2):47–58. <https://doi.org/10.2478/v10102-012-0009-2>
 22. Khanra R, Dewanjee S, Dua TK, Sahu R, Gangopadhyay M, De Feo V, Zia-Ul-Haq M (2015) *Abroma augusta* L.(Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response. *J Transl Med* 13(1):1–14. <https://doi.org/10.1186/s12967-014-0364-1>
 23. Dua TK, Dewanjee S, Khanra R, Joardar S, Barma S, Das S, Zia-Ul-Haq M, De Feo V (2016) Cytoprotective and antioxidant effects of an edible herb, *Enhydra fluctuans* Lour.(Asteraceae), against experimentally induced lead acetate intoxication. *PLoS ONE* 11(2):148757. <https://doi.org/10.1371/journal.pone.0148757>
 24. Tomasetti M, Littarru G, Stocker R, Alleva R (1999) Coenzyme Q10 enrichment decreases oxidative DNA damage in human lymphocytes. *Free Radical Biol Med* 27(9–10):1027–1032. [https://doi.org/10.1016/s0891-5849\(99\)00132-x](https://doi.org/10.1016/s0891-5849(99)00132-x)
 25. Xu Z, Huo J, Ding X, Yang M, Li L, Dai J, Hosoe K, Kubo H, Mori M, Higuchi K (2017) Coenzyme Q10 improves lipid metabolism and ameliorates obesity by regulating CaMKII-mediated PDE4 inhibition. *Sci Rep* 7(1):1–12. <https://doi.org/10.1038/s41598-017-08899-7>
 26. Rivara MB, Yeung CK, Robinson-Cohen C, Phillips BR, Ruzinski J, Rock D, Linke L, Shen DD, Ikizler TA, Himmelfarb J (2017) Effect of coenzyme Q10 on biomarkers of oxidative stress and cardiac function in hemodialysis patients: the CoQ10 biomarker trial. *Am J Kidney Dis* 69(3):389–399. <https://doi.org/10.1053/j.ajkd.2016.08.041>
 27. Omar HA, Almalki WH, Shamardil H, Mahdy AY, Abd El-Latif HA (2016) Lipoic acid and coenzyme Q10 protect against lead-induced toxicity in rats with metabolic syndrome. *Int J Pharmacol* 12(3):146–153
 28. Baranowska-Bosiacka I, Strużyńska L, Gutowska I, Machalińska A, Kolasa A, Kłos P, Czapski G, Kurzawski M, Prokopowicz A, Marchlewicz M (2013) Perinatal exposure to lead induces morphological, ultrastructural and molecular alterations in the hippocampus. *Toxicology* 303:187–200. <https://doi.org/10.1016/j.tox.2012.10.027>
 29. Rauscher FM, Sanders RA, Watkins JB III (2001) Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol*

- 15(1):41–46. [https://doi.org/10.1002/1099-0461\(2001\)15:1%3c41::aid-jbt5%3e3.0.co;2-z](https://doi.org/10.1002/1099-0461(2001)15:1%3c41::aid-jbt5%3e3.0.co;2-z)
30. Amanpour P, Khodarahmi P, Salehipour M (2020) Protective effects of vitamin E on cadmium-induced apoptosis in rat testes. *Naunyn-Schmiedeberg's Arch Pharmacol* 393(3):349–358. <https://doi.org/10.1007/s00210-019-01736-w>
 31. Alian NS, Khodarahmi P, Naseh V (2018) The effect of cadmium on apoptotic genes mRNA expression of Bax and Bcl-2 in small intestine of rats. *Iran J Pathol* 13(4):408–414
 32. Rouhani F, Khodarahmi P, Naseh V (2019) NGF, BDNF and Arc mRNA expression in the hippocampus of rats after administration of morphine. *Neurochem Res* 44(9):2139–2146. <https://doi.org/10.1007/s11064-019-02851-z>
 33. Abdou HM, Hassan MA (2014) Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. *Biomed Res Int*. <https://doi.org/10.1155/2014/435857>
 34. Kojima M, Masui T, Nemoto K, Degawa M (2004) Lead nitrate-induced development of hypercholesterolemia in rats: sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis. *Toxicol Lett* 154(1–2):35–44. <https://doi.org/10.1016/j.toxlet.2004.06.010>
 35. Tarugi P, Calandra S, Borella P, Vivoli G (1982) Heavy metals and experimental atherosclerosis: effect of lead intoxication on rabbit plasma lipoproteins. *Atherosclerosis* 45(2):221–234. [https://doi.org/10.1016/0021-9150\(82\)90140-x](https://doi.org/10.1016/0021-9150(82)90140-x)
 36. Newairy A-SA, Abdou HM (2009) Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. *Food Chem Toxicol* 47(4):813–818. <https://doi.org/10.1016/j.fct.2009.01.012>
 37. El-Boshy ME, Refaat B, Qasem AH, Khan A, Ghaith M, Almasmoum H, Mahbub A, Almaimani RA (2019) The remedial effect of Thymus vulgaris extract against lead toxicity-induced oxidative stress, hepatorenal damage, immunosuppression, and hematological disorders in rats. *Environ Sci Pollut Res* 26(22):22736–22746. <https://doi.org/10.1007/s11356-019-05562-8>
 38. Ohara Y, Peterson TE, Harrison DG (1993) Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91(6):2546–2551. <https://doi.org/10.1172/JCI116491>
 39. Paunović MG, Matic MM, Ognjanović BI, Saičić ZS (2017) Antioxidative and haematoprotective activity of coenzyme Q10 and vitamin E against cadmium-induced oxidative stress in Wistar rats. *Toxicol Ind Health* 33(10):746–756. <https://doi.org/10.1177/0748233717725480>
 40. Tarry-Adkins JL, Fernandez-Twinn DS, Hargreaves IP, Neergheen V, Aiken CE, Martin-Gronert MS, McConnell JM, Ozanne SE (2016) Coenzyme Q10 prevents hepatic fibrosis, inflammation, and oxidative stress in a male rat model of poor maternal nutrition and accelerated postnatal growth. *Am J Clin Nutr* 103(2):579–588. <https://doi.org/10.3945/ajcn.115.119834>
 41. Vašák M, Meloni G (2017) Mammalian metallothionein-3: New functional and structural insights. *Int J Mol Sci* 18(6):1117. <https://doi.org/10.3390/ijms18061117>
 42. Sens MA, Somji S, Garrett SH, Beall CL, Sens DA (2001) Metallothionein isoform 3 overexpression is associated with breast cancers having a poor prognosis. *Am J Pathol* 159(1):21–26. [https://doi.org/10.1016/S0002-9440\(10\)61668-9](https://doi.org/10.1016/S0002-9440(10)61668-9)
 43. Tsui K-H, Hou C-P, Chang K-S, Lin Y-H, Feng T-H, Chen C-C, Shin Y-S, Juang H-H (2019) Metallothionein 3 is a hypoxia-upregulated oncogene enhancing cell invasion and tumorigenesis in human bladder carcinoma cells. *Int J Mol Sci* 20(4):980. <https://doi.org/10.3390/ijms20040980>
 44. Bonaventura G, La Cognata V, Iemmolo R, Zimbone M, Contino A, Maccarrone G, Failla B, Barcellona ML, Conforti FL, D'Agata V (2018) Ag-NPs induce apoptosis, mitochondrial damages and MT3/OSGIN2 expression changes in an in vitro model of human dental-pulp-stem-cells-derived neurons. *Neurotoxicology* 67:84–93. <https://doi.org/10.1016/j.neuro.2018.04.014>
 45. Tahmasbpour E, Ghanei M, Qazvini A, Vahedi E, Panahi Y (2016) Gene expression profile of oxidative stress and antioxidant defense in lung tissue of patients exposed to sulfur mustard. *Mutat Res* 800:12–21. <https://doi.org/10.1016/j.mrgentox.2016.03.006>
 46. Somji S, Garrett SH, Sens MA, Gurel V, Sens DA (2004) Expression of metallothionein isoform 3 (MT-3) determines the choice between apoptotic or necrotic cell death in Cd+2-exposed human proximal tubule cells. *Toxicol Sci* 80(2):358–366. <https://doi.org/10.1093/toxsci/kfh158>
 47. Bousleiman J, Pinsky A, Ki S, Su A, Morozova I, Kalachikov S, Wiqas A, Silver R, Sever M, Austin RN (2017) Function of metallothionein-3 in neuronal cells: do metal ions alter expression levels of MT3? *Int J Mol Sci* 18(6):1133. <https://doi.org/10.3390/ijms18061133>
 48. Wong DL, Merrifield-MacRae ME, Stillman MJ (2017) Lead (II) binding in metallothioneins. *Metal Ions in Life Sciences*, ed A Sigel, H Sigel and RKO Sigel 17:241. <https://doi.org/10.1515/9783110434330-009>
 49. La Guardia PG, Alberici LC, Ravagnani FG, Catharino RR, Vercesi AE (2013) Protection of rat skeletal muscle fibers by either L-carnitine or coenzyme Q10 against statins toxicity mediated by mitochondrial reactive oxygen generation. *Front Physiol* 4:103. <https://doi.org/10.3389/fphys.2013.00103>
 50. El-Tantawy WH (2016) Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *J Tradit Complement Med* 6(4):327–331. <https://doi.org/10.1016/j.jtcm.2015.02.001>

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