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COMPARATIVE TOXICITY OF CADMIUM IN LIVER AND TESTES OF ALBINO MICE FOLLOWING CHRONIC EXPSOURE

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Cadmium (Cd) is an environmental and industrial pollutant that adversely affects human and animal health. This study was carried out to investigate the toxic effects of Cd on liver and testis of albino mice. Cadmium administration led to significant ($P<0.001$) reduction in weight of liver and testes. Histopathological analysis in liver revealed oedema, vacuolation, lymphocytic infilteration and hyperaemia, numerous pyknotic, degenerative and binucleated nuclei, giant cell and kupffer cells at the sites of damage were also visible. Alterations in testis were in the form of degenerated germinal layer, almost complete loss of spermatogenic activity and leydig cell lysis. In the present study testis were found to be more sensitive to Cd than liver.

Key words : Cadmium, histopathology, liver and testis.

INTRODUCTION

Cadmium (Cd) is a ubiquitous environmental pollutant and is widely distributed in the environment due to extensive use of cadmium based products (Shayne, 2005). It has special importance due to its long half life and it can threaten human health both through environmental and occupational exposure (Duruibe *et al.*, 2007). Consumption of contaminated food due to excessive use of phosphate fertilizers (Duruibe *et al.*, 2007) and contaminated water through galvanized pipes have become the major ways by which humans are being exposed to cadmium (WHO, 1992).

Cadmium and cadmium compounds are well known human carcinogens (El-Demerdash *et al.*, 2004). Cd can also cause bone defects, increased blood pressure, myocardiac and hepatic dysfunctions, proteinuria and pulmonary oedema (Jarup, 2003; Young, 2005). It can also accumulate in human ovaries depending upon age and smoking habits, cross placenta and has been found to accumulate in higher amount in placenta of mothers addicted to smoking (Piasek & Kostial, 1996). Inspite of the various applications of cadmium in a variety of consumer and industrial based materials, the metal has been branded as a potent environmental pollutant causing serious health hazards to almost all the organisms of the biosphere including human beings. The extensive mutagenic and carcinogenic spectrum of the metal has warranted the humanity to restrict undesired abuse of the metal so as to save the planet from its dangerous clutches (Acharya *et al.*, 2001).

The main aim of the present study is to evaluate the toxic effects of cadmium on histomorphology of liver and testes in albino mice.

MATERIALS AND METHODS

Animals : Albino mice weighing 23-25g were procured from Central Research Institute, Kasauli (H.P). They were kept and acclimatized to the laboratory conditions for 15 days under optimal conditions of temperature and light. They were fed standard mice

feed and water ad libitum. The animals were handled with humane care in accordance with the guidelines of the 'Institutional Animal Ethical Committee.

Chemicals : Cadmium chloride ($CdCl_2$) was bought from S.D FINE CHEM LIMITED, Mumbai. It was dissolved in double glass distilled water and administered intraperitoneally (ip) to mice.

The mice were divided into two groups. **Group I** were kept as control and were injected only distilled water. **Group II** animals received injection of Cd (0.1mg/kg body wt.) and autopsies were done at 15 and 30 days post treatment. Histopathological studies were done by using standard method of microtomy and slides were stained with haematoxylin and eosin stains. The data was analyzed by using Student's t test.

RESULTS AND DISSCUSSION

In the present study Cd administration does not produce any discernible signs and symptoms of sickness in mice. Also, there was observed no mortality during the entire period of experiment. The comparison of testis and liver weight of Cd treated mice with controls are summarized in Fig. 1 & 2 and showed that Cd treatment caused significant ($P<0.001$) reduction in testicular and liver weight at 15 and 30 days post treatment as compared to control group. This work is in agreement to the work conducted by Sandy *et al.* (2007); Bogiswairy *et al.* (2008); Amara *et al.* (2008) and Zienlinska-Psuja *et al.* (2008). It is revealed that the reduction in liver weight is due to accumulation of Cd which causes hepatocyte injury. Moreover, most of the drugs and toxic chemicals are metabolized in the liver and in turn the hepatocytes get damaged (Samir *et al.*, 2007; Bogiswairy *et al.*, 2008). Further, the regression in weight of testis is attributed mainly to the necrosis of seminiferous tubules induced by Cd cytotoxicity. Also, Cd gets accumulated in testes and hence continuously keeps on damaging its cell population (Bench *et al.*, 1999).

In the present study, liver and testis of control mice showed normal histoarchitectural organization of cells as normal liver tissue showed polyhedral hepatocytes with well defined cell lining and distinctly rounded nuclei (Fig. 3) and a normal testis depicted organized germ cells in seminiferous tubules, normal leydig cells in interstitial mass and cuboidal germinal epithelium (Fig.6). Alterations in both tissues were observed in Cd treated mice. In 15 days post treatment group, hepatocytes showed remarkable oedema, cytoplasmic vacuolation and degranulation. Large number of pyknotic nuclei and giant cells was visible. Marked increase in coalesced nuclei, karyrorrhexis and karyorrhexis could be clearly seen (Fig. 4). Whereas, testis showed histopathological changes in the form of odematous and hypertrophied intertubular spaces, atrophied seminiferous tubules and leydig cell lysis. The germinal layer was also found to be degenerated. The normal stages of the cells which were observed during spermatogenesis, were not seen (Fig.7).

In 30 days post treatment group, liver showed almost similar histomorphological characters as seen in 15 days post treatment group but increased number of pyknotic nuclei, binucleated cells and increased lymphocytic infilteration and hyperaemia were visible (Fig.5). In testis almost all of the spermatogonia showed very much reduced spermatogenic activity. Most of the spermatogonia showed karyorrhexis and karyolysis. Leydig cells showed atrophy (Fig.8). These results are in confirmation with the results of Chiquoine (2005); Karl *et al.* (2005) and Yusuf *et al.* (2008).

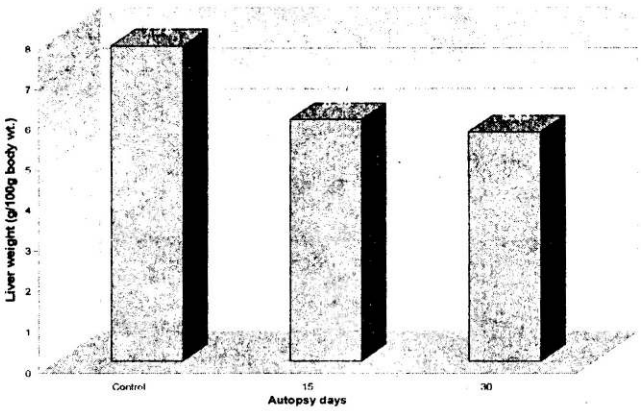


Fig. 1 : Comparison of liver weight of control and cadmium treated mice.

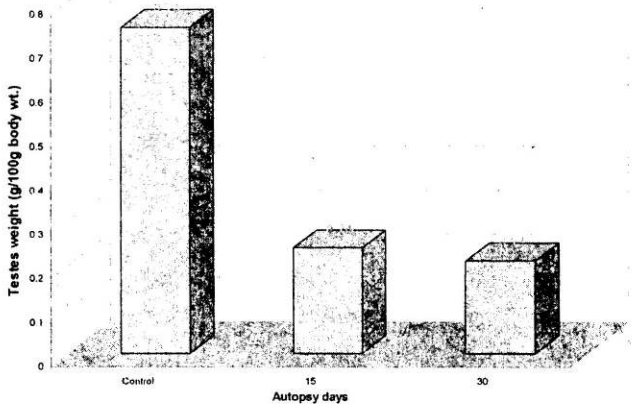
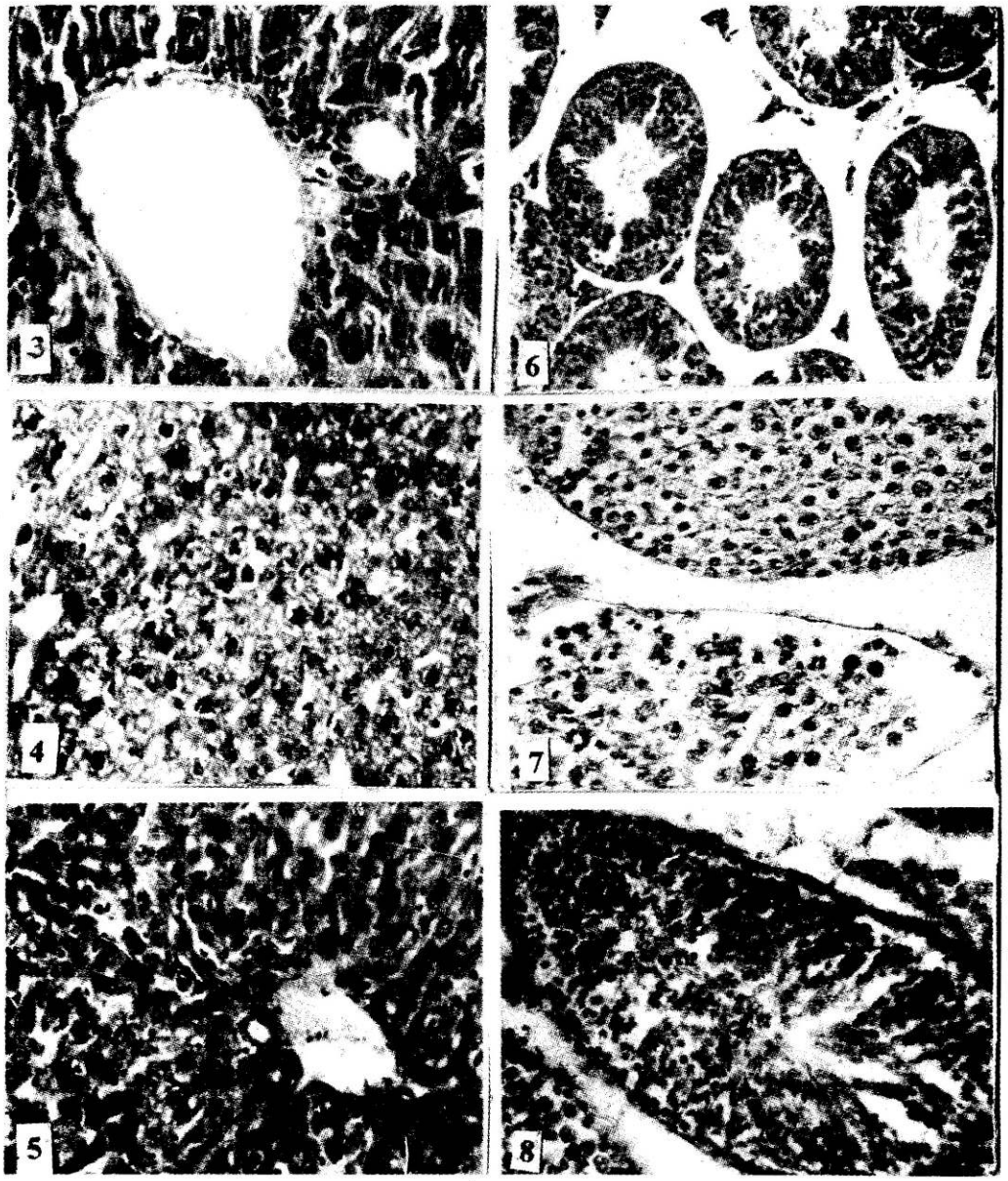


Fig. 2 : Comparison of weight of testes of control and cadmium treated mice.

The histopathological changes seen in liver of treated mice may be due to Cd-induced hepatotoxicity. The mechanism of Cd mediated hepatotoxicity has been the subject of numerous investigations and although some uncertainties persist, sufficient evidence has emerged to provide a reasonable account of the toxic process. Lora & Tetsuo (1999) proposed that cadmium induced hepatotoxicity involves two pathways, one for initial injury produced by direct effects of cadmium and other for subsequent injury produced by inflammation. Primary injury appears to be caused by the binding of Cd to sulphydryl groups on critical molecules in mitochondria resulting in thiol group inactivation which causes oxidative stress, the mitochondrial permeability transition and mitochondrial dysfunction.

Liver is very sensitive to cadmium. This may be due to the fact that most drugs and toxic chemicals are metabolized in it. Liver stimulates the syntheses of metallothionein (MT, a protective protein) which has high affinity for cadmium (Klaassen *et al.*, 1999). Thus liver controls the release of the Cd to other tissues (Sato & Kondoh, 2002). But when the concentration of cadmium over rides the capacity of hepatocytes to produce this



Figs. 3-8 : Photomicrograph of liver and testes. **3.** Normal liver structure. Polyhedr hepatic cells with prominent central nucleus are observed. x200; **4.** Liver of 15 days post treatment (Cd treated) showing atrophy of liver tissue. Some giant cells karyolitic, karyorrhectic nuclei and cytoplasmic vacuolation are also observed. x400; **5.** Liver 30 days post treatment showing large number of damaged cells, coalesced cells, pyknotic nuclei and Kupffer cells. Hypereamia and lymphocytic filertation are also visible. x400; **6.** Testes showing normal structure. Many seminiferous tubules, spermatogonia in different stages of development x200; **7.** Testes 15 days post treatment (Cd treated) showing seminiferous tubule with atrophied testicular tissue and dead cells. x400; **8.** Testis 30 days post treatment of Cd. Testicular tissues showing atrophy and fibrosis. x400.

protective metallothionein, liver cells get injured. Although, cadmium may injure hepatocytes directly, hepatocellular injury can be produced by ischemia caused by damage to endothelial cells (Chiquoine, 2005).

During present study, the damages observed in testes tissue indicate that maturation of spermatogonia through the process of meiosis was completely disrupted following Cd exposure. Lee & Dixon (1973) postulated two possibilities for the primary mechanism of Cd induced toxicity in testes (i) circulatory failure due to vascular damage. (ii) decreased utilization of zinc by spermatogenic cells due to competitive action of cadmium.

According to Karl *et al.* (2005) and Walter *et al.* (2007) the unusual sensitivity of the testes to Cd is related to unique feature of its vasculature namely the pulseless, semistagnant flow of blood in the intratesticular course of the testicular artery, which permit cadmium to alter capillary endothelium permeability, resulting in oedema and pressure effects leading to anoxia.

Leydig cell degeneration observed in the treated groups during present work would have resulted in direct destruction to the hypothalamus-pitutary gonadal axis resulting the decreased synthesis of testosterone, which in turn disturbs the process of spermatogenesis (Habeebu *et al.*, 1998, Stohs *et al.*, 2001). Many studies have revealed that leydig cells play an important role in the structural and functional integrity of seminiferous tubules and synthesis of testosterone which is the one of the main components for regulation of post meiotic stages of spermatogenesis (Stohs *et al.*, 2001).

Many studies proved that most important mechanism responsible for tissue damages by heavy metals is oxidative stress (Klaassen *et al.*, 1999; Garnier *et al.*, 2006). Cadmium induced oxidative stress has been associated with production of reactive oxygen species (ROS) comprising mainly superoxide radical anion, hydrogen peroxide and hydroxyl radicals which lead to lipid peroxidation, membrane protein and DNA damage and can also results in carcinogenesis, apoptosis, necrosis and cell proliferation (Habeebu *et al.*, 1998; Stohs *et al.*, 2001).

In the present study, liver and testes have shown progressive damage after administration of $CdCl_2$. But the testes are found to be more susceptible to Cd toxicity than liver, which can be attributed to fact that Cd exposure subsequently increases hepatic metallothionein (MT) gene expression as observed by Ren *et al.* (2003) but does not increase MT translation in sertoli and spermatogenic cells. Thus, testes show inability to induce the metal detoxicating MT protein in response to Cd, which might account for increased sensitivity of testis to cadmium induced toxicity in comparison to liver.

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REFERENCES

ACHARYA, U.R., DAS, S.S. & MISHRA, M. 2001 Role of vitamin C and E on sperm abnormality and sperm count in cadmium treated swiss mice. *Cytologia.* **67** : 147-152.

AMARA, S., ABDELMELEK, H., GARREL, C., GUIRAUD, P., DOUKI, T., RAVANAT, J-L., FAVIER, A., SAKLY, M. & RHOUMA, K.B. 2008. Preventive effect of zinc against cadmium induced oxidative stress in the rat testes. *J. Reprod. Develop.* **54** (2) : 129-134.

BENCH, G., CORZETT, M.H., MARTINELLI, R. & BALHORN, R. 1999 Cadmium concentrations in the testes, sperm and spermatids of mice subjected to long-term cadmium chloride exposure. *Cytometry.* **35** : 30-36.

BOGISWARIY, S., JEGATHAMBIGAI, R. & MARIMUTHU, K. 2008. Effect of acute exposure of cadmium chloride in the morphology of the kidney of mice. *International Conference on Environmental Research and Technology* (ICERT).

CHIQUOINE, D.A. 2005 Observation on the early events of cadmium necrosis of the testis. *The Anatomical Record.* **149**(1) : 23-35.

DURUIBE, J.O., OGWUEGBU, M.O.C. & EGWURUGWU, J.N. 2007 Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.* **5** : 112-118.

EI- DEMERDASH, F.M., YOUSEF, M.I., KEDWANY, F.S. & BAGHDADI, H.H. 2004 Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of Vitamin E and β-carotene. *Food and Chemical Toxicology.* **42**(10) : 1563-1571.

GARNIER, L., SIMON-PLAS, F., THULEAU, P. AGNEL, J., BLEIN, J., RANJEVA, R. & MONTILLET, J. 2006 Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity. Plant, *Cell and Environment* **29**(10) : 1956-1969.

HABEEBU, S. S. M., LIU, J. & KLAASSEN, C. D. 1998 Cadmium-Induced apoptosis in mouse liver. *Toxicol. Appl. Pharmacol.* **149**(2) : 203-209.

JARUP, L. (2003) Hazards of heavy metals contamination. *Brit. Med. Bull.* **68** : 167-182.

KARL, E. M., JAMES, O. P. & ROBERT, R. N. 2005 Cadmium-Induced injury of the rat testis. *The Anatomical Record,* **149**(1) : 72-78.

KLAASSEN, C. D., LIU, J. & CHOUDHURI, S. 1999 Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annu. Rev. Pharmacol. Toxicol* **39** : 267-294.

LEE, I.P. & DIXON, R.L. 1973 Effects of cadmium on spermiatogenesis studied by velocity sedimentation cell separation and serial mating. *J. Pharmacol. Exp. Ther.* **187** :: 641-652.

LORA,E. & TETSUO, Y. 1999 Mechanism of cadmium mediated acute hepatotoxicity. *J. Biochem. Mol. Toxicol.* **14**(2) : 110-117.

PIASEK, M. & KOSTIAL, K. 1996 Experimental studies on reproductive and perinatal effect of lead and cadmium. *Environ. Mgmt. Health.* 7 : 29-34.

REN, X.Y., ZHOU, Y., ZHANG, J.P., FENG, W.H. & JIA, B.H. 2003 Metallothionein gene expression under different time in testicular sertoli and spermatogenic cells of rats treated with cadmium. *Reprod. Toxicol.* **17**(2) : 219-227.

SAMIR, H., NAIJA, H., MOHAMED, F.N., ABDELHAMID, E., & RACHID, S. 2007 Accumulation of cadmium effects on liver and kidney functions in rats given diet containing cadmium-induced polluted radish bulb. *Environ. and Toxicologic* **59** : 77-80.

SANDY, T., IVO, L., JOHN, M. & EMMY, V.K. 2007 Changes in expression of fibrotic markers histopathological alterations in kidneys of mice chronically exposed to low and high Cd doses. *Toxicol* **238** : 200-210.

SATO, M. & KONDOH, M. 2002 Recent studies on metalothionein protection against toxicity of heavy metals and oxygen free radicals. *Tohoku. J. Exp. Med.* **196** : 9-22.

SHAYNE, C.G. 2005. Cadmium. Toxic *Chemical encyclopaedia.* **1** : 373-377.

STOHS, S.J., BAGCHI, D., HASSOUN, E. & BAGCHI, M. 2001. Oxidative mechanism in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Toxicol. Oncol.,* **20**(2) : 77-88.

WALTER, C.P., JOSHUA, R.E., DANIEL, W.N., JAMES, W.M., AARON, B. & WILLIAM, D.A. 2007 The vascular system as a target of metal toxicity. *Toxicol. Pharmacol.* **23**(1) : 34-40.

WHO 1992 *Environment Health Criteria, 134, Cadmium.* World Health Organisation. IPCS, Geneva.

YOUNG, R.A. 2005 *Toxicity Profiles: Toxicity Summary for Cadmium, Risk Assessment Information System. RAIS,* University of Tennessee.

YUSUF, E., ISMAIL, A.R.I. & EVREN, K.O.C. 2008 Effects of cadmium compounds (cadmium para hydroxybenzoate and cadmium chloride) on the liver of mature mice. *Turk. J. Zool.* **32** : 1-5.

ZIENILSKA-PSUJA, B., LUKASAZYK, A. & SENCZUK, W. 2008 Anti-reproductive effects of long term oral administration of cadmium on the adult male rat. *International Journal of Andrology* **2**(1-6) : 150-161.

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