EFFECTS OF SELENIUM AND CADMIUM ON MALE FERTILITY IN ALBINO RATS

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ABSTRACT

The effect of subcutaneous single dose and chronic cadmium doses, and the effect of selenium to prevent testicular damage was studied. Forty-two mature albino male rats were used. In the experiment I, animals were divided into 4 equal groups each one consists of six rats. The first group considered as control and injected with saline. The second group was given a single subcutaneous injection of 1 mg/kg B.wt cadmium chloride in saline, while the third group was given sodium selenite by stomach tube in a daily dose 0.3 mg/kg B.wt. for two weeks. The fourth group was given the same single dose of $CdCl_2$ and sodium selenite daily for two weeks. In experiment II: Rats were divided into three equal groups each one consists of six rats, the first group served as control, while the second group was given orally $CdCl_2$ in a dose of 1 mg/kg B.wt for 45 days. The third group was given $CdCl_2$ in a dose of 2 mg/kg B.wt for 45 days. Blood samples were collected and sera were used for determination of testosterone and LH by RIA and IRMA methods respectively. Serum urea, creatinine, AST, ALT and alkaline phosphatase were estimated by calorimetric method. The results revealed that, in experiment I, there was a significant decrease in testosterone level in second and fourth groups, while there was no significant change in the third group. Serum LH level showed significant elevation in the second and fourth group. The weight of seminal vesicle and testes were significantly decreased. In Experiment II, the results revealed that, there was significant decline in serum testosterone level in the second and third groups as compared with control. LH level was significantly elevated in the second and third groups. Also the testicular and seminal vesicles weight were significantly decreased. Moreover serum AST, ALT, alkaline phosphatase, urea and creatinine showed a significant increase in the rats treated with $CdCl_2$ in different doses for 45 days.

It is concluded that, a single or chronic doses of cadmium chloride may be directly affected testicular functions through decreasing testosterone secretion from Leydig cells

and subsequent increase in the pituitary gland secretion (LH) (-ve feed back mechanism). Also, selenium may be decrease the effect of single cadmium dose on male testes in albino rats, but not prevent these effects. Moreover, in rats chronic cadmium exposure adversely affect kidney and liver functions. Therefore, environmental pollution with cadmium induce infertility in male animals.

INTRODUCTION

Heavy metals such as cobalt, iron, cadmium, mercury, molybdenum and silver can adversely affect male accessory sex organ function and spermatogenesis. In rodents, testes are one of the most sensitive tissues to acute toxic and chronic carcinogenic effects of cadmium (**Gunn and Gould, 1970; Waalkes and Oberdoster, 1990**). Administration of relatively high doses of cadmium gives rise to testicular necrosis with 24 to 48 hr (**Waalkes and Oberdoster, 1990**). Also, in rats testes, cadmium induces severe necrosis followed by chronic degeneration, a single dose produce high incidence of Leydig cell tumor, (**Waalkes et al. (1997)**. Cadmium administration was associated with significant alkalinization of luminal fluid in seminiferous tubules (**Caflisch and DuBose 1991**). The testicular effect of cadmium may be prevented by means of several specific treatments including, zinc (**Mason et al., 1964; Kolzumi and Waalkes, 1989**). Selenium (**Mason et al., 1964**). In rats selenium prevented the decrease in Zinc in muscles and bone induced by cadmium (**Chmielnicka et al., 1985**). It was concluded that selenium partially improves the antioxidant defense system (AOS) that is insufficient to prevent cadmium induced nephrotoxicity in chronic cadmium exposure (**Staja et al., 1997**). Selenium deficiency is kown to be associated with male infertility and the slenoprotein phospholipid hydroperoxide glutathione peroxidase (PHGPx) has been shown to increase in rat testes after puberty and to depend on gonadotropin stimulation in hypophysectomized rats (**Roveri et al., 1992**).

The aim of the present investigation was to study the effect of selenium pretreatment to prevent effects of single cadmium dose on endocrine testicular functions and release of pituitary gonadotropin "LH". Also to study effect of chronic cadmium administration on testicular, liver and kidney functions.

MATERIAL AND METHODS

Experiment I. Twenty four mature albino male rats weighed 240±5 g were used. Animals were divided into four equal groups each one consists of six rats. First group considered as control, the second group was given a single subcutaneous injection of 1 mg/kg B.wt. cadmium

chloride in saline, the third group was given sodium selenite by stomach tube in a daily dose 0.3 mg/kg B.wt. for two weeks. The fourth group was given the same single dose of cadmium and sodium selenite daily for two weeks. All rats were given diet and water ad libitum, 24 hr after the last treatment, all rats were killed by decapitation and blood was collected and allowed to clot. Serum samples were separated by centrifugation at 2500-3000 r.p.m. for 30 min. The serum samples were stored at -20°C until hormonal assay. Testes, seminal vesicles were removed and weighed.

Experiment II : The experiment was designed to study the chronic effects of $CdCl_2$ on rats testes, kidney and liver functions. 18 mature albino male rats were used in these experiment. Animals were divided into three equal groups each one consists of six rats. the first group served as control group and was given orally saline, the second group was given orally cadmium chloride in a dose of 1 mg/kg body weight for 45 days, while the third group was given cadmium chloride in a dose of 2 mg/kg body weight for 45 days. 24 hr after the last dose all rats were killed by decapitation and blood samples were collected. Serum samples were separated and stored at -20°C until biochemical and hormonal assay. Also Testes and seminal vesicles were removed and weighed.

Hormonal assay :

Serum testosterone level was assayed using Radioimmunoassay kit (RIA) testosterone coated tube supplied by diagnostic systems Laboratories Inc., USA according to methods of **Yalow and Berson (1971)**. Serum LH level was assayed using LH coated tube by immunoradiometric assay kit (LHIRMA) according to **Levine et al. (1985)**.

Biochemical analysis :

To asses the chronic effects of $CdCl_2$ on kidney and liver functions, serum urea, creatinine, were measured according to methods of **Patton and Crouch (1977) and Houto (1985)** using a commercial kits. Moreover AST, ALT and alkaline phosphatase were measured by methods of **Reitman and Frankel (1957), Belfield and Goldberg (1971)** respectively using a commercial kits.

Statistical analysis was done between the control and treated groups by student t test according to **Snedecor and Cochran (1967)**.

RESULTS AND DISCUSSION

The results of the experiment I are shown in Table (1), the results of experiment II are shown in Table (2) and Table (3) respectively.

After a single subcutaneous injection of cadmium chloride in the rats, with 24 to 48 hr, there was degenerative changes occur in the seminiferous tubules, the interstitial tissue and the spermatozoa in the caput epididymis **(Gunn and Gould, 1970). Sakena et al. (1977)** reported that after one week, there was a reduction in androgen out put. The results of the present investigation, revealed that there was a significant decreases in testosterone levels after two weeks in rats treated with cadmium chloride in a single S/C dose 1 mg/kg body weight, and the fourth group treated with the same dose of cadmium plus sodium selenite as a source of selenium there was a significant reduction in testosterone level. These results suggested that the selenium not prevent the effect of cadmium to induce testicular damage but reduce the effect of cadmium. These may be attributed to the dose of selenium given. Heavy metals including mercury, cadmium, cobalt, and copper exerted an adverse effect on the Leydig cells of the testes and there was parallel reduction in luteinizing hormone - stimulated testosterone production by Leydig cells, the results indicated that a direct toxic action of these heavy metals on sterold producing cells in the testes **(Ng and Liu, 1990)**. The results of LH in the present investigation revealed a significant elevation, these may be attributed to the direct effect of cadmium in reduction of testosterone level and results in elevation of LH level. **Laskey and Phelps (1991)** suggested that, in vitro cadmium and other metals cations may act at multiple sites within the Leydig cell and decrease testosterone production. **Caflisch and DuBose (1991)** reported the effect of a single S/C cadmium chloride in dose (2.7 mg/kg B.wt), plasma testosterone concentration was reduced after one day and persisted decline after 11 days postexposure. Selenium deficiency is known to be associated with male infertility, and the Selenoprotein PHGPx has been shown to increase in rat testes after puberty and to depend on gonadotropin stimulation in hypophysectomized rats **(Roveri e al., 1992). Maiorino et al. (1998)** reported that the specific activity of PHGPx in testes, but not of cGPX, correlated with sexual maturation, Leydig cell destruction in vivo by ethane dimethane sulfonate (EDS) resulted in a delayed decrease in PHGPx activity and mRNA that could be completely prevented by testosterone substitution. Therefore in the present investigation the reduction in the serum testosterone level may be reduce the effect of selenium to prevent the toxic effect of cadmium on testicular tissue. **Nemetallah and Bistawroos (1983)** reported that effect of indomethancin and $PGF_2\alpha$ on testosterone replacement of the reproductive tissue of cadmium treated mice, suggested that indomethacin significantly increased the weight of testes, seminal vesicle, penis and epididymal fat body. Also testosterone pretreatment prevents cadmium toxicity in male C57 mice, possibly through enhancement of metallothionein MT synthesis but has no ef-

fect in male C_3H mice **(Shimada et al., 1997). Stajn et al. (1997)** revealed that in rats treated with cadmium and selenium the activities of manganese-containing superoxide dismutase (MnSOD) and Se-dependent glutathione peroxidase (SeGSHPx) were the same as in control rats. It is concluded that selenium only partially improves the antioxidant defense system (AOS) that in insufficient to prevent Cd-induced nephrotoxicity.

Moreover, the effect of cadmium on the plasma selenium of diabetic rats was reported by **(Gumuslu et al., 1997)**. cadmium reduce the plasma levels of selenium and vit. E. Therefore, in the present study, cadmium may be interfere with selenium to reduce the testicular toxic effect in rats.

Also in the present study cadmium reduce the testicular and seminal vesicles weights these may be attributed to decrease in testosterone level, but the cadmium and selenium treated rats showed no significant changes in the testicular or seminal vescile weights. These results was in consistence with the data of **(Caflisch and BuBose, 1991)**, they suggested that a single dose of cadmium induce a significant reducation in the weight of testes and epididymis after 11 days of cadmium exposure.

In the present investigation the chronic effect of cadmium on testicular, seminal vesicles weights and serum testosterone level revealed a significant reduction after 45 days of cadmium exposure. These data was agree with results obtained by **Kawser et al. (1997)** in rats after 8 weeks of cadmium treatment. Moreover, the level of LH was significantly increases also after 45 days of cadmium exposure. In the rodent testes, cadmium induces severe necrosis followed by chronic degeneration at 10 weeks cadmium reduced circulating testosterone level and induced a marked weight loss of the testes. Also cadmium induce testicular tumor, the mechanism of tumor formation is unknown, but pituitary feedback, i.e., increased Lutenizing hormone (LH) production due to low circulating androgen, has been implicated in causation of proliferative lenston within degenerate, hypofunctioning testes **(Waalkes et al., 1997)**. The effects of chronic cadmium exposure on the liver and kidney function was determined in the present study; there were a significant increase in AST, ALT, alkaline phosphatase, urea and creatinine in serum of rats treated with both doses of cadmium for 45 days. These may be attributed to degenerative changes in liver and kidney. Morphologic changes in kidney resulting from long-term cadmium exposure consist mainly of proximal tubule atrophy and degeneration **(Friberg etal., 1974)**. Liver also accumulates substantial amounts of cadmium after both acute and chronic exposure **(Kotsonis and Klaassen, 1978). Dudley et al. (1985)** reported that plasma activities of AST, ALT were elevated after sixth week of cadmium exposure. Moreover, cadmium induce a significant increase in AST, ALT, blood urea, serum creatinine and alkaline phosphatase **(Shiraishi et al., 1993; Kawser et al., 1997; Rana and Rostogi, 1998)**.

It is concluded that a single or chronic doses of cadmium chloride may be directly affect testicular functions through decreasing testosterone secretion from Leydig cells and subsequent increase in the pituitary gland secretion (LH). Also, selenium may be decrease the effect of cadmium on male testes in albino rats, but not prevent these effects. Moreover, in rat chronic cadmium exposure adversely affect liver and kidney functions. Therefore, environmental pollution with cadmium induce infertility in male animals.

Table 1 : Effect of cadmium and selenium on serum levels of testosterone, LH and the weight of testes and seminal vesicles in mature male rats.

Parameter	First group control	Second group cadmium chloride	Third group selenium	Fourth group $CdCl_2$+Se
Testosterone (ng/ml)	3.15 ± 0.16	1.10 ± 0.12**	2.81 ± 0.28	1.65 ± 0.23**
LH (mIU/ml)	2.91 ± 0.21	4.21 ± 0.20*	3.11 ± 0.31	4.50 ± 0.17**
Weight of testes (g)	0.549 ± 0.02	0.396 ± 0.017*	0.576 ± 0.019	0.495 ± 0.02
Weight of seminal vesicles (g)	0.246 ± 0.01	0.176 ± 0.01*	0.255 ± 0.016	0.281 ± 0.012

The weight of testes and seminal vesicles (g/100g B. wt.). Mean + S. E. * P<0.05 ** P<0.005

Table 2 : Effect of chronic cadmium chloride on serum levels of testosterone, LH and on testicular and seminal vesicles weight.

Parameter	Control	Second group	Third group
Testosterone (ng/ml)	3.21 ± 0.17	0.773 ± 0.22**	0.685 ± 0.14**
LH (mIU/ml)	2.80 ± 0 25	5.51 ± 0.76*	6.52 ± 0.56**
Weight of testes (g)	0.645 ± 0.03	0.480 ± 0.02*	0.410 ± 0.018*
Weight of seminal vesicles (g)	0.260 ± 0.02	0.186 ± 0.016*	0.175 ± 0.01*

The weight of testes and seminal vesicles (g/100g B. wt.). Mean + S. E. * P<0.05 ** P<0.005

Table 3 : Chronic effect of cadmium chloride on liver and kidney functions in mature male rats.

Parameter	Control	Second group	Third group
AST (u/ml)	47.3 ± 1.97	69.6 ± 1.33*	84.66 ± 1.47*
ALT (u/ml)	39.16 ± 2 02	62.5 ± 1.30*	71.33 ± 2.15*
Alkaline phosphatase (u/ml)	64.81 ± 1.66	87.7 ± 1.46*	92.0 ± 2.19*
Urea (mg/dl)	21.16 ± 1.40	35.6 ± 1.37*	41.20 ± 2.9*
Creatinine (mg/dl)	1.03 ± 0.11	1.57 ± 0.08*	2.01 ± 0.13*

Mean + S. E. * P<0.05

REFERENCES

Belfield, A. and Goldberg, D. M. (1971) : Enzyme 12, 561.

Caflisch, C. R. and BuBose, T. D. Jr. (1991) : Cadmium-induced changes in luminal fluid pH in testes and epididymis of the rat in vivo. J. Toxicol. Environ. Health, 32: 1, 49-57.

Chmielnicka, J.; Bem, E. M.; Brzeznicka, A. and Kasperek, M. (1985) : The tissue deposition of zinc and copper following repeated administration of cadmium and selenium to rats. Environ. Research, 37: 2, 419-424.

Dudley, R. E.; Gammal, L. M. and Klaassen, C. D. (1985) : Cadmium induced hepatic and renal injury in chronically exposed rats: likely role of hepatic cadmium-metallothionein in nephrotoxicity. Toxicol. Appl. Pharmacol. 77, 414-426.

Fiberg, L.; Piscator, M.; Nordberg, G. F.; and Kjellstrom, T. (1974) : Cadmium in the environment, 2nd ed. CRC Press, Inc., Cleveland, Ohio.

Gumuslu, S.; Yargicoglu, P.; Agar, A.; Edremitlioglu, M. and Aliciguzel, Y. (1997) : Effect of cadmium on antioxidant status in alloxane-induced diabetic rats. Biological trace element Research, 57: 2, 105-114.

Gunn, S. A. and Gould, T. C. (1970) : Cadmium and other mineral elements. In the testes: Influencing factors (A.D. Johnson, W.R. Gomes, and N.L. VanDemark, Eds.) Vol. 3, pp. 377-481 Academic Press, New York.

Houto, O. (1985) : Interpretation of clinical laboratory tests. 220-234, edited by Siest, G. Henny J., Schilde F., and Young, D.S. Biomedical Publications.

Kawser, A. E. H.; Amer, M. S. and Mahmoud, M. M. (1997) : Undesirable effects of long-term administration of zinc and cadmium in rats with special reference to male fertility. Zagazig Vet. J. Vol 25, 1 : 38.

Kotsonis, F. N. and Klaassen, C.D. (1978) : The relationship of metallothionein to the toxicity of cadmium after oral administration to rats. Toxicol. Appl. Pharmacol. 46, 39-54.

Koizumi, T. and Waalkes, M. P. (1989) : Effect of zinc on the distribution and toxicity of cadmium in isolated interstitial cells of the rats testes. Toxicology, 56: 137-146.

Laskey, J. W. and Phelps, P. V. (1991) : Effect of cadmium and other metal cations on in vitro Leydig cell testosterone production. Toxicol. Appl. Pharmacol., 108: 2, 296-306.

Levine, J. E.; Norman, R. L.; Gliesman, P. M.; Oyama, T. T.; Bangsberg, D. R. and Spies, H. G. (1985) : In vivo gonadotropin-releasing hormone and serum Luteinizing hormone

measurements in ovariectomized, estorgen treated rhesus macaques. Endocrinology 117: 711-721.

Maiorino, M.; Wissing, J..B.; Brigelius Flohe, R.; Calabrese, F.; Roveri, A.; Steinert, P.; Ursini, F. and Flohe, L. (1998) : Testosterone mediates expression of the selenoprotein PHGPx by induction of spermatogenesis and not by direct transcriptional gene activation. FASEB J., 12: 13, 1359-70.

Mason, K. E.; Young, J.O. and Borwn, J. A. (1964) : Effectiveness of selenium and zinc in protecting against cadmium-induced injury of the rat testes. Anat. Rec. 148, 309.

Nemetallah, B.R. and Bistawrous, A. E. (1983) : Effect of indomethacin and PGF_2 and on testosterone replacement of the reproductive tissues of cadmium treated male mice. Bull. Zool. Soc. Egypt 33, 61-65.

Ng, T. B. and Liu, W. K. (1990) : Toxic effect of heavy metals on cells isoalted from the rat adrenal and testes. In Vitro Cell Dev. Biol., 26: 1, 24-8.

Patton C. T. and Crouch, S. R. (1977) : Enzymatic determination of urea. Anal. Chem., 49: 464-469.

Rana, S. V. and Rastogi, N. (1998) : Effects of cadmium on liver function in diabetic rats. Toxicol. Ind. Health, 14: 3, 473-7.

Reitman, S. and Frankel, S. (1957) : A calorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminases. Am. J. Clin. Path., 28: 56-63.

Roveri, A.; Casasco, A.; Maiorino, M.; Dalan; Calligaro, A. and Ursini, F. (1992) : Phospholipid hydroperoxide glutathione peroxidase of rat testes. Goandotropin dependence and immunocytochemical identification. J. Biol. Chem., 267: 9, 6142-6.

Saksena, S. K.; Dahlgren, L.; LAU, I. F. and Chang, M. C. (1977) : Reproductive and endocrinological features of male rats after treatment with cadmium chloride. Biology of reproduction 16: 609-613.

Shimada, H.; Bare R. M.; Hochadel, J. F.; Waalkes, M. P. (1997) : Testosterone pretreatment mitigates cadmium toxicity in male C57 mice but not in C3H mice. Toxicology, 116: 1-3, 183-191.

Shiraishi, N.; Barter, R. A.; Uno, H. and Waalkes, M. P., (1993) : Effect of progesterone pretreatment on cadmium toxicity in the male Fischer (F344/Ner) rat. Toxicol. Appl. Pharmacol. 188; 1131 18.

Snedecor, G. W. and Cochran, W. (1967) : Statistical methods 6'h ed., Lowa state Univ. Press,

Ames., Iowa USA.

Stajn, A.; Zikic R. V.; Ognjanovic, B.; Saicic Z. S.; Pavlovic S. Z.; Kostic M. M.; and Petrovic V. M. (1997) : Effect of cadmium and selenium on the antioxidant defense system in rat kidneys. Comp. Biochem. and Physiol. C-Pharmacology 117. 2,167-172.

Waalkes, M. P. and Oberdoster, G. (1990) : Cadmium carcinogenesis. In Biological effects of heavy metals: Mechanisms of Metal Carcinogenesis (E.D. Foulkes, Ed.), Vol. 2, pp. 129-158. CRC Press, Boca Raton, FL.

Waalkes, M. P.; Rehm, S. and Devor, D. E. (1997) : The effect of continuous testosterone exposure on spontaneous and cadmium induced tumors in the male Fischer (F344/Ner rat: loss of testicular response. Toxicol. Appl. Pharmacol., 142: 1, 40-6.

Yalow, R. and Berson, S. (1971) : Introduction and general considerations in Odell W.D., Doughday W. H. eds. Principles of competitive protein binding assays. J.B. Lippencott Co. pp. 1 19. Philadelphia.

الملخص العربى

تأثير الكادميوم والسيلنيوم على خصوبة ذكور الفئران البيضاء

المشتركون فى البحث

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إستهدفت هذه الدراسة معرفة تأثير إعطاء جرعة واحدة تحت الجلد من الكادميوم على وظائف الخصى وأيضاً دراسة تأثير إعطاء السيلنيوم لإيقاف عمل الكادميوم على وظائف الخصى فى فئران التجارب البيضاء. ثم دراسة تأثير جرعات من الكادميوم لمدة طويلة على وظائف الخصى والكبد والكلى. إستخدم فى هذه الدراسة عدد ٤٢ من ذكور الفئران البالغة.

التجربة الأولى : إشتملت على عدد ٢٤ من ذكور الفئران البالغة وقد قسمت هذه الفئران إلى أربع مجموعات. الأولى مجموعة ضابطة والثانية مجموعة أعطيت جرعة واحدة من الكادميوم ١ ملجم لكل كيلو جرام من وزن الجسم تحت الجلد. المجموعة الثالثة أعطيت سيلنيوم بجرعة ٣ر٠ ملجم/ كيلو جرام لمدة إسبوعين والمجموعة الرابعة أعطيت نفس جرعة الكادميوم والسيلنيوم لمدة إسبوعين. التجربة الثانية . إشتملت على عدد ١٨ من ذكور الفئران البالغة وقد قسمت إلى ثلاث مجموعات متساوية. المجموعة الأولى مجموعة ضابطة والمجموعة الثانية أعطيت عن طريق الفم الكادميوم ١ ملجم/كيلو جرام من وزن الجسم يومياً لمدة ٤٥ يوماً والمجموعة الثالثة أعطيت عن طريق الفم جرعة ٢ ملجم/كيلو جرام من وزن الجسم لمدة ٤٥ يوماً. تم قياس مستوى هرمون التستستيرون وهرمون اللوتن وأيضاً معرفة وزن الحويصلات المنوية والخصى. وكذلك دراسة تأثير الكادميوم على وظائف الكلى والكبد.

وأظهرت النتائج أن إعطاء جرعة واحدة من الكادميوم لفئران التجارب أدت إلى نقص معنوى فى مستوى هرمون التستستيرون وزيادة معنوية فى مستوى هرمون اللوتن وأيضاً نقص معنوى فى وزن الخصى والحويصلات المنوية. وأن إعطاء جرعة من الكادميوم والسيلنيوم معاً أدت إلى نقص معنوى فى مستوى هرمون التستستيرون ولكن هذا النقص كان أقل من إعطاء الكادميوم بمفرده. أيضاً حدوث زيادة فى مستوى هرمون اللوتن. أيضاً أظهرت النتائج حدوث نقص شديد فى مستوى هرمون التستستيرون وزيادة فى مستوى هرمون اللوتن بعد إعطاء الكادميوم لمدة ٤٥ يوم وأظهرت نتائج وظائف الكبد والكلى زيادة فى إنزيمات الكبد وخلل فى وظائف الكلى. ومن هنا يتضح لنا أن إعطاء السيلنيوم قد أضعف من تأثير الكادميوم ولكن لم يمنع هذا التأثير على خصوبة الذكور وأيضاً أن إعطاء الكادميوم يؤدى إلى خلل فى وظائف الخصى والكلى والكبد. وأن تلوث البيئة بالعناصر الثقيلة وخاصة الكادميوم يؤدى إلى نقص خصوبة الحيوانات.