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Cover Page Footnote

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HISTOPATHOLOGY OF HEART AND SPLEEN AS A RESULT OF CHROMIUM EXPOSURE IN MICE

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ABSTRACT

Potassium dichromate ($K_2CR_2O_7$) contains hexavalent chromium that was tested for cardiac and splenic histopathology and micro anatomical and morphometric analysis at sub-toxic chronic exposure in drinking water in mice at 50 ppm, 100 ppm and 200 ppm concentration. Its duration of exposure was 30 days. The forty animals were divided into 4 groups as Group I was designated as Control (Ctl) received free normal drinking water, other groups (Group II-IV) were Cr (VI)50 ppm, Cr(VI)100 ppm, Cr(VI)200 ppm received 50, 100 and 200 ppm chromium in drinking water respectively. The animals were sacrificed after 30 days of exposure to obtain the heart and spleen for histological preparation. The digital photographs obtained from selected slides were analyzed histo-anatomically. Considerable decrease in red pulp of spleen was observed in Cr(VI)50 ppm (8.07±0.76), Cr(VI)100 ppm (7.74±0.30) and Cr(VI)200 ppm (7.35±0.65) in a dose dependent manner as compared to Ctl (8.24±0.86). Relative cell abundance in red pulp of Cr(VI)50 ppm (0.81±0.13), Cr(VI)100 ppm (0.65±0.16) and Cr(VI)200 ppm (0.6±0.1) was decreasing as compared to Ctl group (1.47±0.20). However, interestingly white pulp of spleen was increased in Cr (VI)50 ppm (1.31±0.15), Cr(VI)100 ppm (1.47±0.10) and Cr(VI)200 ppm (1.72±0.15) in dose dependent manner as compared to Ctl (1.55±0.15). While heart tissue indicate variation in the mean cross sectional area (CSA) of the cardiac fibers along with damaged to end plates, merger of the adjacent fibers necrosis followed by fibrosis in the myocardial myometrium. The results shows clear impression that persistent low concentration (50 ppm)chromium exposure in drinking water can be dangerous to myometrium leading to congestive heart problems, cardiac failure, brady cardia along with many hematological implications.

Keywords: Chromium; mice spleen pathology; histometry of myocardium; red pulp; white pulp.

INTRODUCTION

Environmental chemicals and drugs effect on the development and maturation of organs (Zeliha et al., 2009). Like, organo selenium disturbs the homeostasis of adrenal medulla in rats (Zeliha et al., 2010, 2013). Synthetic organoselenium compounds effect on the activity of tyrosine hydroxylase, the enzyme in catecholamine synthesis as adrenomedullin in the hypothalamus of rats exposed to 7,12-dimeuiylbenz anthracene (Zeliha et al., 2008; Ilknur et al., 2010).

Heavy metals are present in small amount in environment and diet, many of them are necessary for good health, but large amounts of any of them can cause toxic effects (Rai et al., 2019). Exposure of heavy metals like cadmium, lead etc leads to the formation of free radicals that trigger pathological and toxicological cellular mechanism (Zeliha et al., 2009). Chromium is a mineral that human require in trace

amount. Chromium is widely distributed in the food (Guertin et al., 2016**).** Recent cell culture in vivo rat studies have indicate that chromium probably generate oxidative DNA damage and lipids (Do Nascimento Monteiro et al., 2018) and have also effect on blood. Likewise, Nitric oxide act as endothelium derived relaxing factor used for vascular dilation may cause oxidative stress and damage the liver in rat (Zeliha et al., 2009; Zeliha et al., 2013).

According to Devoy et al., 2016 chromium induced pronounced increase in the population of circulating erythrocytes and also cause reduction in the number of small lymphocytes in fresh water teleost. Uptake of chromium and DNA damage was examined in the liver of chick-embryo (Farag et al., 2017).Trivalent and hexavalent chromium cause reduction of spleen weight, splenocyte number and the percentage of blood lymphocytes in the African mouth breeder *(Oreochromis mossambicus).* There is a time dependency effect of chromium on splenic cells. Studies have demonstrated that rats exposed to chromium (VI) by inhalation for 3-month period and results show an increase in spleen weight and also in macrophage activity. Chromium (VI) effects on lymphocyte and monocyte population of blood (Dai et al., 2018). Exposure of Chromium (VI) reduces content of hemoglobin and enhance WBC's count (Racek et al., 2013).

Low levels of Chromium (VI) in body increased risk of heart attack (Chang et al., 2011). Chromium plays important role in human health and beneficial for those who suffer with diabetes, cholesterol and heart disease (Lewicki et al., 2014). There is very little available literature on the histopathology of chronic chromium exposure on the cardiac tissues. Moreover, only few studies have indicated the effects of Chromium (VI) on spleen histopathology. With keeping these things in mind present research was designed.

MATERIAL AND METHOD

a) Animal Keeping:

Forty Swiss Webster young male mice (*Mus musculus)* were kept in animal house of Department of Biological Sciences, University of Sargodha. Animals randomly divided into 4 groups of 10 mice each. Chromium (VI) dose was given in drinking water via oral route for 30 days in order to investigate the effects of Chromium (VI) dose on heart and spleen.

b) Study Groups

Sr. No	*Groups*	*Dose*	*Day of Recovery*
1	Group I: Control group (Ctl)	Normal drinking water	30^{th} day
2	Group II: Cr(VI)50 ppm group	50 ppm Chromium solution	
3	Group III: Cr(VI)100 ppm group	100 ppm Chromium solution	
4	Group IV: Cr(VI)200 ppm group	200 ppm Chromium solution	

c) Dose Preparation:

The amount of stock solution required

Molecular weight of K2 Cr2 O7 = 294 g

Fractional weight of Chromium in K2 Cr2 O7 = 104 g

1g Chromium will be present in 294/104 = 2.8275 g of K2 Cr2 O7

Weight of Cr in 294 g of K2Cr2O7 = 104.g

As 1000 ppm Chromium solution should contain 1g Cr in 1000 mL of water. By dissolving 2.8275 g of K2Cr2O7 in 1 liter of water, 1000 ppm solution of chromium was prepared. One litter solution of 50 and 100 ppm was calculated by using the following formula

C1 V1= C2 V2

d) Animal Recoveries

The animals were weighted daily to record body weight variations during chronic exposure. Animals were recovered on 30th day for the recovery of heart and spleen and finally fixed in Bouin's for 48 hr and then processed for wax embedding and micrometry.

e) Histology of Spleen and Heart

Spleen and heart sectioning was carried out through rotary microtome (Erma Optical Work Tokyo 422). Clean glass slides were used for stretching and staining (Haematoxylin and Eosin) of the sections.

f) Histological Observations and Photography

Heart sections were studied on 400x magnifications and spleen sections were studied on 1000x under Labomid CXR2 trinocular microscope mechanically fitted with Sony (Model DSC-W35)7.2 mega pixel digital. Digital photographs were obtained for digital cropping, color and contrast improvement. These images were processed in Corel DRAW11.

g) Morphometry:

The final images were digitally sized by means of pre-celebrated stage micrometer images and extra work done in Coral Draw11. CSA of cardiac fibers, red spleen cells, white spleen cells and their relative abundance in spleen/unit area i.e. 100 µm^2 were also calculated. These values were then converted in percentage values for meaningful presentation and statistical application.

h) Statistical Analysis:

Obtained data was analyzed with ANOVA (Single factor).The relative abundance and mean cell size of the red and white pulp cells of spleen were obtained by quadrate method to avoid human error. From each group section quadrate were applied to obtain the average value for cross-sectional area (CSA) of both spleen cells by using the following formula:

$$\boldsymbol{A} = \pi(\boldsymbol{a}^2+\boldsymbol{b}^2)/\,2,\ \textbf{Where } \boldsymbol{a} = (C+D)/2 \quad \boldsymbol{b} = (A+B)/2 \text{ and } \boldsymbol{A} = \text{micron meters.}$$

RESULTS

a) Histological Results of Spleen

Photographical data indicate a gradual decrease in the compactness of cells in spleen sections in the chromium (VI) exposed group as compared to the control. The most bitterly damaged area is the area of red pulp. Photographical data reveals that the compactness in the cardiac fibers decreases dose dependently with wide open gaps in between it seem that the adjacent fibers may also shows merger longitudinally giving an impact of increased thickness of individual fibers at 50 ppm dose level. The fiber texture is bitterly damaged in Cr(IV)100 ppm and Cr(IV)200 ppm exposure with the development of thick deposition of fibrotic mass in between 200 ppm dose groups slides indicate damage of the end plates of cardiac fibers study of slides (Figure 1).

Histological Results of Heart

Photographical data reveals that the compactness in the cardiac fibers decreases dose dependently with wide open gaps in between it seem that the adjacent fibers may also shows merger longitudinally giving an impact of increased thickness of individual fibers at 50 ppm dose level.

The fiber texture is bitterly damaged in Cr (IV) 100 ppm and Cr (IV) 200 ppm exposure with the development of thick deposition of fibrotic mass in between. Cr (IV) 200 ppm dose group's slides indicate damage of the end plates of cardiac fibers study of slides (Figure 2).

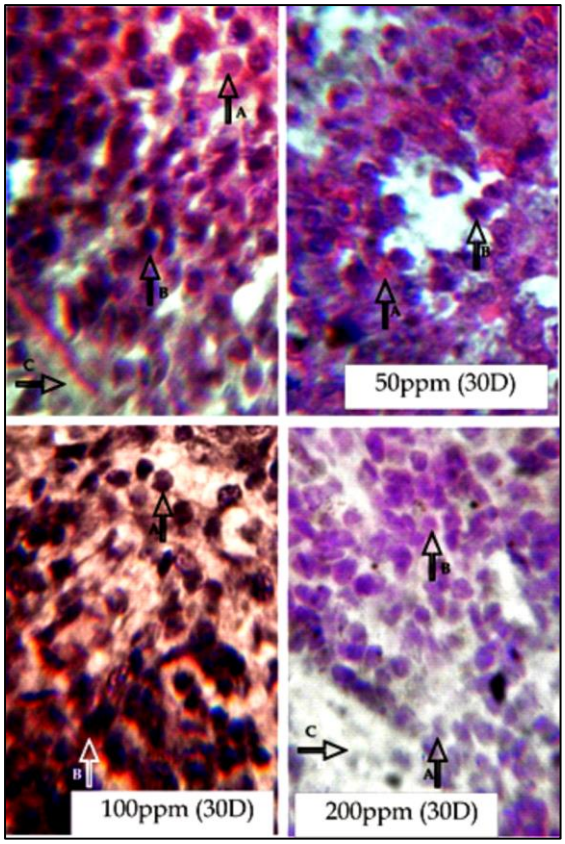

Figure 1: Selected histological sections of spleen in control and chromium treated animals groups at 1000X; Unlabeled Section (Group 1: Control), (Group 2: 50 ppm), (Group 3:100 ppm), (Group 4:200 ppm). ***A:*** *Red pulp cells;* ***B:*** *white pulp cells;* ***C:*** *Splenic Cord.*

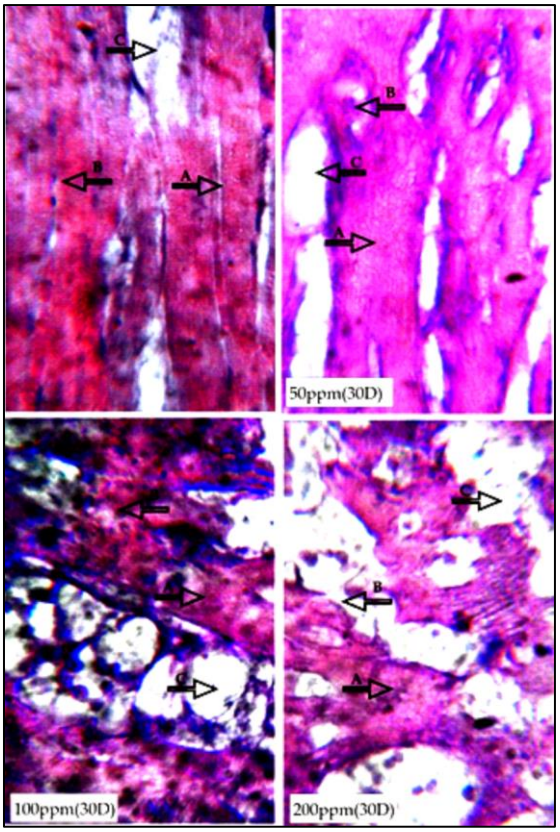

Figure 2: Comparison of heart muscle fiber between control and Cr (VI) treated groups (400x); Unlabeled Section (Group 1: Control), (Group 2: 50 ppm), (Group 3:100 ppm), (Group 4:200 ppm). ***A:*** *Cardiac Fiber;* ***B:*** *End Cardiac Plates;* ***C:*** *Fibrotic Mass.*

Micrometric Results of Spleen and Heart Mean CSA of Red and White Pulp Cells

The mean cell size in red pulp gradually decreases with the increase in chromium exposure. Statistical analysis indicates insignificant variations in the cell sizes. The mean cell size in white pulp shows inverse trend with dose of Chromium exposure groups. Maximum CSA of red pulp cell has been found in Ctrl (8.244±0.86) group followed by Cr (VI)50 ppm (8.07±0.76), Cr(VI)100 ppm (7.74±0.30) and Cr(VI)200 ppm (7.35±0.65) groups respectively (Table 1). Similarly, largest CSA of white pulp cells exist in Ctrl(1.48±0.04) group then followed by Cr(VI)50 ppm (0.82±0.02), Cr(VI)100 ppm (0.66±1.01) and Cr(VI)200 ppm (0.62±1.01) groups respectively (Table 1).

Relative Abundance of the Red Pulp and White Pulp cells per/area (100 µm^2)

The relative abundance of the red pulp cell per 100 µm^2 areas indicates a sharp decline with increase in the concentration of chromium exposed (1.31±0.15) as compared to the Ctrl (1.56±0.15) group. There was no significant variation in the Cr(VI) 100 ppm (1.47±0.10) and Cr(VI)200 ppm (1.72±0.15) groups nevertheless the Posthoc analysis of the mean indicate that the Ctrl group values differ significantly from the treated group mean values.The relative abundance in white pulp shows a dose dependent increase (Table 1).

Mean Cross Sectional Area of the Cardiac Fibers

Average CSA of cardiac fiber in 50ppm is greater to that of Ctrl. It gradually drops with increased in 100 ppm and 200ppm. Mean CSA of the cardiac fibers has been observed in in Cr(VI)50 ppm (133.5±15.65) group followed by Cr(VI)100 ppm (115.5±12.33) as compared to Ctrl (84.57±12.05) group (Table 1).

Table: 1 showing cross sectional area and relative abundance of spleen red & white pulp cells, relative abundance in spleen and heart

Histometric Parameters	Control (Ctrl)	Cr(VI)50 ppm	Cr(VI)100 ppm	Cr(VI)200 ppm
Mean cross sectional area of red pulp cells (CSA) (μm^2) P <0.001	8.244±0.86[a]	8.07±0.76[a]	7.74±0.30[a]	7.35±0.65[a]
Mean cross sectional area of white pulp cells (CSA) (μm^2) P <0.001	1.48±0.04[a]	0.82±0.02[b]	0.66±1.01[ab]	0.62±1.01[ab]
Relative abundance of red pulp and white pulp cells/area (100 μm^2) P < 0.001	1.56±0.15[a]	1.31±0.15 [a]	1.47±0.10 [a]	1.72±0.15 [a]
Mean cross sectional area of the cardiac fibers (CSA) (μm^2) P < 0.05	84.57±12.05[a]	133.5±15.65[b]	115.5±12.33 [ab]	75.47±10.01[a]

(Analyzed by one way ANOVA analysis), *: (P ≤ 0.05), **: (P ≤0.001), *: (P ≤0.0001). *Overall significant (P <0.05) variation (ANOVA-two way) among the groups; the control (Ctrl), Cr(VI)50 ppm, Cr(VI)100 ppm and Cr(VI)200 ppm; [abc]any two groups means not sharing a common lowercase superscript alphabet differ significantly with each other (post hoc comparative analysis through Duncan's multiple range test.**

DISCUSSION

Chromium exposure has been found to be toxic in animals at low dose concentration at chronic exposure (Sun et al., 2015).The major target organs are liver, testes, ovaries, along with hematological and serological implications. The major hematological implications include hypertrophy of spleen decreased level of hemoglobin and increased total leukocyte count (Licina et al., 2010). A significant reduction in the red pulp of the spleen and a corresponding increase in the white pulp cells, suggest a strong effect on immune related response to chromium exposure.

Unfortunately there isn't any research paper directly dealing with effect of hexavalent chromium on cardiac muscle. It is now well documented that hexavalent chromium primarily inflicts its toxicological manifestations by inducing oxidation stress (Sun et al., 2015) Oxidation stress alone can be considered dangerous to the heart tissue. The results obtained in present studies pores a clear spot light on myocardial fiber damages like thinning and the simultaneous merger of the adjacent fibers, loss of sarcoplasm in the interstitial spaces, necrosis of individual fibers and the development of fibrotic mass at their places. These histopathological signs of the cardiac tissue were considered as a logical outcome of the continuous oxidative stress imposed by chronic low dose hexavalent chromium exposure.

Histological studies of spleen in chromium exposed animals have revealed the loss of red pulp and indicative of chromium imposed anemia and hepatotoxicity (Dai et al., 2017). A detailed study on the chromium effects of RBCs, total RBCs count and hemoglobin content is recommended here to unearth the possible causes of diminishing red pulp of spleen with chromium exposure. The simultaneous increase in white pulp indicates immunological instigation of hexavalent chromium exposed it has already been claimed that chromium

exposure leads to increase in leukocyte (Agustin et al., 2012). The macrophage is important in two ways i.e. a general combat with pathogenic disease and with the removal of debris in damaged organs.

Literature shows that long term exposure of Cr(VI) leads to structural and functional changes in spleen. As well as it cause massive loss of spleen cells in rats and leads to oxidative stress (Karaulov et al., 2019). Exposure of Cr(VI) leads to ATP depletion and mitochondria impairment in rats. It inhibits Sesn2 activity in rat heart that causes impairment energy supply to cardiac tissues (Yang et al., 2021). Results obtained in the present studies have led to the author to develop a preview that the increased. TLC has primarily been due to the second reason. A whole lot of histological, hematological and serological study is required to reach to some conclusive end points to the toxicological effects of the hexavalent chromium reported in the present study.

CONCLUSION

Based upon our findings we conclude that *Morus nigra* possess immense mitigating potential thus have a strong medicinal indication against environmental reproductive toxicants.

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AUTHORS CONTRIBUTION

S.N.A. designed the experiment. S.A. conducted the experiment. I.I. provided all the instruments needed for the experiment. M.A.K. helped in lab work. S.S. provided guidance. K.R.A. supervised the complete study and contributed to the scientific discussion and editing of manuscript. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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