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Research Article

Morus Nigra Recover Chromium Induced Hepatic Anomalies in Mice

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ABSTRACT:

Objective: Endocrine disruption by pollutants has raised serious concern in industrial vicinities. In Pakistan Chromium (Cr) contaminated water and food additive are frequently used.The histpatholgical and micrometric anomalies of Cr exposure along their ameliorations by Morus nigra fruit pulp extract (MFE) were focused in this research.
Design: Histopathological experimental study.
Place and Duration: Experiment was design in Sargodha University for duration 2013 to 2015.
Methods: Thirty male mice (n=10) grouped as control (C), chromium (Cr) and chromium morus (CrM) treated group, were given 50ppm Cr for 10days in drinking water but CrM were additionally given 0.2ml MFE/12 h for next 5days.
Results: Cr exposure leads to pathological signs in the hepatic architecture of hepatic cords and necrosis of hepatocytes leading to fibrosis. The numbers of hepatocytes in 46225μ^2area, their cross sectional area (CSA) decreased from 236.38μ^2 to 161.18μ^2 but increasing trend was in hepatocytes nuclei size (32.07μ^2 to 41.94μ^2), mean CSA of central veins (2900.86μ^2 to 3779.05μ^2) and width of sinusoidal spaces (6.16µ to 7.15µ) were observed as compared to control. The lymphocytes, monocytes, eosinophil and hemoglobin were significantly ($p \leq .0001$) decreased while liver fractional weight, RBC, neutrophils and platelets were increased in Cr exposure group. There were signs of histpatholgical proliferation and rehabilitation of hepatic cords statistically confirms the convincingly recovering hepatic pathologies by MFE.
Conclusion: These findings indicate that Morus fruit extract bears nutraceutical capabilities against noxious environmental toxicant particularly heavy metals such as Hexavalent chromium.

Keywords:histpatholgical, amelioration, hepatocyte, necrosis, chromium, Morus.

INTRODUCTION

Chromium (Cr) present in the ground water of Pakistan that continuously enter into the food chain and might be causing bio-accumulation. Performance-enhancing pills, powders and chromium additive tonics, are used without scientific authentications, therefore may suffer from health losses. Potassium dichromate is also being used as colorant of traditional oriental sweets like Gulabjaman, Galabe and Zarda in Asia.

Dietary intake of chromium (Cr) according to National Research Council is 50-200µg/day and its deficiency cause diabetes.[1]Cr (VI) an environmental pollutant, easily taken up by cells to generate reactive species; trigger DNA damages.[2,3]The Cr toxicity cause impairment of

antioxidant defense system, alterations in cytoplasmic signaling,fluctuationsof blood parameters and denaturation of liver-enzymes.[4-7] Exposure to Cr(VI) cause hepatocellular apoptosis, necrosis,lysis of nuclei and atrophy.[8-10]Cr widens the sinusoid space of hepatocytes, inflammation of hepatocytes, lipid peroxidation, oxidative stressand vacuolization.[11,12]

Many plants in Pakistan have been investigated with free radicals scavenger and metal chelating aptitude.[13-15]Medicinal plants like Mulberry possesses antioxidant potential due to presence of anthocyaninwhich protect from lipid peroxidation and inhibit the Low-Density Lipoprotein oxidation.[16]Utilization of Mulberry fruit (Morusnigra)keeps away from liver and kidney damage, strengthens the joints, improves eyesight along with anti-aging effects and ameliorative potentials against lipid related anomalies.[17,18]

The present study was undertaken to investigate Morusnigrapossible ameliorative effects on oxidative damage to liver, resulting from exposure of mice to Cr(VI) toxicity.

MATERIALS AND METHODS

Ripe fruits of Morusnigrawere purchased from market their berries were washed in water, air dried, and 100g of the pulp was blended with electric juicer in 100mL water for 5 minutes. The juicy material was centrifuged (500rpm/10 minutes) to separate the deep purplish supernatants and stored at –30ºC in 5mL ice cubes. For each treatment, extract from a freshly thawed (at room temperature) cube was used.

As 1000ppm Cr(VI) stock solution was prepared by dissolving 2.282g of $K_2Cr_2O_7$ in 1000ml water, which diluted to get 50ppm solution.Thirty Swiss Webster male mice 25-30g, aging 3-4 months were equally divided in 3 groups, provided with Cr free water as control group, 50ppm Cr(VI) solution in drinking water (ad-libitum) for 10 days to Cr and CrM groups while CrM was additionally given MFE(oral gavage 0.2ml/12h daily) for next 5 days at 23±3C˚ and 45% humidity.

Intact liver was removed after dissection and weighed to calculate hepato-somatic index and processed for wax embedding. Serial sections (5-6 µ), on rotary microtome (ERMA TOKYO 422)were treated with HE histopathological and micrometric studies at 40×, 100×, 400× and 1000 × by trinocular research microscope (Labomed CXR_2) with 7.2 mega camera (Sony DSC-W35) and photographs were improved in corelDRAW11. Thus measurement of mean Cross Sectional Area (CSA) of hepatocytes, their nuclei, central hepatic vein, Sinusoidal spaces, number of Kupffer cells and hepatocytes/area and relative area occupied by hepatocytes was measuredand data was analyzed through ANOVA and Duncan's Multiple Range Test as standard protocol[19, 20].

RESULTS

Histological observations: All typical signs of normal histological dispositions such as the hepatic lobules with centrally placed lobular vein and hepatocytes arranged in hepatic cords radiating from the central vein properly lined with Kupffer cells and showing hepatic sinusoids in between were visible in the C group (Fig: 1 C).Signs of extreme hepatic lobular necrosis were evident in Cr group (Fig: 1 Cr).

These include disproportionate hepatic cords, hepatocytes necrosis, presence of debris of hepatocytes and widened intracellular spaces. Signs of connective tissue development at the expense of hepatocytic involutions were also visible.

The signs of necrosis of hepatocytes include disfigured nuclei, irregular cellular margins and cytoplasmic fusions between adjusted hepatocytes. Endothelial cells infestation from the lobular vein into the lobules was also been seen. Obvious signs of liver regeneration that include hepatoblastic mitosis and the rehabilitation of hepatic cords were clearly visible in the CrM group (Fig 1; CrM).

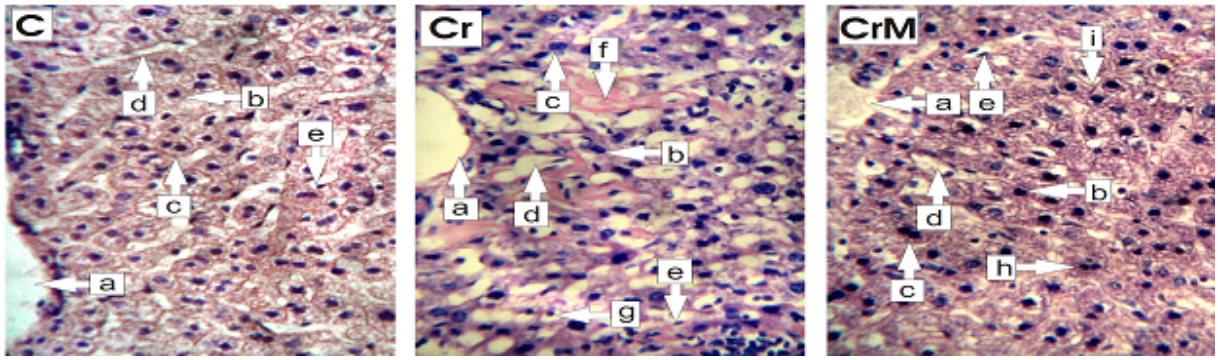

Fig 1: Histological sections of Mice liver Control (C), Chromium (Cr) and Chromium + Morus (CrM) groups at 400×. a: Central vein, b: Uninucleatedhepatocyte, c: binucleated hepatocyte, d: Sinosoidal space, e: Kupffer cell, f: Hepatocytic debris, g: Hepatocyte necrosis, h: Hepatoblast-proliferation, i: Emerging hepatic cord.

Anatomical and Micrometrical ameliorative observations: There was significant variation among the organ index indicate the mean fractional weight of liver have non-significant variation among control and CrM group while Cr group show significant variation from C and CrM groups. The mean CSA of hepatocytes indicate the significant difference between C, Cr and CrM group likewise their nuclei CSA also significantly fluctuate among C, Cr andCrM group seen in Table 1.

Table: 1 Anatomical and Micrometrical amelioration of Morus against chromium exposure in mice

Parameters	C	Cr	CrM
Mean fractional weight of Liver (%) ***	06±0.33^{a}	11.54±1.25^{b}	05±0.139 ^{a}
Mean CSA of hepatocytes (μ^2)***(400×)	236.38±9.82^{c}	161.18±13.01^{b}	133.81±04.04^{a}
Mean CSA of hepatocytic nucleus (μ^2)***(400×)	32.07±01.19^{b}	41.94±01.53^{c}	28.03±01.09^{a}
Mean CSA of central vein(μ^2)** (40×)	2900.86±296.49^{b}	3779.05±605.19^{c}	1956.34±256.32^{a}
Mean width of Sinusoidal spaces (μ)***(400×)	6.16±0.28^{b}	7.15±0.35^{c}	5.17±0.31^{a}
Mean number of Kupffer cells in 14400μ^2 area*** (400×)	10.83±0.34^{a}	15.5±0.85^{b}	11.42±0.48^{a}
Mean number of hepatocytes in 46225μ^2 area***(100×)	112.13±6.08	80.63±3.46	56.75±5.47
MRA of hepatocytes in 46225μ^2 area***(100×)	26504.11±1437.45^{c}	12995.14±558.29^{b}	7593.72±732.36^{a}

C; control group, Cr; chromium group, CrM; chromium + morus group.CSA; cross sectional area, MRA; mean relative area, μ; micrometer, Statistical analysis (ANOVA)*:p≤0.05-0.01; **:p ≤ 0.001; ***:p ≤ .0001, group means ±SEM,$^{a\ b\ c\ d}$: two groups not sharing a lower case letters differ significantly from each other (Duncan's Multiple Range comparison- post hoc analysis).

HEMATOLOGICAL OBSERVATIONS

Preliminary phytochemical protective activities were studied against Cr-induced anomalies in mice with reference to blood profile changes with respect to bilirubin, total protein and globulin changes as shown in (Table: 2).

Table 2: Ameliorative effects of Moruson Cr induced anomalies on Blood Profile.

PARAMETERS	C	Cr	Cr- M
RBC (×10^6/ul)*	7.97±0.16^{a}	8.02±0.01^{b}	7.84±0.08^{a}
TLC (×10^3/ul)*	8.06±0.42^{a}	6.35±0.74^{b}	7.47±0.07^{a}
%Neutrophil***	11.05±0.86^{a}	43.02±2.09^{c}	11.45±0.61^{a}

%Lymphocytes ***	67.06±7.39[a]	42.7±3.9[b]	83.04±1.04[c]
%Monocytes *	2.07±0.15[ac]	2.03±0.15[a]	2.06±0.22[ab]
%Eosinophil *	1.55±0.14[ad]	1.02±0.01[b]	0.95±0.14[c]
Hb(g/dl) **	13.06±0.16[a]	10.39±0.95[b]	12.86±0.29[a]
PCV% **	49.23±1.35[a]	38.28±2.65[b]	46.16±2.37[c]
MCV fl ***	58.93±0.09[a]	51.26±1.17[b]	52.05±0.87[b]
MCH (pg) ***	16.58±0.03[a]	15.2±0.18[b]	16.46±0.28[a]
MCHC(g/dl)***	27.65±1.01[a]	30.39±0.37[b]	31.32±0.24[c]
Platelet($\times 10^3$/ul)***	807.01±16.09[a]	989.2±39.09[c]	907.00±13.07[b]

C: control. Cr: chromium treated, Cr-M: chromium+morus, Values are mean ± SEM, n= 10, RBC: Red Blood Cell, Hb: Hemoglobin, PCV: Pack Cell Volume, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Concentration of Hb in RBC. Statistical analysis (ANOVA)*:p≤0.05-0.01; **:p ≤ 0.001; ***:p ≤ .0001, group means ±SEM,[a b c d]: Any two groups not sharing a lower case letters differ significantly from each other (Duncan's Multiple Range comparison- post hoc analysis).

DISCUSSION

Chromium although is a micronutrient, is toxic at higher doses particularly in its hexavalent form.[21] On the other hand Morus fruit extract has long been used to cure various pathological and allergic conditions.[22]Results indicate that Cr exposure caused rapid weight loss in the exposed animals while the post treatment of MFE was resulted into a secondary weight gain. Simultaneously the mean percentile fractional weight of liver was increased in just 10days of Cr exposure than that of the control, indication hepatic inflammation. This condition was reversed just within 5day of Morus post treatment indication its detoxifying and health promoting capabilities.Histologically it was seen with great concern that Cr exposure bring about various pathological changes in mice liver that include hepatocytic necrosis with an increased deposition of debris. These findings are well in line with the available literature. While the post treatment of MFE led to hepatoblastic proliferation and rehabilitation of the hepatic cords.Micrometric data shows significant decline in the area occupied by the hepatocytes, the number of hepatocytes per unit area and the mean CSA of the hepatocytes on Cr treatment with concurrent increase in sinusoidal spaces and mean caliber of the centrilobular veins. These findings clearly indicate that Cr exposure causes hepatocytic necrosis leading to an increase in the sinusoidal spaces. Cr(VI) treatment significantly increase RBC, neutrophil, MCHC and platelets and a significant decrease in TLC, lymphocytes, monocytes, eosinophil, Hb, PCV, MCV and MCH as other metals exactly like the toxicity of copper.[23]Cr deposition in organ cause the loss of blood plasma and water depletion, result in the appearance of higher levels of RBC also associated with fibrosis.[24] The neutrophils increased by toxification of Cr(VI) treated as compared to control untreated normal as Pb, Cu, Hg, and Cd in human also indicate the causative agents of fibrosis.[25] Cr(VI) inducespericellular fibrosis, vacuolation of hepatocytes and portal tract indicated by dense mononuclear inflammatory cells.[26]The fibrosis causes distortion of the hepatic vessels and lead to an increased intrahepatic resistance and portal hypertention. Damage liver hepatocytes in Cr (VI) treated group causes impair liver function and the liver becomes unable to detoxify the toxicants in blood.

The area occupied by the hepatocytes, the number of hepatocytes per unit area and the CSA of the hepatocytes shows a further decline on post treatment of Morus extract. These finding can be misleading if not seen with conjuncture with the histological findings. The number of functional hepatocytes was lesser than Cr in CrM group, while clear signs of removal of debris with simultaneous hepatoblastic proliferation seen

histologically; indicate liver regeneration upon MFE as post treatment. Secondary decline in sinusoidal breadth and the mean caliber of the centri-lobular veins also indicate rehabilitation of normal physiological status of the liver on post treatment of MFE.

Amelioration of Hepatocellular Fibrosis

The plants are responsible to increase MCV; the same tendency was evident in MFE against Cr-induce fibrosis.[27] Decline of MCV was not only inhibited, but also activates MCV by MFE, indicate the ability to block the toxicity of Cr(VI).The MFE showed excellent results in blocking the heavy metals toxicity by improving MCV and increase the MCH to bring it approximate equal to the normal control values, ameliorate the Cr-induced anomalies and cure the fibrosis by up regulating MCH. The MFE maintain RBCwhich is associated with fibrosis; indicate the clue about detoxification against heavy metals due to presence of medicinal phytochemicals.[15]MFE significantly increase PCV reverse the Cr (VI) induced oxidative thresh and incongruities (Tab: 2).

Mulberry antioxidant play significant role in the treatment of Cr induced oxidative stress as metal chelating activities like other medicinal plants.[28]MFE also have radical scavenging, regulating cell cycle and apoptosis preventing abilities.[29] Blueberry L-carnitine contains anthocyanins; can increase energy and boost metabolism, so there is positive link between fruit extract and carnitine without alteration in normal cecal microbial compositiondue to their non-destructive aptitude against intestinal microbiota, which is essential for carnitinepalmitoyl transferase-1 pathway during rehabilitation and proper lipid metabolism.[30]

Our results clearly indicate that Cr (VI) is injurious to general health and particularly hepatotoxic causing various histopathological and micrometric changes in liver, while MFE surely bears curative potentials against such pathological manifestations.

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