EFFECTS OF CHROMIUM ON TESTES AND PROTECTIVE ROLE OF MULBERRY

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ABSTRACT

Background: The chromium supplements and medicines are used to reduce sugar and boost sexual performance without recommendations. This practice may cause hyperlipidemia and testicular anomalies which can be partially ameliorated by natural phytochemicals of Mulberry (Morus nigra) fruit extract (MFE). **Objective:** To determine the protective effects of mulberry (Morus Nigra) against injury on testes caused by hexavalent chromium. **Methodology:** This experimental study was conducted at DHQ teaching Hospital Sargodha and experimental work was conducted in Sargodha University. Thirty male mice (*Mus musculus*) were equally grouped as: C; Control, Cr; 50ppm in water ad-libitum (10 days) followed withdrawal for 5days, Cr-M; as Cr group but followed by 0.25mL/12h MFE and sacrificed on 16th day. The laboratory work was completed in 3 months. The data was entered and analyzed by using SPSS version 17. **Results:** Cr^{+6} exposure significantly ($p < 0.05$) reduced sugar but caused hyperlipidemia, atrophy, steatosis, cirrhosis and necrosis in testes. The accumulation of debris with tail-less sperms raise cross sectional area of seminiferous tubules (ST) to generate pressure potential to lyse some ST. There significant ($P< 0.001$) reduction of Spermatogonia, primary spermatocytes and dislodged parrot beak headed spermatozoa (PBH) /area specifies sperm deformities and infertility, while Mulberry ameliorates such anomalies. **Conclusion:** Cr contamination in water and food supplementation may produce histpatholgical changes in the testes while Mulberry has rehabilitation and metal chelating capability to recover anomalies.

Keywords: Atrophy, Steatosis, Cirrhosis, Necrosis, Seminiferous tubules, Chromium, Mulberry.

INTRODUCTION

Everyone wish to be fit and want to enhance their muscular performance, by using nutritional supplements, like androstenedione, chromium, vitamins and caffeine without scientific authentications, therefore suffered from health losses.[1] Chromium (Cr) as micronutrient metabolizes sugar, lower the cholesterol but its ridiculous use produces severe toxicities.[2, 3] In Pakistan people irrigate crops, vegetables and fruit plants from unhygienic water and suffered from inevitable diseases. Cr as industrial effluent adversely affects the testosterone and produce cellular impairments by interfering with Leydig cells.[4,5] Hexavalent chromium (Cr^{+6}) exposures alter the size of ST and damage the blood testes barrier; induce accumulation of immature spermatocytes and reduce fertility to enhance the oxidative stress.[6,7]

Antioxidant and β-sitosterol have nitric oxide-scavenging capacity and ferrous ion chelating potency to prevent from free radicals and lipid peroxidation.[8] Berries activate AMP protein kinase in liver to ameliorate the hyperglycemia and insulin sensitivity.[9] The carnitines provide primary fuel for sperm motility and restore the phospholipid composition of mitochondrial membranes.[10, 11] The co-treatment of ginger and L-carnitine improve the male sexual performance.[12] Many plants from Pakistan have radicals which removing and protect cell enzyme from heavy metals.[13] Mulberry (Morus nigra) protects dopaminergic neurons and inhibits lipogenesis and regulate the fatty acid oxidation.[14] It is hepatoprotective due to presence of flavonoids quercetin and prevents from induced systemic lipid peroxidation.[15] Histological changes in testis by heavy metals exposure can be cured by plant and enzymatic changes are same in humans and rodents,[16-18] so the mammalian model mice was selected to investigate the protective effects of common local fruit Mulberry (*Morus nigra*) against injurious effects on testes of hexavalent chromium.

METHODOLOGY

This experiment study was conducted on thirty male mice. Thirty (3-4 months) male mice (25-30g) were kept at 24±3ºC, 46% relative humidity, and a 12-hr dark/light cycle, provided free access of Cr free water and standard mouse diet.

Study duration: 1st August to 30th November 2016.

Preparation of Mullbery (*Morus nigra*) fruit extract: Ripe fruit of *Morus nigra* berries were carefully selected and 100g of the pulp was blended

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in 100mL water for 5min in juicer and resulting juicy material was centrifuged at 500rpm for 10 minutes. The supernatant of centrifuge pulp was immediately placed at –30ºC in 5mL sterilized ice cubes and freshly thawed cube was used.

Preparation of Cr solutions: The stock solution of K_2 Cr_2 O_7 (1000ppm) was prepared by dissolving its 2.81g per liter H_2O and diluted to get required 50ppm dose.

Animal Experimental Groups and Dose Administration: Male animals were equally divided (n=10) and grouped as, Control; without exposure (15days), Cr; provided 50ppm K_2 Cr_2 O_7 at *ad-libitum* for 10days and followed by Cr-free water for next 5days. Cr-M group, animals were given Cr (50ppm) *ad-libitum* for first 10days and MFE 0.25ml/12h by oral gavage for next 5days.

Testes Recovery: All animals were euthanized by cervical dislocation on the 16th day and testes were retracted from visceral chamber and weight. The cardiac blood was used for biochemical analysis. One testis from each animal was cut into two equal halves, gently crushed on a glass slide to make thin smears, air-dried and HE stained for sperm morphology and micrometry.

Sperm micrometry: The parameters of sperm cells like length and width of sperm head, tail length and mid piece diameter were measured by using the protocol.[19]

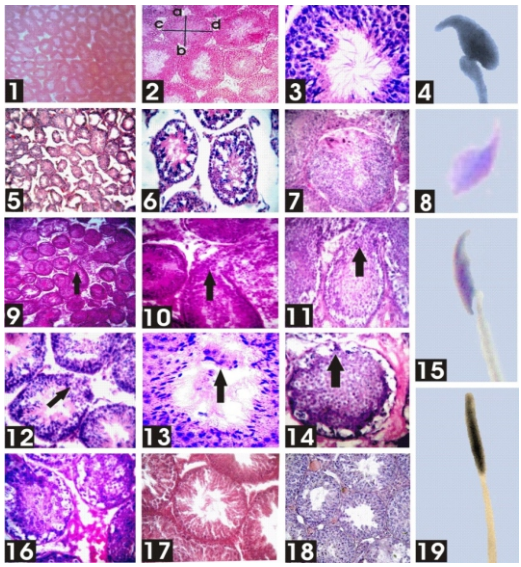
Testicular Histology: Testes fixed in alcoholic Bouin's solution were processed for HE-staining as per standard protocols.[20]

Histometry: Spermatogonia from 10 spermatic cords of 10 randomly selected ST were counted from each individual. These digital values (mm) were used to find out actual diametric values (µ) by means of calibration obtained (means ± SEM) from a photomicrograph of a standard stage micrometer. The data was entered and analyzed by using SPSS version 17.

RESULTS

Histopathological Findings: There were regular boundary of seminiferous tubules (ST) along with prominent basement membranes and interstitial tissues in the histological sections of testes in control group. The lumen of ST contains differentiated spermatozoa; the heads of normal spermatozoa and secondary spermatocytes were properly embedded in the Sertoli cells. Spermatogonia and spermatocyte arranged in whirls while tails of spermatozoa are lashing in the lumen of ST (Fig: 1, 2,and 3). The Cr^{+6} exposure group showed irregular boundaries of ST along with wide inter-tubular spaces (Fig: 5). ST lumen filled with debris and undifferentiated cells (Fig: 6), the extricated spermatocytes particularly "parrot beak headed" (PBH) sperm (Fig 4), similarly partly differentiated spermatozoa (Fig: 15) and embedded "club headed" (CH) spermatozoa (Fig: 19). The PBH spermatozoa were without tail similarly exfoliated degenerated Spermatogonia were scattered in ST lumen (Fig: 6, 7, 8).

Figure (1-19): Testicular Histology and Sperm Morphology of Mice



ST display signs of atrophy with ruptured linings and germinal epithelium disorganization (Fig: 12, 14) filled with debris and undifferentiated cell significantly ($P \leq 0.001$) increased the CSA of ST (Fig: 7, Tab: 1). There were prominent landmarks of apoptosis and steatosis with cytoplasmic vacuolation along with cirrhosis (Fig: 14, 16, 17), while some histological sections have lyse ST appeared as fountain and intra-tubular material seem to come out with a pressure and spread at inter-tubular spaces along with debris (Fig: 9-14, black arrows).

Cr-M group has signs of revival and rehabilitation, numbers of Spermatogonia were increased, PBH and CH spermatozoa were visible embedded in the Sertoli cells. There was no sign of ST lysing activities in Cr-M group, similarly interstial tissues realign and re-establish the intertubular junctions. The bulbous blood vessels and clear peripheral linings of ST indicate interstitial tissue proliferations without vacuolation (Fig: 18).

MICROMETRIC FINDINGS: The highest mean cross sectional study (CSA) of ST at 100× was calculated in Cr^{+6} exposure group (29975.00μ^2) followed by control (27184.48μ^2) and Cr-M (20756.4μ^2) group. The numbers of Spermatogonia, primary spermatocytes and PBH, attach spermatozoa per unit area (264μ^2) at 400× from higher to lower in descending orders were recorded C > Cr-M > Cr group, while CH and dislocated spermatozoa were Cr > Cr-M >C group. (Table: I)

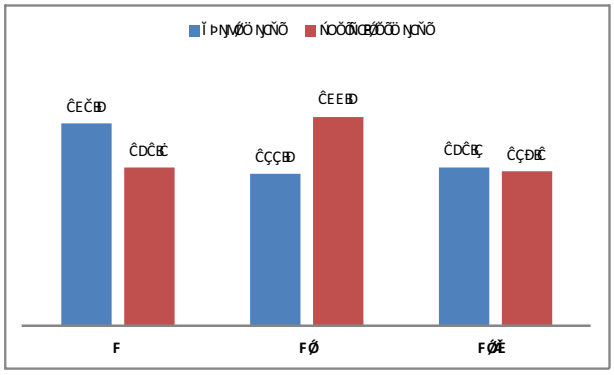
Table I: Amelioration of MFE on Cr induced anomalies at micrometric level.

PERAMETERS	GROUPS		
	C	Cr	Cr-M
CSA of ST(μ^2)***	† 27184.48±488.04[a]	29975.00±2373.01[b]	20756.04±1547.02[c]
‡ Spermatogonia**	65.25±5.21[a]	30.27±3.01[b]	47.08±6.21[c]
‡ Primary spermatocytes***	75.55±7.55[a]	25.05±2.69[b]	28.02±0.72[b]
‡ PBH-spermatozoa***	51.25±5.87[a]	20.35±4.12[b]	47.04±8.20[a]
‡ CH-spermatozoa**	10.25±2.80[a]	33.04±3.99[b]	17.31±1.99[c]
‡ Embedded spermatozoa**	72.25±3.49[a]	14.09±6.51[b]	52.31±9.28[c]
‡ Dislodged spermatozoa**	3.00±1.77[a]	44.37±12.76[b]	6.39±3.70[a]

C: Control. Cr: Chromium, Cr-M: Chromium+ MFE, CSA: Mean Cross Sectional Area (μ^2) of ST at 100×. PBH: Parrot Beak Headed spermatozoa, CH: Club Headed spermatozoa. a b c d : Indicate Duncan's Multiple Range Test (comparison- post hoc). ‡ Relative Abundance per /unit area (264μ^2) in ST at 400×. † Group means ±SEM, μ = μm, *: P ≤ 0.05-0.01, * *: P ≤ 0.001,* * *: P ≤ .0001, {Statistical Analysis (ANOVA: two factors without replication)}.

Table II indicates the biochemical variation of sugar and cholesterol and Table III: indicate animal and testicular weight fluctuations in their respective groups.

Table II: Sugar and cholesterol changes in Mice



C: control. Cr: chromium, Cr-M: Cr+MFE

Statistical analysis (ANOVA) indicate highly significant variation among the groups (P ≤ 0.001) and Duncan's multiple range test revealed the significant (p ≤ 0.05) differences among the control and other groups.

Table III: Variation of mice body and testicular weight

PARAMETERS	GROUPS		
	C	Cr	Cr-M
Animal Body Weight (g)	28.6±0.3	23.8±1.02	26.88±0.4
Weight of Testis (g)	0.08±0.01	0.09±0.03	0.07±0.09

DISCUSSION

The reproductive and metabolic changes are under the influence of neurotransmitters; responsible to coordinate normal cellular physiology and lipid metabolism but their fluctuation may cause disease.[21] Present study was planned to investigate the effects of Cr^{+6} exposures to male mice testes in drinking water (*ad-libitum*) other than force feeding or IP injection. Cr^{+6} like cadmium chloride induced ST atrophy, testicular architecture disorganization and germinal epithelium disruption by necrosis of Leydig's cells.[22]

The oxidative balance system is responsible for the integrity of cellular phospholipids and mitochondrial membrane permeability.[23] The free radical fluxes abruptly change the lipid metabolism by significant reducing sugar and animal weight and diverting acetyl CoA of Krebs cycle by the process of β-oxidation towards Ketonic bodies formation. The intermediates in this pathway converted sugar into another alternative metabolic pathway to elevate cholesterol evident by elevation of testicular weight.

Elevated cholesterol enhance ST cell vacuolation, cirrhosis and steatosis and acidic environment cause sloughing and cell degeneration evident by presence of debris and dislodged spermatids in ST lumen. That significant reduction of normal spermatozoa and alterations in ST epithelium is accordingly other metal induced injuries.[24] The primary hypertrophy is evident by the elevation of CSA by accumulation of undifferentiated cells and absence of normal meiosis during spermatogenesis as spermatogenic arrest. The secondary atrophy and necrosis of Sertoli cells causes the dislocation of dead spermatic cells and the deposition of the fatty droplets. The dislodge spermatozoa, dead necrotic cells and debris slough materials produces a pressure potentials against the basement membrane from inside to outside which

lyse some ST at points where the cell junctions were weak and fragile. The abnormal sperm heads and tail-less spermatids indicate the possible deformities at androgen receptor level in cells during spermatids terminal differentiation. The rodent sperms bear one or more apical regular symmetrical hooks while the Cr^{+6} exposure group have alteration in sperm head with irregular and wavy intermediate appearance along with significant alteration of sperm tail and mid-piece thickness intimate oxidative stress to impair sperm motility.[25] The underdevelopment hypoplastic testis with less numbers of normal spermatozoa specify the anomalies of polymerization of the micro-tubules in sperm tail also intimate the destruction of Leydig's cell. These findings specify the effect of Cr at chromosomal level and ultimately such anomalies increased the risks of infertility.[26] The anabolic androgenic steroids can be modified by the drugs and pharmacological products of plants by regulating acetyl CoA of Krebs cycle and removal of free radicals evident in Cr-M group.[27,28] The infertility can be recovered and regeneration is possible by post-treatment of Morus as our recommendations in recent publications about Jambul.[29,30]

CONCLUSION

Chromium supplementation in food without scientific authentications is not safe while fruit extracts of Mulberry not only ameliorate testicular injuries but also increased the normal spermatozoa.

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