

Potential reproductive health effects and oxidative stress associated with exposure to potassium dichromate ($K_2Cr_2O_7$) and magnesium sulphate ($MgSO_4$) in male mice

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ABSTRACT

Objective: To investigate the potential harmful effects of potassium dichromate and magnesium sulphate causing oxidative stress and reproductive toxicity in adult male mice model.

Methods: The experimental work was conducted on sixty male mice (*Mus musculus*) divided into three groups. Mice in group B and C received potassium dichromate and magnesium sulphate of 5.0 and 500 mg/Kg body weight/ml respectively, for sixty days. The blood sample was analyzed to assess oxidative stress and cellular damage.

Results: Results showed high malondialdehyde (MDA) and low levels of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)] in both potassium dichromate and magnesium sulphate administered groups as compared to control group. Reduced number of sperm count and excessive destruction of testicular follicles, including destruction of spermatids, leydig cells and sertoli cells, were also seen in both groups.

Conclusion: We concluded from present study that potassium dichromate and magnesium sulphate causes oxidative stress by generation of reactive oxygen species (ROS) and causing DNA damage in testicular cells leading to adverse reproductive abnormalities.

KEY WORDS: Magnesium sulphate ($MgSO_4$), Malondialdehyde (MDA), Oxidative stress, Potassium dichromate ($K_2Cr_2O_7$), Superoxide dismutase (SOD).

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INTRODUCTION

Worldwide distribution and extensive use of chemical agents, is associated with concern of the highest priority for environmental and industrial exposure which may have imposing effects on

male reproductive function. Chromium has been identified to be one of these toxic metals. Its common salt, potassium dichromate ($K_2Cr_2O_7$) is most commonly used as an oxidizing agent in various laboratory and industrial applications.^{1,2} Chromium VI is more toxic than in trivalent form because it readily enters the cells producing various pathological conditions, including reproductive dysfunction, induce toxicity provoke lipid peroxidation, DNA damage, cytotoxicity, mutagenesis and carcinogenesis.³ Magnesium sulfate ($MgSO_4$) is a chemical compound often encountered as the heptahydrate epsomite ($MgSO_4 \cdot 7H_2O$) commonly called Epsom salt, is used as a first line of treatment in the majority of cases. It is used in replacement therapy for hypomagnesaemia and as a

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bronchodilator in severe exacerbations of asthma. It is commonly administered via the intravenous route for the management of severe asthma attacks.^{4,5}

Lipid peroxidation is one of the major outcomes of free radical-mediated to cellular injury or beneficial biological effects. $K_2Cr_2O_7$ and $MgSO_4$ both induce oxidative stress due to which high lipid peroxidation occurs and in result MDA level is increased and antioxidant enzymes SOD and CAT activity is decreased.^{6,7} The evidence indicates that the careless use of toxic heavy metals in the past twenty years have shown very alarming trend in male reproductive health.⁸ Testicular tissues are major target organ for metals that induce oxidative damage because of its high contents of polyunsaturated membrane lipids. Ingestion of hexavalent chromium compounds produces uncertain levels of degeneration in the outer most cellular layers in several somniferous tubules, reducing the number of sperm count and spermatogonia per tubule, leading to considerable increases in the morphologically abnormal sperms percentage.^{9,10} Oral administration of vanadyl sulphate for 60 days caused a decrease in the weights of testes, accessory reproductive organs and the diameter of seminiferous tubules and leydig cells nuclei are reduced.¹¹ The aim of this research work was to check the adverse effects of $K_2Cr_2O_7$ and $MgSO_4$ on the testes of adult male mice.

METHODS

Experimental design: Sixty adult male mice were included in the study, divided into three groups, comprising twenty mice in each group with average weight ranging from 25-40 gm. Group A served as control, while in group B mice were treated with $K_2Cr_2O_7$ and in group C mice were administered with $MgSO_4$. All the laboratory work was performed at the Institute of Molecular Biology & Biotechnology, the University of Lahore, during March 2013 to September 2013.

Administration of $K_2Cr_2O_7$ and $MgSO_4$: $K_2Cr_2O_7$ was administrated at the dose of 5mg/Kg body weight while $MgSO_4$ was administrated at the dose of 500mg/Kg body weight.¹² Both the salts were administrated orally for the period of 60 days.

Samples collection and sample analysis: 2 ml blood was taken from each mouse at 1st, 30th and 60th day of the experiment and serum of the sample was separated by centrifugation at 3000 rpm. Then sample were processed and analyzed for the estimation of MDA, SOD, CAT and GPx activity.

Estimation of MDA: Thiobarbituric acid reactive substance test was used for the estimation of MDA

level in serum.¹³ Total 1ml of serum was taken and a 10% (w/v) homogenate was prepared in 10 mM buffer (pH 7.4). The supernatant was used for immediate thiobarbituric acid reactive substances test. In this test 200 μ l of serum sample, 200 μ l of 8.1% sodium dodecyl sulfate (SDS), 1.5ml of 0.8% TBA, 1.5ml of 20% acetic acid solution (pH 3.5) and 4.0ml distilled water and 5.0 ml of n-butanol were used and absorbance at 532nm was recorded.

Estimation of SOD: All procedure for estimation of SOD was performed in ice bath.¹⁴ Blood serum was taken and homogenate was prepared in 50% TCA and centrifuged at 13000 rpm. The supernatant was used for immediate SOD and other enzyme activity evaluation. 100 μ l of serum sample, 1.2ml of sodium phosphate buffer (pH 8.3, 0.052M), 100 μ l of phenazine methosulphate (186 μ m), 300 μ l of nitro blue tetrazolium (300 μ m), 200 μ l of NADH (750 μ m) and 4.0 ml of n- butanol were used and absorbance was recorded at 560nm.

Estimation of CAT activity: Catalase activity was measured by the method of Aebi.¹⁵ The supernatant was used for the estimation of catalase. The rate of decomposition of H₂O₂ was measured at 240nm.

Estimation of GPx activity: Glutathione peroxidase was determined by homogenizing 0.1 ml of serum with 2.4ml of 0.02 M EDTA first and then the test tubes were kept in an ice bath for 10 minutes. 2ml of distilled water and 0.5 ml of TCA (50%) was added and was kept in an ice bath for 10-15 minutes. The mixture was centrifuged at 3000-3500rpm for 10 minutes. 1ml of the supernatant was taken in test tube and 2ml of 0.15M Tris HCL plus 0.05ml DTNB were added in it and absorbance was taken at 412nm.

Histopathology of testicles: Two mice from each group were randomly selected for histopathology of testis at 1st, 30th and 60th day of the experiment. These mice were sacrificed and the specimens from testicular tissues were fixed in 10% neutral buffer formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casted, blocked, cut at 2-5 μ m thickness and stained with hematoxylin-eosin for microscopic examination.¹⁶

Statistical analysis: All the data thus obtained was statistically analyzed by applying one way analysis of variance (ANOVA). The differences of the means were considered significant at $p < 0.05$.

RESULTS

MDA levels in male mice receiving $K_2Cr_2O_7$ and $MgSO_4$: MDA levels in different groups at different days were studied. It was concluded from the

Table-Ia: MDA levels in different groups at different days.

Days	Groups	Means \pm SD ($\mu\text{mol/ml}$)	(P-Value)
1st day	A	24.64 \pm .34	0.426
	B	24.16 \pm .30	
	C	24.48 \pm 0.56	
30th day	A	24.49 \pm 0.31	0.004*
	B	26.80 \pm 0.76	
	C	26.57 \pm 0.20	
60th day	A	24.50 \pm 0.62	0.001*
	B	27.69 \pm 0.25	
	C	26.78 \pm 0.21	

*Significant (p < 0.05)

Table-Ib: SOD levels in different groups at different days.

Days	Groups	Means \pm SD ($\mu\text{g/ml}$)	(P-Value)
1st day	A	28.77 \pm 1.18	0.329
	B	28.70 \pm 0.65	
	C	28.27 \pm 0.64	
30th day	A	28.35 \pm 1.13	0.004*
	B	26.16 \pm 0.43	
	C	19.43 \pm 2.04	
60th day	A	28.51 \pm 0.48	0.001*
	B	22.82 \pm 0.91	
	C	22.44 \pm 5.08	

*Significant (p < 0.05)

descriptive statistics that at first day insignificant differences in MDA levels between all the groups were observed as compared to control group (Table-Ia). While significant differences in MDA levels were observed in all the groups at 30th and 60th day as compared to control group. The results showed that both of these salts cause significant elevation in MDA levels, resulting in high lipid peroxidation. **SOD levels in male mice receiving K₂Cr₂O₇ and MgSO₄:** Further, SOD levels in different groups at different days were studied. The SOD level decreased significantly in the groups B and C at 30th and 60th day as compared to control groups (Table-Ib). It is concluded that both of these salts cause significant depletion of SOD levels, resulting in high lipid peroxidation. SOD level in MgSO₄ treated mice is even lower than with K₂Cr₂O₇ treated mice.

Levels of CAT in male mice receiving K₂Cr₂O₇ and MgSO₄: Table-IIa shows the CAT levels in different groups at different days. Significant differences in CAT levels were observed in all the groups at 60th day as compared to control group. CAT level was found to be more reduced with MgSO₄ as compared to K₂Cr₂O₇.

GPx level in male mice receiving K₂Cr₂O₇ and MgSO₄: Table-IIb shows the GPx levels in different

Table-IIa: Catalase levels in different groups at different days.

Days	Groups	Means \pm SD ($\mu\text{mol/mol of protein}$)	(P-Value)
1st day	A	191.47 \pm 0.62	0.147
	B	190.41 \pm 0.69	
	C	191.29 \pm 0.44	
30th day	A	190.78 \pm 0.98	0.357
	B	183.63 \pm 6.70	
	C	173.30 \pm 2.79	
60th day	A	190.52 \pm 0.94	0.005*
	B	186.52 \pm 1.32	
	C	179.53 \pm 4.08	

*Significant (p < 0.05)

Table-IIb: Glutathione peroxidase levels in different groups at different days.

Days	Groups	Means \pm SD ($\mu\text{mol/g protein}$)	(P-Value)
1st day	A	33.5 \pm 0.62	0.321
	B	32.7 \pm 0.69	
	C	34.9 \pm 0.44	
30th day	A	31 \pm 0.98	0.005*
	B	19.9 \pm 6.70	
	C	09.7 \pm 2.79	
60th day	A	31.4 \pm 0.94	0.001*
	B	15.3 \pm 1.32	
	C	07.7 \pm 4.08	

*Significant (p < 0.05)

groups at different days. Significant differences in GPx levels were observed in all the groups at 30th and 60th day as compared to control group. So it was concluded that both of these salts cause significant decrease of GPx levels, resulting in high oxidative stress. In MgSO₄ treated mice the GPx level reduced substantially, making it more lethal than K₂Cr₂O₇.

Morphological analysis of Testicular tissue: We also studied the morphology of the cross sectional structure of testicles of normal follicles with intact germinal layer and leydig cell and the results showed complete destruction of leydig cells, partially damaged germinal layer and destruction of sperms leading to empty spaces in the lumen of follicle of testicles in which 30% destruction of follicle was observed in the group at 30th day after the administration of K₂Cr₂O₇ and 35% destruction of follicle was observed in the group at 30th day after administration the MgSO₄ (Fig. 1a, b, c). Furthermore, 55% destruction of follicle was observed in this cross section of group B at 60th day after administration the K₂Cr₂O and 64% destruction of follicle was observed in this group at 60th day after administration the MgSO₄ (Fig. 2a, b, c).

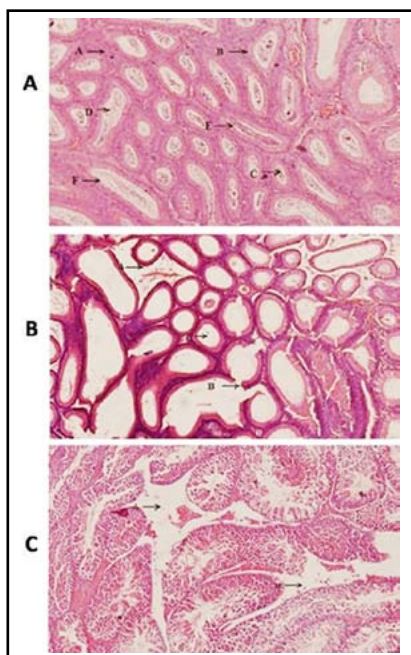


Fig.1: a) Cross section of testicle of mice of control group at 30th day of experiment. A. Leydig Cells, B. Germinal Layer, C. Spermatids, D. Spermatocytes, E. Spermatogonia, F. Sertoli cell.
 b) Cross section of testicle of mice of group B at 30th day. A. Destruction of Leydig cells, B. Partially damaged germinal layer, C. Empty central lumen of follicle of testis without sperms.
 c) Cross section of testicle of mice of group C at 30th day. A. Destruction of Leydig cells B. partially damaged germinal layer.

DISCUSSION

Hexavalent chromium is very toxic and readily enters the cells and induces toxicity provoke lipid peroxidation, DNA damage, cytotoxicity, mutagenesis and carcinogenesis. $MgSO_4$ effects on the lipid peroxidation and significantly increases MDA.^{3,6} The results of lipid peroxidation, MDA, SOD, CAT and GPx showed significant difference in MDA, SOD, CAT and GPx levels in $K_2Cr_2O_7$ and $MgSO_4$ administrated group after 30th and 60th days treatment. The outcomes were in line with the Goulart *et al.*¹⁷. However, further research is needed to estimate the effect of $MgSO_4$ on lipid peroxidation. In $K_2Cr_2O_7$ and $MgSO_4$ administrated group ROS were produced and these reactive molecules are able to remove hydrogen from the lipid membrane and imitating a series of reaction leading to the membrane destruction.

Salts of heavy metals when ingested in high dose produced oxygen reactive species which may lead to adverse clinical outcomes such as DNA damage,

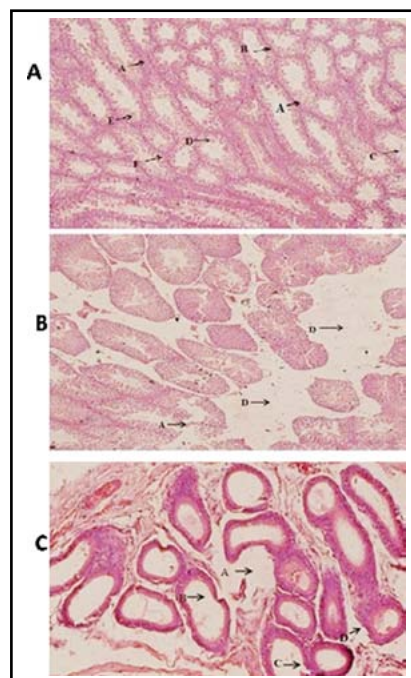


Fig.2: a) Cross section of testicle of mice of control group at 60th day of experiment. A. Leydig Cells, B. Germinal Layer, C. Spermatids, D. Spermatocytes, E. Spermatogonia, F. Sertoli cell.
 b) Cross section of testicle of mice of group B at 60th day. A. Destruction of Leydig cells and D. destruction of complete follicles.
 c) Cross section of testicle of mice of group C at 60th day. A. Destruction of Leydig cells, B. Empty central lumen of follicle of testis without sperms, C. partially damaged germinal layer D. destruction of complete follicle.

destruction of testicular cells and germ line of testis at initial levels. Findings of the present study represent similar results by investigating the destruction of germ cells, spermatocytes, spermatogonia, sertoli cells, sperms and ultimate's whole follicles after administration of the both the salts for two months. However, heavy destruction was observed in the $MgSO_4$ administrated group (being 35% and 66% at 30th and 60th day respectively) as compared to $K_2Cr_2O_7$ administrated group where 30% destruction of follicles were observed at 30th day and 56% destruction of follicle at 60th day of experiment as previously explained by Acharya *et al.*⁹

The destructive effects of both the salts on testicles of male mice could be due to generation of ROS in exposure to the $K_2Cr_2O_7$ and $MgSO_4$. Oxygen reactive species generated are active in causing DNA damage. The DNA damage in soft tissue of follicles leads to excessive destruction of follicles, germ line leading to severe reproductive abnormality resulting in production of sterile

individuals.¹⁸ The significant changes in lipid peroxidation and testicular histology suggested that $K_2Cr_2O_7$ and $MgSO_4$ exposure increase the level of lipid peroxidation leading towards generation of ROS and severe destructive consequences of testicular cells in male mice.

CONCLUSION

We conclude that $K_2Cr_2O_7$ and $MgSO_4$ have a deleterious effect on the histology of the testis of male mice. The effects of $MgSO_4$ found to be more lethal and destructive as compared to $K_2Cr_2O_7$. Thus, we suggest further detailed studies in human to corroborate these findings and assuming that $K_2Cr_2O_7$ and $MgSO_4$ at normal dose could be a potential male anti fertility agent. The self medication involving $K_2Cr_2O_7$ and $MgSO_4$ should also be discouraged.

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Conflict of interest: The authors have no competing financial interests.

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Authors Contributions:

AM, KZ and MR: Designed the study. **UH, AM, and MA:** Collected the data and performed the experiments. **MHQ, MIN, MR and AM:** Analyzed the data critically, performed statistical analysis and wrote the manuscript.

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