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Research Article

Changes in blood Cr level and its oxidative status among leather tannery workers of District Kanpur in North India

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Abstract
Chromium (Cr) sulfate is used in the tannery industry as a basic tanning agent. Workers are directly exposed to the Cr due to unawareness and no safety protocols. Therefore, the present study aimed to investigate the oxidative stress of Cr in tannery workers of District Kanpur in North India. Two groups of the population were for the study, Group I included 50 directly exposed people employed in tannery industries, whereas Group II included 50 healthy people with no previous exposure to Cr. The concentration of total Cr in blood samples was measured by Inductively coupled plasma mass spectroscopy (ICP-MS). Oxidative status was measured by antioxidant enzyme assays such as Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR). Statistical analysis observed a significant ($p<0.001$) increase of Cr concentration in Cr-exposed Group compared to controls which were not exposed to Cr. The level of CAT (9.73 ± 1.68 u/mg) was significantly ($p<0.001$) higher in Cr-exposed group as compared to control (6.00 ± 0.86 u/mg) while GPx (40.02 ± 5.43 u/mg) and GR (5.29 ± 1.59 u/gHg) concentration is significantly ($p<0.001$) lower in Cr-exposed group compared to control (59.71 ± 5.09 u/mg and 10.77 ± 2.32 u/g Hg, respectively. In Pearson correlation analysis, blood Cr level showed a significant correlation ($p<0.05$) with oxidative status. In Pearson correlation analysis, blood Cr level showed a significant correlation ($p<0.05$) with the oxidative status of the exposed population. The outcome of this study may help the early detection of hazardous impact of Cr on tannery workers, which will be crucial for reducing health risk and exposure.

Keywords: Catalase, Chromium (Cr), Oxidative status, Tannery workers

INTRODUCTION

Cr is considered as one of the most commonly used hazardous heavy metals (Zhu and Costa 2020). It is a component of lead ore (Vauquelin in 1798). Cr (III) salt is commonly used in tannery industry as a tanning agent to strengthen the collagen fibers through the cross-linking action of the tanning agents to bring about permanent stabilization of the skin material that is prone to putrefaction (Li *et al.,* 2016). It is estimated that 20,000 workers are exposed to Cr directly or indirectly in their workplace in North India and 5,58,000 in the United States of America are potentially exposed to Cr and Cr-containing compounds in the workplace and about 3,00,000 workers are seriously affected by the Cr compounds across the globe annually (Annangi *et al.,* 2016). It is possible to assert leather and its corresponding downstream sectors as the world's biggest manufacturing field based on a by-product (Khan *et al.,* 2015). A popular centre for leather production in northern India is Jajmau (Kanpur) in Uttar Pradesh. There are about 400 tanneries in the town that account for a large portion of the untreated water that runs into the river. There is a high concentration of Cr in the wastewater discharged by these factories, which contributes to the extensive pollution of different ecosys-

tems (Gowd *et al.,* 2010; Saxena *et al.,* 2016). Common effluent treatment plant (CETP), Jajmau confirmed that their tannery units do not have primary effluent treatment plants in order. Flow metres, which verify the volume of wastewater flowing from tanneries to the drainage do not operate in most areas (Tare *et al.,* 2003; Ghosh, 2019). The same refers to units of Cr recovery that are not set up in compliance with the Uttar Pradesh Pollution Control Board (UPPCB) guidelines (Gupta, 2014). In some places of Kanpur, the content of Cr in aquifers and drinking water is 250 times greater than the WHO allowable limits (0.05 ppm) (CPCB, 1997).

Cr can harm the body by reaching with internal organs through breathing, oral consumption or by cutaneous contact, so this element can be exposed to tannery workers as well as the population living in the industrial tanning region, mostly in the inorganic form of Cr (III) or in the protein-bound form (leather dust). Cr (III) is poorly transported across the membrane, while Cr (VI) can easily cross cell membranes through non-specific anion carriers in neutral aqueous solutions (Danielsson *et al.,* 1982; Vincent, 2013). Cr (VI) is metabolically reduced within the cell to metastable Cr (V), Cr (IV) as well as stable Cr (III) species (Dayan and Paine 2001). The reduction intermediates such as Cr (V) and Cr (III) are capable of coordinating covalent interactions with macromolecules such as DNA, RNA, protein and lipids, while Cr (VI) is unable to react with macromolecules. Cr (III) binds to DNA, leading to a decrease in DNA polymerase fidelity and increase in its processivity that can eventually lead to increased mutations (Estamond *et al.,* 2008). In the presence of biological reductants, Cr (III) decreases to Cr (II) producing oxidative stress (Ozawa and Hanaki, 1990; Chen *et al.,* 2019) and oxidative DNA base modifications such as the synthesis of 8-hydroxyguanosine (8-OH-dG; Snow, 1991; Chaudhary *et al.,* 2005; Wise *et al.,* 2019). The freshly developed Cr (II) responds to hydrogen peroxide to create a hydroxyl radical that causes lipid peroxidation (Setyaningsih *et al.,* 2015). Cells stimulate antioxidant defensive mechanisms in response to lipid peroxidation, where antioxidant enzymes such as CAT, SOD and GSH function synergistically to detoxify the impact of lipid peroxidation (Barber and Harris, 1994; Cheung *et al.,* 2001; Paithankar *et al.,* 2020). Oxidative stress may be found associated directly or indirectly with various pathways, such as autophagy failure, apoptosis resistance, chronic inflammation, gene mutation and epigenetic changes of carcinogenesis and toxicity. Most of these processes have been involved in carcinogenesis induced by Cr (VI) and have the ability to be induced by Cr (III) (Wang *et al.,* 2016). The objective of the present study was, therefore, to assess the hazardous impact of Cr (III) with special regard to its oxidative status in tannery workers in Kanpur district of North India.

MATERIALS AND METHODS

Location

This study was carried out at the Chaudhary Ehsan Kareem Hospital, located near the tannery industry in Jajmau area of Kanpur, Uttar Pradesh, India (Fig.1). There are several tannery industries located in this region that produce many toxic wastes during bating pickling, tanning, colouring, shaving, buffing, and finishing process leather production. These toxic wastes are treated by low capacity Common Effluent Treatment Plants (CFTP). Jajmau CFTP can treat only 9 million litres per day (MLD) discharges from tanneries while approximately 50 MLD is discharged measured from all tanneries in Jajmau, according to the Central Leather Research Institute survey (2012). Therefore, water, soil and air of the nearest area are polluted by high Cr concentrations emitted from these factories (Roy, 2012). Control participants with no known background of Cr toxicity and the same age group (18-55 years of age) were chosen from the general population of an adjacent district Lucknow.

Samples

The study design included a total population of 100 human beings divided into two groups. Group I included 50 Cr-exposed tannery workers with a mean age of 33.11±13.99 years and Group II included 50 control individuals with no history of exposure to Cr. The criterion for including volunteers in the study was average daily exposure of at least 6 hrs/day for not less than 2 years.

Questionnaire

The questionnaire determined the participants' personal data, occupational and medical history, including age, sex, marital status, alcohol, smoking, type and place of work, duration of employment and history of chronic diseases, as the part of the study. Exogenous free radical growth could be a result of exposure of an individual to environmental pollutants, certain drugs and heavy metals, cigarette smoke, alcohol and radiation (Pizzino *et al.,* 2017). Therefore, this questionnaire was important to identify the association between oxidative status and personal characteristics of individuals.

Laboratory investigations

Collection

According to the Recommendations of the Human Ethical Committee of the University of Lucknow (LU/IEC/ZOOL/2020/11/04), qualified paramedic workers obtained blood samples (5mL) in heparinized tubes from Cr-exposed and unexposed persons attending the outpatient clinic at Chaudhary Ehsan Kareem Hospital, Jajmau, Kanpur. All subjects' individuals participating in the proposed study were briefed about the essence of

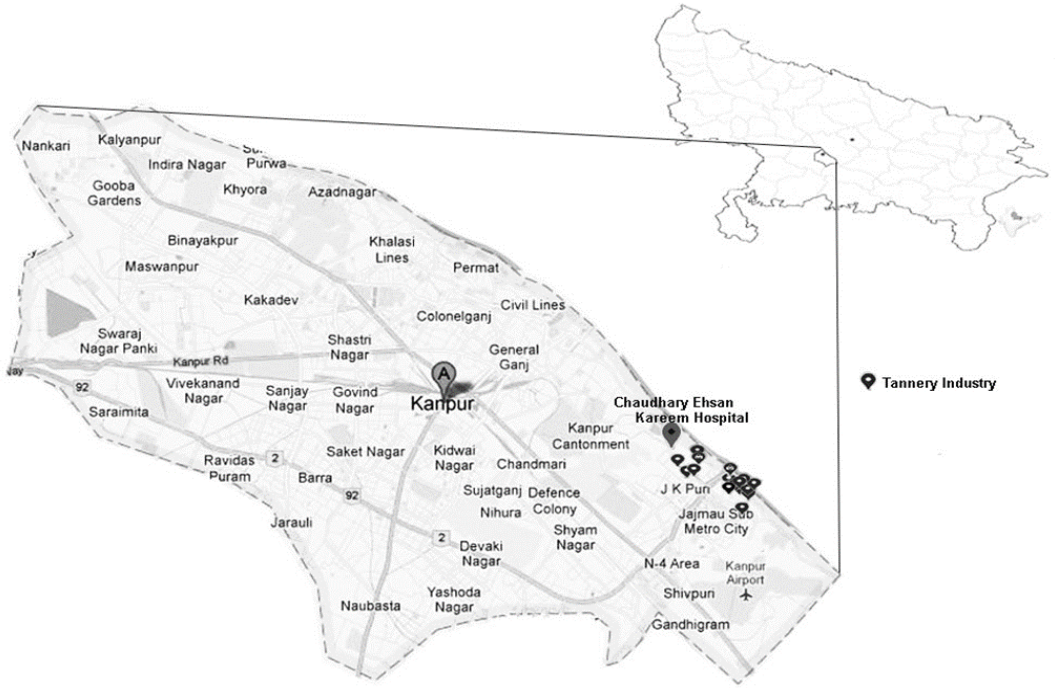

Fig. 1. *Satellite map of Kanpur city with the pointers showing specific location of the tannery industries.*

the study. Written consent of the volunteers was obtained and submitted to the Institutional Ethics Committee of the University of Lucknow.

Blood Cr analysis

Total blood Cr content was measured at the Central research facility of IIT- Delhi, using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) using Agilent Technologies' 7900 ICP-MS process. 0.5 ml of blood samples drawn from all the participants were used to assess Cr concentrations and the results were measured in mg/l.

Erythrocyte lysates preparation

At 2789 rpm (1000 x g) for 10 minutes at 4°C, the EDTA blood was centrifuged. Without disrupting the white buffy coating of WBCs, the upper yellow plasma was taken out. The upper yellow plasma was taken out without interrupting the white buffy coating of the WBCs. Plasma was processed at -80 ° C for biochemical analysis, although the white buffy coating was discarded. The residual lower layer contained RBCs that were lysed with four times their volume of water ice-cold HPLC-grade at 4 °C and centrifuged for 15 minutes at 10,000 rpm (12857 x g). For antioxidant enzyme assays, the supernatant was used. Blood lysate's protein content was measured using the Bradford method (1976).

Catalase (CAT)

The CAT function was estimated with minor modifications by the Spectrum-photometric method as defined (Aebi *et al.* 1984), and H_2O_2 decomposition was estimated at 240 nm. The 0.01M phosphate buffer reaction mixture (pH-7.0), 0.02M H_2O_2 and 20 µl blood lysate were thoroughly mixed according to this 3process and absorption was reported for 3 minutes at 240 nm per 30 seconds. The amount of the enzyme decomposing 1 µmol of H_2O_2 per min at 37°C was described as one unit of CAT. The outcomes were expressed in units/mg protein.

Glutathione peroxidase (GPx)

The activity of glutathione peroxidase was determined by an enzyme kinetic method, according to Flohe and Gunzler (1984). In brief, 1.34 mM GSH (Sigma) and 1.33 U/ml GR (Sigma) were prepared at pH 7.0, containing 1.1 mM EDTA and 1.1 mM NaN3, in a potassium phosphate buffer. In the prepared buffer, a lysate of 1.5 g Hb/l was added. The final concentrations of GSH and GR were 0.94 mM and 0.93 U/ml, respectively. To achieve a final concentration of 0.4 mM and 0.25 mM respectively, NADPH (Sigma) and H_2O_2 were applied. The NADPH reduction was estimated at 340 nm per 30 seconds. The outcomes were expressed as U/g Hb.

Glutathione reductase (GR)

GR operation was measured using the Goldberg and Spooner method (1983). GR reproduces reduced glutathione (GSH) from oxidised glutathione (GSSG) and the role of this enzyme was estimated by the development of NADP from NADPH during the decrease in GSSG. During GSSG reduction, oxidation of NADPH to NADP was reported at 340 nm. The conclusions were

presented as U/g Hb.

Statistical analysis

Obtained data were entered and tested for any anomalies with the Microsoft Excel database software. The findings were described in terms of percentages, mean ± SD (standard deviation). Using the unpaired t-test, two mean values were matched, and the chi-square test was used for comparing dichotomous/categorical variables. The 2-way ANOVA followed by Bonferroni t-test was used to study the influence on oxidative stress parameters of smoking and alcohol intake patterns. Pearson's analysis was conducted to determine the interaction pattern of Cr level with oxidative stress. All tests were performed with the SPSS version -16.0.

RESULTS

The demographic characteristics of the subjects are shown in table 1. No statistically significant differences were found between age, alcohol consumption and smoking habit. We observed a significant difference concerning marital status when we compared to controls and Cr-exposed Group.

Cr Concentration

Group I (Exposed to Cr) showed significantly ($p < 0.001$) higher Cr concentration (71.99 ± 15.71 µg/l) in their blood in comparison to Group II (Control) (13.65 ± 5.80 µg/l). Cr concentrations in blood of smoker and nonsmoker were 18.37 µg/l and 12.48 µg/l respectively in Group II which was significantly ($p < 0.05$) lower to 65.81 µg/l and 74.64 µg/l in smoker and nonsmoker of Group I groups respectively. Cr concentrations of alcoholic and nonalcoholic were 18.09 µg/l and 11.93 µg/l respectively in Group II which was significantly ($p < 0.05$) lower to 70.67 µg/l and 72.41 µg/l in alcoholic and nonalcoholic Group I respectively (Table.2).

Catalase concentration

CAT activity was found significantly ($p < 0.001$) higher (9.73 ± 1.68) in the Group I as compared to group II (6.00 ± 0.86). CAT concentration in smoker and nonsmoker of Group II was found to be 5.78±1.39 u/mg and 6.00±0.87 u/mg respectively, which was significantly ($p<0.001$) increased to 9.70±1.76 u/mg and 9.85±1.88 u/mg in smoker and nonsmoker group II respectively. While the concentration of CAT was found to be 5.50±1.06 u/mg and 6.03±0.86 u/mg in alcoholic and nonalcoholic of group II respectively, which was significantly ($p<0.001$) increased to 9.75±0.57 u/mg and 9.72±0.25 u/mg in alcoholic and nonalcoholic Group I respectively. CAT concentration showed significant ($p>0.05$) positive correlation ($r = 0.98$) within Cr level after adjustment of smoking status and alcohol consumption (Table 3).

Glutathione peroxidase (GPx) concentration

GPx concentration was observed to be significantly ($p < 0.001$) higher in Group II (59.71 ± 5.09) relative to Group I (40.02 ± 5.43). GPx concentration in smoker and nonsmoker of group II was found to be 61.44±3.55 u/mg and 60.23±3.26 u/mg respectively, which was significantly ($p<0.001$) decreased to 41.02±3.11 u/mg and 40.04±3.21 u/mg in smoker and nonsmoker Group I respectively. While the concentration of GPx was found to be 60.07±3.05 u/mg and 61.38±3.89 u/mg in alcoholic and nonalcoholic of Group II respectively, which was significantly ($p<0.001$) decreased to 40.11±1.82 u/mg and 39.99±0.84 u/mg in alcoholic and nonalcoholic Group I, respectively. GPx concentration showed a significant ($p>0.05$) negative correlation ($r = -0.99$) with the Cr concentration after adjustment of smoking status and alcohol consumption (Table 3).

Glutathione reductase (GR) concentration

GR concentration was observed to be significantly ($p < 0.001$) higher in Group II (10.77 ± 2.32) relative to Group I (5.29 ± 1.59). GR concentration in smoker and nonsmoker of group II was found to be 11.25±2.09 u/g Hg and 10.45±2.35 u/g Hg respectively, which was significantly ($p<0.001$) decreased to 5.83±1.87 u/g Hg and 6.02±1.23 u/g Hg in smoker and nonsmoker group I respectively. While the concentration of GPx was found to be 11.13±2.56 u/gHg and 10.41±2.14 u/g Hg in alcoholic and nonalcoholic of group II respectively, which was significantly ($p<0.001$) decreased to 6.23 ± 0.49 u/gHg and 4.99±0.23 u/g Hg in alcoholic and nonalcoholic Group I respectively. GR concentration showed a significant ($p>0.05$) negative correlation ($r = 0.98$) with the Cr concentration after adjustment of smoking status and alcohol consumption (Table 3).

DISCUSSION

In India, about 2200 tanneries are located; out of which nearly 80% are engaged in the tanning process and remaining 20% are involved in non-tanned products production (Goel, 2014; Roy, 2012; Singh and Rajamani, 2011). Two methods of tanning are used all over the world: Vegetable tanning and Chrome tanning (Krishnamoorthy *et al.,* 2012, Dixit *et al.,* 2015). Chromium tanning is a major method of tanning process in India (GCPC, 2015). Workers directly or indirectly remain exposed to Cr while working. The main route of exposure of tannery workers to Cr is either via. inhalation or oral ingestion of polluted water and foodstuff at the factory or by cutaneous contact during the processing of tanned leather (NTP, 2008, Wilbur *et al.,* 2012, Junaid *et al.,* 2016).

There are two stable Cr forms viz., Cr (III) and Cr (VI) (Wilbur et al., 2012). Cr (III) is impermeable to the cellular membrane but by diffusion or phagocytosis, it can

Table 1. Personal characteristics of the individuals of control and Cr-exposed groups.

Variables	Control group (N=50)	Cr-Exposed Group (N=50)	P value
Age (yrs.)	32.40 ± 9.63	33.96 ± 13.21	0.50[1]
Marital Status			
Married (%)	22(44)	35(70)	0.008[2]**
Single (%)	28(56)	15(30)	
Alcohol Consumption			
Yes (%)	14(28)	12(24)	0.20[2]
NO (%)	36(72)	38(76)	
Smoking			
Yes (%)	10(20)	15(30)	0.24[2]
No (%)	40(80)	35(70)	
Years of exposure	-	11.02±8.47	

[1]Unpaired-t test, [2]Chi-square, **: Highly statistically significant

Table 2. Cr concentration in the blood of the control and Cr-exposed Group (Statistics used ANOVA- Analysis of variance).

Characteristics	Control group (Mean±SD) (N=50)	Cr-exposed Group (Mean±SD) (N=50)	ANOVA p values
	Cr Concentration (µg/l)		
Total Cr concentration of groups	13.65 ± 5.80	71.99 ± 15.71	
Smoker	18.37 ± 6.34	65.81 ± 11.47	P < 0.0001
Nonsmoker	12.48 ± 5.09	74.64 ± 16.66	
Alcoholic	18.09 ± 5.69	70.67 ± 16.05	P < 0.0001
Nonalcoholic	11.93 ± 4.92	72.41 ± 15.80	

enter into the cells and eventually solubilize in lysosomes to release Cr^{3+} ions (Eskin 2016). Synthetic Cr (III) complexes with hydrophobic ligands promote chromium penetration through the plasma membrane, is another form of Cr (III) absorption (Warren *et al.,* 1981). Cr (VI) is absorbed more efficiently through the sulfate anion channel transport system than Cr (III) (Zhitkovich, 2005; Chen *et al.,* 2019). Cr (VI) reduces to Cr (III) inside the cell. Further, in presence of bioreductants which reacts with hydrogen peroxide to produce hydroxyl radical through a Fenton type reaction, reduces Cr (III) to Cr (II) (Chen *et al.,* 2019; Shi *et al.,* 1999). Hydroxyl radicals can target lipid proteins, DNA and membranes, thereby interfering with cellular functions and integrity (Bagchi *et al.,* 1997). In the previous study, Katiyar *et al.* (2008), Khan *et al.* (2012) and Ambreen *et al.* (2012) found that total blood chromium concentration was higher in the tannery workers of Jajmau area of Kanpur District in North India. Zhang *et al.* 2014 and Junaid *et al.* (2016) have similarly found higher concentrations of chromium in tannery workers of China and Pakistan, respectively.

In the present study, we observed that increased concentration of Cr (71.99 ± 15.71 µg/l) significantly ($p<0.001$) altered the antioxidant property of Cr-exposed Group. An exposure limit of 0.5 mg/m^3 (8–hour workday) for Cr (II) and Cr (III) in the air is recommended by National Institute for Occupational and Safety Health (NIOSH). NIOSH also recommends an exposure limit of 0.001 mg/m^3 (10-hour workday) for airborne Cr (VI) (ASTDR, 2012). The findings of this study are consistent with another recent study on tannery workers of Jajmau area of Kanpur district in North India that found high levels of total Cr (167.58 ± 23.44 µg/l, 157.59 ± 29.20 µg/l) (Ambreen *et al.,* 2012; Khan *et al.,* 2012) and hexavalent Cr (42.35 ± 0.28 µg/l; Qureshi *et al.,* 2016) recommended by NIOSH in the Cr-exposed Group. The concentration of Cr in the serum of employees from tanning and re-tanning division was estimated to be greater than in workers from Mexico's dying, drying and finishing sectors (Kornhouser *et al.,* 2002).

Table 3. Summary of statistical analysis of catalase (U/mg), GPx (U/mg) and GR (U/gHb) and Pearson correlation coefficient (r) of Cr with catalase, GPx and GR.

Oxidative parameters of Cr		Control group (N=50)	Cr-exposed group (N=50)	t-Value, p- significance	Pearson correlation coefficient(r)
Catalase	Smoker	5.78±1.39	9.70±1.76	6.38***	0.98*
	Nonsmoker	6.00±0.87	9.85±1.88	12.10***	
	Alcoholic	5.50±1.06	9.75±0.57	6.24***	0.98*
	Nonalcohlic	6.03±0.86	9.72±0.25	12.43***	
GPx	Smoker	61.44±3.55	41.02±3.11	9.34***	-0.99*
	Nonsmoker	60.23±3.26	40.04±3.21	15.98***	
	Alcoholic	60.07±3.05	40.11±1.82	9.17***	-0.99***
	Nonalcohlic	61.38±3.89	39.99±0.84	16.31***	
GR	Smoker	11.25±2.09	5.83±1.87	5.86***	-0.97*
	Nonsmoker	10.45±2.35	6.02±1.23	12.41***	
	Alcoholic	11.13±2.56	6.23±0.49	5.52***	-0.97*
	Nonalcohlic	10.41±2.14	4.99±0.23	13.13***	

***p < 0.0001, *Statistical significant (p ≤0.05), r- simple correlation coefficient

Cr (III) is capable of generating free radicals from both hydrogen peroxide and lipid hydroperoxides, which could have important consequences for the mechanism of carcinogenesis caused by chromium (Chen *et al.,* 2019; Shi *et al.,* 1999). Antioxidant mechanisms are triggered in exposed cells in response to oxidative stress and lipid peroxidation. In the present study, CAT, GPx and GR were observed as the three parameters to test the mechanism of antioxidants in response to Cr. We found significantly (p<0.001) higher CAT concentration in Group I as compared to Group II. Similarly, Xu *et al.* (2018), found a higher CAT concentration in exposed Group relative to the non-exposed Group of North China. Our results are also in the same line where CAT shows a significant positive correlation with the Cr concentration after adjustment of smoking status and alcohol consumption. They further observed a positive correlation with the increase of Cr (VI) concentration after adjustment of smoking status and alcohol consumption in North China population. Khan *et al.* (2012) tannery workers of Jajmau area in Kanpur district of North Indian population, found a positive correlation of Cr concentration with antioxidant enzymes GSH and SOD after adjustment of smoking status. A higher concentration of CAT enzyme in Group I suggests a higher concentration of hydrogen peroxide. CAT removes the hydrogen peroxide from the cell and protects against oxidative damage to the cell. We observed that the activity of antioxidant enzymes GPx and GR were significantly (p<0.001) lower in Group I as compared to Group II. Many reports have shown that the activity of GPx was higher in the occupational Cr-exposed populations of North China (Xu *et al.,* 2018), while some have shown that the activity of GPx is decreased in the occupational Cr-exposed Group (Wang *et al.,* 2012). We also observed a significant negative correlation with the Cr concentration after adjustment of smoking status and alcohol consumption. The previous study also showed an interaction effect on GPx with Cr (VI) exposure, smoking and alcohol consumption (Xu *et al.,* 2018). In the present study lower activity of GPx and GR were found significant (p<0.001) to Group I as compared to Group II, also indicates an increased level of oxidative stress in Cr exposed population. In the recycling of GSSH back to GSH, GR is an important enzyme. Similarly, Akhter *et al.* (2012) found that antioxidant molecule GSH and the activity of antioxidant enzymes SOD, CAT, GPx, and GR were significantly lower in ZnO NP-treated cells. The function of GPx during detoxification of organic hydrogen peroxides by GSH may provide useful clues about the intake rate of GSH.

In a previous study, Ambreen *et al.,* (2014) found no significant statistical difference between age, marital status vs alcohol consumption habit whereas they found a significant difference between smoking habit in control vs Cr-exposed groups. But, in the current study, we have found no statistically significant difference between age, alcohol consumption habit vs smoking status but there was a significant difference with the marital status (p<0.05) when compared to control and Cr-exposed groups. The findings also showed that Cr exposure impacts the antioxidant system, such as by stimulating or destroying the antioxidant system as shown by Junaid *et al.* (2016). Besides, oxidative status

was influenced by smoking and alcohol consumption (Khan *et al.,* 2012; Xu *et al.,* 2018).

Conclusion

In the present study, we found significant higher Cr concentration in the Cr-exposed group workers of tanneries located in tannery workers of Jajmau area of District Kanpur in Northern India. Besides, we found higher CAT concentration and low GPx and GR concentration in Cr-exposed Group. The study also showed a positive correlation between Cr and CAT whereas a negative correlation between GPx and GR. Lifestyle such as age, marital status alcohol consumption and smoking status may also be responsible for the alteration of oxidative stress parameters. Therefore, a simple correlation analysis was carried out with modified potential confounders. The present study suggests that the interaction of Cr and alcohol, or Cr and smoking, may affect the antioxidant system. Further studies are required to explore the harmful effects of Cr on tannery workers. The early detection of Cr in tannery workers would help reduce their health risk and exposure to Cr.

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Conflict of interest

The authors declare that they have no conflict of interest.

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