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Metallothionein Protein Concentration in The Liver Tissue of Albino Mice Exposed to Cadmium and Zinc Chloride

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ABSTRACT

Background: Cadmium (Cd), one of the most abundant heavy metals, is extremely toxic to both humans and animals. hIt is well known that zinc (Zn) administration reduces Cd-induced toxicity and that metallothioneins can have a protective effect in biological systems to mitigate Cd toxicity.

Objective: The aim of the current study to determine if Zn administration affected the induction of MT-1 and MT-2 in the liver tissue in mice exposed to Cd.

Materials and methods: Metallothionein protein (MT) level in the tissue of male mice were detected using the anion - exchang high-performance liquid chromatography coupled (HPLC)assay and immunohistochemical staining.

Results: Single treatment to zinc or cadmium increase the level of MT in the liver, but zinc chloride treated significantly increase the level of MT after sub chronic treatment.

Conclusion: Zinc pre-treatment with increasing the concentration of the dose of cadmium used in the co-treatment, and both of them may have worked together to induce a significant increase in protein synthesis to exceed the high toxicity of cadmium, by inducing an increase in MT protein synthesis.

Keywords: Cadmium, Zinc, Metallothionein, Liver, Mice, Experimental study, University of Baghdad.

INTRODUCTION

One of the most pervasive heavy metals, cadmium (Cd), is extremely harmful to both people and animals [1,2,3]. Exposure to Cd is frequently associated with considerable buildup in soft tissue due to its long biological half-life [2,4].

Human and animals require some trace element like Zn. Zn's intracellular participation in enzyme catalysis, protein structure and protein interactions are primarily responsible for its significance in cell physiology [5,6].

It is generally recognized that administering zinc (Zn) can prevent or reduce cadmium toxicity [7], in a recent research, our team proved that treated mice with Zn ameliorative prevent liver tissue damage and oxidative damage caused by Cd.

Metallothioneins (MT) a protein with high capacity to bind heavy metal ions in biological system and protect against toxicity in biological systems. There are at least four main MT isoforms in mammals. The most prevalent isoforms are MT-1 and MT-2, while the MT-3 is mostly found in the brain and MT-4 is mostly present in the stratified epithelium [8]. Induction of MT production is a sensitive indicator of exposure to heavy metal [9].

Many studies showed that different animals tissue might greatly enhance MT expression when exposed to Cd [10,11].

The aim of this study was to determine if Zn administration affected the induction of MT-1 and MT-2 in the liver tissue in mice exposed to Cd.

MATERIALS AND METHODS

Chemicals: Cadmium and Zinc chloride were purchased from Sigma (USA). All other compounds were obtained from standard suppliers.

Animal Model: A total of 36 mature male mices *Mus musculus* (8 weeks old, weighing 28 ±2 gm at the time of the experiments) were kept under controlled ambient and feed *ad libitum.*

Animal Groups: Experimental mice were divided randomly into 6 groups and injected intraperitoneally (i.p) for 21 days as following: control group received distal water (G1), group injected with zinc chloride at 10 mg/kg (G2), groups injected cadmium chloride at two concentrations at 1,5 and 3 mg/kg (G3 and G5) respectively. Two groups received $ZnCl_2$ first then followed by $CdCl_2$ after 30 mints (G4 and G6) respectively.

Liver total Metallothionein (MT1 and MT2) concentration study: Metallothionen protein was separated and quantified according to **Richards and steele** [12]. using UV-Vis 10A-SPD spectrophotometer (USA, Rheodyne, model 7125).

Immunohistochemistry: Fixed liver specimens were sectioned after following histological protocol as described by **AL-Musawi *et al*** [13] and **Ashour *et al*** [14],

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HRP/DAB IHC detection kit (UK, Abcam) was used according to the manufacturer's procedure.

Ethical Approval

The study's approval was given by the ethical council of Baghdad University's College of Education for Pure Sciences.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test/ Mann Whitney test was used to compare between two independent groups. One Way ANOVA test/Kruskal Wallis test was used to compare more than 2 independent groups. P value ≤ 0.05 was considered significant.

RESULTS

The total Metallothionein proteins (MT1 and MT2) content in the liver tissue of mice injected with CdCl4 or/and ZnCl4 for 21 days results were reported in (Table1). The data indicated a significant difference ($P \leq 0.05$) in MT1 and MT2 protein content in all treated groups Vs control, also the difference was significant at ($P \leq 0.05$) in (G2 and G3), and between (G4 and G5) groups. The highest concentrations were seen in G5 group and next in G6 group for MT1 and in G5 group and next in G4 group for MT2 protein Vs control (**Table 1**).

Table 1: The concentration of Metallothionien proteins in the liver of male mice, determined by HPLC assay, after 21 days of injected with $ZnCl_2$ or $CdCl_2$ or both.

Protein concentration (µg/gm wet tissue)	**Treatment groups (Mean±SD)**					
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
	Control (D.W.)	$Zncl_2$(10) Mg/kg b.w.	$Cdcl_2$ (1.5)mg/kg b.w.	$Zncl_2$(10)+ Cd cl_2 (1.5) mg/kg b.w.	Cd cl_2 (1.5)mg/kg b.w.	Zn cl_2 (10)+ Cd cl_2 (3)mg/ kg b.w.
MT1	7.14±0.53	18.18±0.52 [ab]	66.28±0.64 [ab]	70.21±0.61 [ab]	112.18±0.57 [ab]	98.24±0.57 [ab]
MT2	22.1±0.55	41.17±0.59 [ab]	42.09±0.56 [ab]	58.19±0.51 [ab]	65.16±0.61 [ab]	54.21±0.63 [ab]
(N=6) number of animals in each group, a The difference compared to control, b difference within groups at $P \leq 0.05$.						

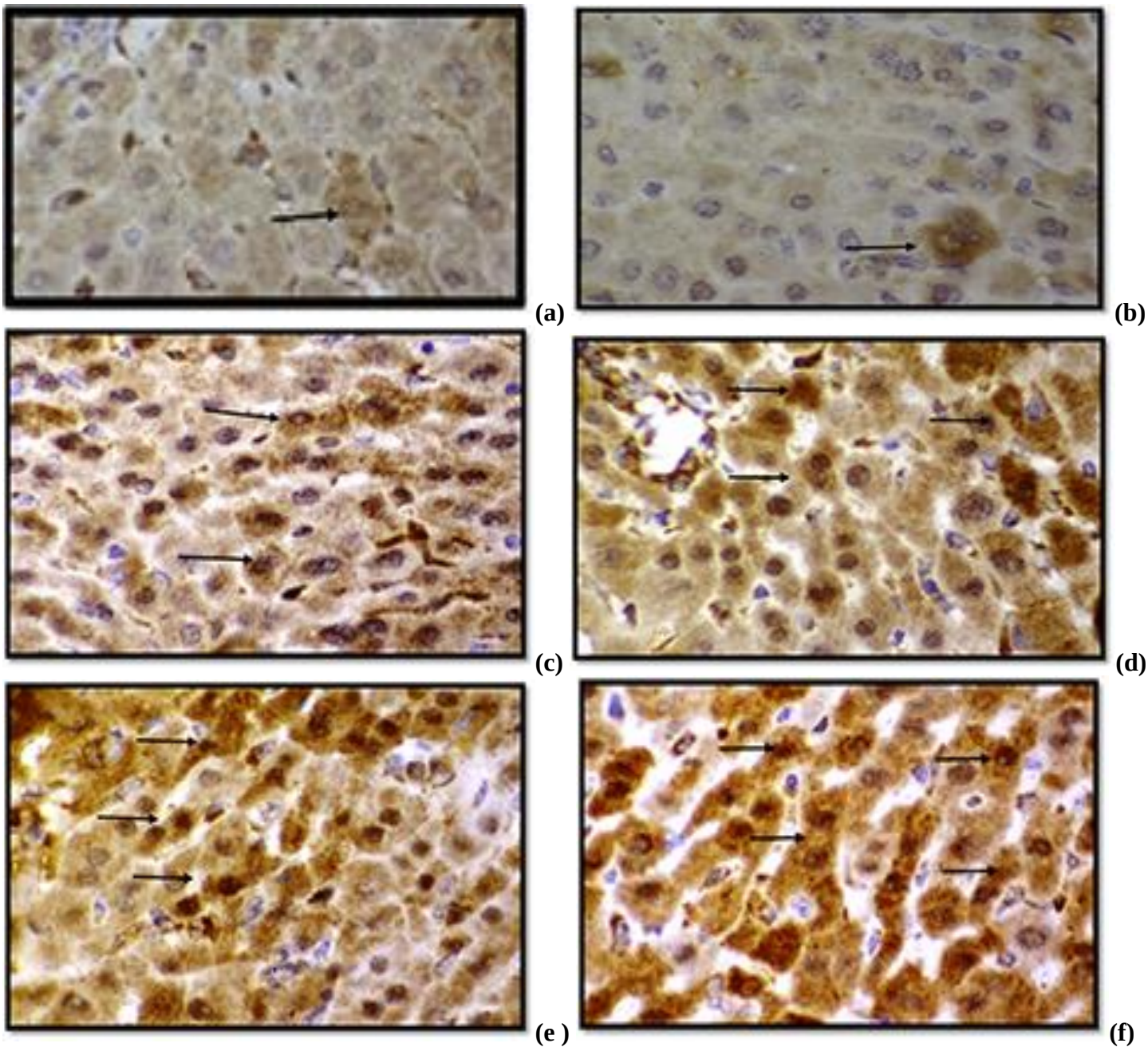

Figure 1. Immunohistochemistry with specific antibody against MT1 in liver tissues, (a) a few positive cells in control G1 group, (b) increased inG2 group, (c) markedly increased inG3 group, (d, e and f) highly increased in G4,G5 and G6 groups, (100X).

DISCUSSION

Results of recent research showed that treatment with zinc chloride solution within group G2 led to a significant increase in MT1 and MT2 proteins concentration in the liver compared to the control G1 group, and compared to the rest of the experimental groups, the significant increase of the current group represented the lowest value among groups G3, G4, G5 and G6. The current result is in agreement with the results of the study by **Bernotiene *et al.*** [15] who showed that treating rats by intraperitoneal injection with ZnSo4 (24 mg/kg body weight) for 14 days led to a significant increase ($P \leq 0.05$) in the concentration of MT protein in the liver compared to the control group. On the other hand, the increase in the group treated with zinc sulfate was less than the increase in the group treated with cadmium chloride solution only, as well as less than the increase in the group treated with zinc and cadmium. Also in a study by **Khudhair and Abass** [7] indicated that there was a significant increase in the concentration of MT2 protein in the liver of mice, which resulted from a doubling of the concentration of MT2 protein by (190%) after oral treatment with zinc sulfate solution concentration (10 mg / kg of body weight), while the treatment group showed with cadmium chloride solution concentration (5 mg/kg of body weight) and the combined treatment group (cadmium chloride and zinc sulfate), an increase resulted in a doubling of the protein concentration by (217% and 330%), respectively, against the control group.

The current result showed that treatment with zinc chloride solution in group G2 led to an increase in protein concentration in the liver of albino mice, but its effect on inducing an increase in protein concentration was found to be less than the effect of the single treatment with cadmium chloride solution, as well as less than the effect of the combined treatment (with zinc then cadmium) after 21 days of treatment. Continuous treatment by intraperitoneal injection. The current result can be attributed to the concentration of the dose and method of treatment and its effect on regulating the balance of zinc within the liver cell, where zinc is one of the basic minerals in vital systems and has many functions, including its presence in the composition of many proteins, and zinc controls many metabolism enzymes. food, building DNA and RNA, gene expression, immune efficiency, and plays an important role in hormone homeostasis, zinc also participates in defense against excess amounts and damage caused by some minerals [16].

Therefore, the current result can be attributed to the cadmium treatment, which led to the stimulation of anti-toxic mechanisms in the liver, including the stimulation of the increase in the processes of building MTs metallothionine proteins due to their anti-heavy metal toxicity role, as it was proven that MTs proteins have a fundamental role in isolating and removing the toxicity of free Cd^{+2} cadmium ions inside the cell by forming the MT-Cd complex and thus accumulating it inside the liver, and MTs proteins are effective scavengers of free radicals generated during oxidative stress resulting from treatment with cadmium [17]. Moreover, the current results showed a dose depended effect and this is consistent with the results of **Bernotiene *et al.*** [15] study which showed that the combined treatment with cadmium and zinc stimulates the formation of MTs proteins in the liver of rats significantly ($P<0.05$) than the single treatment with cadmium only. Therefore, the current result can be attributed to the positive indirect role of the pre-treatment with zinc within the co-treatment, and the interval 30 min. between the two doses within the co-treatment may have contributed to increasing the absorption of zinc and protecting the liver by reducing cadmium toxicity, not by reducing the concentration of cadmium in the liver, However, the effect of zinc may be related to the increase in the construction of stress proteins (MTs) and (hsp) heat shock proteins, to reduce the toxicity of cadmium [18].

CONCLUSIONS

Taking together all the results, zinc pre-treatment with increasing the concentration of the dose of cadmium used in the co-treatment, and both of them may have worked together to induce a significant increase in protein synthesis to exceed the high toxicity of cadmium, by inducing an increase in MT protein synthesis and may enhancing the turnover rate of the liver to the kidneys Cd-MT complex.

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