Original article

The effect of N-acetylcysteine on the levels of copper, zinc and expression of matrix metalloproteinases in the liver of rats exposed to cadmium

M.M. Jaafarzadeh[1], N. Ranji[1*], E. Aboutaleb[2]

[1] Department of Biology, Faculty of Sciences, Rasht Branch,
Islamic Azad University, Lakan Blvd, Rasht, Iran. P.O.Box: 3516-41335
[2] Department of Pharmaceutics, School of Pharmacy,
Guilan University of Medical Sciences, Fuman-Saravan Rd, Rasht, Iran

Abstract

This study was conducted to consider the effect of cadmium (Cd) on the liver and serum levels of zinc (Zn) and copper (Cu), and the role of N-acetylcysteine (NAC) in preserving cells against Cd toxicity. Rats were randomly divided into five groups, including G1 (control), G2 (single dose of Cd), G3 (continuous administration of Cd), G4 (single dose of Cd + continuous administration of NAC), and G5 (continuous administration of Cd + continuous administration of NAC). Rats in G2 and G4 groups were exposed with single dose of Cd on the first day of study. Continuous administration of Cd and NAC was used every day for 4 weeks. Levels of Zn and Cu were measured by atomic absorption spectroscopy. Expression of matrix metalloproteinases-2 (*MMP2)* and *MMP9* genes was evaluated using RT-PCR. The mean level of Cd in serum and liver tissue of G2 group increased significantly by about 26-27%, whereas in G3 group, it increased significantly by about 50-60%. While NAC treatment significantly raised Zn and Cu values, Cd levels significantly decreased in the serum and tissue samples of rats exposed to single or continuous Cd. Exposure to single and continuous administration of Cd caused a significant increase in *MMP2* expression by 10.14-fold (*P*=0.016) and 27.61-fold (*P<0.001*), respectively. Single and continuous administration of Cd led to a significant increase in *MMP9* expression by 3.63-fold (*P*=0.046) and 43.12-fold (*P<0.001*), respectively. NAC treatments decreased the expression of *MMP2* and *MMP9* in rats exposed to single or continuous Cd. Cd exposure was strongly associated with Zn and Cu depletion, and overexpression of *MMP2* and *MMP9*. NAC can protect the liver against Cd toxicity by elevating Zn and Cu contents and down-regulating proteolytic enzymes.

Key words: cadmium, copper, N-acetylcysteine, MMP2, MMP9, zinc

Correspondence to: N. Ranji, e-mail: n_ranji@iaurasht.ac.ir, tel.: +981333424080

Introduction

Cadmium (Cd) is a toxic and heavy metal extremely widespread in our environment. As it can be found in our surrounding area, it has now become a major public health problem worldwide (Godt et al. 2006, Satarug et al. 2010). Human exposure to Cd increases the risk for multiple disorders such as pulmonary diseases, cardiovascular disease, lung and hepatic cancers, neurotoxicity, and diabetes (Fomenko et al. 2017). Nevertheless, the exact cellular and molecular mechanisms in which Cd induces these abnormalities are unclear. Recent studies have proposed that Cd induces cellular toxicity through multiple mechanisms, especially by inhibiting enzymes involved in cellular energy metabolism, DNA synthesis and repair (Giaginis et al. 2006, Skipper et al. 2016). Additionally, oxidative stress and inflammation induced by reactive oxygen species (ROS) are likely the main mechanisms of Cd toxicity (Liu et al. 2009, Nair et al. 2013). However, ROS can directly interact with DNA, proteins and lipids, and cause severe damages, especially DNA breaks or mutations and consequently cell death and apoptosis (Zhou et al. 2013). Exposure to Cd may be associated with the production of different ROS such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot}$), and hydroxyl radicals ($OH^{\cdot}$) (Thijssen et al. 2007). Previous studies reported that chronic exposure to Cd can be associated with overproduction of ROS (Liu et al. 2009), lipid peroxidation (Bagchi et al. 1997), and depletion of antioxidants such as glutathione and Zinc (Dudley and Klaassen 1984). It has been shown to be associated with an increase in proinflammatory cytokines such as interleukin-6 (IL-6) and IL-8 (Jiaxin et al. 2020). Increased expression or activity of matrix metalloproteinases (MMPs) is another possible mechanism of chronic exposure to Cd (Fomenko et al. 2017). MMPs are a group of zinc-dependent endopeptidase enzymes that are responsible for the degradation of most extracellular matrix (ECM) proteins (Sorsa et al. 2004). They are also involved in multiple processes such as embryogenesis, bone growth, wound healing or tissue repair, as well as regeneration, growth, migration, differentiation, inflammatory processes and apoptosis (Jablonska-Trypuc et al. 2016). Although MMPs have a wide range of physiological and pathological functions, their expression pattern must be maintained at a constant low level (Lu et al. 2008).

Since overproduction of Cd-induced ROS and oxidative stress are likely a main reason for overexpression of MMPs, antioxidant therapy may protect tissues against Cd-induced damages. Therefore, it is expected that antioxidant therapy be helpful in mitigating the Cd toxicity in exposed individuals. N-acetylcysteine (NAC) is an acetylated cysteine residue that protects cells against oxidative stress, toxic compounds, and inflammatory conditions (Dekhuijzen 2004, Kerksick and Willoughby 2005). Numerous studies demonstrated that NAC decreases heavy metal-induced toxicities. For example, recent studies have indicated that NAC can attenuate heavy metal-induced hepatotoxicity, renal and cerebral toxicity, DNA damage, oxidative stress, inflammation, and apoptosis (Reddy et al. 2011). Although several studies have evaluated heavy metal-induced toxicity in different tissues, less information is available about the anti-inflammatory and protective effects of NAC during and after Cd treatment of the liver tissue. We hypothesize that treatment with NAC may help maintain liver health by excreting toxic metabolites, improving antioxidants such as zinc and copper, declining inflammation or oxidative stress and consequently MMPs expression. Therefore, the present study was designed to investigate the protective effect of NAC administration on histological alterations, contents of Zn and Cu, as well as inflammatory mediators (including *MMP2*, *MMP9*) in rats exposed to Cd.

Materials and Methods

Animals and treatments

Thirty male Wistar rats (with 8-10 weeks of age and a body weight of 150-200 g) were chosen from the laboratory animal research center at Pasteur Institute of Iran (Tehran). This experimental study was approved by the animal care and use committee at the Islamic Azad University, Rasht branch (Ethics number: IR.IAU.RASHT.REC.1398.008). All rats were adapted to lab environment for one week and then randomly divided into 5 groups, including G1 (control), G2 (single dose of Cd on the first day of study), G3 (continuous administration of Cd every day for 4 weeks), G4 (single dose of Cd on the first day of study + continuous administration of NAC every day for 4 weeks), and G5 (continuous administration of Cd + continuous administration of NAC every day for 4 weeks). Rats in each group were housed 3 per cage (30 × 15 × 15 cm) in a room with standard climate (temperature of 22 ± 2°C, humidity of 50% ± 5%, and a 12:12 light/dark cycle) and had free access to food (10g/kg/day) and tap water.

We selected the Cd and NAC dosages and treatment periods according to previous studies (Grotena JP et al. 1991, Dickey DT et al. 2008, de Oliveira Filho LD et al. 2015, Andjelkovic M et al. 2019). In all experimental groups, NAC and Cd were administrated orally. Rats in G2 group received a single dose of Cd solution (80 mg/kg) on the first day of examination. Rats in G3 group received a continuous administration of Cd solu-

tion (2.5 mg/kg) every day for 4 weeks. Rats in G4 group were treated with a single dose of Cd (80 mg/kg) on the first day of examination and NAC (50 mg/kg) solutions every day for 4 weeks. Rats in G5 group received a continuous administration of Cd (2.5 mg/kg) and NAC (50 mg/kg) solutions every day for 4 weeks. The control group was fed with normal pellet and water for 4 weeks.

Liver tissues collection

Forty eight hours after the final treatment, rats were anesthetized by intraperitoneal administration of xylazine (3-5 mg/kg; Bioveta Sigma Aldrich; K4138, USA) and then ketamine (30-50 mg/kg; Sigma Aldrich; K4138, USA) in the laboratory of animal sciences at the Baqiyatallah University of Medical Sciences. The animals were sacrificed by CO_2 inhalation. For histological examinations, liver tissues were removed and fixed in 10% formalin for at least 48 hours. The specimens were dehydrated in graded series of ethanol, embedded in paraffin and sectioned using an automatic microtome at 4-5 mm thickness. For histological processing, the sectioned tissues were stained with haematoxylin--eosin (H&E) and examined for morphological and histological parameters by light microscopy. A specimen of liver tissue (~50-100 mg) was isolated and homogenized in phosphate buffer (with pH 7.0) at 4°C with homogenizer (Hielscher, UP100H). The homogenized tissue was centrifuged at 12000 rpm/4°C for 15 min (Ma et al. 2017). The supernatants were then collected and stored at -80°C for gene expression analysis.

Metal analysis

For metal analysis, liver tissues (~100 mg) were dried overnight at 75°C and then digested in approximately 10× dry tissue mass of nitric acid. The digested samples were diluted tenfold by deionized water. For the analysis of metals in serum, blood samples were centrifuged at 600×g for 10 min. After centrifugation, supernatants were diluted tenfold by deionized water. Eventually, levels of serum and liver tissue Zn, Cd and Cu were measured by atomic absorption spectroscopy (AAS; *Perkin Elmer model 2380*). For serum metal analysis, concentrations of Zn (1-50 mg/L), Cd (0.001-0.5 mg/L) and Cu (0.1-5 mg/L), and for tissue metal analysis, concentrations of Zn (50-150 μg), Cd (0.01-0.1 μg) and Cu (10-100 μg) were used to plot the standard curve.

Real-time PCR

RNX-Plus (SinaClon; RN7713C) Kit was used for total RNA extraction from homogenized liver tissues. A Nanodrop ND-1000 spectrophotometer (Thermo Sci., Newington, NH) was applied to consider the quantity and quality of extracted RNAs. Electrophoresis on 1% agarose gel was also performed to determine the quality of extracted RNAs. Revert Aid Reverse Transcriptase (Thermo science, Germ any) and random hexamer primers (Thermo science, Germ any) were used for cDNA synthesis at 42°C for 1 h. A Rotor Gene 6000 (Corbett Research, Australia) thermocycler in 40 cycles was applied for amplifications. Each reaction included 5 μl master mix and 100 nM primers. Primer sequences were as follows: *MMP2*, 5'-CCGTCGCCCATCAT CAAGT-3' (forward), 5'-GCAGCCATAGAAAGTGTT CAGGT-3' (reverse); *MMP9*, 5'-ACCACCGCCAACT ATGACCAG-3' (forward), 5'-TGCTTGCCCAGGAA GACGA-3' (reverse); and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), 5'-AAGTTCAACGGCACA GTCAAGG-3' (forward); 5'-CATACTCAGCACCAG CATCACC-3'(reverse). The levels of mRNA were normalized relative to the amount of *GAPDH* mRNA. The relative expression of studied genes was calculated using $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data are presented as means ±SD. One-Way ANOVA with Post Hoc-Tukey test was used to compare the mean of all data between groups. Data were analyzed using SPSS software (version 19). $p<0.05$ was considered as significant.

Results

Histopathological examination of the liver tissue in each group revealed that there were no abnormalities in the control group (G1), while sections of the liver tissue from rats in G3 group showed increased blood in the central vein, elevated number of the inflammatory cells along with inflammation in the liver parenchyma and portal space. The sections of the liver from rats treated with NAC (G4 and G5) showed milder abnormalities compared to the rats in G2 and G3 groups. Combined therapy with NAC decreased the number of inflammatory cells along with mild inflammation in the liver of rats exposed to Cd (Fig. 1).

Comparison of mean levels of Zn and Cu in the liver tissue of rats in all groups can be seen in Fig. 2. Cd significantly affected the liver tissue levels of Zn and Cu. Rats exposed to continuous administration of Cd had significantly lower mean levels of Zn (55.83±5.98 μg/g tissue) and Cu (26.83±3.37 μg/g tissue) compared to the other groups. Furthermore, single dose treatment of Cd caused a significant decrease in the mean of Zn (91.50±6.28 μg/g tissue) and Cu (43.5±6.65 μg/g tissue) values in the liver tissue compared to the control
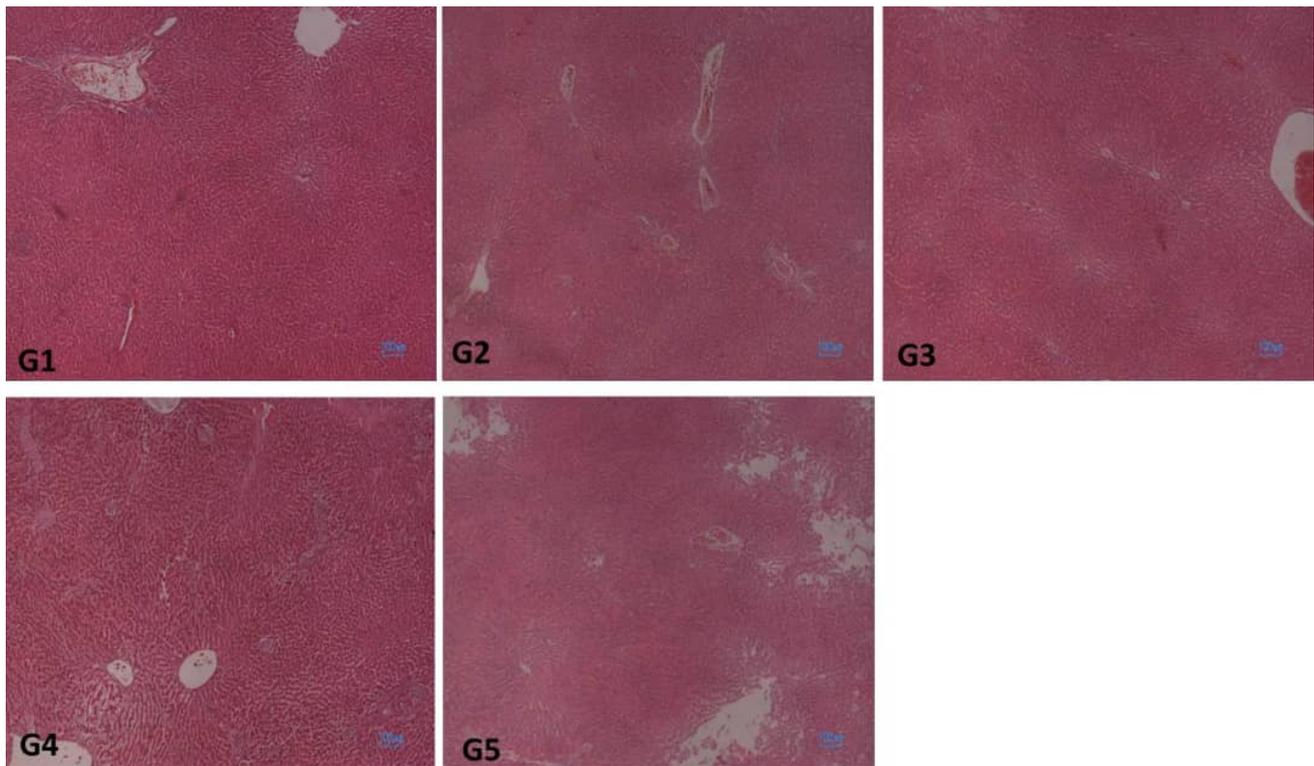
Fig 1. Sections of the liver tissue from different groups. The liver tissue of rats in the control (G1) group was has normal in structure, while sections from rats in continuous group (G3) showed increased blood flow in the central vein, and elevated number of inflammatory cells. Combined therapy with N-acetylcysteine (NAC) resulted in a decrease in the number of inflammatory cells of rats along with mild inflammation in Cd exposed groups (G4 and G5). x20.

group. Interestingly, NAC treatments significantly increased Zn and Cu values in rats exposed to either single dose or continuous administration of Cd. The mean level of Zn and Cu in rats exposed to single dose of Cd + NAC (119.83±11.47 µg/g tissue and 75.16±5.52 µg/g tissue, respectively) and continuous administration of Cd + NAC (85.0±8.98 µg/g tissue and 49.83±8.65 µg/g tissue, respectively) was significantly greater than the ones which only received single dose and continuous administration of Cd (Fig. 2; p<0.05). There was no significant difference in mean level of Cu in the liver tissue of rats in control and single dose of Cd + NAC groups (81.66±5.68 µg/g tissue vs 75.16±5.52 µg/g tissue, respectively).

Comparison of the mean serum levels of Zn and Cu between each group is shown in Fig. 3. Similarly, Cd significantly decreased the serum levels of Zn and Cu in exposed rats. Rats treated with continuous administration of Cd had significantly lower mean levels of Zn (11.16±1.83 mg/L) and Cu (0.70±0.26 mg/L) compared to the other groups. Single exposure to Cd led to a significant reduction in mean serum values of Zn (19.66±2.06 mg/L) and Cu (1.63±0.30 mg/L) compared to those in the control group. NAC treatments significantly increased serum Zn and Cu contents in rats exposed to single dose or continuous administration of the Cd. Mean serum levels of Cu and Zn in rats that

received continuous administration of Cd + NAC (1.99±0.53 mg/L and 20.83±2.99 mg/L, respectively) was significantly greater than in those which receiving continuous administration of Cd (Fig.3; p<0.05). Additionally, a trend for higher levels of serum Cu (p=0.091) and Zn (p<0.05) was observed in rats treated with a single dose of Cd + NAC (2.81±0.47 mg/L and 27.83±1.94 mg/L, respectively) compared to those which were treated with a single dose of Cd (Fig. 3). There was no significant difference in mean serum levels of Zn and Cu between control (30.50±3.93 mg/L and 3.58±0.87 mg/L, respectively) and groups receiving a single dose of Cd + NAC .

The mean levels of Cd in serum and liver tissue of single dose Cd-treated rats significantly increased by about 26-27%, whereas the mean values of Cd in serum and liver tissue of rats exposed to continuous administration of Cd significantly increased by about 50-60% compared to controls (Fig. 4, Fig. 5). The mean levels of Cd in the blood and liver tissue of rats exposed to a single dose of Cd were 0.27±0.045 mg/L and 0.41±0.045 mg/g tissue, respectively, while the mean contents of Cd in the blood and liver tissue of rats exposed to continuous administration of Cd were 0.50±0.05 mg/L and 0.74±0.088 mg/g tissue, respectively (p<0.001; Fig. 4 and Fig. 5). NAC treatments significantly decreased Cd levels in serum and tissue sam-
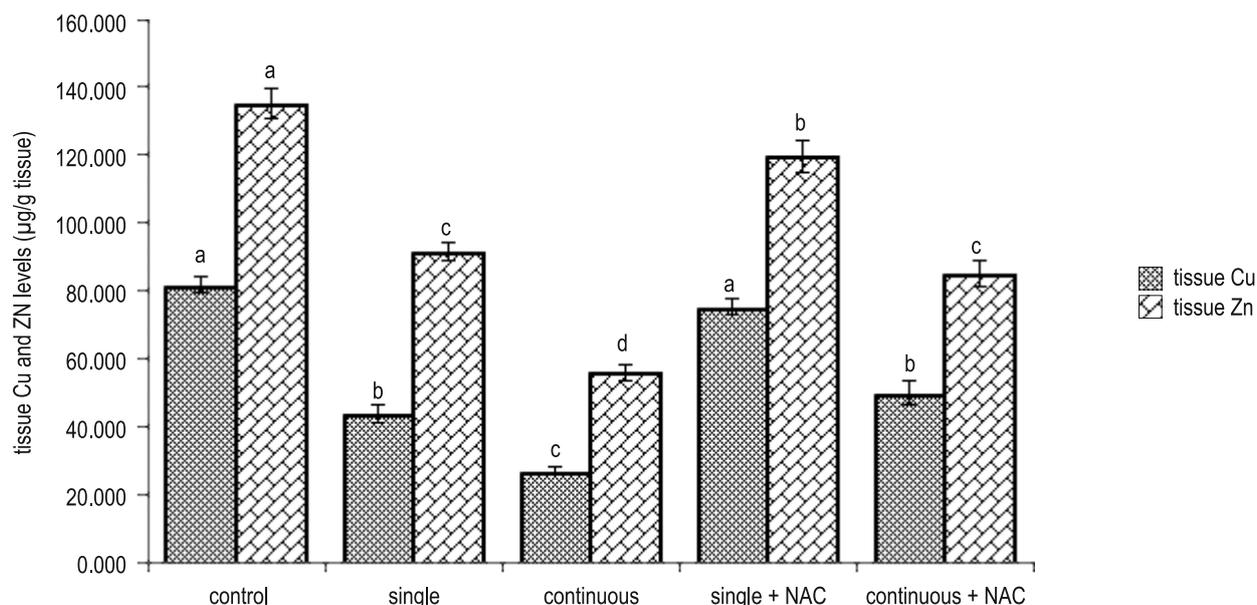
Fig 2. Comparison of the mean Zn and Cu values in the liver tissue of rats in different groups. Data with different superscript letters denote significant differences. One-Way ANOVA: Post Hoc-Tukey test was applied to compare mean value of these elements between all the groups. The mean Cu and Zn serum levels was in the order of a>b>c>d.
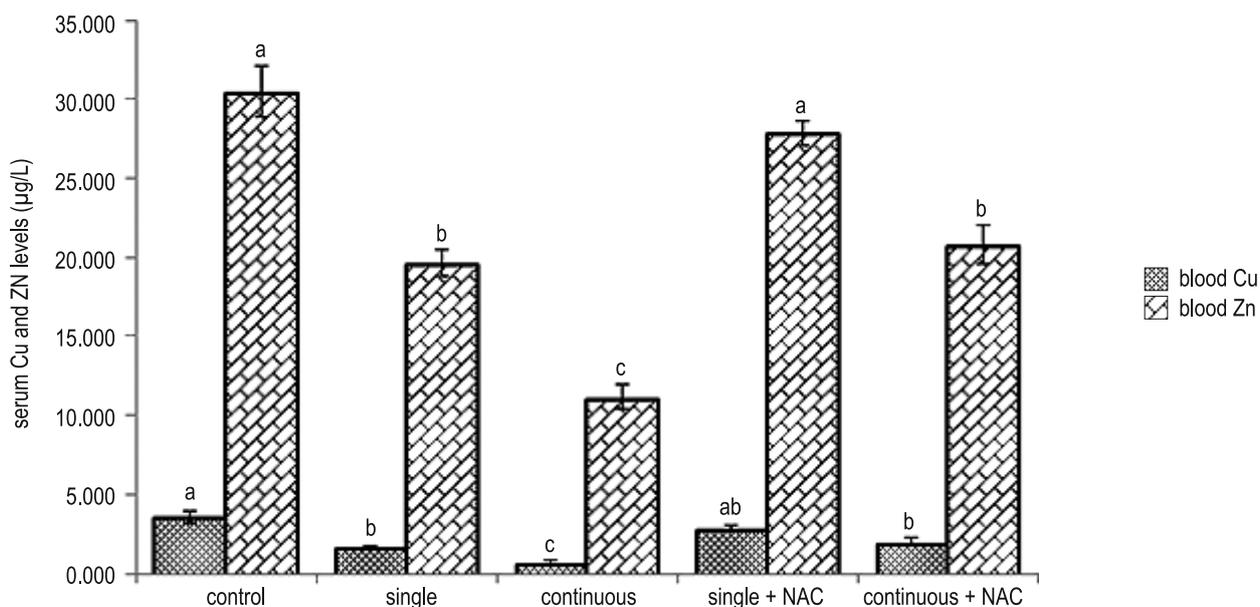

Fig 3. Comparison of the mean Cu and Zn serum levels in rats in different groups. Data with different superscript letters denote significant differences. One-Way ANOVA: Post Hoc-Tukey test was applied to compare mean values of Cu and Zn between all the groups. The mean Cu and Zn serum levels was in the order of a>ab>b>c.

ples of rats exposed to a single dose or continuous administration of Cd. The mean concentration of Cd in the liver and serum of rats treated with a single dose of Cd + continuous administration of NAC (0.19 ± 0.025 mg/g tissue and 0.0079 ± 0.0012 mg/L, respectively) was relatively similar to that in controls. Although the mean levels of Cd in the liver tissue of rats treated with continuous administration of Cd + NAC (0.36 ± 0.075 mg/g tissue) were still higher compared to those in the control group, there was no significant difference in the mean content of blood Cd between both groups.

A significant difference was found in the expression pattern of *MMP2* and *MMP9* genes between groups ($p<0.001$). Continuous or single dose treatment with Cd caused a significant increase in the expression of *MMP2* and *MMP9* in the exposed animals (Fig. 6). NAC treatment significantly decreased the expression of these genes. Compared to the control group, single and continuous administration of Cd caused a significant increase in *MMP2* expression by 10.14-fold ($P=0.016$) and 27.61-fold ($p<0.001$), respectively. Moreover, single dose and continuous administration
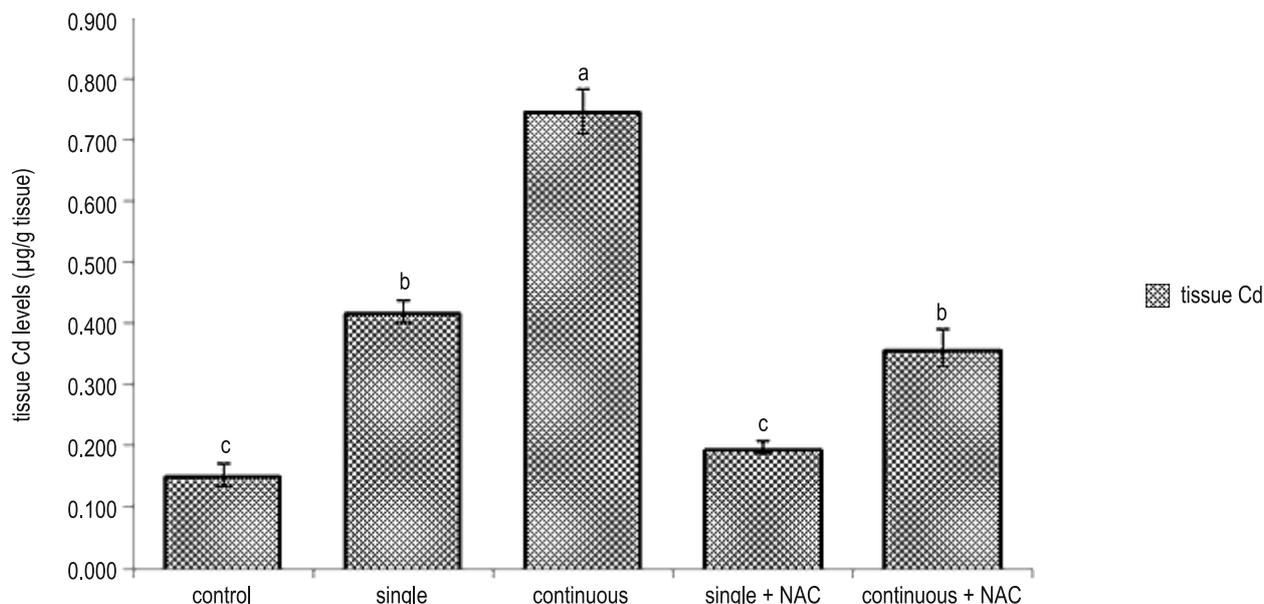
Fig 4. Comparison of mean Cd levels in the liver tissue of rats in different groups. Data with different superscript letters denote significant differences. One-Way ANOVA: Post Hoc-Tukey test was applied to compare mean values of Cd between all groups. The mean Cd level was in the order of a>b>c.
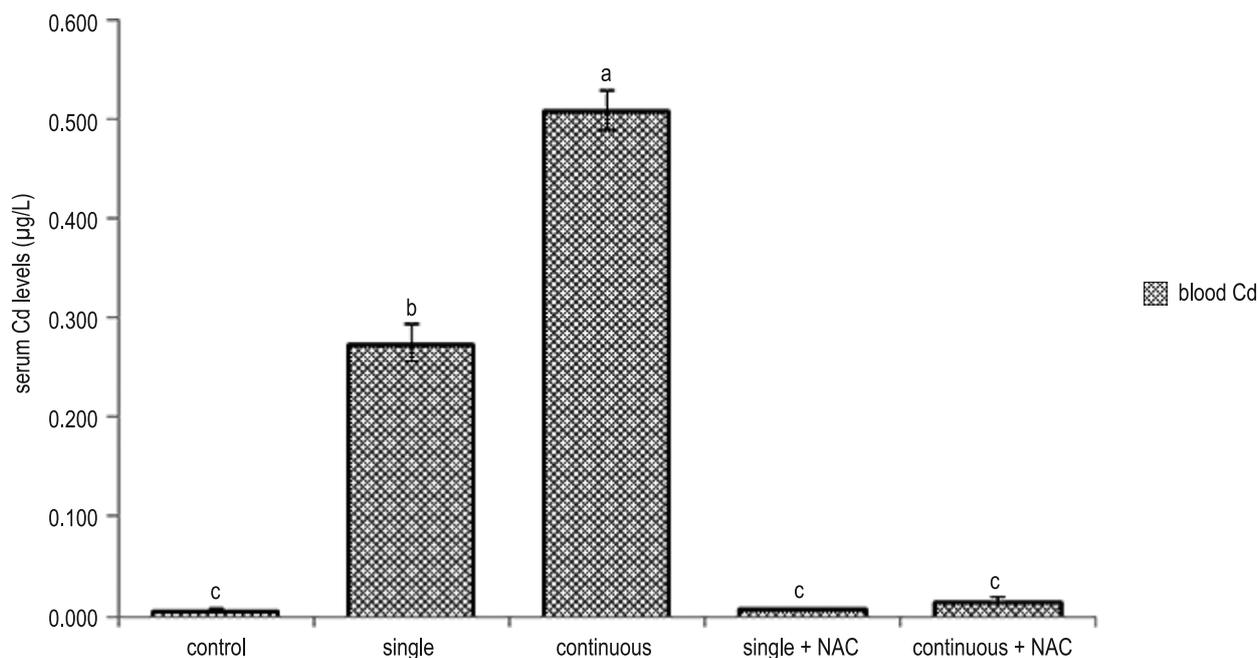

Fig 5. Comparison of the mean Cd Serum levels in rats in different groups. Data with different superscript letters denote significant differences. One-Way ANOVA: Post Hoc-Tukey test was applied to compare mean values of Cd between all the groups. The mean Cd level was in the order of a>b>c.

of Cd led to a significant increase in *MMP9* expression by 3.63-fold (p=0.046) and 43.12-fold (p<0.001), respectively. In contrast, rats exposed to a combination of NAC and Cd showed a significant decrease in *MMPs* expression compared with the animals treated with a single dose or continuous administration of Cd alone. *MMP2* and *MMP9* expression in rats treated with a single dose of Cd + NAC significantly decreased by 5.60-fold (p=0.033) and 6.32-fold (p=0.039),

respectively, compared to rats treated with a single dose of Cd. Additionally, *MMP2* and *MMP9* expression in rats exposed to continuous administration of Cd + NAC was significantly decreased by 3.12-fold (p=0.014) and 5.55-fold (p=0.023), respectively, compared to rats which received continuous administration of Cd.
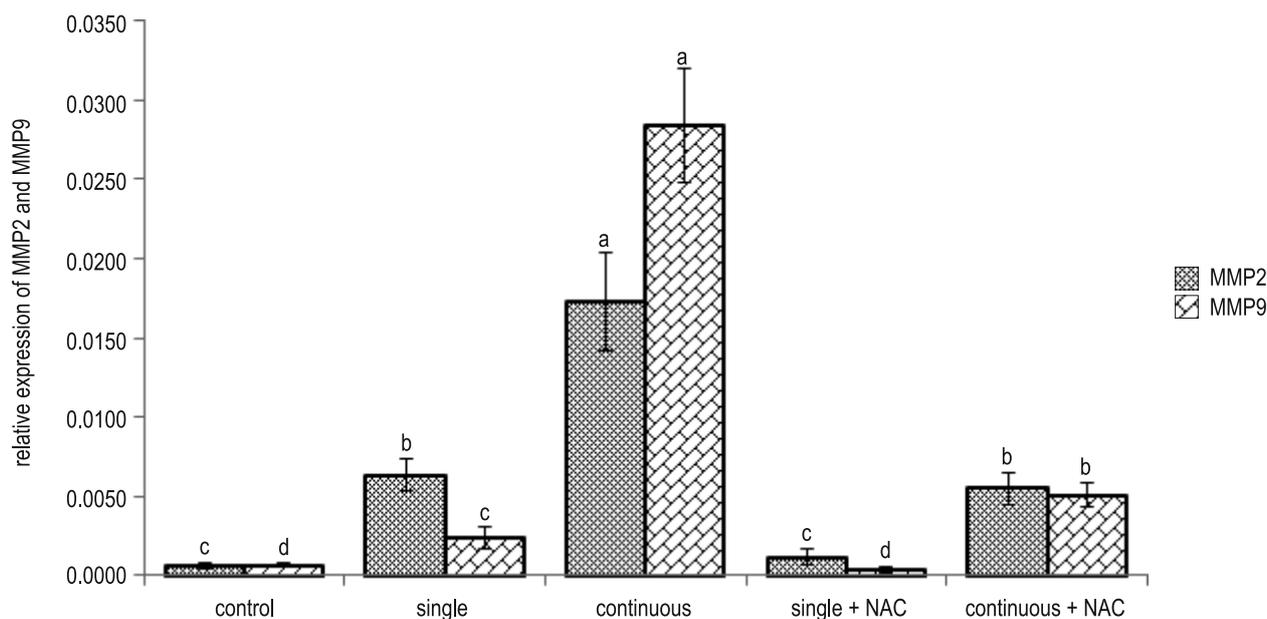
Fig 6. Comparison of the mean mRNA levels of *MMP2* and *MMP9*. The gene expression was determined by Real-Time PCR. Data with different superscript letters denote significant differences. One-Way ANOVA: Post Hoc-Tukey test was applied to compare mean values of *MMP2* and *MMP9* expression pattern between all the groups. The mean mRNA level of *MMP2* and *MMP9* was in the order of a>b>c>d.

Discussion

Our results have demonstrated that Cd exposure, especially the chronic exposure, is significantly associated with depletion of Zn and Cu contents in the serum and liver tissue of exposed rats, while the mean level of liver and blood Cd is significantly increased. These data implicate that accumulation of Cd in the liver tissue is the main mechanism of its toxicity during the chronic phase of injury. We also found that Cd administration, especially the continuous exposure, significantly results in overexpression of *MMP2* and *MMP9* genes in the liver tissue of exposed rats. Our findings support the idea that toxicological effect of Cd on the liver tissue is probably mediated via the depletion of Zn and Cu contents and overexpression of proteinases.

Several lines of studies have demonstrated that Cd exposure decreases the mean level of Zn and Cu in different tissues. For example, Prozialeck et al. (2016) demonstrated that Cd treatment (0.6 mg/kg/BW 5 days per week for 6, 9 or 12 weeks) caused a significant decrease in renal cortical Zn level, but Cu concentration was not significantly changed. In another study, Yaghooti et al. (2012) found that Cd exposure (50 μM) significantly increased *MMP9* expression in lung cells in a dose-dependent manner. Previous studies have reported that the activated resident macrophages in tissues in response to Cd are an important source for inflammatory mediators such as Interleukin-1 (IL-1), IL-6, Tumor necrosis factor-alpha (TNF-α), and IL-8,

which are the mediators of MMPs expression and activation (Sarkar and Yong et al. 2009). Fomenko et al. (2017) revealed that Cd in a higher dose (1 μg/kg for 37 days) caused a decrease in *MMP2* activity but increased that of pro-*MMP9* in the brain.

Given the fact that Cd exposure is associated with antioxidant depletion, inflammatory responses and oxidative stress, antioxidant therapy may be helpful in decreasing its pathological effects. In this study, we considered the protective effect of NAC on the levels of Zn and Cu, as well as on the expression of *MMP2* and *MMP9* genes in the liver of rats exposed to Cd for 4 weeks. A trend was observed toward decreased values of serum and liver tissue of Zn and Cu, in rats exposed to Cd. Additionally, Cd treatment induced liver tissue lesions and structural abnormalities. These data confirmed that the rats suffered from inflammation and proteolytic activity of *MMP2* and *MMP9* after long-term exposure to Cd. Nevertheless, the treatment with NAC mitigated the mean levels of Zn and Cu and decreased the expression of *MMP2* and *MMP9*. These findings suggest that NAC play an important role in protecting liver cell from injury induced by Cd.

Several lines of studies have indicated that NAC plays important role in preventing the harmful effects of heavy metals on different tissues such as the brain, kidney and liver (Tiwari and Rao 2010). For example, da Silva et al. (2016) reported that coadministration of NAC can ameliorate the harmful effects of arsenic on the male genital system. In another study, Reddy

et al. (2011) demonstrated that intraperitoneal injection of NAC increased the weights of reproductive organs, reduced arsenic-induced oxidative stress and improved steroidogenesis in arsenic-exposed mice, suggesting the beneficial role of NAC in counteracting arsenic--induced oxidative stress and in restoring suppressed reproduction in male mice. The anti-apoptotic effect of NAC was also revealed in previous studies (Han et al. 2008). For example, Sanker et al. (2015) have found that heavy metals like arsenic not only increase lipid peroxidation, GSH depletion and decrease the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GSR) in liver, and also increase the activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and cause histological alterations in liver indicating hepatotoxicity. They also showed that all these effects were attenuated with antioxidants such as curcumin treatment (100 mg/kg). These data suggest that antioxidant therapy can protect different organs against Cd toxicity by reducing the ROS generation, DNA damage, lipid peroxidation and increasing the levels of antioxidant activity (Biswas et al. 2010). Therefore, according to our findings and the previously accomplished data, we assume that oxidative stress induced by Cd may be the main mechanism of its toxicity, which is subsequently associated with DNA damage, fatty acid oxidation, cell damage and apoptosis. Cadmium may also damage mitochondria and consequently mitigate ROS overproduction, which is associated with apoptosis. Increased activity of proteases such as MMPs, by ROS and inflammatory mediators is another possible mechanism of Cd action, which causes detrimental effects on tissues. It appears that NAC not only scavenges Cd directly, but also prevents oxidative stress and inflammation (as two inducers of *MMPs* overexpression). Therefore, oxidative stress and inflammatory reactions serve as common mediators of Cd cytotoxicity in the liver tissue. On the other hand, NAC diminishes the genotoxic and cytotoxic effects of Cd.

Conclusion

In conclusion, the present study has revealed that Cd exposure, especially the chronic exposure, is strongly associated with depletion of Zn and Cu levels, and over-expression of *MMP2* and *MMP9* genes in the liver tissue. NAC can help to protect the liver tissue against Cd toxicity by mitigating Zn and Cu capacity and down-regulation of *MMP2* and *MMP9* genes.

References

Andjelkovic M, Djordjevic AB, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z **(2019)** Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int J Environ Res Public Health 16: 1-21.

Bagchi D, Vuchetich PJ, Bagchi M, Hassoun EA, Tran MX, Tang L, Stohs SJ **(1997)** Induction of oxidative stress by chronic administration of sodium dichromate [Chromium VI] and cadmium chloride [Cadmium II] to rats. Free Radic Biol Med 22: 471-478.

Biswas J, Sinha D, Mukherjee S, Roy S, Siddiqi M, Roy M **(2010)** Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal. Hum Exp Toxicol 29: 513-24.

da Silva RF, Borges CS, Missassi G, Kiguti LR., Pupo AS, Barbosa JF, Anselmo-Franci JA, Kempinas WG **(2016)** The coadministration of N-Acetylcysteine ameliorates the effects of arsenic trioxide on the male mouse genital system. Oxid Med Cell Longev 2016: 4257498.

Dekhuijzen PN, **(2004)** Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. Eur Respir J 23: 629-636.

Dickey DT, Muldoon LL, Doolittle ND, Peterson DR, Kraemer DF, Neuwelt EA **(2008)** Effect of N-acetylcysteine route of administration on chemoprotection against cisplatin-induced toxicity in rat models. Cancer Chemother Pharmacol 62: 235-241.

de Oliveira Filho LD, Ruggeri Saad K, Fernandes Saad P, Kiyomi Koike M, Maria da Silva S, de Souza Montero EF **(2015)** Effect of N-acetylcysteine in hearts of rats submitted to controlled hemorrhagic shock. Rev Bras Cir Cardiovasc 30: 173-181.

Dudley RE, Klaassen CD **(1984)** Changes in hepatic glutathione concentration modify cadmium-induced hepatotoxicity. Toxicol Appl Pharmacol 72: 530-538.

Fomenko O, Shiyntum H, Shaulska O, Shevtsova A, Ushakova G **(2017)** Effects of Cadmium on the Activity of Matrix Metalloproteinases and Metallothionein Level in the Rat Brain. Neurophysiology 49: 154-157.

Giaginis C, Gatzidou E, Theocharis S **(2006)** DNA repair systems as targets of cadmium toxicity. Toxicol Appl Pharmacol 213: 282-290.

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, Groneberg DA **(2006)** The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 1: 22.

Grotena JP, Sinkeldam EJ, Luten JB, Bladeren PJ **(1991)**

Cadmium accumulation and metallothionein concentrations after 4-week dietary exposure to cadmium chloride or cadmium-metallothionein in rats. Toxicol Appl Pharmacol 111: 504-513.

Han Y H, Kim S Z, Kim S H, Park W H **(2008)** Suppression of arsenic trioxide-induced apoptosis in HeLa cells by N-acetylcysteine. Mol Cells 26: 18-25.

Jablonska-Trypuc A, Matejczyk M, Rosochacki S **(2016)** Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem 31(Supp1): 177-183.

Jiaxin S, Shengchen W, Yirong C, Shuting W, Shu L **(2020)** Cadmium exposure induces apoptosis, inflammation and immunosuppression through CYPs activation and antioxidant dysfunction in common carp neutrophils. Fish Shellfish Immunol 99: 284-290.

Kerksick C, Willoughby D **(2005)** The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 2: 38-44.

Liu J, Qu W, Kadiiska MB **(2009)** Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238: 209-214.

Lu L, Zhang Q, Pu LJ, Peng WH, Yan XX, Wang LJ, Chen QJ, Zhu Z B, Michel J B, Shen W F **(2008)** Dysregulation of matrix metalloproteinases and their tissue inhibitors is related to abnormality of left ventricular geometry and function in streptozotocin-induced diabetic minipigs. Int J Exp Pathol 89: 125-137.

Ma Z, Chu L, Liu H, Wang W, Li J, Yao W, Yi J, Gao Y **(2017)** Beneficial effects of paeoniflorin on non-alcoholic fatty liver disease induced by high-fat diet in rats. Sci Rep 7: 44819.

Nair AR, DeGheselle O, Smeets K, Van Kerkhove E, Cuypers A **(2013)** Cadmium-induced pathologies: where is the oxidative balance lost (or Not)? Int J Mol Sci 14: 6116-6143.

Prozialeck WC, Lamar PC, Edwards JR **(2016)** Effects of sub-chronic Cd exposure on levels of copper, selenium, zinc, iron and other essential metals in rat renal cortex. Toxicol Rep 3: 740-746.

Reddy P S, Rani G P, Sainath S B, Meena R, Supriya C **(2011)** Protective effects of N-acetylcysteine against arsenic--induced oxidative stress and reprotoxicity in male mice. J Trace Elem Med Biol 25: 247-253.

Sankar P, Gopal Telang A, Kalaivanan R, Karunakaran V, Manikam K, Sarkar S N **(2015)** Effects of nanoparticle--encapsulated curcumin on arsenic-induced liver toxicity in rats. Environ Toxicol 30: 628-637.

Sarkar S, Yong VW **(2009)** Inflammatory cytokine modulation of matrix metalloproteinase expression and invasiveness of glioma cells in a 3-dimensional collagen matrix. J Neurooncol 91: 157-164.

Satarug S, Garrett SH, Sens MA, Sens DA **(2010)** Cadmium, environmental exposure, and health outcomes. Environ Health Perspect 118: 182-190.

Skipper A, Sims JN, Yedjou C, Tchounwou PB **(2016)** Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. Int J Environ Res Public Health 13: 88.

Sorsa T, Tjaderhane L, Salo T **(2004)** Matrix metalloproteinases (MMPs) in oral diseases. Oral Dis 10: 311-318.

Thijssen S, Cuypers A, Maringwa J, Smeets K, Horemans N, Lambrichts I, Van Kerkhove E **(2007)** Low cadmium exposure triggers a biphasic oxidative stress response in mice kidneys. Toxicology 236: 29-41.

Tiwari H, Rao M V **(2010)** Curcumin supplementation protects from genotoxic effects of arsenic and fluoride. Food Chem Toxicol 48: 1234-1238.

Yaghooti H, Firoozrai M, Khorramizadeh MR **(2012)** Acute cadmium exposure augments MMP-9 secretion and disturbs MMP-9/TIMP-1 balance. Asian Biomedicine, 6: 445-451.

Zhou Z, Wang C, Liu H, Huang Q, Wang M, Lei Y **(2013)** Cadmium induced cell apoptosis, DNA damage, decreased DNA repair capacity, and genomic instability during malignant transformation of human bronchial epithelial cells. Int J Med Sci 10: 1485-1496.