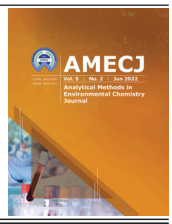
Anal. Methods Environ. Chem. J. 5 (2) (2022) 76-89

AMECJ

Research Article, Issue 2
Analytical Methods in Environmental Chemistry Journal
Journal home page: www.amecj.com/ir



Determination of mercury values in urine and air of chloralkali workers by copper nanoparticles functionalized in carboxylic carbon nanotubes and the effects of mercury exposure on oxidative stress

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ARTICLE INFO:

Received 30 Feb 2022
Revised form 2 May 2022
Accepted 28 May 2022
Available online 30 Jun 2022

Keywords:
Mercury,
Air, and Urine,
Magnetic solid-phase extraction,
Oxidative stress,
Copper functionalized carbon nanotubes,
Cold vapor atomic absorption spectrometer

ABSTRACT

Mercury exposure can produce toxic organic compounds in the body. Also, mercury can potentially cause oxidative damage and cellular disorders. In this study, the determination of mercury values in urine and air of chloralkali workers based on copper nanoparticles functionalized in carboxylic carbon nanotubes (CuNPs@CNT-COOH) were obtained by cold vapor atomic absorption spectrometer (CV-AAS). The urine samples were determined by magnetic solid-phase extraction (MSPE) at pH 8.0. By measuring the mercury level in the air and the urine sample of workers, the level of oxidative stress (Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (Cat)), Interleukin-6 (IL-6), and Tumor Necrosis Factor α (TNF-α) as the proinflammatory cytokines were measured in the subject group. The results revealed statistically significant differences in the mercury level of the urine samples in the case and control groups ($p<0.001$). Similarly, the malondialdehyde (MDA) level was significantly different between the two research groups ($p<0.001$). Catalase concentration was not significantly different in the two groups ($p=0.059$). The LOD and linear range for mercury determination in urine were achieved at 0.012 µg L^{-1} and 0.05-7.0 µg L^{-1}, respectively. Workers' exposure to mercury can significantly increase oxidative stress and inflammatory cell signaling molecules such as cytokines.

1. Introduction

In the biogeochemical system of the earth, there are metallic mercury, organic and non-organic compounds. Exposure to any of the three can produce toxic compounds in the body[1]. Heavy metals are important factors in environmental pollution and mercury is one of the most toxic and threatens human health [2]. The greatest effect of mercury in elemental and organic form in the central nervous system and the greatest effect of mineral mercury on the digestive and excretory systems [3]. Mercury has been extensively investigated due to its wide range of applications, high toxicity, long-term ecological effects, aggregation in the food chain and adverse effects (in exposure to the low concentration of the liquid metal) [1, 4, 5, 6].

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https://doi.org/10.24200/amecj.v5.i02.188

Between 40 and 70% of the existing mercury in the atmosphere is estimated to be induced by human activities. Direct and indirect exposure of more than 2 million occupations to this pollutant is considered a global concern [4, 7, 8]. At work, exposure to mercury vapor through respiration is more common [9]. Yet, the alkali form (methyl/ethyl mercury) is highly soluble in fatty tissues and also highly volatile. Thus, it can be easily absorbed in the lungs and then the blood, and is 10 times as toxic [1]. Occupational mercury exposure can occur in petrochemical, and chloralkali industries, fluorescent lamps, thermometer manufacturing companies, glass production and dentistry (tooth amalgam) [10-12]. Chloralkali processing is a large industry worldwide in electrochemistry. The main products are cholera, sodium hydroxide, carbonate sodium, hydrochloric acid and potash [13]. The common chloralkali processes include mercury cell, membrane cell and diaphragm cell. In the mercury cell process, the anode (carbon electrode) is hung above the cell and the mercury flows on the container surface as the cathode [5, 14].When the electricity is on, the chloride ion dissolved in saline water turns into the chlorine oxide at the anode side. Sodium ions are revived as sodium at the cathode side. Sodium is then solved in mercury and sodium amalgam (sodium-mercury) is produced. Next, the amalgam is analyzed. Thus, mercury returns to the cycle and sodium are turned into sodium hydroxide [14]. Among the disadvantage of this method are ecological issues, low efficiency in terms of the voltage used, exposure to mercury and the high cost [1]. Despite the presence of several metals in the body such as iron, magnesium, zinc, copper, cobalt, molybdenum and selenium, the toxicity of mercury is incomparably high [6]. A body of research explored the threats to health caused by exposure to mercury. Instances are disorders in the nervous system especially the brain, cardiovascular diseases, metabolic disorders, pulmonary issues, damage to the immune system, liver, reproduction system, thyroid, and optical, auditory, tactile and verbal disorders [4, 7, 12, 15-20]. These studies showed that the disorders induced by exposure to mercury produce oxygen radicals in the body. The cytotoxic effects of mercury (Hg^{+2}) can be due to the oxidative stress in cells. Hg^{+2} interacts with thiols and produces mercaptans. Thus, the cellular antioxidant buffers based on glutathione thiol are reduced. Though the exact mechanism of the production of these radicals is yet unknown, probably, an increase in reactive oxygen species (ROS) is the main cause, which results from the reduced rate of glutathione [21]. Several studies show that mercury can cause oxidative damage to multiple organs and systems [21, 22]. Greater production of ROS can lead to oxidative stress and may induce dysfunctions and structural damages such as mutagenesis, carcinogenesis, oxidation, and deterioration of proteins, carbohydrates, lipids and DNA [23]. Numerous studies have also identified a significant positive relationship between the dose of mercury exposure in hair samples and high blood pressure [24]. In the cardiovascular system, the endothelium functioning is essential to the maintenance of the blood flow and the antithrombotic capacity. Vascular endothelial is highly sensitive to oxidative stress. This stress can be the main cause of disorders in this tissue in cardiovascular diseases including hypertension and atherosclerosis [4, 17]. Measuring changes in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) is typically done to act as a biological index in examining cellular oxidant damages [4, 25, 26]. In cells, SOD takes charge of analyzing superoxide anions (O_2) into oxygen and hydrogen peroxide (H_2O_2). Catalase is in charge of analyzing H_2O_2 in water and oxygen [26]. Today, measuring the level of cytokines or low-weight glycoproteins is another index for cellular disorders. These hormones interlink cells and the inner body environment especially the immune and inflammatory systems [21]. Striking a balance between the two groups (i.e., the proinflammatory and anti-inflammatory cytokine groups) is key to human hemostasis. Measuring proinflammatory cytokines is significant, for example, interleukin 6 (IL-6) and the tumor necrosis factor alpha (TNF alpha), both known as major biological indices in

diagnosing cellular damage [27, 28]. The present research aimed to explore the effects of exposure to mercury on oxidative stress and proinflammatory cytokines in the body of workers in the chloralkali industry. In-addition the mercury values were determined in air (NIOSH 6009) and human urine samples based on CuNPs@CNT-COOH by MSPE procedure at pH 8.5.

2. Materials and Methods

2.1. Instrumental and reagents

A cold vapor atomic absorption spectrometer (AAS) was used to determination of mercury in water samples (CV-AAS, HG-3000, GBC, Aus). The background correction (D_2 lamp) the hollow cathode lamp (HCL, Hg), $SnCl_2/NaBH_4$ reagents and a reaction loop were used for the generation of mercury vapor and the mercury concentration determination by CV-AAS. The standard of inorganic mercury [Hg^{2+}, 1000 mg L^{-1} in 1% nitric acid) was prepared from Sigma Aldrich (CAS N: 7487-94-7, Germany). The different standard solutions of mercury were made by diluting deionized water (DW, Millipore, USA).

2.2. Design and Sampling

The present cross-sectional research was case-control in type, and was conducted in 2020 in a chloralkali factory in Tehran. The participants were 179 in number (84 blue-collar workers and 95 white-collar workers). Screening of different units showed that 114 participants were directly exposed to mercury. These workers were significantly exposed to mercury as chlorine was produced by traditional mercury cell processes. Considering the exposure criteria, among the 114 workers, 84 were found to be directly exposed and were, thus, selected as the case. For the control group, 95 white-collar workers were recruited. The inclusion criteria were: full-time work and at least two years' work experience in the unit. The exclusion criteria were: consuming antioxidant supplements (e.g., vitamin E or C) and drugs containing mercury, having renal diseases, and being non-smokers yet being unwilling to participate in the study. According to the inclusion criteria, the final sample was selected to include workers who consumed antioxidant supplements and drugs (n=7), had less than two years' work experience and were non-smokers (n=14). Those unwilling to participate (n=9) were excluded from the study. The final remaining 84 blue-collared workers were included in the research. Thus, the sampling can be called a consensus. The control group consisted of office workers who were not exposed to mercury. All the participants agreed to participate in the study by signing an informed letter of consent. A demographic questionnaire was also filled out by all participants to include their age, weight, height, work experience, smoking status and type of work shift. The human urine samples were collected in 114 participants based on Helsinki Declaration as revised in 2013. The information, including names, initials, and hospital numbers don't publish in text or any other document. (https://www.wma.net/policies-post/wma-declaration-of-helsinkiethical-principles-for-medical-research-involving- ansubjects/).

2.3. Measurement of mercury level in the air sample

Occupational mercury exposure was measured by air samples in the participants' breathing zoon using the NIOSH 6009 method. The solid sorbent tubes with 200 mg Hopcalite in a single section were used as samplers and were connected by Tygon tubing to the personal pumps calibrated before and after sampling. The flow rate was adjusted to 2 L min^{-1} and the sampling duration was set at 3 hours of a normal work shift. The sorbent tubes were capped and packed securely for shipment. The Hopcalite sorbent and the front glass wool of each sample were placed in separate 50 volumetric flasks and 2.5 mL of HNO_3. Then, 2.5 mL of HCl was added to each volumetric flask. The sorbent was dissolved and diluted to 50 ml with deionized water. 20 mL of the sample was transferred to a BOD bottle containing 80 mL of deionized water. All samples were analyzed using a cold vapor atomic absorption (GBC-936, 3000, Australia) at a wavelength of 253.7 nm (Fig. 1). The amount of mercury (C) in

the sampled air volume (V) was calculated using the following Equation 1:

$$C\ (mg/m3\ ) = W\ (\mu g) \times Vs\ (mL)\ Va/(mL) - B\ (\mu g)\ V\ (L) \quad \text{(Eq. 1)}$$

W is the amount of mercury in the sample aliquot from the calibration graph. Vs represents the original sample volume (50 mL). Va stands for the aliquot volume (20 mL), and B is the average amount of mercury in the media blanks.

2.4. Measurement of mercury concentration in the human urine sample

The most practical and sensitive method of measuring the level of mercury in the body is the urine sample. That is because mercury exits the body primarily in the urine. The concentration of the metal in urine samples shows the exposure within the past 2-3 months. In this research, mercury in the urine samples was extracted based on copper nanoparticles functionalized in carboxylic carbon nanotubes (CuNPs@CNT-COOH) by magnetic solid-phase extraction (MSPE) at pH 8.5 before being determined by cold vapor atomic absorption spectrometer (CV-AAS). Urine samples were collected in the field using a 100 mL sterile plastic container before the participants' work shift. The samples were sealed and packed in an ice bath. The mercury in 10 mL of urine samples was extracted with the COOH group of CuNPs@CNT-COOH at pH=8.0 and then the solid phase was separated by an external magnetic accessory in the bottom of the tube. After back-extraction of mercury from CuNPs@CNT-COOH in acidic pH and dilution with DW up to 1 mL, the concentration of mercury in urine samples was determined by CV-AAS (GBC-936, HG-3000, Australia), equipped with a Hg lamp at a wavelength of 253.7 nm. The extraction of toxic mercury with 25 mg of CuNPs@CNT-COOH was obtained more than 95% in 10 mL of urine samples by MSPE (Fig.2)..

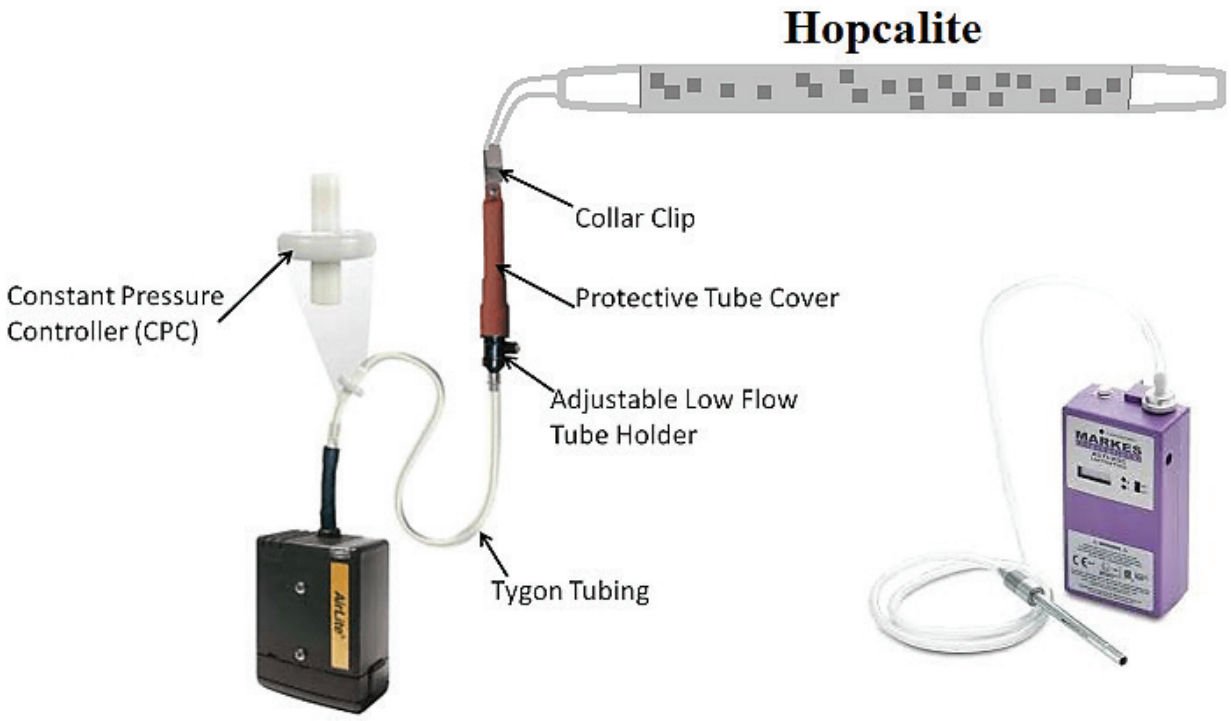

Fig. 1. Measurement of mercury level in the air sample in the participants' breathing zoon using the NIOSH 6009 method.

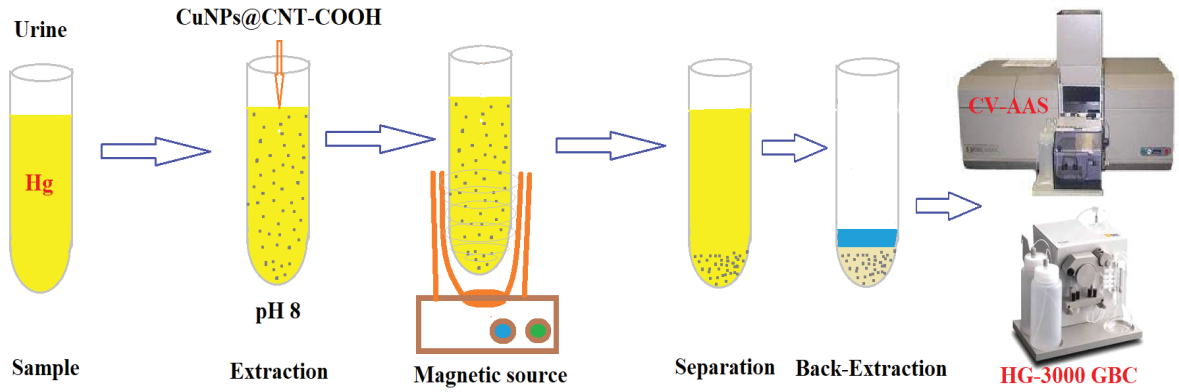

Fig.2. Measurement of mercury concentration in the human urine sample based on CuNPs@CNT-COOH by MSPE procedure at pH 8.5

2.5. Measurement of Oxidative Stress

In the present research, the level of Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (Cat) as the oxidative stress indices and Interleukin-6 (IL-6) and Tumor Necrosis Factor α (TNF-α) as the proinflammatory cytokines were measured in the collected samples. Five milliliters of the venal blood were taken from the participants in both groups before their work shift. These samples were transferred into sterile tubes and were allowed to clot. After tube centrifuging (1600 × g for 10 minutes), the serum samples were separated and stored at -50 °C before analysis. The oxidative stress and proinflammatory cytokines were measured using Hangzhou Eastbiopharm kits (Hangzhou, China) by Double Antibody Sandwich (DAS) ELISA. The mean value of three repetitions for each sample was reported.

2.6. Statistical Analysis

Descriptive statistics were used including frequency (percentage) and median (inter-quartile range) to summarize demographic variables, oxidative stress indices and proinflammatory cytokines. The normality and the equality of variances were analyzed by the Kolmogorov Smirnov test and the Levene's test. Demographic variables were compared in the exposed and unexposed groups via the chi-square test. To compare the median of oxidative stress indices, proinflammatory cytokines and the level of mercury in urine samples (urine Hg) in two groups, the Mann Whitney U-test was run. Predictors of oxidative stress and proinflammatory cytokines were tested using multiple linear regression (backward). Variables with more than two categories entered the regression model after dummy coding. The variables that did not meet the normality assumption were normalized according to the method recommended by Templeton (2011) before entering the final model [29]. All statistical tests were run in SPSS v25 (IBM SPSS, Chicago, IL) at the significance level of < 0.05.

3. Results and discussion

3.1. Optimization and validation of mercury analysis

By the MSPE procedure, the extraction of mercury in urine samples was achieved by CuNPs@CNT-COOH nanoparticles. The various mercury concentration between 0.05–7.0 µg L^{-1} were used for the optimization of parameters. The mercury was extracted and separated in urine samples based on the COOH groups of CuNPs@CNT-COOH adsorbent at optimized conditions. The effective parameters such as the pH, amount of CuNPs@CNT-COOH adsorbent, the eluents, and the sample volume were studied.

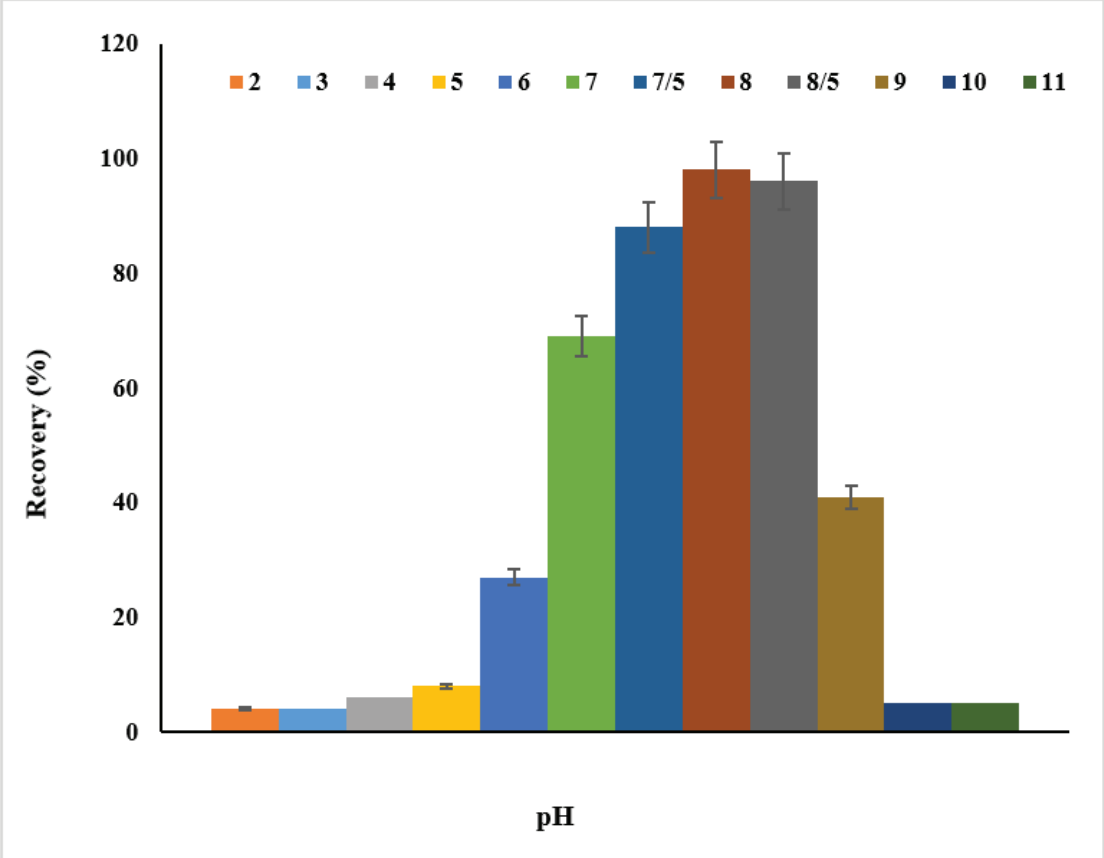

Fig. 3. The effect of pH on mercury extraction in urine samples based on CuNPs@CNT-COOH by MSPE procedure at pH 8.5

3.1.1.pH effect

For efficient extraction of mercury in urine samples, the pH sample must be optimized. So, the different values between 2 and 11 were studied. The pH is the critical parameter that was affected by efficient extraction and absorption capacity by the CuNPs@CNT-COOH adsorbent. Therefore, the various pH was selected for Hg(II) extraction in urine samples using a buffer solution. The results showed that the high recovery based on the CuNPs@CNT-COOH adsorbent for mercury extraction was obtained at a pH of 7.5-8.5. So, the efficient mercury extraction was obtained at pH 8.0 and the recovery was decreased at $8.5 < pH < 7.0$. So, the pH of 8.0 was used as optimized pH in this study (Fig. 3). The extraction mechanism occurred based on COOH groups of CuNPs@CNT-COOH adsorbent as an excellent leaving group

$$(Hg^{2+} \longleftarrow [:COOH\text{-}R])$$

with the positively charged mercury at pH 8. At lower pH the COOH groups have positively charged (+). So, the electrostatic repulsion occurred between Hg^{2+} and +COOH groups. In addition, at more than pH 8.5, the mercury ions participated as $Hg(OH)_2$.

3.1.2.Optimized CuNPs@CNT-COOH amount

For maximum extraction of mercury in water samples, the amount of the CuNPs@CNT-COOH adsorbent must be optimized in mercury concentration between 0.05–7.0 µg L^{-1}. So, the various amounts of the CuNPs@CNT-COOH between 5-40 mg were used for Hg(II) extraction in urine samples by the MSPE procedure. The efficient extraction was obtained at more than 20 mg of the CuNPs@CNT-COOH adsorbent for the extraction of mercury by the proposed procedure. Therefore, 25 mg of the CuNPs@CNT-COOH was used for further work at pH=8 (Fig. 4).

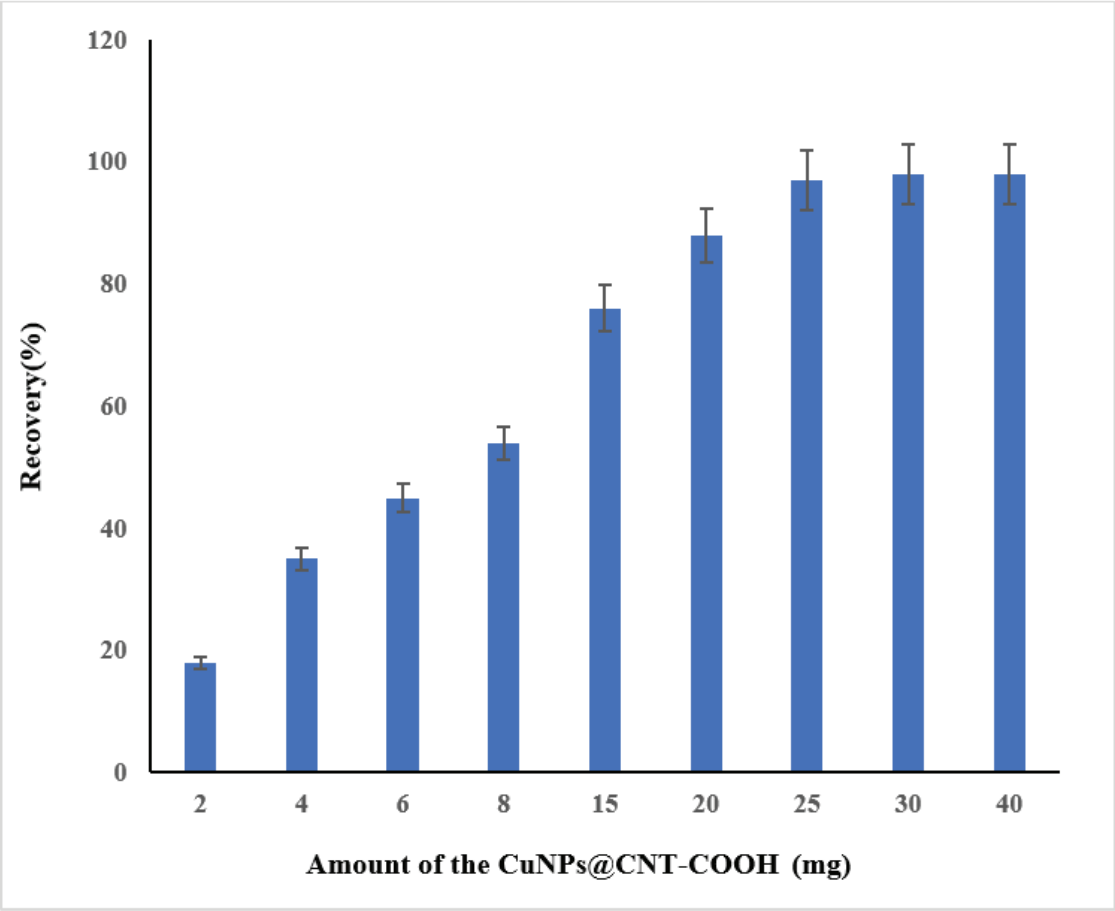

Fig. 4. The effect of amount of adsorbent on mercury extraction in urine samples based on CuNPs@CNT-COOH by MSPE procedure at pH 8.5

3.1.3.Elution process, shaking time and Sample volume

After extraction of mercury by CuNPs@CNT-COOH adsorbent, the mercury loaded on the CuNPs@CNT-COOH must be released from COOH groups by changing pH. Therefore, the different eluents such as, HCl, HNO_3, and H_2SO_4, were used for the back-extraction of mercury (Hg^{2+}) from the CuNPs@CNT-COOH adsorbent. The mercury loaded on the CuNPs@CNT-COOH adsorbent was easily determined by the CV-AAS after back extraction with inorganic acid at low pH. In this study, the different eluents (HCl, HNO_3, H_2SO_4) based on volumes and concentrations were used for back extraction of Hg(II) in urine samples. The results showed us that the Hg(II) ions were back-extracted from the CuNPs@CNT-COOH adsorbent by the nitric acid solution (0.3 mol L^{-1}; 0.5 mL). Also, the shaking time is the main parameter for the extraction of mercury in urine samples. So, the different time was studied from 1 to 10 minute for mercury extraction at pH 8. The maximum extraction was obtained in more than 4 min. So, 5 minutes was used as the optimum shaking time. In addition, the effect of sample volume for mercury extraction was studied at pH=8. The results showed us that the mercury can be extracted in 12 mL at the optimized conditions. So, the 10 mL of urine samples were selected as the optimum volume for mercury extraction for further works.

Table 1. Demographic variables of the exposed group (n=84) vs. unexposed group (n=95)

Variable	Classification	Frequency (%)		P-value*
		Exposed group	Unexposed group	
Age	<30	33 (39.3)	43 (45.3)	0.674
	30-40	37 (44)	36 (37.9)	
	>40	14 (16.7)	16 (16.8)	
Experience	≤10	47 (56)	56 (58.9)	0.686
	>10	37 (44)	39 (41.1)	
BMI	Underweighted	4 (4.7)	7 (7.4)	0.392
	Normal	75 (89.3)	78 (82.1)	
	Obesity	5 (6)	10 (10.5)	
Shift work	Yes	23 (27.4)	20 (21.1)	0.323
	No	61 (72.6)	75 (78.9)	

* Chi-square

3.2. Comparing exposed and unexposed groups

Table 1 summarizes the two research groups' demographic information. More than half of the participants in both groups had less than 10 years of work experience. Most of the participants had a normal BMI. There was no statistically significant difference between demographic variables in the exposed and unexposed groups. The median (inter-quartile range) of mercury concentration in air and urine samples, oxidative stress indices and proinflammatory cytokines are shown in Table 2. The level of mercury in the urine samples of the exposed group was significantly different from the unexposed group (p<.001). In addition, the lipid peroxidation products were measured as MDA and showed to diverge significantly between the two groups (p≤.001). The results also showed that the level of all oxidative stress indices (except for catalase) and inflammatory cytokines were significantly higher in the exposed group than the unexposed. Catalase concentration did not account for any statistically significant difference between the two groups (p=.059). The concentration of mercury in urine samples was the most significant

Table 2. Mercury level in air and urine samples, oxidative stress and proinflammatory cytokines in the exposed vs. unexposed groups

Variables	Median (inter-quartile range)		P-value*
	Exposed group (n=84)	Unexposed group (n=95)	
Air Hg ()	18.49 (13.75)	-	-
Urine Hg (μg L^{-1})	15.44 (19.85)	4.62 (3.64)	<0.001
Malondialdehyde (μmol L^{-1})	6.65 (4.88)	2.41 (3.06)	<0.001
Superoxide Dismutase (U L^{-1})	312.97 (244.67)	242.82 (144.35)	0.004
Catalase (U L^{-1})	1.16 (1.68)	1.31 (0.32)	0.059
Interleukin 6 (pg mL^{-1})	1.79 (1.41)	0.51 (0.62)	<0.001
Tumor Necrosis Factor α (pg mL^{-1})	8.13 (7.88)	4.77 (3.89)	<0.001

* Mann Whitney U

Table 3. Predictors of oxidative stress and proinflammatory cytokines in the exposed group

Variable		β	95% CI		P-value
Independent	**Dependent**		**Lower**	**Upper**	
MDA	Age Group (>30 vs 30-40)	1.33	0.066	2.61	0.039
	Urine Hg	0.123	0.069	0.178	<0.001
SOD	BMI Group (Underweight vs Normal)	182.22	54.21	310.23	0.006
	Urine Hg	4.22	1.51	6.92	0.003
Cat	Shift Work (No vs Yes)	-1.39	-2.72	-0.057	0.041
	Urine Hg	0.094	0.041	0.146	0.001
IL-6	Urine Hg	0.028	0.12	0.45	0.001
TNF-α	Urine Hg	0.145	0.056	0.233	0.002

predictor of oxidative stress and proinflammatory cytokines based on a multiple backward linear regression. As the results showed, an increase for 1 mg of mercury in the urine was followed by significant changes in the oxidative stress and proinflammatory cytokines. The regression analysis results (Table 3) show that a 1 mg L^{-1} of increase in urine mercury was followed by about a 12% of the increase in MDA level. Also, any 1 mg of increase in urine mercury showed to be followed by a four-fold increase in SOD. Similarly, any 1 mg of increase in urine mercury was found to predict a 14% of the increase in TNF-a. However, the same amount of increase in mercury showed to a predict 9% and 2% of increase in CAT and IL-6, respectively. These were the lowest levels of predicted variance in the present findings.

3.3. Discussion

The overall findings showed that among the chloralkal unit workers, the levels of oxidative stress and proinflammatory cytokines were higher in the exposed group than the control. All the variables except for the catalase were significantly different between the two groups (Fig.5). These findings point to the increase in oxidative stress and body immune responses in this population. The maximum permitted level of mercury in blood and urine is 3 and 4-5 mg L^{-1}, respectively [30]. The present findings, however, showed that the mercury concentration was more than these limits in the sampled population. Similarly, in their research, Neghab et al. found a higher (than the standard level) concentration of mercury in the exposed group, and they found a statistically significant difference between the two groups with this regard. This study not only measured and compared the mercury concentration but also the oxidative stress and proinflammatory cytokines [22]. Different mechanisms have been suggested to explain the biological toxicity of mercury, such as the oxidative stress and inflammatory mechanisms. Yet, the precise mechanism of producing ROS and inflammatory mediators by mercury is unknown. Oxidative stress is a primary lead-induced mechanism. The present findings attested to the capability of mercury to generate free oxidative species through increasing the level of LPO. MDA is a main product of non-oxidized unsaturated fatty acids. An increase in MDA content is a key indicator of LPO [31]. Mahboub et al. investigated this issue and showed that HgCl2 manages to increase the MDA level in tissues [32]. In this research, the MDA level was significantly different between the exposed and non-exposed groups. Moreover, the urine mercury level was a strong predictor of the MDA level. In another study, Hasan et al. showed that the MDA level was significantly increased along with the increased mercury concentration [33].

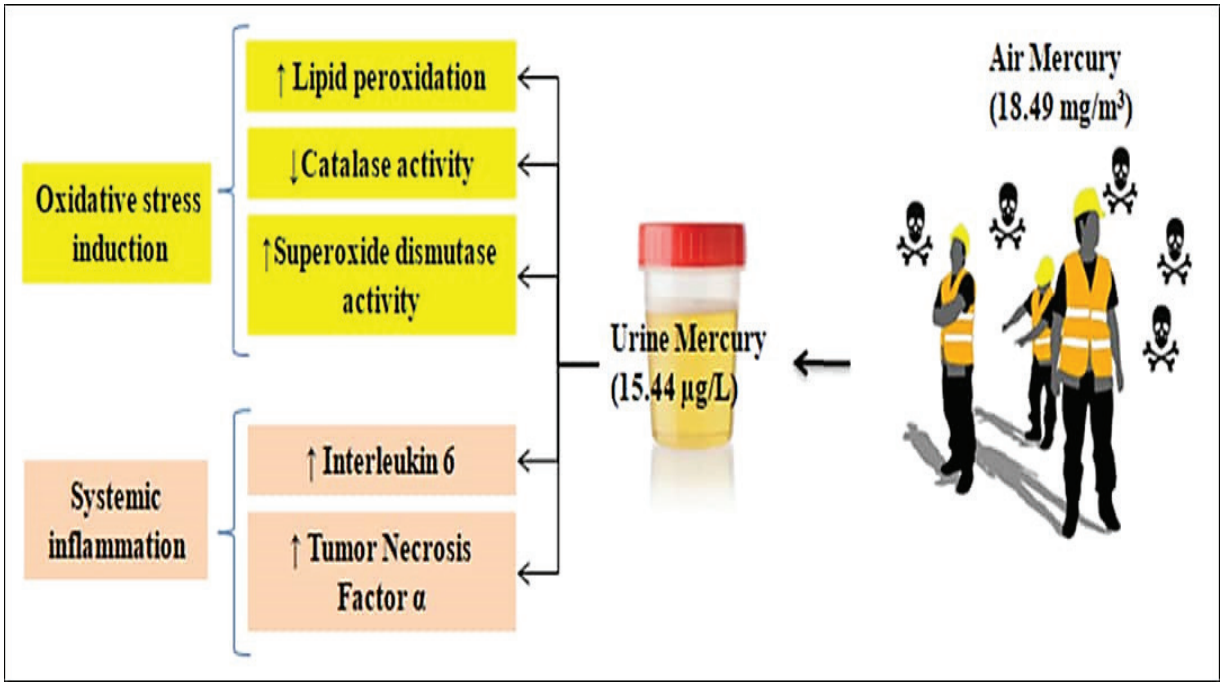

Fig.5. Flow diagram effects of mercury exposure on oxidative stress and proinflammatory cytokines

Lipid peroxidation is a chemical mechanism that can disrupt the structure and functioning of biological membranes by the free radicals attacking the lipids. The higher peroxidation rate of serum lipids in exposed workers to mercury is indicative of serious oxidative damages [31]. As a metallic compound, mercury reacts to thiols (SH-) and leads to chelate antioxidant proteins such as glutathione. Finally, reducing the antioxidant capacity of the tissues induces oxidative stress [34] the overproduction of ROS by mercury indicates its capability of making mitochondrial changes by blocking the mitochondrial permeability transition pore [35]. The Overabundance of ROS induced by mercury correlates to the incidence of neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's, or Alzheimer's. The recent research showed that within the past decades, the toxic effects of mercury have been correlated, probably, with the central nervous system [36]. Several studies, similar to the present research, showed that exposure to lower concentrations of mercury, firstly, induces oxidative stress and increases the number of free oxygen radicals compared to the existing serum antioxidant mechanisms [6, 37]. An increased number of free oxygen species can be one reason for the analysis of proteins, lipid peroxidation, and cellular damage or mortality [23]. As also raised by Neghab et al., mercury can damage cellular membrane through lipid peroxidation and finally disturbs the balance of synthesis and, consequently, leads to enzymatic protein deterioration [22]. The present research also revealed that the level of inflammatory mediators, (e.g. IL-6 and TNF alpha) was significantly higher in the exposed group of workers than the non-exposed. This finding is consistent with Gardner et al.'s epidemiologic investigation of the mercury level among 94 workers exposed to mercury at work. Investigating the level of proinflammatory cytokines (e.g. TNF-alpha and IL-6) in the mercury exposed workers in gold mines showed that the urine mercury level correlates with an increase in IL-1B, TNF-a, and IFN-Y in the gold miner population. Exposure to mercury in these mines can disrupt the immune body and inflammatory systems [40]. Furthermore, the research findings of animal models showed a significant correlation between the mercury level and proinflammatory cytokines such as TNF-a, IL-6, and IFN-y [37-39].

Several empirical and epidemiologic studies showed that mercury level was correlated with different cytokine profiles. Results of the experimental study of PBMC Gardner et al. showed that in the presence of LPS, the antigenic stimulus of non-organic mercury can increase the propagation of proinflammatory cytokines IL-1B and TNF-a. Simultaneously, it reduces the propagation of anti-inflammatory cytokines, IL-1Ra, and IL-10 [40]. Yet, in another study, Monastero et al. aimed to explore the correlation between exposure to a low mercury concentration, immunologic indices, and several cytokines such as TNF-a, IL-10, IL-4, IL-1B, IL-1ra, IFN-y, and IL-17. Results showed that the serum mercury level and the antinuclear antibody (ANA) or cytokine did not correlate in seafood consumers in the U.S. The Association between exposure to low concentrations of mercury and immunologic indices is unknown. Monastero et al. found a high mercury concentration in urine and blood samples of subjects exposed to a low level of mercury. However, in this research, the concentration of mercury in workers' blood and urine exceeded the recommended level [41, 42].

4. Conclusion

The present findings revealed that workers exposed to mercury have significantly more oxidative and inflammatory mediator damages. These observations highlight the essentiality of preventive measures at the workplace and checking the state of pollutants at work. Many studies confirmed that an increase in oxidative stress and inflammatory factors is followed by a higher risk of affliction with other diseases. The mercury in urine samples was determined based on CuNPs@CNT-COOH adsorbent by the MSPE procedure coupled to CV AAS. The absorption capacity of CuNPs@CNT-COOH for mercury was achieved at 167.5 mg g^{-1}. Also, the mercury in the air was obtained by the NIOSH method. The recovery and RSD for mercury extraction in urine were more than 96% and 1.65%, respectively.

5. Conflict of Interest

The authors have declared no conflict of interest.

6. Acknowledgment

The work supported by the Kerman University of Medical Sciences is based on a number of projects PN: 401000242, for mercury determination in air and human urine samples by supervisors.
The ethical code obtained by Kerman University of Medical Sciences (E.C.:IR.KMU.REC. 1400.144).

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