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Histopathological and biochemical changes in rat thyroid following acute exposure to hexavalent chromium

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Summary. Chromium in hexavalent form is highly toxic and a known carcinogen, although its effects on thyroid structure and function are relatively unexplored. Workers in an industrial environment can be, at times, exposed to this form of chromium. The present study was, therefore, designed using laboratory rats as a model system to investigate the effect on thyroid structure and function following two acute intraperitoneal doses of 30 mg/kg b.w. potassium dichromate administered within 48 hours. The results showed that hypothalamic chromium concentration increased ($p<0.05$) while thyroid chromium concentration decreased ($p<0.01$).The excretion of chromium in urine increased ($p<0.05$). The treated thyroid sections revealed hyperplasia. Follicles were disorganized, clustered and collapsed, while some of them were fused. Interfollicular spaces widened. Morphometrical analysis showed significantly ($p<0.001$) increased number of follicles whereas the follicular size significantly decreased ($p<0.001$). Nuclei were regressed ($p<0.001$); nuclear shapes were irregular; round, oval and shrunken. The membrane on the apical as well as the basal lamina side showed disruption. Colloid retraction within the follicles was noticeable in some sections stained with Periodic acid Schiff (PAS). Serum free tetra-iodothyronine (FT_4) and free tri-iodothyronine (FT_3) levels decreased ($p<0.01$ and $p<0.001$, respectively), while serum thyroid stimulating hormone (TSH) concentration increased ($p<0.01$). Ultrastructural analysis showed disrupted basal laminae of the follicles, regressed nuclei and disrupted cell organelles. Acridine orange stained thyroid cells demonstrated excessive dead cells, whereas DNA fragmentation assay demonstrated percent decrease of hypothalamic, pituitary and thyroidal total DNA.

Key words: Hexavalent chromium, Rat, Hypothyroidism, Hyperplasia, Histological changes

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Introduction

Toxicity due to heavy metals is of major concern in Pakistan. The concentrations of metals like mercury, cadmium, chromium, arsenic, lead, zinc and copper have increased to alarming levels in the water, soil and air, which have become large dumps of industrial effluents. Physiologically, heavy metals can profoundly affect the functioning of the endocrine system. In Pakistan, endocrine disorders, especially thyroid related diseases are very common, and acute exposure to a heavy metal such as chromium (VI) may occur in the occupational environment.

Chromium, a transition metal, exists in various oxidation sates but trivalent [Cr (III) or Cr^{3+}] and hexavalent [Cr (VI) or Cr^{6+}] forms are predominant. Cr (III) is an essential trace element for animal bodies, required for carbohydrate, protein and fat metabolisms (Cefalu and Hu, 2004). In contrast, Cr (VI) has widespread industrial applications. It is used for chrome plating, steel alloys, cast iron, metal finishes and glassware cleaning solutions, leather tanning, wood treatment and production of pigments (Stohs et al., 2001). Accordingly, industrial refuse contains Cr (VI) which finds its way into biological life forms; plants, animals and humans through contaminated soils and

water. Hexavalent chromium compounds are approximately 1000-fold more cytotoxic and mutagenic than trivalent compounds (Biedermann and Landolph, 1990).

Many toxic effects of Cr (VI) are well known, including allergic dermatitis and carcinogenicity in humans and other animals (Bagchi et al., 2002; Kawanishi et al., 2002). Similarly, acute and chronic neurotoxicity, dermatotoxicity, genotoxicity, immunotoxicity and general environmental toxicity due to soluble and insoluble hexavalent chromium salts have been reviewed extensively (Von Burg and Liu, 1993; Barceloux, 1999; Paine, 2001). Cr (VI) inhalation also causes DNA damage which is induced by the generation of reactive oxygen species (ROS) and by inhibition of base excision repair activity during the earlier phase of exposure (Maeng et al., 2003). It has also been demonstrated that exposure to chromium compounds at the workplace can result in nephrotoxicity. Similarly, dose-dependent ultrastructural damage to the rat kidneys on exposure to Cr (VI) is already known (Chmielnicka et al., 2002).

The authors could not find any specialized studies conducted on the toxic effects of hexavalent chromium on the endocrine glands, except for a few studies that addressed gonads and pancreas, whereby Cr (VI) was shown to cause reproductive toxicity of the testes in bonnet monkeys. It disrupts spermatogenesis, leading to accumulation of prematurely released spematocytes, spermatids and uni- and multinucleate giant cells in the lumen of seminiferous tubules (Aruldhas et al., 2005).

Existing information regarding the toxic effects of chromium on the endocrine glands, in general and thyroid in particular, is very limited. We managed to find only one study in the literature that directly focused on the effects of chromium on the thyroid gland (Goncharov and Ametov, 1977). The thyroid gland is one of the most important endocrine organs of animal bodies and is required for normal growth and maturation of the body, increases oxygen consumption and helps regulate lipid and carbohydrate metabolism. Therefore, pathological conditions in relation to over and under activity of this particular gland could be very dangerous (Besser and Thorner, 2002). The present study was undertaken basically to investigate the hexavaent chromium induced histopathological changes in the thyroid gland that might occur as a result of an accidental exposure in occupational environment. The cellular architecture of the thyroid gland at light and ultrastructural levels, serum FT_4, FT_3 and TSH concentrations, as well as DNA damage to the thyroid cells were investigated after exposure to an acute intraperitoneal dose of Cr (VI).

Materials and methods

Animals and housing

Adult male Sprague Dawley rats (20-24 weeks old) with an average body weight of 225±1.35 g were obtained from the National Institute of Health (NIH) Islamabad and housed in the Department of Animal sciences, Quaid-i-Azam University Islamabad, Pakistan. The rats were maintained on a semi-synthetic rat chow and water *ad libitum* for 15 days prior to experimentation. Photoperiods were maintained at 12L:12D. The ambient temperature was kept at 27±2.00°C through the air-conditioning system that conforms to the average day time temperature. Moreover, the rats were monitored for temperature related stress or discomfort before the start of the experiment. All animal handling and experimental procedures followed were strictly according to the guidelines given by the local ethics committee of the Department of Animal Sciences.

Experimental design

The rats were divided into control and treatment groups. Since it was not humanly possible to test and analyze each parameter simultaneously, experiments were conducted in different sets to test more closely related parameters at one time. The estimation of hormones and determination of chromium concentration in the glands were conducted at one time while experiments for evaluating tissue damage at light and ultrastructural levels were carried out in the second set of experiments. Investigation of percent DNA damage in the hypothalamus, pituitary and thyroid glands was done in the third set of experiments. All experiments were run either in duplicate or, where possible, in triplicate.

The control and treatment groups of rats used in each set of experiments were of the same age group with similar body weights. Similar ambient temperature and photoperiods were maintained for each set of experiments. Throughout, standard diet was provided to the rats twice a day, except when the treatment had to be made, when food was withheld 4 hours (hrs) prior to administering the toxicant (chromium) in order to make sure that there was no dietary chromium in the animal's body that could bias the results. The rats had full access to water *ad libitum* during all the experiments. To eliminate the confounding factor of adding any extra quantity of chromium, the animal feed and water samples were analyzed for chromium content for each batch of animals. The concentrations were found to be in agreement with the Environment Protection Agency (EPA) of Pakistan (1-10 μg^{-L} or μg^{-kg}).

Because of the environmental and strain differences of the experimental animals, trial experiments were done to determine the lethal dose (LD_{50}). For this purpose, ten rats were injected with a single dose of 60 mg/kg b.w potassium dichromate ($K_2Cr_2O_7$) intraperitoneally (i.p.) and it caused 100 % mortality after 12 hrs. To achieve the sublethal concentration, the original dose of 60 mg/kg b.w. was then split into two doses of 30 mg/kg b.w. i.p. given within 48 hrs. In this case, fifty percent rats (n=5 out of n=10) survived for more than 48 hours

(hrs) following the split dose, thereby reducing the mortality to 50%. For all subsequent experimentation, therefore, a split dose of 30 mg/kg b.w. was selected. The rats were injected the first dose of 30 mg/kg b.w. at 09:00 hr in the morning and this was considered the first day. After an interval of 24 hrs (on the 2nd day), they were injected with the second dose, again at 09:00 hr. The control groups of rats maintained in parallel received 0.9% w/v physiological saline (Geofman Pharmaceuticals, Pakistan) at the same quantity and at the same time.

Collection of blood and tissue samples

The rats were sacrificed 48 hrs post administration of the toxicant after injecting sodium pentobarbital. Blood was drawn from the left ventricle of the heart, allowed to stand for 1 hr at room temperature and later centrifuged at 1258 xg (Eppendorf centrifuge, 5810 R, Germany) for 15 min to obtain serum. Serum samples of control (n=10) and treatment rats (n=10) were obtained and aliquoted for the estimation of hormones. Urine was first collected in steel trays kept beneath the cage of each individual rat for this purpose and was then transferred to sterilized plastic vials. Where urine was produced in less than required amounts, urinary bladders were gently pressed with the help of thumb to collect the urine. Whole blood, serum, urine and tissues including the hypothalamus, pituitary and thyroid were collected for the estimation of chromium. The standard method for atomic absorption requires at least 0.5 g of tissue for estimation of any metal concentration. Since rat hypothalamus, pituitary and thyroid tissues are too small in size, five tissues each of control and treatment rats were pooled to make one sample. Five such samples each for the hypothalamus, pituitary and thyroid glands, were obtained and processed for atomic absorption spectrophotometry. For light microscopy, the thyroid glands of control (n=10) and treatment rats (n=10) were fixed in 4% paraformaldehyde (PFA) solution prepared in phosphate buffered saline (PBS) for further processing. While for electron microscopy, thyroid tissues were fixed in 5% gluteraldehyde solution prepared in pipes buffer. Hypothalamus, pituitary and thyroid tissues of control (n=10) and treated rats (n=10) were also collected and processed for the investigation of DNA damage. In all experimental groups, the mortality rate of the treated rats remained between 4-10% while no mortality was recorded in all the control rats.

Estimation of metal concentration

Digests of the tissues, whole blood, serum and urine samples were prepared according to Mascia et al. (1990) with some modifications. The samples (0.5 g each) were digested with 5 ml HNO_3 (69% pure, Merck, Germany) on Microwave Accelerated Reaction System (MARS 1200 W Power CEM Matthews, USA). The maximum temperature set was 200°C in ramping mode and the power was 1200 Watt. The samples were run for 5 min and then filtered with 0.45 μm filter paper. Then, the digests were diluted to 8 ml with dH_2O. The processed samples were subjected to air-acetylene flame in an atomic absorption spectrophotometer (Varian, AA240 FS, USA) for the estimation of chromium.

$$\text{Metal concentration (g/g)} = \frac{\text{Absorbance of the sample (ppm)}}{\text{Weight of the tissue (g)}} \times 8.0 \text{ ml}$$

Radioimmunoassay (RIA)

Serum TSH (Thyroid stimulating hormone), FT_4 (Free thyroxine) and FT_3 (Free triiodothyronine) concentrations were determined using commercially available hormone kits (Biocode- Hycel, rue E, Solvay, Liege Belgium and Immunotech a.s.-Radiova 1-102 27 Prague 10- Czech Republic, respectively for TSH, FT_4 and FT_3). Serum TSH, FT_4 and FT_3 were assayed using standard competitive radioimmunoassay (RIA). Bound radioactivity was measured as counts per minute (cpm) in a gamma counter (Oakfield Sourcerer RIA Counter, No.238, Type SD 16, UK). Mean sensitivities of the assays were 1.03ng/ml, 0.4 pmol/l and 0.5 pmol/l for serum TSH, FT_4 and FT_3 respectively. Mean intra-assay coefficients of variation were 3.7%, 6.7% and 6.4% for serum TSH, FT_4 and FT_3 respectively.

Iodine concentrations

For the determination of iodine concentration, free iodine was estimated in the urine while protein-bound iodine (PBI) was measured in the serum.

Free iodine in the urine

Free iodine excreted in the urine was estimated according to the method of Zak et al. (1952) with slight modifications. Chloric acid (3 ml) was added to each tube containing 0.5 ml of urine sample. The tubes were placed in an electrically heated sand bath set to a temperature of 105-110°C for 1 hr to prepare digests. The tubes were kept under observation for color changes such as orange-yellow to light green until changed to colorless. Chloric acid was added drop-wise wherever necessary to prevent the color changes and to avoid the loss of iodine. The tubes were allowed to cool until red crystals of chromium trioxide appeared. In tubes in which red crystals did not appear, the digestion was allowed to continue until the red crystals appeared on cooling. The samples were analyzed at 420 nm in a UV visible spectrophotometer (Schimadzu, UV-120-01, Japan).

Calculations: μg iodine found in the sample x 100 = μg iodine / 100 ml

Serum Protein- bound Iodine (PBI)

The protein-bound iodine in the serum samples of control and treated rats was estimated according to the method of Bird and Jackson (1962). For all experiments,

chemicals were purchased from Sigma Chemical Company, St. Louis, Missouri, USA and Merck and BDH, Germany.

For the estimation of protein-bound iodine (PBI), 5 ml of 5% TCA was added to 0.2 ml of each serum sample, the contents were mixed, allowed to stand for 30 min and then centrifuged at 1510xg (3000 rpm) for 5 min. The supernatant was poured off and the tubes were decanted for 5 min on a filter paper (Whattman International Ltd. England). The precipitated protein was washed with 5 ml of 5% TCA, centrifuged again and supernatant was decanted as above. 0.2 ml each of blank, standards (I, II and III) and samples were treated identically. 2 ml of chloric acid and 0.1 ml of sodium chromate solutions were added to samples which were then allowed to hold in a sand bath at 140 to 150°C for 1 hr. Two drops of chloric acid were added to each sample at 20, 30, 40, 50, and 55 min times during 1 hr duration, after which the tubes were removed. The reaction was complete in those tubes in which red crystals of dichromate appeared on cooling. In those tubes where no red crystals formed, two more drops of chloric acid were added and the tubes were returned to the sand bath for an additional 5 min.

After the digestion was completed, the tubes were cooled to room temperature and 10 ml of 1% NaCl was added to each tube, followed by 2 ml of 0.2 N arsenious acid. 0.5 ml of 2% ceric sulphate was then added to each sample at 1 min. intervals, starting with the 0.03 μg standard, followed by 0.02 μg, 0.01 μg, blank and serum tubes. The contents were mixed after adding 2% ceric sulphate. The time of addition of 2% ceric sulphate to the first tube was noted and reaction was allowed to proceed at ambient temperature for 1 hr. The absorbance of each sample was read in a spectrophotometer at 415 nm. The absorbance values of the standards and blank were plotted on semi-log paper, against the iodine concentration.

Calculation: μg PBI in 0.2 ml serum x 500 = μg PBI / 100 ml

Light microscopy and staining

Thyroid tissues were processed for light microscopic studies. Standard methods were followed for fixation and staining. Paraffin wax embedded tissues were cut with a rotary microtome (Shandon, Finesse 325, UK) at 5 μm thickness and stained with conventional Hematoxylin (for 2-3 minutes) and eosin (for 1 min) (Bancroft and Stevens, 1990; Riddel, 1996). Sections were then dehydrated and mounted in DPX mountant medium (BDH, Germany).

Periodic Acid and Schiff reagent were prepared to differentially stain the colloid inside the thyroid follicles. Sections were dewaxed, oxidized in 1% Periodic acid for 5 min and treated with Schiff reagent for 15 min. These were stained with Harris's hematoxylin for 1 min, and mounted with DPX mountant medium.

Morphometry

Thyroid follicles and follicular cells were counted according to the method of Abercrombie and Johnson (1946), following the formulae given therein. The cells were counted for their relative number and size by placing a graticule in the eyepiece of the microscope (Nikon, Optiphot BH-2, Japan). Follicle numbers were estimated by counting them in each box of the graticule. The size of the follicles and follicular cells were measured by using standard methods of microscopic measurements. Follicular length (FL) was taken at the two farthest points in the follicle while follicular width (FW) was then measured perpendicular to the FL measurement. Four measurements of epithelial cell height (ECH) per follicle were made at each of the four extremities.

The follicular size (FS) was calculated using the formula:

$$FS = \frac{FL + FW}{2}$$

Once FS was calculated, the epithelium-follicular index was determined using the formula:

$$EFI = \frac{ECH \times 100}{FS}$$

The sizes of nucleus and cytoplasm were measured in a similar way.

Transmission Electron Microscopy (TEM)

Small pieces (1mm^3) of thyroid glands were fixed in 5% glutaraldehyde. Post fixation was achieved with 1% osmium tetraoxide for 18 hours at room temperature. The tissues were stained with 5% uranyl acetate solution and then infiltrated with a mixture of Spur embedding medium. The ultra thin (120 nm) sections were doubly stained with 5% uranyl acetate and lead citrate. Tissue sections were examined and photographed on a transmission electron microscope (JEOL JEM1010, Japan) operating at 80 KV.

Nuclear abnormalities

Nuclear abnormalities of the thyroid glandular cells were studied using a slightly modified method of Singh et al. (2003). About 20 mg each of thyroid, pituitary and hypothalamic tissues were homogenized in 1 ml PBS to make the cell suspension. After centrifugation, the supernatant was discarded, and then 140 μl of low melting point (LMP) agarose was added in the 20 μl cell suspension and mixed well. Two drops of 70 μl were put on to a precoated NMP agarose glass slide (0.5%), and cover slipped. The slides were placed in a refrigerator for 20 min. The cover slips were removed and slides were placed in lysing solution and again kept at 4°C overnight in the dark. Slides were electrophoresed for 30

min at 25 V and 300 mA in an electrophoresis chamber containing denaturation buffer. Slides were neutralized with a neutralizing buffer, left overnight to be air dried, then stained with acridine orange (2 μl per slide), and scored under fluorescent microscope (Nikon, Optiphot, AFX-II, Japan).

Quantification of DNA Fragmentation

DNA damage was estimated according to Wu et al. (2005); 30 mg each of hypothalamus, pituitary and thyroid tissues were ground and left in TTE solution overnight and then centrifuged at 12000 x g for 5 min. The supernatant was separated and named as S. To the remaining pellet, 1 ml TTE solution was added and centrifuged at 12000xg for 5 min. The supernatant was again separated and called T. To the remaining pellet, 1 ml TTE was added and named B. Then 600 μl of 5% Trichloroacetic acid was added to S, B and T supernatants and vortexed vigorously. After overnight precipitation at 4°C, DNA was recovered by pelleting for 10 min at 18,000xg at 4°C. Supernatants were discarded by aspiration. DNA was hydrolyzed adding 160 μl of 5% TCA to each pellet and heating for 15 min at 90°C in a heating block. To each tube 320 μl of freshly prepared diphenylamine solution was added, then vortexed and allowed to develop color for about 4 hrs at 37°C. Absorbance was read at 620 nm in a spectrophotometer (SmartSpec™ plus Spectrophotometer) and finally the amount of % fragmented DNA was calculated using the formula:

$$\%\text{Fragmented DNA} = \frac{T \times 100}{T + B}$$

Statistical analysis

The results obtained were analysed and compared through Microsoft Excel 2007 software for Microsoft Windows (Version XP 2008, NY, USA) and also by using the software "Statistica" (Version, Inc. NY, USA). Comparisons between control and treated samples were made using student's unpaired t-test. Correlations among different variables were determined by Pearson's Correlation analysis and values for coefficient of correlation (r) were determined. P<0.05 was considered a significant difference.

Results

Chromium concentration

Hypothalamic chromium concentration increased (p<0.05), while pituitary chromium concentration showed a non-significant increase (p=n.s) as compared to the control rats. On the contrary, thyroid chromium concentration decreased significantly (p<0.01). The whole blood showed significantly increased chromium concentration (p<0.001), while serum Cr increased non-significantly (p=n.s) in treatment groups. In the urine samples, chromium concentration showed a significant increase (p<0.001) (Table 1).

Hormone concentrations

Serum FT_4 and FT_3 concentrations significantly decreased (p<0.01 and p<0.001, respectively); while serum TSH concentration increased (p<0.01) in chromium treated rats as compared to the non-treated control animals. The ratio of FT_4 to FT_3 showed an increase from 4.865 to 5.142 (Table 2).

Iodine concentrations

Urine analysis in pre- and post-treatment rats showed that the free iodine removal in the urine samples decreased as compared to the control rats (p<0.001). It was also noticeable that the volume of the urine excreted also decreased with the passage of time post treatment of the toxicant. The concentration of protein-bound iodine in the serum also decreased when compared to non-treated control rats (p<0.05) (Table 2).

Table 1. Chromium concentrations (μg/g wet weight of tissue) in the hypothalamus, pituitary and thyroid glands, whole blood, Serum and urine (μg/ml) of control and treated rats.

Tissues	Control	Treated	t-Value
Hypothalamus	4.28±0.24	8.59±1.33*	3.174
Pituitary	10.31±1.59	12.14±1.15	0.933
Thyroid	9.87±1.59	3.28±0.24**	4.080
Whole Blood	5.98±0.40	161.24±4.16***	6.032
Serum	4.34±0.16	4.68±0.27	1.073
Urine	53.27±7.78	175.18±2.38***	14.97

Values are expressed as mean ± SEM. (n=25 rats in each case). *: p<0.05; **: p<0.01; ***: p<0.001

Table 2. Shows FT_4, FT_3 and TSH levels and iodine concentrations, in control and treated rats.

	Control	Treated	t-Value
Thyroid hormones (pmol/l)			
SerumFT_4	19.46±0.57	9.72±2.16*	4.33
SerumFT_3	4.00±0.18	1.89±0.29**	6.09
Pituitary-derived Thyrotropin (ng/ml)			
Serum TSH	4.52±0.23	15.80±2.28*	4.91
Iodine Concentrations (μg/dl)			
Free Iodine in urine	16.50±1.81	2.36±0.58*	7.41
Serum PBI	18.50±0.91	15.08±0.79***	2.82

Values are presented as mean ± SEM. (n=10 rats in each case). *: p<0.01; **: p<0.001; ***: p<0.05

Light microscopy and morphometry

Control non-treated thyroid gland

Control thyroid tissue consisted of small and large variable size follicles with intact basal laminae. Normal secreted and light stained colloid was found near the apical border of the cells. Interfollicular spaces were normally formed. The cells were desquamated with normal cell height; follicular cell nuclei were spherical (Fig. 1A).

Treated thyroid gland

Treated thyroid gland showed follicular hyperplasia with darkly stained colloid (Figs. 1B, 2A,B). The sections overall demonstrated hemorrhagic picture and many red blood cells were frequently observed. Follicular density increased significantly ($p<0.001$) as compared to control thyroid, while the follicular size showed a significant decrease ($p<0.001$) (Table 3, Fig. 1B-D). Average sizes of the largest as well as smallest follicles were significantly decreased as compared to

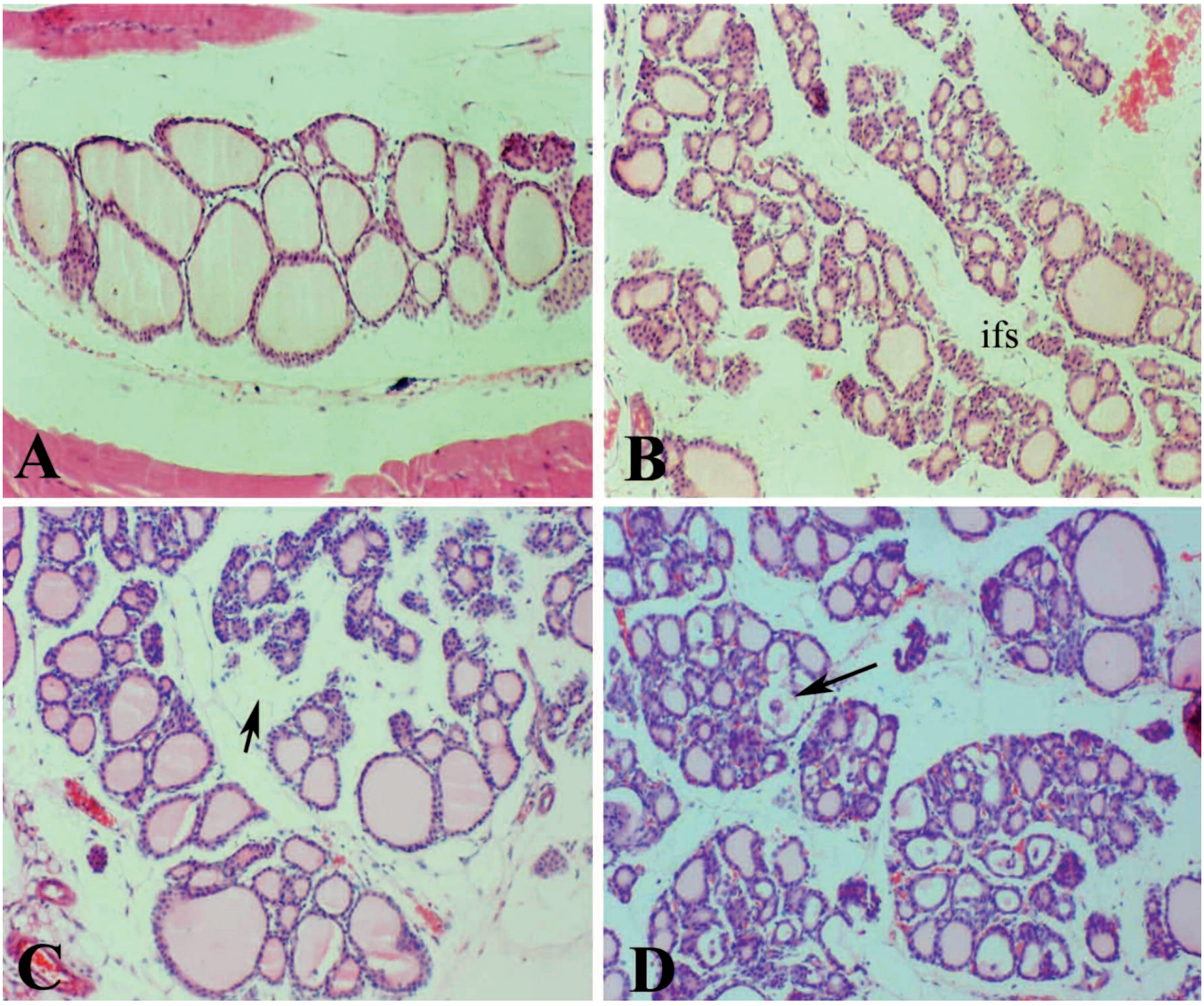

Fig. 1. Photomicrographs of control and chromium treated rat thyroid sections stained with conventional haematoxylin and eosin. **A.** Normal follicles with abundant colloid and peripherally positioned normal epithelial cells. **B.** Treated thyroid showing follicular hyperplasia, abnormal, and disorganized follicular architecture with large interfollicular spaces (ifs). **C.** Treated rat thyroid having dissolute connective tissue (arrow) and abnormal and scattered follicles. **D.** Treated thyroid showing abnormal aggregations of follicles in the form of various groups, arrow indicating the dissolution of follicular walls as a result of fusion. Dead tissue is also evident in the interfollicular space behind the arrow tail. x 32

control rats (Table 3).

The follicles were disorganized, collapsed and irregularly shaped (Fig. 1B,C). Invagination of epithelial cells into the colloid was also noticeable (Fig. 1D). The connective tissue was also disrupted demonstrating collapsed follicles (Fig. 1C). Interfollicular spaces enlarged (Fig. 1B-D). Colloidal space was reduced in most follicles (Fig. 1B,D). Abnormal nuclear aggregations due to follicular disruption were readily noticeable (Fig. 1C, D). In the central portion of one thyroid section, dead tissue was also evident (Fig. 1D). The apical membranes as well as basal laminae were disrupted (Fig. 2A,B).

The epithelial cell height (ECH) was increased as compared to the control ($p<0.001$) (Table 4, Fig. 2). There was a significant reduction in the size of the nucleus of the epithelial cells ($p<0.001$) (Table 4). The nuclear shapes were irregular; some nuclei were round and shrunken while others were oval, elongated and pyknotic (Fig. 2). On the other hand, the size of the cytoplasm showed a significant increase ($p<0.01$) (Table 4). In some sections of the treated rat thyroid, which were stained with periodic acid Schiff (PAS), colloid retraction was noticeable (Fig. 3) whereas in few other sections, colloid showed a significant reduction /and resorption (Fig. 4). Among thyroid follicles of the treated rats, 10% showed colloid retraction whereas 7.6% follicles showed colloid resorption (Table 3).

The extent of damage to the thyroid gland as a result of exposure to hexavalent chromium was quantified morphometrically. The number of follicles with ruptured membranes, desquamated epithelial cells inside the follicles, follicles lined with high-toned epithelium, follicles showing colloid retraction and also follicles showing colloid resorption / reduction, were morphometrically analysed (Table 5).

Table 3. Average morphometric measurements (μm) of the control and chromium treated rat thyroid gland.

Groups	Follicular density	Follicular Size (μm)	Mean size of largest follicles (μm)	Mean size of smallest follicles (μm)	Follicles showing colloid retraction (%)	Follicles showing colloid resorption (%)
Control	64.78±1.64	125.8±3.51	125.8±3.00	69±1.81	0.00	0.00
Treated	156.51±4.06*	53.6±3.17* (t=15.25)	112.6±3.41*** (t=2.90)	29.8±1.93** (t=14.77)	10.19	7.64

Values expressed as mean ± SEM (n=30 sections). *: $p<0.001$; **: $p<0.01$; ***: $p<0.05$

Table 4. Morphometrical parameters (μm) of the control and treated rat thyroid follicular epithelial cells.

	ECH (μm)	EFI	Nuclear size (μm)	Cytoplasmic size (μm)	Nuclear / cytoplasm ratio
Control	4.6±0.14	51.01	5.46±0.16	8.2±0.26	4.52±0.23
Treated	8.5±0.24** (t=13.96)	42.65	4.52±0.08** (t=5.09)	9.74±0.37* (t=3.35)	15.80±4.91

Values expressed as mean ± SEM (n=30 sections) *: $p<0.01$; **: $p<0.001$; ECH: epithelial cell height; EFI: epithelium - follicular index

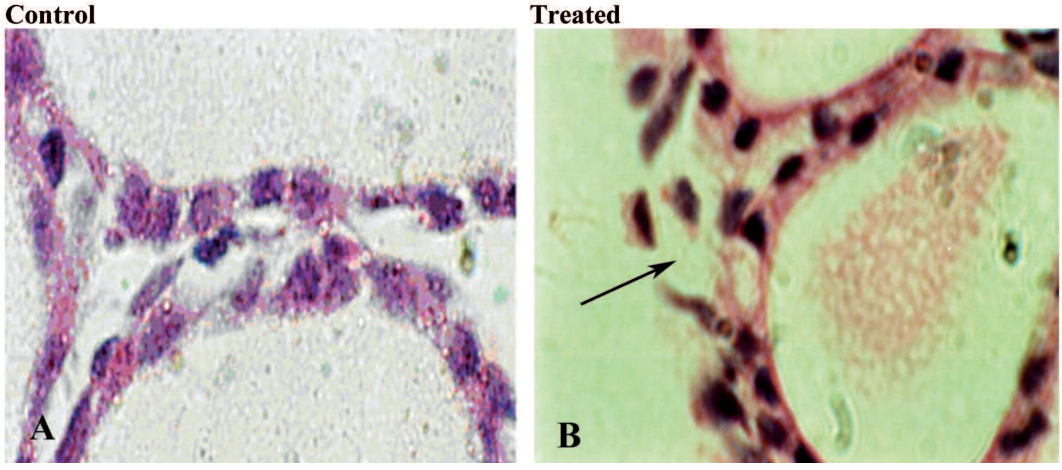

Fig. 2. High magnification photomicrographs of H&E stained control and chromium treated thyroid sections. **A.** Showing control thyroid with normal epithelial cells having intact follicular membranes. **B.** Treated thyroid having ruptured follicular epithelium (arrow) that resulted in the evasion of nuclei. Nuclei were reduced in size and elongated in shape. x 320

Electron microscopy

The control ultrathin sections (120 nm) showed normal follicles with abundant colloid. Basal laminae were intact. The epithelial cells were normally organized and contained round nuclei, endoplasmic reticulum, mitochondria, a large number of lysosomes, secretory granules and collagen fibres etc. (Fig. 5A,B).

Chromium treated sections (120 nm) of the thyroid glands showed significant changes when compared to the control;

Follicular organization

Follicular architecture was irregular and damaged. The follicles became more irregularly shaped and

Table 5. Morphometrical analysis of the extent of damage to the thyroid follicles of treated rats.

Follicle Parameters	+	++	+++
Follicles with ruptured epithelium	-	4.72±0.10	-
Desquamated epithelial cells inside the follicles	-	4.60±0.49	-
Follicles lined with high-toned epithelium	2.64±0.24	-	-
Follicles with colloid retraction	2.44±0.11	-	-
Follicles with colloid resorption	2.92±0.32	-	-

Values are expressed as mean ± SEM. (n=10 in all cases). +: Change observed in few follicles; ++: Change observed in many follicles; +++: Change observed in all the follicles.

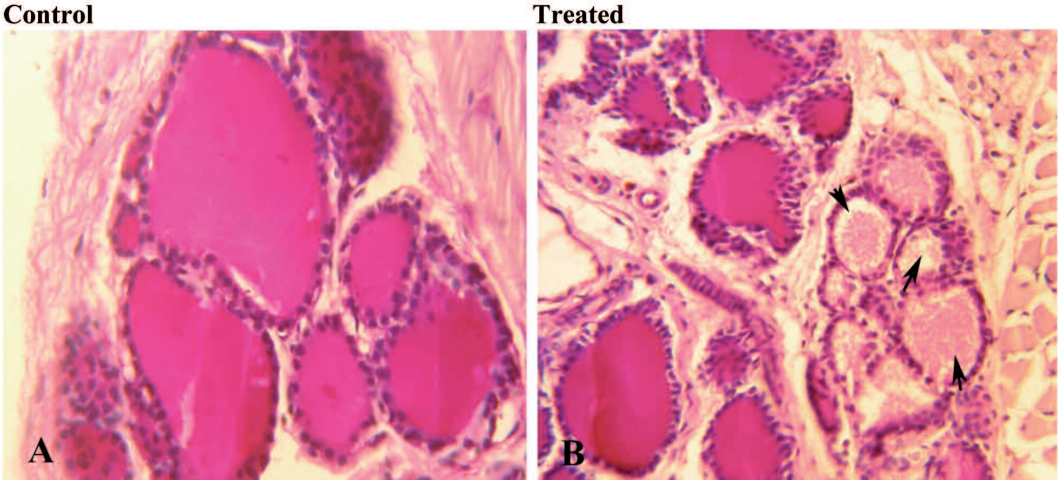

Fig. 3. Photomicrographs of rat thyroid sections stained with Periodic acid Schiff (PAS). **A.** Showing control thyroid having normal follicles and abundant colloid inside stained magenta in color. **B.** Treated thyroid section showing reduced sized follicles and colloid retraction (arrow heads), tracheal cartilage is also visible on the right hand corner. x 128

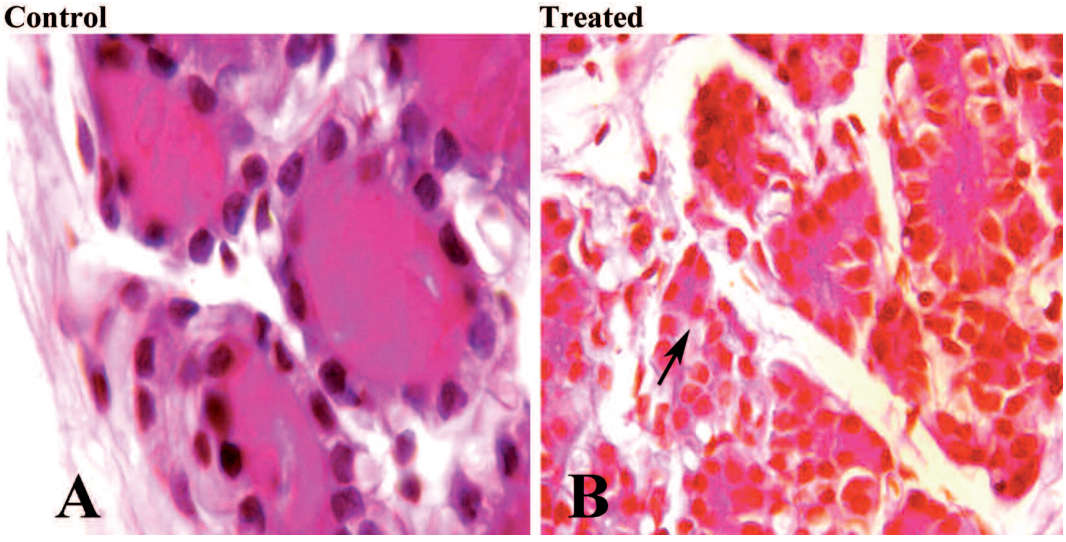

Fig. 4. High magnification photomicrographs of PAS stained control and chromium treated rat thyroid sections. **A.** Follicles having normal colloid content in a control thyroid. **B.** Shows reduced colloid (arrows) inside follicles with large interfollicular spaces. x 320

reduced in size (Fig. 5C).

Basal lamina

The basal laminae of the follicles were disrupted; follicles were collapsed and fused with one another (Fig. 5C).

Follicular epithelial cells and Nuclei

The epithelial cells showed noticeable shrinkage and appeared disrupted. Their nuclei appeared pyknotic as compared to those in control. Nuclear shapes were oval while nuclear membranes were irregular and deformed (Fig. 5D).

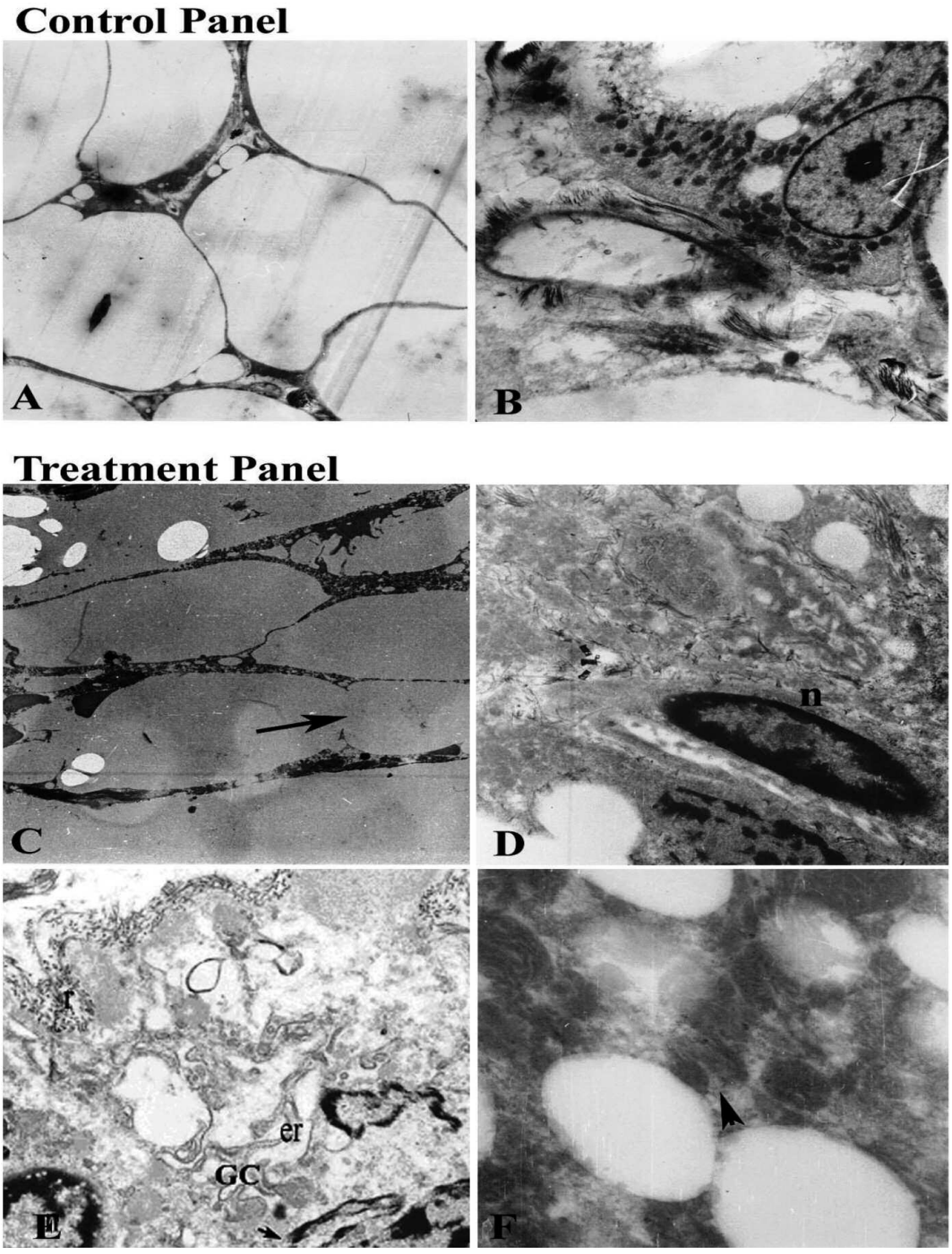

Fig. 5. Electron micrographs of control and chromium treated rat thyroid. **A.** Showing control thyroid having normal follicles with intact membranes. **B.** Showing normal sized and normal shaped nucleus and other cellular organelles in a control thyroid. **C.** Treated thyroid having elongated follicles with disrupted membranes (arrow). **D.** A treated thyroid epithelial cell showing regressing nucleus (n) and other cell organelles. **E.** Shows shrunken nucleus, many ribosomes (r) and disruption and depletion of Golgi complex (GC) and endoplasmic reticulum (er). **F.** Shows mitochondria in the thyroid epithelial cells visible at higher magnification having membrane notched from the middle (arrow). A, C, x 2,000; B, D, E, x 6,000; F, x 30,000

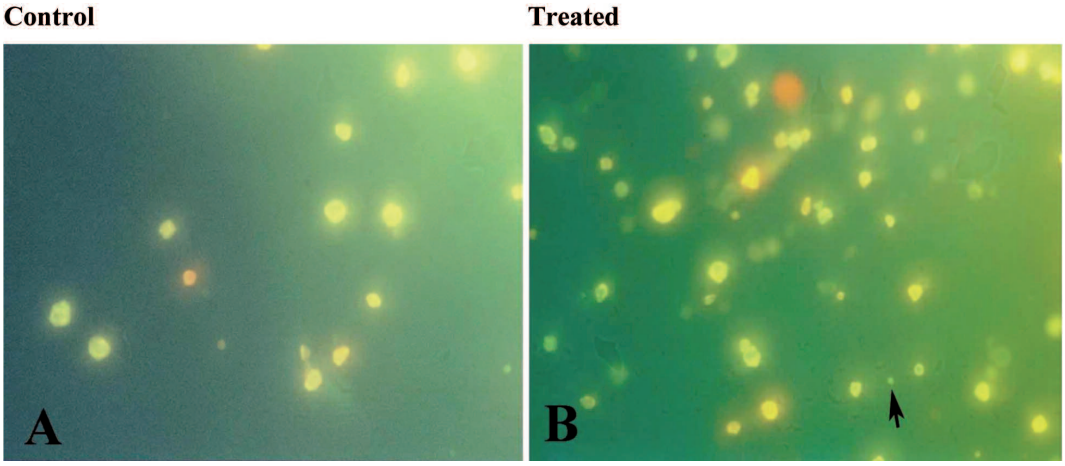

Fig. 6. Fluorescent Photomicrographs of rat thyroid gland sections stained with acridine orange. **A.** Control thyroid section with normal nuclei. **B.** Showing many dead nuclei (arrow) as a result of exposure to hexavalent chromium. x 128

Lysosomes and collagen fibers

Lysosomes appeared less abundant in treated sections than in control, while collagen fibers increased (Fig. 5D).

Endoplasmic reticulum and Golgi apparatus

The endoplasmic reticulum, as well as Golgi apparatus, appeared disrupted disorganized or depleted (Fig. 5E).

Mitochondria

Mitochondria appeared less abundant in treated sections than in the control. Moreover, their membranes appeared notched from the middle (Fig. 5F).

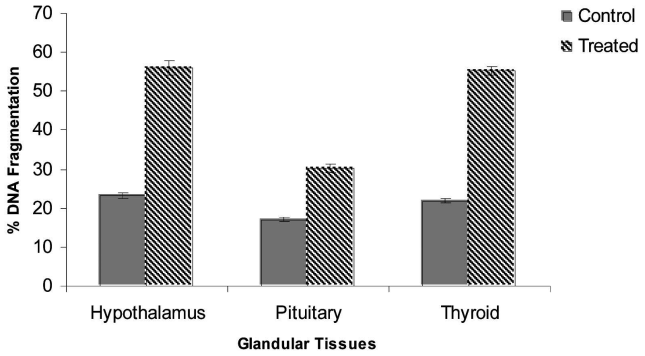

Fig. 7. Shows the quantitative analysis of DNA damage (fragmented DNA %) caused to hypothalamus, pituitary and thyroid tissues of rats as a result of acute exposure to hexavalent chromium. Sample size, n=10 in each group.

Nuclear abnormalities

Acridine orange stained fluorescent photomicrographs of the thyroid gland demonstrated several dead cells with brightly stained pyknotic nuclei in the treatment sections compared to those in the control group (Fig. 6).

Quantification of the DNA fragmentation

Damage that occurred to cell DNA as a result of exposure to hexavalent chromium was quantified by using diphenylamine and the results demonstrated 56.03±1.78% DNA fragmentations in the hypothalamus, 30.23±1.26% in the pituitary and 55.22±1.21% in the thyroid tissues of treatment groups compared to control (Fig. 7).

Correlation analysis

A positive correlation was found between thyroid Cr concentration and serum FT_4 and FT_3 concentrations (r=0.875 and r=0.925, respectively). Follicular density was negatively correlated with the thyroid chromium concentration (r=-0.523). Pituitary chromium concentration was positively correlated with the serum FT_3 (r=0.538), as was also the case with nuclear size of the thyroid follicular cells (r=0.731). On the other hand, there was negative correlation between pituitary chromium and the chromium in the whole blood, as well as the number of thyroid follicular (r=-0.809 and -0.937, respectively).

Serum FT_4 and FT_3 concentrations were negatively correlated with the chromium concentration in the hypothalamus (r=-0.765 and -0.575, respectively). Similarly, there was a negative correlation between hypothalamic chromium and the nuclear size of the thyroid cells (r=-0.572). Chromium concentration in the whole blood was also negatively correlated with nuclear size of the thyroid follicular cells as well as chromium

excreted through urine (r=-0.841 and 0.738, respectively). Also a negative correlation was found between urine chromium concentration and FT_4 and FT_3 (r=-0.628 and -0.575, respectively) and between urine chromium, whole blood chromium, as well as follicular size of the thyroid gland (r=-0.738 and -0.669, respectively). Serum chromium concentration was negatively correlated with the TSH concentration, as was thyroid follicular size with epithelial cell height (r= -0.668 and -0.907, respectively).

Discussion

Widespread effects of heavy metal pollution on all bodily organs and on a few endocrine glands have been well documented. Several adverse effects on endocrine systems due to occupational and environmental chemical agents, in particular heavy metals on human health have also been described (Baccarelli et al., 2000). The thyroid gland is one of the most important endocrine glands of animal bodies and toxic effects of aluminium (Galle, 1987; Zaidi et al., 2001; Aktaç and Bakar, 2002) and cadmium on this gland have been investigated (Pilat-Marcinkiewicz et al., 2003). The current study was concerned particularly with chromium, due to the reason that chromium salts; chromium sulphide and chromium sulphate, are the most common leather tanning agents (Venier et al., 1985), and Pakistan is home to Asia's largest tanneries e.g. "Leather Field" in Sambrial-Wazirabad, with an estimated hundred processing units (Bhalli and Khan, 2006). According to recent statistics published by the Ministry of Industries and Production, there are 650 registered leather industries throughout major cities: Peshawar, Lahore, Kasur, Sialkot, Wazirabad, Hyderabad and Karachi (Kazmi, 1995). At present the country produces 7.4 million hides and 36.2 million skins, with an average annual growth of 2.9 and 1.47% respectively (Tahir and Naseem, 2007). The rampant discharge of untreated tannery effluents is, therefore, a growing environmental and national problem for Pakistan.

A careful review of the literature of the past fifty years as regards heavy metal toxicity revealed only few studies with reference to chromium and thyroid gland (Goncharov, 1964; Kucher, 1973; Goncharov and Ametov, 1977). Even these studies did not particularly address the toxic effects of hexavalent chromium. As people working in the chrome plating and stainless steel industries can at times be accidentally exposed to huge concentrations of chromium directly (Stern et al., 1987), in these occupational environments an acute dose was quite logical to be administered into rats. It is well known that hexavalent chromium, irrespective of its route of entry in the body, is readily taken up by red blood cells in much larger quantities than trivalent chromium (Wiegand et al., 1984). In the present study also, whole blood chromium concentration significantly increased. In the serum, it competes with the blood for binding to plasma transferrin (Hopkins and Schwarz, 1964). Once in the blood, chromium is immediately transported to the tissues where it is reduced in sequential steps to its trivalent form, in which form it then resides in the body (Wiegand et al., 1984; Anderson, 1998; Bagchi et al., 2002). Also, chromium (III) compounds are cleared rapidly from the blood and more slowly from the tissues (Aghdassi et al., 2006). Presently, a non-significant increase of chromium concentration in the serum indicates that as the chromium burden increased in the blood, it was soon transported to the tissues. Ingested or injected chromium leaves the blood rapidly; hence, blood chromium levels do not reflect the overall chromium content of tissues, except after a glucose load (Anderson et al., 1985).

Urine analysis of the animal can provide better information as to the removal of the toxicant or the level of toxicity. In the current study, a significant increase in urine chromium concentration is not surprising because the kidney removes maximal load of chromium to get rid of the chromium burden from the body (Behari and Tandon, 1980); however, tissue concentration of chromium increases non-linearly with dose, and concentrations in the kidneys increase with duration (Tandon et al., 1979). The kidney, in doing so, fails due to tubular necrosis and low-molecular-weight proteinuria (Abdulkader et al., 2008, Hanji et al., 2008). Barrera et al. (2003) demonstrated that $K_2Cr_2O_7$ induced renal damage in rats 24 hour after treatment. Noticeably, the decreased urine volume and urine color that varied from pale yellow to dark brown to red in the present study indicates damage to the renal capillary.

Elevated hypothalamic chromium concentration and a decreased thyroid chromium concentration, and at the same time no significant change in the chromium concentration of pituitary gland, indicates different tissues bioavailability of the chromium. Significant uptake or accumulation of chromium by the hypothalamus indicates transportation by the blood, possibly due to the damage caused to the blood-brain barrier. On the other hand, decreased chromium concentration in the thyroid gland attributes likely to a depletion of the chromium content. How this could possibly have occurred is not clear from the present study, but the most plausible explanation is selective disruption of the thyroid follicles and follicular cell membranes, which is evident from the histological examination of the thyroidal tissue. Thus, chromium would most likely have leaked into the interstitial spaces. A significant accumulation of chromium occurs in most tissues; brain, kidney, intestine, spleen, lungs, heart, skin, and blood on both 24 hours acute and 90 days chronic exposure to potassium dichromate (unpublished observations from the lab) and also known from earlier studies (Jacobsen et al., 2007; Rubio et al., 2008), which suggest that chromium readily enters into most body tissues.

Currently, decreased FT_4 and FT_3 and elevated TSH levels typically suggest hypo-functioning of the thyroid gland, which is a significant finding of the present study.

Circulating T_4 and T_3 levels act as useful biomarkers of potential effect on thyroid gland as a result of exposure to contaminant and as surrogate measures of health in species of marine and terrestrial mammals (Beland et al., 1993; Shumacher et al., 1993; De Guise et al., 1995). Serum levels of T_4 and T_3 act as reliable indicators of the thyroid function in both human and experimental animals. Any change in their levels reflects disturbance in the glandular synthesis and/or secretion, as well as disorders in the extrathyroidal metabolism (Rolland, 2000).

Unavailability of the iodide may account for the decreased FT_4 and FT_3 concentrations. There is a possibility that iodide was available to the thyroid gland but it was probably unable to interact with the tyrosine residues, leading to an impairment of the process of organification. Sodium iodide symporter pump (NIS), however, appears not to be affected because protein-bound iodine in the serum was decreased, indicating that at least iodine uptake took place normally, whereas decreased iodine in the urine was quite possible due to kidney failure (Chemielnicka et al., 2002).

Chromium combines actively with globulins present in the animal body and it is possible that it enhances the synthesis of thyroglobulin; however, at the same time it hinders the process of proteolysis of thyroglobulin and consequent decrease of serum FT_4 concentration (Goncharov and Ametov, 1977). Thus, failure of thyroglobulin proteolysis because of binding with the chromium might be responsible for decreased levels of concentrations of FT_4 and FT_3. At the ultrastructure level, damage to lysosomes and other cellular organelles of the chromium treated thyroid epithelial cells further support this hypothesis. Collins and Capens (1980) and Gerber et al. (1985) demonstrated that in response to long term stimulation of the follicular cells by TSH, as occurs with chronic iodine deficiency, both lateral lobes of the thyroid are uniformly enlarged due to intense hypertrophy and hyperplasia of follicular cells. Endocytosis of colloid usually proceeds at a rate greater than synthesis, resulting in progressive depletion of colloid. During the present study, small size thyroid follicles and a partial collapse of follicles might have occurred due to the lack of colloid. Such data are available regarding cadmium and lithium (Gupta and Kar, 1999). Cadmium interferes with the thyroid function at the glandular level, as well as at the peripheral level by inhibiting the conversion of T_4 to T_3 (Chaurasia et al., 1996; Gupta et al., 1997; Gupta and Kar, 1999). Cadmium at a dose of 50 mg/l does not influence T_3, but led to a decreased T_4 concentration and increased T_3/T_4 ratio and non-significantly increased TSH concentration (Pilat-Marcinkiewicz et al., 2003). In spite of low retention in the thyroid, exposure to cadmium causes serious damage to the thyroid follicular cells (Gupta and Kar, 1999). Since only thyroid gland synthesizes T_4 and T_3, the decrease in the serum level of these hormones in the cadmium treated rats suggests that it influences the production and secretion of T_4 and T_3 by follicular cells. In this respect, the effects of chromium observed in the present study are very similar to cadmium.

According to Christian and Trenton (2003), decreased levels of serum T_4 and T_3 and increased levels of serum TSH with sustained release of TSH and resultant follicular cell hypertrophy/hyperplasia in rodents, are typical hormonal and histopathological findings attributable to compounds altering thyroid function". Lopez et al. (2000) and Singh et al. (2000), have shown that depending on duration of adverse influence, chronic exposure of humans at the work place to small amounts of lead can result in significant reduction of blood thyroxine and tri-iodothyronine level, as well as a marked reduction of blood TSH level. The present study in rats appears similar to that of Erfurth et al. (2001), who demonstrated that human exposure to lead causes significant toxicity to the hypothalamic-pituitary-thyroid axis, resulting in higher concentration of thyrotrophin-releasing hormone and TSH. Presently, thyrotropin (TRH) releasing hormone concentrations were not determined due to certain limitations although raised TSH levels indicate similarly elevated TRH levels. Among other metals, lithium has been associated with hypothyroidism. The inhibitory effect of lithium occurs mainly at the level of hormone secretion, although effects on iodide trapping, release and coupling have also been described (Lazarus, 1998).

Thyroid hormones have been studied with reference to some other metals also. For example, Pilat-Marcinkiewicz et al. (2003) have shown that cadmium influences dose dependent structural and functional changes of the thyroid follicular cells in female rats. At low exposure, only structural changes in the thyroid follicular cells occur, whereas at the highest exposure, cadmium causes both structural and functional damage to these cells. Similarly, Yoshizuka et al. (1991) have shown that cadmium accumulates in the mitochondria of the thyroid follicular epithelial cells and it can inhibit the synthesis and release of thyroid hormones, influencing the oxidative phosphorylation of these organelles. Presently, marked histopathological findings such as collapsed and disintegrated follicles, colloid retraction or absorption, hyperplasia, alteration of the cellular architecture and damaged or reduced number of cell organelles, indicate severe impairment of the thyroid gland both at the structural and functional level. In the current study, depressed levels of thyroid hormones and hyperplasia of the thyroid strongly indicate that the chromium administration induced suppression of the thyroid hormone synthesis and release, and in turn stimulated the pituitary to secrete more TSH because of the negative feedback mechanism.

In the current study, depressed levels of thyroid hormones and hyperplasia of the thyroid strongly indicate that the chromium administration induced suppression of the thyroid hormone synthesis and release in turn stimulated the pituitary to secrete more TSH because of the negative feedback mechanism. Decreased

protein-bound iodine in the treated rats indicates that the thyroid gland, in order to synthesize T_4 and T_3 perhaps attempted to trap a normal or larger quantity of iodine from the blood under the influence of the hypothalamus and the pituitary through increased TSH concentration. Since the concentration of FT_4 and FT_3 were depressed, this suggests that iodide was possibly trapped in the thyroid gland and was not available for the synthesis of the hormones. Wolff (1998) has also demonstrated that the presence of excess iodide inhibits the thyroid function by multiple mechanisms and, as result, thyroid activity slows down. Similar to the present study, Siegel et al. (1989) demonstrated that apart from alteration of the endocrine system, occupational exposure of adult human males to inorganic lead is associated with impaired uptake of iodine by the thyroid tissue, as well as depressed free thyroxine level and altered morphology of the gland.

It seems that the anterior pituitary gland was under stress of the toxicant and was trying to cope with this condition by secreting greater quantities of TSH. The signal for the pituitary, and quite possibly for the hypothalamus, to release more TRH employed both the short and long negative feedback loops. This observation is supported by a parallel increase of TSH concentration determined by radioimmunoassay. Similar effects due to the stressors have been shown by Bailey (1984), who demonstrated that, depending upon the intensity, stressors act indirectly on the pituitary gland and stimulate it to release adrenocorticotropic hormone (ACTH). If the stress continues, the adrenal cortex enlarges and maintains the production of corticoids. At this resistance stage, adrenal cortex is large but not depleted. Continuation of excessive levels of stress causes the exhaustion phase, in which adrenal cortex is both large and depleted. This stage is associated with kidney damage. Presently also, the hexavalent chromium acted as a stressor on the pituitary gland to make it hyperactive. Observations like increased epithelial cell height, decreased nuclear size and increased cytoplasmic size in the thyroid follicles all indicate a state of acute stress. Exposure of rats to 3.0 and 30 mg/kg/day doses of ammonium perchlorate in drinking water led to an increase of relative thyroid weights, hypertrophy, hyperplasia, and statistically significant differences in TSH, T_4 and T_3 in the 30 mg/kg/day dosage group (Christian and Trenton, 2003). Aktaç and Bakar (2002) demonstrated similar findings in rats exposed to aluminium for a longer period. They showed that most abundant degenerative changes in the thyroid gland occurred in the 5% $AlCl_3$ dosage group.

Degenerative changes such as follicle destruction, hypertrophy and hyperplasia, nuclear, cytoplasmic and organelle abnormalities suggest accumulation of chromium in the cellular organelles. Yoshizuka et al. (1991) have shown that cadmium accumulates in the mitochondria of the thyroid follicular epithelial cells and it can inhibit the synthesis and release of thyroid hormones, influencing the oxidative phosphorylation of these organelles. Cobo and Castineira (1997) have also demonstrated oxidative stress-induced mitochondrial dysfunction. However, a lower thyroid chromium concentration seen at present apparently does not bring these observations closer to the above studies. Meager numbers of mitochondria with morphological abnormalities were readily noticeable in ultrathin sections of thyroid gland. Thus, altered thyroid morphology is quite possibly attributed to the toxicant, as no such changes were visible in the control thyroid. Currently, ultrastructural analysis of the thyroid gland demonstrated regressed and collapsed follicles with disrupted basal laminae; pyknotic nuclei; disorganized Golgi complex and endoplasmic reticulum; less abundant mitochondria; abundant collagen fibers and lysosomes in the thyroid stroma, all indicating abnormal status of the gland. A possible decrease of thyroglobulin synthesis can also be attributed to these cellular changes. Severe alterations were evident in the nuclei. It can be safely assumed because of the appearance of a large number of acridine orange positive pyknotic nuclei that the process of transcription of mRNA was either very much reduced or halted, eventually leading to an abnormal working of the thyroid. According to Aktac and Bakar (2002) aluminium produces similar effects, as damaged nuclei within follicle lumen and increased fibers within dispersed stroma of rats exposed to aluminium.

Determination of the Percent DNA fragmentation demonstrated greater damage in the chromium treated sections of hypothalamus, pituitary and thyroid glands. From the results it appears as if chromium interacted with the DNA and possibly caused single and double strand breaks. Hexavalent chromium is genetically active because of its ability to cross the membranes and enter the cells. If its reduction takes place inside the nucleus (near or at, the target DNA molecules) alterations in DNA can occur, depending upon the oxidation power of hexavalent chromium or the formation of trivalent chromium complexes with nucleophilic sites of DNA. Thus, trivalent chromium could be the ultimate mutagenic form of chromium (Levis and Bianchi, 1982; De Flora et al., 1984). Izzoti et al. (1998) injected Sprague-Dawley rats intratracheal instillations of sodium dichromate at the rate of 0.25 mg/kg b.w. for three consecutive days. They showed localized DNA lesions in the lung but not in the liver tissue and this was ascribed toxicokinetics and metabolic characteristics of chromium (VI). DNA alterations included DNA-protein crosslinks, DNA fragmentation, nucleotidic modifications, and 8-hydroxy-2'-deoxyguanosine.

The current study indicates that exposure to hexavalent chromium leads to hypo-function of the thyroid gland. If considered as hypothyroidism, besides other physiological disturbances it may lead to infertility (Elbetieha and Al-Hamood, 1997; Aruldhas et al., 2005). Thus, if people living in areas where there is greater environmental concentration of hexavalent or other

oxidative forms of chromium are exposed to such high concentrations, would this present an infertility risk? Moreover, dangerously elevated levels of even trivalent form are now being recognized as highly toxic. Several methods are on trial to remove environmental chromium (Tahir and Naseem, 2007).

Hexavalent chromium is a known human carcinogen (Kimura, 2007). Increased mortality caused in particular from stomach cancer is associated with exposure to high concentrations of hexavalent chromium in well water in Liaoning Province, China (Beaumont et al., 2008; Smith, 2008). Presently, there was no direct indication of tumor formation in the thyroid or pituitary gland, probably due to the very short exposure, although severe hyperplasia of the thyroid and pituitary hypertrophy suggest that if such a stress had continued, hyperplasia would have transformed into tumor formation during the shortest exposure. Molecular damage caused by chromium may be due to its intracellular reduction to the even more highly reactive and short-lived chemical species chromium (III) and chromium (V). Exposure to chromium (VI) can result in point mutations in DNA and to chromosomal damage, as well as to oxidative changes in proteins and to adduct formation (Paine, 2001). If TSH levels remain elevated chronically, there is an association of such high levels with an increased risk of thyroid tumors in the rat, which may be due, in part, to the high turnover rate of circulating T3 as compared to humans, who have a lower T3 turnover rate (Capen, 1997).

The current study suggests that the thyroid is sensitive to chromium toxicity. Since no extensive studies have been done on rat thyroid from this perspective, accurate comparisons are not possible at present. However, it is pertinent to further investigate the hexavalent chromium toxicity in this gland using low but chronic doses. Moreover, thyroidal enzymes TPO and 5´-deiodinase should also be investigated. There is also a need to conduct large-scale epidemiological studies on humans as regards the effect of chromium (VI). Whether exposure to hexavalent or trivalent chromium actually leads to thyroid related abnormalities in humans remains to be established.

References

Abercrombie M. and Johnson M.L. (1946). Quantitative histology of Wallerian degeneration-I: Nuclear population in rabbit sciatic nerve. J. Anat. Lond. 80, 37-50.

Abdulkader R.C., Libório A.B. and Malheiros D.M. (2008). Histological features of acute tubular necrosis in native kidneys and long-term renal function. Ren. Fail. 30, 667-73.

Aghdassi E., Salit I.E., Fung L., Sreetharan L., Walmsley S. and Allard J.P. (2006). Is chromium an important element in hiv-positive patients with metabolic abnormalities? J. Am. Col. Nutr. 25, 56-63.

Aktac T. and Bakar E. (2002). The histopathological changes in the mouse thyroid depending on the aluminium. J. Cell. Mol. Biol. 1, 69-72.

Anderson R.A. (1998). Chromium, glucose intolerance and diabetes. J. Am. Coll. Nutr. 17, 548-555.

Anderson R.A., Bryden N.A. and Polansky M.M. (1985). Serum chromium of human subjects: effects of chromium supplementation and glucose. Am. J. Clin. Nutr. 41, 571-577.

Aruldhas M.M., Subramanian S., Sekar P., Vengatesh G., Chandrahasan G., Govinda-rajulu P. and Akbarsha M.A. (2005). Chronic chromium exposure-induced changes in testicular histoarchitecture associated with oxidative stress; study in non-human primate (*Macaca radiata* Geoffroy). Hum. Reprod. 20, 2801-2813.

Baccarelli A., Pesatori A.C. and Bertazzi P.A. (2000). Occupational and environmental agents as endocrine disruptors: experimental and human evidence. J. Endocrinol. Invest. 23, 771-781.

Bagchi D., Stohs S.J. Downs B.W., Bagchi M. and Preuss H.G. (2002). Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology 180, 5-22.

Bailey J.A. (1984). Stress. In: Principles of wildlife management. John Wiley and Sons, Inc. NY. USA. pp 160-161.

Barceloux D.G. (1999). Chromium. J. Toxicol. Clin. Toxicol. 37, 173-194.

Barrera D., Maldonado P.D., Medina-Campos O.N., Hernandes-Pando R., Ibarra-Rubic M.E. and Pedraza-Chaverri J. (2003). Protective effect of $SnCl_2$ on K_2Cr2O_7-induced nephrotoxicity in rats: the indispensability of HO-^{1} preinduction and lack of association with some antioxidant enzymes. Life Sci. 73, 3027-3041.

Beaumont J.J., Sedman R.M., Reynolds S.D., Sherman C.D., Li H., Howd R.A., Sandy M.S., Ziese L. and Alexeeff G.V. (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. Epidemiology 19, 12-23.

Behari J.R. and Tandon S.K. (1980). Chelation in metal intoxication. Removal of chromium from organs of potassium chromate administered rats. Clin. Toxicol. 16, 33-40.

Beland P., De Guise S., Girard C., Lagace A., Martineau D., Michaud R., Muir D., Norstrom R., Pelletier E., Ray S. and Shugart L. (1993). Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. J. Great Lakes Res. 19, 766-775.

Besser C.M. and Thorner M.O. (2002). Comprehensive clinical endocrinology. 3rd ed. St. Louis. Mosby.

Bhalli J.A. and Khan Q.A. (2006). Pollution level analysis in tannery effluents collected from three different cities of Punjab, Pakistan. Pak. J. Biol. Sci. 9, 418-421.

Biederman K.A. and Landolph J.R. (1990). Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis and anchorage independence in diploid human fibroblasts. Cancer Res. 50, 7835-7842.

Bird R. and Jackson D.J. (1962). A routine method for estimation of serum protein bound iodine. Clin. Chem. 8, 389-398.

Capen C.C. (1997). Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol. Pathol. 25, 39-48.

Cefalu W.T. and Hu F.B. (2004). Role of chromium in human health and in diabetes. Diabet. Care 27, 2741–2751.

Chaurasia S.S., Gupta P., Kar A. and Maiti P.K. (1996). Free radical mediated membrane perturbation and inhibition of type-I iodothyronine 5'-monodeiodinase activity by lead and cadmium in rat liver homogenate. Biochem. Mol. Biol. Int. 39, 765-770.

Chmielnicka J., Swietlicka E. and Nasiadek M. (2002). Essential elements as early indicators of hexavalent chromium nephrotoxicity. Ecotoxicol. Environ. Safe. 53, 20-26.

Christian M.S. and Trenton N.A. (2003). Evaluation of thyroid function in

neonatal and adult rats: The neglected endocrine mode of action. Pure Appl. Chem.. 75, 2055-2068.

Cobo J.M. and Castineira M. (1997). Oxidative stress, mitochondrial respiration, and glycemic control: clues from chronic supplementation with Cr^{3+} or As^{3+} to male Wistar rats. Nutrition. 13, 965-970.

Collins W.T. and Capen C.C. (1980). Biliary excretion of 1251-thyroxin and five structural alterations in the thyroid glands of Gunn rats feed polychlorinated biphenyls (PCB). Lab. Invest. 43, 158-164.

De Flora S., Bianchi V. and Levis A.G. (1984). Distinctive mechanisms for interaction of hexavalent and trivalent chromium with DNA. Toxicol. Environ. Chem. 8, 287-294.

De Guise S., Martineau D., Béland P. and Fournier M. (1995). Possible mechanisms of action of environmental contaminants on St. Lawrence beluga whales (*Delphinap-terus leucas*). Environ. Health Perspect.103, 73-77.

Elbetieha A. and Al-Hamood M. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. Toxicology 116, 39-47.

Erfurth E.M., Gerhardsson L., Nilsson A., Rylander L., Schutz A., Skerfving S. and Borjesson J. (2001). Effects of lead on the endocrine system in lead smelter workers. Arch. Environ. Health 56, 449-455.

Galle P. (1987). The toxicity of aluminium. World Sci. 13, 26-35.

Gerber H., Studer H. and Von Grunigen C. (1985). Paradoxical effects of thyrotropin on diffusion of thyroglobulin in the colloid of rat thyroid follicles after long term thyroxin treatment. Endocrinology 116, 303-310.

Goncahrov A.T. (1964). Information for a hygiene-related description of chromium as a trace element (the role of chromium in the pathogenesis of endemic goiter). Author's extract from candidate's dissertation, Leningrad.

Goncharov A.T. and Ametov A.S. (1977). Thyroid gland: Morphological and functional indices in the experimental action of chromium. Probl. Endokrinol. (MOSK) 23, 59-61.

Gupta P., Chaurasia, S.S., Maiti P.K. and Kar A. (1997). Cadmium induced alterations in extrathyroidal conversion of thyroxine to tri-iodothyronine by type-I iodothy-roxine 5'-monodeiodinase in male mouse. Horm. Metabol. Res. 29, 151-152.

Gupta P. and Kar A. (1999). Cadmium induced thyroid dysfunction in chicken: hepatic type-I iodothyroxine 5'-mondeoiodinase activity and role of lipid peroxidation. Comp. Biochem. Physiol. Part C. 123, 39-44.

Hanji I., Matsuo K., Kimura I., Kosugi T., Saito M., Yoshihara K. and Suga N. (2008). A case of serious renal failure induced by ingesting the large volume of makiron. Chud. Kenk. 21, 311-315.

Hopkins L.L. and Schwarz K. (1964). Chromium (III) binding to serum proteins, specifically siderophidin. Biochim. Biophys. Acta 90, 484-491.

Izzotti A., Bagnasco M., Camoirano A., Orlando M. and De Flora S. (1998). DNA fragmentation, DNA-protein crosslinks, postlabeled nucleotidic modifications, and 8-hydroxy-2'-deoxyguanosine in the lung but not in the liver of rats receiving intratracheal instillations of chromium (VI). Chemoprevention by oral N- acetylcysteine. Mutat. Res. 400, 233-244.

Jakobsen S.S., Danscher G., Stoltenberg M., Larsen A., Bruun J.M., Mygind T., Kemp K. and Soballe K. (2007). Cobalt-chromium-molybdenum alloy causes metal accumulation and metallothionein up-regulation in rat liver and kidney. Basic. Clin. Pharmacol. Toxicol. 101, 441-446.

Kawanishi S., Hiraku Y., Murata M. and Oikawa S. (2002). The role of metals in site-specific DNA damage with reference to carcinogenesis. Free Radic. Biol. Med. 32, 822-832.

Kazmi S. (1995). "Leather Garments", Pakistan & Gulf Economist, 29th July – 4th August. pp 10.

Kimura T. (2007). Molecular mechanisms involved in chromium (VI) toxicity. Yaku. Zassh. 127, 1957-1965.

Kucher I.M. (1973). Morphological and histochemical changes in the endocrine glands with experimental chromium intoxication, Author's extract from candidate's dissertation, Aktyubinsk.

Lazarus J.H. (1998). The effects of lithium therapy on thyroid and thyrotropin-releasing hormone. Thyroid 8, 909-913.

Levis A.G. and Bianchi V. (1982). Mutagenic and cytogenetic effects of chromium compounds. In: Biological and environmental aspects of chromium. Lagand S. (ed). Elsevier Science Publishers. Amsterdam, Oxford. pp 171-208.

Lopez C.M., Pineiro A.E., Nunez N., Avagnina A.M., Villaamil E.C. and Roses O.E. (2000). Thyroid hormone changes in males exposed to lead in the Buenos Aires area (Argentina). Lead poisoning alters the levels of T3 and T4. Pharmacol. Res. 42, 599-602.

Maeng S.H., Chung H.W., Yu I. J., Kim H.Y, Lim C.H., Kim K.J., Kim S.J., Ootsuyama Y. and Kasai H.. (2003). Changes of 8-OH-dG levels in DNA and its base excision repair activity in rat lungs after inhalation exposure to hexavalent chromium. Mutat. Res. 539, 109-116.

Mascia C., Capone W., Melis M. and Valenti D. (1990). Determination of heavy metals in animal tissues. Farmaco. 45 (6 Suppl), 777-781.

Paine A.J. (2001). Mechanisms of chromium toxicity, carcinogenicity and allergicity: review of the literature from 1985 to 2000. Hum Exp. Toxicol. 20, 439-451.

Pilat-Marcinkiewicz B., Brzoska M.M., Sawicki B. and Moniuszko-Jakoniuk J. (2003). Structure and function of thyroid follicular cells in female rats chronically exposed to Cadmium. Bull. Vet. 47, 157-163.

Rolland R. (2000). A review of chemically-induced alterations in thyroid and vitamin-A status from field studies of wildlife and fish. J. Wildl. Dis. 36, 615-635.

Rubio J.C., Garcia-Alonso M.C., Alonso C., Alobera M.A., Clemente C., Munuera L. and Escudero M.L. (2008). Determination of metallic traces in kidneys, livers, lungs and spleens of rats with metallic implants after a long implantation time. J. Mat. Sci. Mat. Med. 19, 369-375.

Schumacher U., Zahler S., Horny H., Heidemann K., Skirnisson K. and Welsch U. (1993). Histological investigations on the thyroid glands of marine mammals (*Phoca vitulina, Phocoena phocoena*) and the possible implications of marine pollution. J. Wildl Dis. 29, 103-108.

Siegel M., Forsyth B., Siegel L. and Cullen M.R. (1989). The effect of lead on thyroid function in children. Environ. Res. 49, 190-196.

Singh B., Chandran V., Bandhu H.K., Mittal B.R., Bhattacharya A., Jindal S.K. and Varma S. (2000). Impact of lead exposure on pituitary-thyroid axis in humans. Biol. Met. 13, 187-192.

Singh N.P., Muller C.H. and Berger K.E. (2003). Effects of age on DNA-double strand breaks and apoptosis in human sperm. Fertil. Steril. 80, 1420-1430.

Smith A.H. (2008). Hexavalent chromium, yellow water and cancer: A convoluted saga. Epidemiology 19, 12-23.

Stern F.B., Beaumont J.J., Halperin W.E. Murthy L.I., Hills B.W. and Fajen J.M. (1987). Mortality of chrome leather tannery workers and chemical exposures in tanneries. Scand. J. Work Environ. Health.

13, 108-117.

Stohs S.J., Bagchi D., Hassoun E. and Bagchi M. (2001). Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. 20, 77-88.

Tahir S.S. and Naseem R. (2007). Removal of Cr (III) from tannery wastewater by adsorption onto bentonite clay. Separat. Purific. Technol. 53, 312-321.

Tandon S.K., Behari J.R. and Kachru D.N. (1979). Distribution of chromium in poisoned rats. Toxicology 13, 29-34.

Venier P., Montaldi A., Busi L., Gava C., Zentilin L., Tecchio G., Bianchi V. and Levis A.G. (1985). Genetic effects of chromium tannins. Carcinogenesis 6, 1327-1335.

Von Burg R. and Liu D. (1993). Chromium and hexavalent chromium. J. Appl. Toxicol. 13, 225-230.

Wiegand H.J., Ottenwalder H. and Bolt H.M. (1984). The reduction of chromium (VI) to chromium (III) by glutathione: an intracellular redox pathway in the metabolism of the carcinogen chromate. Toxicology 33, 341-348.

Wolff J. (1998). Perchlorate and the thyroid gland. Pharmacol. Rev. 50, 89.

Wu B., Ootani A., Iwakiri R., Sokata Y., Fujisi T., Amemori S., Yokoyema F., Tsunda S., Toda S. and Fujimoto K. (2005). T-cell deficiency leads to liver carcinogenesis in Azoxymethane-treated rats. Exp. Biol. Med. 23, 91-98.

Yoshizuka M., Mori N., Hamasaki K., Tanaka I., Yokoyama M., Hara K., Doi Y., Umezu Y., Araki H. and Sakamoto Y. (1991). Cadmium toxicity in the thyroid gland of pregnant rats. Exp. Mol. Pathol. 55, 97-104.

Zaidi S.S.A., Kumar S., Gandhi S.J. and Saiyed H.N. (2001). Preliminary studies on thyroid function in Welders. J. Occup. Health 43, 90-91.

Zak B., Willard H.H., Myers G.B. and Boyle A.J. (1952). Chloric acid method for determination of protein bound iodine. Anal. Chem. 24, 1345-1348.

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