Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/4832
DOI URL: http://dx.doi.org/10.21474/IJAR01/4832
ISSN NO. 2320-5407

RESEARCH ARTICLE

PROTECTIVE/ POTENTATIVE ACTION OF CAFFEINE IN CHROMIUM TREATED SWISS MICE MALE GERM LINE CELLS.

Manoj Kumar Panda[1], Rabi Narayan Pradhan and *U. R. Acharya[3].

1. Lecturer in Zoology, U.P. Science College, Sheragada, Ganjam, Odisha.
2. Lecturer in Zoology, Nuapada College, Ganjam, Odisha.
3. Retd. Professor, Department of Zoology, Berhampur University Berhampur-760007.

Manuscript Info

Manuscript History

Received: 13 May 2017
Final Accepted: 15 June 2017
Published: July 2017

Key words:-
Chromium, Swiss Mice, testis, lipid peroxidation, sperm count, sperm abnormality, Oxidative stress, ROS, Caffeine.

Abstract

Chromic acid, a hexavalent compound of chromium is known to induce reproductive dysfunctions in male laboratory animals and humans. Reduced semen quality, formation of morphologically abnormal sperm population and significant reduction in sperm count are the chief reproductive deformities resulted due to significantly higher oxidative stress accompanied with marked decrease in antioxidant enzymes in the testes of Cr-exposed individuals. In the present study, an attempt has been taken to investigate the Protective/Potentiate effect of caffeine, a plant product, in reducing Chromium induced testicular cytotoxicity in chromium treated Swiss mice. In this contest, three sub-lethal doses of CAF 25, 50 & 100 mg/kg body weight were tested in mouse followed by Chromic acid injection. Results indicate that CAF pretreatment in 25 mg/kg potentially reduced oxidative stress in terms of lipid peroxidation (LPP), marked increase in sperm count and significant decline in abnormal sperm population. Contrarily, Cr- treated mice pretreated with 100 mg of CAF reflected in higher LPP compared to controls. Also, population of aberrant sperm was significantly higher with a significant decrease in sperm count compared to controls. Furthermore, the efficacy of CAF is also compared with other antioxidant vitamins like Vit-C &Vit-E. It is also inferred that lower dose of CAF is comparable to VIT-E rather that Vit-C in ameliorating the Cr-induced testicular dysfunction in mice.

Introduction:-

Chromium (Cr) is metal of transitional element found in different oxidation states. Chromium (IV) compounds are mainly used in the preparation of industrial and household based Products including welding, painting and steel manufacturing (Katz and Salim, 1994). Occupational, industrial, environmental, therapeutic and dietary exposures to a wide range of chemicals and heavy metals have harmful effects on male fertility (Cheek and McLachlan, 1998; Pant *et al*., 2003). A number of investigations using laboratory animals have pointed out testicular toxicity of Cr VI (Behari*et al*., 1978; Ernst, 1990; Saxena*et al*., 1990; Murthy*et al*., 1991; Ernst and Bonde, 1992; Chowdhury and Mitra, 1995; Sutherland *et al*., 2000).

Corresponding Author:-U. R. Acharya.
Address:-Retd. Professor, Department of Zoology, Berhampur University Berhampur.

Chromium (Cr) is one of the major industrial wastes produced from many industries like textiles, tanneries, electroplating, metallurgical which causes health issues in humans and animals and also affects marine life (Ajmal et al., 1996, Moncur et al., 2005). These compounds are also proven mutagens & carcinogens affecting animals including humans; due to their oxygen mediated cytotoxicity (Stoh and, Bagchi, 1995). Chromic acid, a hexavalent compound of Chromium (Cr) widely used in different industries has been associated with reproductive abnormalities in male Swiss mice. Metal induced Reactive Oxygen Species (ROS) may impair spermatogenesis or alter sperm morphology and defective sperm formation (Xu *et al.*).

In the present study Chromium treated animals show a significant increase in a lipid peroxidation, an index of oxidative stress was measured in the form of malondialdehyde content (Bagchi*et..al*. 2002), significant decrease in enzyme activity of catalase and peroxidase, significant decrease in sperm count and increased formation of abnormal sperms. In this study, an attempt has been taken to ameliorate the deleterious effects of chromium in the testes of Swiss mice by supplementation of caffeine (CAF), the 1,3,7 trimethyl xanthine, a biologically important constituent of coffee, green tea and coca that neutralize the action of chromium. Polyphenols are the most significant group of tea components and have wide range of pharmaceutical properties including antioxidative, anticarcinogenic (Dufresne and Franworth 2001; Atoul*et.al*. 2005). The study explained the oxidative stress related testicular dysfunction identifying the possible adverse effect of chromium on sperm count and sperm abnormality and focus on the protective/potentative activity of CAF in different doses treated to chromic acid induced mice.

Material and Methods:-

Swiss albino male mice at 10 week of age were used in the present study which was acclimated to laboratory condition with balanced diet and water. Chromic acid at 99% purity manufactured by Qualigens Glaxo India ltd. and CAF manufactured by LOBA Chime Pvt. Ltd. were used as the test chemicals.

Experimental Protocol:-

From among the acclimated stock of mice, 48 nos. of healthy male mice were used for the study. The control groups of mice were intraperitoneally injected with normal distilled water. Experimental groups of mice (n=6nos.) were injected with chromic acid 1mg/kg body weight through the same route. Three chromic acid treated groups of mice were injected with Caffeine @25, 50,100mg/kg body wt. prior to one hour of chromic acid treatment. Both treated and control mice were cased in the laboratory conditions and were sacrificed by cervical dislocation at 24 hrs, 4^{th} week and 8^{th} week of post treatment. After sacrifice, testes were recovered cleaned of accessory tissues and subjected to the estimation of lipid peroxidation, enzyme activity studies of catalase and peroxidase. Sperms were collected from vas deference and utilized for sperm count and sperm head abnormalities studies following standard procedures.

Biochemical Parameters:-

The testes were dissected from the accessory tissue under ice cold normal saline. A portion was processed for lipid peroxidation potential (LPP) by thio-barbituric acid (TBA) test (Stroev and Mokarova 1989). LPP was expressed in terms of µ mol/g wet weight. Tissue samples were gently homogenized in phosphate buffer, pH 7.4 at 0-4^{0}C using glass potter type homogenizer at 500-800rpm and the resultant supernatant was immediately used for measuring enzyme activities like Catalase (CT) and Peroxidase (PD). Data are expressed as units (U) of enzyme/mg of tissue protein. PD activity was determined by following a standard method. CT activity was estimated spectrophotomatri cally at 570 nm.

Sperm Parameters:-

Starting from the 24^{th} , 4^{th} week and 8^{th} week of post treatment a group of six mice were scarified by cervical dislocation for preparation of slides to analyze sperm head abnormalities and sperm counting following the procedure as follows:

The vas deference were dissected out and kept in phosphate buffer saline (PBS) solution. The sperm were squeezed out from the vas deferens in PBS at room temperature aspirated gently by pasture pipette and left for five minutes. It was centrifuged for one minute at 1000 rpm and the supernatant was discarded. A small amount of PBS was added and aspirated gently to prepare a thick homogenous suspension of sperm in PBS. A small drop of sperm suspension was taken on clean grease-free slide smeared gently with a glass rod and left overnight for natural drying. The dry slides were stained with 10% Giemsa diluted in fresh Sorenson's Buffer (pH-6.8) for one hour. The stained slides were washed in tap water and observed under microscope. About 1000 sperms for each specimen were scanned.

Morphologically abnormal sperm were recorded following Wyrobeck and Bruce (1975). For sperm counting, sperm suspension was taken on the haemocytometer and the number of sperm heads was counted on R. B. C. counting chamber. The slides prepared at different end points were coded separately. The students 't' test was utilized for comparison of data between control and experimental groups. The difference was considered significant at the $P \leq 0.05$ level. The data are reported here as mean ± SEM.

Results:-

The results of the present study indicate significantly higher malondialdehyde content, an index of oxidative stress in the testes of chromium treated Swiss mice at 24 hrs, 4th week and 8th week of post treatment. Activity of catalase and peroxidase enzymes also decline significantly (Graph I, II and III). In CAF pretreated mice group (25mg/kg body wt.) oxidative stress declined with a significant increase in catalase and peroxidase enzyme activity. However in chromium treated mice groups pre-treated with 100mg/kg body wt. CAF, increased oxidative stress and significantly decline catalase and peroxidase activity absorbed. But, in chromium treated mice group pre treated with 50 mg/kg body wt. CAF, an insignificant rise in oxidative stress and significantly increased ($p \leq 0.05$) catalase and peroxidase enzyme activity were noticed.

Sperm count studies indicated a significant ($p \leq 0.01$) fall in chromium treated mice in all post treatment phases compared to control groups. However chromic acid treated mice group pretreated with 25mg/kg b.w. of CAF indicated significant rise ($p \leq 0.01$) in sperm count in all post treatment stages. Significant ($p \leq 0.01$, $p \leq 0.05$) sperm count decline were observed in chromic acid treated mice groups pretreated with 100 & 50mg/kg b.w. CAF respectively (Graph IV).

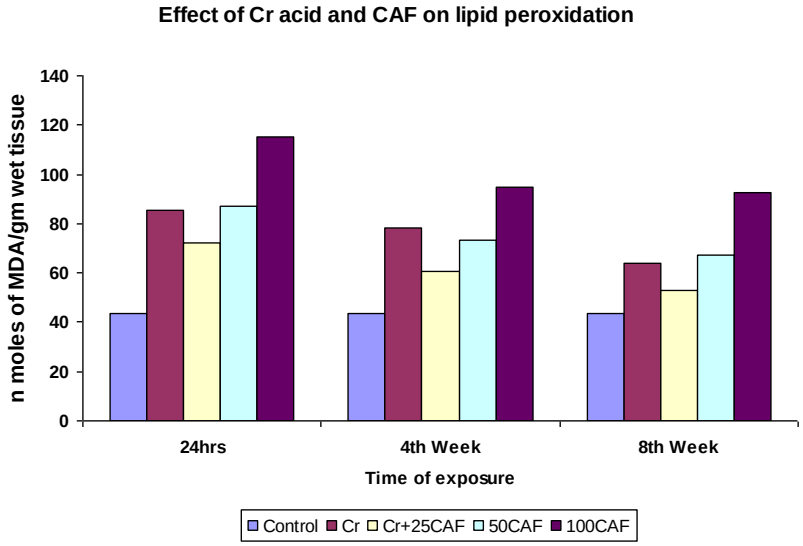
Study of sperm head abnormalities was done following standard procedure (Wyrobek and Bruce, 1975). Percentage of abnormal sperm population increased significantly ($p \leq 0.01$) in Cr treated mice compared to control groups. However, CAF treatment (25mg/kg b wt.) significantly ($p \leq 0.01$) declined abnormal sperm population chromium treated mice, 100mg of CAF treatment increased significantly ($p \leq 0.01$) the occurrence of deformed sperms (Graph V).

Discussion:-

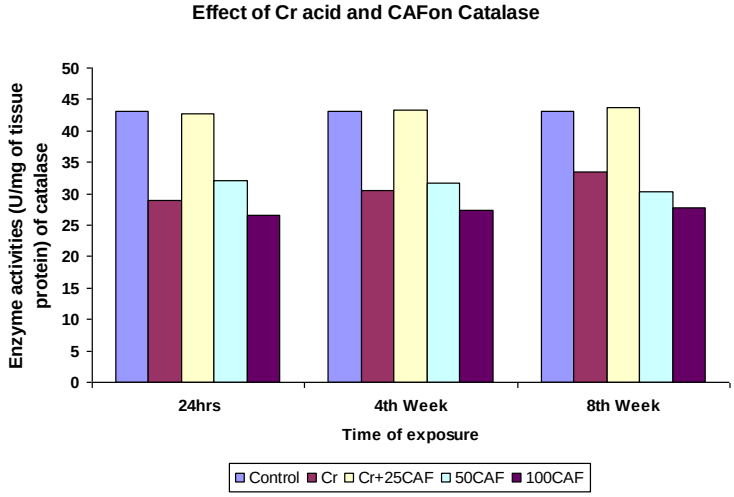
Increased oxidative stress in chromic acid treated mice indicates the generation of singlet oxygen species which allegedly known to damage the membrane system and detrimentally affected the macromolecules. As a result of this the activity of catalase and peroxidase enzymes are drastically affected. The reactive oxygen species also damaged the membrane system of the spermatogonial cells, spermatocytes and mature sperm membranes indicating a significant decline in sperm count that cause partial infertility (Acharya et. al. 2004). The membrane of post meiotic sperm population was also affected which is noticed in the drastic fall in sperm count at 24hrs of post treatment. Similarly the reactive radicals have possibly destroyed the spermatogonial stem cells resulting in a drastic sperm count decline at 8th week post treatment. The reactive oxygen species generated through chromium catalysis also have induced mutations in spermatogonial stem cell population resulting in structural transformation as abnormal sperm which is indicated in the significant rise in sperm abnormality percentage at 8th week. The presence of abnormal sperm population during 24hrs and 4th week of post treatment demonstrate the steady detrimental effects of oxyradicals on developing spermatogonial cells. Alternatively, free radicals can initiate apoptosis within the sperm, leading to caspase-mediated enzymatic degradation of the DNA (Kemal Duru et al., 2000; Wang et al., 2003; Moustafa et al., 2004; Villegas et al., 2005). Several investigators (Kodama et al., 1997; Aitken et al., 1998; Saleh et al., 2002b; Oger et al.,2003; Wang et al., 2003; Henkel et al., 2005; Kao et al., 2007)have now confirmed the link between oxidative stress and sperm DNA damage using various techniques such as terminal deoxynucleotidytransferase-mediated dUTP nick-end labeling (TUNEL), sperm chromatin structure assay (SCSA) and measurement of the byproduct of DNA oxidation, 8-hydroxydeoxyguanosine(8-OHdG).

CAF pretreatment particularly in lower doses seems to be affective in neutralizing the chromium induced oxyradicals, by protecting the antioxidant enzymes like catalase and peroxidase. It is supposed that those antioxidant activities may be due to high level of total phenolic compounds (Hwang et. al. 2010). The protective behavior of CAF is also visualized in the increase of sperm count and decrease in sperm abnormality percentage. The probable mutational affects incurred by chromium induced oxyradicals are neutralized by lower dose of CAF hence significant decline in sperm abnormality is observed. However higher doses of CAF seems to be detrimental in increasing oxidative stress in Cr treated mice. The activity of catalase and peroxidase were also declined.

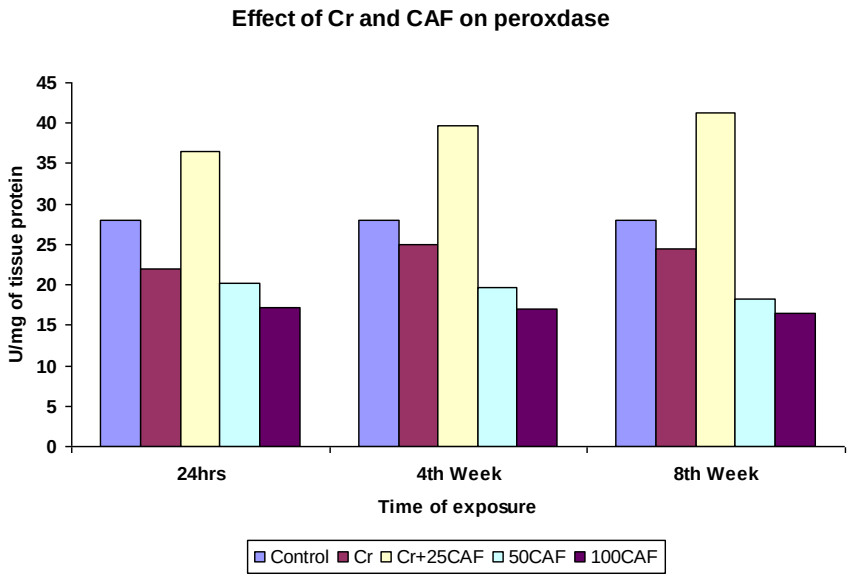
Furthermore, the potentative activity of CAF at higher doses is linked with increased sperm abnormality and decreased sperm count profile.



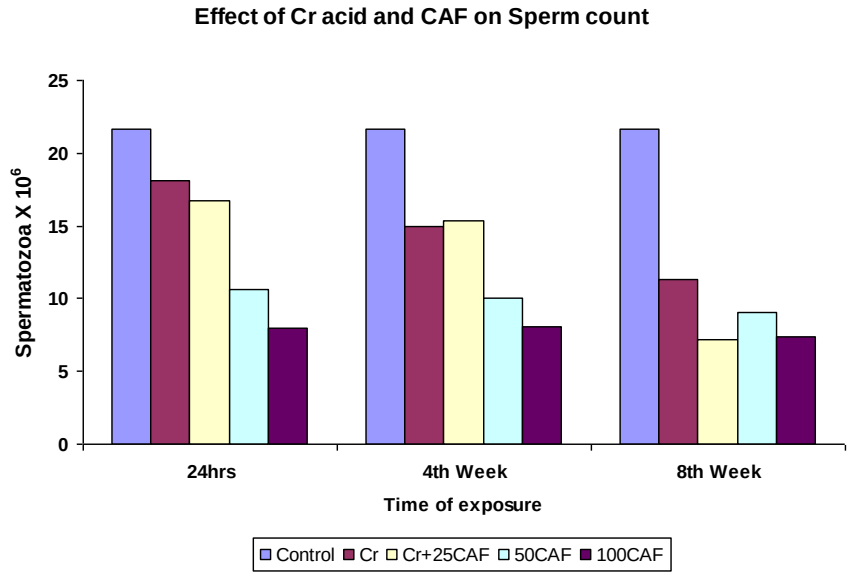
Graph I: -Effect of Cr acid (1mg/kg b wt.) and Cr acid + CAF (25mg/50mg and 100mg/kg b wt.) on lipid peroxidation (n moles of MDA/gm wet weight) of the testes of Swiss mice.



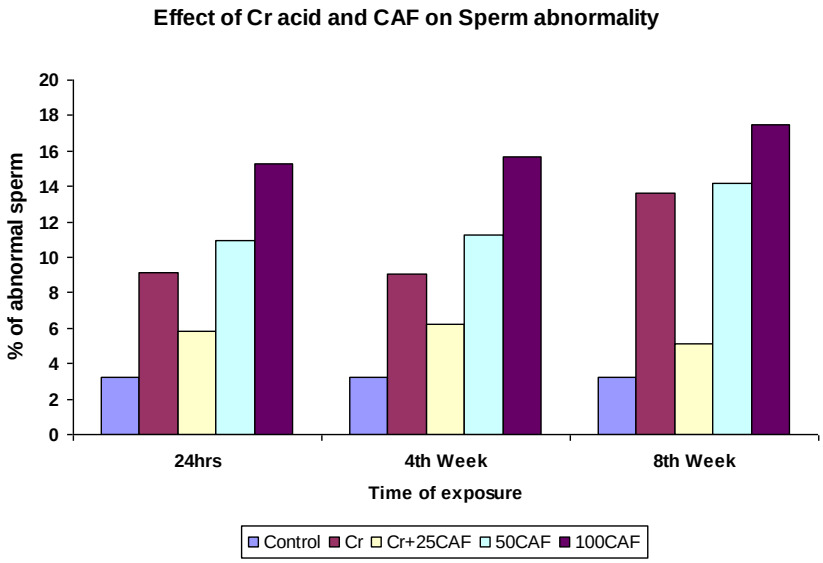
Graph II: - Effect of Cr acid (1mg/kg b wt.) and Cr acid + CAF (25mg/50mg and 100mg/kg b wt.) on the activity of Catalase enzyme (U/mg of tissue protein of the testes of Swiss mice.



Graph III: - Effect of Cr acid (1mg/kg b wt.) and Cr acid + CAF (25mg/50mg and 100mg/kg b wt.) on the activity of Peroxidase enzyme (U/mg of tissue protein) of Swiss mice.



Graph IV: - Effect of Cr acid (1mg/kg b wt.) and Cr acid + CAF (25mg/50mg and 100mg/kg b wt.) on sperm count (in millions) the testes of Swiss mice



Graph V:-Effect of Cr acid (1mg/kg b wt.) and Cr acid + CAF (25mg/50mg and 100mg/kg b wt.) on sperm abnormality (%) of Swiss mice.

Acknowledgements:-

Authors are thankful to Head, Dept. of zoology for providing laboratories facilities.

References:-

1. Acharya UR, Acharya S, Mishra M. Lead acetate induced cytotoxicity in male germinal cells of Swiss mice. Ind Health 2003; 41:291–294.
2. Acharya UR, Mishra M, Mishra I, Tripathy RR, Potential role of vitamins in chromium induced spermatogenesis in Swiss mice. Environ ToxicolPharmacol 2004.
3. Acharya UR, Mishra M, Tripathy RR, Mishra I. Testicular dysfunction and antioxidative defense system of Swiss mice after chromic acid exposure. ReprodToxicol 2006; 22:87–91
4. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. BJU Int. 2005; 95:503–507.
5. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, Irvine DS. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. BiolReprod 1998; 59:1037–1046.
6. Ajmal, M., Rao, W.A. and Peyton, J.N.: Effect of carbon and energy sources on bacterial chromate reduction. Bioremed. J., 1996; 6: 205-215.
7. Atoul AK, Mansouri A, Boskou G, Kerflas P. Tea and herbalinclusions: Their antioxidant activity and phenolic profile. Food chem., 2005; 89: 27-36
8. Bagchi D, Stohs Downs BW, Bagchi M, preuss HG. Cytotoxicity and oxidative mechanism of different forms of chromium. Toxicol 2002; 180: 5-22
9. Behari J, Chandra SV and TandonSK . Comparative toxicity of trivalentand hexavalent chromium to rabbits. III. Biochemical and histologicalchanges in testicular tissue. ActaBiol Med Ger. 1978; 37:463–468
10. Cheek AO and McLachlan JA. Environmental hormones and the malereproductive system. J Androl 1998; 19:5–10.
11. Chowdhury AR and Mitra C. Spermatogenic and steroidogenic impairmentafter chromium treatment in rats. Indian J ExpBiol 1995; 33:480–484.
12. Dufresne and Franworth ER. A review of latest research findings on the health promotion properties of tea. J. Nur.Biochem., 2001; 12; 404-421.
13. Ernst E and Bonde JP. Sex and epididymal sperm parameters in rat followingsub chronic treatment with hexavalent chromium. Human EnvironToxicol 1992; 11:255–258.
14. Ernst E. Testicular toxicity following short-term exposure to tri- andhexavalent chromium: an experimental study inthe rat. ToxicolLett 1990; 51:269–275.

15. Henkel R, Kierspel E, Stalf T, Mehnert C, Menkveld R, TinnebergHR,Schill WB, Kruger TF. Effect of reactive oxygen species produced by spermatozoa and leukocytes on sperm functions in nonleukocytospermic patients. FertilSteril 2005; 83:635–642.
16. Hwang P, Wu CH, Gau SY, Chien SY, Hwang DF. Antioxidant and immune-stimulating activities of hot water extract from seaweed sargassumhemiphyllum J. Mar. Sci. Technol, 2010; 18(1) 41-46.
17. Katz SA, Salim H, Editors manufacture, use and distributionof chromium compound: biological and environmental chemistry of chromium. New York:VCH; 1994.
18. Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. FertilSteril 2000; 74:1200–1207.
19. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. FertilSteril 1997; 68:519–524.
20. Moncur, M.C., Ptacek, C.J., Blowes, D.W. and Jambor, J.L.: Release, transport and attenuation of metals from an old tailings impoundment. Appl. Geochem., 2005; 20: 639-659.
21. Moustafa MH, Sharma RK, Thornton J, Mascha E, Abdel-Hafez MA, Thomas AJ, Jr, Agarwal A. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Hum Reprod 2004; 19:129–138.
22. Murthy RC, Saxena DK, Gupta SK and Chandra SV. Ultrastructuralobservations in testicular tissue of chromium-treated rats. ReprodToxicol 1991; 5:443–447.
23. Oger I, Da Cruz C, Panteix G, Menezo Y. Evaluating human sperm DNA integrity: relationship between 8-hydroxydeoxyguanosine quantification and the sperm chromatin structure assay. Zygote 2003; 11:367–371.
24. Pant N, Upadhyay G, Pandey S, Mathur N, Saxena DK and Srivastava SP. Lead and cadmium concentration in seminal plasma of men ingeneral population: correlation with sperm quality. ReprodToxicol 2003; 17:447–450.
25. Saxena DK, Murthy RC, Lal B, Srivastava RS and Chandra SV. Effectof hexavalent chromium on testicular maturation in the rat. ReprodToxico l990; 4:223–228.
26. Saxena DK, Murthy RC, Lal B, Srivastava RS and Chandra SV. Effectof hexavalent chromium on testicular maturation in the rat. ReprodToxicol 1990; 4:223–228.
27. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in thetoxicity of chromium and cadmium ions. J Environ PatholToxicolOncol 2001; 20:77–88.
28. Stohs, S.J., Bagchi, D.Oxidative Mechanisms in the toxicity of Metal ions. Free radical Biol. Med. 1995; 18:321-336.
29. Stroev EA. and Mokarova VG. Metabolismofxenobiotics in laboratory mammal. In: Biochemistry. Moscow: Mir publishers: 1989; 178.
30. Sutherland JE, Zhitkovich A, Kluz T and Costa M. Rats retainchromium in testis following chronic ingestion of drinking water containinghexavalent chromium. Biol Trace Elem Res. 2000, 74:41–53.
31. Villegas J, Schulz M, Soto L, Iglesias T, Miska W, Sanchez R. Influence of reactive oxygen species produced by activated leukocytes at the level of apoptosis in mature human spermatozoa. FertilSteril 2005; 83:808–810.
32. Wang X, Sharma RK, Sikka SC, Thomas AJ, Jr, Falcone T, AgarwalA.Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. FertilSteril 2003; 80:531–535.
33. Wyrobek AJ. Bruce WR. Chemical induction of sperm abnormalities in mice.proc. Natl. 1975; 72(1):4425-9.
34. Xu L, Sun H, Wang S, Song L, Chang H, Wang X. The roles of metallothionein on cadmium-induced testes damages in Sprague–Dawleyrats.EnvironToxicolPharmacol 2005; 20:83–7.
35. Zahid ZR, Al-Hakkak ZS, Kadhimm AHH, Elias EA and Al-Jumaily IS. Comparative effects of trivalent and hexavalent chromium on spermatogenesisof the mouse. Toxicol. Environ. Chem. 1990; 25:131–136.