

PROTECTIVE EFFECT OF SOME CHELATING AGENTS AND ANTIOXIDANTS ON THE BIOHAZARDS PRODUCED FROM WATER POLLUTION BY HEAVY METALS IN WISTAR RATS: BIOLOGICAL, GENETIC AND HISTOPATHOLOGICAL STUDY

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**Abstract**

**Background:** Heavy metals that normally cause problems are mercury (HgCl<sub>2</sub>) and lead acetate (LA). Chelating and inhibitor agents are the target to treat and overcome metal toxicity. The current study has been carried out to evaluate the protective effects of N-acetyl cysteine (NAC) and meso 2,3 dimercaptosuccinic acid (DMSA) against HgCl<sub>2</sub> and LA toxicity.

**Materials and Methods:** Ninety male Wistar rats were divided into nine equal groups. The groups were administered NAC and/or DMSA in presence or absence of LA (LA; 0.2% in drinking water) or HgCl<sub>2</sub> (2 mg/kg BW) for 2 consecutive months. Serum and organs were collected for biochemical, genetic and histopathological changes.

**Results:** Biochemical results revealed that LA and HgCl<sub>2</sub> significantly increased the levels of liver and kidney biomarkers. Administration of NAC and DMSA considerably improved these altered changes. LA and HgCl<sub>2</sub> decreased serum levels of antioxidants and were ameliorated in NAC and DMSA administered rats. LA and HgCl<sub>2</sub> administration upregulated expression of IL-1 $\beta$  and IL-8 that were normalized by NAC and DMSA. Kidneys of LA and HgCl<sub>2</sub> groups showed intraluminal hyaline casts. Kidneys of DMSA-administrated rats showed mild hydropic degeneration of renal tubular epithelium in LA and HgCl<sub>2</sub> groups. Kidneys of NAC administrated rats showed atrophy of capillary tufts. Kidneys of LA and HgCl<sub>2</sub> administered rats which received DMSA and NAC showed normal glomerular structure. Liver histopathology showed sever changes that were ameliorated by NAC and DMSA.

**Conclusion:** Taken together, usage of NAC and DMSA provide significant protection against LA and HgCl<sub>2</sub>-induced hepatotoxicity and nephrotoxicity in male Wistar rats.

**Keywords:** Lead, Mercury, Histopathology, DMSA, n-acetyl cysteine, gene expression.

**Introduction**

Metal toxicity occurs because of essential metal overload or exposure to heavy metals from different sources. Most metals are capable of forming covalent bonds with carbon, forming metal-organic compounds. Metals and metal compounds interfere with functions of varied organ such as central nervous system, liver, kidneys and heart (Swaran & Vidhu, 2010). Metal poisoning may be acute, sub-acute or chronic. Typically, acute poisoning is well defined with serious and fast manifestations that will be recovered by immediate medical attention. Moreover, the chronic toxicities are also reversible or irreversible inflicting development of symptoms like cancer or teratogenesis (Swaran & Vidhu, 2010).

The most common types of toxicity in humans are lead and mercury toxicity. Children are more vulnerable to lead exposure than adults because of the frequency of hand-to-mouth activity, and a higher rate of intestinal absorption and retention. Blood lead has been reported to impair normal metabolic pathways in children at very low levels

(Finkelstein et al., 1998). Lead (Pb) binds to sulfhydryl and amide group components of enzymes diminishing their activities. It's going to additionally contend with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations as calcium (Flora et al., 2007). Lead produces a range of effects, totally on the vascular system, nervous system, liver and kidneys (Jaffe, 1995). Oxidative stress has been involved as a pathologic condition in lead toxicity. Lead causes oxidative stress by inducement of the generation of reactive oxygen species (ROS) and weakening the antioxidant defense system (Flora, 2002; Ercal et al., 2001). Patients heavily exposed to lead toxicity should be removed from the site of positioning and chelation therapy should be administered (Klaassen, 2006).

Mercury (Hg) is a naturally occurring component of the Earth's crust and is found in many chemical and physical forms. In recent years, elemental mercury has evidenced to be a possible supply of toxicity in kids (Amler, 2002; Risher et al., 2003). Elemental mercury is often made from dental amalgam restorations (Patterson et al., 1985) and might then be born-again into inorganic mercury in the body which may accumulate in the brain (Bjorkman, 2007). Exposure to high levels of metallic mercury may result in neurologic, respiratory, renal, hepatic, immunologic, dermatologic, and a variety of different effects (Goyer & Clarkson, 2001). Mercury toxicity induces molecular interactions with sulfhydryl groups on various molecules as GSH, metallothionein and albumin (Geier et al., 2007). The skeletal muscle is a crucial deposit for mercury (Gonzalez et al., 2005). However, it has a more neurotoxic result (Sarafian et al., 1996). It's been shown that uses of some clearing agents are with success tested for treatment of mercurial compounds toxicity (Blanusa et al., 2005).

Chelating agents are organic or inorganic compounds that connect metal ions to make complicated ring-like structure referred to as chelates. These chelating agents have ligand binding atoms that type either two covalent linkages or one covalent and one co-ordinate or two co-ordinate linkages (Gonzalez-Ramirez et al., 1998). Metal toxicity is tormented by chelating agents by mobilizing the toxic metal to go by urine. A chelating agent forming a stable complicated with a toxicant metal can shield biological targets from the metal particle, thereby reducing the local toxicity (Andersen, 1999). Historically, chelation therapy has been used to reduce the body burden of toxic metals in highly symptomatic patients with elevated biological markers (Fournier et al., 1988). Calcium disodium ethylene diamine tetra acetic acid (CaNa<sub>2</sub>EDTA) and alternative new known materials are identified as thiol-containing chelating agents such as meso 2,3 dimercaptosuccinic acid (DMSA) are the most frequently used chelating agent for its safe and less side effects (Klaassen, 2006; Guldager et al., 1996; Alcaraz-Contreras et al., 2016).

Combinational therapies with antioxidants like N-acetyl cysteine (NAC) have shown considerable promise in improving clinical recoveries in animal models (Flora et al., 2004). NAC is derived from the amino acid L-cysteine, and is a precursor in the formation of the antioxidant glutathione in the body. NAC is known to have metal chelating properties and has been used in numerous clinical conditions (Banner et al., 1986). Thiol groups found in NAC act to supply chelating sites for metals and reduce free radicals. Combined administration of NAC and succimer post arsenic exposure led to a noteworthy turnover in the variables indicative of oxidative stress and elimination of toxic metal from the soft organs (Flora, 1999). Because of the dangerous effects of toxicity by heavy metals, we need to recognize and protect our health from the threats induced by such toxicity. Therefore, this study is outlined to examine the protective effects of some chelating agents such as NAC and DMSA on the biohazards induced by lead and mercury toxicity at the biochemical, molecular and histopathological levels.

## **Materials and Methods**

### **Animals and experimental design**

All animal procedures were approved by the Ethical Committee Office of the Scientific Dean of Taif University, Saudi Arabia for project # 4790-437-1. Rats were purchased from King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Ninety male Wistar rats, 3 months old (250–280 g) were used for this study. Animals were kept under observation for one week, for acclimatization. The animals were kept at 12:12-h light/dark cycle and gained free access for water and food. Healthy rats were randomly divided into nine equal groups as follows: first group was used as a control. Group 2 administered lead acetate (LA; 0.2% in drinking water) daily for 2 months, Group 3 administered LA and NAC, Group 4 administered LA and DMSA, Group 5 administered LA plus NAC and DMSA, Group 6 administered mercuric chloride (HgCl<sub>2</sub>) in a dose 2 mg/kg BW orally daily for 2 months, Group 7 administered HgCl<sub>2</sub> and NAC, Group 8 administered HgCl<sub>2</sub> and DMSA, Group 9 administered HgCl<sub>2</sub> plus NAC DMSA. The doses used in this study were used based on previous studies (Sivaprasad et al., 2003; Sharma et al., 2015; Rao et al., 2010). The dose of NAC was 50 mg/kg BW orally and DMSA was 20 mg/kg BW intraperitoneally. All animals were sacrificed after anesthetization by diethyl ether inhalation. Tissues from the livers and kidneys were collected from slaughtered rats. Serum was extracted after blood centrifugation for 10 min at 5000 rpm. Liver and kidney tissues were preserved in Qiazol reagent at -80 °C for gene expression, and in 10 % neutral buffered formalin (NBF) at room temperature for histopathological examination.

### **Chemistry assessments**

Kidney (creatinine and urea) and liver (GPT and GOT) biomarkers were measured calorimetrically using commercial available. Superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and catalase were measured spectrophotometrically in the laboratory of faculty of veterinary medicine Benha University using commercial ELISA kits based on manufacture instruction manual. All kits were purchased from Biodiagnostic Co, Dokki, Giza, Egypt.

## RNA extraction, cDNA synthesis and PCR analysis

RNA from liver and kidney tissues (50 mg per sample) was extracted based on protocol designed by Soliman et al (2015). Tissue samples were homogenized in polytron (Brinkman Instruments, Westbury, NY) in 1 ml Qiazol (Valencia, CA, USA). Using chloroform and isopropanol, RNA was pelleted, then washed with 70% ethanol then dried and dissolved in Diethylpyrocarbonate (DEPC) water. RNA purity and concentration were calculated spectrophotometrically at 260/280 nm. For reverse transcription, a mixture of 2 µg total RNA and 0.5 ng oligo dT primer were incubated in 11 µl sterilized DEPC water and run in the PeX 0.5 thermal Cycler at 70°C for 5 min for early denaturation. Using 5X RT-buffer (4 µl), 10 mM dNTPs (2 µl) and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase in a total volume of 20 µl, reverse transcription was carried. To complete reverse transcription, the mixture was re-incubated in the thermal Cycler at 37°C for 1h, then at 90°C for 10 min. Specific primers for used genes were designed using Oligo-4 computer program, synthesized and ordered by Macrogen (Macrogen Company, GAsa-dong, and Geumcheon-gu. Korea). PCR was conducted as stated in table 1, using specific primers, 1 ul cDNA as template and 12.5 µl PCR master mix (Promega Corporation, Madison, WI). The volume was adjusted to 25 µl using sterilized, deionized water. The cycle sequence of PCR reaction was carried out at 95 °C for 4 minutes one cycle, 28 cycles consisted of initial denaturation at 95 °C for a minute, annealing at the specific temperature (table1) and extension at 72 °C for a minute. Reaction was fulfilled with additional final extension at 72 °C for 7 minutes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as a reference for PCR quality and integrity. PCR products were run on 1.5% agarose (Bio Basic Inc., Markham, ON, Canada) in gel marked with ethidium bromide in TBE (Tris-Borate-EDTA) buffer. PCR products were detected using UV light and photographed using gel documentation system (Gel Doc-It Imaging system; UVP, LLC, Upland, CA, USA). The intensity of bands was quantified densitometrically using ImageJ software version 1.47 <http://imagej.en.softonic.com/>.

## Histopathological examination

Kidney and liver tissues samples were fixed in 10% NBF solution, washed in tap water, dehydrated through an upgraded series of ethanol, cleared by xylene and then embedded in paraffin. The paraffin-embedded samples were cut into 4 µm sections, which were then routinely stained with eosin and hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) consistent with Bancroft and Gamble (2008).

## Statistical analysis

Results and data are expressed as means that ± S.E. for ten totally different rats per group. Statistical examination was done using ANOVA and Fischer's post hoc check, with p<0.05 being take into account as statistically significant.

**Table 1:** PCR conditions and primer sequence for examined genes.

Gene	Product size (bp)	Annealing (°C)	Direction	Sequence (5'-3')
GPx	406	57	Sense	AAGGTGCTGCTCATTGAGAATG
			Antisense	CGTCTGGACCTACCAGGAACTT
GST	575	55	Sense	GCTGGAGTGGAGTTTGAAGAA
			Antisense	GTCCTGACCACGTCAACATAG
IL-1β	497	61	Sense	ATGGCAACCGTACCTGAACCCA
			Antisense	GCTCGAAAATGTCCCAGGAA
IL-8	308	56	Sense	CTCCAGCCACACTCCAACAGA
			Antisense	CACCCTAACACAAAACAGAT
α-2MG	325	56	Sense	GCTCCTGTCTGTTTCCTTAGTT
			Antisense	ATTGGCCTTTCGTGGTTTAG
AGP	230	55	Sense	GCTTTCCTCCTGACAACGCTG
			Antisense	GGCTTTTTGTTGTTTGCTTCTATTTT
GAPDH	309	52	Sense	AGATCCACAACGGATACATT
			Antisense	TCCCTCAAGATTGTCAGCAA

## Results

### Protective effects of NAC and DMSA on changes on Liver and Kidney Biomarkers

Lead acetate and mercuric chloride considerably amplified serum levels of hepatic transaminases (GPT and GOT), urea and creatinine. Administration of NAC and DMSA notably ameliorated changes in liver and kidney

biomarkers (Table 2). Co-administration of NAC with DMSA showed slight additive restoring on altered biomarkers compared to NAC and DMSA effect alone (Table 2).

### Protective effects of NAC and DMSA on changes in antioxidants levels induced by lead acetate and mercuric chloride toxicity

The serum levels of MD after lead acetate and mercuric chloride intoxication were significantly increased due to tissue destruction and decreased serum levels of GSH, catalase and SOD. Administration of NAC and DMSA notably improved the antioxidant activity and normalized it (Table 3).

### NAC and DMSA Protective effects on changes in mRNA expression of antioxidants and acute phase proteins induced by lead acetate and mercuric chloride toxicity

As seen in figure 1, administration of LA and HgCl<sub>2</sub> induced down regulation in the mRNA expression of liver GST and SOD. NAC and DMSA administration showed amelioration in down regulated genes. Additive ameliorative effect and normalization in GST and SOD expression was reported in LA and HgCl<sub>2</sub> groups administered both NAC and DMSA (Fig.1). Parallel to changes in antioxidants expression, both LA and HgCl<sub>2</sub> induced upregulation in mRNA expression of both  $\alpha$ 2-MG and  $\alpha$ 1-AGP (figure 2). Administration of NAC and DMSA prior to LA and HgCl<sub>2</sub> counteracted this upregulation and normalized it. Co-administration of NAC and DMSA induced additive anti-inflammatory and inhibitory effect for acute phase proteins expression.

**Table 2:** Protective effects of NAC and DMSA on changes in renal and hepatic biomarkers altered by lead acetate and mercuric chloride toxicity in Wistar rats

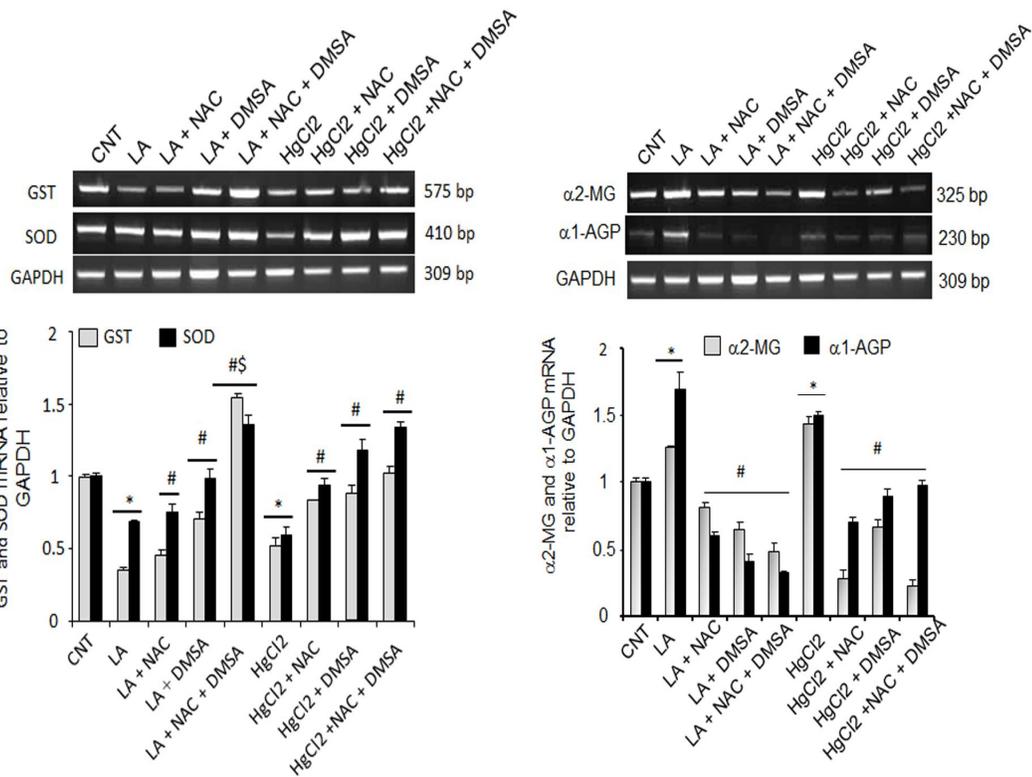
	Urea (mg/dL)	Creatinine (mg/dL)	GPT (U/L)	GOT (U/L)
Control	37.7 ± 6.2	0.6 ± 0.04	66.2 ± 12.7	56.25 ± 12.8
LA	114.2 ± 9.4*	1.6 ± 0.1*	132.5 ± 9.6*	156.2 ± 8.7*
LA+NAC	57.3 ± 1.8#	0.5 ± 0.01#	94.7 ± 6.7#	116 ± 15.7#
LA+DMSA	62.7 ± 6.1#	0.58 ± 0.09#	57.75 ± 4.9#	92.75 ± 6.6#
LA+ NAC + DMSA	43.5 ± 4.1#	0.5 ± 0.01#	58.5 ± 5.3#	86.5 ± 4.3#
HgCl <sub>2</sub>	204.5 ± 18.9*	1.6 ± 0.01*	147.7 ± 12.5*	154.5 ± 10.5*
HgCl <sub>2</sub> +NAC	64.2 ± 7.6#	0.5 ± 0.05#	84.7 ± 6.3#	87.5 ± 11.9#
HgCl <sub>2</sub> + DMSA	77.5 ± 0.5#	0.4 ± 0.04#	94 ± 10.03#	99.5 ± 18.6#
HgCl <sub>2</sub> + NAC + DMSA	63.2 ± 5.1#	0.3 ± 0.01#	54.5 ± 13.4#	77.7 ± 5.1#

Values are means ± standard error (SEM) for 10 different rats per each treatment. Values are statistically significant at \* $p < 0.05$  Vs. control; # $p < 0.05$  Vs. either LA or HgCl<sub>2</sub> alone injected rats and #  $p < 0.05$  Vs. NAC or DMSA injected groups

**Table 3:** Protective effects of NAC and DMSA on changes in antioxidants levels induced by lead acetate and mercuric chloride toxicity

	MDA (nmol/ml)	GSH (U/dL)	Catalase (U/dL)	SOD (U/ml)
Control	5.1 ± 0.3	1.7 ± 0.08	0.7 ± 0.04	397.9 ± 37.8
LA	30.1 ± 0.7*	0.4 ± 0.08*	0.33 ± 0.09*	198.5 ± 9.3*
LA+NAC	13.5 ± 1.9#	1.35 ± 0.04#	0.59 ± 0.03#	409 ± 73#
LA+DMSA	15.2 ± 2.4#	1.6 ± 0.08#	0.65 ± 0.04#	407 ± 65#
LA+ NAC + DMSA	9.5 ± 0.6#	1.12 ± 0.03#	0.76 ± 0.3#	461 ± 67#
HgCl <sub>2</sub>	39.2 ± 1.6*	0.70 ± 0.08*	0.64 ± 0.3*	209 ± 11*
HgCl <sub>2</sub> +NAC	20 ± 2.4#	1.1 ± 0.1#	0.6 ± 0.05#	343 ± 35#
HgCl <sub>2</sub> + DMSA	19.7 ± 0.5#	1.20 ± 0.1#	0.7 ± 0.06#	322 ± 24#
HgCl <sub>2</sub> + NAC + DMSA	13.2 ± 1.3#	1.3 ± 0.23#	0.66 ± 0.06#	402 ± 46#

Values are means ± standard error (SEM) for 10 different rats per each treatment. Values are statistically significant at \* $p < 0.05$  Vs. control; # $p < 0.05$  Vs. either LA or HgCl<sub>2</sub> alone injected rats and #  $p < 0.05$  Vs. NAC or DMSA injected groups.

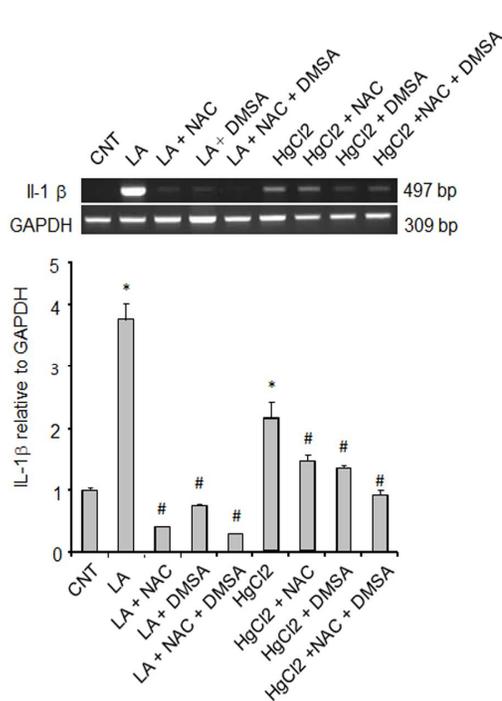


**Figure 1:** Protective effect of NAC and DMSA on changes in  $\alpha 1$ -AGP and  $\alpha 2$ -MG expression induced by LA and HgCl<sub>2</sub> intoxication. Intoxicated rats were administered by NAC or DMSA or a combination of both for 2 consecutive months. Total RNA was extracted from liver tissues and the expressions of  $\alpha 1$ -AGP and  $\alpha 2$ -MG were analyzed by semi-quantitative RT-PCR analysis. Values are means  $\pm$  SE of 10 rats. \*P < 0.05 Vs control group; # P < 0.05 VS LA or HgCl<sub>2</sub> group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

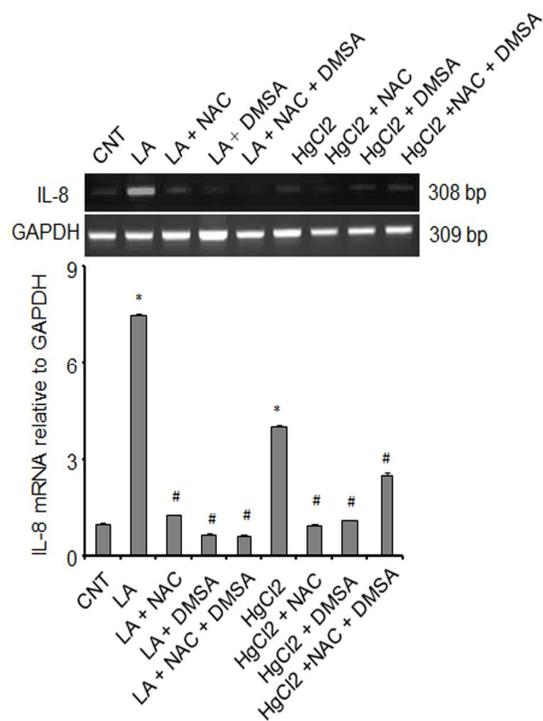
**Figure 2:** Protective effect of NAC and DMSA on changes in antioxidants expression induced by LA and HgCl<sub>2</sub> intoxication. Intoxicated rats were administered by NAC or DMSA or a combination of both for 2 consecutive months. Total RNA was extracted from liver tissues and the expressions of GST and SODS were analyzed by semi-quantitative RT-PCR analysis. Values are means  $\pm$  SE of 10 rats. \*P < 0.05 Vs control group; # P < 0.05 VS LA or HgCl<sub>2</sub> group; \$ P < 0.05 VS LA +NAC or LA+ DMSA group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

**Protective effects of NAC and DMSA on changes in mRNA expression of IL-1 $\beta$  and IL-8 induced by HgCl<sub>2</sub> and LA intoxication**

LA and HgCl<sub>2</sub> administration significantly upregulated both IL-1 $\beta$  and IL-8 expression compared to control group. Administration of NAC and DMSA normalized and downregulated their expression respectively (figure 3 and 4). Co-administration of NAC of DMSA induced additive downregulatory result for in IL-1 and IL-8 for LA and HgCl<sub>2</sub> administered rats.



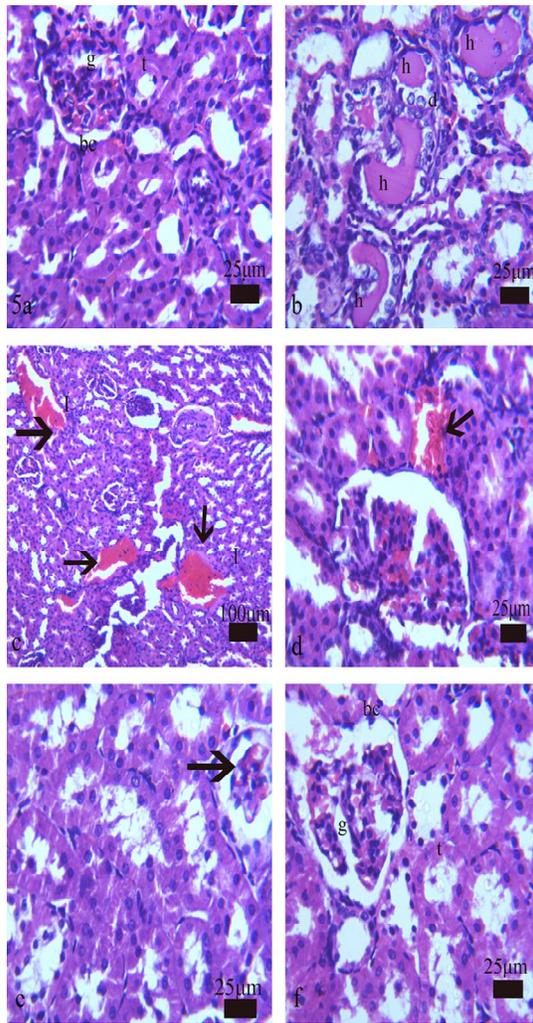
**Figure 3:** Protective effect of NAC and DMSA on changes in IL-1 $\beta$  expression induced by LA and HgCl<sub>2</sub> intoxication. Intoxicated rats were administered by NAC or DMSA or combination of both for 2 consecutive months. Total RNA was extracted from liver tissues and the expression of IL-1 $\beta$  was analyzed by semi-quantitative RT-PCR analysis. Values are means  $\pm$  SE of 10 rats. \*P < 0.05 Vs control group; # P < 0.05 VS LA or HgCl<sub>2</sub> group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.



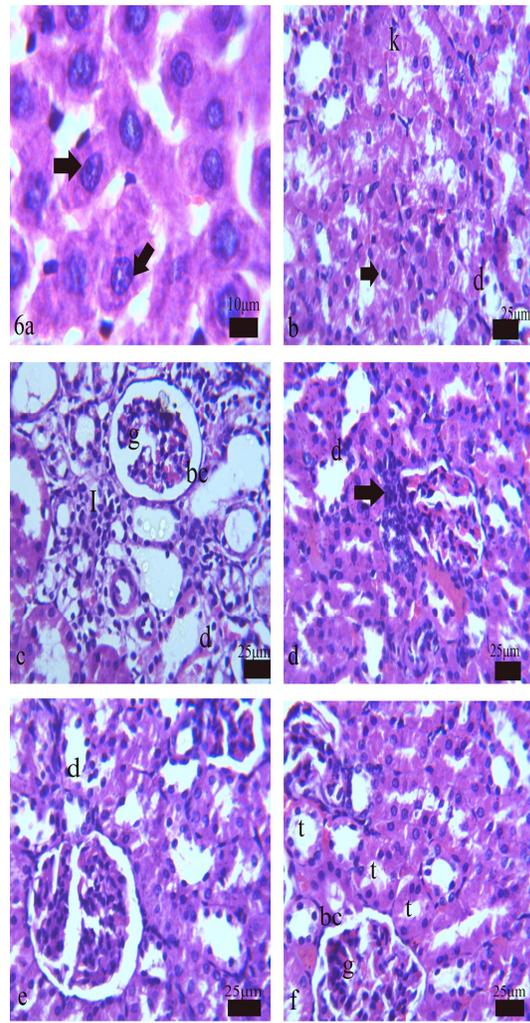
**Figure 4:** Protective effect of NAC and DMSA on changes in IL-8 expression induced by LA and HgCl<sub>2</sub> intoxication. Intoxicated rats were administered by NAC or DMSA or a combination of both for 2 consecutive months. Total RNA was extracted from liver tissues and the expression of IL-8 was analyzed by semi-quantitative RT-PCR analysis. Values are means  $\pm$  SE of 10 rats. \*P < 0.05 Vs control group; # P < 0.05 VS LA or HgCl<sub>2</sub> group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

### Protective effects of NAC and DMSA on histopathological changes in renal tissues induced by HgCl<sub>2</sub> and LA intoxication

Control rats showed normal glomerular structure with intact Bomans capsule and normal renal tubular structures (Fig. 5A). Kidney of HgCl<sub>2</sub>-intoxicated rats showed eosinophilic intraluminal hyaline casts, hydropic degeneration of tubular epithelium with sever congestion of nephritic blood vessels and perivascular round cells infiltrations (Figs. 5B, C). HgCl<sub>2</sub>-intoxicated rats given DMSA showed delicate congestion of peri-glomerular blood vessel (Fig. 5D). HgCl<sub>2</sub>-intoxicated rats given NAC showed normal renal tubules with atrophy of glomerular tufts (Fig. 5E). HgCl<sub>2</sub> intoxicated rats given both NAC and DMSA showed normal glomerular structure with intact Bomans capsule and normal renal tubular structures (Fig. 5F). LA-intoxicated rats showed intranuclear inclusion bodies in tubular epithelium, necrosis of tubular cells represented by pyknosis and karolysis, hydropic degeneration of tubular epithelium and periglomerular round cells infiltrations (Figs. 6 A-C). LA-intoxicated rats given DMSA-showed delicate periglomerular round cells infiltrations with mild hydropic degeneration of renal tubular epithelium (Fig. 6D). LA-intoxicated rats given NAC showed normal renal tubules with mild hydropic degeneration of renal tubular epithelium (Fig. 6E). Co-administration of DMSA and NAC for LA-administered rats showed normal glomerular structure with intact Bomans capsule and normal renal tubular structures (Fig. 6F).



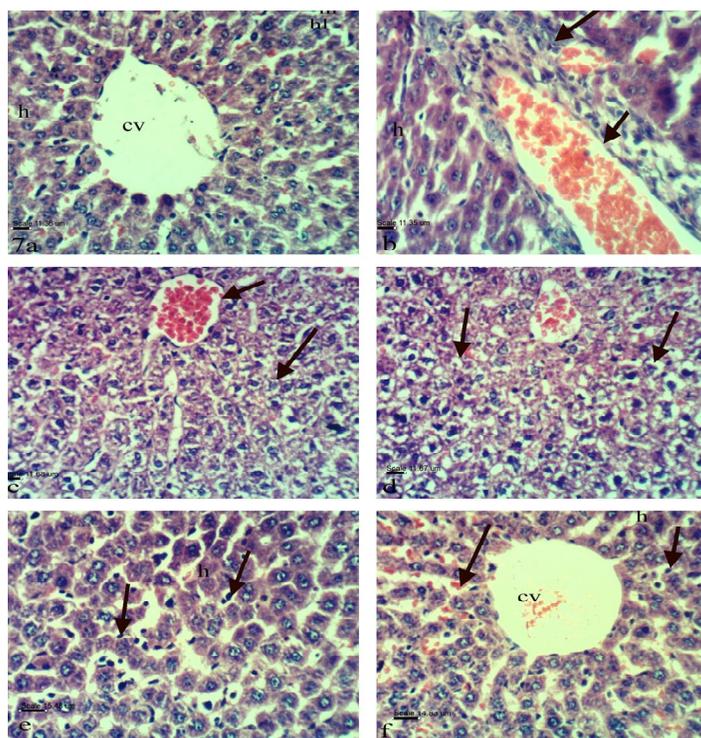
**Figure 5:** Renal photomicrographs show the protective effect of NAC and DMSA on HgCl<sub>2</sub> intoxication. A) Control rats showed normal glomerular structure (g) with intact Bomans capsule (bc) and normal renal tubular structures (t): H&E (bar=25µm). B) Kidney of HgCl<sub>2</sub>-intoxicated rats showed eosinophilic intraluminal hyaline casts (h) and hydropic degeneration of tubular epithelium (d): H&E (bar=25µm). C) Kidney of HgCl<sub>2</sub>-intoxicated rats showed sever congestion of renal blood vessels (arrows) and perivascular round cells infiltrations (I): H&E (bar=100µm). D) Kidney of HgCl<sub>2</sub> intoxicated given DMSA-administrated rats showed mild congestion of periglomerular blood vessel (arrow): H&E (bar=25µm). E) Kidney of HgCl<sub>2</sub> intoxicated rats given NAC-administrated rats showed normal renal tubules with atrophy of glomerular tufts (arrow): H&E (bar=25µm). F) Kidney of HgCl<sub>2</sub> intoxicated rats given co- administration of DMSA and NAC showed normal glomerular structure (g) with intact Bomans capsule (bc) and normal renal tubular structures (t) (bar=25µm).



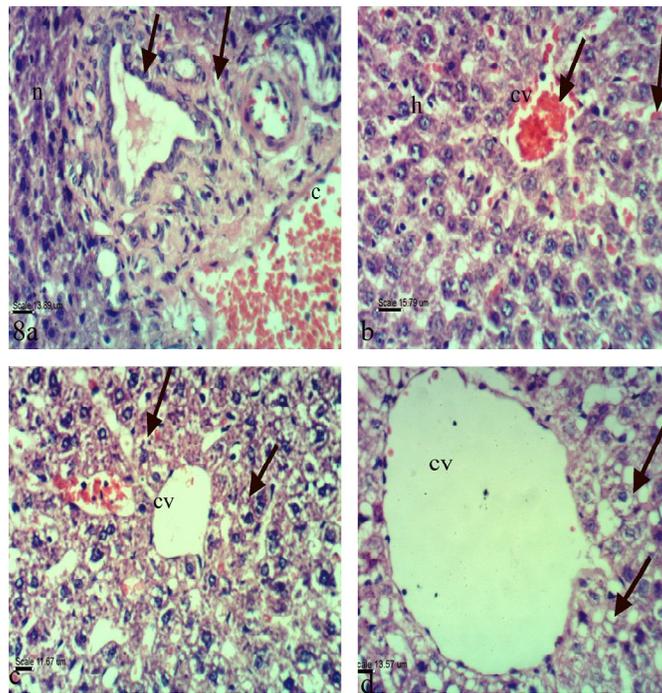
**Figure 6:** Renal photomicrographs show the protective effect of NAC and DMSA on LA intoxication. A) Kidney of lead-intoxicated rats showed intranuclear inclusion bodies in tubular epithelium (arrows): H&E (bar=10µm). B) Kidney of lead- intoxicated rats showed necrosis of tubular cells represented by pyknosis (arrow) and karyolysis (k) and hydropic degeneration of tubular epithelium (d): H&E (bar=25µm). C) Kidney of lead -intoxicated rats showed periglomerular round cells infiltrations (I) with hydropic degeneration of renal tubular epithelium (d): H&E (bar=25µm). D) Kidney of lead- intoxicated rats given DMSA showed mild periglomerular round cells infiltrations (I) with mild hydropic degeneration of renal tubular epithelium (d): H&E (bar=25µm). E) Kidney of lead- intoxicated rats given NAC showed normal renal tubules with mild hydropic degeneration of renal tubular epithelium (d): H&E (bar=25µm). F) Kidney of lead- intoxicated rats given both DMSA and NAC showed normal glomerular structure (g) with intact Bomans capsule (bc) and normal renal tubular structures (t) (bar=25µm).

## Protective effects of NAC and DMSA on histopathological changes in hepatic tissues induced by HgCl<sub>2</sub> and LA intoxication

Liver of control rats showed normal architecture, with hepatic lobules around the central vein and every lobule consisting of hepatic cords of hepatocytes (Fig. 7A). HgCl<sub>2</sub>-intoxicated rats showed sever congestion of hepato-portal vessel and central vein, oval cell proliferation and hydropic degeneration of hepatocytes (Fig. 7B, C). HgCl<sub>2</sub>-intoxicated rats given DMSA-administrated rats showed hydropic degeneration of hepatocytes and gentle congestion of central vein: (Fig. 7D). HgCl<sub>2</sub>-intoxicated rats given NAC-administrated rats showed normal hepatocytes with kupffer cell activation (Fig. 7E). HgCl<sub>2</sub>-intoxicated rats co-administered each NAC and DMSA showed normal hepatocytes represented by hepatic cords with kupffer cell activation and really gentle congestion of hepatic sinusoids (Fig. 7F). Liver sections of LA-intoxicated rats showed sever congestion of hepatoportal vessel, hyperplasia of epithelial lining bile duct, fibroplasias in portal tract and necrosis of hepatocytes (Fig. 8A). LA intoxicated rats given DMSA, showed moderate congestion of central vein and hepatic sinusoids (Fig. 8B). LA intoxicated rats given NAC, showed hydropic degeneration of hepatocytes (Fig. 8C). LA intoxicated rats given both DMSA and NAC showed normal hepatocytes and central vein with cytoplasmic vacuolization of centrolobular hepatocytes (Fig. 8D).



**Figure 7:** Hepatic photomicrographs show the protective effect of NAC and DMSA on HgCl<sub>2</sub> intoxication. A) Control rats showed normal histological structure of hepatic lobule (hl) with normal hepatocytes (h) and normal central vein (cv): H&E (bar=11.36 $\mu$ m). B) Liver of mercury- intoxicated rats showed sever congestion o hepatoportal blood vessel (short arrow) and oval cell proliferation (long arrow): H&E (bar=11.35 $\mu$ m). C) Liver of mercury -intoxicated rats showed sever congestion o central vein (short arrow) and hydropic degeneration of hepatocytes (long arrow): H&E (bar=11.68 $\mu$ m). D) Liver of HgCl<sub>2</sub> intoxicated rats given DMSA-administrated rats showed hydropic degeneration of hepatocytes (arrows) and mild congestion of central vein: H&E (bar=11.67 $\mu$ m). E) Liver of HgCl<sub>2</sub> intoxicated rats given NAC showed normal hepatocytes with kupffer cell activation (arrows): H&E (bar=15.48 $\mu$ m). F) Liver of HgCl<sub>2</sub> intoxicated rats given DMSA and NAC showed normal hepatocytes (h) with kupffer cell activation (short arrow) and mild congestion of hepatic sinusoids (long arrow) (bar=14.83 $\mu$ m).



**Figure 8:** Hepatic photomicrographs show the protective effect of NAC and DMSA on lead acetate intoxication. A) Liver of lead- intoxicated rats showed sever congestion o hepatoportal blood vessel (c), hyperplasia of epithelial lining bile duct (short arrow), fibroplasias in portal tract (long arrow) and necrosis of hepatocytes (n): H&E (bar=13.89 $\mu$ m). B) Liver of lead- intoxicated rats given DMSA showed sever congestion of central vein (cv) and hepatic sinusoids (long arrow): H&E (bar=15.79 $\mu$ m). C) Liver of lead- intoxicated rats given NAC showed hydropic degeneration of hepatocytes (arrows): H&E (bar=11.67 $\mu$ m). D) Liver of lead- intoxicated rats given both DMSA and NAC showed normal hepatocytes and central vein (cv) with cytoplasmic vacuolization of centrolobular hepatocytes (arrows) (bar=13.57 $\mu$ m).

## Discussion

The current study confirmed the protective effect of NAC and DMSA on biohazards induced by chronic toxicity of LA and HgCl<sub>2</sub> at the biochemical, histopathological and genetic levels. Chronic LA and HgCl<sub>2</sub> intoxication showed (a) liver and kidney damage as indicated by increase liver and kidney biomarkers, decrease in antioxidant gene expression, upregulation in  $\alpha$ 1-AGP and  $\alpha$ 2-MG expression, and changes in kidney and liver histopathology, (b) upregulation in IL-1 $\beta$  and IL-8. All were ameliorated by NAC and DMSA administration.

LA and HgCl<sub>2</sub> are very dangerous to human health and are the most toxic metals (Ercal et al., 2001). Exposure to Pb is related to cancer, neurotoxicity, hepatotoxicity, and cardiovascular disorder (Kumar et al., 2013). The method by which NAC and DMSA ameliorated Pb and HgCl<sub>2</sub> toxicity is not examined well till now. Possibly NAC and DMSA act as chelating agents to create a molecular complex and to prevent and therefore hinder their entry and gathering within the liver and renal tissues. As known, kidney is the major excretory and also the final organ of clearance for most of the xenobiotics. Furthermore, kidney is the most vulnerable organ to lead toxicity (Jarrar, 2003). It is known that mercury can cause unplanned and occupational exposures and poisoning will created from inhalation, ingestion, and absorption through the skin (Ercal et al., 2001). Oxidative stress, incredibly damage renal and hepatic activity. It causes degradation in proteins, lipids, and DNA (Halliwell & Gutteridge, 1990). Like kidney, liver is one of the targets for lead and mercury; it reacts by increasing the activity of transaminases (Mehana et al., 2012; Agarwal et al., 2007) in acute intoxication. Here, in chronic study both LA and HgCl<sub>2</sub> attenuated antioxidants levels and expression and increased liver transaminases.

Liver and kidney damage, gastrointestinal diseases, and alterations in central nervous system, blood pressure and vitamin D metabolism occurred after exposure to LA and HgCl<sub>2</sub> (Ansari et al., 2013; Ahn et al., 2002). Furthermore, antioxidants, plant extracts and chelating agents have the potential to inhibit these effects (Kumar et al., 2013; Jarrar, 2003; Mehana et al., 2012; Ekor et al., 2010). Our findings showed that both NAC and DMSA ameliorated the decrease in serum and mRNA expression of antioxidants. These findings were confirmed in previous reports for both LA and HgCl<sub>2</sub> using curcumin and selenium, respectively (Agarwal et al., 2007; Ahn et al., 2002; Abu El-Saad et al., 2016). NAC interacts most powerfully with the free radicals, it exerts an indirect effect on antioxidant status, since it restores catalase and is a precursor for synthesis of GSH, (Arakawa & Ito, 2007) thus providing enhanced defense against toxin-induced oxidative insult (Abu El-Saad & Elgerbed, 2010). DMSA also has an

important role in decreasing the oxidative stress, either by removing the toxic metal from the target organ and/or by directly scavenging ROS via its sulfhydryl groups (Abu El-Saad & Elgerbed, 2010).

$\alpha$ 1-AGP is a marker for acute phase proteins. It is formed in liver and is affected by inflammation and certain diseases (Colombo et al., 2006). It has been found that some medications and natural products mediate their action by  $\alpha$ 1-AGP during hepatic toxicity and inflammation in some tissues (Anderson et al., 1999). Our findings confirmed that both LA and HgCl<sub>2</sub> increased  $\alpha$ 1-AGP expression and both NAC and DMSA normalized and control the degree of inflammation. In parallel,  $\alpha$ 2-MG is upregulated after chronic LA and HgCl<sub>2</sub> intoxication and is downregulated by NAC and DMSA. The increase in  $\alpha$ 2-MG is a counteract mechanism to reduce plasmin synthesis occurred after hepatic intoxication and to facilitate the growth factors transportation that help in hepatocyte renewal that strengthened by antioxidants and chelating agents (Ekor et al., 2010; Arakawa & Ito, 2007; Lyoumi et al., 1998).

Oxidative stress induced alteration in cytokines expression and consequently promotes Th cell dysregulation, and alters the accessibility of Th1 and Th2 cytokines (Hsiao et al.; 2011; Srosiri et al., 2010). Intoxication by lead and mercury stirred up localized immune responses in liver and excretory organ, signifying gentle inflammatory changes and leading to increased cytokine expression as confirmed by our findings and others (Zhang et al., 2016; Kim et al., 2003). Immune perform is taken into account as biological marker to evaluate the effects of contaminants, however only a few studies have ventured to assess the immunotoxic effects of LA and HgCl<sub>2</sub> in wildlife (Rodriguez-Estival et al., 2013). Most of cytokines are regulatory proteins, can be affected differentially by serious metals, that successively could cause the dysregulation of innate and/or adaptational immune responses. Moreover, it's been rumored that both LA and HgCl<sub>2</sub> have the prospective to induce immunotoxicity (Agarwal et al., 2007; Zhang et al., 2016). Among these cytokines are IL-1 $\beta$  and IL-8. IL-8 is an important mediator of host and inflammation (al-Dalaan et al., 1995). It has diverse functions as a neutrophil activator and a chemoattractant for neutrophils, T cells, and basophils (Wang et al., 1997). IL-8 is created by a range of cell sorts including monocytes/macrophages, T cells, neutrophils, endothelial cells, fibroblasts, and keratinocytes (al-Dalaan et al., 1995; Ozoran et al., 1995). Therefore, the elevation of IL-8 mRNA expression could also be the initial sign of the acute inflammatory response due to LA and HgCl<sub>2</sub> exposure and play a role in the initiation or pathogenesis of hepatic toxicity and both NAC and DMSA regulate it to retain normal hepatic activity and regeneration. As known, IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 are the main inducers of acute phase response (Ganjali et al., 2014; Yang et al., 1998; García-Niño & Pedraza-Chaverri, 2014). They proceed as hepatotropic factors as proven by expression levels of IL-1 $\beta$  and IL-8 that are inflated in rats with liver injury (Yang et al., 1998; García-Niño & Pedraza-Chaverri, 2014). Therefore, both LA and HgCl<sub>2</sub> upregulated IL-1 and 8 expressions and its expression were normalized by NAC and DMSA.

The histopathological alternations in the present study, caused by lead acetate and mercury in the kidney and liver tissues and possible protection by DMSA, are in agreement with previous studies (Abdelghaffar et al., 2015; Tugbobo et al., 2016; Goyer & Clarkson, 2001; Mesquita et al., 2016). The current study proved that DMSA compact the toxic effects of lead and mercury on the renal and hepatic tissues. It is supposed that chelating agents reduce the lead and mercury toxicity in soft organs via its chelating activity, while antioxidants protect the cells from persuade of oxidative damage by scavenging the free radical generation and inhibiting of lipid peroxidation (El-Neweshy & El-Sayed, 2011; Buchet & Lauwerys, 1989; Janle et al., 2015; Brandon et al., 2016). It has been indicated that chelating agent, like DMSA, decrease the toxic and apoptotic effects of lead and mercury on the histological sections of soft tissues (Goyer & Clarkson, 2001; El-Neweshy & El-Sayed, 2011; Brandon et al., 2016). However, some in vitro studies exhibited that NAC is directly associated with inhibition of cytokines expression and encouragement of cell growth (Yan & Greene, 1998).

## Conclusions

Current findings strongly conclude that both NAC and DMSA have protective effect against LA and HgCl<sub>2</sub> induced hepatic and renal toxicity. Both regulate the antioxidants expressions and levels and are immunomodulators to normalize renal and hepatic activity. Moreover, administration of NAC and DMSA was valuable in improving and restoring histological changes of renal and hepatic tissues. NAC and DMSA are beneficial chelating agents against heavy metals toxicity. Both are good to protect humans against metal toxicity.

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