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OXIDATIVE DNA DAMAGE AND MODULATION OF P53 TUMOR SUPPRESSOR GENE AS POSSIBLE MECHANISMS OF CHROMIUM CARCINOGENICITY (PART II)

By

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Abstract

Introduction: Chromium, like many transition metal elements, is essential for life at low concentrations yet toxic to many systems at higher concentrations.Toxic effects of chromium can be classified into sensitizing, caustic and carcinogenic effects. Chromium is one of the best known sensitizing agents; it causes allergic dermatitis, allergic rhinitis and occupational asthma. There is sufficient evidence of the carcinogenicity of various chromium compounds in humans encountered via inhalation in industrial applications. **Aim of Work:** To assess the health effects and carcinogenic potential of chromium exposure in tannery workers with special emphasis on the different job categories for better and detailed evaluation of adverse effects and for more targeted efforts of safety and prevention. **Materials and Methods:** This work was carried out in twelve small-to medium-sized tanneries, in Misr Al Kadema district in Cairo. The study comprised of two groups, an exposed group (45 male workers) and a matched control group (30 male workers). Equal number of workers (15 workers) representing the different industrial stages of the tanning process were studied in three subgroups of exposed workers, named as preparation, tanning and finishing subgroups. All workers were subjected to a detailed history including present, past, family and occupational history. Clinical examination was performed with special emphasis on chest and skin examination. Laboratory investigations were performed in the form of kidney function test (blood urea and serum creatinine), blood level of Total Anti-oxidant Capacity (TAC), P53 and

chromium. Also, urinary 8-hydroxydeoxyguanosine (8-OHdG) was done. **Results:** A significant increase of chromium level coupled with a significant decrease of TAC was found in the tanning subgroup of exposed workers. However, there was non-significant difference as regards p53 and urinary 8-OHG between the three subgroups. **Conclusion:** Our results revealed that workers in the tanning job category have increased level of blood chromium, and this increase is associated with oxidative stress damage especially oxidative DNA damage reflected by significant decrement of TAC serum level and increase (non significant) in urinary 8-OHG.

Key words: Chromium, Total Anti-oxidant Capacity (TAC), urinary 8-hydroxydeoxyguanosine (8-OHdG) , p53 and Carcinogenic effect.

Introduction

Tanning is the chemical process that converts animal hides and skin into leather. The term hide is used for the skin of large animals e.g. cows or horses, while skin is used for that of small animals e.g. sheep. Hides and skin are mostly by products of slaughterhouses, although they may also come from animals that have died naturally or have been hunted or trapped (Baker, 1998).

Tanning involves a complex combination of mechanical and chemical processes. The heart of the process is the tanning operation itself in which organic or inorganic materials become chemically bound to the protein structure of the hide and preserve it from deterioration. The substances generally used to accomplish the tanning process are chromium or extracts from bark of trees, such as chestnut.These tanning agents give rise to the two predominant types of tanning operations chrome and vegetable tanning (Bacardit et al., 2014)

Indeed, 90 per cent of the world's leather is chrome tanned. Chrome tanning has a strong impact on the environment due to the pollution of wastewater and the difficulty to get rid of the solid waste containing chrome. A great variety of research has been carried out in order to explore how to minimize this impact, including work on: recycling of pickle-tanning floats (Thanikaivelan et al., 2004), management of solid waste containing chrome (Muralidharan et al., 2001) and processes with high-exhaustion floats (Morera et al., 2007).

Leather tanning and finishing process can be divided into three stages, as shown in figure (A) (Marshall and Rutland, 1996, Sundar et al., 2013 and Bacardit et al., 2014):

The beamhouse operation (preparation): they include trimming, soaking, fleshing, and unhearing. Tanyard processes (tanning) which include bating, pickling, tanning, wringing, and splitting and Finishing processes include conditioning, staking, dry milling, buffing, spray finishing, and plating.

The generic tanning process involves several steps, depending on the type of skin used and its desired end product:

1-Curing: this process involves salting and/or drying the skin once it's been stripped from the dead animal. This is an immediate step upon removal. Skins can be cured in one of two ways:

A-Wet salting: salting the skin and then piling many skins together until a moist bunch is formed. Skins are then left for a month allowing the salt to be completely absorbed.

B-Brine curing: more common than wet-salting since it is considered faster and easier. During brine-curing, skins are placed in vats and smothered with a mixture of salt and disinfectant. This process takes up to 16 hours for the skins to be completely cured to proceed to the next stage.

2-Soaking: Once cured, the skins are then soaked in water for several hours to several days. The water aids in the removal of salt, dirt, debris, blood and excess animal fats.

3-Hair Removal: At this stage the animal hair is still present. The skins are transported to another large vat and immersed in a mixture of lime and water, which loosens the hair from the skin. After soaking from 1 to 10 days, the hair is then mechanically removed from the skin.

4-Scudding: Any stray hairs and fat which were missed are removed with a plastic tool dull knife or by hand.

5-Deliming: After the hair, debris and excess fats has been cleaned from the skin, the skins are delimet in a vat of acid. After the lime has been pulled from the skin, skins are then treated with enzymes, which smooth the grain of the leather allowing for a product that is soft and flexible.

6-Skins, finished leather or sole leather.

Vegetable tanning: skins are tanned with a vegetable tanning agent solution to produce flexible, but stiff leather. This process involves stringing the skins on large frames, situated in large vats, and exposing them to tannin, a natural product found in wood, bark, leaves and fruits from oak, chestnut and hemlock trees. Skins are treated repeatedly and soaked in a stronger solution of tanning. This type of leather is used in luggage, furniture, leashes, belts, hats and harnesses.

Mineral tanning (Chromium): The most common tanning type in the world. Mineral or chrome tanning is carried out on skins needed for softer, stretcher leathers. Skins are pickled first in an acid and salt mixture then soaked into a chromium-sulphate solution. A faster process than vegetable tanning since it usually takes 1 day. This type of leather is found in purses, bags, briefcases, gloves, shoes, boots, pants, jackets, and sandals.

7-Dyeing process: Depending on the desired product, the skins go through a dying process which involves replacing moisture back into the skin. skins that have been vegetable tanned are bleached and then soaked with oils, soaps, greases and waxes to make them more pliable.

8-Rolling: This involves running the skins through a machine to produce leather that is firm and stronger. After the rolling process, the leather is stretched and dried out in a heat controlled room.

9-Finishing compound: This is the final step in the tanning process. The process involves covering the grain surface with a chemical compound and then brushing it. Light leathers are buffed and sandpapered to cover any imperfections. Leathers that have been buffed for long periods of time become suede. To make the leather more appealing to the buyer, waxes, pigments, dyes, oils, glazes and other solutions are added to the leather.

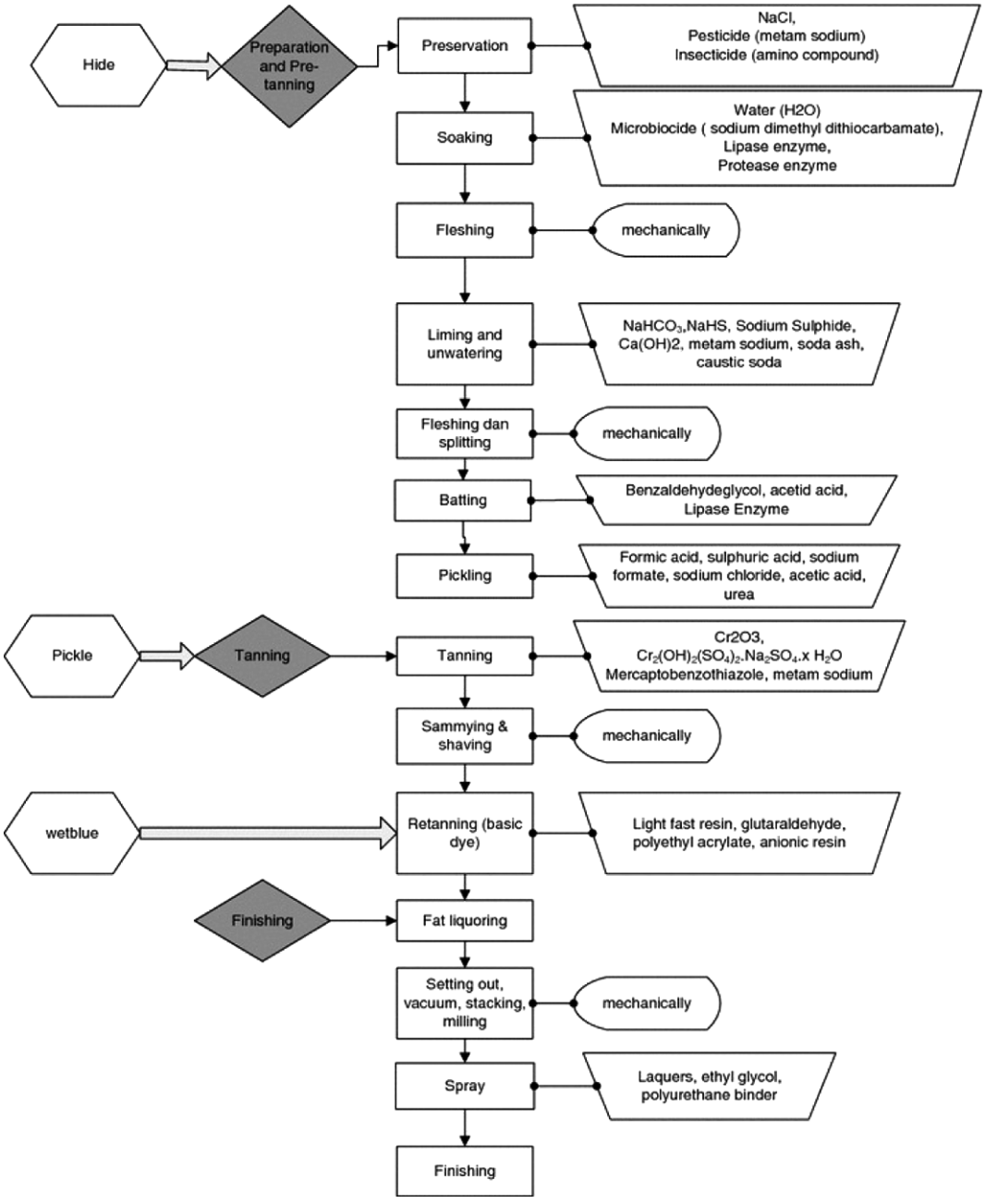

Figure (A): The tanning process (Marshall and Rutland, 1996)

Employment in the leather and leather tanning industry has been associated with various diseases caused by biological, toxicological and carcinogenic agents.Chromium (III) and (VI) salts are used in tanning stage with other chemicals such as ammonium chloride, sodium sulfide(Stern, 1998 and Boonyaratanakort, 2000).

Chromium emissions may occur from chromate reduction, handling of basic chromic sulfate powder, and from the buffing process, dust containing chromium may be emitted during storage, handling, and mixing of the dry chromic sulfate (Marshall and Rutland, 1996).

Chromium, like many transition metal elements, is essential to life at low concentrations yet toxic to many systems at higher concentrations. As a general rule chromium (VI) is much more toxic than chromium (III) (Dayan and Paine, 2001). Chromium (VI) enters many types of cells and under physiological conditions can be reduced by hydrogen peroxide (H2O2), glutathione (GSH) reductase, ascorbic acid and GSH to produce reactive intermediates, including chromium (V), thiylradicals, hydroxyl radicals and ultimately, chromium (III). Any of these species could attack DNA, proteins, and membrane lipids, thereby disrupting cellular integrity and functions (ATSDR, 2008).

Effects of chromium on the skin include ulcerations, dermatitis, and allergic skin reactions. Inhalation of hexavalent chromium compounds can result in ulceration and perforation of the mucous membranes of the nasal septum, irritation of the pharynx and larynx, asthmatic bronchitis, bronchospasms and edema (Antonini et al., 2004).

It is widely recognized that hexavalent chromium is a carcinogen and that exposure significantly increases the risk of respiratory tract cancer. Moreover, Cr (VI) causes chromosome aberrations, sister chromatid exchanges, gene mutation and cell death (Kuo et al., 2003).

Aim of Work

To assess the health effects and carcinogenic potential of chromium exposure in tannery workers with special emphasis on the different job categories for better and detailed evaluation of adverse effects and for more targeted efforts of safety and prevention.

The possible mechanisms of chromium carcinogenicity will be studied using 8-hydroxydeoxyguanosine (8-OHdG) which is one of the major oxidative DNA adducts, TAC (total antioxidant capacity) as a biomarker of cellular oxidative stress and p53 as apoptotic regulatory protein.

Material and Methods

Study design: it is a case-control study.

Study place and duration: This work had been conducted at twelve small-to medium-sized Egyptian tanneries, in Misr Al kadema area in Cairo, an area which contains most of the private sector for leather tanning plants in Egypt. The work was performed during the period from June 2012 to June 2014.

Study sample: The exposed group comprised forty five male workers that fulfilled eligibility for inclusion. The control group comprised thirty male workers randomly selected from administration and security personnel from an insurance company and never occupationally exposed to tanning and fulfilled eligibility for inclusion. The control group was matched to the exposed group as regards age, sex and socioeconomic status. None of the studied personnel were current or previous smokers.

Inclusion criteria for exposed group were working in tanneries for at least the proceeding five years. While exclusion criteria for both exposed and control subjects were occupational exposure to oxidants or genotoxins, smoking, chronic liver or kidney diseases, diabetes or immunological disorders. Also, regular intake of antioxidants or immunosuppressive drugs.

Methods:

Studied groups were subjected to the following:

1-A self designed questionnaire including inquiries about age, sex, occupational history and special habits. Also, present, past and family history were taken.

2-Clinical examination for:

Vital signs: pulse, blood pressure, respiratory rate and temperature.

Eye: redness and tearing

Skin: redness, dryness, cracks and scaring.

Neurological: Mood, intelligence, cooperation, attention, orientation of time, place & persons, equilibrium and sensory affection.

Chest: cough and wheezes.

3-Laboratory investigations:

A- Blood sample collection:

From each subject 10 cc of venous blood were taken through a vein puncture using a dry plastic disposable syringe under complete aseptic conditions. The blood was kept in a tube and allowed to clot then centrifuged for separation of the serum for determination of the following biochemical parameters serum chromium level, serum p53 and TAC (total antioxidant capacity).

B- Urine sample collection:

A urine sample was collected from each subject, in a sterile container for measuring of 8-hydroxydeoxyguanosine (8-OHdG).All subjects washed their hands with soap and water prior to sample collection to avoid contamination.

The samples for blood chromium level were prepared by dilution of 0.5 ml of blood with 2 ml deionized water. The chromium in blood was measured by graphite furnace atomic absorption spectrophotometer (Perkin-Elmer model 5100PC, Norwalk, CT).

Assement of P53 protein in serum using ELISA principle: An anti-human p53 coating antibody is adsorbed onto Human p53 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human p53 antibody is added and binds to human p53 captured by the first antibody. A spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards (www.eBioscience.com).

8-hydroxydeoxyguanosine principle: 8-OHdG ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with a monoclonal antibody specific for 8-OHdG. Standards or samples are then added to the microtiter plate wells and 8-OHdG if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of 8-OHdG present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for 8-OHdG are added to each well to "sandwich" the 8-OHdG immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solutions are added to each well. The

enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain 8-OHdG and enzyme-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm (www.bluegene.cc).

Total Antioxidant Capacity (TAC) principle: Fast colorimetric/ photometric test:The determination of the total antioxidative capacity is based on the reaction of peroxides with peroxidase followed by a color reaction of the chromogenic substrate tetramethylbenzidine. Its blue color turns to yellow after addition of the stop solution and can be measured photometrical at 450 nm (Alternatively kinetic measurements at 600 nm are possible, if end point measurement is not wanted. In case of end point measurement (use of stop solution) samples with absorptions at 450 nm like sera have to be assayed by subtraction of initial absorption. Quantification is achieved by serial dilutions of a standard Antioxidant-solution (www. Idn.de).

Statistical analysis:

Data obtained from the study was coded and entered using the statistical package SPSS version 15. The mean values, standard deviation (SD) and ranges were then estimated for quantitative variables. Qualitative data was expressed as frequency distribution. Comparisons between exposed and control groups were done using Chi Square ($\chi 2$) test for qualitative variables and using the independent simple t-test as well as the analysis of variance (ANOVA test) followed by Post Hoc test for normally distributed quantitative variable. The non-parametrical Mann-Whitney test was used for quantitative variables not normally distributed. Correlations were done to test for the presence of linear relations between quantitative variables. P-values less than 0.05 and less than 0.005 were considered statistically significant and highly significant, respectively.

Consent:

Authors declare that a verbal consent was taken from the studied group,confidentiality was maintained.

Ethical approval:

The study protocol was approved by Occupational and Environmental Department Ethical Committee, Faculty of Medicine, Cairo University.

Results

As regards the demographic characteristics of the study participants (data not presented), they were all males, with no significant difference between the exposed and control groups as regards age. Mean age of exposed workers was 33.07±11.05years [ranging from (17-49) years]. Mean duration of exposure was 15.64 ±7.63 years. The control group mean age was 31.90±10.81years, ranging from (20-55) years.

Table (1): Age and duration of work in the exposed subgroups:

	Preparation N:15	**Tanning N:15**	**Finishing N:15**	**F+**	**P**
Age in years	31.13±12.42	37±9.45	31.07±10.75	1.45	***<0.05**
Duration of work	14.47 ±8.5	17.93± 7.5	14.53±6.7	3.6	>0.05

* Significance ≤0.05.

*F+=Analysis of variance (ANOVA).

As shown in table (1), there was no statistically significant difference between the exposed subgroups as regards duration of work (p>0.05). However as regards age, workers of the tanning stage were the oldest (37.0±9.45), and using ANOVA test, there was a statistically significant difference between the three subgroups (p<0.05).

Table (2): Prevalence of respiratory manifestations among exposed subgroups

	Preparation 15(3 3.3)	**Tanning 15(33.3)**	**Finishing 15(33.3)**	**P**
Allergic rhinitis	1(6.7)	15(100)	0(0)	****<0.01**
Dry cough	6(40)	7(46.6)	7(46.6)	>0.05
Expectoration	6 (40)	7(46.6)	5(33.3)	>0.05
Chronic bronchitis	6(40)	12(80)	12(80)	**<0.05**
Asthmatic attacks	0(0)	13(86.7)	7(46.7)	****<0.01**

* Significance ≤0.05.

** High significance ≤0.01.

Table (2) shows the prevalence of respiratory manifestations among the exposed subgroups. Allergic respiratory manifestations (allergic rhinitis and asthmatic attacks) were significantly higher in the tanning job category of exposed workers, compared to the other two categories (preparation and finishing subgroups). As regards chronic bronchitis equal prevalence was noted in the tanning and finishing subgroups [12(80%)] which was significantly higher than preparation.

Table (3): Non-respiratory manifestations among exposed subgroups

	Preparation N (%)	Tanning N (%)	Finishing N (%)	P
High blood pressure	15(100)	1(6.7)	0(0)	**<0.01
Eye manifestations	10(66.7)	5(33.3)	2(13.3)	>0.05
Skin manifestation (Contact dermatitis)	0(0)	13(86.7)	7(46.7)	**<0.01

* High significance ≤0.01.

Table (3) shows highly significant difference among the three stages of tanning as regards high blood pressure. All exposed workers in the preparation job, one worker in the tanning and no worker in the finishing job had hypertention ($p<0.01$). More than two thirds of the preparation workers complaints of eye manifestation (n=10, 66%). However, the majority of tanning workers (86.7%) and almost half of finishing workers had skin manifestation (46.7%) $p<0.01$.

Table (4): Mean (SD) of kidney function tests, serum chromium, TAC, 8-OHG and P53 in exposed subgroups.

	Preparation (n=15) Mean ±SD	Tanning (n=15) Mean ±SD	Finishing (n=15) Mean ±SD	P
Chromium (µg/L)	0.72±0.39	1.01±0.27	0.52±0.27	****<0.01(a,b)**
TAC(mmol/L)	1.14±0.66	1.11±0.58	1.54±0.47	**<0.05 (b)**
8OHG(ng/mL)	1.67±0.37	1.93 ±0.55	1.59±0.21	>0.05
P53(U/mL)	3.46±2.6	5.15±3.34	5.05±3.65	>0.05
Urea(mg/dl)	34±4.17	33.27±4.92	34.6±4.55	>0.05
Creatinine(mg/dl)	0.94±0.12	0.93±0.13	0.93±0.10	>0.05

a=significance between preparation and tanning groups.

b=significance between tanning and finishing groups.

* Significance ≤0.05

** High significance ≤0.01.

Table (4) shows the results of the different laboratory parameters investigated among the exposed subgroups. Using analysis of variance (ANOVA), there was a statistically significant difference between the three subgroups as regards serum chromium level ($p<0.01$) and TAC ($p<0.05$).Post hock test, showed that as regards serum chromium level, it was significantly higher among the tanning group compared to both the preparation and finishing groups. However, TAC level was only significantly lower in the tanning subgroup compared to finishing subgroup ($p<0.05$).

Discussion

Our study showed statistically significant higher chromium level in blood among workers in the tanning department, compared to other departments ($p<0.001$). This finding agrees with Kornhauser et al., 2002 (2002), who studied three groups of subjects, group (1) included 15 male tannery workers highly exposed to chromium from tanning and retanning departments. Group (2) included 14 male tannery workers with moderate chromium exposure from dying, drying and finishing departments. Group (3) included 11 healthy, male subjects without direct chromium exposure. Higher serum chromium levels were observed in group one compared to the other groups. Urine chromium levels in group (1) were higher than those in controls.

As regards correlation between both age and duration of exposure in tanning industry and chromium level in blood, we found that it was non-significant. This could be explained by the fact that chromium has no cumulative effect in the body.

However, in Muttamara and Leong study in 2004, they assessed the occupational exposure to chromium in alloy manufacture and its health impact; the results demonstrated that blood and urinary levels among workers were associated with increasing age and duration of exposure, Hara andTakahashi (2012) decided that the age at first exposure to chromium may be a more important factor than the duration of exposure as regards increased risk of lung cancer and malignant lymphoma among chromium exposed workers.

In this study, prevalence of respiratory tract disorders is significantly higher among tannery workers as compared to the control group (table 2). The results showed that allergic respiratory manifestations were significantly higher with tanning job category subgroup compared to the other two categories (preparation and finishing subgroups). This agrees with Elhosary and her colleagues in 2014, who found that about one third of cement and tannery workers had severe skin and asthmatic chest manifestations and severe nasal allergic manifestations in 22.7% and 20% of cement and tannery workers, respectively.

Similarly, results of Khan et al., study in 2013 on tannery workers in Pakistan revealed that 15(13%) had skin rashes, 14(12%) had chronic bronchitis and theses results were associated with high blood and urinary chromium levels.

As regards bronchial asthma there was a significantly higher prevalence in tanning subgroup compared to control. This is in accordance with Adams et al.,2006 and Schneideret al.,2012 who observed association between high chromium levels in blood and elevated incidences of asthma.

There was significant difference among the three exposed subgroups, where all preparation and tanning workers subgroups were hypertensive and one worker in tanning subgroup was hypertensive.This observation may be explained by the use of excess salt during curing of the hides in preparation stage. This finding was previously observed by Kripa et al., (2005), whose cross-sectional study was conducted among salt workers in India. They concluded that inhalation of salt particles in salt workers may be an occupational cause of increased blood pressure.

Almost half of the exposed workers (44.4%) complaint of contact dermatitis, which was mainly observed in tanning subgroup followed by the finishing subgroup with no cases in the preparation group. This agrees with Abdel Aziz (1995), who found that the prevalence of contact dermatitis in Asir tanning factory in the Southern region in the Kingdom of Saudi Arabia was (45.2%).

A number of studies have noted that chromium exposure resulted in the up regulation and/or activation of p53 (Carlisle et al. 2000, Russo et al., 2005 andYao et al., 2008).The results were consistent with reactive chromium species as important drivers of that activation.About half of cement and one third of tannery groups expressed high grade of p53 expression in a study done by Elhosaryet al., 2014.

In the present study, statistically significant increase in P53 was detected among exposed group compared to their control. With non-significant difference among the exposed subgroups, and this increase was positively correlated to level of chromium.

Moreover, we observed that there was a significant increase in the level of 8-Hydroxydeoxyguanosine in urine and significant reduction in the serum TAC, this reduction of TAC was only significantly lower in the tanning subgroup compared to other subgroups ($p<0.05$), with non-significant difference among subgroups as regards 8-Hydroxydeoxyguanosine, presence of both parameters indicate a state of oxidative stress.

Interestingly this decrease of TAC was associated with a significant positive correlation between TAC and serum level of chromium. As the TAC has a protective role against oxygen free radical-induced damage, its decrement can be understood as a response to overconsumption due to oxidative stress. However the significant positive correlation with chromium could be explained as an adaptive response with prolonged exposure to chromium and as a defense against chromium-induced oxidative stress.

Reactive oxygen species (ROS) have been implicated in the toxicity of chromium (VI) by several authors (Miesel et al., 1995 and O'Brien et al., 2003). Their formation with subsequent cellular damage is considered as the common molecular mechanism of Cr (VI)-induced toxicity and carcinogenicity. According to this hypothesis, chromium (VI) itself is not a cytotoxic agent but rather an oxygen free radical generator through cellular reduction to chromium (VI) (Kadiiska et al., 1994). Chromium reduction intermediates are believed to react with hydrogen peroxide to form the hydroxyl radical (HO) (Yao et al., 2008), which may finally attack proteins, DNA, and membranes lipids thereby disrupting cellular functions and integrity (O'Brien et al., 2003).

Comparable to our results, blood chromium levels ranging from 4.42 to 10.6 μg/L, induced oxidative stress with marked significantly increased lipid peroxidation, decreased plasma antioxidant capacity (PAC) and plasma total thiol (SH groups) in exposed compared to controls (Patlolla et al., 2009).

Also, our results agree with another study in 2014, they studied effect of repeated chromium inhalation in rats. The study deduced overproduction

of cellular ROS due to chromium exposure resulting in genomic DNA oxidative damage appeared in form of increased urinary 8-OHdG level(Zhao et al., 2014).

The results of the current study throw some light on the adverse health effects of chromium exposure among tannery workers with special emphasis on the carcinogenic potential and the possible role of oxidative stress and oxidative DNA damage in pathogenesis. Also, the different job categories of workers was investigated for better and detailed evaluation of effects and targeted efforts of safety and prevention.

Conclusion and Recommendations:

From our study, we can conclude that tannery workers are exposed to markedly higher level of chromium which is associated with significantly higher prevalence of respiratory disorders such as rhinitis, asthma and chronic bronchitis. Also, there was a higher prevalence of occupational dermatoses in the form of contact dermatitis. The prevalence of chronic bronchitis, asthmatic attacks and contact dermatitis were statistically significantly higher among workers in tanning stage than the preparation and finishing stages.

Moreover, both urinary level of 8-OHG an index of oxidative DNA damage, blood level of P53 (a tumor suppressor protein) were also markedly elevated among workers than controls, with marked decrease of serum TAC (index of antioxidant).Reduction of TAC was only significantly lower in the tanning subgroup compared to other subgroups ($p<0.05$), with non-significant difference among subgroups as regards 8-Hydroxydeoxyguanosine, indicating the relation between higher blood chromium level and lipid peroxidation among tannery workers.Indeed urinary 8-OHG and serum TAC can be used as indices of oxidative damage induced by occupational exposure to chromium in leather tanning industry.

Regular health education and training of workers about health hazards of tanning and the benefit of safe work practices is mandatory. Encourage the use of personal protective equipments. Environmental and biological monitoring should be regularly done. Periodic medical examination for exposed workers with emphasis on the

health effects of exposures in tanning should be performed.

Conflict of interest:

Authors have declared that no conflict of interests exists.

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