Toxicology and Environmental Health Sciences
https://doi.org/10.1007/s13530-020-00061-7

ORIGINAL ARTICLE

Protective effects of Co-Q10, *Ginkgo biloba*, and L-carnitine on brain, kidney, liver, and endocrine system against sub-acute heavy metals toxicity in male rats

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Accepted: 29 June 2020

Abstract

Objective Since occupational exposure to heavy metals is associated with diverse disorders, the present study evaluates the ameliorative and protective capacity of Co enzyme Q10, L-carnitine and *Ginkgo biloba* against systemic sub-acute cadmium (Cd) and lead (Pb) toxicity in male rats.

Methods We randomly divided 24 adult male rats into four groups of six rats each; they were placed in pairs in plastic cages. The intoxicated (Group 1) received a mixture of Cd (2 mg/kg) and Pb (60 mg/kg) orally for 30 days. The protected (Group 2) received Co-Q10 (100 mg/kg), L-carnitine (100 mg/kg), and *G. biloba* (100 mg/kg) orally once daily for 30 days before oral administration of Cd and Pb mixture. The comparison (Group 3) received Co-Q10 (100 mg/kg), L-carnitine (100 mg/kg), and *G. biloba* (100 mg/kg) administered orally once daily for 30 days. The control group received normal saline orally in volumes equal to those of the substances administered to the other groups. At the end of the study period, all rats were killed, blood was withdrawn, and organs were excised for biochemical and histopathological analyses.

Results Group (1) showed the least weight gain, hyperthyroidism (high free T4, while TSH was suppressed), decreased testosterone secretion, abnormal liver and renal functions that were associated with damaged cells. Heavy metals adversely affect lipid profile and significantly decreased glutathione levels in serum and tested organs tissues. The administration of Co-Q10, L-carnitine, and *G. biloba*) showed significant protection against endocrine disorder and organs damage, including improvements in liver and renal functions and a significant increase in glutathione level.

Conclusion Rat exposure to Co-Q10, L-carnitine, and *G. biloba* is associated with protective effects on Pb- and Cd-mediated toxicity of brain, kidney, liver, and endocrine system.

Keywords Heavy metals · Lead · Cadmium · Antioxidant · Endocrine disruptor · Glutathione

Introduction

Protective mechanisms against oxidative damage and free radical formation are essential objectives when production of toxic reactive oxygen species (ROS) exceeds the capacity to remove them. Heavy metals (HM), as widespread toxicants, may affect physiological functions at systemic levels: commonly causing loose bowels and stomatosis in the gastrointestinal tract; ataxia and mental tension in the central nervous system; and insulin resistance, infertility and thyroid disorders as endocrine disorders [1, 2]. HM toxicity commonly causes hypertension and thrombotic disorders as well as anemia and infections [3]. Epidemiologic and experimental studies show occupational exposure to HM to be associated with diverse consequent disorders including cancer, osteoporosis, and hepatic and renal diseases. Mechanistically, free radical generation and its binding with the nucleus and protein with adducts formation are considered the major causes of HM toxicity leading to the degradation of cellular macromolecules and the disruption of cellular functions [2, 4]. HM are dispersed throughout the atmosphere by several

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Published online: 21 July 2020

processes, including anthropogenic activities such as fuel combustion and industrial waste, as well as agriculture activities and feeding through diet appears to be prominent reasons [5].

Cadmium (Cd) has the potential to harm the oxidizing DNA-repair system and may also alter mitochondrial functions through lipid peroxidation and membrane integrity disruption [6]. Lead is a generalized contaminant of the atmosphere; it may substitute calcium and deposit in the bone an effect which is particularly hazardous in children. Lead-induced oxidative damage in the brain disturbs endogenous homeostasis [7]. There is extensive work as yet to be undertaken to test the many natural antioxidants for their therapeutic role in heavy metal toxicity. Flavonoids, polyphenols, vitamins, and carotenoids are the main natural antioxidants known to react safely with free radicals and prevent harmful chain reactions which damage cell organelles [8].

Co enzyme Q10 (Co-Q10) is a natural hydrophobic compound that is not only a vital component of mitochondrial respiration but also a powerful antioxidant. It can suppress the generation of ROS by disrupting NADPH oxidase expression, and it scavenges product of lipid peroxidation during free radical reactions. Co-Q10 also can suppress excess nitric oxide development and avoid nitrative tissue stress [9, 10].

L-carnitine (LC) is an amino acid (β-hydroxy-γ-trimethyl-amino-butyric acid) synthesized from methionine and lysine. LC promotes β-oxidation of long-chain fatty acids and takes part in branched-chain amino acid metabolism which results in cell membrane stabilization [11]. *Ginkgo biloba* (Gb) is a natural products used in the management of various pathologies. As phytotherapeutic products Gb control antioxidant enzymes positively contributing to lipid peroxidation reduction. It also regulates anti-inflammatory cytokines negatively when used in inflammatory disorders [12]. The present study evaluates the ameliorative and protective capacity of Co-Q10, LC and Gb against systemic sub-acute lead and cadmium toxicity in male rats.

Results

Subacute exposure to HM (lead and cadmium) for 30 days was found to be associated with significant differences in weight changes between groups. Compared to other groups, Group 1 experienced the least weight gain (Fig. 1). In Group 1, changes in body weight in gm (mean ± SEM) measured every 10 days were: 197.1 ± 22.2/218.16 ± 25.6/232 ± 29.5 and 236.66 ± 27.8. The changes in weight throughout the experiment in the remaining groups are comparable to that of the control group.

With regard to HM effects on thyroid functions (Fig. 2a, b), TSH (thyroid-stimulating hormone) levels were

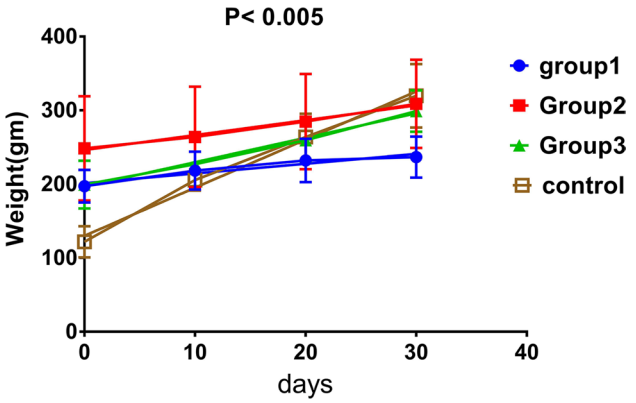

Fig. 1 Effect of CoQ10, GB and LC against sub-acute administration of cadmium and lead on body weight changes. The values are expressed as mean ± SEM in each group. $P > 0.005$

significantly lower in Group 1 compared to other groups (< 0.005 μIU/l). Daily administration of Co-Q10, LC, and GB increased the level of TSH significantly, with a concentration comparable to that observed in the control group. Furthermore, Group 1 showed significantly higher FT4 (free thyroxine) levels were significantly higher compared to rats in other groups. Figure 2c illustrates the effect of heavy metals on testosterone levels. The lowest testosterone concentrations were observed in Group 1 (0.866 ± 0.266 ng/ml). This reduction in testosterone level was associated with severe sloughing of the germinal epithelium with disarrangement of basement membrane (Fig. 3). In comparison, testosterone levels in Group 2 and Group 3 were significantly higher and were associated with significant improvements in spermatogonia in seminiferous tubules; however, such improvements were relatively lower than those observed in the control group. Group 3 showed the largest amount of spermatozoa and spermatogonia at all stages of the spermatogenesis of some seminiferous tubule. There was no significant effect on blood glucose levels across treated groups compared to control group (Fig. 2d).

The effects on liver toxicity are illustrated in Fig. 4, where the level of AST was significantly higher in Group 1 compared to other groups (Fig. 4a); however, ALK phosphatase levels were not significantly different across treated groups compared to control group (Fig. 4b). Liver section of Group 1 revealed hepatic tissue damage including congested central vein enlargement. Compared to Group 1, Group 2 showed some improvement and less hepatic tissue damage (Fig. 5).

Renal function was deteriorated in Group 1 compared to other treated and control groups. Group 1 showed significantly higher urea and serum creatinine levels (Fig. 6a, b, respectively). On histopathological section, dilatation of Bowman's space with atrophy and disappearance of glomeruli were observed in Group 1. Moreover, hemorrhage in the glomeruli and interstitial tissues was associated with

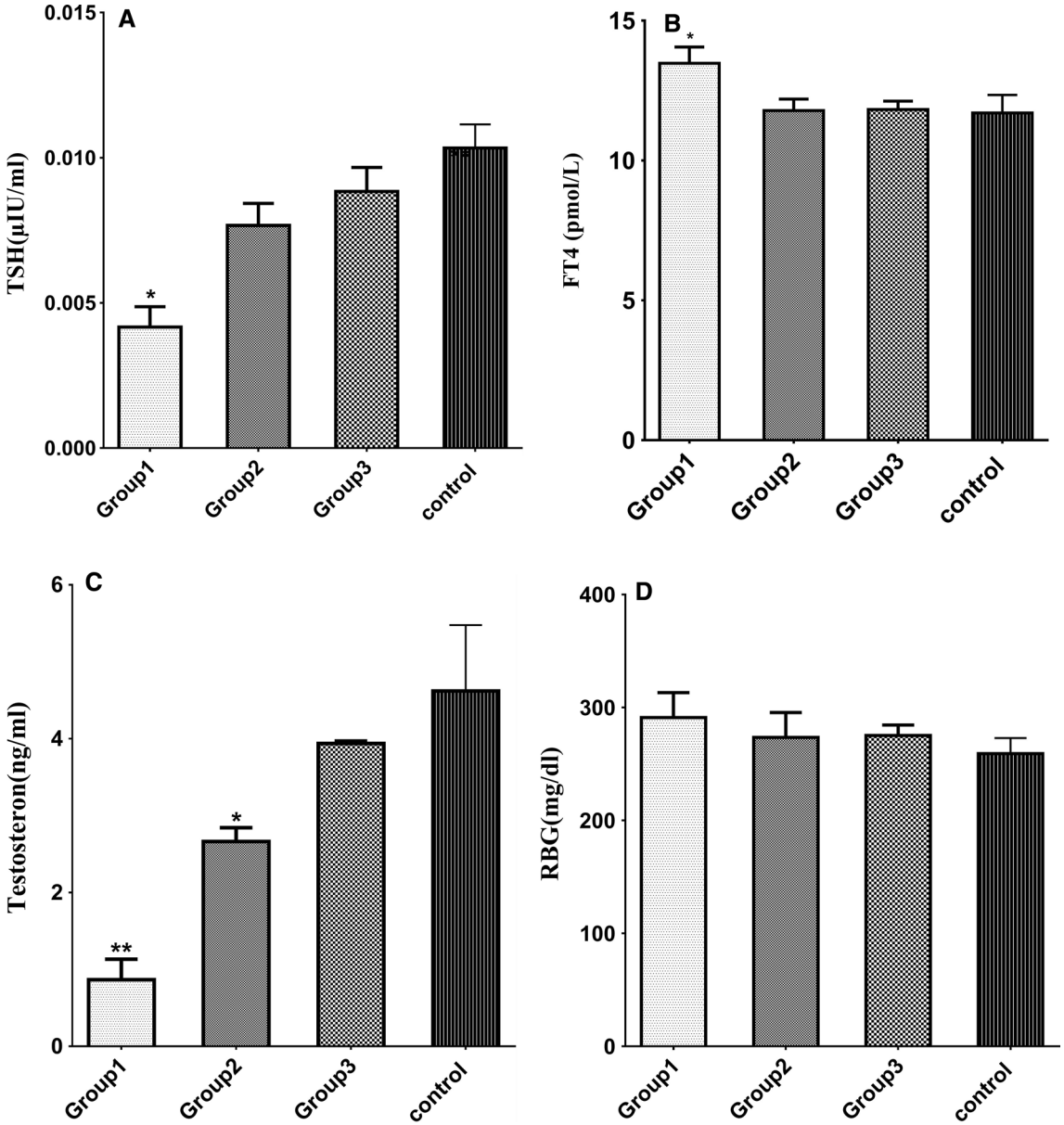

Fig. 2 Effect of CoQ10, GB, and LC against sub-acute administration of cadmium and lead on endocrine function tests. **a** Serum TSH concentration, **b** serum FT4 levels, **c** serum testosterone levels, **d** RBG (random blood glucose) levels. Values are expressed as mean ± SEM in each group. *Significant difference compared to other groups $P > 0.05$; **Significant difference compared to control group $P > 0.0001$

degeneration of tubular epithelial cells and renal tissue edema (Fig. 7).

Prolonged oral exposure to Cd and Pb had adverse affects on lipid profile of Group 1 (Table 1). The levels of TG, VLDL, and LDL exploded significantly in Group 1 compared to other groups. Meanwhile, Group 2 showed some protection against heavy metals effect on lipid profile when compared to Group 1 supported by significant reduction in TG, VLDL, and LDL levels, and an increase in HDL level); nonetheless, the effect was less than that observed in the control group.

Group 1 observed significant reduction in serum and tissue glutathione levels after 30 days of exposure to heavy metals mixture. Corresponding glutathione levels were significantly increased after the administration of protective compounds (Co-Q10, LC, and GB). However, such increment appears to be less significant when compared to that in Group 3 and control group, where the highest concentration

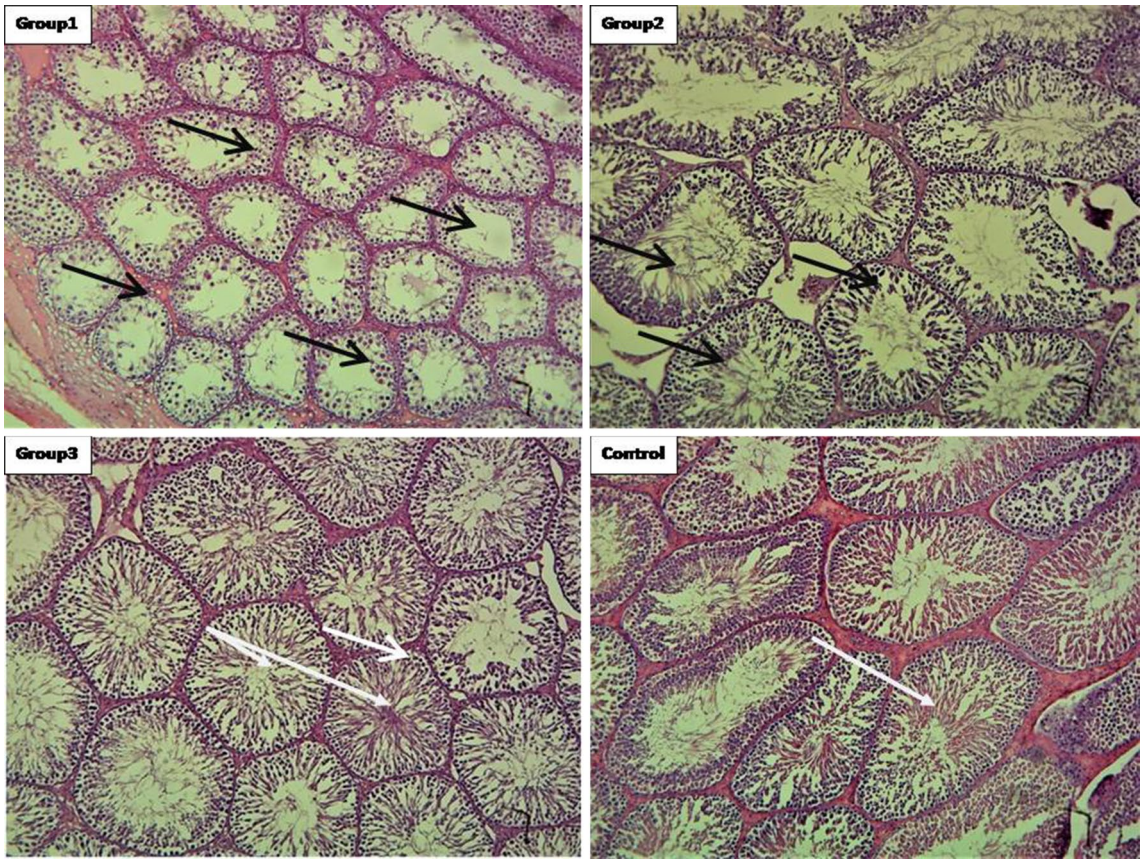

Fig. 3 Light micrographs of the testes section stained with H and E ×10. Group 1: Sever degeneration and sloughing of germinal epithelium with disarrangement of basement membrane; there are no spermatozoa in lumens of the seminiferous tubule associated with atrophy of seminiferous and irregularity (black arrows). Group 2: vacuolization with few numbers of spermatogonia in lumen of seminiferous tubules (black arrows). Group 3: normal structure of seminiferous tubules contains spermatogenic with an enormous amount of spermatozoa and normal arrangement of germ cells (white arrows). Control: normal tissues structure and sperm (white arrows)

of glutathione was observed in the control group followed by Group 3. Compared to other treated groups, Group 3 exhibited highest average glutathione concentration, which was a statistically significant difference (Table 2).

Figure 8 illustrates the effects on brain tissue, where subacute administration of Pb and Cd to the rats in Group 1 was associated with multiple necrosis and atrophy of multi polar neurons well as hemorrhage. On the other hand, significant improvement was seen in the brain of rats in Group 2 who was treated with CoQ10, GB, and LC combination, which was reflected by less or moderate necrosis with minor improvement on neuron structure compared to control group.

Discussion

Heavy metals may adversely affect the oxidative and free radical scavenger balance. Free radicals being beyond the degree of being barred through antioxidants use, at this stage, the side effects caused by increased amounts of oxidants continue to manifest in distinct types of cellular and biochemical disorders. In the present study, Group 1 (the intoxicated group of rats) exhibited decreased weight gain. The established decrease in weight gain appears to be less significant compared to the corresponding values of the other groups. These results are comparable to those of Lopotych et al. [13] who showed that chronic Pb and Cd administration are associated with decreased weight gain in rats. Several studies have provided possible explanations for how heavy metals may cause nausea, anorexia, and vomiting associated with muscle wasting and oxidative stress which typically follow continuous exposure [14–16].

Despite well-known associations of heavy metals with different diseases and health problems, their effect as endocrine disruptors is still to be explored. The level of free thyroxin (FT4) was significantly increased in the intoxicated rats (Group 1). The explanation of this finding is slightly difficult since numerous articles support a negative association between thyroid hormone levels and chronic exposure to heavy metals [17]. However, exposure to high doses of

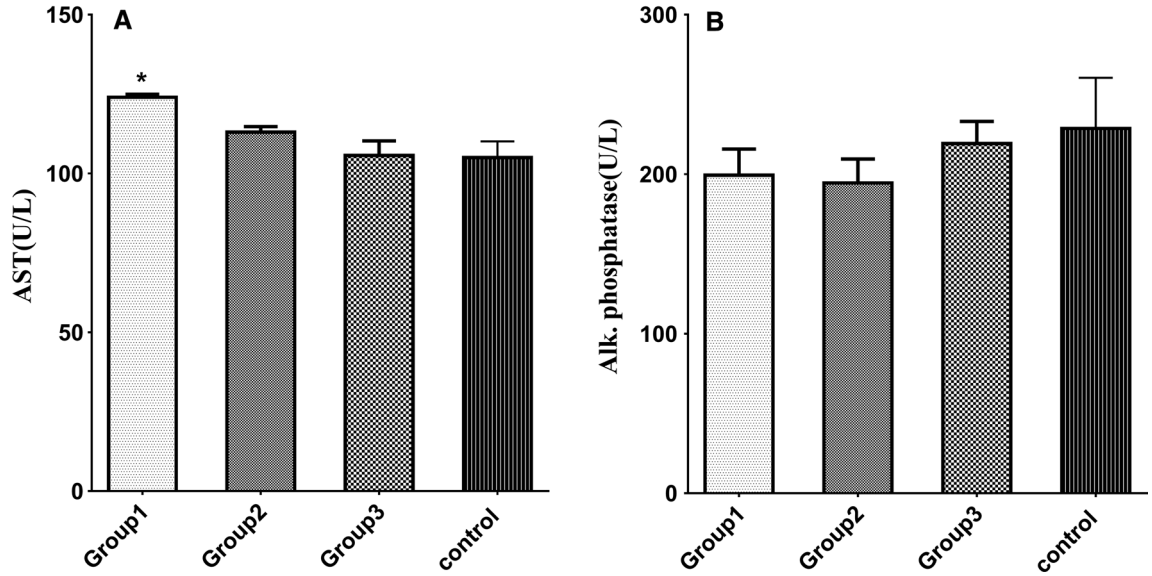

Fig. 4 Effect of CoQ10, GB, and LC against sub-acute administration of cadmium and lead on liver enzymes. **a** serum AST (aspartate transaminase) level, **b** serum ALK (alkaline phosphatase) levels. Values are expressed as mean ± SEM in each group. *Significant difference compared to other groups $P < 0.05$

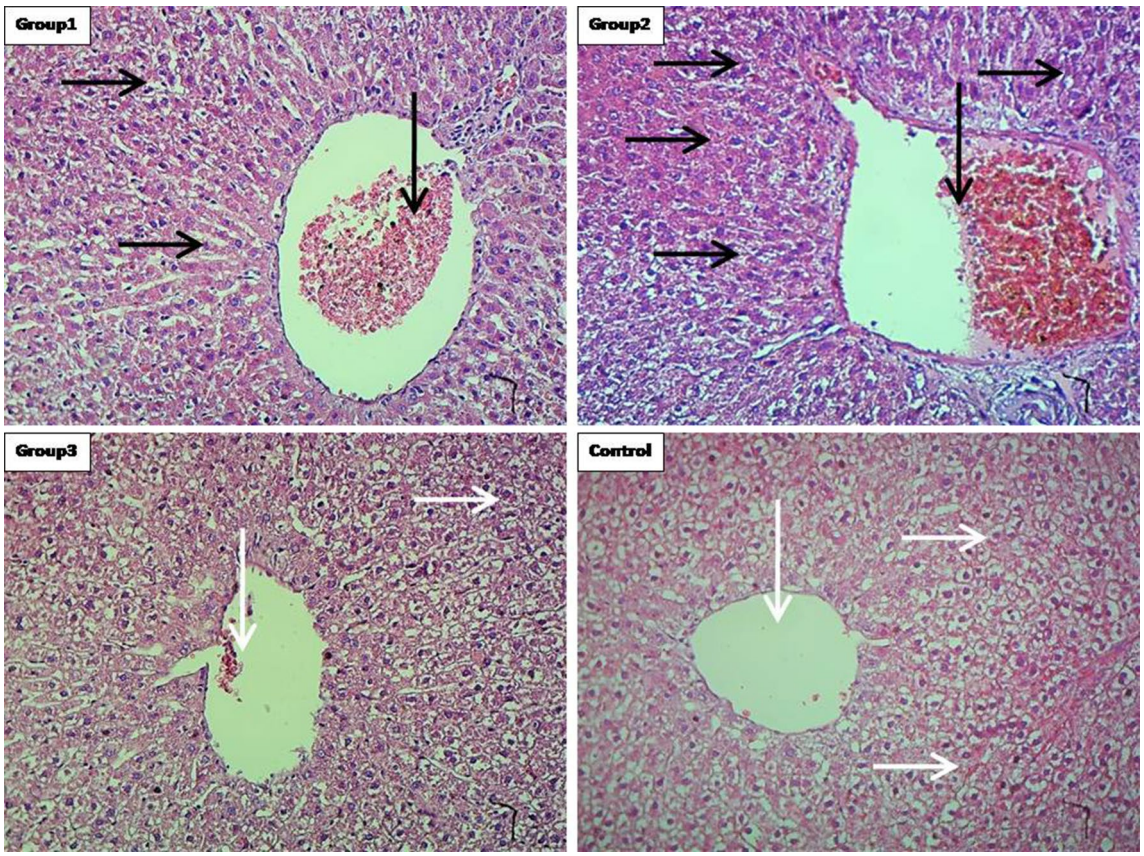

Fig. 5 Light micrographs of the liver section stained with H and E ×10. Group 1: central vein congestion with enlarged, pyknotic with light chromatinic stained and enlarged sinusoids (black arrows). Group 2: enlarged central vein with congestion, pyknotic with light chromatinic stained, and enlarged sinusoids with congested spaces (black arrows). Group 3: normal central vein and normal hepatocyte (white arrows). Control: normal tissue structure of liver: central vein, hepatocytes cord, and sinusoid (white arrows)

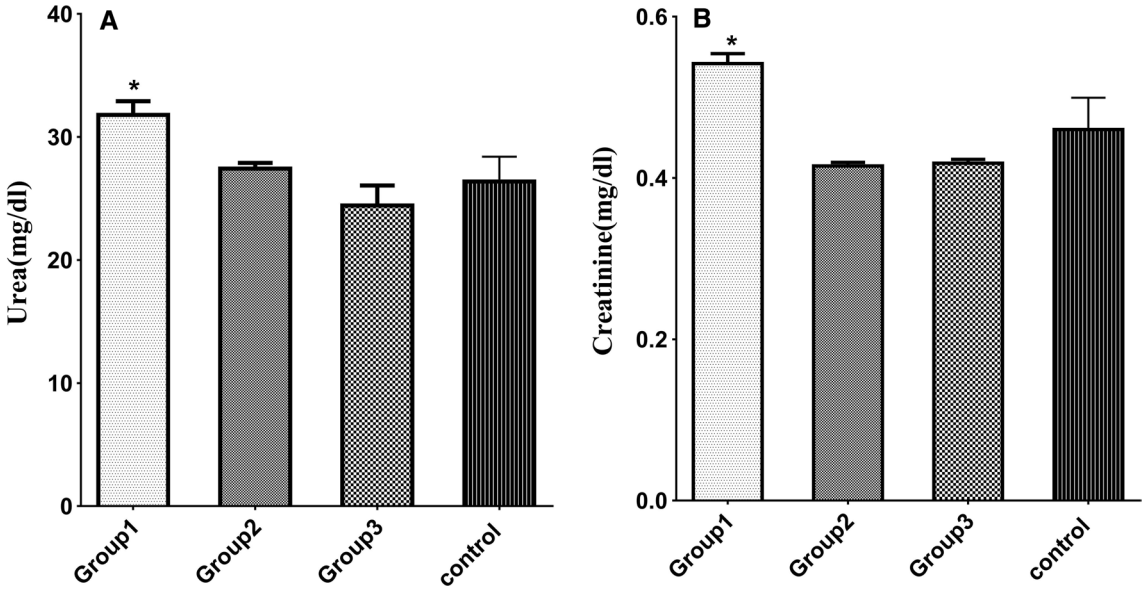

Fig. 6 Effect of CoQ10, GB, and LC against sub-acute administration of cadmium and lead on renal function. **a** Serum urea level, **b** serum creatinine level. Values are expressed as mean ± SEM in each group. *Significant difference compared to other groups $P > 0.05$

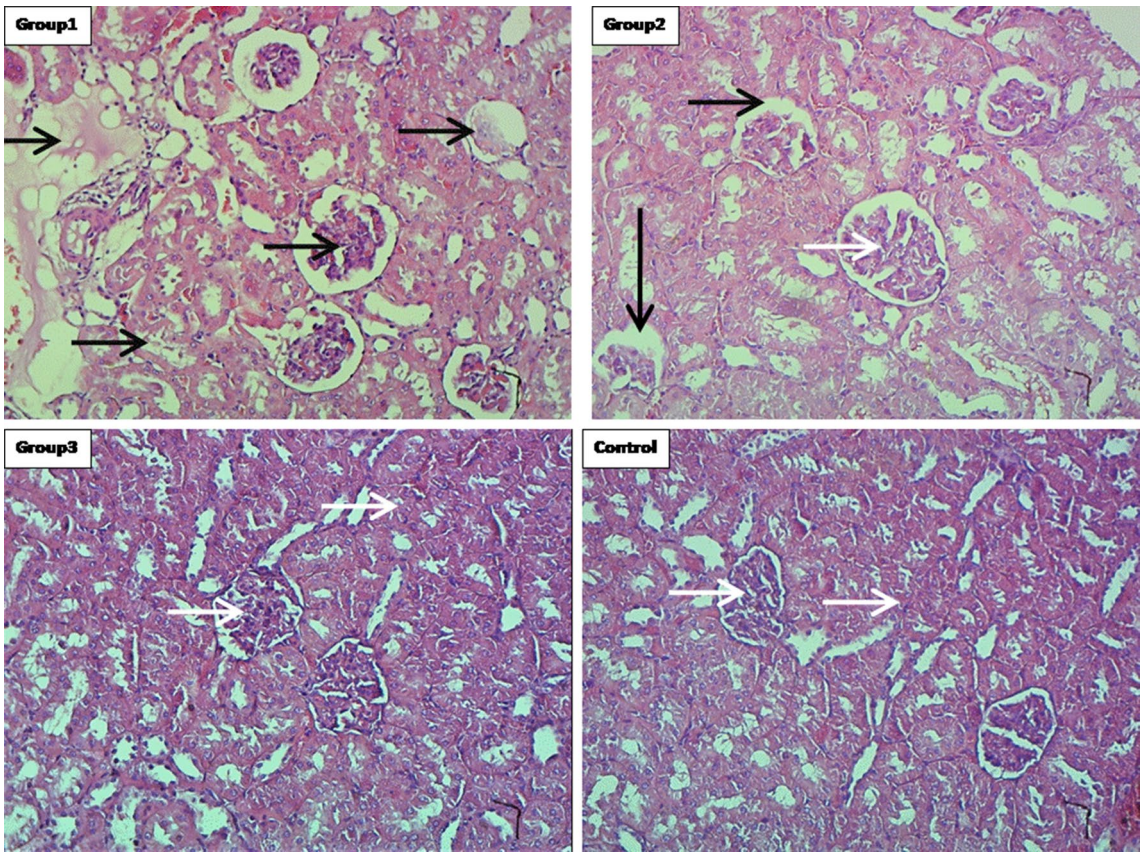

Fig. 7 Light micrographs of the kidney section stained with H and E ×10. Group 1: dilated of Bowman's space, atrophy disappearance of glomeruli, hemorrhage in the glomeruli as well as interstitial tissue, degeneration of tubular, and edema (black arrows). Group 2: normal glomeruli (white arrow), dilated of Bowman's space (black arrows). Group 3: normal tissue, glomeruli, and renal tubules (white arrows). Control: normal renal glomeruli, Bowman's capsule and tubules (white arrows)

Table 1 Effect of CoQ10, GB, and LC against sub-acute administration of (cadmium and lead) on lipid profile in rats

Groups	LDL (mg/dl) Mean ± SEM	VLDL (mg/dl) Mean ± SEM	HDL (mg/dl) Mean ± SEM	Chol (mg/dl) Mean ± SEM	TG (mg/dl) Mean ± SEM
Group 1	9.8 ± 0.4#	12.38 ± 1.023#	17.83 ± 0.70#	60 ± 3.65	64.5 ± 5.365#
Group 2	7.16 ± 0.40	6.83 ± 0.307	22.83 ± 1.57*	60.83 ± 4.21	34.17 ± 1.424*
Group 3	7 ± 0.93	7.16 ± 0.6	22.67 ± 1.25*	60.17 ± 4.64	33.17 ± 1.276*
Control	7.16 ± 0.47	9 ± 0.683	28 ± 0.57	64.67 ± 1.70	46.33 ± 1.726
P value	**< 0.05**	**< 0.0001**	**< 0.05**	**0.2368**	**0.0026**

Values are expressed as mean ± SEM in each group

*,# Different characters represent highly significant difference compared to remaining groups $p < 0.05$

Table 2 Effect of CoQ10, GB, and LC against sub-acute administration of cadmium and lead on serum and tissues glutathione levels in rats

Groups	Glut. serum Mean ± SEM	Glut. brain Mean ± SEM	Glut. liver Mean ± SEM	Glut. kidney Mean ± SEM	Glut. testes Mean ± SSE
Group 1	16.24 ± 1.87#	1.78 ± 0.29#	0.713 ± 0.07#	0.79 ± 0.04#	1.85 ± 0.10#
Group 2	23.11 ± 1.71	2.81 ± 0.104	1.76 ± 0.09	1.41 ± 0.05*	4.33 ± 0.7
Group 3	28.83 ± 0.52*	3.47 ± 0.33	2.12 ± 0.2	1.94 ± 0.13	5.09 ± 0.26
Control	22.54 ± 0.55	3.51 ± 0.14	2.18 ± 0.07	2.13 ± 0.18	3.69 ± 0.07
P value	**< 0.05**	**< 0.05**	**< 0.05**	**0.0078**	**< 0.0001**

Values are expressed as mean ± SEM in each group

*,# Different characters represent highly significant difference compared to remaining groups $p < 0.05$

Pb and Cd mixture may increase the thyroxin release in a positive manner, leading to subsequent TSH suppression. Free radical generation and oxidative stress can explain such results satisfactorily [18]. The reactive nature of oxygen and its intermediates may contribute to autoimmune diseases such as Graves' disease, which is characterized by an overproduction of thyroid hormones [19]. Thus, free radical-mediated lipid peroxidation plays a pivotal role in our results; it illustrates the protective impact of the administered combination (CoQ10, GB, and LC) against heavy metals toxicity. The present work also indicates that sub-acute exposure to the Pb and Cd mixture decreased the serum testosterone level significantly. This result is associated with decreased sperm count and increased oxidative stress evident by decreased glutathione level; all these findings explained testicular damage and failure of antioxidant protective mechanisms observed in the intoxicated group. Our results agree with those of Pandya et al. [20] who found that decreased testicular enzyme activity, low serum testosterone level, low sperm count, and loss of motility were associated with oxidative damage of the epididymis and testis after exposure to heavy metals. The combination of antioxidants (CoQ10, GB, and LC) can protect and ameliorate HM-induced testicular oxidative damage. Similar articles have also reported the treatment and protective role of antioxidants such as LC, vitamins C and E, Gb, and CoQ10 in Cd- and Pb-induced male reproductive toxicity [21, 22]. In this study, no significant differences were found in the serum concentrations of RBG among the tested and control groups. In contrast to our finding regarding RBG, HM exposure deemed as a risk factor for the development of mellitus [23]. However, the measurement of the serum glucose level gave an idea about liver and pancreas functions.

Liver and kidneys are the major metabolizing organs responsible for the different detoxification processes against drugs and toxins. Thus, the significant liver and kidney damage due to heavy metal exposure is well documented, and an ameliorating effect is essential [24, 25]. A variety of herbal medicines, natural products, and supplements have been studied extensively to protect these organs against heavy metal-induced-damage. Hepatic injury followed by exposure to heavy metals is well indicated by the high levels of the liver enzymes marker, which exhibit cellular leakage and loss of the functional integrity of the hepatic membranes architecture. In the present study, we revealed increased serum levels of AST in the intoxicated rats (Group 1) compared to the control. This increment reflects deterioration in the liver function after sub-acute administration of the Pb and Cd mixture. Our finding is similar to that of Eltayeb et al. [26] who showed a significant increase in liver enzyme activity because of the release of heavy metals mixture from Pepsi and Atamonia cans throughout 3 months study. The level of AST decreased significantly in the present work with the use of a protective combination of (CoQ10, GB, and LC) in Group 2 to level comparable to that in the control group. Gb, a herbal medicine, reportedly protects and improves liver function against external insults and many pathogenic factors [27]. LC effectively minimized hepatic

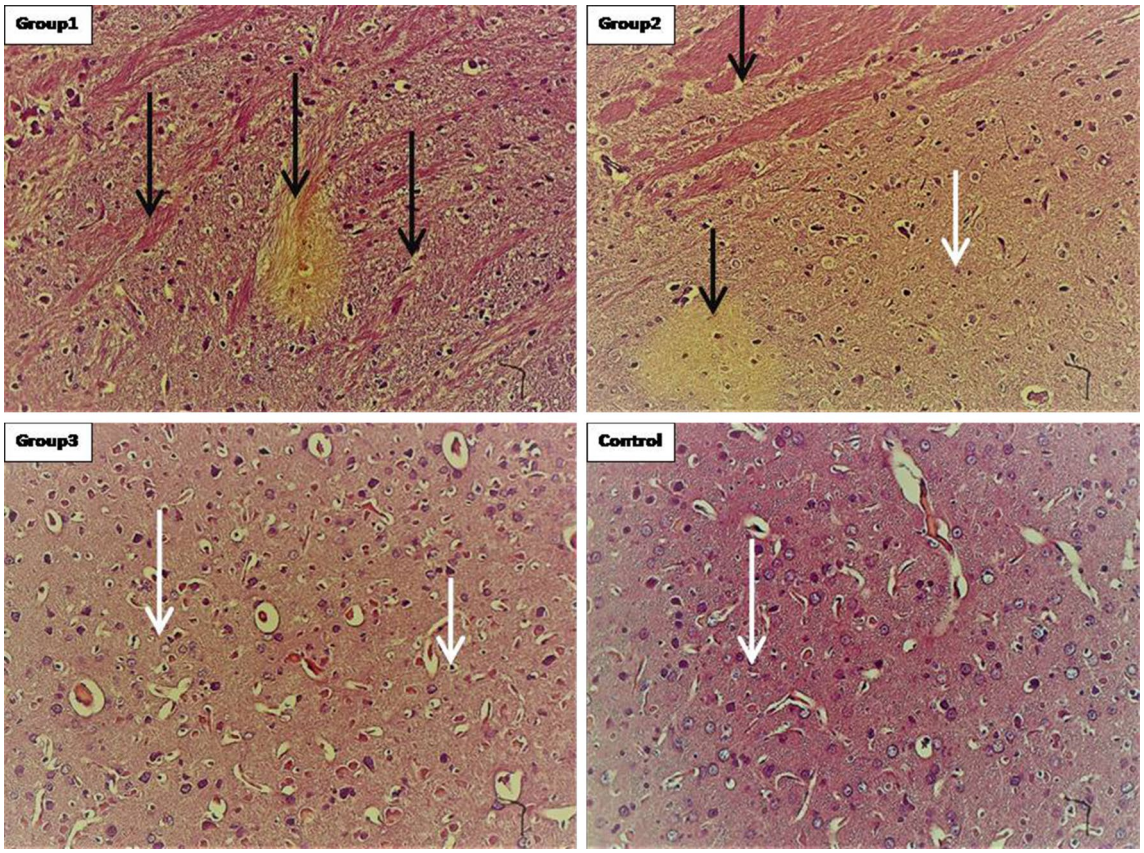

Fig. 8 Light micrographs of the brain section n stained with H and E ×10. Group 1: atrophy and degeneration, necrosis, with hemorrhage of brain tissues (black arrows). Group 2: slight hemorrhage with necrosis (black arrow) and clear some area with no change (white arrow). Group 3: unremarkable any change of the brain tissue (white arrows). Control: normal structure (white arrows) with preserved tissue components

damage caused by Pb injection for 60 days in rats [28]. As a potent antioxidant, CoQ10 significantly decreases AST enzyme levels against CCL4 induce hepatic degeneration and inflammation [29]. In contrast to AST, no obvious effect on the ALP level was observed in our study. It is difficult to explain this finding, as the enzyme is considered as a tool to study varying hepatic cell viability and cell membrane permeability [30]. However, ALP increases predominantly in cases of canalicular or bile duct obstruction [31].

Significant deterioration in kidney function was observed in our work, and the increase in the serum urea and creatinine can explain it in the Group 1 compared with the control. HM lead to deterioration in renal functions. In reality, the renal system can accumulate HM at higher levels in comparison with other organs resulting in nephrotoxicity [4]. Pb can affect cell polarity and renal transporters leading to loss of lumen microvilli as a consequence of changes to the cell junction structure. Moreover, renal accumulation of Pb and Cd leads to depletion in glutathione, which increases renal oxidative stress [32, 33]. The current study displayed that Pb and Cd exposure induced progressive glomerular and tubular alterations resulting in hydraulic changes in the renal tissue. These results suggest that the intoxication provides partial failure in the transport of kidney tubular cells by the ion pump, which in turn producing tubular swelling and causing tubular necrosis and vacuolization. This explanation may also account for the increased levels of serum urea and creatinine [34, 35]. Our results also revealed that concomitant administration of a combination of (CoQ10, GB, and LC) in Group 2 led to significant protective effects against biochemical alterations caused by HM induced renal injury. This finding agrees with that of Ali et al. [36] demonstrated that natural products such as ascorbic provide ameliorative effect against HM-induced renal toxicity.

Oral administration of heavy metals in the present study increased significantly TG, VLDL, and LDL levels and decreased significantly HDL level. Previous articles confirmed our results reported that heavy metals generate excess reactive oxygen species that have a pathological role contributing to adverse lipid profile, lipid peroxidation, and dyslipidemia [37]. High TG and VLDL levels may be due to decreased activity of lipoprotein lipase which impaired

TG metabolism and subsequent higher VLDL level. Also, decreased HDL levels may contribute to increased LDL and VLDL as an indirect effect [38]. Searching for natural supplements that can combat oxidative stress-induced dyslipidemia is the millstone in atherosclerosis therapy. In this study, the use of combination (Co-Q10, GB, and LC) showed a promising effect. Actually, many products had been evaluated for a possible antihyperlipidemic effect like curcumin, zinc, and vitamin C [39, 40]; they showed significant improvement in lipid profile similarly to our study.

It is well accepted that glutathione has an important role in detoxification of various heavy metal and other toxicants for their maintenance of redox balance and antioxidant properties. In this study, there were significant decreases in serum and tissue levels of glutathione in Group 1 when measuring after 30 days exposure to heavy metals mixture. This is associated with significant brain damage, hemorrhage, atrophy, and multiple necroses. Different HM induce adverse neurological effects and brain damage mainly due to oxidative stress [16]. This effect can be ameliorated by protective administration of natural and synthetic antioxidants to restore glutathione and other antioxidants to the normal level. Glutathione is also considered as a biomarker associated with disease and health status. Today, glutathione level modification is an important target where many natural products are used [41].

Materials and methods

Materials

Co-Q10 200 mg soft gelatin capsule (Nitrol company), L-carnitine tablet 500 mg (Basic Nutrition), Ginkgo Biloba tablet 120 mg (ADREN GAHNON), Cadmium Chloride (Monohydrate) 500 mg CDH (china company), Lead Acetate BDH (British company).

Methods

After an acclimatization period, twenty-four adult Sprague male rats (from Basra College of Veterinary Medicine) (weighing 200–300 mg) were involved in the study. We placed the animals in pairs in plastic cages, in normal laboratory conditions regarding humidity and at a temperature of 28 ± 3 °C, housed in a 12-h light/12-h dark cycle and fed a standard pellet diet and provided drinking water ad libitum. The rats were randomly divided into four groups of six rats each. Group 1 the experimental group for heavy metal toxicity received a mixture of Cd (2 mg/kg) and Pb (60 mg/kg) administered orally once per day for 30 days. Group 2, the protective group, received Co-Q10 (100 mg/kg), LC (100 mg/kg), and Gb (100 mg/kg) administered orally once per day for 30 days before oral administration of HM mixture Cd (2 mg/kg) and Pb (60 mg/kg). Group 3 received Co-Q10 (100 mg/kg), LC (100 mg/kg), and Gb (100 mg/kg) administered orally once per day for 30 days. Group 3 acted as a comparison group for the unexpected effects of protective compounds. Control group (Group 4) received normal saline orally in volumes equal to those of the substances administered to the tested groups (Groups 1, 2, and 3). The rats' weights were measured at the onset of the experiment, then once every 10 days thereafter for 30 days, and again before killing.

At the end of the study period on the 31 days, all rats were killed and blood was withdrawn by intracardiac puncture and then centrifuged for serum collections. For histopathological examination, the thyroid gland and only small parts of the brain, liver, kidney, and testes were excised for fixation, and other were homogenized for glutathione measurements.

Samples preparations and biochemical analysis

Serum concentrations of thyroid hormones (TSH, FT4) and testosterone level were measured by electrochemiluminescence method using. Liver enzymes (AST and ALK) were evaluated employing ELISA technique. Renal functions and other biochemical parameters such as lipid profile (LDL, VLDL, HDL, cholesterol and triglycerides) and serum glucose were analyzed using the spectrophotometric technique. All these parameters were evaluated according to the manufacturer's protocol with a diagnostic automated laboratory analyzer (Abbott architect 4000c, USA).

Glutathione levels are analyzed in tissues (brain, liver, kidney, and testes) and serum. The tissues were cut and washed with ice-cold saline, stored at − 20 °C, and then homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was obtained and serum was used for determination of glutathione (GSH) levels using Elisa kite according to the manufacturer's instructions.

Histopathological examination of tissues

For histopathological examination, tissues were fixed in 10% formalin solution for 4 days, sectioned, and then embedded in paraffin. Histological sections were cut at 4–5 µm and then stained with routine hematoxylin (H) and eosin (E), for examination under a light dissection microscope by a histopathologist for analysis of tissue damage, surface epithelium disruption, and infiltration of the epithelium.

Statistical analysis

We interpreted the results of this study as mean ± SEM. The data were analyzed using a one-way analysis of variance,

and then post hoc analysis was performed to compare the tested groups. Values of $P < 0.05$ were significantly different. The study was carried out using version 6.0 of the GraphPad Prism program.

Conclusion

The study demonstrated the ameliorative role of (Co-Q10, LC, and Gb) combination against Cd- and Pb-mediated oxidative damage in the brain, liver, kidney, testes, and thyroid gland evident by histological sections, diminishing high liver enzymes, serum urea and creatinine and improve glutathione level. Meanwhile, there are protective role against HM-induced endocrine disorders, dyslipidemia, and neurotoxicity with general improvement in overall physiological functions. Our results support the use of such combination as protective compounds to nullifying the toxic effect of heavy metals and suggest using therapeutic or antidote regimen.

Suggestion for future work

1. Evaluating the possible protective role of Co-Q10, LC, and Gb combination on HM-induced cardiovascular disorder.
2. Identifying the molecular mechanisms behind the ameliorative effect of combination (Co-Q10, LC, and Gb) against HM chronic toxicity.
3. Possible role of Co-Q10, LC, and Gb as anticancer adjuvant treatment.

Acknowledgements The presented study was abstracted from M.Sc. thesis submitted to the College of Pharmacy, Basrah University/Department of Pharmacology and Toxicology. Authors thank the college for continuous support.

Compliance with ethical standards

Conflict of interest Noor Ali Abdulidha, Ausama Ayob Jaccob, and Muhsin S.G. AL-Moziel declare that they have no conflict of interest.

Ethical statement All experiments were performed in compliance with the National Institute of Health Guidelines for the Treatment and Use of Laboratory Animals (86/609/EEC) and approved by the College of Pharmacy/Basrah University, Ethics Committee.

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