

**ENDOCRINE DISRUPTION, GENOTOXICITY AND BIOCHEMICAL
ABNORMALITIES IN HUMAN POPULATION EXPOSED TO
CHROMIUM IN EFFLUENTS OF TANNERIES IN PAKISTAN**



By

MUHAMMAD ATEEQ

A Dissertation Submitted to the University of Peshawar in Partial
Fulfillment of the Requirements for the Degree of

**DOCTOR OF PHILOSOPHY IN
CHEMISTRY**

**INSTITUTE OF CHEMICAL SCIENCES
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DECLARATIONS

This is to certify that this dissertation prepared by Muhammad Ateeq entitled “Endocrine Disruption, Genotoxicity and Biochemical Abnormalities in Human Population Exposed to Chromium in Effluents of Tanneries in Pakistan” is accepted in the present form by the Institute of Chemical Sciences, University of Peshawar as fulfilling this part of the requirements for the degree of “ Doctor of Philosophy in Chemistry”

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DEDICATED
to
My loving Parents
and
younger brother Qaiser Shehzad Qureshi



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ABSTRACT

Chromium is one of the toxic heavy metals, which is extensively discharged from tanning industry to the environment. Chromium has binding potential to biomolecules in living systems and cause toxicity to biological life. Leather industry has been categorized as one of the highly polluting industry. Tanning industry is polluting the environment at a higher rate with huge amount of harmful material. These pollutants are also harmful for environment, humans and aquatic organisms.

The aim of the present study was to investigate the effects of chromium on selected endocrine hormones, oxidative stress, DNA damage, biochemical and haematological parameters of tannery workers and environmental parameters. Two major cities, Peshawar and Sheikhpura, were selected for the sampling sites. Samples of effluents, ground water, soil and vegetables were collected from the neighboring vicinity of tanning industries. The background area and adjoining villages were considered as control areas. Seven trace metals (Cr, Cu, Ni, Cd, Pb, Fe, and Zn) were investigated in tannery effluents, ground water, soil and dietary vegetables (*Spinacia oleracea*, *Solanum tuberosum* and *Solanum melongena*) samples using Graphite Furnace Atomic Absorption Spectrophotometer. It was observed that both soil and groundwater of study area were badly affected by the toxic elements discharged by tanning industries. The maximum level of Cr in tannery effluents was 350.15 mg/l, while in the soil sample it approached to 31.13mg/l. The values of chromium, cobalt, cadmium, lead and iron in the tannery effluents from Sheikhpura and Peshawar were above the permissible limits set by WHO (World health organization) and FAO (Food and Agriculture organization). The concentration of chromium, cadmium, nickel and lead in study area ground water from Sheikhpura and Peshawar exceeded the

standards of NSDWQ/Pak (National standard for drinking water quality). Vegetables irrigated with tannery effluents showed elevated level of Cr, Zn, Ni and Cd, which were above the permissible limits of WHO/FO. There was a significant positive correlation between all the trace metals in three media, tannery effluent, soil and ground water ($p < 0.001$). The metal to metal association was supported by dendrograms using cluster analysis.

A total of 240 smoking and nonsmoking individuals were registered in the study, including 120 chromium exposed tannery workers and 120 controls. The workers were selected from the tanneries of Sheikhpura and Peshawar, Pakistan. The average age of exposure group I was 20-35 years, exposure group II was 36-50 years and controls were 20-50 years. Hormonal parameters in the blood samples were determined by commercial kit, Chemiluminescent Microparticle Immunoassay (CMIA). Oxidative stress parameters were measured in the blood samples by spectrophotometric methods. DNA damage in lymphocytes was measured by comet assay (single-cell gel electrophoresis). Blood samples were collected from different age group subjects (chromium exposed tannery workers and controls).

The results revealed that both smoking and nonsmoking exposed groups showed significantly higher chromium levels in blood and urine than those of unexposed groups. The levels of testosterone (326.238 ± 12), (306.636 ± 13.8) and growth hormones (0.1030 ± 0.03), (0.0734 ± 0.01) were significantly decreased in both age groups of male tannery workers exposed to chromium.

The level of progesterone (2.416 ± 1.1), estradiol (48.879 ± 40.1), luteinizing (1.9692 ± 0.82) and growth hormones of female tannery workers (0.7983 ± 0.1) were significantly decreased while follicle stimulating hormone (9.2857 ± 0.7) was significantly increased as compared to unexposed population. Thyroid stimulating

hormone, triiodothyronine and tetraiodothyronine hormones were not affected in tannery workers. The hormones in tannery workers in group II aged (36-50) were more affected due to long-term Cr exposure as compared to group 1 with short term chromium exposure and controls.

This was further supported by correlation and regression analyses of the data. Pearson correlation showed that the levels of sex hormones (testosterone, progesterone, estradiol and luteinizing hormone) and growth hormone had negative correlation with blood chromium concentration in tannery workers.

The present study revealed that occupational exposure to Cr (VI) and Cr (III) can lead to a detectable DNA damage in tannery workers. DNA damage (28.79 ± 2.154), (25.41 ± 7.199) in smoking and nonsmoking tannery workers were significantly higher than those of unexposed groups. In addition, DNA damage was significantly associated with concentration of chromium in blood.

Malondialdehyde (MDA) levels (11.00 ± 0.7), (8.77 ± 2.3) and superoxide dismutase (SOD) concentrations (75.178 ± 2.3), (79.52 ± 1.9) of smoking and nonsmoking exposed groups were significantly higher than those of unexposed groups. While glutathione (GSH) levels (59.926 ± 1.5), (55.702 ± 2.3) in smoking and nonsmoking exposed groups were significantly lower when compared with unexposed groups. Oxidative stress parameters and DNA damage in group II tannery workers aged (36-50) with long-term Cr exposure were more affected as compared to tannery workers in group I with short term chromium exposure and controls. This investigation was further supported by correlation analysis. There was positive correlation between levels of blood chromium and DNA damage, MDA and SOD levels, while negative correlation was observed between blood chromium concentration and GSH levels in

tannery workers. The results showed that duration of exposure and smoking has significant effect on DNA damage and oxidative stress parameters in tannery workers.

The present study also described biochemical and haematological defects in tannery workers exposed to hexavalent chromium. From the findings, it is evident that white blood cells (WBC) (8.373 ± 0.7), red blood cells (RBC) (4.12 ± 0.5), haemoglobin (Hb) (10.02 ± 1.14), mean corpuscular haemoglobin (MCH) (25.91 ± 1.50), and packed cell volume (PCV) (42.62 ± 2.0) were lower in exposed groups than that of controls.

The values of liver function tests of tannery workers were found to be within the normal range in both the age groups except alkaline phosphatase (AP) activity (121.28 ± 8.3), which was significantly increased in exposed groups. All other biochemical parameters were found to be within the normal range except the albumin (Alb) levels which was significantly decreased in exposed groups.

The present study found that occupational exposure to chromium can lead to health hazards including hormonal abnormalities, oxidative stress, DNA damage and hematological and biochemical defects in tannery workers.

INTRODUCTION

Industrial effluent contains a variety of toxic chemicals, including heavy metals, which are considered mutagenic and carcinogenic (1, 2). Initially, heavy metals combine with proteins and may not cause any poisoning but when their concentration exceeds the threshold level, they begin having adverse effects on human's health (3). The heavy metals directly react with cellular components making ionic and covalent bonding. At high concentration, both essential and non essential metals can alter enzyme specificity, damage cell membrane, disrupt cellular function and damage genetic material (4, 5).

Thus tanning industry has gained a negative image on the human with respect to its pollution potential due to the loads of chemicals released. The industry is considered a primary polluter of the environment and has a strong potential to cause soil and water pollution owing to the discharge of untreated effluent. In accordance with the threat posed by the unsafe discharge of tannery effluent on the biosphere, the present study was formulated. Large amount of toxic metals are discharged from various industries into the environment which causes various diseases in humans and other living organisms, including both animals and plants (6). The existing tendency of industrialization and urbanization might contribute extraordinarily to the poor quality of water through indiscriminate transfer of industrial effluents posing danger to the health of human being (7). Different industries discharge different types of pollutants into the surrounding ecology which are linked with sewerage system, open spaces and

agricultural irrigation lines. Primarily the rivers and natural drains are used as waste disposal means by the industries (8).

The effluents from industries are responsible for contamination of rivers, canals and ponds which increase the level of pollutants in water leading to high chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), toxic metals such as, chromium (Cr), cadmium (Cd), lead (Pb) and nickel (Ni). (9). Ground water is not only important for potable water supply but also used in industrial processes and for agricultural purposes. Solid and liquid wastes entering into water bodies are mostly discharged from industries and domestic activities, as a result water systems receiving these partially treated and untreated industrial wastes become polluted. This polluted water has adverse effects on public health, environment and aquatic resources (10).

Soil is a very important component of the environment, but it is mostly misused and undervalued (11). Plants and animals depend on soil for their growth and development. Soil has been contaminated due to rapid discharge from industrial processes. In many instances, the life on soil matrix is badly affected by the presence of high concentration of heavy metals and contaminants from the discharge of untreated waste disposal by industries (12).

The contamination of soil with micronutrients in higher concentration generates detrimental effects on human health and plants (13). Soil is considered as an ultimate sink for the contaminants released into the environment. In addition, soil pollution by heavy metals has become a serious problem in industrialized areas. Toxic metals adversely affect the plants grown on heavily polluted areas and high accumulation of contaminants increases the production of reactive oxygen species in plants and also affects their growth and metabolism (14, 15).

Use of raw effluents for irrigation of crops adversely affects the plant growth and development. The accumulation of toxic pollutants in plant cell changes the physiological processes, which in turn affects the growth rate and metabolic processes. Moreover, the process of respiration, mitotic activity and photosynthesis are also affected (16). High accumulation of heavy metals may lead to stress in plants (17). Effluents are used as irrigation water in areas where there is a shortage of water for agriculture purposes and an excess amount of effluents are produced from the industries. In many cities diluted forms of tannery effluents are also used to overcome this problem (18, 19).

1.1 Tanneries

Leather tanning is a common industry all over the world and is considered as a serious environmental threat. The tanning operation converts the skin or raw hides into leather, which is used in the manufacturing of various products. The process includes soaking, liming, dehairing, deliming, bating, degreasing and pickling. Chemicals used in the process include ammonium chloride, hydrogen peroxide, sodium sulphide, sodium sulphite, format, chromate, chloride and sodium bicarbonate. Due to multifarious operations with toxic chemicals, the leather processing industry is considered as a primary polluter for the environment (20).

Waste water from tanneries adversely affects the surrounding environment. Leather industry uses a lot of water; the waste water released during the processes ranges between 30 and 50 litre per kilogram of the processed skin (21). Water consumption in tanneries depends upon different processes like raw material used and the manufactured products. In addition to, large amount of fresh water used to treat raw leather, many toxic chemicals such as synthetic tannins, resins, oil, chromium, detergents and biocides are also used and discharged during the process. Careless

disposal of untreated tannery waste into the environment results in air and water pollution (22).

Leather industry has been categorized as one of the highly polluting industry. Discharge of leather industry wastes into the agricultural fields causes indicative changes in nutrient cycling and organic matter processing (23, 24).

Common heavy metals in tannery effluents are chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn) and manganese (Mn). Tannery effluents discharge large amount of chromium (25). Tanning industry is polluting the environment at higher rate with huge amount of harmful material. These pollutants are also harmful for aquatic organisms. Heavy metals accumulate in the tissues of plants and animals. As food products, these aquatic animals are health hazards for humans and animals which depend on this source of food (26).

The majority of global leather production is chrome-tanned. Using complex salts of trivalent chromium in the process of tanning effectively influenced the desired functional properties of leather. Trivalent chromium stabilizes a hide by crosslinking the collagen fibers and supply required qualities. The possibility of the spontaneous oxidation of Cr (III) into Cr (VI) can occur both in alkaline and acidic medium in the wide range of pH what complicate the proper conditions specification (22).

The known prerequisite factors for the formation of hexavalent chromium in leather are (27):

- Oxidizing agents: the fundamental for the conversion is presence of oxygen.
- Energy in a form of heat and ultraviolet light, because the conversion requires energy supply.
- Fatty acids as catalysts for peroxide formation and high pH.



Figure 1.1 Unhairing process in tannery of Sheikhupura

Source: (Photo taken on 10 September)



Figure 1.2 Beamhouse process used for unhairing and liming of leather in tannery of Sheikhupura

Source: (Photo taken on 10 September 2013)



Figure 1.3 Raw waste produced in tanning process in tannery of Sheikhpura

Source: (Photo taken on 10 September 2013)

Despite the fact that leather tanning industry is one among the leading economic sector in several countries, there has been an increasing environmental concern about the discharge of tannery waste (27). Tannery effluent characteristically contains a complex mixture of both organic and inorganic pollutants. Chromium as inorganic pollutant exists in several oxidation states, with Cr (III) and Cr (VI) species being the most common forms. While chlorinated phenols (e.g. 3, 5-dichlorophenol) as organic pollutant associated with tannery have been found to be highly toxic and affect the cellular compounds of organisms (28-31).

In Pakistan, there are more than 650 tanning industries (32). The major pollutants of tanning industry include arsenic, selenium, barium, copper, zinc, nickel, cobalt, mercury, lead, azodyes, antimony, cadmium compounds, formaldehyde resins, pesticides residues and polychlorinated biphenyls (PCB). Leather industries in Pakistan discharge their untreated effluents directly into water resources which lead to severe environmental problems (33, 34). Most of the leather industries in Peshawar

are located in residential neighborhoods of the city due to which chromium and other toxic metals from tanneries have polluted the ground water in the surrounding area, where tube wells, hand pumps and domestic motor pumps are the main sources of drinking water. In addition, the tannery operation in Peshawar is also contaminating the Kabul River, which badly affects the water used for irrigation and domestic purposes (35, 36)

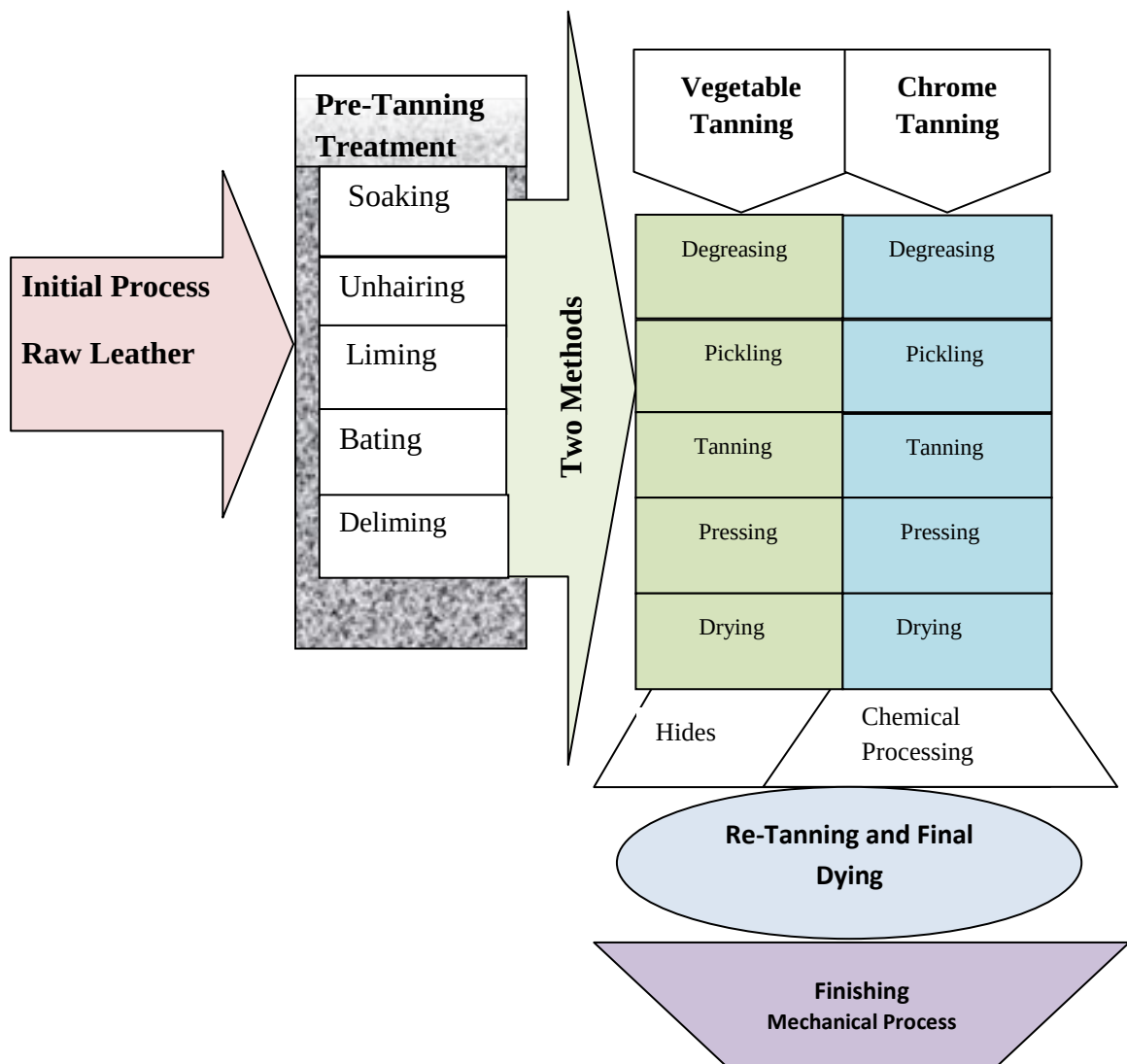


Figure 1.4 Flow sheet of typical process in leather industry (246).

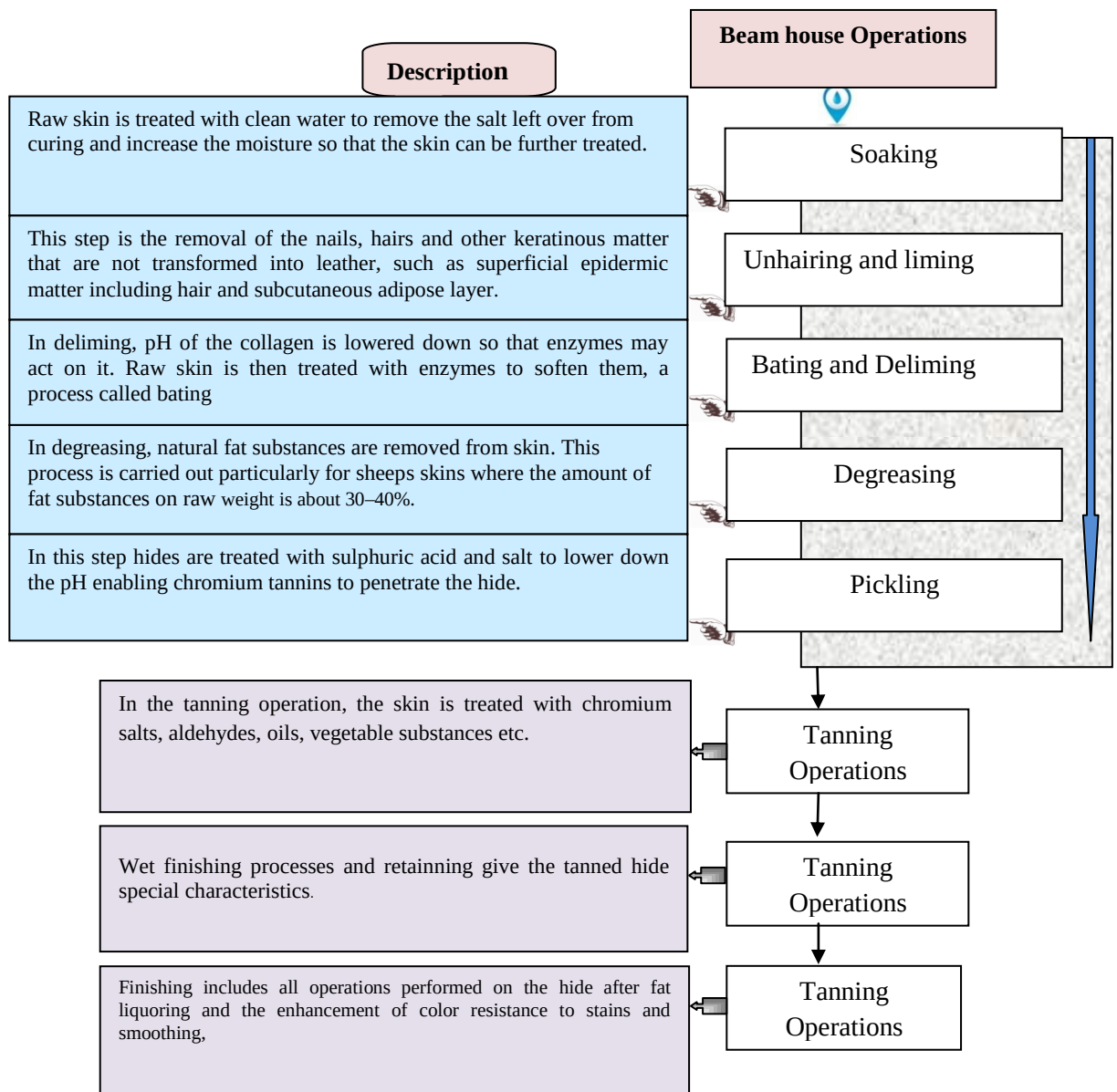


Figure 1.5 Description of unit processes in leather production (246).

Tanning industry in Pakistan is the third important foreign exchange sector. In Pakistan, tanneries discharge three different types of wastes: solid waste, liquid waste and air emissions. However, wastewater is the major environmental problem caused by tanneries (37, 38).

1.2 Chromium occurrence and pathways in the environment

Chromium was first reported as an essential mineral for animals by Schwarz and Mertz. It is naturally occurring metal present in rocks, soils, volcanic dust, and gases, plants as well as animals. Chromium is 21st most abundant mineral in the earth crust; it is normally present in various oxidation states ranging from chromium (II) to chromium (VI). It is mostly found in 0, +2, +3 and +6 oxidation states. However, trivalent (III) and hexavalent (VI) forms are biologically important. Trivalent chromium occurs naturally, while the hexavalent form is mostly of industrial origin. Most of the chromium compounds are sulphides, oxides and halides (39).

Hexavalent chromium is the 2nd most stable form and considered as strong oxidizing agent particularly in acidic media. Hexavalent chromium is many times more toxic than trivalent chromium. It is reduced to chromium (III) that is less toxic and important nutrient for humans. It is usually used in four basic industries: tannery, chemical, refractory and metallurgy which cause environmental pollution. Cr (IV) is bound to oxygen with a strong oxidative capacity as chromate (CrO_4^{2-}) or dichromate ($\text{Cr}_2\text{O}_7^{2-}$). Inside the cell, hexavalent chromium easily crosses biological membrane and reacts with protein component and nucleic acid. The reaction between Cr (IV) and genetic material shows its carcinogenic properties (40).

Cr (III) is the most stable oxidation state of chromium which is also found in living organisms. It cannot cross cell membrane easily and shows low reactivity and this feature distinguishes it from the hexavalent form. Cr (III) can form a number of coordination complexes. Some forms of trivalent chromium are also used as markers in the experiments of digestive processes due to their low reactivity and absorption in gastrointestinal system (41, 42).

Chromium (III) occurs naturally in many fruits, grains, meat, yeast and fresh vegetables. It is the most common form in surface soils where oxidation processes mostly convert chromium from Cr (VI) to Cr (III). Cr (III) formed from hexavalent chromium inside the cell can complex with organic compounds and interferes at high concentration with metallo-enzyme systems (43).

Hexavalent chromium compounds are of tremendous industrial importance. Chromium (VI) is used in leather tanning, chrome plating, manufacture of dyes and pigments and wood preservation. Increased levels of chromium in anthropogenic polluted ecosystem are serious threat to human beings and have been shown to cause carcinogenic and allergenic effects in humans and animals (44-46).

For the optimum function of insulin in mammalian tissues, chromium acts as a cofactor. It is also an important constituent of glucose tolerance factor GTF and needed for the normal metabolism of carbohydrates, lipid and protein.

Chromium is reported to enhance the action of insulin by promoting insulin-receptors binding at cell surface and thus increasing the sensitivity of insulin and pancreatic β cells. Despite increasing insulin sensitivity, chromium cannot replace insulin. But in the presence of chromium, even low level insulin is enough to fulfill similar biological response (42, 46).

Chromium is an essential nutrient for glucose and fat metabolism. Insufficient dietary intake of chromium leads to various diseases like diabetes and cardiovascular problems including elevated circulating glucose, insulin, total cholesterol, triglycerides and impaired immune function. A low glycemic index (GI) diet may also elevate insulin sensitivity and decrease postprandial glucose levels but it keeps blood glucose level within a normal range (47).

1.3 Dietary chromium requirement and intake

Chromium (III) is considered one of the safest nutrients found in food and nutrient supplements. A reference dose has been established by Environmental Protection Agency defined as “an estimate of daily dose exposure to human” and it has no adverse effects over a lifetime. The estimated safe and adequate daily dietary intake (ESADDI) of chromium as recommended by the Food and Nutrition Board of the US National Academy of Science in 1989 is shown in Table (1.1). European Research Network on Trace Elements studied the chromium content in various foodstuffs. Most dairy products provide <0.6 µg Cr/serving. Wheat flour and whole wheat contain 5 to 10 µg Cr/Kg. Certain foodstuffs such as seeds, pulses, black pepper and dark chocolates contain higher concentration of chromium. Some brands of beer contain higher chromium content, some of which probably comes from the brewing containers. Glucose tolerance factor found in brewer’s yeast is considered the best known chromium complex. The average daily dietary chromium intake estimate representing various populations living in 14 different countries ranges between 30-60 µg (48-50).

Table 1.1 The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of Cr as recommended by the Food and Nutrition Board of the US National Academy of Science in 1989, (42).

Category	Age, Year	ESADDI, µg Cr
Infants	0-0.5	10-40
Infants	0.5-1	20-60
Children	1-3	20-80
Children	4-6	30-120
Children	7-10	50-200
Children and adolescents	>11	50-200
Adults		50-200

1.4 Chromium absorption via gastrointestinal tract

Chromium is found in both inorganic and organic forms in diet. Inorganic chromium form is absorbed at lower rate, from 0.4-3% depending on the daily dose taken. Absorption of chromium mainly takes place through intestinal mucosa. Lesser the dose of chromium ingestion, higher is the rate of absorption and vice versa, while at certain higher dose the rate of absorption becomes constant. Absorbed chromium is then bound to transferrin in blood possessing two binding sites: A and B, which is also a carrier for Fe. Thus there is competition between chromium and Fe for this carrier. It is reported that chromium binds to site B. Its most common supplemental forms are chromium chloride, chromium pinacolate and chromium polynicotinate (48, 51).

Higher amount of chromium is absorbed in the form of organic compounds, while a very little amount of chromium (< 2%) is absorbed in the inorganic form. Once chromium is absorbed, it is rapidly distributed to various tissues of the body but higher concentration is found in kidney, liver and muscles (52). In addition to oxidation state and route of administration, carbohydrates, ascorbic acid, aspirin, oxalate, indomethacin and antacid also change chromium absorption (53).

1.5 Chromium excretion

The chromium absorbed in the body is excreted mainly through urine and in a small amount in hair, bile, sweat and faecal. Chromium levels in the blood and urine serve as biomarkers of exposure, which indicate the amount of chromium present in the body. Moreover, chromium concentration in erythrocyte serves as recent exposure to hexavalent chromium. On the basis of urinary chromium level, it is impossible to differentiate between exposure of hexavalent chromium and trivalent chromium (54).

For healthy adults, the average urinary excretion is 0.22µg/day (55). Urinary chromium excretion is 0.5% for the subjects, whose diet includes 40 µg of chromium. The negative relationship is observed between dietary chromium and percent urinary chromium excretion (56).

1.6 Toxicity of chromium

Toxicity of chromium depends on its valence state. Cr (VI) is not present in foodstuff and has higher toxicity than trivalent chromium, while Cr (III) is present in foods. In addition, ingested Cr (III) has shown low level of toxicity due to its poor absorption (57). Li *et al.*, have reported that exposure to hexavalent chromium induces reproductive abnormalities in laboratory animals (58). Marouani *et al.*, have documented increased concentration of follicle stimulating hormone (FSH) in serum and reduced sperm cell count in electroplating industry workers (59). Aruldas *et al.*, has reported testicular as well as ovarian toxicity caused by chromium (VI) in laboratory mammals (60).

Occupational exposure to sublethal Cr (VI) affects the pituitary-ovarian-liver axis (60, 61). Marouani *et al.*, found that testosterone and LH levels were significantly decreased in rats treated with chromium (VI). Moreover, occupational exposure to chromium also affects the sertoli cells which have a negative impact on impact on FSH secretion (59, 61).

Chowdhury *et al.*, and Meeker *et al.*, reported a significant decrease in sperm counts of human and animals exposed to chromium, which may cause to male infertility (62, 63). The use of chromium in chromium-tanned leather represents a significant health problem including skin dermatitis and respiratory infections. Srivastava *et al.*, and Van *et al.*, documented that allergic contact dermatitis caused by Cr (VI) results from

oxidation of Cr (III) during the tanning process (64, 65). Krejpcio *et al.*, documented that occupational exposure to high doses of chromium, usually airborne, can cause skin cancer, allergic dermatitis, bronchogenic carcinoma. As chromium is potent sensitizer, skin contacts with chromates and dichromates can cause eczema (48, 66).

Various toxic effects of chromium depend on route of exposure, as chromium is considered an irritant, it can cause respiratory problems, perforation in nasal septum, dermatitis, hepatic and renal impairments. In industries, chromium binds with the skin protein of workers, induce complex antigen causing hypersensitivity. Cr (VI) mainly affects respiratory tract which is related to both acute and chronic inhalation. The toxic effect of chromium is produced by generation reactive oxygen species (ROS). These ROS cause chromosomal damage and various point mutations in DNA (64, 67).

1.6.1 Chronic oral toxicity

Hexavalent chromium can be ingested with food, beverages and drinking water. Kerger *et al.*, documented that hexavalent chromium is frequently reduced when drinking water is utilized to prepare beverages (68). Human can also be exposed to hexavalent chromium by swallowing through the airways via the muco-ciliary escalator by inhalation (69). Oral hexavalent chromium may not cause genotoxic effect at the low dose. Ingestion of 5mg hexavalent chromium by human volunteers didn't cause DNA-protein crosslink in peripheral blood lymphocytes (70). Kuykendall *et al.*, reported that ingestion of 20mg hexavalent chromium in drinking water didn't cause any adverse effect in mouse bone marrow (71).

1.6.2 Dermal toxicity

Workers can be exposed to chromium through dermal contact. The prevalence of dermatitis was observed among construction workers occupationally exposed to chromium present in cement. In addition, positive correlation has been documented between the incidence of dermatitis among construction workers and chromium content in cement (72).

Workers exposed to chromium containing material in industries mostly suffer from chronic skin ulcers or contact irritative dermatitis. Allergic contact dermatitis is caused by direct contact with chromates and hexavalent chromium, which is released from chromium alloy and objects plated with chromium. Both hexavalent chromium and trivalent chromium have been shown to cause allergic dermatitis in exposed individual (73).

1.6.3 Hexavalent chromium metabolism

Hexavalent chromium is known to be a pro carcinogen before entering into the cell. The structural resemblance of chromates to phosphate ions and physiological sulphates enables molecular mimicry and easy entrance of hexavalent chromium into cells via nonspecific ion channels (74, 75). Once inside the cell, hexavalent chromium is reduced to chromium (V), chromium (IV) and finally to its trivalent form. These reduced forms of chromium have been reported to cause genomic DNA damage, which induces DNA replication inhibition. At physiological pH, several molecules like glutathione (GSH), ascorbate, cysteine, lipoic acid, hydrogen peroxide, ribose and fructose facilitates intracellular reduction. It has been reported that ascorbate and glutathione (GSH) reduce hexavalent chromium. However, most of the studies

documented that ascorbate is the biological reductant which plays a significant role in vivo hexavalent chromium metabolism (76).

Moreover, glutathione (GSH) is found at milli molar concentration, has been recognized as a modulator of cell stress caused by hexavalent chromium cytotoxicity. Trivalent chromium is the final product of chromate reduction and has greater efficiency to bind DNA than hexavalent chromium. When hexavalent chromium is reduced to the trivalent form in the cell, the trivalent chromium binds to molecules intercellularly due to weak membrane permeability. Hence, the formation of stable trivalent chromium complex takes place with nucleic acid and protein, leading to DNA damage (77-79).

1.7 DNA damage

Chromium is found at workplace in two oxidation states: Cr (VI) and Cr (III). Trivalent chromium cannot enter the cell and travel in the serum bound to transferrin, while hexavalent chromium can pass through the cell membrane through the anion transport system. Once inside the cell, hexavalent chromium undergoes reduction through the endogenous reducing agents such as glutathione and ascorbate. The reduction of hexavalent chromium produces various reactive species of chromium namely Cr (V), and possibly Cr (IV), Cr (II) and the stable Cr (III). Moreover, other reactive species including hydroxyl radical and singlet oxygen are also produced leading to DNA damage (80-82). Chromium (VI) is considered carcinogen and human mutagen which is not directly reactive with DNA. DNA damage by Cr (VI) involves a reduction step producing lower valence forms which form stable complexes with intracellular macromolecules. The Cr (III) produced as a result of reduction mechanism may directly react with the DNA leading to generate oxidative damage in vitro (80, 81, 83).

Trivalent chromium is considered more efficient than hexavalent chromium in causing DNA damage in cell free systems. Interaction of trivalent chromium with DNA leads to DNA strand breaks and oxidative DNA base modification such as production of 8-hydroxydeoxyguanosine (8-OH-dG) (83, 84). Oxidative DNA damage has been associated with reduction in intracellular antioxidants (85). GSH and cysteine are the main thiol molecules involved in this process (86).

Evidence received from in-vitro cell-free systems reported that once inside the cell, trivalent chromium forms complexes with various intracellular macromolecules, while hexavalent chromium is inert towards hereditary materials in the absence of reducing systems (87, 88). However, DNA damage caused by hexavalent chromium requires a reducing system, producing (ROS) and lower valence forms which readily make complexes with intracellular macromolecules (89).

Chromium has potential to inhibit macromolecular synthesis and induce various types of DNA lesions and gene mutation. Chromium induced mutations in the tumor suppressor gene (p53) are the important factors that play a key role in determining genotoxicity (80, 90).

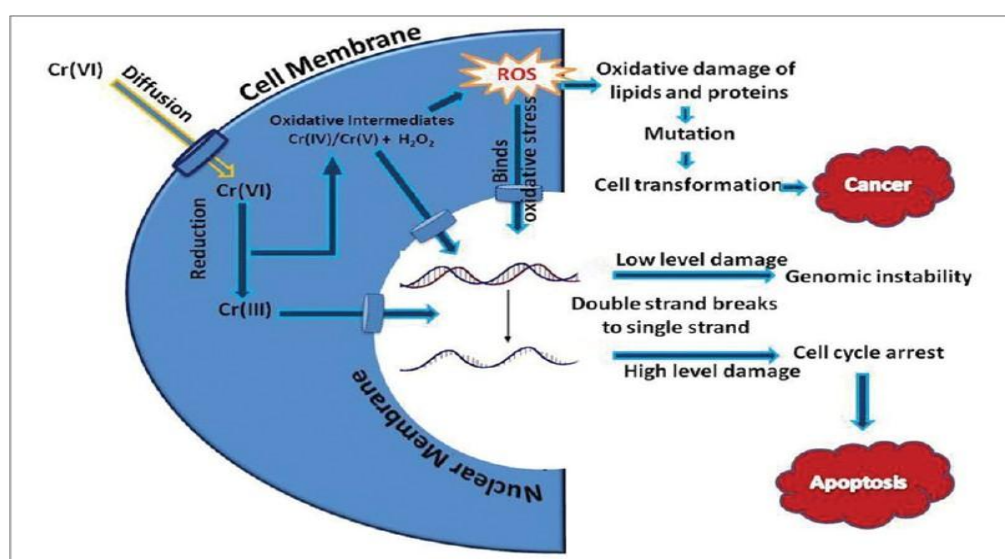


Figure 1.6 Reduction of Cr (VI) and DNA damage inside cell (167).

Hexavalent chromium causes mutations, chromosomal aberrations and transformation in cultured mammalian cells (91, 92). Chromium (VI) compounds cause a variety of DNA lesions like alkali labile-sites and DNA single-strand breaks in liver, kidneys, Chinese hamster ovary and mouse embryo fibroblast (93-95). Both the particulate and soluble form of hexavalent chromium has been shown to induce DNA double strand breaks in animals and cultured cells. In addition, trivalent chromium also causes physicochemical alterations of nucleic acids by interacting with phosphate groups and nitrogen bases (96-98).

1.8 Selected male and female endocrine hormones

1.8.1 Selected male endocrine hormone

Biosynthesis of testosterone hormones is shown in Fig 1.7. In men, sex hormones are produced in the testis (99). Testosterone is a steroid hormone and is the principal male androgen produced by the interstitial cells of Leydig. It is the major male sex hormone and an anabolic steroid. In mammals, testosterone is primarily secreted in the testes of males and ovaries of female (100, 101). In men, testosterone plays an important role in the development of male reproductive tissues (102). Moreover, testosterone is essential for health as well as for the prevention of osteoporosis (103). Testosterone is converted to potent hormone, dihydro-testosterone. Aromatase converts part of testosterone to estrogen in brain, testis, adipose tissues and this activity increases with obesity, age and hyperinsulinemia (104).

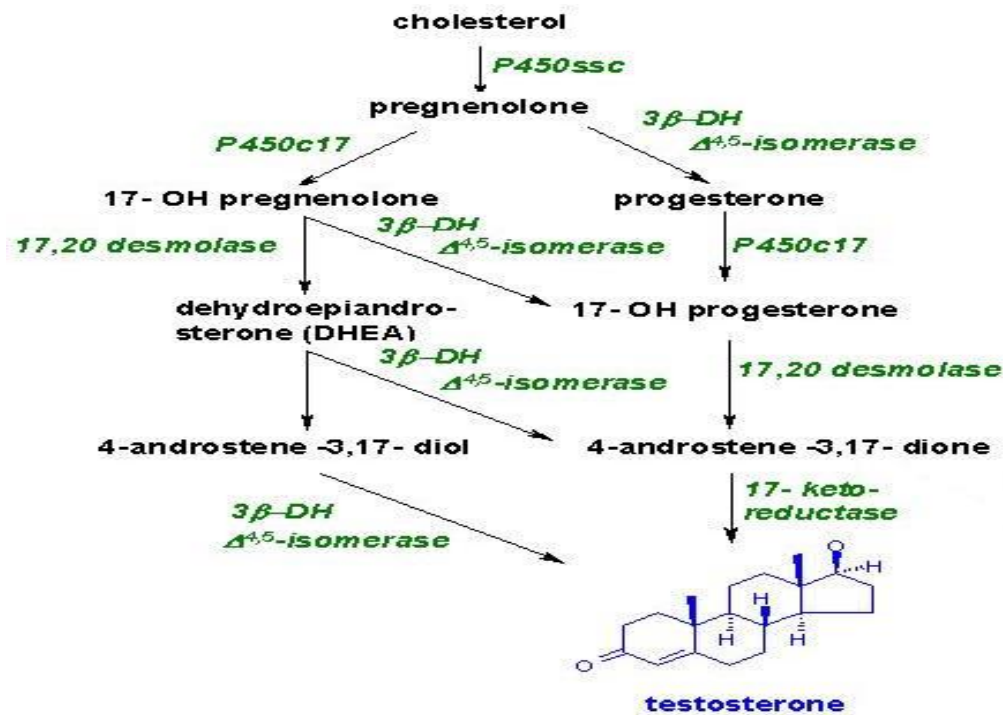


Figure 1.7 Biosynthesis of testosterone hormones.

(Bledar Daka, (2014) Sex hormones and cardiovascular risk in men and women)

Testosterone and estradiol are liposoluble hormones and circulate in blood bound to sex hormone-binding globulin (SHBG) and albumin (Figure 1.8) (105).

Pre-hormone dehydroepiandrosterone (DHEA) is produced in the adrenals and converted into sex hormones by a local enzyme in target tissues. DHEA is responsible for about 50% of sex hormones in men aged 60 years or older (100, 106). DHEA and sulphated-DHEA are easily converted into active sex hormones and also serve as reservoirs for sex hormone in the blood stream due to high concentrations.

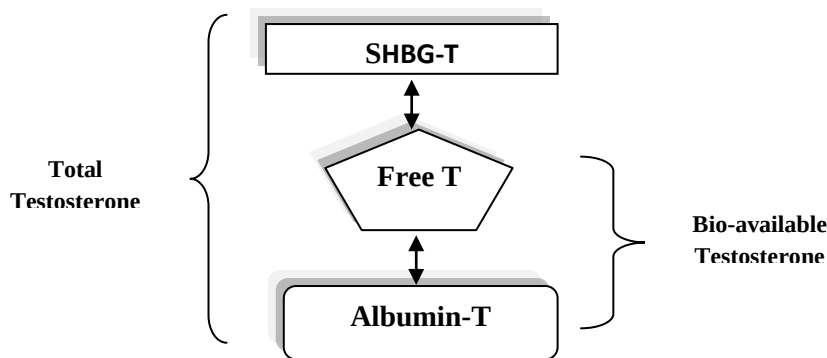


Figure 1.8 Circulation of testosterone bound to SHBG and albumin.

(Bledar Daka, (2014) Sex hormones and cardiovascular risk in men and women)

Lower testosterone levels are associated with an increased risk of developing type 2 diabetes mellitus and metabolic syndrome (104, 106).

In men, endogenous testosterone levels are inversely associated with mortality due to cardiovascular problems. Thus low testosterone level can be a predictive marker for those at high risk of cardiovascular disease (107).

1.8.2 Selected female endocrine hormones

The hormones controlling the female reproductive system, estradiol and progesterone, are produced by the ovaries and the corpus luteum, while follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are produced in the brain.

Progesterone is a steroid hormone secreted by the corpus luteum: Also known as P4 (pregn-4-ene-3, 20-dione) is a C-21 steroid hormone synthesized by the ovaries, testes and adrenal glands. Progesterone is also synthesized from cholesterol molecule characterized by cyclo-pentano-phenanthrene skeleton consisting of three hexagonal cycles, A, B, C and one pentagonal cycle D (108-110). Biosynthesis of progesterone hormone is shown in Figure 1.9. Desmolase enzyme converts cholesterol into pregnenolone, which is then converted to progesterone by isomerase/dehydrogenase enzymes.

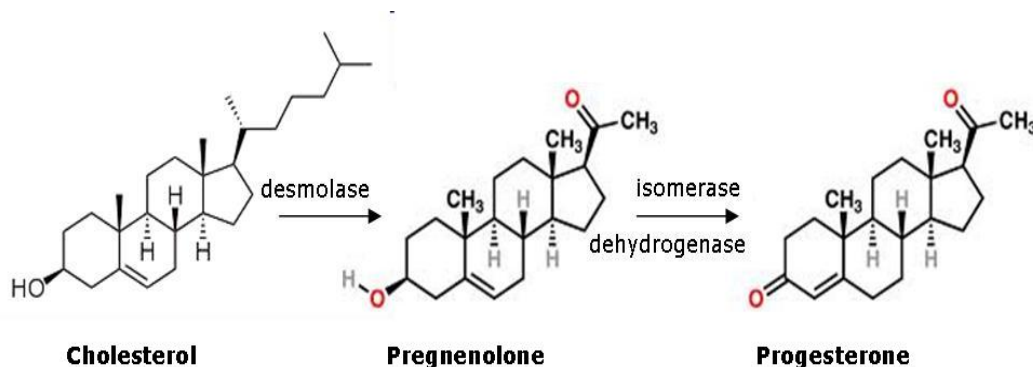


Figure 1.9 Biosynthesis of progesterone hormone

(Junaid ali khan, (2011) Progesterone receptor isoforms : Functional selectivity and pharmacological targeting)

Due to its physical and chemical properties and abundance in tissues, it plays a structural and functional role in the luteal cell membrane. It controls the functions of female reproductive organs. It also actively participates in the regulation of blood pressure and other cardiovascular regulations (111).

Progesterone is an essential regulator of normal human female reproductive functions in the ovary, uterus, mammary gland and brain. It is also important for normal functioning of several non-reproductive tissues such as central nervous system and cardiovascular system (112-114).

Estradiol in women is synthesized from cholesterol. The major part of it is produced in granulosa cells of the ovaries and involves the conversion of androstenedione into estrone and then estrone is further converted into estradiol (115). Estradiol and progesterone are not only involved in human sexual maturation and differentiation but also in the regulations of bone maintenance, cognition, mood and memory (116-118). Cholesterol is the precursor for progesterone and estradiol. Their biosynthetic pathways are depicted in Figure 1.10 (119).

globulin (SHBG), while the fraction of estradiol which is not bound to SHBG shows bioactivity (120, 119). Due to lipophilic behaviour, estradiol can diffuse through the cell membrane without a specialized transport protein (121). The gonadotropins include luteinizing hormone (LH) and follicle stimulating-hormone (FSH) which are produced by the same pituitary cells. At the anterior pituitary, gonadotropins releasing hormone stimulates the secretion of FSH and LH, both play a central role in ovarian function (122). FSH plays a key role in regulating the development, growth, pubertal maturation and reproductive processes of the body, while LH stimulates the production of progesterone and estradiol from the ovary via luteal cells and theca interna cells (123, 124).

1.8.3 Mechanism of Action

Steroid hormones travel in blood stream (*e.g.*, estradiol, progesterone) bound to proteins, but also exist in free form in a complex equilibrium. The free hormones cross cell membranes in all tissues in the body.

When the hormone reaches the target tissues, it either acts on the plasma membrane of the cells (peptide hormones and epinephrine) or in the cytosol (steroid, thyroid hormones, active vit D3, retinoic acid). When the hormone binds into the receptor's active site, a series of mechanism takes place which at last produces the intended action (120).

When testosterone reaches its target cells, an enzyme called 5 α -reductase converts it to 5 α -dehydrotestosterone, a more potent form of the hormone, which then binds to the intracellular androgen receptor. The receptor then travels to the nucleus where it acts as a Transcription Factor, thus activating or deactivating the expression of specific genes. Estradiol travel the bloodstream bound to plasma proteins (Sex-hormone binding globulin). Because steroids are fat-soluble molecules, estradiol can

diffuse across plasma membranes. Once inside the cell, they can bind to one of two classes of intracellular estradiol receptors (Estrogen Receptor (ER), which is present in the nucleus and G-Coupled Protein Receptor 30 (GPR30), which is membrane-bound. These Hormone-Receptor Complexes act as DNA-binding transcription factors: they bind to specific DNA sequences called Hormone Response Elements, located in promoters of their target genes, and modulate genes' transcription, and therefore expression, thereby producing a response in their target cells. Progestogens travel the bloodstream bound to plasma proteins (Transcortin molecule and albumin). After diffusion through the plasma membranes of their target cells, progestogen hormones can bind to their corresponding intracellular Progesterone Receptor for which they have a very high affinity. Estradiol can have an up-regulating effect on the number of progesterone receptors present in the cell (121-124).

The hypothesis was formulated to test toxic effect of tannery environment and chromium in this study. First hypothesis posits that physical health of the workers is positively related to exposure of hexvalent chromium and duration of work. The goal of this study is to delineate the association between occupation and disease. In the tannery workers study, it was hypothesized that exposure to the various known or suspected occupational carcinogens among the workers in the tannery environment (hexavalent chromium salts, benzidine-based azo dyes, aromatic organic solvents, formaldehyde, airborne leather dust, and nitrosamines) could be associated with an increased risk of mortality due to various cancers (lung, larynx, bladder, kidney, nasal cavity, etc) as reported by others for these exposures.

1.9 Aims and Objectives

In accordance with the threat posed by the unsafe discharge of tannery effluents on the land and in water bodies, the present study was formulated with following objectives.

- assessment of the growth and sex hormones (estrogen, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone) abnormalities in tannery workers exposed to chromium.
- evaluation of the oxidative stress parameters (malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD)) and DNA damage in tannery workers.
- evaluation of biochemical and haematological abnormalities in tannery workers.
- determination of chromium and other heavy metals in the blood serum and urine of tannery workers.
- investigation into the prevalence of reproductive abnormalities, infertility, abortion, survival rate of children, respiratory problems and contact dermatitis among tannery workers.
- study into chromium contamination and presence of other heavy metals in tanneries wastewater, ground water, soil and vegetables from tannery polluted area.
- analysis of the significance and integrity of biological and environmental parameters through statistical analysis of data.

Literature Review

Heavy metals are important for proper functioning of biological systems, but their deficiency or excess could lead to a number of disorders (125). Effluents discharged from tanning industry contain a higher amount of metals especially chromium, copper and cadmium. These effluents are discharged into surface water as well as the agricultural fields which ultimately seepage to the ground water and leads to environmental pollution due to accumulation of toxic metals (126). The open-air waste disposal areas have been reduced due to the rapid industrialization. The disposals of untreated industrial wastes into the open lands and water affect both the soil and ground water quality. Careless disposal of wastes has affected the soil and water resources. The environmental risk assessment and its consequences on human health have been of great interest in the modern era of industrialization (127).

Among the environmental issues, two basic problems are the disposal of large amount of effluents and wastes that are produced continuously from industries and the removal of waste at the dump sites on the land and in the water system (128).

2.1 Previous investigation into the tanning process

In the tanning process, the skins or raw hides are converted into leather. The tannic acid ($C_{76}H_{52}O_{46}$) and other chemicals are used in this process. The skins and hides are treated with chemicals to prevent them from moldy process and keep them durable and supple. The three types of skins mostly used in leather manufacturing are; cattle, sheep and goat (129). The tanning process consists of reactions of collagen fibers of the hides with chromium, tannins, alum and other chemical agents. Chrome and

vegetable tannins are the most common tanning agents used in this process. There are other tanning agents, including syntans (man-made chemicals), alum, glutaraldehyde, formaldehyde and heavy oils used in the tanning process (130). The tanning industry produces 80-90 percent toxic pollutants during pre-tanning processes and also discharges chrome sludge, harmful gases such as hydrogen sulphide as well as sulphuric acid and solid wastes (131). About 20-30 litres of effluents are discharged in the processing of one kilogram skin/hide, while this quantity increases to 40 litres per kilogram in finishing units (132).

Different tanning processes are carried out for desired leather products (133). In the transformation of raw skins into marketable goods, large quantity of acids, chromium salts, solvents, tannins, alkalis, sulphides, dyes and many other complex chemicals are used, which are fully absorbed by skins and discharged with effluents (134, 135). During re-tanning procedures, synthetic tannins (syntan), resins and oils are added at varying quantities to form softer leather (136). Syntans are composed of various chemicals such as formaldehyde, phenol, naphthalene, and acrylic resins (137-139).

2.2 Chemical analysis of tannery effluents

An average of 30–35 m³ of wastewater is produced per ton of raw hide (140). In the tanning process beam-house wastewater is characterized by an alkaline pH and the tanning effluents by acidic pH as well as high COD. In addition, the highest concentration of the salt load occurs in the beam-house area. About 15% (w/w) to 40% (w/w) of sodium salt is used for preserving animal skins and this salt is removed during the soaking stage (141). Sodium sulphide or sulfhydrate and lime are routinely used during liming-unhairing operation. In addition, organic solvents are commonly used chemicals in degreasing step, which leads to environmental pollution by emission of volatile compounds (142). Tannery wastewater contains the highest

concentration of total chromium (up to 4950 mg/L), as compared to this, the tanning industry which process salted hides discharges largest amount of sodium chloride in wastewater (143, 143). Coloring process usually involves combining dyes with the tanned skin fibers to form an insoluble compound (144). Fabbicino *et al.*, reported that chrome tanning is the most useful tanning method for the tanning industry and represents over 80% of the leather production world-wide. Chromium sulphate is commonly used in tanning processes. The issue related to toxicity of hexavalent chromium in leathers industries started to develop around 1994-1995, when Cr (VI) was found in leather articles. The first findings were made in a French study and very soon the same results were also seen in Germany (145-147).

Trivalent and hexavalent chromium are in mutual dynamic equilibrium. Cr (III) is unable to cross the cell membrane due to which it is considered less toxic than hexavalent chromium (148).

2.2.1 Heavy metals in tannery effluents

According to Veliappan *et al.*, pollution caused by heavy metals is due to increased usage of chemicals in industries and agriculture (149). The environmental contamination by heavy metals through discharge of effluents is one of the main health problems. Metal contaminants can easily enter into the food chain if polluted water and soils are used for crops production. The industrial wastewater is generally polluted by organic compounds, inorganic complexes and other non-biodegradable substances (150). Tiwari *et al.*, studied that environmental pollution by toxic metals has accelerated dramatically in recent years due to rapid industrialization, which contributed to the highly polluted biosphere and atmosphere (151). Tanning industries discharge only chromium but also release other toxic metals (152). Ogbonna *et al.*, observed that the waste from the tanneries consists of tanned solids and sludge (153).

The sludge derived from tanning industries effluents varies in composition and usually contains water, chromium, lime and residual sulphides. Significant quantities of common mineral elements such as Al, Fe, Ca, Na, K and Si are present in the sludge (154). The effluents and sludge from tanning operations are discharged into agricultural land and surface water. The tannery sludge contains high sulphide content which creates serious odour problems in the environment. The tannery effluents also affect the quality surface and ground water. There is also a health risk to human and livestock, particularly from the discharge of high chromium content in water and the environment (155).

2.3 Heavy metals induced pollution in soil and ground water

Heavy metals released either from anthropogenic or natural activities can disperse in the environment and may ultimately get deposited in the soil. Plants grown in such areas can easily absorb heavy metals. Soil pollution is different from water pollution due to the persistence and accumulation of heavy metals in soil. Moreover, the removal of toxic metals from polluted soil is difficult, because certain heavy metals once deposited on the soil such as chromium may be virtually permanent (156).

Javed *et al.*, studied that ground water is contributing about one-third of total water resources of Pakistan and is a sole source of water supplies in major municipalities (157). In Pakistan, the main toxic contributors to the surface and ground water pollution are the by-products of various industries such as leather, dyeing metals, pesticides, cement, steel, engineering, mining and construction (158). The discharge of industrial effluents, municipal sewage and urban wastes carried by drains and canals to rivers has further worsened the water pollution (159). In Pakistan, especially in Khyber Pakhtunkhwa, no facilities are available for the treatment of industrial

effluents and wastes. Hence the effluents are dumped into various water sources causing water contamination and affecting agriculture production (160).

The groundwater in the premises of industrial areas is badly affected by tannery effluents, industrial waste and municipal sewage discharged indiscriminately without any regulatory system (161, 162). Groundwater is less contaminated directly by wastes as compared to surface water. However, due to recent industrialization and fast urbanization the quality of surface and groundwater has become an increasing concern due to contamination by various toxic chemicals (163).

2.4 Effect of tannery effluents on human health

Fabiana *et al.*, reported that tannery workers were exposed to various hazardous chemicals; such as chromium salts, benzidine based azo dyes, organic solvents (e.g. benzene and formaldehyde), penta-chlorophenol, arsenic, dimethyl formamide, N-nitroso compounds, and airborne leather dust (164). These chemicals are potential irritants and sensitizers for the workers who are frequently exposed to these chemicals for prolonged periods of time (165, 166). Tannery workers are handling more than 250 chemicals used in the leather tanning process. Chemicals enter into the worker's body by 3 routes: inhalation, ingestion and skin contact. Inhalation takes place through the nose, while skin contact occurs while handling the chemicals during various processes such as wetting, liming, skinning, tanning and drying (167). Tannery workers are exposed mainly to chromium salts, e.g. potassium dichromate, especially in the leather tanning section and they are under constant threat of chromium in the tanning industry. As chromium is an irritant, it can cause perforations in the nasal septum, respiratory problems, dermatitis, gastrointestinal, hepatic and renal impairments (168). Lockman *et al.*, reported that during skin contact, chromium has the potential to bind with the skin proteins of the workers,

producing complex antigen which leads to hypersensitivity and dermatitis (169). Chromium enters the cells in hexavalent form and is rapidly reduced to trivalent form. The health effects of chromium are primarily related to the valence state at the time of exposure, but trivalent and hexavalent forms are thought to be the most biologically significant valence states. Cr (VI) is generally considered 1,000 times more toxic than Cr (III) (46). Grabrant *et al.*, (1984) reported that (Cr VI) is a well-known corrosive, cytotoxic and carcinogenic element. Workers exposed to Cr are at a high risk of developing nasal and lungs cancer (170). Chromium produces most of its toxic effects by the generation of free radicals. Free radicals cause oxidative changes in proteins, chromosomal damage, point mutations in DNA and adduct formation (171, 172). The respiratory tract is a target organ for Cr toxicity associated with both acute and chronic inhalations (169). Many studies reported high blood Cr concentration in occupationally exposed tannery workers (142, 168, 173). Other studies also showed many fold increase of Cr levels in plasma and urine of the exposed tannery workers in comparison with controls (173-175). Hassanein *et al.*, reported that steel workers occupationally exposed to Cr had significantly higher levels of urinary Cr in comparison with control (176).

2.4.1 Endocrine disruption in industrial workers

It has been reported that exposure to chromium (VI) causes reproductive toxicity in humans and laboratory animals (177-178). Thonneau *et al.*, has reported a decrease in sperm cell count and motility as well as increased follicle stimulating hormone (FSH) levels in male workers employed in electroplating (179). Moreover, a decreased concentration of sperm cells and increase in abnormal spermatozoa were observed in mice, bonnet monkeys and rabbits treated/exposed to chromium (180-183). Chandal

and Jain (2014) reported decrease in testis weight and an increase in the seminal vesicles and prostate weights in male rats after chromium treatment (184). In addition, a decreased sperm motility and number of epididymal spermatozoa were found in chromium-treated rats in comparison to controls (185).

Li *et al.*, reported that sperm count and sperm motility of workers was significantly decreased exposed to Cr (VI) as compared to controls (177). According to Danadevi *et al.*, occupational exposure to Cr (VI) affects the semen parameters and reproductive success of welders (178).

2.4.2 DNA damage in industrial workers

Cr (VI) is the most toxic form of chromium. It can be absorbed by the lungs, gastrointestinal tract and even, to a certain extent, by skin contact and easily crosses the cell membrane, while Cr (III) is unable to pass the cell membrane.

The reduction of hexavalent chromium is considered to serve as a detoxification process when it takes place at a distance from the target site for the genotoxic effect, but the reduction of hexavalent chromium may serve to activate chromium toxicity if it takes place in or near the cell nucleus of the target organs (186-188).

Cr (VI) is mutagenic and carcinogenic and causes respiratory cancers in humans (189-190). The mechanism of Cr (VI) induced carcinogenesis is unclear. Workers occupationally exposed to Cr are at a higher risk for developing cancer (191).

Friedman *et al.*, reported chromosomal aberrations in human lymphocytes induced by CrCl_3 via indirect action and Cr (III) induces chromosomal aberrations in human lymphocytes and also causes tumor-promoter-like properties (192).

Elias *et al.*, documented sister chromatid exchanges (SCEs) in Chinese hamster V79 cells exposed to soluble CrCl_3 and insoluble Cr_2O_3 (193). According to Snow and Xu, (1991) trivalent Cr has been shown to decrease the fidelity of DNA synthesis (194).

Neto *et al.*, documented that occupational exposure to chromium causes the health problems in tannery workers that are associated with genetic damage (195). Zhang *et al.*, reported that Cr (III) is more effective than Cr (VI) in causing DNA damage in a cell free system (196).

2.4.3 Oxidative stress (MDA, GSH, SOD) in industrial workers

Toxicity of Cr has been demonstrated in various human cells, such as gastric mucosa cells and peripheral blood lymphocytes (197, 198). The major cellular targets of Cr toxicity are lung epithelial cells and lung fibroblasts; however, human Cr intoxication is also associated with hepatotoxicity, nephrotoxicity, cardiotoxicity and immunotoxicity (199, 200). Studies related to chromium exposure have reported nasal dysfunction, chromosome abnormality, immunological effects and renal tubular dysfunction during chrome plating process (201-204). Several studies have reported increased levels of lipid peroxidation in plasma, blood and urine samples of workers exposed to chromium during chromium plating process (201, 205). The mechanism of Cr toxicity is possibly activated by oxidative stress, when the production of reactive oxygen species (ROS) overwhelms antioxidant defence mechanism (206). Excess production of ROS induces lipid peroxidation, which destroys cell structures, lipids, proteins and nucleic acids (207). According to Harris and Shi., higher MDA concentration in exposed groups causes oxidative stress induced by heavy metals (208). Goulart *et al.*, reported that tannery workers exposed to Cr (III), showed higher concentration of urinary MDA than those of controls (174).

GSH is a major endogenous antioxidant, produced by the cells. GSH participate directly in the neutralization of reactive oxygen compounds and plays an important role in protecting against oxidative stress (209, 210). The concentration of GSH was found to be significantly reduced in industrial workers than those of unexposed

population. Goulart *et al.*, reported no significant decrease in GSH concentration in leather industry workers exposed to Cr (III) than that of unexposed population (174).

Lenton *et al.*, observed a significant decrease in GSH concentration with increased rate of oxidative DNA damage (211). It has been reported that GSH depletion impairs the cell defence system against the toxic actions of reactive oxygen species and might lead to cell injury and death (212).

Beltowski *et al.*, reported that the intensity of oxidative stress is determined not only by the free radical production but also by antioxidants (enzymatic and non enzymatic) defence (213).

Alberdg, and Bayil *et al.*, reported decreased antioxidant enzyme SOD in the smoking and non-smoking shoe workers as compared to controls. This is due to the production of ROS that overwhelm the endogenous antioxidant defence system. The combined effect of cigarette smoking and occupational exposure plays the major role in the induction of oxidative stress in exposed workers. Although, duration of exposure is an important factor inducing reductions in ventilatory function, yet there is no significant relationship between duration of exposure and the tested ventilatory function (214, 215). Heba *et al.*, reported that there is no significant relationship between the ventilatory function and the levels of SOD, MDA in shoe workers (216).

2.4.4 Biochemical characteristics of population exposed to chromium

Hexavalent chromium compounds are considered to be mutagenic and carcinogenic in a variety of test systems. Chromium is also a compound of biological interest. It plays an important role in glucose and lipid metabolism as an essential nutrient (217). The workers exposed to leather dust, which contains chromium in the protein-bound form,

showed a higher level of chromium in blood and urine than those of controls. Tannery workers showed high morbidity (40.1%) in comparison to the unexposed population. The increased morbidity in the tannery workers could be due to high respiratory illness (16.7%) in comparison with that of 4.27 % in the control group. The higher values of chromium in blood and urine among the workers could be explained by atmospheric pollution caused by the leather dust in the workplace. The increased pulmonary morbidity has been associated with certain symptoms such as dry cough (5.6%), throat irritation (3.6%) and lung congestion in 3.0% of the tannery workers (171, 217).

MATERIAL AND METHODS

3.1 Sampling sites

Sampling sites were selected according to demographic factors like location of tanneries discharging their effluents directly into open land and agriculture fields without pretreatment and extent of human exposure to toxic effect of these effluents.

Two major cities, Peshawar and Sheikhupura, were selected for the sampling sites. A small industrial area on Charsadda road near Peshawar city and Sheikhupura road Lahore (35 kilometer northwest of Lahore) are known for tanneries. These areas and surrounding population were taken as sampling sites. Pabbi, Mathra and Serdheri were considered as control sites from Peshawar and Ladheke Mallian, Kot Pindi Dass, Gulwere and adjoining villages were taken as control sites for the sampling sites of Sheikhupura.

3.1.1 Sheikhupura City

Sheikhupura is an industrial city of Punjab province, Pakistan. The city has a harsh climate with winter season starts from November to March. The summer season commences from April and finishes in October. The sole objective of Sheikhupura industrial estate is to develop and accelerate the industrial pace on heavy and large scale industries including leather, paper, stainless steel, pharmaceuticals, chemicals, rice, marble grinding, stone, soap, flour, poultry, animal feed, textile and many other industries. According to previous studies, leather, ceramics, textile, steel, fertilizer, pharmaceuticals are major industries that cause water pollution (218). Muridke is one

of the Tehsils of district Sheikhpura, known as an industrial city of Punjab, a number of tanneries are situated here (219, 220).

3.1.2 Peshawar city

Peshawar is the capital of the Khyber Pakhtunkhwa province of Pakistan. The agricultural fields of the city are irrigated by River Kabul (221). In Peshawar, most of the tanneries are situated in residential neighbourhoods of the city. The effluents from tanneries are disposed off into open land, water bodies and agricultural fields. Moreover, tannery effluents are polluting the River Kabul directly or indirectly making its water unsuitable for irrigation (222).

PHASE I

3.2 Collection and assessment of trace metals in tannery effluent, water, soil and vegetable samples

3.2.1 Selection of the tanning industry

3.2.2 Selection of the target area

3.2.3 Collection of the tannery effluents, ground water, soil and vegetable samples

3.2.4 Assessment of trace metals in tannery effluents, ground water, soil and vegetable samples

3.2.1 Selection of the tannery industry

The tanning industry generates waste which contains higher concentration of suspended solids, total dissolved solids, chromium, chlorides, phenols, ammonia and heavy metals (223). Besides these, chemicals like mercuric chloride, zinc chloride and formaldehyde are used as disinfectants; sodium fluoride is used to prevent putrefaction, lime is used in liming and sodium chloride is used in curing and as bleaching powder and ammonium chloride, sodium sulphate, hydrochloric acid and borax are used in deliming (224).

In the present study, the tanning industries located in Sheikhpura and Peshawar were selected for the assessment of trace metals in effluents, ground water, soil and vegetable samples and their effect on component of the biosphere.

3.2.2 Selection of target area

Soil and water samples were collected from the area within one kilometer radius of the effluent discharge site. The samples of unpolluted soil, ground water and vegetables were also collected from the control site 15 km away from the tanning area.

3.2.3 Collection of the tannery effluents, ground water, soil and vegetable samples

About 500ml sample was collected in a plastic bottle from each site. Standard procedure for sample preservation and further processing were applied (225). The effluent samples were collected from the outlet of tannery.

Potable water samples were collected from the outlets of tube well and mechanically driven hand pump (within one kilometer radius) near the target area. To avoid contamination, empty plastic bottles used for water sampling were acid-washed with 5% HNO₃ then rinsed with deionized water.

The site selection for the soil samples were identified by the visual interpretation of the area located around the outflow of waste water up to which effluent has distressed the contiguous soil. The surface soil samples were collected manually using plastic shovel from the top layer of soil with 0-15cm depth. These samples were collected within a distance of 50m and 100m from the tannery effluent so that average effect, spread and effluent mixing in soil can be assessed. The soil samples were collected in closed zip plastic bags. The soil samples were manually cleaned prior to sampling for any other alien matter of leaves, debris or rocks by the subsequent standard sampling

procedure (226). Samples of seasonal dietary vegetables *S. oleracea* (Spinach), *S. melongena* (Brinjal) and *S. tuberosom* (potato) were collected from cultivated fields irrigated with tannery effluents. The samples of vegetables were put in plastic bags and transported to laboratory. These vegetables were arranged in different sections before air and oven drying and then crushed separately. The samples of vegetables powder for each part were preserved for analysis.

3.2.4 Assessment of trace metals in tannery effluents, ground water, soil and vegetables

All the effluents, ground water, soil and vegetable samples were analyzed for trace metals by using Graphite Furnace Atomic Absorption Spectrometer (GFAAS, Analyst 700, Perkin Elmer) at the Centralized Resource Laboratory, University of Peshawar.

Effluent samples were filtered through Whatman 42 filter paper and then acidified with concentrated nitric acid to bring down the pH up to 2.0. 100 ml of sample was taken in a test tube and 5 ml concentrated HNO_3 was added and then digested in a closed chamber, digestion was completed within 30 minutes and made up the volume to 100 ml with distilled water. Digested samples were analyzed for metals concentration including chromium (Cr), lead (Pb), iron (Fe), zinc (Zn), cadmium (Cd), Nickel (Ni) and copper (Cu). (227).

50ml water samples were digested with 10 ml concentrated nitric acid at 80°C. The digested samples were filtered through Whatman 42 filter paper and filtrate was diluted to 50 ml with deionized water. The filtrate was analyzed for metals concentration (228).

The concentration of trace metals in 1.0g soil samples were placed into 100 ml beakers, to which, 15 ml of tri-acid acid mixture (70% high purity HNO_3 , 65% HClO_4 and 70% H_2SO_4 in 5:1:1 ratio) was added. The digested solution of samples was

heated to 80°C temperature till the solution became transparent (229). These samples were cooled to 4°C and filtered through Whatman No.42; the volume of samples was maintained at 100 ml by adding distilled water. The soil samples were tested for the determination of metals concentration.

The samples of vegetables were first washed with tap water, and then cleaned with distilled water. The vegetables samples were sliced into small uniform pieces for drying in an oven at 100°C. After grinding into powder form, The concentration of trace metals in 1.00g vegetable samples were placed into 100 ml beakers, to which, 15 ml of tri-acid acid mixture (70% high purity HNO₃, 65% HClO₄ and 70% H₂SO₄ in 5:1:1 ratio) was added. The digested solution of samples was heated to 80°C temperature till the solution became transparent (229). These samples were cooled to 4°C and filtered through Whatman No.42 filter paper; the volume of samples was maintained at 100 ml by adding distilled water. The samples of vegetables were tested for the determination of metals concentration.

3.2.5 Enrichment Factor

The enrichment factor (EF) was calculated to find the enrichment index value for the metals in all vegetables and soil samples. The EF was calculated using the following formula (230).

$$EF = \frac{\{[Metal]_{PV} - [Metal]_{PS}\}}{\{[Metal]_{CV} - [Metal]_{CS}\}} \quad [1]$$

Where [Metal]_{PV} = Metal in polluted vegetable, (Metal)_{PS} = Metal in polluted soil
[Metal]_{CV} = Metal in control vegetables, and (Metal)_{CS} = Metal in control soil

The results of the enrichment factor were plotted using an agglomeration tree diagram in S-Plus.

PHASE II

3.3 Biomonitoring the health status of the tannery workers

3.3.1 Selection of the individuals for the study

3.3.2 Grouping of the individuals

3.3.3 Collection of blood and urine samples from the individuals

3.3.4 Assessment of metal contents in blood and urine samples

3.3.5 Assessment of selected endocrine hormonal parameters

3.3.6 Measurement of DNA damage

3.3.7 Assessment of the oxidative stress parameters

3.3.8 Assessment of the selected haematological parameters

3.3.9 Assessment of the selected biochemical parameters

3.3.1 Selection of individuals for the study

A total of 240 individuals were registered in the study which included 120 chromium exposed tannery workers and 120 controls, with age ranging 20-50 years. These individuals were subdivided, on the basis of the duration of chromium exposure, into exposed group I and exposed II. Exposed group I was subdivided into 30 smoking and 30 nonsmoking workers with 1-10 years chromium exposure in tannery. While exposed group II was subdivided into 21 smoking and 39 nonsmoking workers with 10-20 years chromium exposure in tannery. Control population for group I and II included 28 smoking and 32 nonsmoking individuals and 20 smoking and 40 nonsmoking individuals respectively (Table 3.1). The 120 tannery workers were selected from tanneries of Sheikhpura and Peshawar, Pakistan. Tannery workers worked for at least 8–10 hours a day for six days per week. All the workers were actively involved in the chrome tanning process.

Ethical clearance for the conduct of study was obtained from Ethical Committee, University of Peshawar. A written consent was obtained from each participant after explaining the aim of the study and only those individuals were included who agreed

with the objective of the study. A detailed medical examination was performed on every worker. A questionnaire was obtained to record clinical manifestations, demographic data and past medical histories of all the participants. The workers were selected on the basis of following criteria.

3.3.1.1 Exclusion criteria

Tobacco chewing, drug addicted, diabetic and hypertensive workers were excluded from the study to minimize the effect of other factors on blood samples.

3.3.1.2 Inclusion criteria

1. Workers had been working in tanning industry for at least five years.
2. Workers had not been exposed to any other kind of pollution.
3. Workers had not suffered from any serious disease.

Control population belonging to a similar age group and socioeconomic strata, who never had any occupational exposure in the leather tanneries were selected from Pabbi, Mathra, Serdheri villages, 15 km away from Peshawar city, Ladheke Mallian, Kot Pindi Dass, Gulwere villages, 18 km away from Sheikhupura, was also taken as control sites.

3.3.2 Grouping of the individuals

Based on the age and duration of exposure in tanning industry, the workers were categorized into the following two groups.

Group I– Sixty workers in the age of group 20-35 years with 1-10 years of exposure.

Group II- Sixty workers in the age of group 36-50 years with 10-20 years of exposure.

Control Group- 120 participants in the age of group 20-50 years.

Table 3.1. The age groups, duration of exposure, gender and smokers status of population

Total Groups		Age (Years)	Exposure time in tannery (Years)	Smokers		
Groups	Numbers of people	Mean $\pm$ S.D	Mean $\pm$ S.D *	Numbers of smokers	% of smokers from total tanners	Cigarette/day (Mean $\pm$ S.D)
Exposure I	60	31.71 $\pm$ 2.228	6.12 $\pm$ 2.403	30	50	16.7 $\pm$ 3.62
Control I	60	29.17 $\pm$ 4.503	-	28	46	15.82 $\pm$ 3.87
Exposure II	60	45.02 $\pm$ 4.61	15.86 $\pm$ 3.673	21	35	18.19 $\pm$ 3.93
Control II	60	42.61 $\pm$ 4.107	-	20	33	16.3 $\pm$ 3.09

* $\pm$ S.D = Standard Deviation

3.3.3 The workplace environment

The exposed workers were selected from the tanning department. All the tannery workers were working in ill-ventilated environment. The workers were exposed to mixture of solvents, dyes, chromium salts and processing chemicals. Chromium may enter the body by breathing and by direct skin contact; therefore, workers are exposed to this element, mainly in the inorganic Cr (III) form. They are also exposed to leather dust which contains chromium in the protein-bound form and toxic irritant gases. In addition to that, chromium emission may also occur from chromate reduction and from handling of basic chromium sulphate. Leather industries that purchase chromic sulfate in powder form, dust containing Cr (III) may be emitted during handling and mixing of dry chromic sulphate.

3.3.4 Collection of blood and urine samples

3-5 ml blood samples were collected from tannery workers and control population in a metal-free safety vacutainer blood collecting tubes containing 1.5 mg K₂EDTA. The

samples were stored at -20°C until required for analysis. For hormonal analysis of female participants, the blood was collected during luteal phase.

A spot morning urine sample was collected in acid-washed, decontaminated 100 ml polyethylene tubes. Urine samples were acidified with ultrapure concentrated HNO_3 (1% v/v) and stored at -4°C until analysed. Before sub sampling for analysis, the samples were shaken vigorously for 1 min to ensure a homogeneous suspension.



(A)



(B)

Figure 3.1 (A) and (B) Skin dermatitis symptoms on worker body

Source: (Photo taken on 22 September 2013)

3.3.5 Assessment of metal contents in blood and urine samples

3.3.5.1 Microwave-Assisted Acid Digestion (MD)

For blood and urine, 0.5 ml of each sample was transferred into Teflon polytetrafluoroethylene (PTFE) flasks. 2 ml of concentrated HNO₃ and H₂O (2:1, v/v) was added to each flask, and kept for 10 min and placed in covered PTFE container. Flasks were heated following a one-stage digestion program at 80% of total power (900 W). After this, digestion flasks were cooled down. For the removal of excess acid, resulting solutions were evaporated to semidried residue. 5.0 ml of 0.1 M HNO₃ were mixed with residue and filtered through a Whatman no. 42 filter paper and diluted to 10 ml in volumetric flask. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix (231). Heavy metals in blood and urine samples were analyzed using Graphite Furnace atomic absorption spectrophotometer Perkin Elmer model 700 equipped with HGA 400 and an auto sampler AS 800 (Perkin Elmer, CA, USA).

The concentrations were obtained directly from calibration plots after correction of the signal obtained from blank. All experiments were performed at room temperature.

3.3.5.2 Determination of chromium in erythrocytes and lymphocytes

Red blood cells (RBC) were separated and prepared according to the method described by Lukanova *et al.*, (232). Briefly, haematocrit 1 (Ht 1) was first determined soon after collection, using a haematocrit centrifuge. Samples were then left for 30–35 min at room temperature to let the blood to separate into two fractions: fraction 1, the supernatant, contained the plasma and white blood cells, and fraction 2 the residual, contained predominantly red blood cells. Three milliliters of supernatant (fraction 1) was layered on 3ml of histopaque 1077 (Sigma, St. Louis, Mo., USA) and centrifuged for 30min at 1800 rpm. The lymphocytes were removed and washed three

times with phosphate buffered saline. The lymphocyte pellet was shifted to an eppendorf tube, brought up to a final volume of 1 ml, and centrifuged for 5min at 4000 rpm. The supernatant was discarded and the lymphocytes were resuspended in 0.1ml deionized distilled water.

The second fraction was diluted with 3ml of sodium chloride (0.9%) up to the initial volume of blood collected, allowed to stand at room temperature for 10min, and centrifuged for 10min at 3000 rpm. Haematocrit 2 measurement (Ht 2) and red blood cells were made just before centrifuging for the last washing. The erythrocyte pellet was diluted to the final volume of the blood sample with Triton X-100 at 0.1%.

Chromium levels in erythrocytes and lymphocytes were measured by graphite furnace atomic absorption spectrophotometer Perkin Elmer model 700 (Perkin Elmer, CA, USA) with Zeeman background correction based on the method described by Gao *et al.*, (233).

3.3.6 Assessment of selected endocrine hormonal parameters

Hormonal parameters namely testosterone, progesterone, estradiol, follicle stimulating hormone, luteinizing hormones were determined in the blood samples.

3.3.6.1 Assessment of testosterone hormone

Testosterone in the blood samples was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

Testosterone reagent kit specifications

Micro particles: 6.6 ml anti-testosterone coated micro particles in BIS-TRIS buffer with protein (bovine) stabilizers. Preservative: antimicrobial agent.

Conjugate: testosterone acridinium-labeled conjugate in citrate buffer with surfactant stabilizer. Minimum concentration: 0.1nM.

Assay diluent: 5.9ml testosterone assay diluent containing surfactant in citrate buffer.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide.

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

Serum was mixed with anti-testosterone coated paramagnetic micro particles. Then testosterone acridinium-labeled conjugate and assay diluent were added to create a reaction mixture. Testosterone in serum competes with testosterone acridinium-labeled conjugate for binding anti-testosterone coated microparticle to form an antigen-antibody complex. After washing with Wash Buffer, Pre-Trigger and Trigger solutions were added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship was found between the amount of testosterone in the sample and the RLUs detected. Intra-assay coefficients of variation were between 2.3% and 6.2%, while Inter-assay variations were between 1.4% and 4.7%. Detection limit was 10ng/dl. Calibration range was 20 to 1600 ng/dl. Results were expressed in nanograms per deciliter (ng/dl).

3.3.6.2 Assessment of progesterone hormone

Progesterone in blood samples was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

Progesterone reagent kit specifications

Microparticles: 6.6 ml anti-fluorescein, fluorescein progesterone complex coated

microparticles in TRIS buffer with protein (bovine and murine) and surfactant stabilizers. Concentration: 0.1% solids. Preservatives: sodium azide and proclin.

Conjugate: 17.0 ml anti-progesterone acridinium-labeled conjugate in MES buffer with protein stabilizers. Minimum concentration: 7 ng/ml.

Assay diluent: 5.9 ml estradiol assay diluent containing surfactant in citrate buffer. Preservatives: proclin.

Specimen Diluent: 10.0 ml estradiol specimen diluent containing TRIS buffer with protein (bovine) stabilizers. Preservative: Sodium azide

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

Serum was mixed with anti-fluorescein and fluorescein-progesterone complex coated paramagnetic micro particles. Then anti-progesterone acridinium labeled conjugate was added to create the reaction mixture. Progesterone present in the sample competes with the anti-fluorescein and fluorescein-progesterone complex coated micro particles for binding with anti-progesterone acridinium-labeled conjugate to form antibody-antigen-antibody complexes. After washing with wash buffer, Pre-Trigger and Trigger Solutions were mixed with the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship existed between the amount of progesterone in the sample and the RLUs detected.

Intra-assay varied from 1.9% to 5.7% and inter-assay varied from 3.7% to 12.7%, respectively. The assay range was 0.20–40 ng/ml. Detection limit was 0.2ng/ml. Calibration range was 0.2 to 40ng/ml. Results were expressed in nanograms per deciliter (ng/dl).

3.3.6.3 Assessment of estradiol hormone

Estradiol in blood samples was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

Estradiol reagent kit specifications

Microparticles: 9.9 ml anti-Estradiol coated micro particles in TRIS/BIS-TRIS buffer with protein stabilizers. Minimum Concentration: 0.0657% solids. Preservatives: sodium azide and proclin.

Conjugate: 5.9 ml estradiol acridinium-labeled conjugate in citrate buffer with protein stabilizers. Minimum concentration: 63.36 ng/ml. Preservatives: proclin.

Assay Diluent: 8.0 ml progesterone assay diluent containing surfactant in citrate buffer. Preservatives: sodium azide.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

In the first step, serum, specimen diluent, assay diluent, and anti-estradiol coated paramagnetic microparticles were mixed. Estradiol present in the sample binded to the anti-estradiol coated microparticles. After incubation, estradiol acridinium labeled conjugate was mixed to the reaction mixture. After a second incubation and washing, Pre-Trigger and Trigger Solutions were mixed to reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship existed between the amount of estradiol in the sample and the RLUs detected. Intra-assay variation varied from 4.5% to 8.1% and inter-assay varied from 6.3% to 12.1%, respectively. Calibration Range was 20 to 2,000pg/ml. Detection limit was 10 pg/ml. Results were expressed in pg/ml.

3.3.6.4 Assessment of follicle stimulating hormone (FSH)

Follicle stimulating hormone in blood samples was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

FSH Reagent kit

Micro particles: 6.6 ml/27.0ml anti- β FSH coated microparticles in MES buffer with protein stabilizers. Preservatives: antimicrobial agents.

Conjugate: 5.9 ml/26.3 ml anti- α FSH acridinium-labeled conjugate in MES buffer with protein stabilizers. Minimum concentration: 45 ng/ml.

Multi-Assay Manual Diluent: containing phosphate buffered saline solution. Preservative: Antimicrobial Agent.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

In the first step, sample and anti- β FSH coated paramagnetic microparticles were combined. FSH present in the sample binded to the anti- β FSH coated microparticles. After washing, anti- α FSH acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship was found between the amount of FSH in the sample and the RLUs detected. Intra-assay variation varied from 0.3% to 2.7% and inter-assay varied from 2.2% to 2.9%. Calibration range was 170mIU/ml. Detection limit was 0.1mIU/ml. Results were expressed in milli international units per milliliter (mIU/ml).

3.3.6.5 Assessment of luteinizing hormone (LH)

Luteinizing hormone in blood sample was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

FSH Reagent kit

Micro particles: 6.6 ml/27.0 ml anti- β LH coated microparticles in HEPES buffer with sucrose stabilizers. Preservative: antimicrobial agents.

Conjugate: 5.9ml/26.3ml anti- α LH acridinium labeled conjugate in MES buffer with protein stabilizers. Minimum concentration: 85ng/ml.

Multi-Assay Manual Diluent: containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

In the first step, sample and anti- β LH coated paramagnetic microparticles were combined. LH present in the sample binded to the anti- β LH coated microparticles. After washing, anti- α LH acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship was found between the amount of LH in the sample and the RLUs detected. Intra-assay variations varied from 1.5% to 2.9% and inter-assay varied from 2.3% to 3.0%. Calibration Range up to 200mIU/ml. Detection Limit: 0.7 mIU/ml. Results were expressed in milli international units per milliliter (mIU/ml).

3.3.6.6 Assessment of growth hormone (GH)

Growth hormone in blood sample was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay on an IMMULITE 2000 Analyzer) (SIEMENS). Reagent kits were supplied by (Diagnostic Products Corporation, 5700 West 96th Street, Los Angeles, USA).

In the first step, sample and solid phase bead were coated with murine monoclonal anti-hGH antibody. The reagent contained alkaline phosphatase conjugated with anti-hGH polyclonal antibody. The reagent and hGH in the sample were incubated together with bead coated along with a murine anti- hGH monoclonal antibody to form an antibody sandwich complex. Unbound enzyme conjugate was removed by a centrifugal wash. Finally, chemiluminescent substrate was mixed with the bead and signal was generated in proportion to the bound enzyme. Calibration range was 40ng/ml. Detection limit was 0.01ng/ml. Result was expressed in ng/ml.

3.3.6.7 Assessment of thyroid stimulating hormone (TSH)

Thyroid stimulating hormone in blood sample was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

TSH Reagent kit

Micro particles: 6.6 ml/27.0ml anti- β TSH coated microparticles in TRIS buffer with protein (bovine) stabilizers. Preservative: antimicrobial agents.

Conjugate: 5.9 ml/26.3ml anti- α TSH acridinium-labeled Conjugate in MES buffer with protein stabilizers. Minimum concentration: 60ng/ml.

Assay Diluent: 8.0 ml/40.7ml TSH assay diluent in TRIS buffer. Preservative: antimicrobial agents.

Multi-Assay Manual Diluent: containing phosphate buffered saline solution.

Preservative: antimicrobial agent.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

TSH assay is a two-step immunoassay for the determination of thyroid stimulating hormone (TSH) in serum.

In the first step, sample, anti- β TSH antibody coated paramagnetic microparticles and TSH assay diluent were combined. TSH present in the sample binded to the anti-TSH antibody coated microparticles. After washing, anti- α TSH acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship was found between the amount of TSH in the sample and the RLUs detected. Intra-assay variation varied from 2.41%-2.48% and inter-assay coefficients of variation were between 2.05%-5.31%. Calibration range was up to 75 μ IU/ml. Detection limit was 0.01 μ IU/ml. Results were expressed in micro international unit per milliliter (μ IU/ml).

3.3.6.8 Assessment of triiodothyronine Free T₃

Triiodothyronine Free T₃ hormone in blood sample was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay) (CMIA) (Abbott-ARCHITECT). Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

Free T3 Reagent kit

Micro particles: 6.6 ml/27.0ml coated microparticles in MES buffer with IgG stabilizers. Preservative: antimicrobial agent.

Conjugate: 5.9ml/26.3ml T₃ acridinium-labeled Conjugate in citrate buffer with NaCl and Triton X-100 stabilizers. Minimum concentration: 0.33 ng/ml.

Assay Diluent: 8.0 ml/40.7ml TSH assay diluent in TRIS buffer. Preservative: antimicrobial agents.

Multi-Assay Manual Diluent: containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution.

TSH assay is a two-step immunoassay for the determination of thyroid stimulating hormone (TSH) in serum.

Triiodothyronine assay is a two-step immunoassay for the determination of triiodothyronine Free T₃ serum.

In the first step, sample and anti-T₃ coated paramagnetic microparticles were combined. Free T₃ (unbound) present in the sample bind to the anti-T₃ coated microparticles. After washing, T₃ acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship was found between the amount of triiodothyronine in the sample and the RLUs detected. Intra-assay variation varied from 1.81-2.57% and inter-assay coefficients of variation were between 1.48-3.48 %. Results were expressed in ng/dl.

3.3.6.9 Assessment of tetraiodothyronine FreeT₄

Tetraiodothyronine FreeT₄ hormone in blood sample was determined by commercial kit, (Chemiluminescent Microparticle Immunoassay (CMIA) (Abbott-ARCHITECT).

Reagent kits were supplied by (Abbott Laboratories, Abbott Park, Illinois, USA).

Free T₄ Reagent kit

Micro particles: 6.6 ml/27.0ml anti T₄ coated microparticles in TRIS buffer with IgG stabilizers. Minimum concentration: 0.08% solids. Preservative: antimicrobial agent.

Conjugate: 5.9 ml/26.3 ml T₃ acridinium–labeled conjugate in MES buffer with NaCl and Triton X-100 stabilizers. Minimum concentration: 0.2ng/ml. Preservative: proclin.

Pre-Trigger Solution: containing 1.32% (w/v) hydrogen peroxide

Trigger Solution: containing 0.35N sodium hydroxide.

Wash buffer: containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Tetraiodothyronine assay is a two-step immunoassay for the determination of tetraiodothyronine Free T₄ serum.

In the first step, sample and anti-T₄ coated paramagnetic microparticles were combined. Free T₄ (unbound) present in the sample binded to the anti-T₄ coated microparticles. After washing, T₃ acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger solutions were added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship was found between the amount of triiodothyronine in the sample and the RLUs detected. Detection Limit: 0.18ng/dl. Calibration Range: 0.2 to 6ng/dl. Results were expressed in ng/dl.

3.3.7 Measurement of DNA damage

Human lymphocytes were analyzed using comet assay according to the method of Singh *et al.*, (1988) with slight modifications (234). A whole blood sample of 20 μ l was used to carry out single gel electrophoresis (comet assay). The slides were prepared in duplicate for every person. These slides were first coated with 50 ml regular melting point agarose (1% DW) to prepare a base layer and air dried. The base layer second coat was prepared by placing 200 μ L agarose (1% in phosphate-buffered saline (PBS)) on the slides and covered with cover glass.

After applying cover glass, the slides were allowed to gel at 4°C for 10 min. After removing cover slip, second layer was prepared by mixing 20 μ L of the whole blood with 80 μ L low melting point agarose (0.5% in PBS) and covered with cover slip and kept at 4°C for 10 min.

Cover slip was again removed and third layer of 100 μ L low melting point agarose (0.5% in PBS) was pipetted out on the second layer and covered with cover glass. The slides (without cover slip) were immersed in a tank filled with 50ml cold lysing solution (2.5 M NaCl, 200 mM Na₂ EDTA, 10 mM Tris-HCl, 10% dimethyl sulfoxide (DMSO) and 1% triton-X 100) for 24 hours.

To allow unwinding of DNA, the slides were immersed in alkaline buffer (0.3 M NaOH, 1 mM Na₂ EDTA, pH 12) for 20 min. The slides were further subjected to electrophoresis 18V (0.7-1.0 V/cm), 300 mA for 20 min at 4°C (dim light). After electrophoresis, the slides were placed in neutralizing solution with Tris-HCl buffer (400 mM, pH 7.4) for 5min and this step was repeated. Slides were then dehydrated in absolute methanol for 5 min and kept at 30°C to dry and stain with 75 μ L EtBr (ethidium bromide).

A total of 50 individual cells were screened per sample (25 cells from each slide). An undamaged cell appears an intact nucleus without a tail and a damaged cell has the resemblance of a comet.

For visualization of DNA damage, fluorescent microscope (Nikon Eclips 80i) equipped with an excitation filter of 450-490nm was used. DNA damage was expressed as micrometer.

3.3.8 Assessment of oxidative stress parameters

Oxidative stress parameters namely malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) were determined in the blood samples. Catalyse enzyme is not determined in blood samples as chromium does not affect this enzyme.

3.3.8.1 Assessment of malondialdehyde (MDA)

The blood plasma MDA level was determined by the method of Ohkawa *et al.*, (1979) (235). Briefly, 200 μ L sample of blood plasma was added to 1ml of 20% acetic acid followed by 200 μ L of 8% SDS (sodium dodecyl sulfate) (pH adjusted to 4). Thereafter, 1.5 ml of 0.8% thiobarbutric acid (TBA) and 1.1 ml of distilled water (DW) was added. Reaction mixture was incubated for 1 hour at 35°C. After cooling, 3 ml of n-butanol was added. A clear butanol fraction obtained was used for measuring the absorbance at 532 nm and expressed as nano mole per milliliter ($\text{nmol} \cdot \text{mL}^{-1}$) of blood plasma.

3.3.8.2 Assessment of glutathione (GSH)

Blood GSH level was measured according to the method of Beutler *et al.*, (236). A 200 μ L sample of the whole blood was added to 1.8 ml of cold distilled water (DW) and incubated for 10 min at 37°C. 600 μ L of sulpha salicylic acid was mixed with the reaction mixture and centrifuged at 2000 r/min for 15 min. 200 μ L of supernatant was

mixed with 400 ml of phosphate buffer and 80 ml of 3, 5-dithiobis-2-nitrobenzoic acid. The yellow color developed was measured at 412 nm and expressed as microgram per milliliter ($\mu\text{g}\cdot\text{ml}^{-1}$).

3.3.8.3 Assessment of superoxide dismutase (SOD)

SOD activity was assayed according to the method of McCord and Fridovich with minor modifications (237). A 200 μl of the whole blood sample was washed thrice with ice cold normal saline. Blood sample was centrifuged three times at 10000 r/min. Red blood cells were collected and hemolyzed by adding 1.5 volume of water and temperature was kept at 0–4°C. Hemoglobin (Hb) was precipitated by adding chloroform (180 ml) and cold ethanol (300 ml) followed by vigorous shaking and then centrifuged. The resulting supernatant containing SOD was taken for the measurement of its activity. Two reaction setups were performed in parallel. The first setup tube received 1.2 ml (0.052 M) of sodium pyrophosphate, 0.3 ml (186 μM) of phenazine methosulphate, 0.3 ml (300 μM) of nitro blue tetrazolium and 0.2 ml of enzyme source. The tubes in the second setup (reference) received all the above reagents except the enzyme source. After adding 0.8 ml and 1 ml distilled water (DW) in both sets and finally the reactions were started by the addition of 0.2 ml (780 mM) of reduced nicotinamide adenine dinucleotide (NADH) disodium salt. 1 ml of glacial acetic acid was added after an interval of 90 s to each reaction tube and absorbance was measured at 560 nm against a blank on spectrophotometer. The SOD activity was expressed as units per gram of hemoglobin ($\text{unit g}^{-1}\text{Hb}^{-1}$).

3.3.9 Assessment of selected haematological parameters

The following haematological parameters were determined in blood samples.

3.3.9.1 Haemoglobin (Hb) count

3.3.9.2 Red blood cells (RBC) count

3.3.9.3 White blood cells (WBC) count

3.3.9.4 Packed volume cells (PCV)

3.3.9.5 Mean corpuscular volume (MCV)

3.3.9.6 Mean corpuscular Haemoglobin (MCH)

3.3.9.7 Mean corpuscular haemoglobin concentration (MCHC)

3.3.9.8 Platelets (Plt)

3.3.9.9 Erythrocyte sedimentation rate (ESR)

Haematological parameters were determined at the department of Biochemistry, Fatima Jinnah medical college, Lahore. Haemoglobin (Hb) count, red blood cells (RBC) count, white blood cells (WBC) count, packed volume cells (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (Plt), and erythrocyte sedimentation rate (ESR) were determined by using the Sysmex model KX-21 automated haematology analyzer.

3.3.9 Assessment of selected biochemical parameters

The following biochemical parameters were determined in the blood serum.

3.3.10.1 Total Protein

3.3.10.2 Albumin

3.3.10.3 Alkaline phosphatase

3.3.10.4 Alanine aminotransferase

3.3.10.5 Aspartate transaminase

3.3.10.6 Total bilirubin

3.3.10.7 Direct bilirubin

3.3.10.8 Serum glucose

Biochemical parameters were determined at the department of Biochemistry, Fatima Jinnah medical college, Lahore. Total protein, albumin, alkaline phosphatase, alanine

aminotransferase, aspartate transaminase, total bilirubin, direct bilirubin and serum glucose were determined by using the Architect Ci8200 (Abbott Diagnostic).

3.11 Statistical analysis

The samples of blood, urine, soil, water and vegetables were analyzed in terms of mean, percentages and standard deviations. The data was analysed using "IBM SPSS software" 20.0 version. For comparison of the two means, independent t-test was used, while ANOVA was applied to identify variation in the data. The association pattern of chromium concentration with hormonal parameters, DNA damage, oxidative stress, biochemical and haematological parameters were analysed using Pearson's correlation analysis. P-value <0.05 was considered as statistically significant.

3.12 Medical history questionnaire

Reference Number: _____ Dated: _____

Name: _____

S/O, D/O, W/O: _____

Age: _____

Gender: _____

Occupation: _____

Marital Status: _____

Reproductive Abnormality: _____

Abortions: _____

Sterility: _____

Still Birth: _____

Survival rate of Children: _____

Number of Children: _____

Clinical Data:

Proforma used for collection of data on medical history and reproductive abnormalities of the tannery workers.

RESULTS AND DISCUSSION

Phase I

4.1 Study of environmental parameters

Trace metals were investigated in effluents, ground water, soil and selected vegetables in the tanning areas of Sheikhpura and Peshawar.

4.1.1 Trace metals in tannery effluents

4.1.1.1 Chromium in tannery effluents

The concentration of chromium in effluents of Sheikhpura tanneries was found to be 350.15 mg/l followed by 228.5 mg/l in effluents of Peshawar tanneries (Table 4.1, 4.2). The chromium level in Sheikhpura tanneries effluents was found to be higher than that of Peshawar tanneries. Chromium concentration was detected to be the highest among all the trace metals in tannery effluents. Tariq *et al.*, (2006) reported high concentration of chromium in effluents of Peshawar tanneries (238). Chromium is a major chemical used in tanneries and hence its discharge in tannery effluents was found to be high. The presence of chromium in tannery effluents has been reported to disrupt the aquatic food chain and found to be toxic to aquatic life (239). It has been observed that the transfer of chromium from tannery effluents into ground water exceeded the permissible limits set by WHO/FAO (240). It has been documented that 50-70% chrome is taken up by skin collagen during tanning process, while the rest of chromium is discharged in the effluents (241, 242). The Peshawar tannery effluents are discharged directly or indirectly into Kabul River rendering its water useless for irrigation (243). Chromium discharge in the tannery effluents can be toxic to human

population, animals and plants; therefore it must be removed from the effluents before discharge (244).

The above result showed that tannery effluents discharged into environment contain chromium above the permissible limits set by WHO/FAO.

4.1.1.2 Cobalt, manganese, cadmium, nickel, lead, zinc, iron in tannery effluents

Cobalt, manganese, cadmium, nickel, lead, zinc and iron in the tannery effluents from Sheikhpura were found to be 1.519mg/l, 0.35mg/l, 0.07mg/l, 0.53mg/l, 0.84mg/l, 0.31mg/l, 15.92mg/l respectively, while in the tannery effluents from Peshawar the concentration was found to be 0.554mg/l, 0.345mg/l, 0.0617mg/l, 0.478mg/l, 0.515mg/l, 0.295mg/l and 11.423 mg/l respectively (Table 4.1, 4.2). However, higher value of Fe was detected in the both tannery effluents followed by a meager concentration of Co and Pb. The concentrations of cobalt, manganese, cadmium, nickel, lead, zinc and iron in Sheikhpura tannery effluents were found to be higher as compared to tannery effluents from Peshawar. The tannery effluents are contaminated with higher concentration of chromium, cadmium, nickel, iron, copper and manganese. According to Verma *et al.*, irrigation with tannery effluents pollute the food crops and vegetables, which when consumed can cause health disorder in humans (245). According to Lofrano *et al.*, toxic effects of cadmium and other heavy metals in environment are considered major threat to human life, animals and plants, therefore their removal from effluents are imperative (246).

Table 4.1 Mean and standard deviation of trace metals concentration in the samples of environmental media in Sheikhpura

Metal	Effluent (mg/l)			Groundwater (mg/l)			Soil (mg/kg)		
	Mean	S.D	Kurtosis	Mean	S.D	Kurtosis	Mean	S.D	Kurtosis
Cr	350.15	61.97	9.34	0.06	0.05	-0.21	31.13	36.09	-0.70
Co	1.519	1.84	-0.47	0.20	0.15	-0.39	3.82	2.38	0.32
Mn	0.35	0.51	-0.84	0.13	0.20	2.37	2.97	2.65	0.53
Cd	0.07	0.08	0.19	0.02	0.01	-1.07	0.36	0.26	1.28
Ni	0.53	0.79	-0.04	0.11	0.16	-0.03	4.21	2.65	-0.33
Pb	0.84	1.23	1.25	0.11	0.15	-0.14	4.15	1.98	0.34
Zn	0.31	0.51	1.16	0.15	0.20	-0.64	3.20	2.61	-0.25
Fe	15.92	16.74	-0.20	0.19	0.31	1.75	17.64	26.89	1.84

The value of $p < 0.05$ $n=50$ for each medium soil, groundwater and effluent

Table 4.2 Mean and standard deviation of trace metals concentration in the samples of environmental media of Peshawar

Metal	Effluent (mg/l)	Groundwater (mg/l)	Soil (mg/kg)
Cr	228.5±67	0.056±0.042	*17.99±18.70
Co	0.554±0.765	0.169±0.144	3.38 ±1.7
Mn	0.345±0.493	0.073±0.178	2.2±2.4
Cd	0.061±0.087	0.168±0.009	0.29±0.15
Ni	0.478±0.719	0.140±0.170	3.86±2.1
Pb	0.515±0.634	0.070±0.133	3.95±1.7
Zn	0.295±0.476	0.145±0.203	2.9±2.2
Fe	11.42±8.86	0.151±0.316	14.3±19.5

$p < 0.05$

* Mean ± standard Deviation, $n=50$ (Soil and groundwater) $n=30$ (Effluent)

4.1.2 Trace metals in ground water samples of target area

4.1.2.1 Chromium in ground water

The concentration of chromium in ground water from Sheikhpura was found to be 0.06 mg/l followed by 0.056 mg/l in ground water from Peshawar (Table 4.1, 4.2). The chromium level in ground water taken from Sheikhpura was found to be higher than that of Peshawar. Tariq *et al.*, also reported high concentration of chromium (0.089mg/l) in drinking water near Peshawar tanneries (238). According to the Duruibe *et al.*, immediate concern metals are chromium, manganese, aluminum, iron, copper, cobalt, zinc, nickel, cadmium, lead and mercury (247). When these metals are discharged into ground water sources, the water becomes useless for drinking purpose (248). The presence of heavy metals in industrial effluents is one of the main causes of ground water and soil contamination (249, 250)

4.1.2.2 Cobalt, manganese, cadmium, nickel, lead, zinc and iron in ground water

The concentration of cobalt, manganese, cadmium, nickel, lead, zinc and iron in the ground water from Sheikhpura was found to be 0.20 mg/l, 0.13mg/l, 0.02mg/l, 0.11mg/l, 0.19mg/l, 0.15mg/l, 0.19mg/l respectively, while in the ground water from Peshawar the concentration was found to be 0.169mg/l, 0.073mg/l, 0.168mg/l, 0.140mg/l, 0.070mg/l, 0.145mg/l, and 0.151mg/l respectively (Table 4.1, 4.2). The concentration of trace metals in target area ground water measured during the study was found to be higher in Sheikhpura as compared to ground water used in Peshawar. High concentration of heavy metals was also detected in the ground water from Peshawar (238). The higher concentration of heavy metals in ground water recorded in Sheikhpura was due to the non-point sources, surface run-off, agricultural and the discharges from the town and various tanneries.

The major cause of water pollution is wastewater disposal from various industries which is being released into common drainage. The waste water not only affects the surrounding land, but also changes the biochemical nature of ground water. This could be due to the presence of chemicals in industrial wastewater which contaminate and change the biochemical nature of ground water (251). The organic chemicals in drinking water are also the major cause of pathogenic diseases like cholera and diarrhea (252).

The findings of this study showed that the ground water near the tannery area was significantly contaminated with chromium, iron, cadmium, nickel and zinc due to careless and unsafe discharge of tannery effluents into bare land.

4.1.3 Trace metals in target area soil

4.1.3.1 Chromium in soil samples

The concentration of chromium in soil of Sheikhpura was found to be 31.13mg/l followed by 17.99mg/l in the soil of Peshawar (Table 4.1, 4.2). The chromium level in the soil of Sheikhpura was found to be higher than that of Peshawar. This may be due to greater effluent discharge of chromium from Sheikhpura tanneries.

The tanneries discharge large amount of toxic substances like chromium, sodium carbonate, ammonium sulphate and sodium sulphide in their effluents which cause soil pollution (253). Unsafe disposal of effluents containing chromium at industrial site has polluted the soil and resultantly ground water, which has a negative impact on plants growth (254).

4.1.3.2 Cobalt, manganese, cadmium, nickel, lead, zinc and iron in soil samples

The concentration of cobalt, manganese, cadmium, nickel, lead, zinc and iron in the soil of Sheikhpura and Peshawar is summarized in Table 4.1, 4.2.

The concentration of cobalt, manganese, cadmium, nickel, lead, zinc and iron in the soil of Sheikhpura was found to be 3.82mg/l, 2.97mg/l, 0.36mg/l, 4.21mg/l, 4.15mg/l, 3.20mg/l, 17.64mg/l respectively, while in soil samples from Peshawar the concentration was found to be 3.381mg/l, 2.20mg/l, 0.29mg/l, 3.86mg/l, 3.95mg/l, 2.90mg/l, 14.30mg/l respectively. Trace metals discharged from industries or natural activities may disperse in the environment and ultimately accumulated in the soil and finally transferred to the plants growing in this area. Metals like iron, manganese, molybdenum, copper, zinc, magnesium, selenium, nickel and copper play important role in the growth and development of plants, but they are toxic beyond a certain level (255). According to Altieri *et al.*, the dissolved chemicals in the industrial effluents react with clay complex of the soil leading to accumulation of salts and increase in exchangeable sodium and other nutrient in the soil (256).

The presence of heavy metals in soil has toxic impact on human health and environmental quality via surface and ground water. Moreover, high concentration of heavy metals also renders soil less fertile and also causes phytotoxicity in plants and bioaccumulation in human body (257).

4.1.4 Trace metals in selected vegetables from Peshawar

The concentration of trace metals in selected vegetables from tanneries affected area in Peshawar is summarized in Table 4.3. The whole plant of *Spinacia oleracea* (Spinach) bioaccumulated high amount of chromium (Cr) 10.16µg/g, while the concentration of Fe, Zn, Pb, Ni, Cu and Cd was found to be 302.10µg/g, 144.2µg/g, 18.43µg/g, 37.80µg/g, 25.09µg/g and 2.56µg/g respectively (Fig. 4.1).

The tuber and shoot of *Solanum tuberosum* (Potato) bioaccumulated high amount of Cr with the mean values of 38.20µg/g and 21.16µg/g respectively (Fig. 4.2). The tuber of *Solanum tuberosum* has the highest concentration of Cr (38.20 µg/g) and is

significantly ($p > 0.001$) correlated with chromium concentration in soil. There is considerable discrepancy in bioaccumulation of trace metals in leaves and fruits. The elevated concentration of metals observed in whole plant of spinach is iron, zinc and nickel. The fruit of brinjal has accumulated the least amount of heavy metals in all the three tested vegetables. Okoronkwo *et al.*, reported that the porous cells of tuber have the capacity to accumulate heavy metals at a faster rate (258). The presence of high concentration of trace metals in vegetables and fruits may build up excessive toxic metals in human body. Copper, chromium, iron, zinc and manganese are necessary for normal functioning of the body, but in case of overexposure, they cause serious health problems (259, 260). According to Shanker *et al.*, bioaccumulation of heavy metals in fruits and vegetables depend on the type, and nature of the plant (261).

The concentration of Cr in the roots of *Solanum melongena* (brinjal) was found to be $16.26\mu\text{g/g}$, while $11.80\mu\text{g/g}$ and $7.80\mu\text{g/g}$ in shoot and fruit respectively (Fig. 4.3). The texture and cells in roots of *Solanum melongena* have the potential to store high concentration of metals (259, 262).

In the enrichment index (Table 4.4), values greater than 1.0 showed significantly higher rate of metals concentration in plants and soil. The values between 0.5 and 1.0 indicated normal enrichment of metals, while values less than 0.5 in enrichment index signify negligible loading of particular heavy metals in plants and soil.

The tree diagram showed the significance of the metal enrichment factor (Fig 4.4) using hierarchical agglomerative cluster analysis (HACA) for metal association in plants and soil. The enrichment rate of Cr and Fe is similar in *Solanum tuberosom*, while the transfer rate of Cr is higher than that of Fe in *Solanum melongena*. The high enrichment factor of Zn has been observed in all the three vegetables with values of 14.36, 2.32 and 4.57 for *S. oleracea*, *S. melongena* and *S. tuberosom* respectively

(Table 4.4). All the plants have varied capacities to absorb metals. Zn and Pb showed high enrichment values. The metals Cd, Fe, Cu, Co, Zn, Ni and Cr have higher concentrations in vegetables than permissible limits, leading to serious food contamination. According to Wang *et al.*, Zn has the potential to induce toxicity in plants at a greater rate (263).

Oxidative stress is produced in plants exposed to heavy metals, which damages the cell function and causes genetic disturbance in cellular homeostasis (264-266). Moreover, the heavy metals in vital food chain displace the important nutrients and accumulate in the human body, leading to adverse health effects (267-269).

The present study revealed the toxic effect of tannery effluents on vegetables grown near tanning industrial area. The irrigation practices using contaminated water have caused enrichment of toxic metals in soil which is eventually transferred to vegetables. The vegetables grown in the contaminated soil have significantly elevated levels of trace metals (Fe, Zn, Cr, Pb, and Ni). In addition, the farmers are apparently unaware of the potential threat of contaminated vegetables to the local population.

Table 4.3 Summary of statistical analysis of trace metals in vegetables irrigated by tannery effluents ($\mu\text{g/g}$)

Vegetable	Part of vegetable	Cr	Fe	Zn	Pb	Ni	Cu	Cd
Spinacia oleracea (Spinach)	Whole plant	10.16a $\pm$ 1.b	302.1 $\pm$ 6.8	144.2 $\pm$ 6.9	18.43 $\pm$ 1.2	37.8 $\pm$ 5.03	25.09 $\pm$ 2.8	2.56 $\pm$ 85
Solanum melongena (Brinjal)	Root	16.26 $\pm$ 1.4	138.69 $\pm$ 3.03	49.05 $\pm$ 2.25	14.87 $\pm$ 1.9	11.50 $\pm$ 1.43	11.50 $\pm$ 1.6	1.19 $\pm$ 9
	Shoot	11.8 $\pm$ 1.5	133.66 $\pm$ 3.7	20.45 $\pm$ 1.9	12.45 $\pm$ 1.7	11.45 $\pm$ 2.2	10.68 $\pm$ 1.9	1.02 $\pm$ 56
	Fruit	7.8 $\pm$ 1.7	96 $\pm$ 2.3	15.18 $\pm$ 2.1	8.79 $\pm$ 1.6	6.9 $\pm$ 1.8	6.07 $\pm$ 2.15	1.11 $\pm$ 6
Solanum tuberosom	Tuber	38.2 $\pm$ 2.08	158.03 $\pm$ 2.4	47.01 $\pm$ 2.68	31.05 $\pm$ 2.73	17.02 $\pm$ 2.13	15.9 $\pm$ 2.13	3.44 $\pm$ 1.71
	Shoot	21.16 $\pm$ 2.9	134.97 $\pm$ 3.07	34.97 $\pm$ 3.08	22.04 $\pm$ 3	12.73 $\pm$ 2.3	12.53 $\pm$ 2.5	3.65 $\pm$ 1.7

*a = Mean and b= Standard Deviation (SD), (n) total number of samples = 10

Table 4.4 Enrichment factor for the loading of trace metals in vegetables

Vegetable	Part of vegetable	Cr	Fe	Cu	Zn	Ni	Pb	Cd
Spinacia oleracea (Spinach)	Whole Plant	0.24157	0.56956	2.4215	14.366	0.3036	4.4594	0.5104
Solanum melongena (Brinjal)	Root	0.39480	0.27020	1.0701	2.3274	0.2444	1.0816	0.2023
	Shoot	0.28779	0.23353	1.0526	1.9282	0.2058	1.1162	0.2034
	Fruit	0.18895	0.25370	0.7454	1.4228	0.1453	0.5708	0.1056
Solanum tuberosom	Tuber	0.92746	0.76616	1.2017	4.5751	0.5111	1.2849	0.2522
	Shoot	0.51347	0.82313	0.9916	3.3814	0.3630	0.7163	0.2204

Values with > 0.5 EF leads to more loading of heavy metals in plants n=10

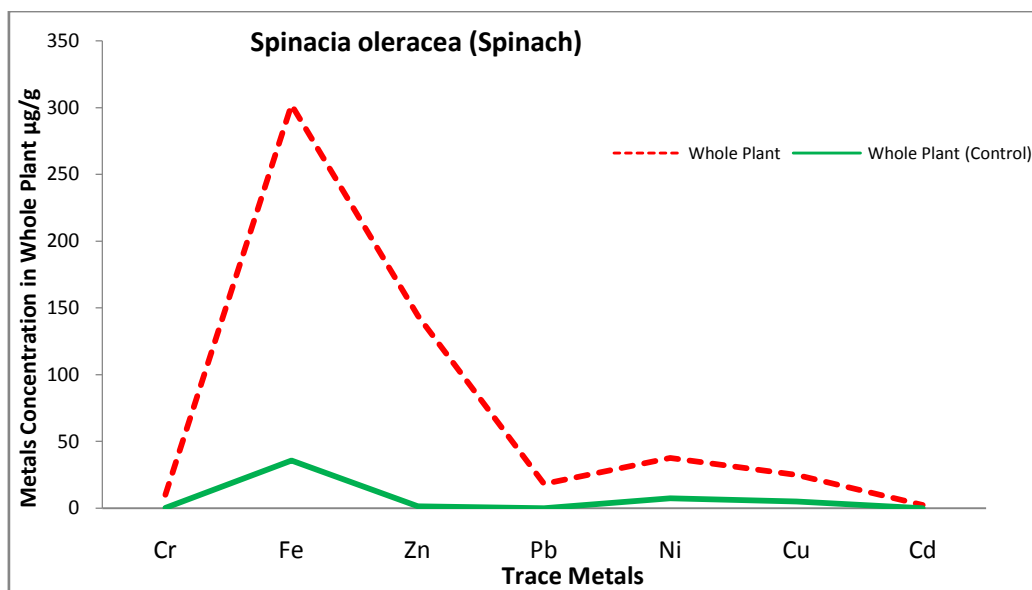


Figure 4.1 Variation of trace metals in control and tannery water irrigated whole plant of *Spinacia oleracea* (Spinach)

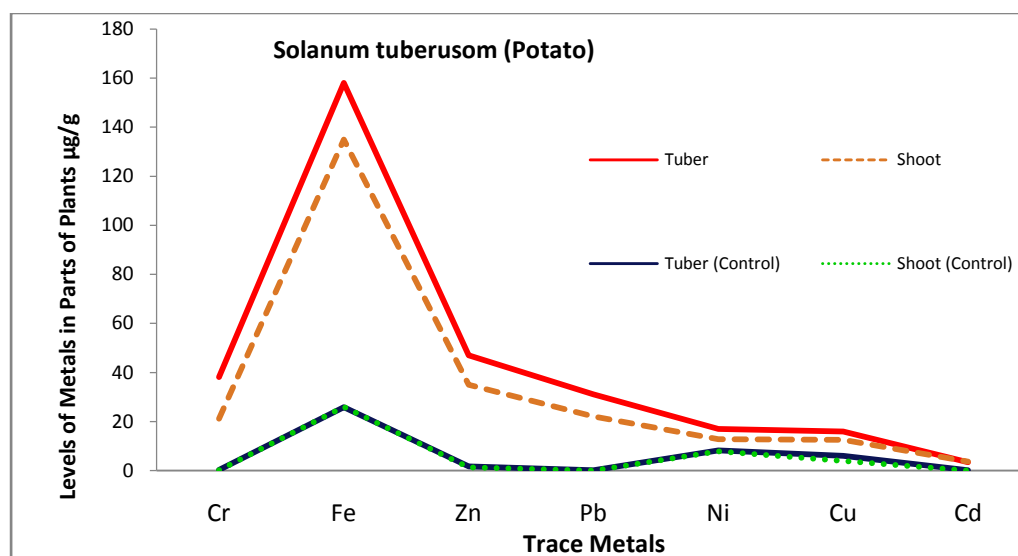


Figure 4.2 Variation of trace metals in control and tannery water irrigated plant parts of *Solanum tuberosum* (Potato)

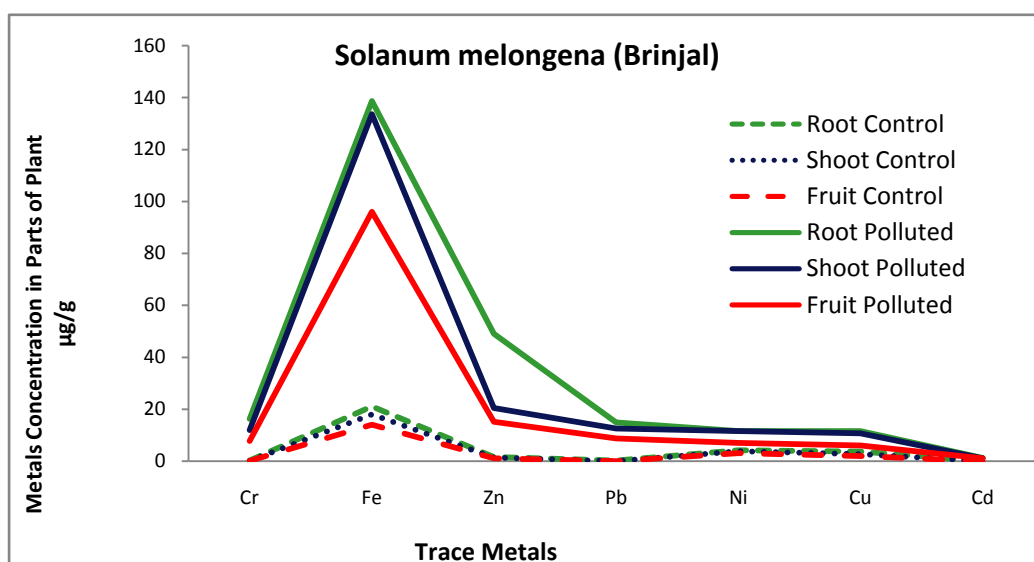


Figure 4.3 Variation of trace metals in control and tannery water irrigated plant parts of *Solanum melongena* (Brinjal)

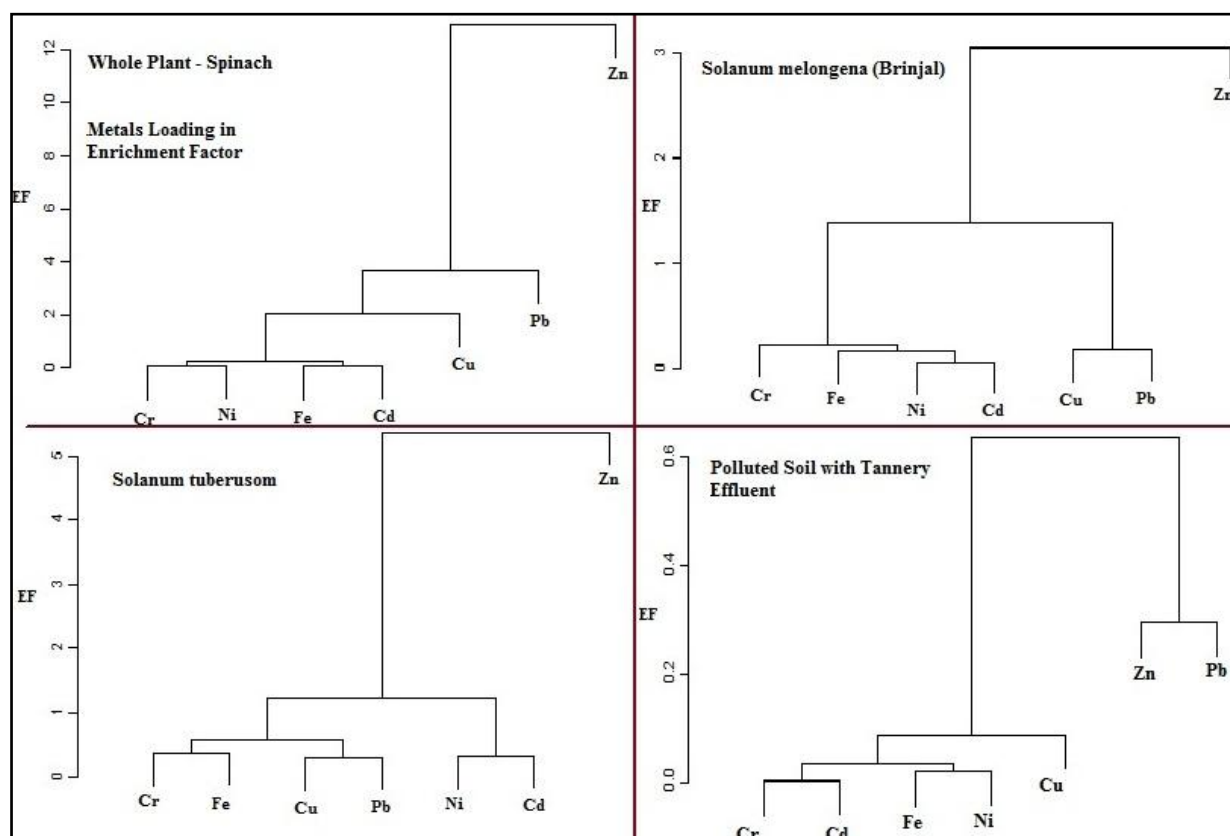


Figure 4.4 The hierarchical diagram showing metal loading in soil and vegetables by enrichment factor

4.1.5 Effect of trace metals from tannery effluents on the soil and groundwater using multivariate analysis

A complete statistical summary of the distribution of the trace metals in samples belonging to the three media is given in Table 4.5. The data shows that in the effluent samples, Na, Cr, Mg, K, and Ca were among the dominant metals. The order of the metals' abundance in tannery effluents on the basis of mean values is $\text{Na} > \text{Cr} > \text{Mg} > \text{K} > \text{Ca}$ at 1017.28, 350.15, 216.71, 201.17 and 83.60 (mg/L) respectively. Cd is at the lowest position having mean 0.07 (mg/L). In the effluent samples, Zn, Mn, Ni, and Pb have low mean concentration of 0.310, 0.350, 0.530 and 0.840 (mg/L) respectively. It was observed that values were randomly spread around the mean of metals concentration. No symmetry was observed in the skewness factor of data; furthermore, the kurtosis values show non normal and significant random distribution in effluent samples.

The most prominent metals in the ground water were Na, K, Ca and Mg, with their means values of $356.22 > 69.76 > 35.71 > 9.65$ (mg/L). The data spread is random and does not show normal distribution pattern. Cd, Cr, Pb, Ni, Mn, Zn, Fe and Co have lowest mean values of 0.02, 0.05, 0.11, 0.19, 0.13, 0.15, 0.19 and 0.20 (mg/L) respectively, showing lower concentration compared to the Na, K, Ca and Mg.

In the soil samples the distribution of metal concentration follows a decreasing order of $\text{Na} > \text{Mg} > \text{Ca} > \text{K} > \text{Cr} > \text{Fe}$ with respect to mean concentration; 11947.3, 3359.15, 951.86, 692.08, 31.13 and 17.64 (mg/L) respectively. While Cd, Mn, Zn, Co, Pb and Ni have many fold lower concentration than that of Na and Mg. The most dominant metal in the samples belonging to the three media (effluents, water and soil) is Na which in many folds higher than that of the metals having lower mean values. The metal data is random around their mean and shows non normal distribution in three

media. Na, K, Ca and Mg are those common metals having highest ranking with respect to mean in the samples of effluents, water and soil.

Table 4.5 Summary of statistical analysis of twelve metals level in all the three media of tannery effluent, groundwater and soil

Metal	Tannery Effluent			Groundwater			Soil		
	Mean	S.D	Kurtosis	Mean	S.D	Kurtosis	Mean	S.D	Kurtosis
Na	1017.28	191.31	0.17	356.22	342.99	0.71	11947.3	802.01	0.81
Ca	83.60	133.15	1.32	35.71	12.64	-0.25	951.86	652.79	-0.13
K	201.17	312.23	0.01	69.76	98.80	1.95	692.08	434.66	-0.97
Mg	216.71	343.24	0.49	9.65	10.43	0.25	3359.15	6534.90	3.00
Fe	15.92	16.74	-0.20	0.19	0.31	1.75	17.64	26.89	1.84
Mn	0.35	0.51	-0.84	0.13	0.20	2.37	2.97	2.65	0.53
Cr	350.15	661.97	9.34	0.05	0.05	-0.21	31.13	36.09	-0.70
Co	1.51	1.84	-0.47	0.20	0.15	-0.39	3.82	2.38	0.32
Cd	0.07	0.08	0.19	0.02	0.01	-1.07	0.36	0.26	1.28
Ni	0.53	0.79	-0.04	0.11	0.16	-0.03	4.21	2.65	-0.33
Pb	0.84	1.23	1.25	0.11	0.15	-0.14	4.15	1.98	0.34
Zn	0.31	0.51	1.16	0.15	0.20	-0.64	3.20	2.61	-0.25

n=50 for each medium soil, groundwater and effluent

The effluent versus ground water trace metals relationship is shown in Table 4.6. There is significant correlation between the two media as $p < 0.001$. The data confirms that presence of Na and Ca in ground water has strong relationship with all metals in effluents. The Na and Ca in the effluents are strongly correlated with metals in water (Na, Ca, K, Mg, Fe, Mn, Cr, Co, Cd, Ni, Pb and Zn). Cr in the effluents also exhibits positive correlation with Fe. The amount of Co in the effluent and K in the ground water have positive correlation but lower as compared to all other metals in two media.

Table 4.6 Correlation coefficient (r) matrix for trace metals in tannery effluents vs. ground water samples (n=50)

Metal	Na (e)	Ca (e)	K (e)	Mg (e)	Fe (e)	Mn (e)	Cr (e)	Co (e)	Cd (e)	Ni (e)	Pb (e)	Zn (e)
Na(w)	0.909	0.887	0.816	0.629	0.648	0.878	0.739	0.584	0.880	0.627	0.677	0.848
Ca(w)	0.955	0.931	0.838	0.739	0.704	0.853	0.683	0.626	0.842	0.707	0.828	0.907
K(w)	0.768	0.746	0.774	0.567	0.609	0.772	0.645	0.325	0.800	0.513	0.644	0.704
Mg(w)	0.988	0.977	0.867	0.755	0.668	0.887	0.743	0.598	0.863	0.677	0.829	0.950
Fe(w)	0.959	0.994	0.853	0.794	0.686	0.810	0.846	0.541	0.824	0.703	0.873	0.968
Mn(w)	0.950	0.986	0.847	0.820	0.688	0.780	0.863	0.542	0.837	0.728	0.892	0.969
Cr(w)	0.979	0.953	0.856	0.694	0.673	0.922	0.698	0.584	0.861	0.623	0.787	0.931
Co(w)	0.897	0.842	0.820	0.822	0.669	0.788	0.621	0.633	0.870	0.718	0.842	0.844
Cd(w)	0.852	0.759	0.763	0.684	0.682	0.803	0.473	0.661	0.835	0.641	0.715	0.753
Ni(w)	0.979	0.968	0.862	0.696	0.667	0.910	0.720	0.557	0.842	0.631	0.800	0.938
Pb(w)	0.976	0.961	0.854	0.678	0.661	0.915	0.706	0.561	0.839	0.620	0.784	0.930
Zn(w)	0.946	0.918	0.858	0.627	0.637	0.940	0.635	0.588	0.827	0.573	0.737	0.866

All the correlation values are significant as $p < 0.001$ $n = 50$

The correlation between trace metals in tannery effluent (e) and soil (s) are shown in Table 4.7. All the metals in two media are highly correlated. The data confirmed that Na and Ca are significantly correlated with all other metals. In the two media, there is a positive correlation between Pb and Zn. Mn in the effluent has the highest correlation with Ca and K in soil. Zn in effluent is significantly correlated with Pb present in the subsoil and cause toxicity in it. The data shows positive significant correlation between the two media. In order to study the interrelationship of metals in three media; cluster analysis is applied in the form of dendrograms. The positive significant correlations between metals in effluents, water and soil samples are also supported by clustering in the dendrograms.

Table 4.7 Correlation coefficient matrix for trace metals in tannery effluents vs. soil samples (n=50)

Metal	Na (e)	Ca (e)	K (e)	Mg (e)	Fe (e)	Mn (e)	Cr (e)	Co (e)	Cd (e)	Ni (e)	Pb (e)	Zn (e)
Na(s)	0.872	0.874	0.725	0.800	0.631	0.711	0.738	0.529	0.774	0.788	0.739	0.892
Ca(s)	0.979	0.957	0.881	0.709	0.641	0.924	0.713	0.587	0.863	0.623	0.796	0.925
K(s)	0.947	0.893	0.888	0.661	0.612	0.949	0.616	0.581	0.870	0.578	0.725	0.850
Mg(s)	0.762	0.744	0.642	0.474	0.641	0.711	0.718	0.413	0.750	0.488	0.573	0.727
Fe(s)	0.832	0.857	0.698	0.654	0.579	0.719	0.770	0.617	0.751	0.646	0.673	0.830
Mn(s)	0.967	0.946	0.850	0.818	0.726	0.833	0.741	0.621	0.880	0.729	0.875	0.941
Cr(s)	0.682	0.681	0.687	0.614	0.621	0.699	0.547	0.450	0.651	0.496	0.660	0.672
Co(s)	0.876	0.877	0.682	0.852	0.702	0.658	0.697	0.557	0.734	0.779	0.899	0.918
Cd(s)	0.932	0.949	0.780	0.843	0.699	0.743	0.790	0.536	0.798	0.728	0.913	0.956
Ni(s)	0.843	0.805	0.709	0.804	0.606	0.604	0.624	0.585	0.766	0.776	0.779	0.826
Pb(s)	0.945	0.946	0.787	0.769	0.618	0.772	0.743	0.500	0.828	0.711	0.812	0.933
Zn(s)	0.931	0.883	0.838	0.803	0.718	0.824	0.636	0.660	0.859	0.713	0.859	0.878

All the correlation values are significant as $p < 0.001$ n=50

The dendrogram of effluent samples in Fig. 4.5 (a) shows that Ca-Zn made a cluster with Na by using maximum pearson correlation (270). Other metals are making clusters but with low strength of association. The dendrogram of ground water samples in Fig. 4.5(c) shows that metals like Pb, Ni, Cr and Mg are making a cluster by similar correlation in Ca and Na. Dendrogram of selected metals in soil samples presented in Fig. 4.5(b) shows that Mn-Zn and Ca-K have highest level of association. The metals analysis shows that Mn, Zn, Co, Cd and Pb have inter-metal correlation in soil.

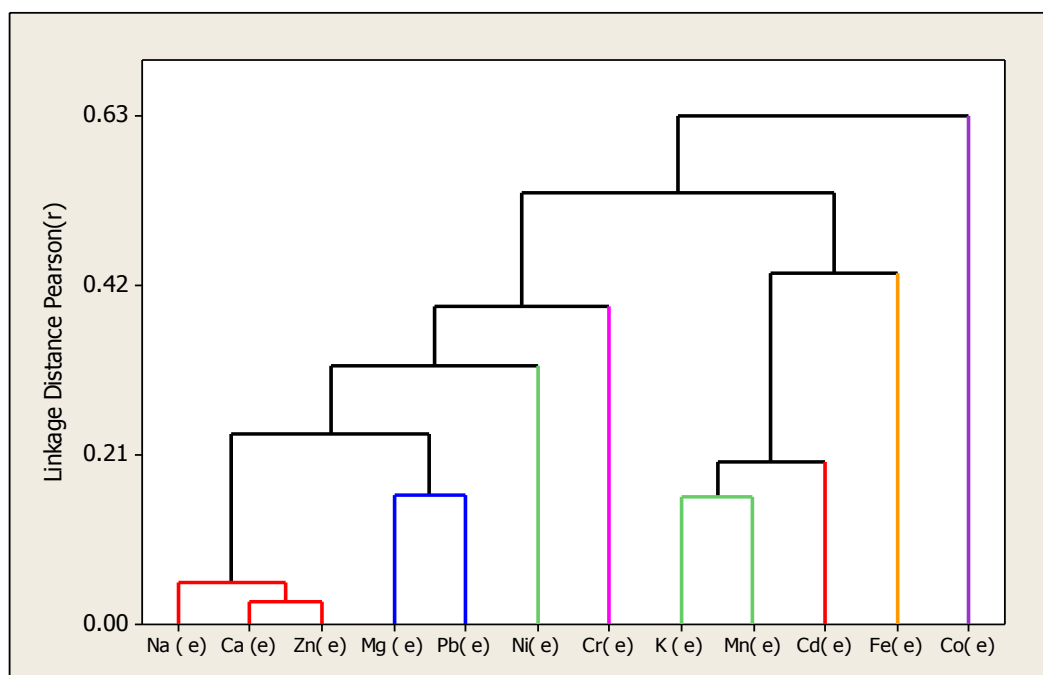


Figure 4.5 (a) Dendrogram of selected metals in tannery effluent

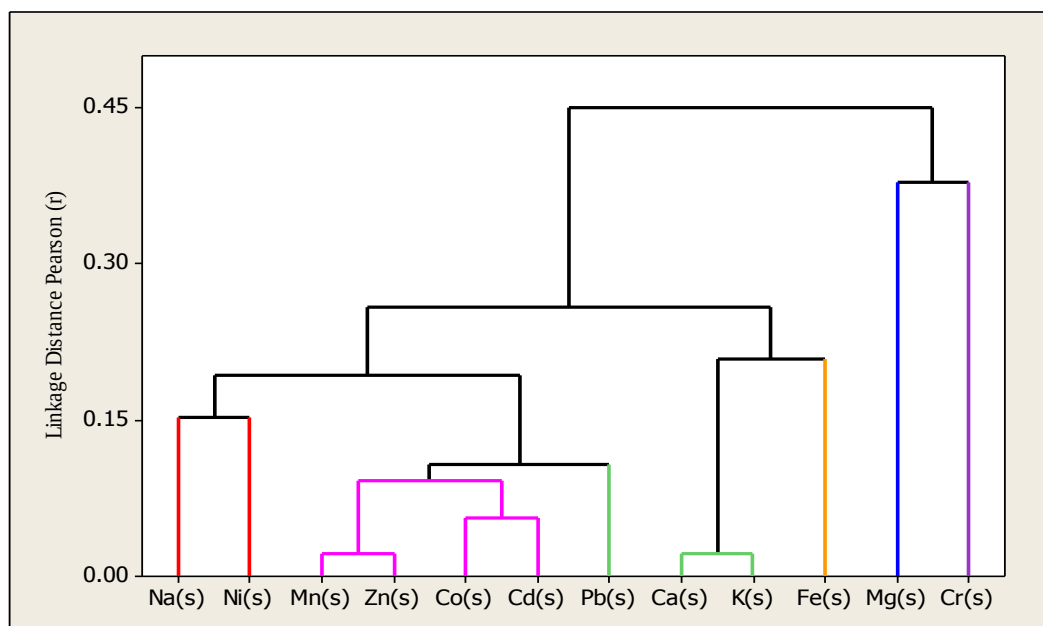


Figure 4.5 (b) Dendrogram of selected metals in soil

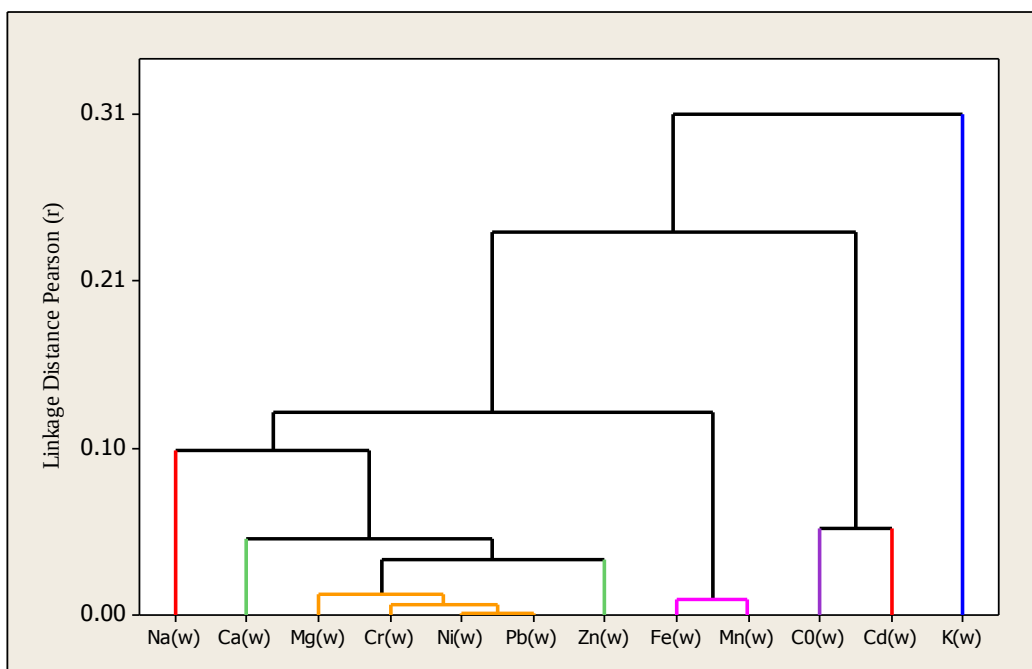


Figure 4.5 (c) Dendrogram of selected metals in groundwater

This research has revealed that both soil and groundwater of the study area have been badly affected by the toxic elements discharged by tanning industries. The levels of Cr, Cd, and Na were being increased from the standard levels due to the leaching of hazardous material from tanning waste water. The maximum level of Cr in the effluent was 3266 mg/L, while in soil sample it approached to 102 mg/L. It was indicated that Cr has exceeded the standard levels. The poisonous pollutants adversely affect the environment in the premises of tannery areas. The high intensity of Cr amount, particularly Cr (VI) in soil and ground water is harmful for human health. The prominent increase in Na causes salinity in the water and affects the utilization of the ground water for domestic and agricultural purposes. The observations revealed the consequences of tannery effluent on the increasing trend of hazardous metals in soil and water in the vicinity of tanneries. There is need to implement the standards for disposal of tanning industrial waste through environmental friendly methods.

Phase II

4.2 Study of health status of the tannery workers

4.2.1 Heavy metals in the blood and urine samples of the tannery workers

The total chromium level in the whole blood of the exposed and unexposed population is summarized in Table 4.8. The exposed population showed significantly ($p<0.001$) higher Cr concentration than that of controls. Smoking and nonsmoking tannery workers in group I (aged 20-35, 1-10 years exposure) and group II (aged 36-50, 11-20 years exposure) showed significantly higher blood Cr levels than that of controls (Fig. 4.6). The smoking and nonsmoking tannery workers in group II with long-term chromium exposure have significantly higher blood Cr level than that of smoking tannery workers in group I with short term chromium exposure. These findings have shown that the duration of exposure has significant effect on blood chromium level in tannery workers. There was a significant ($p<0.001$) effect of smoking and duration of exposure on blood chromium level of control and exposed tannery workers.

The smoking tannery workers in group I and II showed 27% higher Cr concentration as compared to control populations. In addition, smoking tannery workers in group II showed 4% increase in blood Cr level than that of group I. Similarly nonsmoking tannery workers in group I and II showed 27% and 30% higher Cr levels respectively than that of unexposed population. Nonsmoking tannery workers in group II showed 5% increase in blood Cr level than that of group I.

Chromium concentration in urine samples of the exposed and unexposed groups is summarized in Table 4.9 and Fig. 4.7. Smoking and nonsmoking tannery workers in group I and II showed significantly ($p<0.001$) higher Cr level in urine than that of the control population. Smoking tannery workers in group I and II showed 30% and 27%

higher urine Cr levels respectively than that of controls. Moreover, smoking tannery workers in group II showed 5% increase in urine Cr levels when compared with group I. Similarly nonsmoking tannery workers in group I and II showed 34% and 33% increase in urine Cr levels respectively than that of unexposed groups. Nonsmoking tannery workers in group II showed 4% increase in urine Cr concentration than that of group I.

Ahsan, M. *et al.*, also reported very high chromium concentration (1228 ± 34.3 nmol/L) in tannery workers than that of controls (420 ± 68 nmol/L) in Kasur, Pakistan (271). High blood Cr level (569nmol/L) and urinary Cr level (131 nmol/L) was also reported in tannery workers in Sialkot, Pakistan (272).

Chromium is toxic and mutagenic to human and animals, which causes lungs carcinoma and respiratory tract infection in humans (273). Chromium is considered an important health risk factor for tannery workers, as it is used as a basic tanning agent. The tannery workers are exposed to leather dust in working place containing chromium, which increases the blood chromium level of workers (274).

Table 4.8 Concentration of chromium in blood ($\mu\text{g/l}$) samples of male tannery workers

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistics	Controls	Tanners (exposed group II)	t-statistic
Smoker	25.55 ± 1.00	43.038 ± 1.12	49.58*	27.76 ± 1.65	49.308 ± 1.96	40.72*
Non Smokers	22.26 ± 1.36	39.156 ± 1.43	47.66**	23.89 ± 1.24	46.448 ± 1.43	46.47*

*Values are significant at $p < 0.01$ ** $p < 0.001$ n = 240

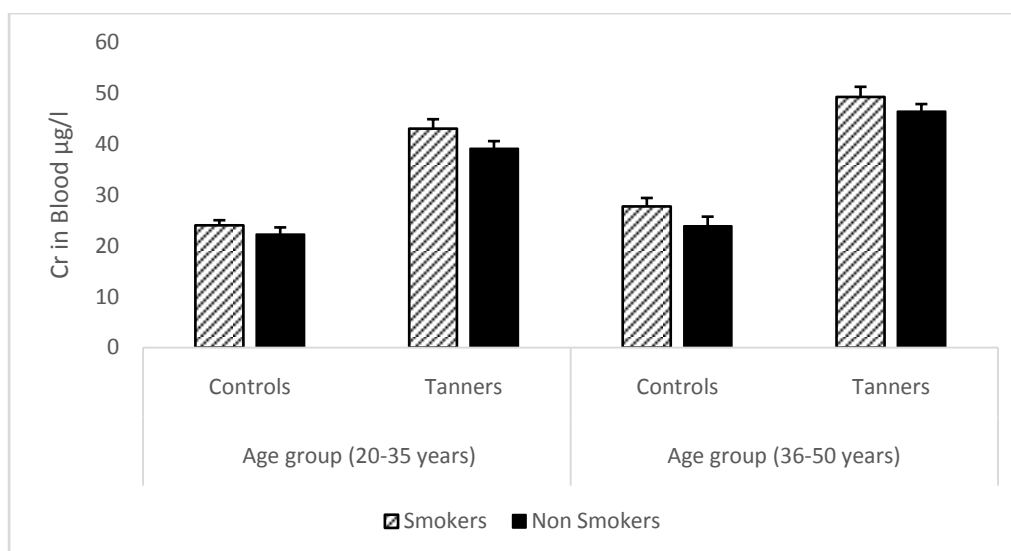


Figure 4.6 The levels of chromium in blood of male smoking, nonsmoking tanners and control groups in error bar graph ($p < 0.001$)

Table 4.9 Concentration of chromium in urine (µg/l) of tannery workers and control Population

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	11.52 ± 1.182	21.461 ± 0.50	42.356*	13.50 ± 0.84	23.82 ± 1.08	37.507*
Non Smokers	9.39 ± 0.492	19.425 ± 0.79	58.613*	10.54 ± 1.23	21.06 ± 1.95	21.811*

*Values are significant at $p < 0.001$ $n = 240$

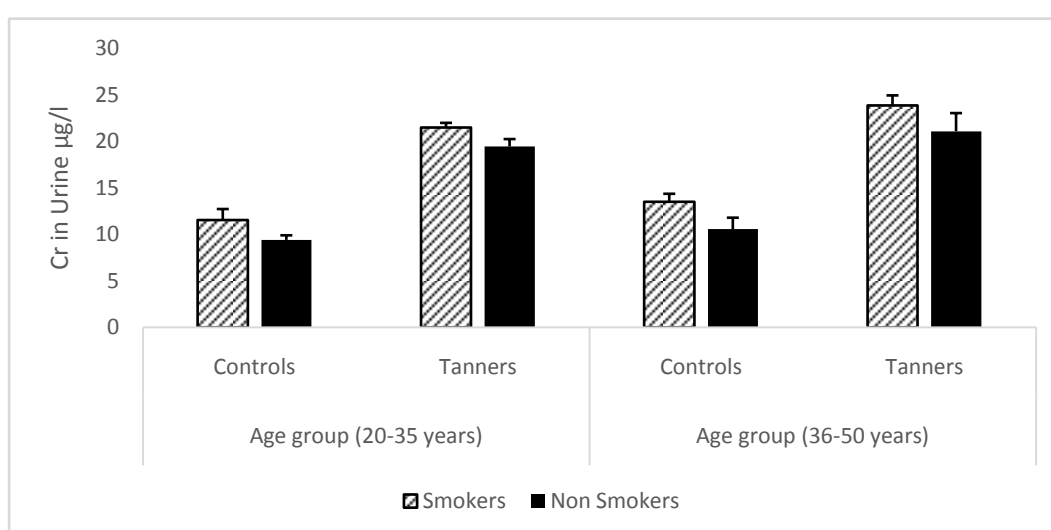


Figure 4.7 The levels of chromium in urine of smoking, nonsmoking tanners and control groups in error bar graph ($p < 0.001$)

Hexavalent chromium levels in erythrocyte of the exposed and control groups are shown in Table 4.10, Fig. 4.8. The exposed groups showed significantly ($p<0.001$) higher erythrocyte Cr concentration than that of controls. Cr concentration in erythrocytes were found to be 0.79 $\mu\text{g/l}$ and 1.16 $\mu\text{g/l}$ in smoking control groups I and II respectively, which was increased significantly to 4.20 $\mu\text{g/l}$ and 5.06 $\mu\text{g/l}$ in smoking exposed groups I and II respectively, while in nonsmoking control groups I and II were found to be 0.54 $\mu\text{g/l}$ and 0.68 $\mu\text{g/l}$, which was increased significantly to 3.78 $\mu\text{g/l}$ and 4.71 $\mu\text{g/l}$ in nonsmoking exposed groups I and II respectively.

The smoking tannery workers in groups I and II showed 68% and 62% higher erythrocyte Cr (VI) concentration respectively than those in control populations, while nonsmoking tannery workers in groups I and II showed 75% and 74% increase in erythrocyte Cr (VI) concentration respectively than that of controls. Smoking tannery workers in group II showed 9% increase in erythrocyte Cr (VI) concentration than group I. While nonsmoking tannery workers in group II showed 10% increase in erythrocyte Cr (VI) level than those in group I.

Ahsan *et al.*, reported high level of blood Cr (VI) (12.34 ± 1.12) in tannery workers in kasur, Pakistan (271). Occupational exposure to hexavalent chromium is known to cause increased incidences of certain tumors, skin ulcers, lungs cancer, allergic dermatitis and also damages major organs as liver, lungs and kidney (275, 276). Chromium concentration in erythrocytes has been proposed as useful indicator of internal dose, in relation to the ability of chromium (VI) to penetrate the cell membrane. The chromium concentration in erythrocytes is a sensitive indicator of recent Cr exposure. Considering the erythrocyte lifespan and turnover rate, intra-cell chromium content represents approximately the amount accumulated over the previous 8-10 weeks (277).

Table 4.10 Concentration of hexavalent chromium in erythrocytes ($\mu\text{g/l}$) of tannery workers and control population

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	0.79 ± 0.10	4.20 ± 0.36	41.82*	1.16 ± 0.17	5.062 ± 0.67	30.50*
Non Smokers	0.54 ± 0.05	3.78 ± 0.30	57.84*	0.686 ± 0.09	4.712 ± 0.28	51.16*

*Values are significant at $p < 0.001$ n = 240

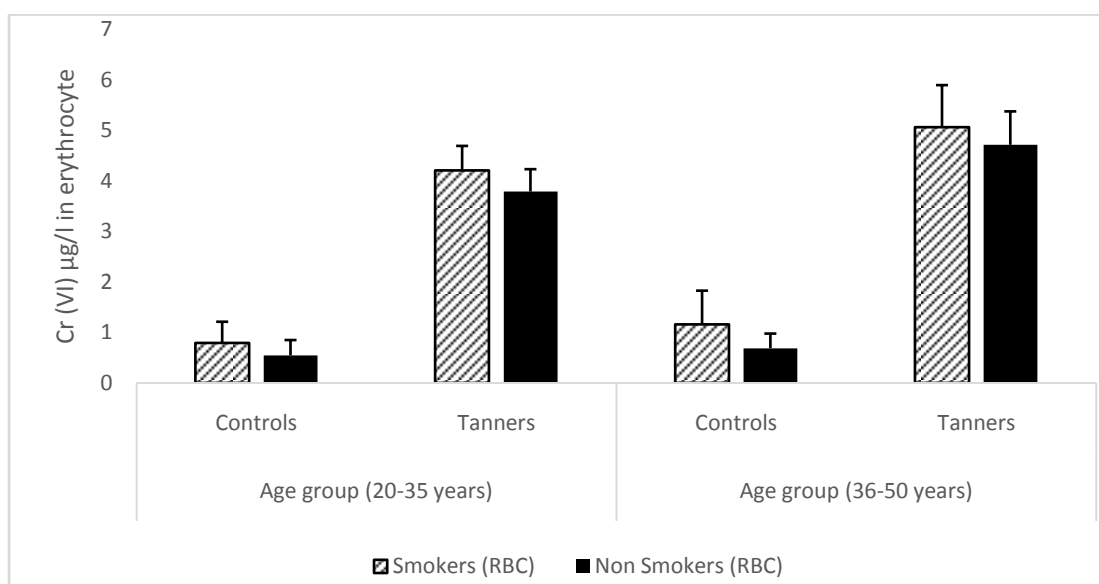


Figure 4.8 The concentration of hexavalent chromium in erythrocyte of smoking, nonsmoking tanners and control groups in error bar graph ($p < 0.001$)

Hexavalent chromium concentration in lymphocytes of the exposed and control groups is shown in Table 4.11, Fig. 4.9. The exposed groups showed significantly ($p < 0.001$) higher hexavalent Cr concentration than those of controls. Cr concentration in lymphocytes was found to be $29.23 \mu\text{g}/10^{12}$ cells and $33.14 \mu\text{g}/10^{12}$ cells in smoking control groups I and II respectively, which were increased significantly to $42.35 \mu\text{g}/10^{12}$ cells and $61.56 \mu\text{g}/10^{12}$ cells in smoking exposed groups I and II respectively, while in nonsmoking control groups I and II were $27.15 \mu\text{g}/10^{12}$ cells and

30.98 $\mu\text{g}/10^{12}$ cells respectively, which were increased significantly to 38.12 $\mu\text{g}/10^{12}$ cells and 57.38 $\mu\text{g}/10^{12}$ cells in nonsmoking exposed group I and II respectively.

The smoking tannery workers in groups I and II showed 18% and 30 % higher concentration of Cr in lymphocytes respectively than those of controls. Similarly nonsmoking tannery workers in group I and II showed 16% and 29% higher Cr concentration in lymphocytes respectively than those of controls. Moreover, smoking tannery workers in group II showed 18% increase in Cr level in lymphocytes than that of tannery workers in group I. Nonsmoking tannery workers in group II showed 20% increase in Cr concentration in lymphocytes than those of workers in group I.

Lymphocytes are also capable of great uptake, intracellular reduction and accumulation of chromium (VI) (277). As their life span ranges from a few months to years, their chromium content represents the amount accumulated over a long period of time and may express the blood cell body burden more accurately.

In addition to urinary chromium values, the measurement of chromium concentration in erythrocytes and lymphocytes may provide information about Cr uptake at different time points after exposure.

Table 4.11 Concentration of hexavalent chromium in lymphocytes ($\mu\text{g}/\text{l}$) of tannery workers and control population

20-35 years age group				36-50 years age group		
Controls		Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	29.23 $\pm$ 0.05	42.35 $\pm$ 0.28	32.428*	33.14 $\pm$ 0.52	61.568 $\pm$ 0.72	21.215*
Non Smokers	27.15 $\pm$ 0.23	38.12 $\pm$ 0.22	26.037*	30.98 $\pm$ 0.32	57.388 $\pm$ 0.54	25.254*

*Values are significant at $p < 0.001$ n = 240

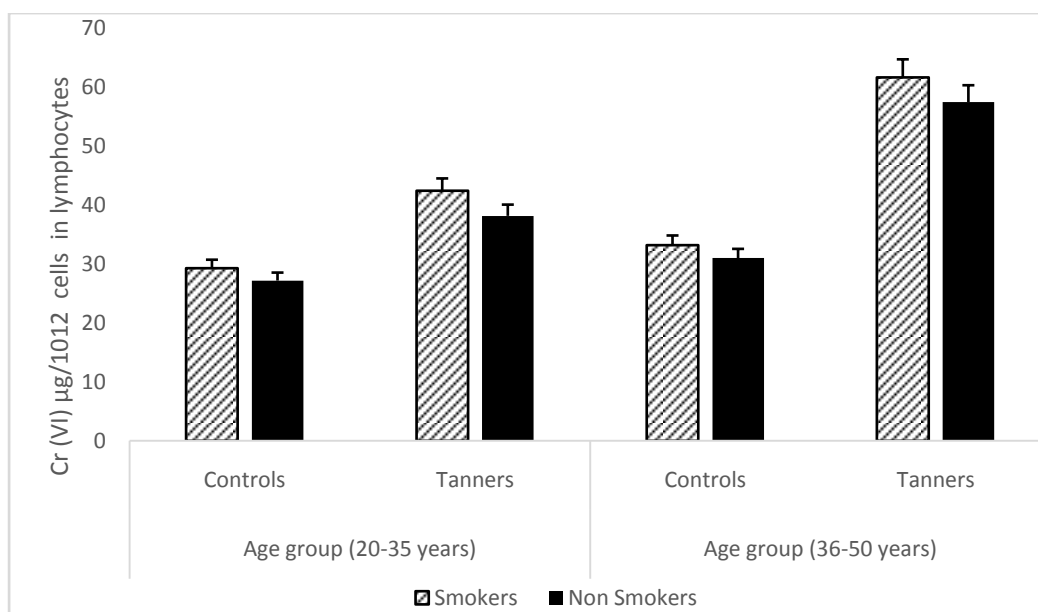


Figure 4.9 The concentration of hexavalent chromium in lymphocytes of smoking, nonsmoking tanners and control groups in error bar graph ($p < 0.001$)

The total chromium levels in the whole blood of the female exposed and unexposed groups are summarized in Table 4.12 and Fig. 4.10. The exposed groups showed significantly ($p < 0.001$) higher Cr concentration than that of unexposed groups. Female tannery workers in group I and II showed significantly higher blood Cr levels than that of control populations. The tannery workers in group II with longer duration of chromium exposure have significantly higher Cr levels in blood than those of tannery workers in group I with short term chromium exposure. The tannery workers in group II showed 5% higher Cr concentration than that of group I.

Table 4.12 Concentration of total chromium and hexavalent chromium in blood ($\mu\text{g/l}$) of female tannery workers

20-35 years age groups				36-50 years age groups		
Controls		Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Total Cr	24.03 ± 2.398	43.039 ± 3.018	39.32568*	25.04 ± 2.64	47.054 ± 3.330	42.78569*
Hexavalent Cr	4.10 ± 0.56	7.194 ± 0.718	20.19947*	4.32 ± 0.548	8.127 ± 1.351	15.69412*

*Values are significant at $p < 0.001$ n = 240

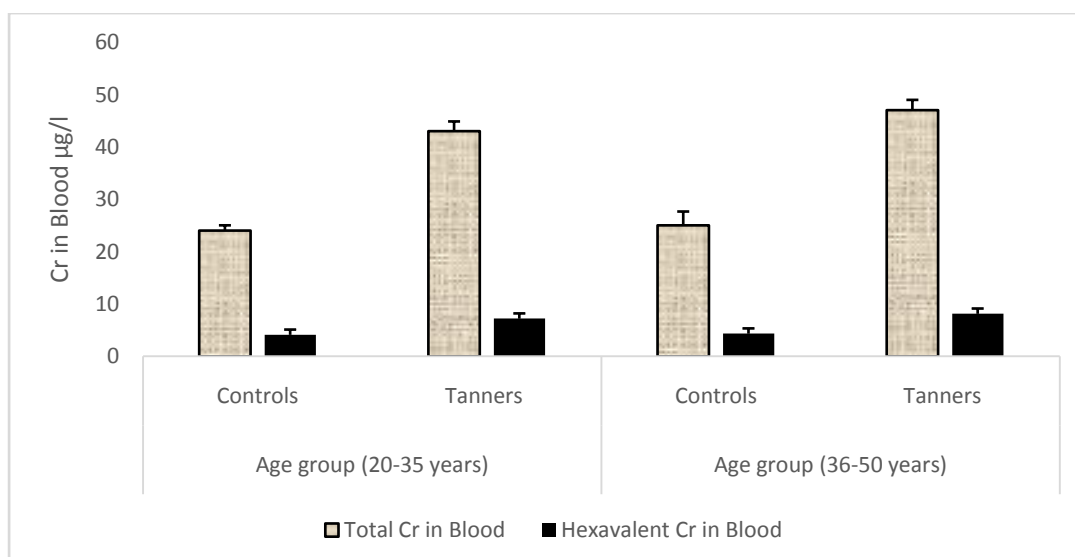


Figure 4.10 The levels of chromium in blood of female tanners and control groups in error bar graph ($p < 0.001$)

Concentration of Ni in blood samples of tannery workers was found to be $1.344\mu\text{g/l}$ and $1.501\mu\text{g/l}$ in smoking control groups, which was increased insignificantly to $1.637\mu\text{g/l}$ and $1.658\mu\text{g/l}$ in smoking exposed groups I and II respectively, while in nonsmoking control groups it was found to be $1.065\mu\text{g/l}$ and $1.101\mu\text{g/l}$ which was increased insignificantly to $1.15\mu\text{g/l}$ and $1.27\mu\text{g/l}$ in nonsmoking exposed groups I and II respectively Table 4.13, Fig. 4.11. There was no significant increase in both smoking and nonsmoking groups. Ni was not detectable in the urine sample. Ni is a potential carcinogen for the respiratory tract and may cause lungs fibrosis, allergies and lungs cancer in occupationally exposed populations (278).

Table 4.13 Concentration of Ni in blood ($\mu\text{g/l}$) of tannery workers and control Population

20-36 years age group				36-50 years age groups		
	Controls	Tanners (exposed group I)	P value	Controls	Tanners (exposed group II)	P value
Smokers	1.344 ± 0.171	1.637 ± 0.121	0.05	1.501 ± 0.140	1.658 ± 0.159	0.07
Non Smokers	1.065 ± 0.105	1.15 ± 0.0715	0.21	1.10 ± 0.0813	1.27 ± 0.0969	0.23

n=240

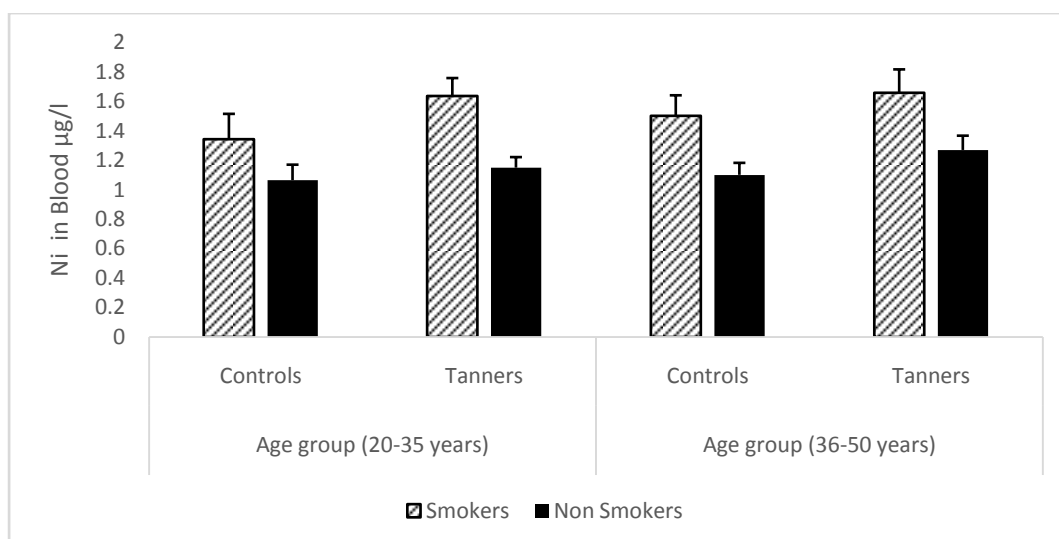


Figure 4.11 The levels of Ni in blood of smoking and non-smoking tanners and control groups in error bar graph

Concentration of zinc in blood samples of tannery workers was found to be 16.767µg/l and 15.818µg/l in smoking control groups, which was increased insignificantly to 17.134µg/l and 16.238µg/l in smoking exposed groups I and II respectively, while in nonsmoking control groups it was found to be 17.316µg/l and 16.002µg/l, which was increased insignificantly to 17.804µg/l and 16.352µg/l in nonsmoking exposed group I and II respectively (Table 4.14, Fig 4.12). There was no significant increase in both smoking and nonsmoking groups. There was no detectable Zn in the urine samples. Acute zinc toxicity in humans includes drowsiness, lethargy, vomiting, dehydration, renal failure and lack of muscular coordination. Prolonged exposure to zinc can cause short-term illness called fern-fever (279).

Table 4.14 Concentration of zinc in blood (µg/l) of tannery workers and control Population

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	P value	Controls	Tanners (exposed group II)	P value
Smokers	16.76 ± 0.269	17.134 ± 0.4	0.77	15.81 ± 0.333	16.238 ± 0.229	0.016
Non Smokers	17.31 ± 0.486	17.804 ± 0.501	0.48	16.00 ± 0.207	16.352 ± 0.318	0.09

n = 240

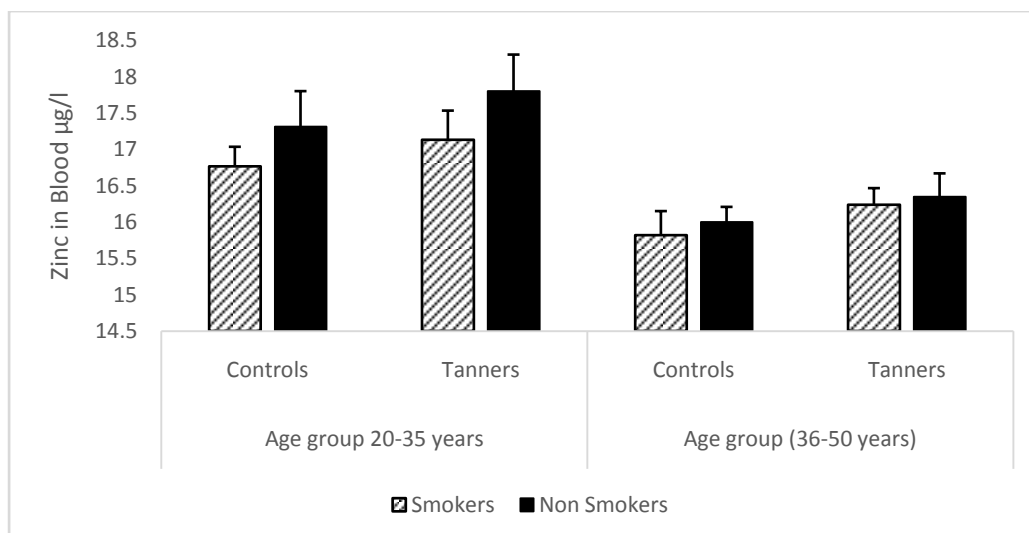


Figure 4.12 The level of zinc in blood of smoking and non-smoking tanners and control groups in error bar graph

Cadmium concentration in blood samples was found to be 1.17µg/l and 1.22µg/l in smoking control groups, which was increased insignificantly to 1.302µg/l and 1.387µg/l in smoking exposed groups I and II respectively, while in nonsmoking control groups it was found to be 1.06µg/l and 1.10µg/l which was increased insignificantly to 1.15µg/l and 1.27µg/l in nonsmoking exposed groups I and II respectively (Table 4.15, Fig. 4.13). There was no significant increase in both the smoking and nonsmoking groups. There was no detectable Cd in the urine sample. According to Meena *et al.*, occupational exposure to cadmium can cause yellow stains on neck and teeth, while prolonged exposure to cadmium may lead to adverse health effects in humans (280, 281).

Table 4.15 Concentration of cadmium in blood (µg/l) of tannery workers and control Population

20-35 years age group				36-50 years age group		
Controls		Tanners (exposed group I)	P value	Controls	Tanners (exposed group II)	P value
Smokers	1.17 ± 0.096	1.302 ± 0.082	0.32	1.22 ± 0.095	1.387 ± 0.1	0.24
Non Smokers	1.06 ± 0.105	1.15 ± 0.071	0.5	1.10 ± 0.081	1.27 ± 0.069	0.11

n = 240

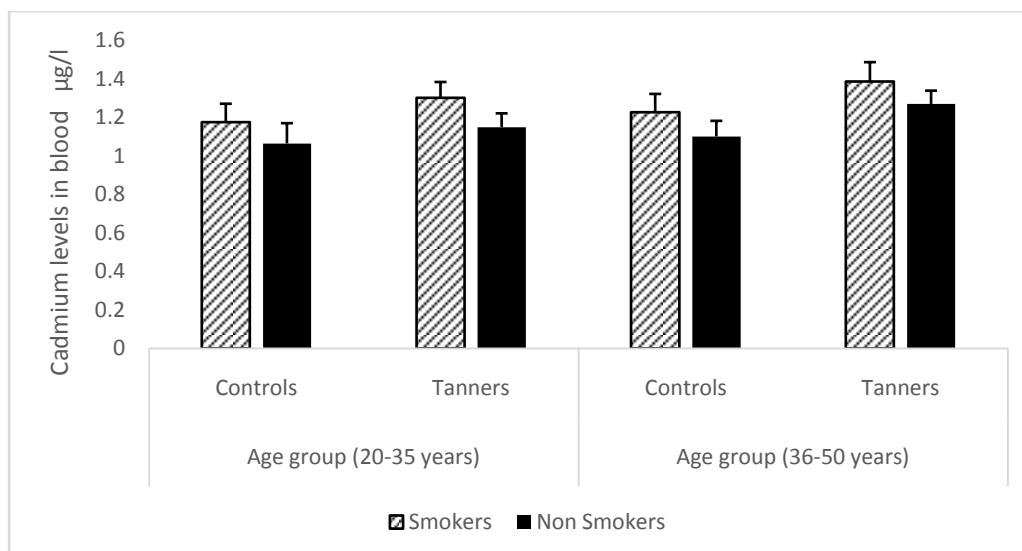


Figure 4.13 The levels of cadmium in blood of smoking and non-smoking tanners and control groups in error bar graph

4.2.2 Assessment of selected endocrine hormonal parameters

4.2.2.1 Testosterone hormone

Concentration of testosterone in blood samples of control groups of male tannery workers was found to be 491.721ng/dl, which was decreased significantly to 326.238/dl and 306.636ng/dl in both the exposed groups I and II respectively (Table 4.16, Fig. 4.14). The exposed groups showed significantly ($p < 0.001$) lower testosterone level than those of unexposed groups. Tannery workers in group II were more affected and showed lower testosterone level due to long-term Cr exposure than that of group 1 and control. This effect was due to the reduction of chromium (VI) in cells, which ultimately produces chromium (V). Chromium (V) affects the functional properties of sertoli cells and disrupts the blood-testis barrier (282) which also decreases inhibin production (283).

The tannery workers showed 20% and 23% decrease in testosterone concentration in groups I and II respectively than that of unexposed population. In addition, tannery workers in group II showed a further 3% decrease in testosterone levels than that of group I. The tannery workers in group II have significantly lower testosterone levels

than that of group I with short-term chromium exposure. These findings have shown that the duration of exposure has a significant effect on testosterone level in tannery workers.

Bonde *et al.*, reported low levels testosterone in welders exposed to hexavalent chromium (284). Another study revealed that testosterone levels were significantly decreased in rats treated for two weeks with chromium (VI) (285). According to Basaria *et al.*, low testosterone can lead to obesity and diminishes insulin sensitivity in men (286).

Barud *et al.*, documented that low testosterone level has been associated with decreased level of high density lipoprotein (HDL) and affects the cardiovascular system in workers (287). According to Nettleship *et al.*, low testosterone concentration has been associated with increased expression of mediators of the atherosclerotic process and other cardiovascular risk factors (288). Khaw *et al.*, reported that low testosterone concentration may lead to hypertension in men (289).

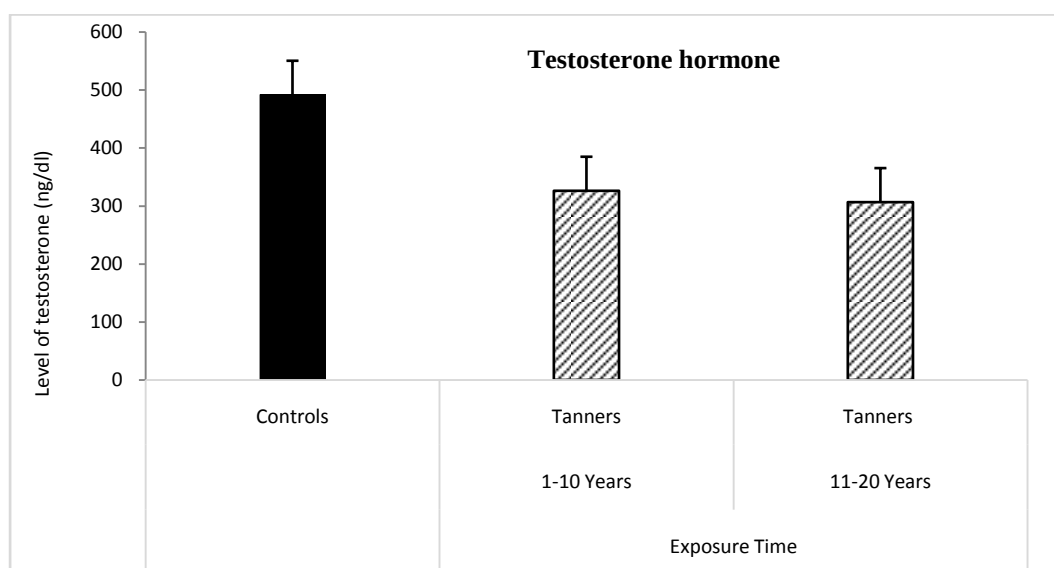


Figure 4.14 The levels of testosterone hormone in male tannery workers and controls in error bar graph ($p < 0.001$) ($n = 100$)

4.2.2.2 Progesterone hormone

Progesterone concentration in blood samples of female controls was found to be 9.806ng/dl, which was decreased significantly to 4.236ng/dl and 2.416ng/dl in exposed group I and II respectively (Table 4.16, Fig. 4.15). The exposed groups showed significantly ($p<0.001$) lower progesterone levels than that of controls. Tannery workers in group II were more affected and showed lower progesterone levels in response to long-term Cr exposure than that of group I and controls.

The tannery workers showed 39% and 60% decrease in progesterone concentration in groups I and group II respectively than that of control population. Moreover, tannery workers in group II showed 27% decrease in progesterone levels than group I. The tannery workers in group II have significantly lower progesterone levels in response to long-term chromium exposure as compared to group I with short-term chromium exposure. These findings showed that the duration of exposure has a significant effect on progesterone concentration in blood. Low levels of progesterone have been associated with placental abnormalities, increased incidences of pregnancy, prematurity, fetal growth restriction and fetal distress (290, 291). Hinshaw *et al.*, reported that low progesterone levels have been associated with ectopic pregnancies and miscarriages (292).

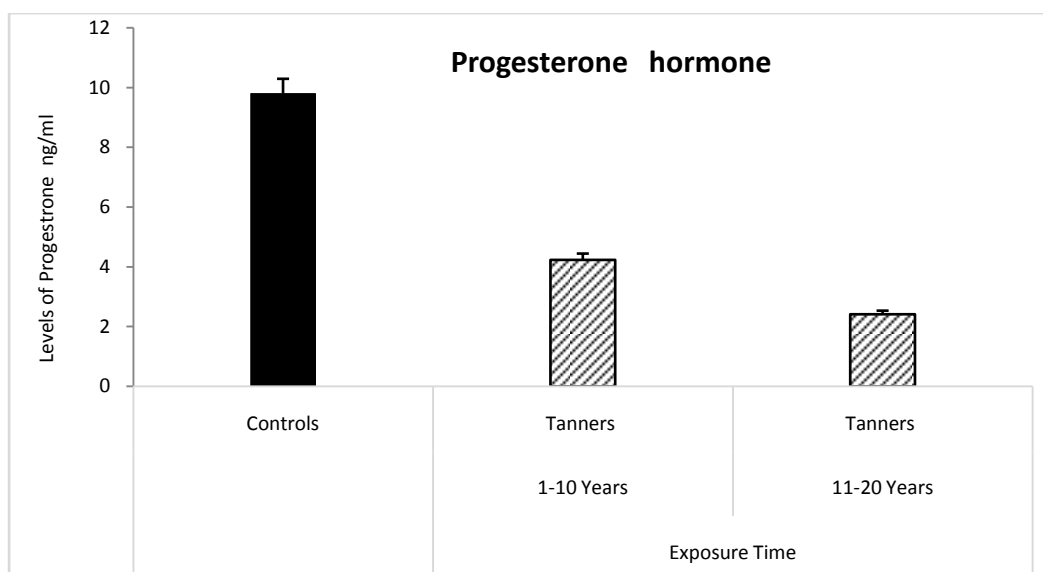


Figure 4.15 The levels of progesterone hormone in female tanners and control in error bar graph ($p < 0.001$)

4.2.2.3 Estradiol hormone

The levels of estradiol in the whole blood of the exposed and unexposed groups are shown in Table 4.16 and Fig. 4.16. Estradiol concentration in blood samples of female control group was found to be 94.89pg/ml, which was decreased significantly to 58.404pg/ml and 48.879pg/ml in exposed group I and II respectively. The exposed groups showed significantly ($p < 0.001$) lower estradiol levels than that of unexposed population.

The tannery workers in group I showed 23% and 32% decrease in estradiol level as than that of unexposed population. Moreover, tannery workers in group II showed 8% decrease in estradiol level than that of group I.

The tannery workers in group II with longer duration of chromium exposure have significantly lower estradiol level than that of group I with short-term chromium exposure. It is observed from the present study that duration of exposure has a significant effect on estradiol levels.

Turgeon *et al.*, reported that reduced estradiol level has adverse effects on bones, skeletal muscles, cardiovascular and brain functions (293). The lower level of estradiol has been associated with the onset of metabolic diseases (294, 295). Chronically decreased estradiol level may increase in visceral fat mass in women that leads to shift in body shape from a gynoid to an android pattern, which is the major cause of developing cardiovascular diseases in women (293).

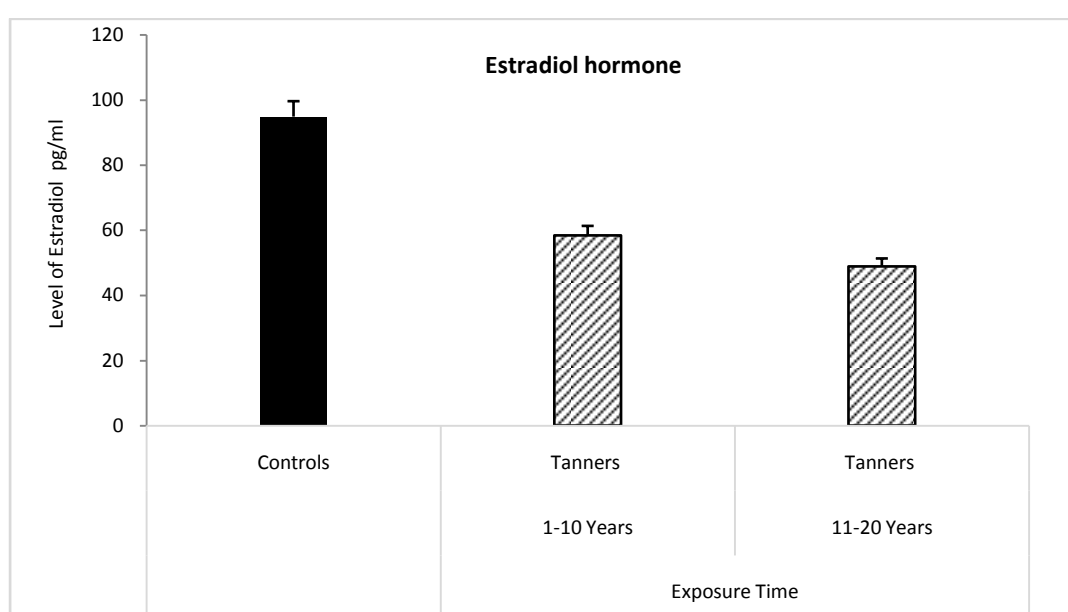


Figure 4.16 The levels of estradiol hormone in female tanners and control group in error bar graph ($p < 0.001$)

Table 4.16 The levels of male and female endocrine hormones (ng/dl) in tannery workers and control population

Parameters	Controls	Group-I (1-10years exposure)	Group-II (11-20years exposure)
Testosterone (ng/dl)	491.721 ± 11.707	326.238 ± 12.478*	306.636 ± 13.808*
Progesterone (ng/ml)	9.806 ± 1.417	4.236 ± 1.106*	2.416 ± 1.111*
Estradiol (pg/ml)	94.89 ± 15.462	58.404 ± 10.186*	48.879 ± 40.141*

*Values are significant at $p < 0.001$ n = 240

4.2.2.4 Follicle stimulating hormone (FSH)

FSH levels in blood samples of female controls were found to be 3.158mIU/ml in the control group which was decreased significantly to 7.8607mIU/ml and 9.285mIU/ml in exposed groups I and II respectively (Table.17, Fig. 4.17). The exposed groups showed significantly ($p<0.001$) higher FSH level than that of unexposed group. The tannery workers in groups I and group II showed 42% and 49% increase in FSH level respectively than that of unexposed population. In addition, tannery workers in group II showed 8% increase in FSH level than that of group I.

Long-term chromium exposed tannery workers in group II exhibit significantly higher FSH level than that of group I with short term chromium exposure. It is concluded from the present study that duration of exposure has significant affect on FSH level in tannery workers.

High level of FSH (7.34×10^{-3} IU/ml) was also reported in workers exposed to Cr (VI) in electroplating industry (296). It is reported that high level of FSH has been associated with significantly lower chances of pregnancy (297) and poor response to gonadotrophin stimulation (298). Cahill *et al.*, reported that high FSH levels caused severe testicular damage in women (299).

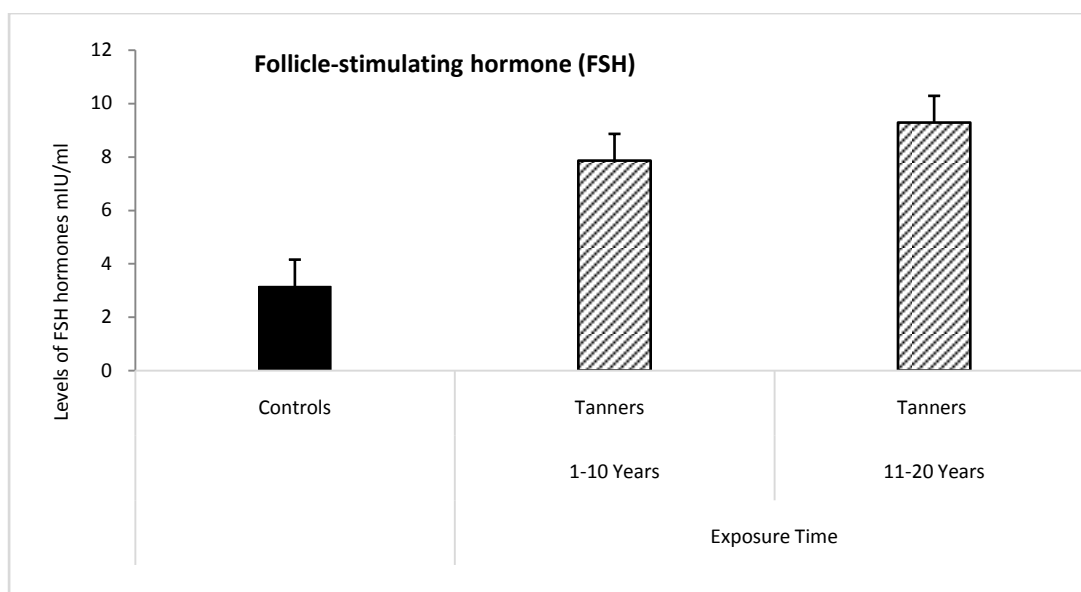


Figure 4.17 The levels of FSH hormone in female tanners and control groups in error bar graph ($p < 0.001$)

4.2.2.5 Luteinizing hormone (LH)

The levels of luteinizing hormone (LH) in the whole blood of the exposed and unexposed groups are shown in Table 4.17 and Fig. 4.18. LH concentration in blood samples was found to be 6.789mIU/ml in female control group, which was decreased significantly to 3.0781mIU/ml and 1.9692mIU/ml in exposed groups I and II respectively. The exposed groups showed significantly ($p < 0.001$) lower LH levels than unexposed group.

The tannery workers in group I and group II showed 37% and 55% decrease in LH levels respectively than that of unexposed population. Moreover, tannery workers in group II showed 21% decrease in LH level than that of group I.

The tannery workers in group II with long-term chromium exposure have significantly lower LH level than that of group I with short-term chromium exposure. It is observed from the present findings that duration of exposure has a significant effect on the LH levels in blood. The low LH levels are reported in chromium treated rats than those of controls. Low serum LH levels have been associated with decreased

activities of 17 β -hydroxy steroid dehydrogenase (17 β -HSD) and testicular Δ^5 3 β hydroxy steroid dehydrogenase (3 β –HSD) enzymes, responsible for the synthesis of testosterone (285).

Table 4.17 Levels of female endocrine hormones in blood (mIU/ml) of tannery workers and control population

Parameters	Controls	Group-I (1-10years exposure)	Group-II (11-20years exposure)
FSH (mIU/ml)	3.158 $\pm$ 0.6049	7.8607 $\pm$ 0.7277*	9.2857 $\pm$ 0.7658*
LH (mIU/ml)	6.789 $\pm$ 0.9188	3.0781 $\pm$ 1.0411*	1.9692 $\pm$ 0.8201*

*Values are significant at p<0.001 n = 240

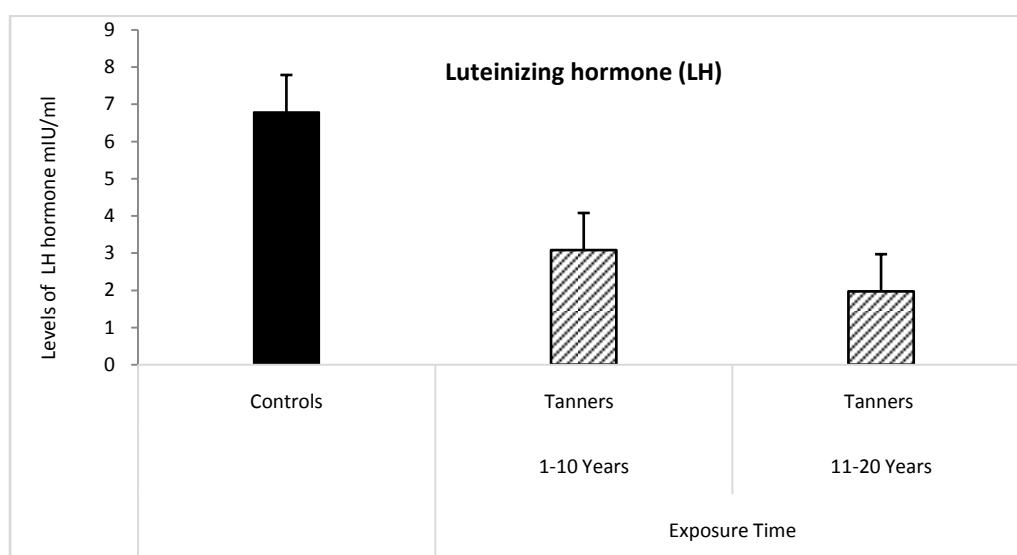


Figure 4.18 The levels of LH hormone in female tanners and control groups in error bar graph (p<0.001)

4.2.2.6 Growth hormone (GH)

The levels of growth hormone in male workers in the whole blood samples of the exposed and unexposed groups are shown in Table 4.18 and Fig. 4.19. GH levels in blood samples of controls were found to be 0.7014ng/ml, which was decreased significantly to 0.1030ng/ml and 0.0734ng/ml in exposed groups I and II respectively.

The exposed groups showed significantly ($p < 0.001$) lower GH levels than that of unexposed group.

The tannery workers in group I and II showed 74% and 81% decrease in GH levels in male tannery workers than that of unexposed population. Moreover, tannery workers in group II showed 16% further decrease in GH level than that of group I.

The levels of growth hormone in female workers in the whole blood of the exposed and unexposed groups are shown in Table 4.18 and Fig. 4.20. GH level in blood samples of female tannery workers was found to be 2.6775ng/ml in unexposed population, which was decreased significantly to 1.0817ng/ml and 0.7938ng/ml in exposed groups I and II. The exposed groups showed significantly ($p < 0.001$) lower GH levels than that of control group. Long-term chromium exposed male and female tannery workers in group II exhibit significantly lower GH level than that of group I with short term chromium exposure. It is observed that the duration of exposure has significant effect on GH levels in blood of tannery workers.

The tannery workers in groups I and II showed 42% and 54% decrease in GH levels respectively of female tannery workers than that of unexposed population. In addition, tannery workers in group II showed a further 15% decrease in GH level than that of group I. Growth hormone deficiency can cause several abnormalities, and body composition is also altered due to reduced muscle mass and increased fat mass. It also affects the plasma lipid profile and increases the cardiovascular morbidity (300). Gupta *et al.*, reported that growth hormone deficiency (GHD) is associated with cardiovascular, neuropsychiatric-cognitive, metabolic, neuromuscular and skeletal abnormalities (301).

Table 4.18 The level of male and female growth hormones in blood of tannery workers and control population

Parameters	Controls	Group-I (1-10years exposure)	Group-II (11-20years exposure)
GH in male(ng/ml)	0.7014 $\pm$ 0.1875	0.1030 $\pm$ 0.0311*	0.0734 $\pm$ 0.0194*
GH in female(ng/ml)	2.6775 $\pm$ 0.4783	1.0817 $\pm$ 0.4473*	0.7938 $\pm$ 0.1024*

*Values are significant at $p < 0.001$ n = 240

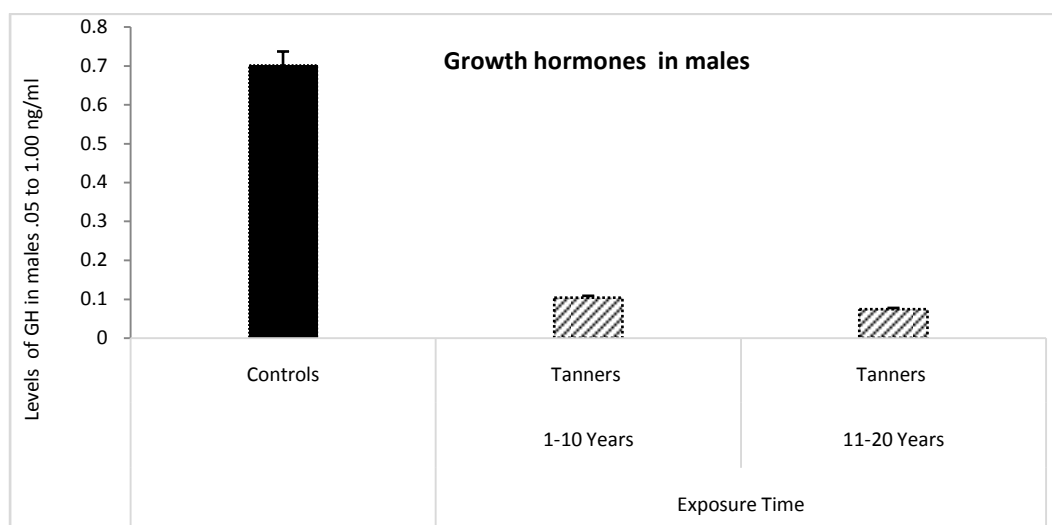


Figure 4.19 The levels of growth hormone in male tanners and control groups in error bar graph ($p < 0.001$)

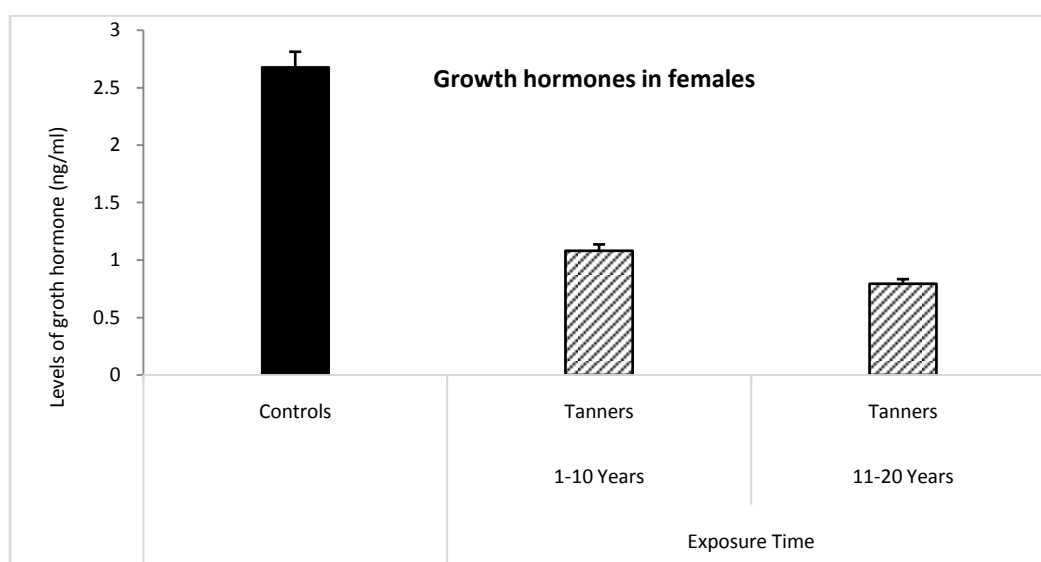


Figure 4.20 The levels of growth hormone in female tanners and control group in error bar graph ($p < 0.001$)

4.2.2.7 Thyroid stimulating hormone (TSH)

The levels of thyroid stimulating hormone (TSH) in the blood samples of the tannery workers and controls are shown in Table 4.19 and Fig. 4.21. TSH levels in blood samples of control was found to be 2.64 μ IU/ml, which was decreased insignificantly to 2.44 μ IU/ml and 2.28 μ IU/ml in exposed groups I and II respectively. There was no significant decrease in TSH levels in both the exposed groups I and II.

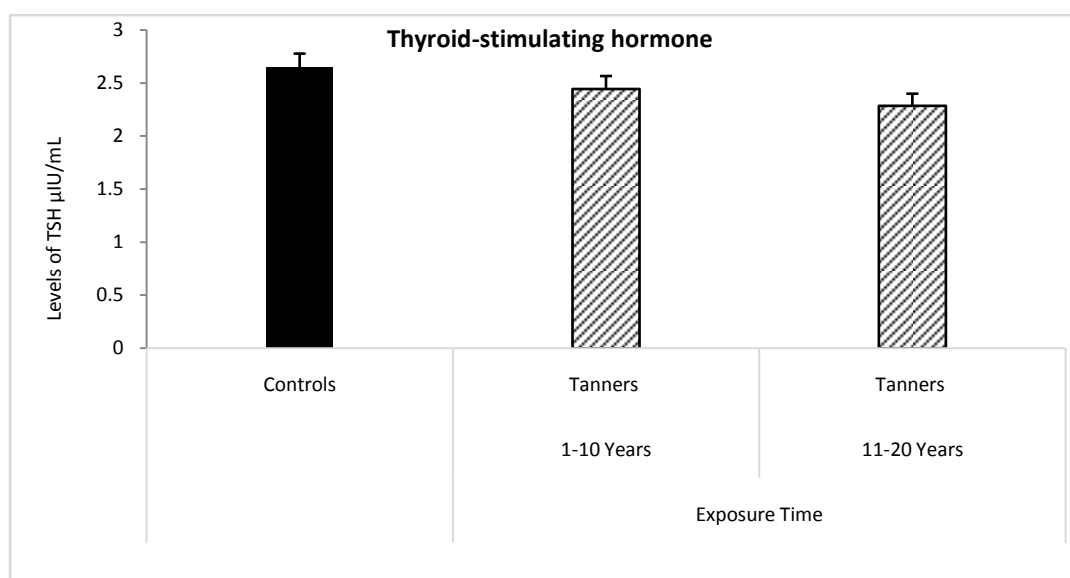


Figure 4.21 The levels of TSH in tanners of different exposure times and control group in error bar graph

4.2.2.8 Triiodothyronine (Free T₃)

The levels of triiodothyronine (Free T₃) hormone in the whole blood of the tannery workers and controls are shown in Table 4.19 and Fig. 4.22. The level of Free T₃ hormone in blood sample of controls was found to be 95.87ng/dl, which was decreased insignificantly to 94.68ng/dl and 94.04ng/dl in exposed groups I and II respectively. There was no significant decrease in the levels of Free T₃ hormone in both the exposed groups I and II.

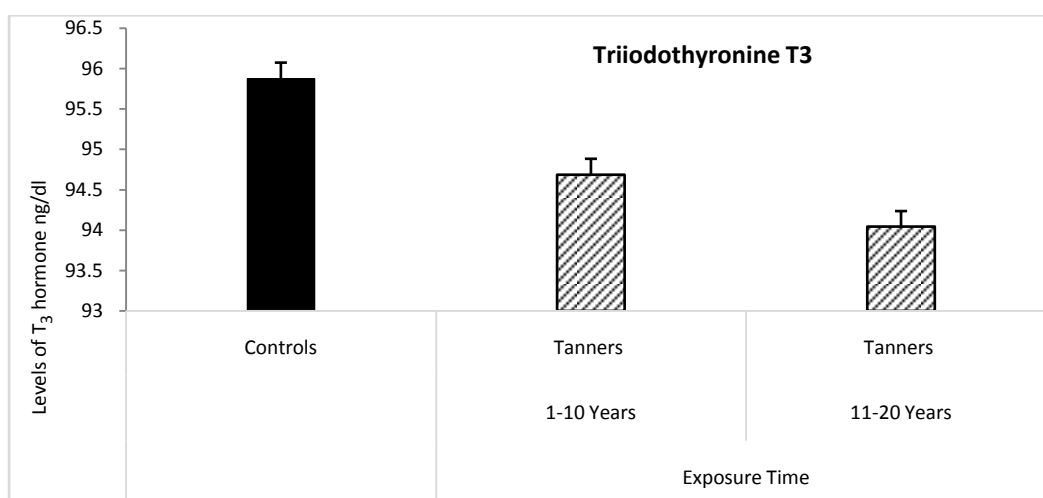


Figure 4.22 The levels of Free T₃ hormone in tanners and control in error bar graph

4.2.2.9 Tetraiodothyronine (FreeT₄)

The levels of tetraiodothyronine (FreeT₄) in the whole blood of the tannery workers and controls are shown in Table 4.19 and Fig. 4.23. The level of Free T₄ hormone in the blood samples of controls was found to be 5.7986ng/dl, which was increased insignificantly to 6.0746ng/dl and 6.1516ng/dl in exposed groups I and II respectively. There was no significant increase in (Free T₄) levels in both the exposed groups I and II.

Table 4.19 The levels of TSH, T3 and T4 in blood of tannery workers and control population

Parameters	Control	Group-I (1-10years exposure)	Group-II (11-20years exposure)
TSH (μ IU/mL)	2.64 $\pm$ 0.81	2.44 $\pm$ 0.76	2.28 $\pm$ 0.79
T₃ (ng/dl)	95.87 $\pm$ 9.95	94.68 $\pm$ 9.92	94.04 $\pm$ 9.65
T₄ (ng/dl)	5.79 $\pm$ 0.55	6.074 $\pm$ 0.93	6.15 $\pm$ 1.17

n = 240

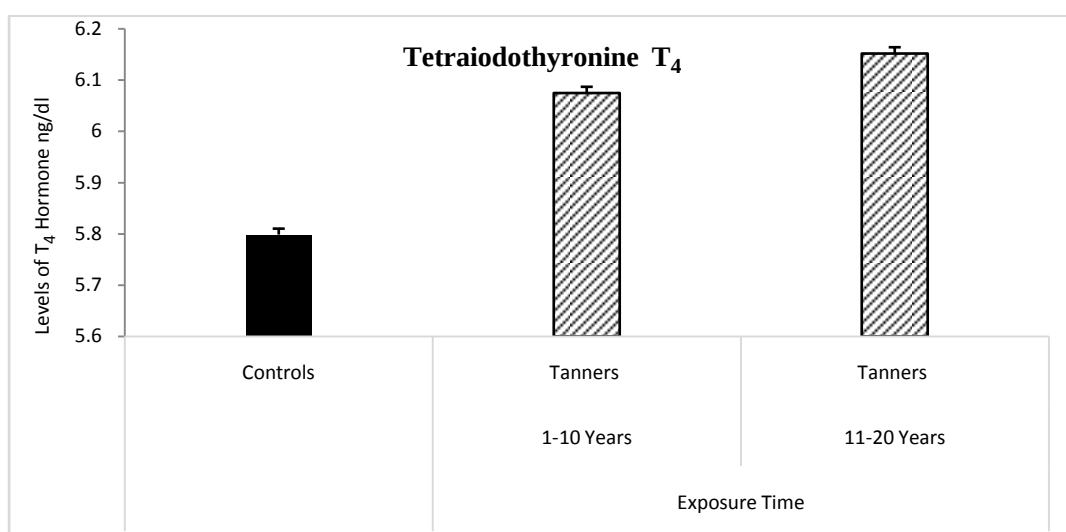


Figure 4.23 The levels of T₄ hormone in tanners of different exposure times and control group in error bar graph

4.2.2.10 Correlation and regression analysis for hormonal parameters from tannery workers.

In pearson correlation Table 4.20, testosterone concentration showed significant negative correlation with Cr concentration ($r = -0.78$, $p < 0.001$) ($r = -0.68$, $p < 0.001$) in exposed groups I and II respectively. There is no significant correlation ($r = 0.023$, $p > 0.05$) observed between the levels of testosterone and Cr concentration in unexposed population. While in multiple regression, there is a significant association between the levels of testosterone and Cr concentration ($r^2 = 0.606$) ($r^2 = 0.64$) in both the exposed groups I and II respectively Fig. 4.24. There was a significant negative correlation between GH concentration and Cr levels ($r = -0.79$, $p < 0.001$) ($r = -0.52$, $p < 0.05$) in exposed groups I and II respectively. GH concentration showed no significant correlation ($r = -0.412$, $p > 0.05$) with Cr levels in unexposed population. In multiple linear regression, there is significant dependency level ($r^2 = 0.628$) between the concentration of GH and Cr levels in exposed groups I, while there is no dependency level ($r^2 = 0.268$) in exposed group II Fig. 4.25.

TSH levels showed no significant positive correlation with Cr concentration ($r = 0.19$, $p > 0.05$) ($r = 0.06$, $p > 0.05$) in exposed groups I and II respectively. While, in multiple regression, there is no significant association between the levels of TSH and Cr concentration ($r^2 = 0.036$) and ($r^2 = 0.004$) in both the exposed groups I and II respectively Fig 4.26.

Moreover, there is no significant correlation observed between T_3 levels and Cr concentration ($r = -0.22$, $p > 0.05$) ($r = 0.12$, $p > 0.05$) in exposed groups I and II respectively. While In multiple regression, there is no significant dependency between the T_3 and Cr concentration ($r^2 = 0.208$) and ($r^2 = 0.21$) in both exposed groups I and II respectively Fig. 4.27.

T_4 level showed no significant correlation with Cr concentration ($r = 0.36$) ($r = 0.43$) in exposed groups I and II respectively. While In multiple regression, there is no significant level of dependency between the T_4 and Cr concentration ($r^2 = 0.11$) ($r^2 = 0.06$) in both exposed groups I and II respectively Fig 4.28.

Table 4.20 The correlation coefficient and multiple regression between the blood chromium levels and selected male hormones

Worker Groups	T		G.H		TSH		T3		T4	
	r	r ²	r	r ²	r	r ²	r	r ²	r	r ²
Controls	0.023 ^a	0.001	-0.412 ^a	0.11	0.16 ^a	0.025	-0.29 ^a	0.089	0.38	0.141
Exposed 20-35 years	-0.78 ^{**}	0.606	-0.79 ^{**}	0.628	0.19 ^a	0.036	-0.22 ^a	0.208	0.36	0.11
Exposed 36-50 years	-0.68 ^{**}	0.64	-0.52 ^{**}	0.268	0.06 ^a	0.004	0.12 ^a	0.21	0.43	0.06

T = Testosterone

G.H = Growth hormone

TSH = Thyroid-stimulating hormone

T3 = Triiodothyronine

T4 = Tetraiodothyronine

* $p < 0.05$ ** $p < 0.001$ ^a $p > 0.05$

r = Simple Correlation r^2 = Multiple regression

In Pearson correlation analysis Table 4.21, a significant negative correlation is observed for progesterone concentration ($r = -0.97$, $p < 0.001$) ($r = -0.83$, $p < 0.001$) and Cr levels in exposed groups I and II respectively. While in multiple regression significant association is observed between the levels of progesterone ($r^2 = 0.96$) ($r^2 = 0.69$) and Cr concentration in exposed groups I and II respectively Fig. 4.29.

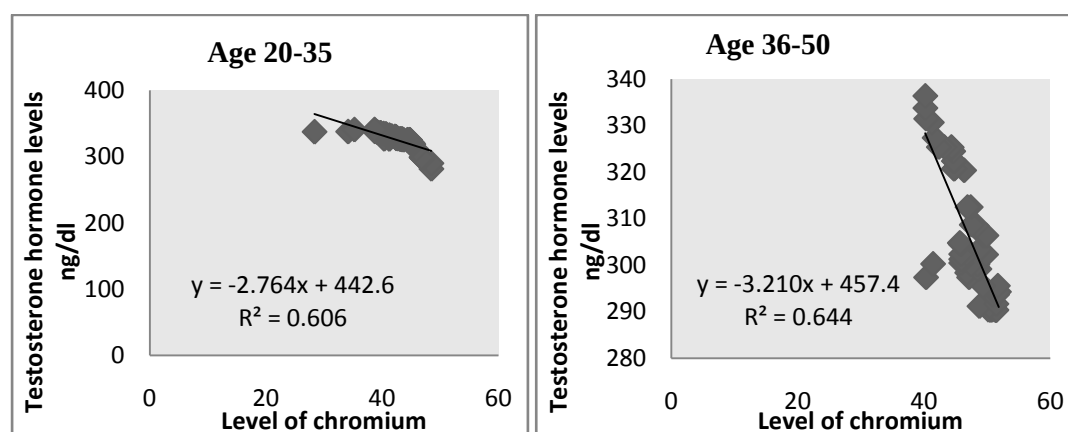


Figure 4.24 The linear multiple regression between the levels of testosterone hormone and Cr concentration in blood of tanners ($p < 0.001$)

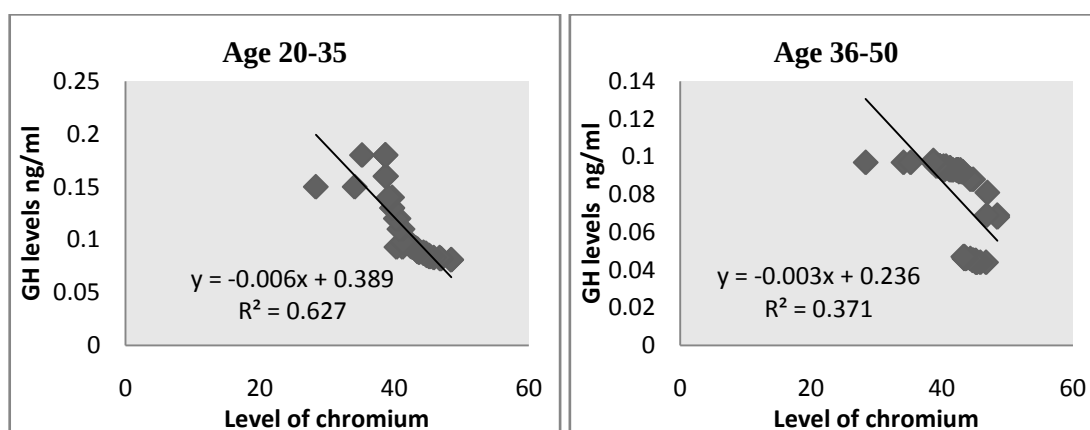


Figure 4.25 The linear multiple regression between the levels of growth hormone and Cr levels in blood of tanners ($p < 0.001$)

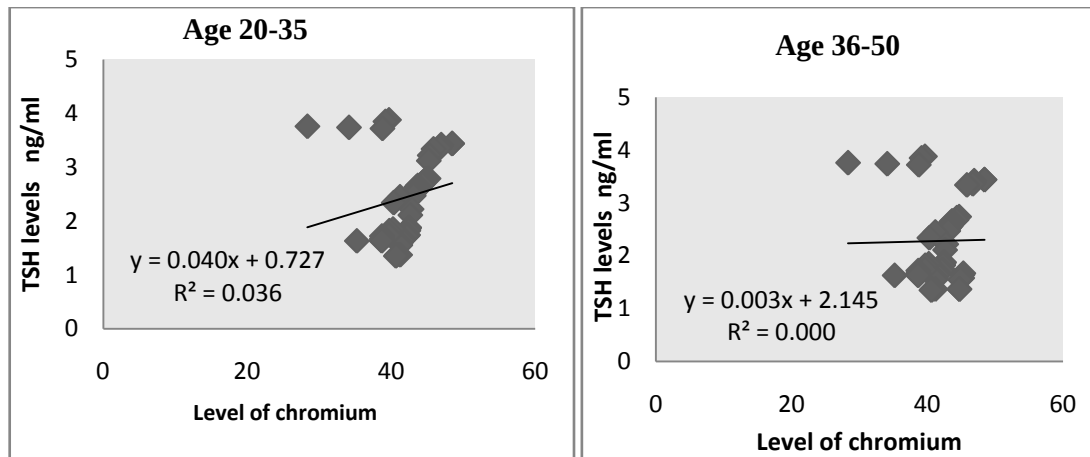


Figure 4.26 The linear multiple regression between the levels of TSH and Cr concentration in blood of tanners

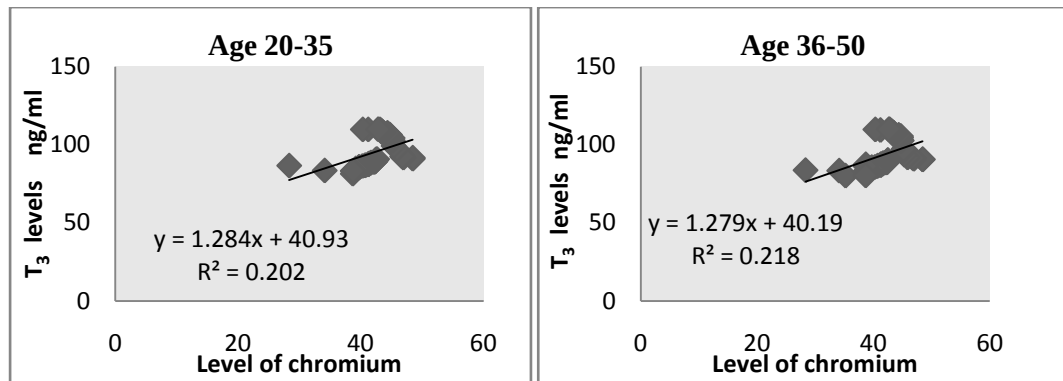


Figure 4.27 The linear multiple regression between the levels of T₃ and Cr concentration in blood of tanners

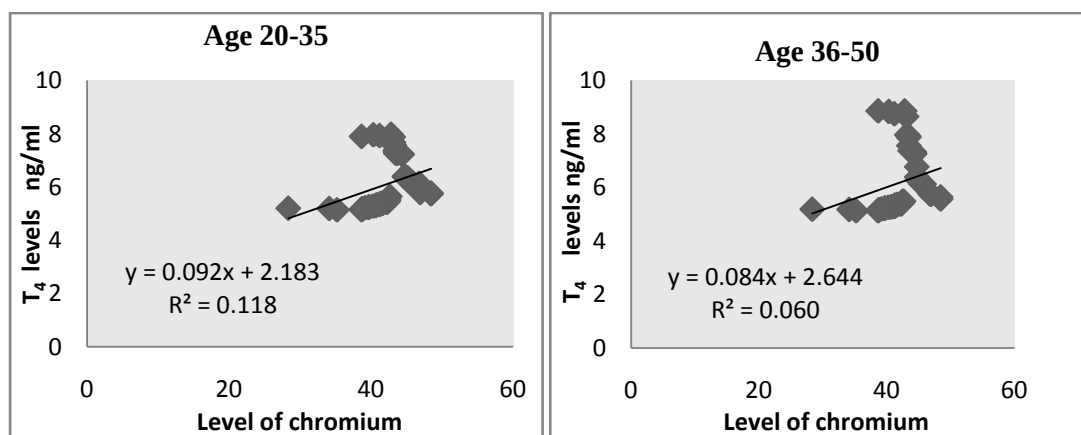


Figure 4.28 The linear multiple regressions between the levels of T₄ and Cr concentration in blood of tanners

Estradiol concentration showed significant negative correlation with Cr concentration ($r = -0.90$, $p < 0.001$) ($r = -0.86$, $p < 0.001$) in exposed groups I and II respectively. In multiple regression analysis, there is a significant level of dependency between the level of estradiol and Cr concentration ($r^2 = 0.816$) ($r^2 = 0.746$) in exposed groups I and II respectively Fig. 4.30.

There is a significant positive correlation between levels of FSH and Cr concentration ($r = 0.91$, $p < 0.001$) ($r = 0.87$, $p < 0.001$) in exposed groups I and II respectively. There is no significant correlation ($r = 0.21$, $p > 0.05$) observed between the levels of FSH and Cr concentration in unexposed population. While in multiple regression, there is significant association between the level of FSH and Cr concentration ($r^2 = 0.845$) ($r^2 = 0.746$) in exposed groups I and II respectively Fig. 4.31. A significant positive correlation was observed between the level of LH and Cr concentration ($r = -0.98$, $p < 0.001$) ($r = -0.89$, $p < 0.001$) in exposed groups I and II respectively. While in multiple regression, there is significant dependency between the levels of LH and Cr concentration ($r^2 = 0.97$) ($r^2 = 0.80$) in exposed groups I and II Fig. 4.32.

Table 4.21 The correlation coefficient and multiple regression between the blood chromium levels and selected female hormones

Worker Groups	Pg		Est		FSH		LH	
	r	r ²	r	r ²	r	r ²	r	r ²
Controls	-0.23 ^a	0.17	-0.38 ^a	0.23	0.2 ^a	0.04	-0.15 ^a	0.77
Exposed 20-35	-0.97*	0.96*	-0.90*	0.816	0.91*	0.845	-0.98*	0.97
Exposed 36-50	-0.83*	0.69*	-0.86*	0.746	0.87*	0.74	-0.89*	0.80

Pg. = Progesterone hormone
 Est. = Estradiol hormone
 FSH = Follicle-stimulating hormone
 LH = Luteinizing hormone

* $p < 0.001$ a $p > 0.05$

r = Simple correlation r^2 = Multiple regression

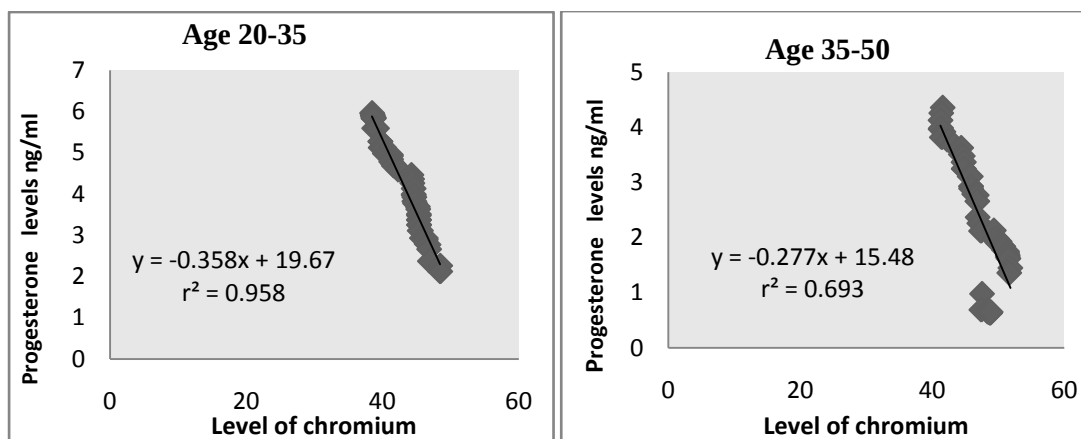


Figure 4.29 The linear multiple regression between the levels of progesterone hormone and concentration in blood of tanners ($p < 0.001$)

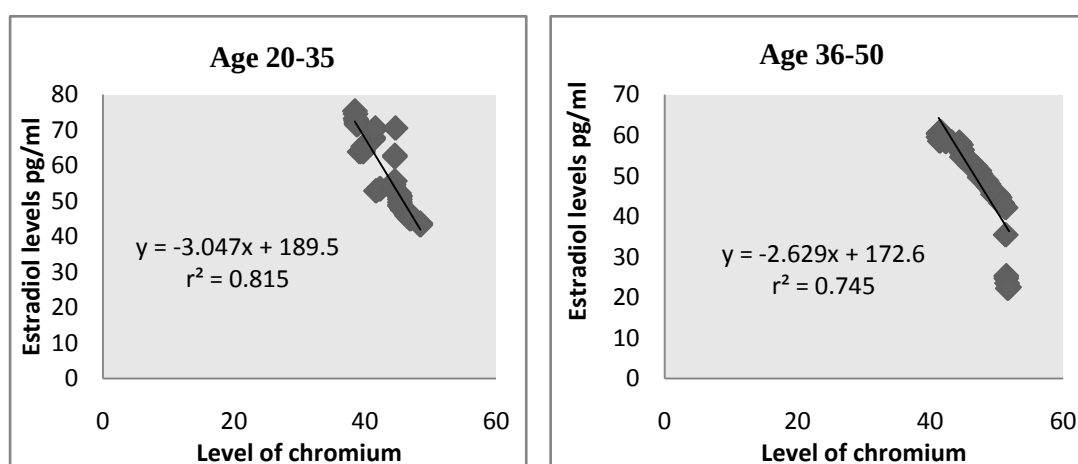


Figure 4.30 The linear multiple regression between the levels of estradiol and Cr concentration in blood of tanners ($p < 0.001$)

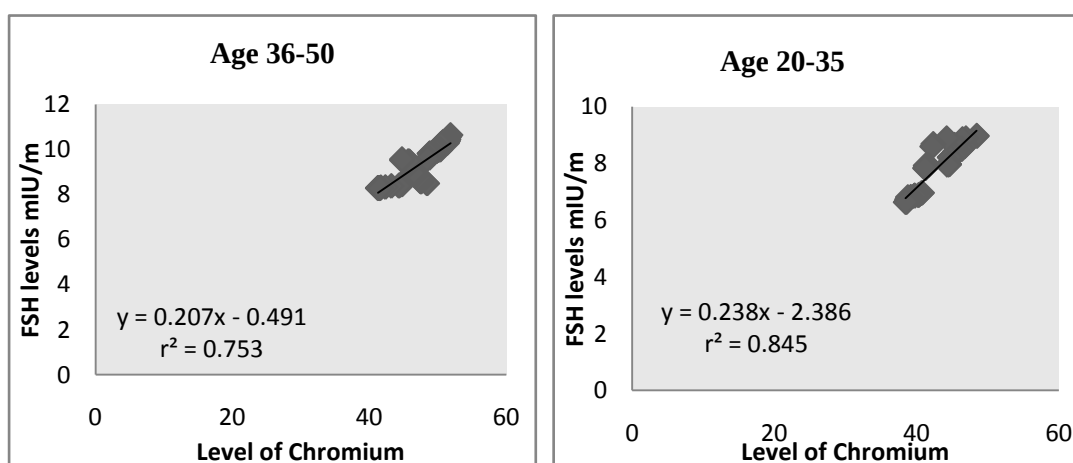


Figure 4.31 The linear multiple regression between the level of FSH and Cr concentration in blood of tanners ($p < 0.001$)

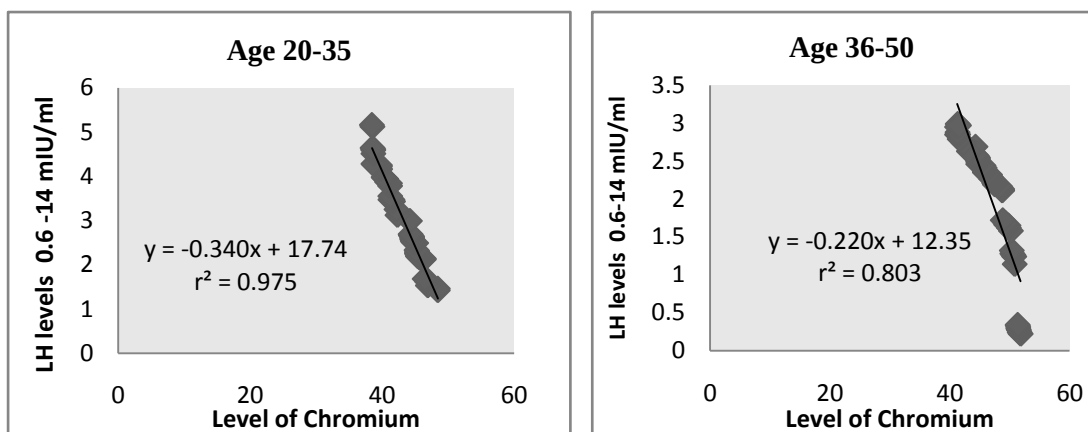


Figure 4.32 The linear multiple regressions between LH levels and Cr concentration in blood of tanners ($p < 0.001$)

4.2.3 Measurement of DNA damage in tannery workers

DNA damage was measured in terms of the comet tail length in exposed and unexposed population Table 4.22, Fig. 4.33 and 4.34. Comet tail lengths for smoking control groups I and II were found to be $9.03\mu\text{m}$ and $9.90\mu\text{m}$ in smoking control groups I and II respectively, which were increased significantly to $26.99\mu\text{m}$ and $28.79\mu\text{m}$ in smoking exposed group I and II respectively. Comet tail lengths were found to be $7.18\mu\text{m}$ and $8.51\mu\text{m}$, which were increased significantly to $23.32\mu\text{m}$ and $25.41\mu\text{m}$ in nonsmoking exposed groups I and II respectively. Comet tail length of exposed groups was significantly ($p < 0.001$) higher than those of unexposed groups. Tannery workers in group II with long-term Cr exposure were more affected and showed larger comet tail length than that of group 1 and controls.

Tannery workers are continuously exposed to Cr (III) which has been shown toxic effect on health. Cr (III) is unable to cross the cell membrane. While hexavalent chromium has the ability to cross cell membrane in the presence of phosphate and sulfate anion-exchange carrier pathway. Hexavalent chromium is inert towards DNA. Once inside the cell, Cr (VI) reduces to reactive intermediates and is eventually converted into kinetically stable Cr (III), which is more reactive than hexavalent

chromium toward DNA and protein. After reduction this trivalent chromium causes DNA damage.

Zhang *et al.*, reported DNA damage in chromium exposed workers in electroplating industry (302). Patlolla *et al.*, also documented DNA damage in hexavalent chromium induced Sprague-Dawley rats (303). During reduction of hexavalent chromium, various reactive species of chromium namely Cr (V), and possibly Cr (IV), Cr (II) and the stable Cr (III) are generated. In addition, other reactive oxygen species including hydroxyl radical or singlet oxygen are also produced leading to oxidative damage to cell (304). However, according to evidence received from in vitro cell-free systems, once inside the cell, trivalent chromium form complexes with various intracellular macromolecules and causes DNA damage (305, 306).

Chromium has potential to inhibit macromolecular synthesis and induce various types of DNA lesions and gene mutation. Chromium induced DNA–DNA interstrand crosslinks and mutations in the tumor suppressor gene (*p53*) are the important factors that play a key role in determining DNA damage (307).

Trivalent chromium is considered more efficient than hexavalent chromium in causing DNA damage in cell free systems. Interaction of trivalent chromium with DNA leads to DNA strand breaks and oxidative DNA base modification such as production of 8-hydroxydeoxyguanosine (8-OH-dG) (307, 308). Reactive oxygen species induced DNA damage can result in base modifications, single-double strand breakage and deoxyribose modification. DNA mutation, cell death, genomic instability and replication errors may occur if DNA damage is unable to repair prior to DNA replication (309-311).

These findings showed that during long-term chromium exposure, chromium is absorbed in the body, which may be distributed in the various tissues and organs of exposed workers. Our data demonstrated the fact that trivalent chromium causes oxidative stress and DNA damage in exposed tannery workers.

The results of the comet assay in the present study showed that comet mean tail lengths for exposed groups I and II were significantly increased than that of unexposed population, indicating that high genetic damage has occurred in tannery workers.

Table 4.22 Summary of statistical analysis of DNA tail lengths (μm) in blood of tannery Workers

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	9.03 $\pm$ 0.270	26.99 $\pm$ 2.651	-57.541*	9.90 $\pm$ 0.522	28.79 $\pm$ 2.154	-64.878*
Non smokers	7.18 $\pm$ 0.574	23.32 $\pm$ 6.021	-34.411*	8.51 $\pm$ 0.592	25.41 $\pm$ 7.199	-30.113*

*Values are significant at $p < 0.001$ $n = 240$

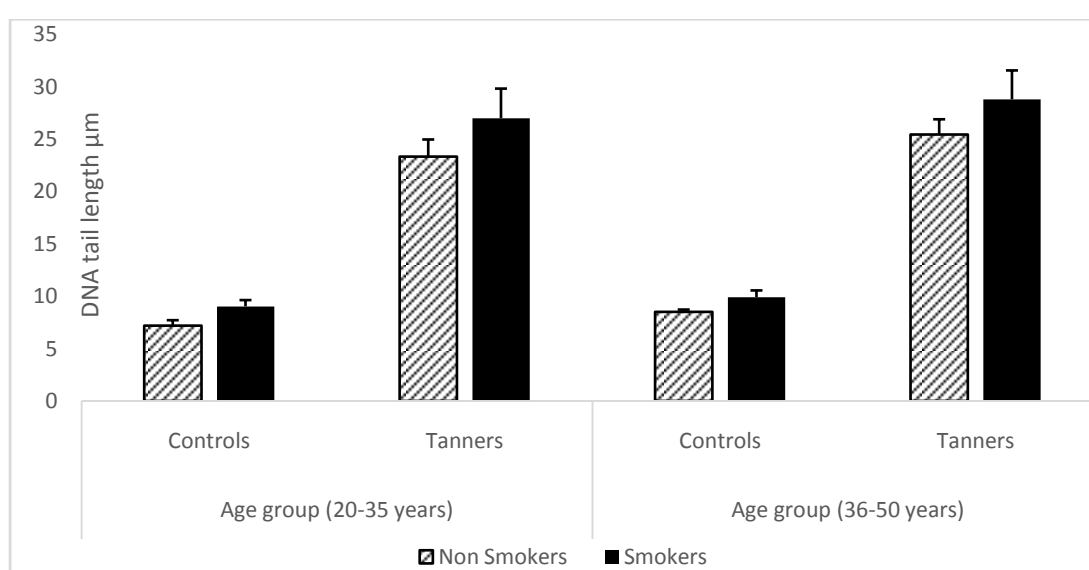


Figure 4.33 DNA tail lengths in tanners and control groups in error bar graph ($p < 0.001$)

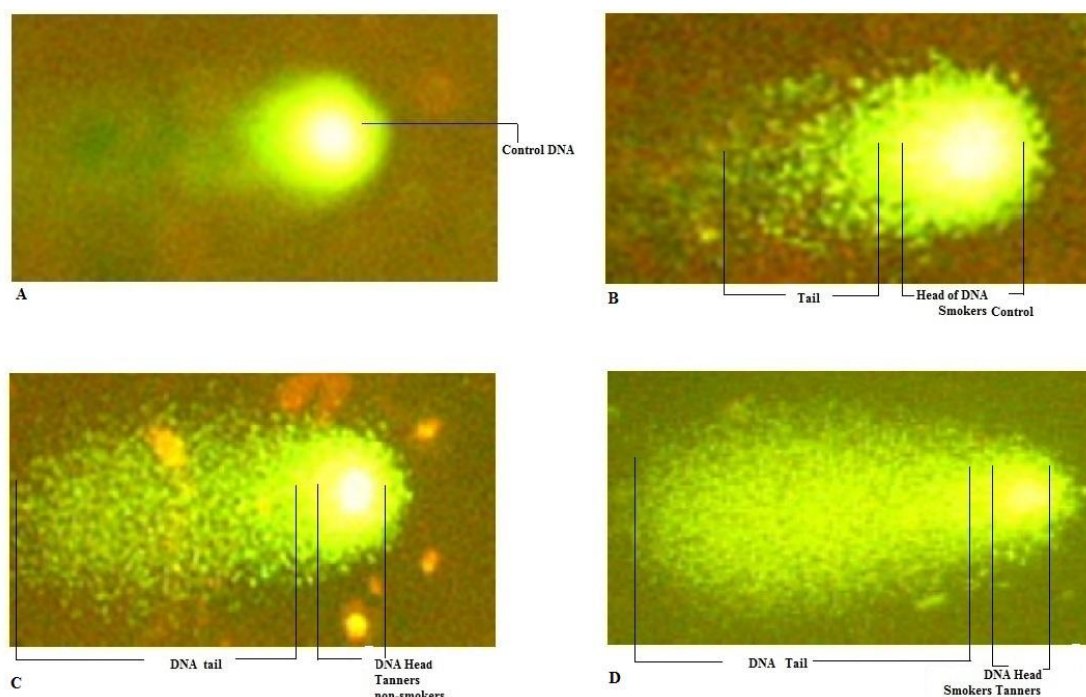


Figure 4.34 Representative photographs of DNA damage in tannery workers: (A) unexposed nonsmokers (B) unexposed smokers (C) exposed nonsmokers and (D) exposed smokers.

4.2.4 Assessment of oxidative stress parameters in tannery workers

4.2.4.1 Malondialdehyde (MDA)

The concentration of malondialdehyde (MDA) in the whole blood of the exposed and unexposed groups is shown in Table 4.23 and Fig. 4.35. The concentration of MDA in blood samples of smoking control groups I and II were found to be 8.35nmol/ml and 8.73nmol/ml respectively, which were increased significantly to 10.12nmol/ml and 11.00nmol/ml in smoking exposed groups I and II respectively. MDA concentration in blood samples of nonsmoking control groups I and II were found as 5.27nmol/ml and 6.11nmol/ml respectively, which were increased significantly to 6.62nmol/ml and 8.77nmol/ml in nonsmoking exposed groups I and II respectively.

MDA concentration of exposed groups were significantly ($p < 0.001$) higher than that of unexposed groups. In addition, the tannery workers in group II with long-term chromium exposure have significantly higher MDA level than that of group I with short-term chromium exposure. It is evident from the present findings that duration of exposure has significant effect on blood MDA levels. This higher MDA level in tannery workers may be due to occupational exposure to high level of chromium in workplace. Higher level of MDA induced oxidative stress in tannery workers.

Caglieri *et al.*, reported high concentration of MDA in chromium exposed workers of chrome-plating plants than those of unexposed groups (312).

The mechanism of Cr toxicity is associated with oxidative stress, which is an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanism (313). Excess production of reactive oxygen species causes lipid peroxidation, which destroy cell structure, nucleic acid, proteins and lipids (314). According to Leonard and Harris higher MDA concentration showed increased rate of oxidative stress in exposed population induced by the metals (315).

In this study, MDA concentration was significantly higher in exposed workers than that of unexposed population. These findings strongly support that higher concentration of MDA in exposed tannery workers was due to chromium exposure in work place environment in the tanning industry.

Table 4.23 Summary of statistical analysis of MDA levels in tannery workers

20-35 years age group				36-50 years age group		
Controls		Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	8.35 ± 0.519	10.12 ± 1.354	7.01437*	8.73 ± 0.725	11.00 ± 0.727	12.0750*
Non Smokers	5.27 ± 1.365	6.62 ± 1.533	3.64156*	6.11 ± 0.772	8.77 ± 2.323	5.9512*

*Values are significant at $p < 0.001$ n = 240

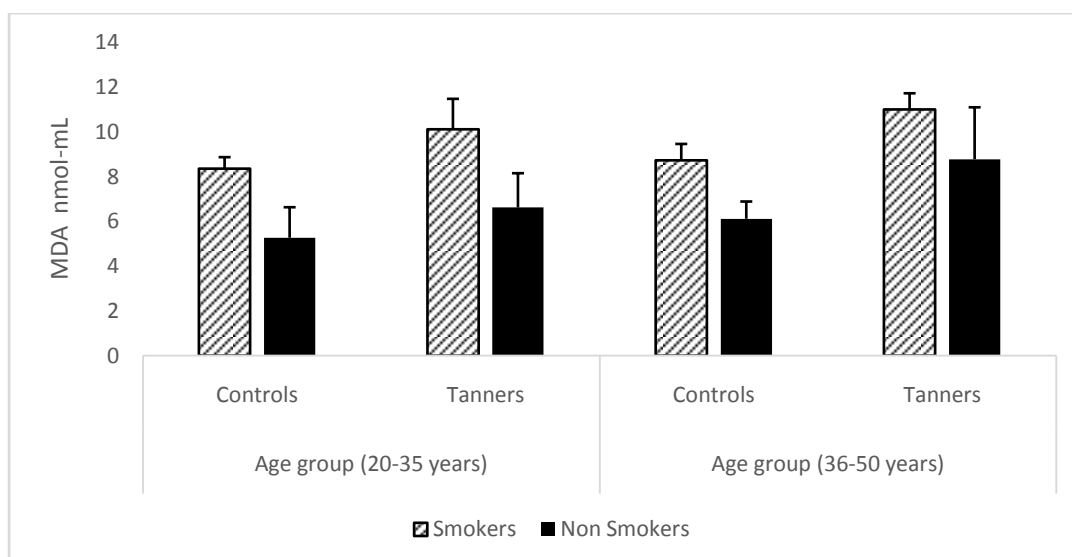


Figure 4.35 The MDA levels in tanners and control groups in error bar graph ($p < 0.001$)

4.2.4.2 Glutathione (GSH)

The concentration of glutathione (GSH) in the whole blood of the exposed and unexposed groups is shown in Table 4.24 and Fig. 4.36. The concentration of GSH in blood samples of smoking control groups I and II were $64.32\mu\text{g/ml}$ and $72.16\mu\text{g/ml}$ respectively, which were decreased significantly to $54.49\mu\text{g/ml}$ and $59.92\mu\text{g/ml}$ in smoking exposed groups I and II respectively. GSH concentration in blood samples of nonsmoking control groups I and II were found as $61.75\mu\text{g/ml}$ and $69.56\mu\text{g/ml}$, which were decreased significantly to $49.71\mu\text{g/ml}$ and $55.70\mu\text{g/ml}$ in nonsmoking exposed groups I and II respectively. GSH concentration of exposed groups was significantly ($p < 0.001$) lower than that of unexposed groups.

The tannery workers in group II have significantly lowered GSH level due to long-term Cr exposure as compared to group I with short-term chromium exposure. It is evident from the present findings that duration of exposure has significant effect on GSH level in blood.

The decreased concentration of GSH in tannery workers was due to its involvement in the inhibition and detoxification of lipid peroxidation by scavenging free radicals. The

marked depletion in GSH concentration impairs cells defense against the toxic action of reactive oxygen species and can cause cell injury and death (316).

The disturbance in GSH homeostasis has been associated with a number of human diseases including cystic fibrosis, cancer, cardiovascular, metabolic, and neurodegenerative diseases. In addition, an imbalance in GSH concentration impairs the function of the immune system and plays a key role in the aging process. According to Ballatori *et al.*, low intracellular GSH concentration affects the cellular antioxidant capacity (317).

Table 4.24 Summary of statistical analysis of GSH levels in tanner workers

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Smokers	64.32 ± 1.053	54.499 ± 2.006	23.7705*	72.16 ± 0.379	59.926 ± 1.594	44.0002*
Non Smokers	61.75 ± 0.537	49.711 ± 2.094	30.5173*	69.56 ± 1.009	55.702 ± 2.303	27.8928*

*Values are significant at p<0.001 n = 240

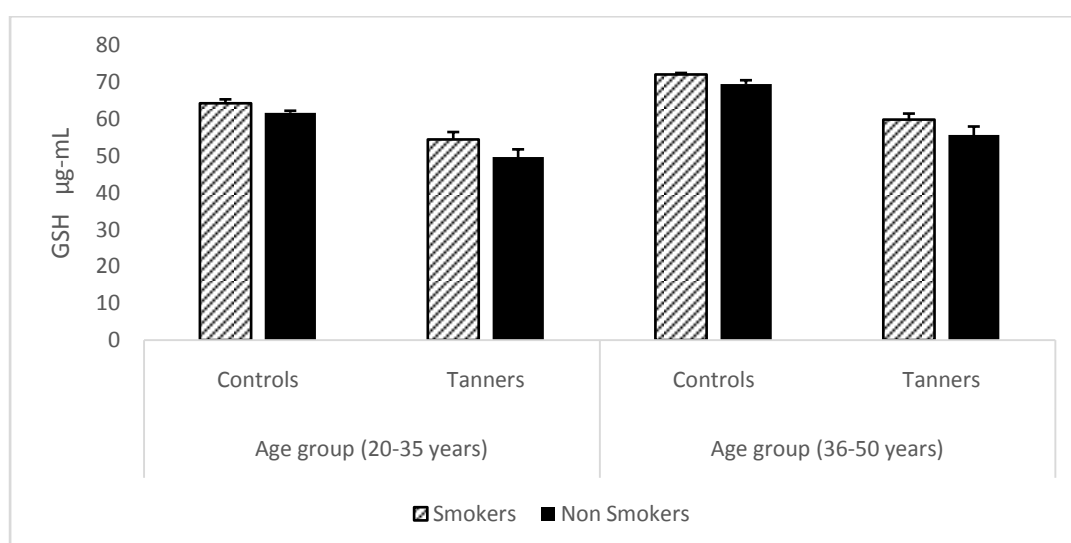


Figure 4.36 The GSH levels in tanners and control groups in error bar graph (p<0.001)

4.2.4.3 Superoxide dismutase (SOD)

The concentration of superoxide dismutase (SOD) in the whole blood of the exposed and unexposed groups is shown in Table 4.25 and Fig. 4.37. SOD levels were found to be 56.67U/gHb and 64.75U/gHb in smoking control groups I and II respectively, which were increased significantly to 65.99U/gHb and 75.17U/gHb in smoking exposed groups I and II respectively. While the levels of SOD were found to be 59.69U/gHb and 67.93U/gHb in nonsmoking control groups I and II respectively, which were increased significantly to 70.04U/gHb and 79.52U/gHb in nonsmoking exposed groups I and II respectively. SOD level of exposed groups was significantly ($p < 0.001$) higher than those of unexposed groups. Long-term chromium exposed tannery workers in group II exhibit significantly higher SOD level than that of group I with short term chromium exposure. It is concluded from the present study that duration of exposure has a significant effect on the SOD level in tannery workers.

SOD catalyzes the dismutation of superoxide radicals to hydrogen peroxide. Thus, it also plays a protective role against free radical-induced damage. SOD enzyme also boosts up the primary antioxidant defense system of the body (86). Kowald *et al.*, reported that high SOD level may lead to increased lipid peroxidation and hypersensitivity to oxidative stress (318).

Table 4.25 Summary of statistical analysis of SOD activity (unit/g) in tannery workers

20-35 years				36-50 years		
	Controls	Tanners (exposed group I)	t-statistics	Controls	Tanners (exposed group II)	t-statistic
Smokers	56.67 $\pm$ 0.939	65.99 $\pm$ 2.347	20.2005*	64.75 $\pm$ 0.981	75.178 $\pm$ 2.397	22.990417*
Non Smokers	59.69 $\pm$ 0.968	70.04 $\pm$ 1.337	34.3304*	67.93 $\pm$ 1.153	79.52 $\pm$ 1.947	26.46469*

*Values are significant at $p < 0.001$ n = 240

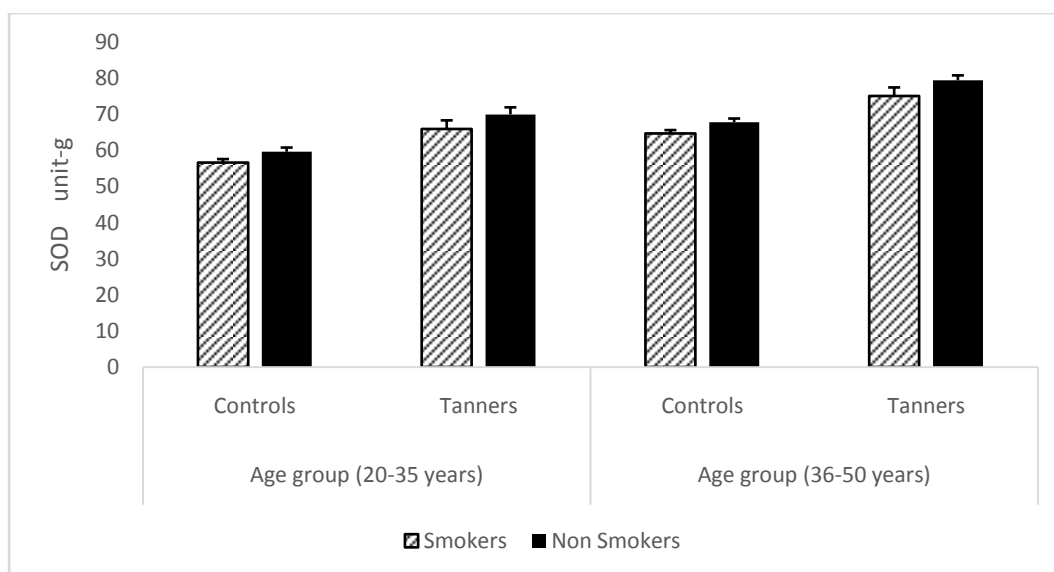


Figure 4.37 The SOD levels in tanners and control groups in error bar graph $p < 0.001$)

4.2.5 Correlation analysis for DNA damage and oxidative stress parameters in tannery workers

In pearson correlation analysis Table 4.26, a significant positive correlation was observed for DNA damage with Cr concentration ($r = 0.907$, $p < 0.001$) ($r = 0.700$, $p < 0.01$) in smoking and nonsmoking exposed group I respectively. Similarly DNA damage also showed significant positive correlation with level of Cr ($r = 0.976$, $p < 0.001$) ($r = 0.877$, $p < 0.01$) in smoking and nonsmoking exposed group II respectively.

A significant positive correlation was observed for MDA concentration with Cr level ($r = 0.837$, $p < 0.01$) ($r = 0.801$, $p < 0.01$) in smoking and nonsmoking exposed group I respectively. Similarly MDA concentration showed significant positive correlation with level of Cr ($r = 0.939$, $p < 0.001$) ($r = 0.822$, $p < 0.01$) in smoking and nonsmoking exposed group II respectively. A significant negative correlation was observed for GSH with Cr concentration ($r = -0.870$, $p < 0.01$) ($r = -0.792$, $p < 0.01$) in smoking and nonsmoking exposed group I respectively. GSH also showed significant negative correlation with Cr level ($r = -0.961$, $p < 0.001$) ($r = -0.753$, $p < 0.01$) in smoking and nonsmoking exposed group II respectively. SOD level showed

significant positive correlation with Cr concentration ($r = 0.754$, $p < 0.01$) ($r = 0.731$, $p < 0.01$) in smoking and nonsmoking exposed group I respectively. There was also significant positive correlation observed for SOD level and Cr concentration ($r = 0.979$, $p < 0.001$) ($r = 0.743$, $p < 0.001$) in smoking and nonsmoking exposure group II respectively.

Table 4.26 Simple correlation analysis between DNA damage, oxidative stress parameters and Cr concentration in tannery workers

Chromium	Correlation groups			DNA	MDA	GSH	SOD
	20-35 years	Controls	Smokers	0.704	0.764*	-0.087	0.670*
			Non smokers	-0.179	0.303	0.001	0.278
		Tanners (exposed group I)	Smokers	0.907**	0.837*	-0.870*	0.754*
			Non smokers	0.700*	0.801*	-0.792*	0.731*
	36-50 years	Controls	Smokers	0.726*	0.795*	-0.719*	0.743*
			Non smokers	0.480	0.321	-0.523	0.732*
		Tanners (exposed group II)	Smokers	0.976**	0.939**	-0.961**	0.979**
			Non smokers	0.877*	0.822*	-0.753*	0.743**

* $p < 0.01$ ** $p < 0.001$

4.2.6 Regression analysis for DNA damage and oxidative stress parameters in tannery workers

In multiple regression (Table 4.27), there is an association between the DNA damage and levels of Cr ($r^2 = 0.820$, $p < 0.01$) ($r^2 = 0.510$, $p < 0.05$) in smoking and nonsmoking exposed group I respectively. DNA damage also showed association with Cr concentration ($r^2 = 0.950$, $p < 0.01$) ($r^2 = 0.763$, $p < 0.001$) in smoking and nonsmoking exposed group II respectively. MDA concentration showed dependency on Cr concentration ($r^2 = 0.701$, $p < 0.01$) ($r^2 = 0.640$, $p > 0.05$) in smoking and nonsmoking exposure group I respectively. There is also an association between MDA level and Cr concentration ($r^2 = 0.520$, $p > 0.05$) in smoking exposed group II

respectively, but there is no dependency observed between MDA level with Cr concentration ($r^2=0.467$, $p < 0.05$) in nonsmoking exposure group II respectively.

GSH concentration showed association with Cr concentration ($r^2=0.757$, $p < 0.01$) ($r^2=0.628$, $p < 0.05$) in smoking and nonsmoking exposed group I respectively.

Similarly, there is an association between GSH level and Cr concentration ($r^2=0.924$, $p < 0.001$) ($r^2=0.567$, $p < 0.05$) in smoking and nonsmoking exposed group II respectively.

SOD level also showed association with Cr concentration ($r^2=0.568$, $p < 0.05$) ($r^2=0.534$, $p < 0.05$) in smoking and nonsmoking exposed group I respectively.

SOD level also showed an association with Cr concentration ($r^2=0.959$, $p < 0.001$) in smoking exposure group II, but there was no association observed between SOD level and Cr concentration ($r^2=0.439$, $p > 0.05$) in nonsmokers exposure group II.

Table 4.27 The regression analysis between oxidative stress and blood chromium levels in tannery workers

Sample parameters				DNA (r^2)	MDA (r^2)	GSH (r^2)	SOD (r^2)
Chromium	20-35 years	Controls	Smokers	0.500*	0.580*	0.007	0.449
			Non smokers	0.032	0.083	0.000	0.075
		Tanners (exposed group I)	Smokers	0.820**	0.701*	0.757**	0.568*
			Non smokers	0.510*	0.640*	0.628*	0.534*
	36-50 years	Controls	Smokers	0.528*	0.424	0.648*	0.304
			Non smokers	0.230	0.165	0.589*	0.501*
		Tanners (exposed group II)	Smokers	0.950***	0.520*	0.924** *	0.959** *
			Non smokers	0.763**	0.467	0.567*	0.439

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.2.7 Assessment of selected haematological parameters in blood of tannery workers

4.2.7.1 White blood cell (WBC) counts

The mean value of WBC counts estimated in exposed and unexposed groups are shown in Table 4.28 and Fig. 4.38. WBC counts in tannery workers were found to be $9.60 \times 10^3/\mu\text{l}$ and $9.22 \times 10^3/\mu\text{l}$ in control groups I and II respectively, which were decreased significantly to $8.54 \times 10^3/\mu\text{l}$ and $8.37 \times 10^3/\mu\text{l}$ in exposed groups I and II respectively. WBC counts of exposed groups were significantly ($p < 0.001$) lower than that of unexposed groups. Tannery workers in group II with long-term Cr exposure have lower WBC count than that of group 1 and unexposed groups.

Shaheen and Akhtar reported 7-8% decrease in WBC count in *Cyprinus caprio* treated with chromium (319). Afify *et al.*, also reported decreased WBC ($7.7 \pm 0.7 \times 10^3/\mu\text{l}$) counts in tannery workers in Egypt (320). WBC can accumulate large amount of hexavalent chromium as compared to RBCs. WBC can also accumulate trivalent chromium both in vivo and vitro (321). According to Munshi and Montgomery low WBC count has been associated with severe neutropenia (322).

4.2.7.2 Red blood cell (RBC) counts

The mean value of RBC counts, estimated in exposed and unexposed groups are shown in Table 4.28 and Fig. 4.38.

RBC counts in control groups I and II were found to be $5.17 (\times 10^6/\mu\text{l})$ and $5.06 (\times 10^6/\mu\text{l})$ respectively, which were decreased significantly to $4.46 (\times 10^6/\mu\text{l})$ and $4.12 (\times 10^6/\mu\text{l})$ in exposed groups I and II respectively. RBC counts of exposed groups were significantly ($p < 0.001$) lowered than that of unexposed groups. Tannery workers

in group II with long-term Cr exposure had lowered RBC count than that of group 1 and unexposed groups.

The decrease in RBC count in tannery workers may be due to the chromium induced erythrocytes damage or reduction in red cell glutathione leading to increase free radical inside RBC which causes cell death (323-325). Afify *et al.*, reported low RBC count (4.5 ± 0.6) in tannery workers in Egypt (320). Ahsan *et al.*, (2006) has also documented low RBC counts (5.39 ± 0.06) in tannery workers in kasur (271). Studies on human blood showed that RBC fraction has the capacity to reduce the hexavalent chromium at level up to $15000 \mu\text{g/l}$ in blood and the rate of hexavalent chromium uptake into RBC cannot exceed the rate of intracellular reduction at this level (326).

4.2.7.3 Erythrocyte sedimentation rate

The mean value of ESR rate estimated in exposed and unexposed groups are shown in Table 4.28 and Fig. 4.38. The ESR rate in the blood of tannery workers was not affected by chromium exposure. ESR rates in tannery workers were found to be 7.56mm/hr and 8.15mm/hr in unexposed group, which were decreased to 7.25mm/hr and 7.90mm/hr in exposed groups I and II respectively. ESR rate of exposed groups were insignificantly lowered than that of unexposed groups.

Table 4.28 White blood cell counts, red blood cell counts and erythrocyte sedimentation rate in blood of tannery workers

20-35 years				36-50 years		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
WBC x103/ μl	9.60 ± 0.96	8.543 ± 0.91	6.19*	9.22 ± 1.03	8.373 ± 0.747	5.16*
RBC x106/ μl	5.17 ± 0.27	4.46 ± 0.31	13.24*	5.061 ± 0.32	4.12 ± 0.530	11.72*
ESR mm/hr	7.56 ± 1.59	7.25 ± 1.17	1.24	8.15 ± 1.85	7.90 ± 1.51	0.82

*Values are significant at $p < 0.001$ n = 240

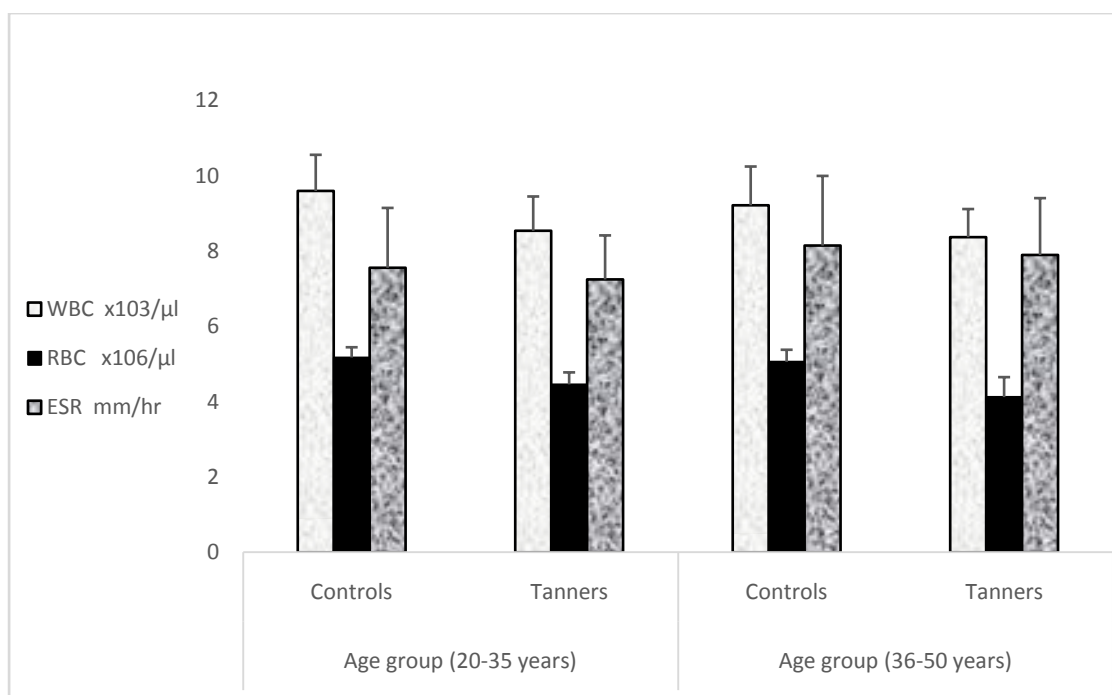


Figure 4.38 The WBC, RBC and ESR levels in blood samples of tanners and control groups in error bar graph ($p < 0.001$)

4.2.7.4 Haemoglobin

The mean values of haemoglobin levels determined in exposed and unexposed groups are shown in Table 4.29 and Fig. 4.39. Haemoglobin levels were found to be 14.483g/dl and 14.25g/dl in control groups respectively, which were decreased significantly ($p < 0.001$) to 10.409g/dl and 10.02g/dl in exposed groups I and II respectively. Tannery workers in group II with longer duration of Cr exposure were more affected and showed lower haemoglobin level than that of group I and unexposed population.

Mathivanan *et al.*, reported that reduction in haemoglobin level might be due to the effect of toxic pollutants on hematopoietic system which may cause anemic condition in human (327).

Ahsan *et al.*, reported that haemoglobin content of population residing in industrial area exposed to toxic pollutants showed 4-5% decrease as compared to unexposed

population (271). Chromium absorbed through lungs into the blood easily passes through cell membranes and binds to the haemoglobin in the RBCs, thereby affects the oxygen carrying capacity and impairs the lungs function (328, 329).

4.2.7.5 Mean corpuscular haemoglobin (MCH)

The mean values of mean corpuscular haemoglobin (MCH) level determined in exposed and unexposed groups are shown in Table 4.29 and Fig. 4.39. The mean corpuscular haemoglobin (MCH) is the average mass of haemoglobin per RBC in a blood sample (330). MCH levels in tannery workers were found to be 28.61pg and 28.02pg in control groups I and II respectively, which were decreased significantly ($p < 0.001$) to 26.38pg and 25.91pg in exposed groups I and II respectively. Tannery workers in group II with longer duration of Cr exposure had lowered MCH level than that of group 1 and unexposed population. The decrease in MCH might be due to additive effects of chromium and age (330).

Ahsan *et al.*, reported 8-10% decrease in MCH level in industrial workers exposed to chromium as compared to exposed population (271). Jadhav *et al.*, reported a decrease in MCH value in albino wistar rats exposed to metals mixture (331). In addition, microcytic and hypochromic anemia has been observed by decrease in concentration in MCH, haemoglobin and RBC count.

4.2.7.6 Mean corpuscular hemoglobin concentration (MCHC)

The mean values of mean corpuscular hemoglobin concentration (MCHC) level determined in unexposed population and exposed groups are presented in Table 4.29 and Fig. 4.39. The mean corpuscular haemoglobin concentration (MCHC) is a measure of the concentration of haemoglobin in a given volume of packed RBCs (99). MCHC levels in tannery workers were found to be 29.09g/dl and 29.35g/dl in control

groups I and II respectively, which were decreased 28.62g/dl and 28.89g/dl in exposed groups I and II respectively. There was no significant difference of MCHC level between exposed groups and control. Cr (VI) seems to have no effect on MCHC in the blood sample of tannery workers. Ramzan *et al.*, also reported no significant decrease in MCHC level of tannery workers exposed to hexavalent chromium (330). Liu *et al.*, reported that decrease in MCV do not support that iron deficiency contributed to development of anemia (332).

Table 4.29 Haemoglobin, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in blood samples tannery workers

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
Hemoglobin (g/dl)	14.48±0.71	10.409±0.733	30.78*	14.25±1.00	10.02±1.14	21.64*
MCH (pg)	28.61±1.45	26.38±1.48	8.35*	28.02±1.44	25.91±1.50	7.86*
MCHC (g/dl)	29.09±1.55	28.62±2.74	1.15	29.35±1.52	28.89±2.32	1.28

*Values are significant at $p < 0.001$ n = 240

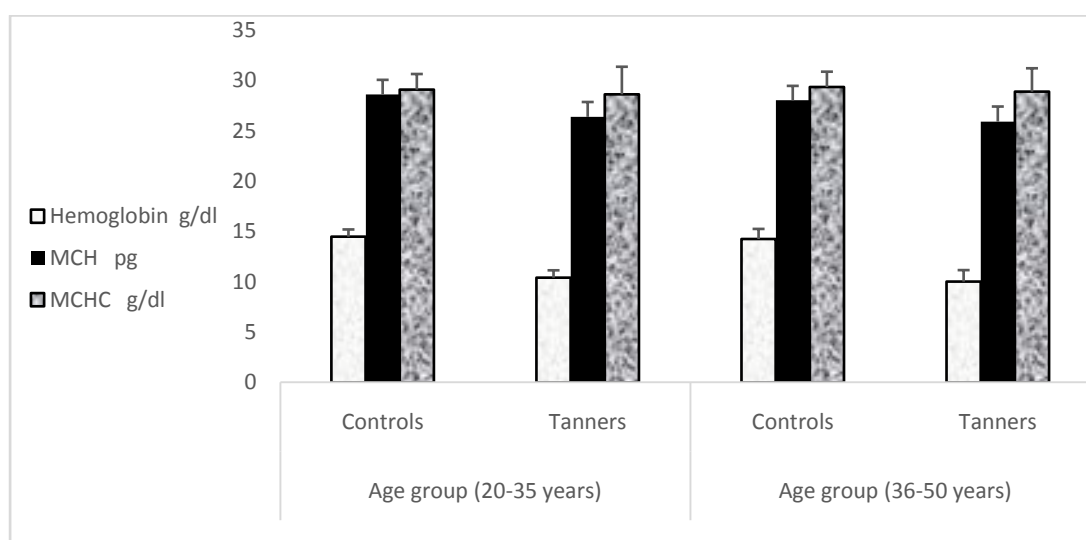


Figure 4.39 The haemoglobin, MCH and MCHC levels in blood samples of tanners and control groups in error bar graph ($p < 0.001$)

4.2.7.7 Packed cell volume (PCV)

The mean values of packed cell volume (PCV) in unexposed population and exposed groups are shown in Table 4.30 and Fig. 4.40. PCV is the proportion of blood volume that is occupied by RBCs (330). It is normally 38% for women and 46% for men (331). PCV levels in tannery workers were found to be 44.57% and 45.07% in control groups I and II respectively, which were significantly decreased to 43.24% and 42.62% in exposed groups I and II respectively. PCV levels of exposed groups were significantly ($p<0.001$) lower than that of unexposed groups. Tannery workers in group II with long-term Cr exposure were more affected and showed lower PCV levels than that of group 1 and unexposed population. It is observed from the present study that duration of exposure has significant affect on PCV level in blood.

Low PCV level was also observed in albino wistar rats exposed to metals mixture of different concentration (331). According to Emmanuelchide *et al.*, PCV level has been associated with Sick cell disease. Microcytic and hypochromic anemia was reported by the reduction in PCV value (333).

Table 4.30 Packed cell volume (PCV), Mean corpuscular volume (MCV) and Platelets (Plt) in blood samples of tannery workers and control groups

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
PCV %	44.57±1.35	43.24±1.60	4.96*	45.07±1.23	42.62±2.00	8.08*
MCV (fl)	85.08±1.35	82.24±1.73	10.03*	85.74±1.35	81.87±1.52	14.79*
Plt x103/μl	260.58±1.99	259.91±3.28	1.35	252.65±3.91	251.90±4.47	0.98

*Values are significant at $p<0.001$ n = 240

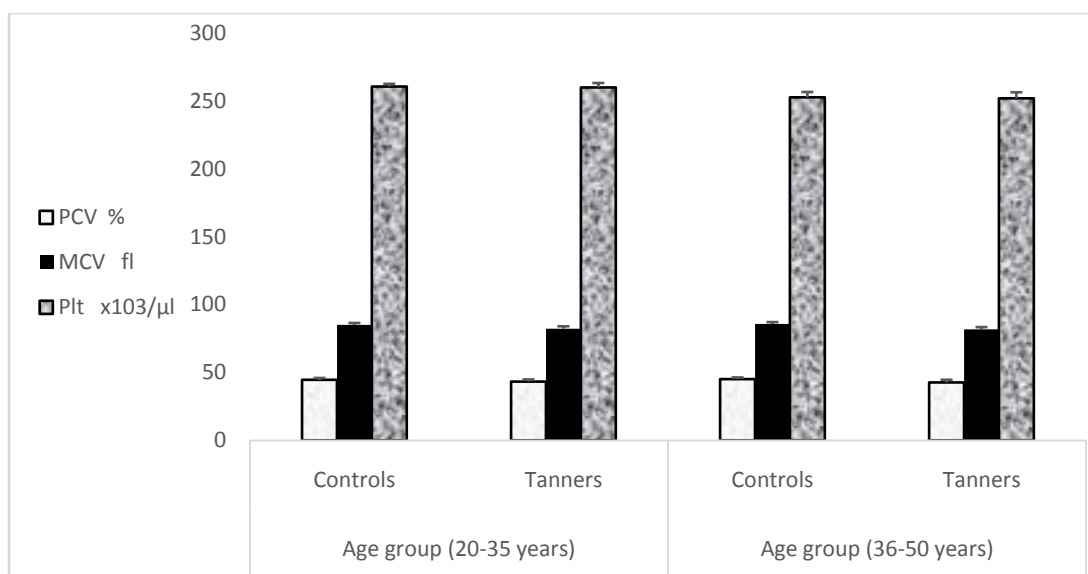


Figure 4.40 The PCV, MCV and Plt levels in blood of tanners and control groups in error bar graph ($p < 0.001$)

4.2.7.8 Mean corpuscular volume (MCV)

MCV levels in tannery workers were found to be 85.08fl and 85.74fl in unexposed groups, which were increased to 82.24fl and 81.87fl in exposed groups I and II respectively Table 4.30, Fig. 4.40. MCV levels of exposed groups were significantly ($p < 0.001$) lower as than that of unexposed groups. Tannery workers in group II with longer duration of Cr exposure showed lowered MCV level than that of group 1 and unexposed groups. It is concluded from the present study that duration of exposure has significant effect on MCV level in tannery workers.

Jadhav *et al.*, reported low level of MCV in male wistar rats treated with different metal mixture concentration (331). The mean corpuscular volume (MCV) is a measure of the average RBC volume (i.e. size). In patient, anemia is classified on the basis of MCV measurement as either a macrocytic anemia (MCV above normal range) or microcytic anemia (MCV below normal range) (330). Microcytic and hypochromic anemia were observed by decrease in concentration of MCV, MCH and RBC counts (331).

In this study, the decrease in concentration of MCV, MCH and red blood cells showed that tannery workers exposed to hexavalent chromium are at high risk for microcytic and hypochromic anemia.

4.2.7.9 Platelet counts

The mean value of Platelet counts determined in exposed and exposed groups is shown in Table 4.30 and Fig. 4.40. Platelet concentrations in tannery workers were found to be $260.58 \times 10^3/\mu\text{l}$ and $252.65 \times 10^3/\mu\text{l}$ in unexposed groups respectively, which were decreased to $259.91 \times 10^3/\mu\text{l}$ and $251.90 \times 10^3/\mu\text{l}$ in exposed groups I and II respectively. There was no significant difference of platelet count between exposed groups and unexposed population.

Despite being anucleate, platelets fulfill a variety of functions: They not only play a key role in hemostasis and in vascular disease; but also contribute to tumor angiogenesis inflammation, immunologic responses and embryonic development. Platelets also contain various biologically active molecules, such as growth factors, factors triggering platelet aggregation and other compounds which are secreted on platelet activation (334).

4.2.7.10 Pearson correlation coefficient between levels of blood chromium and haematological parameters in tannery workers

In Pearson correlation Table 4.31, hemoglobin concentration showed negative correlation with Cr concentration ($r = -0.908$, $p < 0.001$) ($r = -0.959$, $p < 0.001$) in exposed groups I and II respectively.

WBC concentration showed significant negative correlation with Cr concentration ($r = -0.932$, $p < 0.001$) ($r = -0.957$, $p < 0.001$) in exposed groups I and II respectively. While there was no significant correlation ($r = -0.218$, $p > 0.05$) ($r = 0.154$, $p > 0.05$)

observed between WBC count and Cr concentration in control groups I and II respectively.

RBC count showed significant negative correlation with Cr concentration ($r = -0.965$, $p < 0.001$) ($r = -0.898$, $p < 0.001$) in both exposed groups I and II respectively. While RBC count showed no significant correlation with Cr concentration ($r = 0.101$, $p > 0.05$) ($r = -0.211$, $p > 0.05$) in controls.

PCV concentration showed significant negative correlation with Cr concentration ($r = -0.965$, $p < 0.001$) ($r = 0.972$, $p < 0.001$) in exposed groups I and II respectively. While PCV concentration showed no significant correlation with Cr concentration ($r = -0.397$, $p > 0.05$) ($r = -0.119$, $p > 0.05$) in controls.

MCV concentration showed significant negative correlation with Cr concentration ($r = -0.954$, $p < 0.001$) ($r = 0.965$, $p < 0.001$) in exposed groups I and II respectively. While MCV concentration showed no significant correlation with Cr concentration ($r = 0.096$, $p > 0.05$) ($r = 0.040$, $p > 0.05$) in controls.

MCH concentration showed significant negative correlation with Cr concentration ($r = -0.951$, $p < 0.001$) ($r = -0.869$, $p < 0.05$) in exposed groups I and II respectively. While MCH concentration showed no significant correlation with Cr concentration ($r = 0.055$, $p > 0.05$) ($r = -0.178$, $p > 0.05$) in controls.

MCHC concentration showed significant negative correlation with Cr concentration ($r = -0.231$, $p > 0.05$) ($r = -0.234$, $p > 0.05$) in exposed groups I and II respectively. While MCHC concentration showed no significant correlation with Cr concentration ($r = 0.035$, $p > 0.05$) ($r = 0.148$, $p > 0.05$) in controls.

Platelets concentration showed significant negative correlation with Cr concentration ($r = 0.122$, $p > 0.05$) ($r = -0.050$, $p > 0.05$) in exposed groups I and II respectively.

While Platelets concentration showed no significant correlation with Cr concentration ($r = -0.104$, $p > 0.05$) ($r = -0.031$, $p > 0.05$) in controls.

ESR concentration showed no significant correlation with Cr concentration ($r = -0.101$, $p > 0.05$) ($r = -0.186$, $p > 0.05$) in exposed groups I and II respectively. While ESR concentration showed no significant correlation with Cr concentration ($r = 0.257$, $p > 0.05$) ($r = -0.193$, $p > 0.05$) in controls.

Table 4.31 Pearson correlation coefficient between level of blood chromium and hematological parameters in tannery workers and controls

Hematological Parameter	Age 20-35 age group		Age 36-50 age group	
	Controls	Tanners (exposed group I)	Controls	Tanners (exposed group II)
Hemoglobin g/dl	-0.395	-0.908**	0.028	-0.959**
WBC $\times 10^3/\mu\text{l}$	-0.218	-0.932**	0.154	-0.957**
RBC $\times 10^6/\mu\text{l}$	0.101	-0.965**	-0.211	-0.898*
PCV %	-0.397	-0.965**	-0.119	-0.972**
MCV fl	0.096	-0.954**	0.040	-0.965**
MCH pg	0.055	-0.951**	-0.178	-0.869*
MCHC g/dl	0.035	-0.231	0.148	-0.234
Plt $\times 10^3/\mu\text{l}$	-0.104	0.122	-0.031	-0.050
ESR mm/hr	0.257	-0.101	-0.193	-0.186

* $p < 0.05$ ** $p < 0.001$

4.2.8 Assessment of biochemical parameters in blood of tannery workers

4.2.8.1 Assessment of liver function

Table 4.32 and Fig. 4.41 show the activities of liver enzymes alkaline phosphatase (AP), Aspartate aminotransferase (AST), Alanine transaminase (ALT) of tannery workers.

4.2.8.2 Alkaline phosphatase (AP)

AP activity in tannery workers was found to be 95.79U/l and 98.35U/l in unexposed groups I and II respectively, which was increased significantly to 117.27U/l and 121.28U/l in exposed groups I and II respectively Table 4.32, Fig. 4.41. AP activity of exposed groups was significantly ($p<0.001$) higher than that of unexposed groups. Tannery workers in group II with long-term Cr exposure were more affected and showed higher AP level compared with group 1 and unexposed population.

Chromium is reported to cause severe elevation in liver enzyme levels, hepatic effects, hepatic failure and hepatomegaly in four of five workers exposed to chromium in leather tanning industry. Human serum contains various forms of AP. Elevated activity of AP without bone disease is a sign of biliary disturbance. Low AP levels are found in diseases of the liver parenchyma like cirrhosis and hepatitis. Higher elevations are found in intrahepatic functional cholestasis and extra hepatic mechanical biliary obstruction (335, 336).

Elevated level of AP has been associated with bile duct obstruction, primary biliary cirrhosis, primary sclerosing cholangiti, metastatic liver disease and bone disease. The concentration of AP may be raised in malignancies without liver or bone involvement. (336).

4.2.8.3 Alanine transaminase (ALT)

The activity of alanine transaminase (ALT) determined in exposed and unexposed groups is shown in Table 4.32 and Fig. 4.41. ALT level was found to be 33.91U/l and 37.51U/l in unexposed groups I and II respectively, which was increased to 34.20U/l and 38.05U/l in exposed groups I and II respectively. ALT level in exposed groups was insignificantly higher than that of unexposed groups.

Wu *et al.*, documented that a 33 year old woman was taking 6-12 times more chromium picolinate as compared to the recommended dose. The woman presented with raised liver enzymes, anemia, thrombocytopenia, weight loss and hemolysis (337). According to Ahsan *et al.*, there was no significant increase in ALT in tannery workers exposed to chromium (271). According to Colletta *et al.*, serum ALT serves as an indirect marker of hepatitis C (338). Aminotransferases include ALT and AST, which are an excellent marker for hepatocellular injury. ALT, a cytosolic enzyme is located in its highest concentration in the liver and is considered more specific to the liver. Hepatocellular injury is the trigger to release of these aminotransferases into the circulation. Elevated levels of aminotransferases cause chronic hepatitis B and C, haemochromatosis, autoimmune liver disease, Wilson's disease, α - antitrypsin deficiency, alcoholic liver disease, and coeliac disease (336).

4.2.8.4 Aspartate aminotransferase (AST)

The activity of Aspartate aminotransferase (AST) determined in exposed and unexposed groups is shown in Table 4.32, and Fig. 4.41. AST level was found to be 32.89 U/l and 35.61 U/l in unexposed groups I and II respectively, which was increased 33.53 U/l and 36.05 U/l in exposed groups I and II respectively. AST level of exposed groups was insignificantly higher than that of unexposed groups.

Ahsan *et al.*, reported that there is no change in AST activity in tannery workers (271). Afify *et al.*, documented that there was no significant difference between exposed tannery workers and controls (320).

Elevated levels of AST and ALT caused cholestatic liver disease, fatty liver, cirrhosis and hepatic neoplasms. Elevated levels of serum aminotransferase are also reported in autoimmune hepatitis, chronic and milder cases of acute viral or drug-induced hepatitis and alcoholic liver disease (339). According to Munagala *et al.*, chromium is

found to cause many liver diseases in workers. Increase in alanine aminotransferase and aspartate aminotransferase is an indication of leakage of enzymes from liver leading to alterations in the cell permeability (340).

Table 4.32 AP, ALT and AST levels in blood samples of tannery workers and control Groups

20-35 years age group				36-50 years age group		
	Controls	Tanners Exposed group I	t-statistic	Controls	Tanners Exposed group II	t-statistic
AP (U/l)	95.79±4.76	117.27±8.84	-1.51*	98.35±6.52	121.28±8.371	0.28*
ALT (U/l)	33.91±2.35	34.20±2.20	-0.69	37.51±4.81	38.05±4.60	-0.64
AST (U/l)	32.89±2.60	33.53±3.49	-1.15	35.61±2.61	36.05±2.31	-0.98

*Values are significance at $p < 0.05$ n = 240

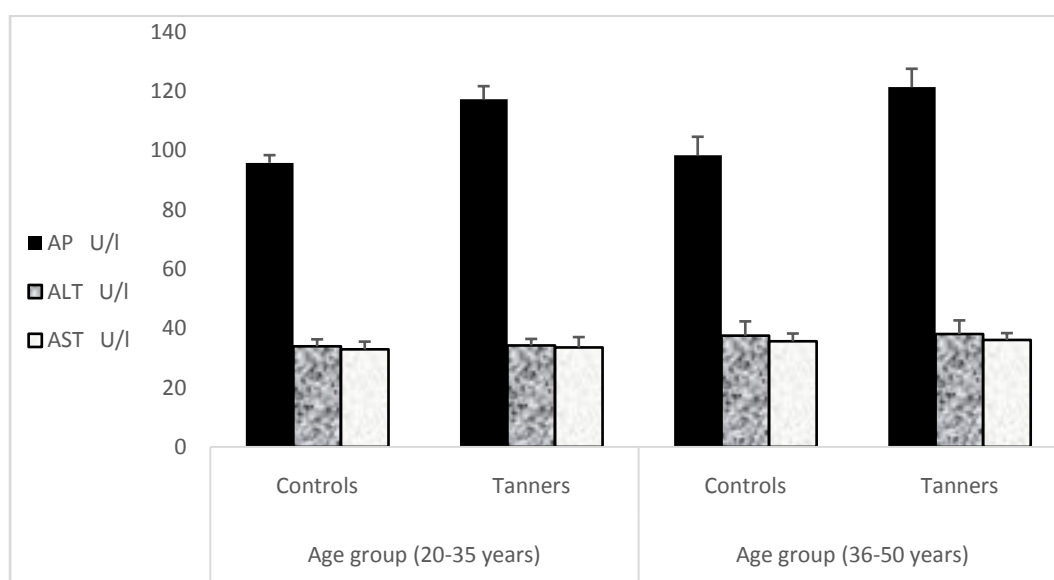


Figure 4.41 The AP, ALT, AST levels in blood of tanners and control groups in error bar graph

4.2.8.5 Albumin level in tannery workers

Albumin level was found to be 5.20g/dl and 5.08g/dl in unexposed groups I and II respectively, which was decreased significantly to 3.81g/dl and 3.75g/dl in exposed groups I and II respectively Table 4.33 and Fig. 4.42. Albumin level of exposed groups was significantly ($p<0.001$) lower than that of unexposed groups. Tannery workers in group II showed lower albumin level than that of group 1 and control. It is observed from the present study that duration of exposure has significant effect on albumin level in tannery workers.

Ahsan *et al.*, reported low level albumin in tannery workers exposed to hexavalent chromium (271). Albumin synthesis is an important function and characteristic feature of the liver. 10g albumin is synthesized and secreted daily from the human body. The low level of albumin is an indication of liver disease. Low albumin concentration has been associated with prognosis in chronic liver disease (336). According to Schillinger *et al.*, low albumin level is a powerful predictor of cardiovascular adverse events in humans (341). Low serum albumin concentration has also been associated with a 2-fold increased risk of cardiovascular disease. Albumin can scavenge peroxy radicals and inhibits the production of free radicals from a system containing H_2O_2 and copper ions (342). Albumin also possesses antioxidant properties and low albumin concentration can cause hemolysis (343). According to Djousse *et al.*, low albumin concentration also appears to be a risk factor for myocardial infarction (MI) in women (342).

4.2.8.6 Total Protein

Total Protein level estimated in exposed and unexposed groups is shown in Table 4.33 and Fig. 4.42. Total protein levels were found to be 7.84 g/dl and 7.59 g/dl in unexposed groups I and II respectively, which were increased to 7.92 g/dl and 7.70

g/dl in exposed groups I and II respectively. Total protein in the exposed groups was insignificantly higher than that of unexposed groups. There was no significant change in total protein concentration in tannery workers exposed to chromium. Low levels of albumin in tannery workers could be because of reduction in protein synthesis due to hexavalent chromium intoxication (271, 319). Serum total protein is related to the immune system and maintains transportation of various metabolites, hydrogen potential, intravascular colloid osmotic pressure and regulation of numerous physiological functions (344,345).

Table 4.33 The Albumin and total protein levels in blood of tanners and control groups

20-35 years age group				36-50 years		
	Controls	Tanners (Expose group I)	t-statistic	Controls	Tanners (Expose group II)	t-statistic
Alb g/dl	5.20±0.33	3.81±0.288	24.42*	5.08±0.38	3.75±0.282	21.69*
T. Protein g/dl	7.84±0.380	7.92±0.443	-0.98	7.59±0.44	7.70±0.496	-1.37

*Values are significant at $p < 0.001$ n = 240

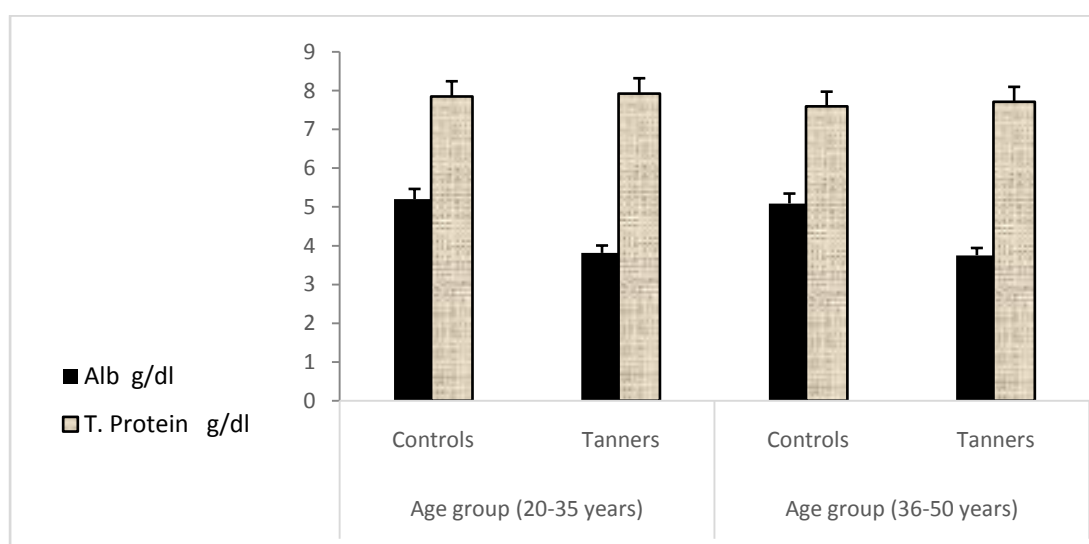


Figure 4.42 The Alb and T. Protein levels in blood of tanners and control groups in error bar graph ($p < 0.001$)

4.2.8.7 Direct bilirubin in tannery workers

Direct bilirubin level determined in exposed and unexposed groups is shown in Table 4.34 and Fig. 4.43. Direct bilirubin level was found to be 0.21mg/dl and 0.24mg/dl in unexposed groups I and II respectively, which was increased insignificantly to 0.22mg/dl and 0.25mg/dl in exposed groups I and II respectively. Direct bilirubin of exposed groups was insignificantly higher than that of unexposed groups.

According to Ahsan *et al.*, no significant difference in direct bilirubin was reported in tannery workers exposed to chromium as compared to control (271). Limdi *et al.*, reported that direct bilirubin level has been associated with primary biliary cirrhosis, alcoholic hepatitis and in acute liver failure (336). Serum bilirubin possesses an anti-inflammatory property and also contributes to total antioxidant capacity as well as acts as scavengers of reactive oxygen species (346,347)

4.2.8.8 Total bilirubin in tannery workers

Total bilirubin level determined in unexposed and exposed groups is shown in Table 4.34 and Fig. 4.43. Total bilirubin level was found to be 0.52mg/dl and 0.54mg/dl in unexposed groups I and II respectively, which was increased insignificantly to 0.55mg/dl and 0.56mg/dl in exposed groups I and II respectively. Total bilirubin of exposed groups was insignificantly higher than that of unexposed groups.

There was no significant difference observed in total bilirubin in tannery workers exposed to chromium as compared to unexposed group (271).

Serum bilirubin is mainly in an unconjugated form maintaining balance between production and hepatobiliary excretion. Bilirubin production (unconjugated form) increases in ineffective erythropoiesis, resorption of a haematoma, haemolysis, and rarely in muscle injury (336).

Table 4.34 The D. Bilirubin and T. Bilirubin levels in blood samples of tanners and control Groups

20-35 years age group				36-50 years age group		
	Controls	Tanners (exposed group I)	t-statistic	Controls	Tanners (exposed group II)	t-statistic
D. Bilirubin (mg/dl)	0.21±0.01	0.22±0.018	-1.19	0.24±0.02	0.25±0.017	-0.51
T. Bilirubin (mg/dl)	0.52±0.10	0.55±0.148	-1.21	0.54±0.16	0.56±0.187	-0.69
Glucose (mg/dl)	86.59±2.86	85.82±3.14	-1.4	89.48±3.32	88.92±3.28	-0.94

n = 240

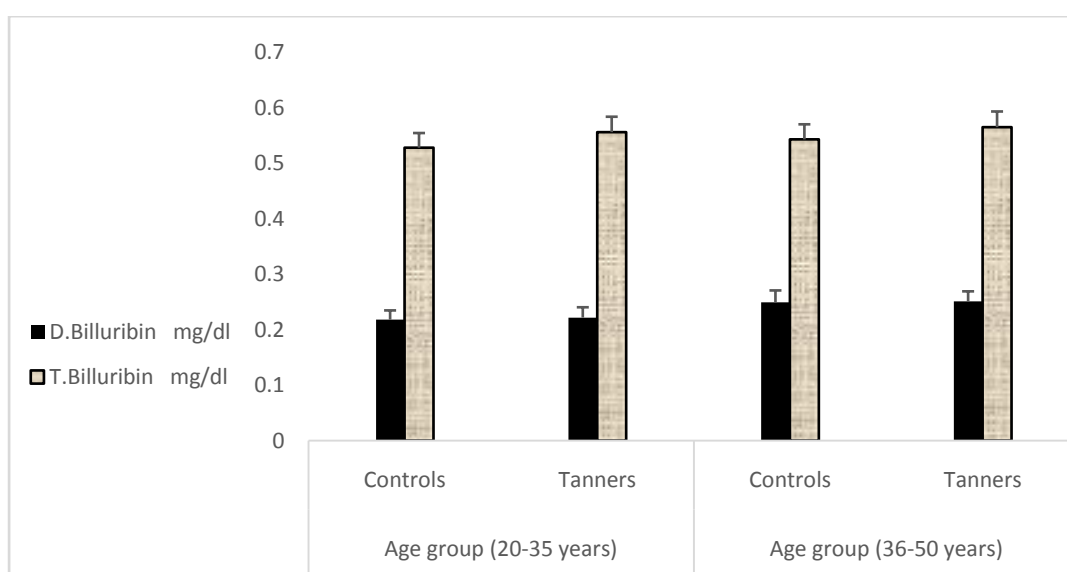


Figure 4.43 The D. Bilirubin and T. Bilirubin levels in blood samples of tanners and control groups in error bar graph

4.2.8.9 Blood glucose in tannery workers

Blood glucose level determined in unexposed and exposed groups is shown in Table 4.34 and Fig. 4.44. Blood glucose level was found to be 86.59mg/dl and 89.48mg/dl in unexposed groups I and II respectively, which was decreased to 85.82mg/dl and 88.92mg/dl in exposed groups I and II respectively. Blood glucose level of exposed groups was insignificantly lower than that of unexposed groups.

In the present study, tannery workers showed lower blood glucose level as compared to unexposed population. Ahsan *et al.*, reported that there was no significant difference in blood glucose levels in tannery workers exposed to chromium as compared to unexposed population (271). Fujimoto *et al.*, reported that chromium containing glucose tolerance factor, showed lower blood glucose levels in patients with severe complications such as nephropathy and retinopathy (348). Therefore, it shows that chromium plays a key role in advancing diabetes mellitus. Vincent *et al.*, has documented that chromium binding oligopeptide chromodulin play an important role in the auto amplification of insulin signaling (349).

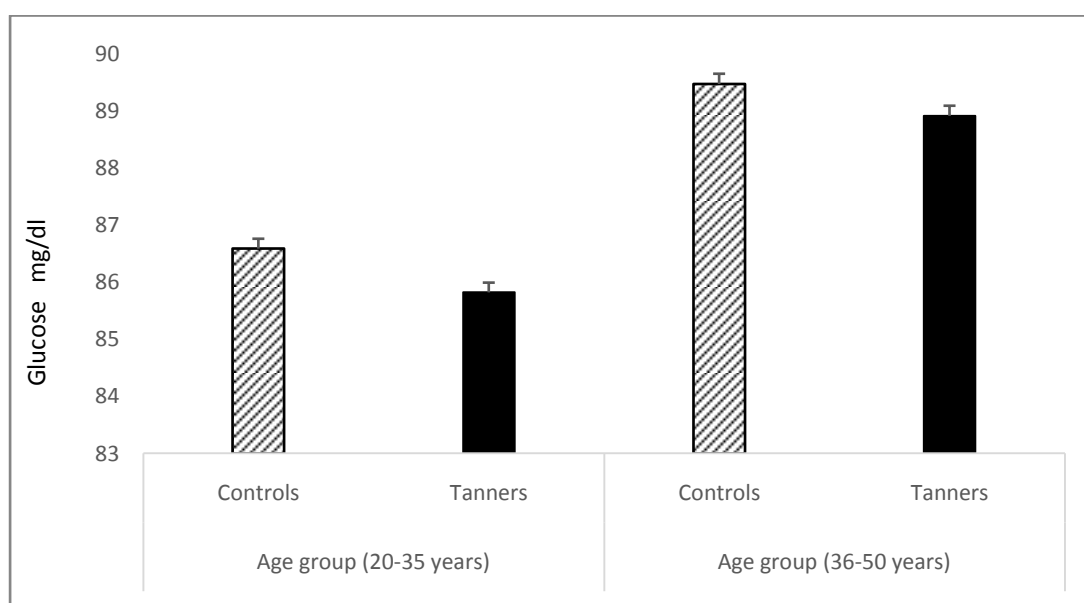


Figure 4.44 The glucose levels in blood of tanners and control groups in error bar graph

4.2.8.10 Pearson correlation coefficient between levels of blood chromium and biochemical parameters in tannery workers

In Pearson correlation Table 4.35, albumin level showed significant negative correlation with Cr concentration ($r = -0.728$, $p < 0.05$) ($r = -0.983$, $p < 0.001$) in exposed groups I and II respectively. There was no significant correlation ($r = -0.474$, $p > 0.05$) ($r = -0.052$, $p > 0.05$) observed between albumin and Cr concentration in control groups I and II respectively.

ALP concentration showed significant positive correlation with Cr concentration ($r = 0.725$, $p < 0.001$) ($r = 0.967$, $p < 0.05$) in exposed groups I and II respectively. While, no significant correlation ($r = -0.036$, $p > 0.05$) ($r = 0.228$, $p > 0.05$) was observed between ALP and Cr concentration in control groups I and II respectively.

ALT showed no significant correlation with Cr concentration ($r = 0.110$, $p > 0.05$) ($r = -0.016$, $p > 0.05$) in both exposed groups I and II respectively. AST concentration also showed no significant correlation with Cr concentration ($r = 0.201$, $p > 0.05$) ($r = 0.142$, $p > 0.05$) in exposed groups I and II respectively.

There was no significant correlation observed between D.Bilirubin and Cr concentration ($r = 0.233$, $p > 0.05$) ($r = 0.287$, $p > 0.05$) in exposed groups I and II respectively.

T.Bilirubin showed no significant correlation with Cr concentration ($r = 0.161$, $p > 0.05$) ($r = 0.200$, $p > 0.05$) in both exposed groups I and II respectively.

T. Protein concentration showed no significant correlation with Cr concentration ($r = 0.353$, $p > 0.05$) ($r = 0.386$, $p > 0.05$) in exposed groups I and II respectively. There was also no significant correlation observed between the level of glucose and Cr concentration ($r = 0.343$, $p > 0.05$) ($r = 0.158$, $p > 0.05$) in exposed groups I and II respectively.

Table 4.35 Pearson correlation coefficient between the levels of blood chromium and biochemical parameters in tannery workers and controls

Biochemical Parameter	20-35 years age group		36-50 years age group	
	Controls	Tanners (exposed group I)	Controls	Tanners (exposed group II)
Alb	-0.474	-0.728*	-0.052	-0.983**
Alp	0.036	0.725**	0.228	0.967**
ALT	0.404	0.110	0.012	-0.016
AST	0.416	0.201	-0.067	0.142
D.Bilirubin	0.456	0.233	0.215	0.287
T.Bilirubin	0.392	0.161	0.199	0.200
T. Protein	0.298	0.353	0.471	0.386
Glucose	0.284	0.343	0.129	0.158

* p<0.05 ** p< 0.001

4.3 Health problems in tannery workers occupationally exposed to chromium

In this study of 332 tannery workers from Sheikhpura and Peshawar, both male and female with age ranging from 20 to 50 were selected for the assessment of health risks. Tannery workers were interviewed and underwent clinical examination. A control group of 112 individuals from the same age without chromium exposure was selected. Table 4.35 shows the reproductive abnormalities, i.e. abortion, sterility, still birth and survival rate of children. A total of 164 tannery workers with age ranging 20-35, were included. The sterility problem was found to be 22% in male and 25 % in female in contrast to the 3% in the male controls and 1% in female controls. While the survival rate of children was observed to be 69% in male and 67% in female in contrast to that observed in controls, which was 94% in male and 92% in female. In addition, 22% females had an abortion problem, while 2% were found following birth control procedures. Still birth problems were found to be 23% in female in contrast to that 3% were found in controls.

While in the age group 36-50, a total of 168 tannery workers were served the questionnaire, out of which 32% male and 34% female had sterility problems. While

the survival rate of children was found to be 67% in male and 65% in female. Moreover, 31% female had abortion problem and 27% female had still birth problems.

In health questionnaire Table 4.37 cough related problems like upper respiratory tract infection and productive cough were reported by 15.3% in tannery workers. In addition, 31% tannery workers had phlegm problems while 32% had a stuffy nose problem. Red eyes problems were found to be 49% in tannery workers. Skin problems like dermatitis and eczema was observed to be 55% in tannery workers, while stomach problems like diarrhea and irregular bowl habits were found to be 29% in tannery workers. Similar results were also reported by Issever *et al.*, (350). 40% asthma problems were found in tannery workers. Shahzad *et al.*, also documented 10% asthma in tannery workers (351).

In this cross sectional study, reproductive abnormalities, respiratory and stomach problems in tannery workers have been examined. However, skin problems took first place as compared to other health problems, which is due to direct skin contact with chemicals used in tanning processes. Tannery workers were questioned regarding reproductive abnormalities, skin problems and asthma. In addition to that, there were other signs and symptoms of obstruction including wheezing. During the tanning process, occupational exposure to toxic chemicals and microbial agents: mycotoxins and endotoxins are one of the major reasons for these health hazards. These findings suggest that occupational exposure to chromium can cause reproductive abnormalities, skin dermatitis and asthma related problems in tannery workers.

Table 4.36 The demographic information of tannery workers and controls

No of Subjects	Age Groups	Gender	Reproductive Abnormality	Abortions	Sterility	Still Birth	Survival rate of children
112	Exposed group I 20-35 years	Male	yes	-	22	-	69
52		Female	yes	22	25	23	67
122	Exposed group II 36-50 years	Male	yes	-	32	-	67
46		female	yes	31	34	27	65
62	Controls 20-35 years	Male	yes	-	3	-	94
43		Female	yes	2	1	3	92
61	Controls 36-50 years	Male	yes	-	3	-	93
46		female	yes	3	2	4	90

Table 4.37 The health questionnaire from tannery workers at their work place

Questions asked from the tannery workers	Answers	Frequency	Percent
Do you usually cough on getting up in the morning in winter?	Yes	51	15.3
	No	281	84.6
Do you usually have phlegm on getting up in the morning in winter?	Yes	106	31.9
	No	226	68.9
Do you usually have a stuffy nose or drainage at the back of your nose?	Yes	107	32.3
	No	225	67.7
During the past 12 months, have your eyes been red, itchy or watery more than twice?	Yes	164	49.3
	No	168	50.9
During the last 12 months, have you had a skin rash, dermatitis, hives or eczema?	Yes	183	55.1
	No	149	43.8
During the last 12 months, have you had stomach problem, diarrhea, and irregular bowl habits?	Yes	98	29.5
	No	234	70.4
During the last 12 months, have you had asthma problem?	Yes	135	40.6
	No	197	59.3

n = 240

Final multivariable logistic model included age, educational status, ethnicity, smoking status, glove use and an interaction between perceived allergy and duration of the work is shown in Table 4.38. The effect of allergy was evaluated at a mean duration of work (10 years); also the effect of allergy with 10 additional years of work duration (20 years of work duration) was also evaluated. Illiterate were more likely to have asthma compared to those who were literate (adjusted OR = 2.12; 95% CI: 1.17-3.88). Pathan workers were more likely to have asthma compared to workers of Punjabi ethnicity (adjusted OR = 2.32; 95% CI: 1.32-5.26). The workers who did not report the use of gloves were more likely to have asthma compared to those who at least rarely used them during different tanning tasks (adjusted OR = 3.29; 95% CI: 1.72-6.26). The smokers were more likely to have asthma compared to those who had never smoked (adjusted OR = 2.50; 95% CI: 1.25-4.22). As noted earlier, final model included a significant interaction ($p = 0.02$) between perceived allergy and duration of work in the tannery. The workers who perceived to have allergy, were more likely to have asthma if their duration of work was 10 years (adjusted OR = 2.21; 95% CI: 0.40 - 3.12) and this relationship was even stronger if duration was 20 years (adjusted OR = 3.75; 95% CI: 1.99 - 6.80). However, there was non-significant relationship between duration of work and asthma for those who did not have perceived allergy.

Table 4.38 Multiple logistic regression analysis of factors associated with asthma health disorders among leather tannery workers, in Sheikhpura and Peshawar tanneries

Variables	Adjusted R	95% CI	p alue	Standard error
Educational status				
Literate	1.30	0.45, 3.23	< 0.001	0.026
Illiterate	2.12	1.17, 3.88	< 0.001	0.177
Ethnicity				
Punjabi	1.20	0.39, 3.12	< 0.001	0.032
Pathan	2.32	1.32, 5.26	< 0.001	0.129
Others	1.67	0.62, 4.56	< 0.001	0.058
Smoking status				
Smokers	2.50	1.25, 4.22	< 0.001	0.122
Non-Smokers	1.01	0.02, 2.98	< 0.001	0.001
Glove use				
User****	1.12	0.29, 2.22	< 0.001	0.023
Non-User††††	3.29	1.72, 6.26	< 0.001	0.169
Allergy†††† and duration of work§§§§				
<i>Non-allergic</i>				
10 years duration of work	1.21	0.40, 3.12	< 0.001	0.021
20 years duration of work	0.99	0.73, 1.33	< 0.001	0.065
<i>Allergic</i>				
10 years duration of work	2.34	1.21, 4.29	< 0.001	0.112
20 years duration of work	3.75	1.99, 6.80	< 0.001	0.189

(n = 120^{†††})

^{†††} The final model included 120 subjects

^{††††} All variables are mutually adjusted for each other

^{§§§} Other ethnicity includes Urdu, Sindhi, Balochi and Brahvi

^{****} Worker who has at least rarely used gloves during different tanning tasks

^{††††} Worker who has never used gloves

^{††††} Perceived allergy to at least one of the following substances: food, metals, chemicals, medicine, dusts or animals

^{§§§§} The interaction was analyzed by calculating odds ratio relative to a common reference i.e. the duration of work at mean duration of 10 years and non-allergic status

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Phase I

From the findings of this research, it is evident that both soil and groundwater of the study area were adversely affected by tannery effluents. The levels of chromium in effluents from Sheikhpura and Peshawar tanneries were found to be 350.15 mg/l and 228.50 mg/l respectively. While in target area ground water from Sheikhpura and Peshawar, concentration of chromium was found to be 0.06 mg/l and 0.056 mg/l respectively and in target area soil from Sheikhpura and Peshawar, it was found to be 31.13mg/l and 17.99mg/l respectively. The values of chromium, cobalt, cadmium, lead and iron in the effluents from Sheikhpura and Peshawar tanneries were above the permissible limits set by WHO/FAO (World health organization)/(Food and Agriculture organization). The concentration of chromium, cadmium, nickel and lead in ground water from Sheikhpura and Peshawar was above the NSDWQ/Pak (National standard for drinking water quality). In addition to that, the concentration of trace metals in tannery effluents, ground water and soil of Sheikhpura were higher than that of Peshawar. The tannery effluents were contaminated with higher concentration of trace metals. These effluents had a clear deleterious effect on the water quality. The higher concentration of trace metals reported in tannery effluents, study area water and soil in Sheikhpura city could be due to that of the non-point sources, surface run-off, agricultural and the discharges from the town and various tanneries.

Long term irrigation with tannery effluents has caused trace metal contamination in cultivated vegetation. Vegetables irrigated with tannery effluents showed elevated levels of Cr, Zn, Ni and Cd, which were above the permissible limits of WHO/FAO.

Phase II

The present results have demonstrated that total chromium and hexavalent chromium in the blood of tannery workers aged 20-35 and 36-50 were significantly higher than that of controls. Occupational exposure to hexavalent chromium has badly affected the endocrine hormones of tannery workers. From the findings it can be concluded that levels of testosterone and growth hormones have significantly decreased in both the age groups of male tannery workers exposed to chromium. Progesterone, estradiol, luteinizing and growth hormones of female tannery workers were significantly decreased while follicle stimulating hormone was significantly increased than that of unexposed population. TSH, Free T₄ and Free T₃ hormones were not affected in the tannery workers. This was further strengthened by the correlation and regression study between chromium in blood and endocrine hormones in tannery workers. Negative correlation between blood chromium level and testosterone and growth hormones was observed in male tannery workers and negative correlation was also observed between blood chromium concentration and progesterone, estradiol and luteinizing hormone in female tannery workers. Tannery workers in group II aged (36-50) were more affected due to long-term chromium exposure as compared to group I.

The results showed that DNA damage in smoking and nonsmoking tannery workers was significantly higher than that of unexposed groups. Smoking tannery workers showed higher DNA damage than those of nonsmoking tannery workers. Tannery

workers in group II with long-term Cr exposure showed larger comet tail length than that of group 1 and controls.

Malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations in smoking and nonsmoking exposed groups were significantly higher than those of unexposed groups. While glutathione (GSH) level in smoking and nonsmoking exposed groups were significantly lower than that of unexposed groups. Oxidative stress parameters in tannery workers in group II aged (36-50) with longer duration of Cr exposure were more affected.

These findings demonstrated the fact that trivalent chromium causes oxidative stress and DNA damage in exposed tannery workers. This was further strengthened by correlation and regression study between blood chromium level and DNA damage and oxidative stress parameters in tannery workers. Positive correlation was observed between level of chromium and DNA damage, MDA and SOD levels, while negative correlation was observed between concentration of chromium and GSH levels in tannery workers.

From the results it is clear that no definite pattern has been observed in different haematological and biochemical parameters. The difference observed in various parameters is mostly insignificant. White blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and packed cell volume (PCV) are lower in exposed groups than that of controls.

The findings showed that alkaline phosphatase (ALP) activity was significantly increased in exposed groups than that of unexposed population. Albumin (Alb) was significantly decreased in exposed groups than that of controls.

The present study found that occupational exposure to chromium can lead to health hazards including hormonal abnormalities, oxidative stress, DNA damage and hematological and biochemical defects in tannery workers.

5.2 RECOMMENDATIONS

1. Cleaner leather processing and treatment plants for tanneries could be the most appropriate theme for sustainable plant growth in the leather industry and safe environment.
2. Tannery workers should be encouraged to follow the health protection procedures and strictly observe workplace safety rules. When leaving the workplace in tannery, they should follow decontamination procedures to avoid wearing contaminated clothes that could potentially expose other people.
3. Regular health checkup of tannery workers should be performed in tanning industries.
4. Workers education and awareness about the workplace health risks in tanneries should be a mandatory program in the leather industry throughout the country.

5.3 LIMITATIONS

While this study provided an opportunity to collect workers' health data, there was a problem to collect information from tannery workers.

1. The administration of tanning industries was not willing to cooperate.
2. The management and distribution of the large amount of data was a very intensive process in which a significant amount of effort and time were required.
3. Certain uncontrollable factors such as weather made the survey process problematic, although the accuracy may or may not have been affected.

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