Original Article

Chromium Induced Reproductive Anomalies

Ameliorative Role of Jamul (Syzygiumcumini) against Chromium Induced Histopathological Reproductive Anomalies

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ABSTRACT

Objectives: Heavy metals are global environmental pollutants and one of them chromium (Cr); frequently used in Pakistani traditional sweets without medical authentications; may induce reproductive anomalies.
Study Design: Observational Study.
Place and Duration of Study: That study was conducted at the Medicine Deptt Sh. Zayed Hospital, Lahore and Department of Biology, Punjab University Lahore from Oct-2016 to Dec-2016.
Materials and Methods: A randomized study was conducted on 30 male mice (Musmusculus), maintained for 15days as,G-1 (C) control, G-2 (Cr)$K_2Cr_2O_7$ (50ppm for 10days ad-libitum)andG-3 (Cr-J) as G-2 but received Jamul (Syzygiumcumini) fruit pulp extract (JFE) 0.2ml/12h for next 5days(oral gavage). At 16th days, animals were sacrificed and testes were processed for micrometrical and histopathological study.
Results: Cr exposure significantly ($p \leq 0.001$)reduced testicular weight, produce necrotic changes in seminiferous tubules (ST) along with vacuolization. The CSA of ST was increased while Spermatogonia and spermatozoa were decreased. There were apoptic and exfoliated cells while some ST showed desquamation and multinucleated giant cells. The parrot beak headed (PBH) spermatozoa werecommon in testicular smear of Cr group.
Conclusions: Histopathological examination of the specimens showed Cr compounds are highly toxic, while JFE ameliorate toxicities by inhibiting oxidative stress and metal chelating ability.
Key words: necrotic, apoptic, exfoliated, desquamation, chromium.

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INTRODUCTION

Pakistan is rich in medicinally important flora; about 600-700 plants species have metal chelating properties with potential health implications than synthetic drugs[1]. Natural antioxidants have more ameliorative abilities against the histopathologies of toxicants[2]dietary intake of Cr in U.S. was 50µg/d, Food and Nutrition Board recommendationis 20-25µg/d (females) and 30-35µg/d (male) for humans[3]. Cr boost the sexuality, muscular performance and working capabilities but Gunton[4]claim that Cr supplementation does not positively improve the glucose toleranceand insulin sensitivity.

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Beaumont[5] notice the cancer mortality due to presence of hexavalent chromium (Cr^{+6}) in drinking water in China. The environmental pollutants are the causative agents of oxidative stress and their main targeting is central nervous and reproductive systems.Exposure to free heavy metal ions and radicals produced from the industrial effluents damage and reduce antioxidant melatonin activities in testes[6].$Cr^{+6}$are more toxic than Cr^{+3}, because of reducibility properties of Cr^{+6} to Cr^{+3}in cells. Theirserial conversion becomescytotoxic, genotoxic and carcinogenic. Oral administration of Cr^{+6} affect lipid peroxidation within membranous organellesespeciallymitochondriato releasecytochromes[7].$K_2Cr_2O_7$ exposures inhibit the function of polymerase during replication and DNArepairing processes. Lipid peroxidation and oxidative stress inhibit the proliferation of cell and accumulate the cell in G_2 phase of mitosisfurther it may initiate apoptosis, shrinkage**and cellular**injuries[8].
Oxidative anomalies are regulated by p53 and tyrosine kinase; which regulatetestosterone.Multinucleate formations of young spermatids in rat testes results from a failure of intercellular bridges during spermiogenesis[9]. Temporary ligation of the testicular artery cause the formation of multinucleated spermatogenic cells involves karyokinesis devoid of

cytokinesis which degenerate and germ-cell loss at spermatocyte stage[10].

Medicinal plants containcarotenoids, vitamin C, anthocyanin and polyphenols which diffuse into blood and enhance the anti-oxidative activities to reestablish the cellular capacity, prevent leakage of cytochromes and restored the mitochondrial membranes[11]. Plant steroids are recommended as dietary modifiers of serum lipids; analogous to human cholesterol. Vit-C attenuates $K_2Cr_2O_7$ induced nephrotoxicity and alterations in renal brush border membrane enzymeswhileVit-E maintains a balance in metal ions during exposure to heavy metals[12].

Syzygiumcuminialso known as Jamun or Jambul, containellagic acids,β-sitosterol, vit-C, gallic acid, anthocyanins and malvidin-diglucosides used as anti-hyperglycemic[13,14].

Cr is toxicant to humans in Pakistan where contaminated sewage water used for cultivation so it was decided mammalian modelmice should be allowed to consume Cr in drinking water, and their rehabilitation should be studied by cheapest local fruit Jamul.

MATERIALS AND METHODS

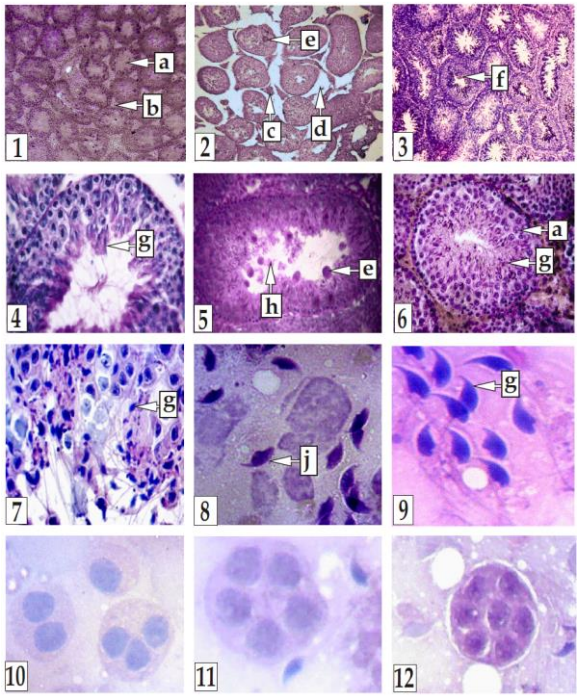
Thirty male albino mice (25-30g) were maintained under controlled conditions with free water access and standard diet, equally dividedasG1:C; control,G2: -Cr; 50ppm $K_2Cr_2O_7$ for 10days in drinking water and withdrawal for 5 days, G3: Cr-J;asG2 but given JFE by gavage (0.2 ml/12h) for next 5days. Animal were sacrificed at 16th day by cervical dislocation and testes were HE-stained and micrometry as protocols[15]. The results were expressed as mean ± SD and evaluated by ANOVA/Duncan′s multiple comparison tests.

RESULTS

Histological and Morphometric analysis: Testeshistological sections in control group specify clear boundary of ST. Spermatogoniaandspermatocytes are regularly distributed in whirls and inner most area filled with spermatozoa; heads and prominent tail were visible (Fig: 1, 4, 7). Cr exposure cause ST epitheliumdisruption, marked reduction in spermatozoa, dilatation of intercellular spaces, detachment of Sertoli cells, necrotic Leydig cells (Fig; 1, 2, 4, 5).ST showed cytoplasmic vacuolation of atrophied Sertoli cells, shrinkage and pyknotic nuclei of spermatogonia and primary spermatocytes (Fig; 2-c).

There wereprominentapoptic and exfoliated cells in Cr group(Fig; 5-e,h), while some ST showed desquamation and destruction of nuclei with multinucleated giant cells (Fig; 11,12).Spermatids losing their location and attachment oriented in different directions between the spermatogenetic cellsalong with tail-less sperm(Fig; 8-j).Cellular debris fill ST lumen with reduction of germinal lineage and dilated basement membrane (Fig. 2, 5-h). JFE ameliorate the histopahological alterations of Cr exposure with signs of restoration and rejuvenation. Spermatogonia, spermatocytes and spermatids were increased, interstial tissues rearrange and re-establish the intertubular junctions as evidenced by normal spermatozoa in testicular smear(Fig; 9, 3-f, 6-a,g).

Highest mean CSA of ST at 100×**,** mean number of Spermatogonia/area (264μ^2)at 400×**,**highest mean PBH/area, mean attached and dislocated Spermatozoa with tail length, middle piece thickness was recorded.Cr group have majority of tail-less heads of PBH, large number of spermatozoa and head of sperm were more elongated. Significant reduction of Spermatogonia and enrichment of undifferentiated dislodged spermatozoa were observed. Primary spermatocytes, PBH spermatozoa, CSA of PBH head, PBH tail length, attached embedded were decreased, but CSA, PBH middle piece thickness and testicular weight were increasedCr group (Tab; 1).



Figures: ST-mice testes (100x, 400x, 1000x, 1000x+oil emulsion).1, 4; Control, 2, 5; Cr-treated, 3,6;Cr-J, 7, 10; Testicular-smear of Control, 8; smear Cr-treated, 9; smear Cr-J, 11, 12; multinucleated spermatocytes Cr-treated. a; ST, b; interstialspace,c; Leydig's cell, d; interstial tissue, e; apoptosis, f; spermatozoa, g; PBH- tail, h; debris, j; tail-less sperm head.

DISCUSSION

Environmental chemicals exposure in the induction of various diseases is significant[16].Heavy metal concerning to reproductive toxicity in male is one of the areas of current issue and reproductive changes are

under the impact of neurotransmitters while Cr^{+6} interact and interrupt the feedback interaction. That study probes the effects of Cr (50ppm) on male mice, instead of 100ppm in drinking water accordingly Holstein and Eckmann[17].

Table No. 1:Histomorphometry, Body and Testicular Weight of Mice

Perameters	Groups		
	C	Cr	Cr-J
CSAof ST (μ^2)**	†27183.49±478.04[a]	29974.05±2373.01[b]	25702.75±633.91[a]
‡Spermatogonia /area **	65.55±5.31[a]	30.67±3.51[b]	49.97±7.81[c]
‡ Primary spermatocytes/area***	75.94±7.56[a]	25.05±2.78[b]	47.36±5.68[c]
‡PBH-spermatozoa/area**	51.26±5.87[a]	20.55±4.12[b]	22.81±0.38[b]
‡PBH CSAhead (μ^2)**	14.31±0.63[a]	8.31±0.65[b]	13.76±1.92[a]
‡PBH tail length (µ)*	85.57±10.69[a]	76.81±11.89[a]	86.69±2.45[a]
‡PBH Middle piece. DM (µ)**	0.09±0.56[a]	0.56±0.97[b]	0.93±0.14[a]
‡ Attached spermatozoa/area***	72.25±3.39[a]	14.09±6.54[b]	23.45±2.69[c]
‡Dislodged spermatozoa/area**	3.00±1.78[a]	44.37±12.70[b]	3.15±1.89[a]
Mice Weight(g)**	27.99±0.39[a]	22.78±1.70[b]	26.90±0.23[a]
Testicular Weight(g)**	0.07±0.29[b]	0.08±0.49[b]	0.04±0.80[a]

C: Control. Cr: Chromium Exposure, Cr-J: chromium-JFE.CSA(μ^2) of ST at 100×.

a, b, c, d. Duncan's Multiple Range-post hoc analysis. ‡ Relative Abundance per /264μ^2 at 400×.†means ±SEM, n=10, µ = µm, *: p≤0.05-0.01, **: p≤0.001,***: p≤0.0001, ANOVA: two factors without replication.

Cr^{+6} damage testes by two ways: one mediated by apoptosis and other by necrosis of Leydig cells like cadmium[18]. The energy deficiency due to sugar unavailability during metabolism and destruction of mitochondria in Cr^{+6} exposure groups cause the diminution of germinal epithelium and elevate desquamation. Sertoli cells necrosis leading to the supplanting spermatogenic cells, indicate the sign of oxidative stress. The tight junctions of Sertoli cells may temporarily open to permit the passage of spermatogenic cells. Sertoli cellsare responsible to bind testosterone to androgen receptor and activate the spermatogenesisalong with regulation of apoptosis. The loss of interstitial cells brings about the permanent loss of androgenic steroids; affects spermatogenesis andspermiogenesis. Crexposure (2mg/kg-15days) led to Cr accrual, leakage of Sertoli-cell junctions, with cytoplasmic vacuolations and mitochondrial deteriorations.

Abnormal sperm heads, tail-less spermatozoa, and extricate appearance indicate the possible distortions at androgen receptor during terminal differentiationand annihilation of Sertoli cells[19]. Normal rodent sperm contain one or more apical regular symmetrical hooks[20]and significant abnormality in sperm head were more recurrently in Cr^{+6} exposure animals, than control, specify possible alteration to the spermatogenic process. Analysis at micrometric level of spermatozoa specified the alterations in CSA, thickness of mid-piece, length of the tail and loss of polymerization of micro-tubular array into sperm microtubules indicate the mark of Cr^{+6} toxicities. There is damage to endothelial cells of capillaries and Leydig cells at intertubular spaces of ST, which revealeddamaged blood vessels and degeneration of Leydig cells. Spermatocytes appeared to be arrested necrotic spermatocytes and germ cell shedding as debris in ST lumen accordingly[21,22].

Multinucleated cells indicateimpaired spermatogenesis, spermatocytes and spermatid giant cells with oligozoospermia, due to defects of intercellular bridges, germ cell degeneration and absence of G_1 and S phases or may be associated with spermatogonial stem cell apoptosis[8]. Anabolic androgenic steroids can be adapted and ameliorated by drugs and pharmacological plants products. These findings are accordingly Abdallaet al.,[23]who observed Syzygiumcumini**have antagonistic behavior**against methylmercury induced systemic toxicity. The JFE has been found convincingly addressed the histopatholgical and micrometric derangement of Cr^{+6} exposures. The above mention results strongly support the use of JFEupon the pathological manifestation of environment toxicants mainly heavy metals and most specifically of Cr^{+6} exposures as immense mitigating potential due to anthocyanin, β-sitosterol and α-tocopherol. Vit-E is lipid-soluble antioxidant molecules and interjects the chain reactions of lipid peroxidation and cause the augmentation of free radicalsscavengesactivity. JFEas blue-green algae normalized the ST and testicular weight, moderately improved the sperm head CSA and increased the length of sperm tails[24]. Debris from ST lumen and interstitial portion removed, spermatogoniaredevelop and significantly elevate thenormal spermatozoa which indicate the ameliorative actions of JFE.

CSA of ST normalized specifies the ameliorative effect against testis impairments and this may be arbitrated by its potent antioxidant activities.The development of spermatozoa tail in spermatids depends upon signals provided by Sertoli cells, may activated by the JFE pharmacological components. The rehabilitated Sertoli cell keeps the spermatozoa till the completion of normal

spermiogenesis.JFE administration showed a remarkable protective effect in all aspects as evident from significant recovery from histological alterations and has ability to inhibit ROS by preventing testicular tissue peroxidation. JFE protect the Sertoli cells by stimulating Leydig cells to produce testosterone. Antioxidants in JFE work against metals and ultimately remove toxicities by diminishing their oxidative flux as efficient metal chelators and apoptosis preventing abilities. Syzygiumcuminilike L-carnitine capsule contain anthocyanins which can boost metabolism without any alteration in cecal microbial composition and not destroying the intestinal microbiota; essential for rehabilitation[25]

CONCLUSION

The modern allopathic medicines have limited therapeutic options and have less successful results due to their side effects while pure herbal compounds are harmless and must be used as an alternative way to cure diseases.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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