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Cadmium acetate induced hematotoxicity, sperm abnormality and mutagenicity in rats

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Summary

Cadmium (Cd) is a dangerous occupational and environmental toxin. The aim of this study is to assess the long – term uptake of cadmium acetate on blood parameter, sperm numbers, sperm abnormality and mutagenic index of male and female rats. Male and femle wistar rats were administrated orally with 200 ppm and 400 ppm cadmium acetate for six weeks. After the end of administration, the animals were anaesthetized and blood samples were collected from their hearts for blood parameters. Right and left epidydimus were collected for study of sperm numbers and sperm abnormality. Mutagenic index of male rats was determined after six weeks of the treatments of male rats with cadmium acetate by mating it with normal female rats. The results showed the significant decreasd of all blood parameters of male and female rats treated with cadmium acetate compared with control group. Sperm concentration was decreased significantly, while sperm abnormality was increased significantly in male rats treated with 200 ppm and 400 ppm cadmium acetate. Mutagenic index was increased in male rats treated with 200 ppm cadmium acetate compared with control group. No pregnancy was noted when male rats were treated with 400 ppm cadmium acetate mated with normal female rats.

Key words : Cadmium acetate, Hematoxicity, Sperm abnormality, Mutagenic index

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Introduction

Cadmium is one of the most toxic environmental and industrial pollutants , the general population may be exposed to cadmium through consumption of food and drinking water inhalation of cadmium containing particles from ambient air or cigarette smoke or ingestion of contaminated soil and dust (Bako *et al.*,1982). cadmium is ubiquitous toxic heavy metal and unlike organic compounds , it's not biodegradable and has a very long biological half life (Goyer , 1995) .

Cadmium induces several alterations in the tissue of laboratory animals and humans (Foulkes , 1986) , in the blood Cadmium mainly accumulated in the red cells and binds to a low molecular weight protein (Cherian and Nordberg , 1983) and in kidney cadmium affects tubular epithelium resulting increased cadmium in urine , amino acid urea , glucosuria and decreased renal tubular reabsorption of phosphate (Goyer , 1995) . Cadmium has also been demonstrated to inhibit many enzyme and competes with calcium metabolism and alter phosphorylation patterns (Vallee and Ulmer , 1972 ; Moshtaghie *et al.,* 1991). Koizumi *et al.* (1996) indicated that cadmium caused H2O2 accumulation and H+ , cadmium and H2O2-related permeability changes of the plasma membrane. Egwurugwu *et al.*(2007) showed that cadmium accumulated highly in rat livers, and raised serum GOT and GPT, while ginger lowered these parameters.

Exposure to high Cd concentration have been found to be carcinogenic , mutagenic and teratogenic for a large number of animal species (Degraeve , 1981).In several studies , it is indicate that Cd damaged the nucleolar structure, DNA and RNA in both animal and plant cells (Misra *et al.*,1998;Hartwig and Schwerdtle,2002 ; Jomak *et al*., 2004).

The major aim of the present study was to investigate the effects of Cadmium on some blood parameters , concentration of sperm ,sperm abnormal and mutagenic index in rats .

Material and Methods

The present study was performed in animal house of Biology department in College of Education , University of Thi-Qar during the first half of 2008.This study included the examination of the effect of cadmium acetate on the following:

Blood parameters

Sixty male and female wistar rats weighting (250 – 300) grams and 9 weeks old were used in this experimental study .Rats (male and female) divided in to three groups (n = 10) :-

1. The first group (Control group) were given normal tap water for 6 weeks .

2. The second group received 200 ppm cadmium acetate in their drinking water for 6 weeks .

3. The third group received 400 ppm cadmium acetate in their drinking water also for 6 weeks .

Rats were housed under controlled conditions of ambient temperature (22 ± 1 ˚C) , with 14 h – 10 h light / dark cycle . Food and water provided *ad libitum.*

After experimental period (6 weeks) male and female rats were anaesthetized and blood samples were withdrawn directly from their hearts. All blood parameters (Red blood corpuscles count, packed corpuscular volume , Hemoglobin concentration and White blood

corpuscles count) were determination by routine laboratory methods (Baker and Silverton , 1976 ; Lewis *et al.*,2001) .

Numbers and abnormality of sperms

Thirty male wistar rats weighting (250 – 300) grams and 9 weeks old were used in this experimental study. To determination the effect of Cadmium in sperm concentration Soto's method (1983) was used , and Wyrobek and Bruce's method (1975) used to determination sperm abnormalities .

Mutagenic index

The laboratory male rats with age (8-9) weeks and (250-300) gram body weight were used in this test . These males were divided in to three groups (control group , 200 ppm of Cadmium acetate group and 400 ppm of Cadmium acetate group) each group contains ten animals.

The procedure which was used in determination of mutagenic index was described by Green *et al.*(1985) followed : After the treatment , each male was mated with two virgin females , which were replaced weekly for seven consecutive weeks . All females were killed 11 day after the day of their separation from the males and their reproductive status as dead , living implant and mutagenic index was determined .

The mutagenic index was calculated as follows :

$$\text{Mutagenic Index} = \frac{\text{Number of dead implants}}{\text{Total number of implants}} \times 100$$

Statistical analysis

Statistical analysis of the results of blood parameters and sperm concentration and abnormality was performed by SPSS test, data are presented as Mean ± Stander Deviation .The mutagenic index result was analyzed by Chi-Square test.

Results

Blood parameters

Table (1) showed the effect of cadmium acetate on blood parameters of male rats. All blood parameters (R.B.C. , P.C.V. , Hb and W.B.C.)of male rats treated with 200 ppm and 400 ppm cadmium acetate in drinking water (second and third groups) were decreased significantly compared with the first group (control group).The results indicated that there no significant difference between second and third groups.

Table (1): Effect of Cadmium acetate on blood parametersof male rats

Treatments	R.B.C. ($\times 10^4$ Cell /mm^3blood)	P.C.V. (%)	Hb (mg / dl)	W.B.C. ($\times 10^3$ Cell /mm^3blood)
First group (control)	8.57 ±1.17	46.00 ±2.94	12.95 ±0.79	7.71 ±0.79
Second group (treated with 200 ppm cadmium acetate)	5.73* ±0.29	30.60* ±2.06	9.35* ±3.00	5.20* ±0.38
Third group (treated with 400 ppm cadmium acetate)	5.11* ±0.28	29.25* ±1.79	7.95* ±0.02	4.94* ±0.17

* There is significant difference compare with control group at P<0. 01.

Cadmium acetate caused significant decreased (P< 0.01) of all blood parameters (R.B.C. , P.C.V. , Hb and W.B.C.) of female rats treated with 200 ppm and 400 ppm cadmium acetate for six weeks compared with the first group(control group) , and the results indicated that R.B.C. and Hb. Of female rats treated with 400 ppm cadmium acetate were decreased significantly (P< 0.05) (P<0.01) respectively compared with female treated with 200 ppm cadmium acetate .

Table (2): Effect of Cadmium acetate on blood parameters of female rats

Treatments	R.B.C. ($\times 10^4$ Cell /mm^3blood)	P.C.V. (%)	Hb (mg / dl)	W.B.C. ($\times 10^3$ Cell /mm^3blood)
First group (control)	8.20 ±1.30	44.30 ±3.70	13.19 ±0.75	7.66 ±1.20
Second group (treated with 200 ppm cadmium acetate)	5.85* ±0.40	31.35* ±2.00	11.20* ±0.78	4.96* ±0.30
Third group (treated with 400 ppm cadmium acetate)	5.00*[b] ±0.34	30.10* ±1.00	9.75*[a] ±0.92	4.69* ±1.12

* There is significant difference P< 0.01 compared with control group.

a There is significant difference P< 0.01 compared with second group .

b There is significant difference P< 0.05 compared with second group.

Sperm concentration and abnormality

Table (3) showed that cadmium acetate caused significant decreased (P<0.01) of sperm concentration and significant increased (P<0.01) of male rats treated with 200 ppm and 400 ppm cadmium acetate compared with control group. The results indicated the significant decreased (P<0.01) of sperm concentration of male rats treated with 400 ppm cadmium acetate (third group) compared with male rats treated with 200 ppm cadmium acetate (second group), and there was no significant different in sperm abnormality between second and third groups.

Table (3): Effect of Cadmium acetate on sperm concentration and sperm abnormality of male rats

Treatments	SPERM CONCENTRATIOM (X 10)	SPERM ABNORMALITY (%)
First group (control)	90.80 ±2.74	90.90 ±1.96
Second group (treated with 200 ppm cadmium acetate)	53.80 * ±5.61	26.9 * ±4.97
Third group (treated with 400 ppm cadmium acetate)	33.9 *a ±3.47	23.3 * ±5.92

* There is significant difference P< 0.01 compared with control group.

a There is significant difference P< 0.01 compared with second group .

Mutagenic index

Table (4) explained the mutagenic index of male rats treated with 200 ppm and 400 ppm cadmium acetate compared with control group. The results indicated that the mutagenic index of male rats treated with 200 ppm cadmium acetate was 46% compared with zero in control group. The results showed that there was no pregnancy in female rats mated with male rats treated with 400 ppm cadmium acetate.

Table (4) Effect of Cadmium acetate on mutagenic index of male rats.

TREATMENT	NUMBER OF MALES TREATED	NUMBER OF FEMALES TREATED	NUMBER OF FEMALES PREGNANT	TOTAL DEAD IMPLANTS	TOTAL IMPLANTS (DEAD+LIVE)	DEAD IMPLANTS/ PREGNANT FEMALES (MEAN±S.D)	TOTAL IMPLANTS/ PREGNANT FEMALES (MEAN±S.D)	MUTAGENIC INDEX %
First group (control group treated with tap water)	10	20	18	-	132	0.00	7.33 ±1.32	0.00
Second group (treated with 200 ppm of cadmium acetate)	10	20	12	38	84	3.16 ±0.71	7.0 ±0.85	45.00*
Third group (treated with 400 ppm of cadmium acetate)	10	20	-	-	-	-	-	0.00

* There is significant difference compared with control group (Chi Square)

Discussion

Mechanism of Cadmium toxicity remainin completely understood , but elevated lipid peroxidation in tissue is observed soon after exposure to Cadmium (Hussain *et al.,* 1987 ; Bagchi *et al.* , 1997 ; Grudzimski *et al.* , 2001) there also is a positive correlation between cadmium intake and the cell injury . Cadmium induced alteration in the phospholipids and protein content of the blood cell membrane which are accepted normally as evidence of disturbed membrane fluiclity were associated in this case with unaltered membrane fragility on the other hand changes in cellular membrane of red blood cell leading to decreased of hemoglobin and packed cell volume (Demir and Oner , 1995) . A supportive finding for our results comes from the study of Garty *et al.* (1994) who exposed rat blood cells to cadmium in vitro and found that cadmium uptake by the red blood cells occurs by passive transport .

Cadmium have been reported to reduce male fertility in both humans and rodents (Schrg and Dixon , 1995 ; Bench *et al.,* 1999) . There are several hypotheses that suggest how reduced male fertility may result from incorporation of heavy metals in to sperm chromatin by replace or compete with the zinc that is normally bound to the cysteine residues in protamine forming more stable metal , cadmium may prevent normal disulfide bound formation within and among protamines during the final stages of sperm maturation leading to increased sperm abnormal (Johansson and Pellicciari , 1968 ; Shelby *et al.*,1986) , and the cadmium may have a determinate effect on testicular function (the cadmium could be toxic to the supporting testicular tissue or to the earlier stage of spermatogenesis) that could result in reduced sperm production leading to decreased sperm concentration (Battersby *et al.*,1982 ; Krichah *et al.*, 2003 ; Meistrich *et al.*,1976) .

The toxicity of Cadmium on blood and sperm formation (concentration and abnormal) increased as soon as increased of cadmium concentration (it has been reported that pre-treatment of experimental male and female rats with small doses of cadmium prevent acute toxic effects of large doses of cadmium) (Nordberg *et al.*,1975).

In many studies , Cd has been shown to be a genotoxic metal , and cadmium enhanced the effects of other mutagens (Zhang and Xiao , 1998 ; Fojtova and Kovarik , 2000 ; Fatur *et al.* , 2003). Rojas *et al.* (1999) reported that cadmium exerts pronounced indirect genotoxic effects ; it enhanced mutagenity of UV light in several cells. Some researchers reported that the Cd salts are not directly genotoxic in rodent cell lines. According to the International Agency for Research on Cancer classified Cd is suspected as co-mutagen and human carcinogen(IARC, 1993).

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تاثير خلات الكادميوم في معايير الدم ، عدد وتشوهات الحيامن ، الدليل التطفيري لذكور واناث الجرذان المختبرية

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الملخص

هدفت الدراسة الحالية لبحث تأثير التجريع الفموي لخلات الكادميوم بالجرعتين ٢٠٠ جزء بالمليون و٤٠٠ جزء بالمليون ولمدة ستة أسابيع في معايير الدم ، عدد وتشوهات الحيامن والدليل التطفيري لذكور وإناث الجرذان المختبرية . شرحت الحيوانات في نهاية مدة التجريع وجمعت عينات الدم والبربخين الأيمن والأيسر ، فيما حدد الدليل التطفيري لذكور الجرذان بعد نهاية مدة التجريع بخلات الكادميوم ومزاوجتها مع إناث جرذان سليمة . أظهرت النتائج انخفاضا معنويا في جميع المعايير الدموية لذكور وإناث الجرذان (عدد كريات الدم الحمراء ، حجم الخلية المضغوط ، تركيز الهيموكلوبين، العدد الكلي لخلايا الدم البيض) مقارنة مع مجموعة السيطرة . كما انخفضت أعداد الحيامن بشكل معنوي بينما ازدادت أعداد الحيامن المشوهة معنويا بتأثير الجرعتين أعلاه . أظهرت النتائج كذلك زيادة الدليل التطفيري لذكور الجرذان المعاملة ب٢٠٠ جزء بالمليون من خلات الكادميوم مقارنة مع مجموعة السيطرة ، فيما لم يحصل حمل في إناث الجرذان السليمة التي زوجت مع ذكور الجرذان المعاملة بـ٤٠٠ جزء بالمليون من خلات الكادميوم .

الكلمات المفتاحية : خلات الكادميوم ، المعايير الدموية ، عدد وتشوهات الحيامن ، الدليل التطفيري ، الجرذان المختبرية