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EFFECT OF LEAD AND CADMIUM ON REPRODUCTIVE PERFORMANCE OF MALE RABBITS

(With 6 Tables)

By

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تأثير الرصاص والكادميوم على الكفاءة التناسلية لذكور الأرانب

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أجريت هذه الدراسة على عدد ٣٢ ذكر أرنب بالغ (كاليفورنيا) وعدد واحد أنثى استخدمت لتجميع السائل المنوى. جعلت هذه الحيوانات تشرب من ماء ملوث بكل من الرصاص والكادميوم بتركيزات مختلفة الرصاص (٥ جزء فى المليون و ١٠ جزء فى المليون) والكادميوم (٢ جزء فى المليون و ٤ جزء فى المليون) لمدة ٦ أسابيع ثم أوقفت التغذية بالماء الملوث وتركت هذه الحيوانات لمدة ٤ أسابيع أخرى تشرب ماء غير ملوث (من الصنبور) فى خلال التجربة. تم تجميع السائل المنوى أسبوعيا لمتابعة تأثير هذه العناصر على نوعية السائل المنوى وكذلك أخذت عينات دم وتم تحليل الهرمونات (هرمون الذكورة والهرمونات المحفزة للأقناد L.H., F.S.H. Testosterone) لمعرفة صورة هذه الهرمونات ومدى تأثير هذه العناصر على تركيزها فى الدم وكذلك مدى أحتمال حدوث تحسن فى نوعية السائل المنوى وتركيز هذه الهرمونات فى الدم بعد توقف التغذية بالماء الملوث. بعد أنتهاء التجربة ثبت ان تلوث المياه بهذه العناصر له تأثير سئ على نوعية السائل المنوى وكذلك خفض لمستوى هذه الهرمونات فى الدم. ومن المحتمل حدوث تحسن فى كل من نوعية السائل المنوى وتركيز هذه الهرمونات فى الدم بعد توقف عملية التغذية بالماء الملوث.

SUMMARY

A total of 32 adult male rabbits were used in this study. They were left for one month for training on semen collection and then classified into four groups (8 animals in each). The 1st and 2 nd group were allowed to

drink lead polluted water with low and high levels respectively (5 ppm &10 ppm) while the 3 rd and 4 th group were allowed to drink Cadmium polluted water with low and high level respectively (2ppm & 4ppm) for 6 weeks, then the treatments were stopped and the animals were allowed to drink pollution free water (normal tap water) for 4 weeks. Blood and semen samples were taken weekly before beginning, during and after stopping the treatments for evaluation of semen picture and analysis of testosterone, LH and F.S.H. hormones. The results showed that, the levels of testosterone, LH and F.S.H. hormones significantly decreased in treated animals and began to be significantly improved after stopping the treatment in both pollution levels of both elements. Also, the semen picture (volume, concentration, live/dead %, motility %, abnormalities) significantly decreased in treated animals starting from the 3 rd week of treatment in case of low level of both elements and at the 2 nd week in case of high level of both elements. There were significant improvement in semen picture at the 4 th week post treatment when compared with that of the last week of treatment in all cases of pollution Both Cadmium and Lead pollutant are able to produce drastic effects on semen picture and reduction of hormonal levels, after stopping of pollution some improvement on semen picture and hormonal levels have occurred.

Key Words: *Lead, Cadmium, Performance.*

INTRODUCTION

The pollution of the environment with different sources is the vital talk allover the scientific media and the governmental and non governmental organization (N.G.O.). Thus cadmium and lead water pollution and its effects on reproductive capacity on different animals species has got great attention in different researches (Gabbiani et al, 1974, Suter-Ke, 1975 Aoki and Hoffer, 1978, Bryant and Rose, 1985, Alexaki and Tsafaris, 1991, Monsi, et al, 1993, and Ruby, et al, 1993) .The recommended permissible level of cadmium in water is 10 Mg /l according to United States Environmental Protection Agency (1980)# and 5 Mg/l according to the World Health Organization guidline values for drinking water quality (1984)#.

The interpretation of the damage produced by cadmium and lead on the testis is not clearly identified but some suggestions and theories mentioned that the cadmium makes its effect on the vasculature of the testes, (Doh and Foster1972). Also Gazdik and Kaminski (1984) recomended that the cadmium and other metals can activate calmodulin

(calcium-binding protein) which make disturbances in normal calcium control mechanism and by turn induced the toxiciy of the metal. These results were supported by Shore, et al (1995), Moreover, Gunn and Gould (1970) discussed a theory # that cadmium caused a disruption of tight junction associated with microflaments in rat Sertoli cell. # cited by Fayed and Abdallah 1997.

MATERIAL and METHODS

Animals preparation:

Thirty two male adult rabbits (Californian) of an average weight 4 kg were used in this experiment. Adult female were used in semen collection (as a teaser). These animals were kept in healthy, air refreshed room inside well prepared cages. Animal care including nutrition,watering health inspection were done with great attention. Eight males were used for each treatment. The animals were put under training program for semen collection for one month before the beginning of the experiment in a rate of twice a week,Each animal was kept in a separate cage.

Semen collection:

The semen was collected using artificial vagina for rabbits according to the method described by salisbury et al (1985).

Chemicals and its preparation:

Two heavy metals have been chosen, cadmium and lead in two successive concentrations from each .Tow concentrations of lead acetate were prepared. A low concentration, which is 5 ppm and the high concentration is 10 ppm dissolved in distilled water. Similarly two concentrations of cadmium chloride were prepared 1st is 2 ppm (low concentration) and the 2nd (high concentration) was 4 ppm in distilled water.

Watering of the animals:

The animals were classified into 4 groups, 8 male animals in each group. The 1st and 2nd groups were allowed to drink day and night lead polluted water (low and high concentration respectively) and the 3rd and 4 th groups were allowed to drink day and night cadmium polluted water (low and high concentration respectively) for 6weeks. The males of each treatment were allowed to drink normal water for four weeks. Through this period regular semen collection was carried out to follow up the changes in semen picture (according to Salisbury et al, 1985)

Hormonal evaluation:

Blood samples were collected before, (before starting the treatment), during, (at the 6th week from the beginning of the treatment period), and after treatment, (4th week after treatment) using sterile disposable syringe through jugular puncture. Serum was obtaind and preserved in deep freezer at (-20 °c) for hormonal analysis. Gonadotropine hormones according to ELISA Voller, et al, (1979), in this respect, F.S.H. (gestyl, Nile drug company) and L.H., (pregnyl, Nile drug company) were used as antigen to prepare their corresponding rabbit anti sera Testosterone using radio-imunoassay adopted by Vermuelen, and Verdonck, (1976) Statistical analysis was done according to Snedecor and Cochran (1980)

RESULTS

The results are presented in Tables 1,2,3,4,5 and, 6. Table 1 shows hormonal analysis of lead 1 (5ppm) and lead 2 (10 ppm) treated animals. The data cleared that the levels of the three hormones (LH &F.S.H. and testosterone) were significantly reduced in treated animals and began to be significantly improved after stopping of treatment in both pollution levels of both elements (tables 1,2). Tables 3,4,5 and, 6 illustrate the semen picture of the animals drank polluted water with lead and cadmium. The data cleared that the semen picture began to significantly affected (decreased motility % & sperm cell concentration and increased dead % & abnormalities %), from the 3 rd week of treatments in animals kept to drink polluted water with low level of both elements and from the 2 nd week in animals drank polluted water with high level of both elements. Also the data cleared that there were significant improvement in semen picture at the 4 th week post-treatment when compared with that of the last week of treatment (6 th week) in all cases of pollution.

DISCUSSION

The obtained results of semen picture, revealed the drastic effect of treatment by both cadmium and lead treatments and control is very clear tables 3,4,5,6. and these results agree with Zavos and Cohen (1980) and Hew, et al (1993), However the results obtained by Monsi, et al (1993) on turkey differed with obtained results. This disagreement may due to species used (turkey) and route of injection (subcutaneous). Ruby,

jaroslawsi, and Hull (1993) recorded that lead toxicity on the rainbow lead to drastic effect on semen picture and these results agreed with our results. Also the results obtained on bull revealed drastic effect on semen picture of bulls due to lead toxicity, Mankiewicz, et al (1975), similarly the obtained results in lead treatment was in accordance with those results obtained by Jaczewski, et al (1977) who reported that lead toxicity significantly reduced sperm cell concentration, sperm cell survival time in male rabbit.

These clearly identified deteriorating picture of semen in our results could be considered as an evidence of the damage effect on the seminiferous tubules these results agreed with Fayed and Abdalla (1997) who recorded that toxicity of male rats with cadmium chloride lead to degeneration in seminiferous tubules. Also the results agreed with Gunn and Gould (1970) who reported that cadmium toxicity in rat lead to degeneration in seminiferous tubules. In addition Ruby, et al (1993) recorded that the lead toxicity in rainbow suppress the spermatogenesis by acting on sublethal level on the cycle. These result appeared in accordance with our results.

The obtained results point out that there are reduction in both testosterone, L.H. and F.S.H. in comparison with control readings, (Tables 1,2). The available literature concerning the effect of cadmium and lead on the level of these hormones are very scarce. These results appeared in accordance with those obtained by Kuo, et al (1995) that the L.H &F.S.H. & testosterone are significantly reduced .In mice fertility and libido were lost after treatment with cadmium 5mg /kgr and 2mg/kgr body weight thus may be the effect of these heavy metals on the vascularity of the testis and the Leydig cells. Doh and Foster (1972), Gabbiani, et al (1974) Aoki and Hoffer (1978).

Unfortunately the recovery result which have been obtained in both semen picture and hormonal level, a little references were available. Thus after stopping of treatment there are some improvement in semen characters and libido also hormonal level. These results agreed with Kuo, et al (1995), who claimed that the L.H &F.S.H. & testosterone are significantly reduced and the infertility and libido were lost in mice treated with cadmium and recovered again after 6 weeks from stopping of treatment.

According to the results obtained in this study pollution with both lead (5ppm & 10ppm) and cadmium (2 ppm & 4 ppm) adversly affected fertility and libido which is a warning from pollution with both metals.

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Table 1: Levels of L.H., F.S.H. and Testosterone in male rabbits drunk polluted water with both levels of lead acetate.

Group	Lead 5 ppm L.H i.u./ml	5 ppm F.S.H i.u./ml	5 ppm Testosterone ng/ml	10 ppm L.H i.u./ml	10 ppm F.S.H i.u./ml	10 ppm Testosterone ng/ml
Control	b 3.17 ± 0.44	b 2.78 ± 0.26	b 4.75 ± 0.51	b 3.17 ± 0.44	b 2.78 ± 0.26	b 3.17 ± 0.5
Treated	a b 1.05 ± 0.35	a b 1.017 ± 0.29	ba 1.98 ± 0.15	bc 0.99 ± 0.09	ba 1.12 ± 0.21	d b 1.59 ± 0.17
Post. Treated	ba 2.64 ±0.51	a 2.13 ± 0.32	a 3.65 ± 0.49	c 2.52 ± 0.33	ba 2.18 ± 0.23	B 3.8 ± 0.52

Within the same column values having the same letter differ significantly at :a (p(0.05) ,b and c: (p(0.01) and d: at (p(0.001)

Table 2: Levels of L.H., F.S.H. and Testosterone in male rabbits drunk polluted water with both levels of Cadmium chloride.

Group	Cadmium 2 ppm L.H. i.u./ml	2 ppm F.S.H. i.u./ml	2 ppm Testosterone n.g/ml	4 ppm. L.H. i.u./ml	4 ppm. F.S.H i.u./ml	4 ppm. Testosterone n.g/ml
Control	a 3.17 ± 0.44	c 2.78 ±0.26	c 4.75 ± 0.51	a 3.17 ± 0.44	c 2.78 ±0.26	c 4.75 ± 0.51
Treated	ab 1.01 ±0.12	cd 0.75 ±0.11	ca 1.45 ±0.16	ac 0.88 ±0.11	cd 0.66 ±0.10	ca 1.44 ±0.15
Post-treated	b 2.22 ±0.19	d 2.01 ± 0.15	a 3.98 ±0.56	c 2.44 ± 0.22	d 2.18 ± 0.20	A 3.87 ± 0.55

Within the same column values having the same letter differ significantly at a and b: (p(0.01) and c and d: at (p(0.001)

Table 3: The results of semen characters of male rabbits of both, control, (pretreatment) and during treatment with low and high levels of Lead acetate.

Semen characters of lead 1 treated animals (5 ppm)

Characters/ treatments	* Volume (ml)	Motility (%)	Dead (%)	Concentration $X10^6$ (ml)	** Abnormalities (%)
Control	0.72±0.007	bc 74.5±1.167	abc 8.5±0.5	bc 188.0±2.49	c 9.2±0.44
1st Weak	0.74±0.026	73.75±2.39	11.75±2.32	180±19.7	11.0±4.05
2nd Weak	0.73±0.012	71.25±2.39	13.0±2.517	169.±15.3	13.0±2.17
3rd Weak	0.718±.012	b 65.0±2.041	a 15.0±2.11	c 150.0±4.08	c 20.0±2.04
4th Weak	0.718±0.024	c 55.0±3.146	c 22.5±2.5	c 135.0±8.66	c 26.5±3.15
5th Weak	0.73±0.012	c 47.5±4.787	c 30.0±2.887	c 102.5±8.5	c 31.5±1.25
6th Weak	0.735±0.009	cd 30.0±4.082	cd 31.25±2.39	cd 70.0±8.16	cd 37.5±1.44
1st Weak post-treatment	0.735±0.009	c 33.5±2.394	c 31.25±2.39	c 82.5±2.5	c 25.0±2.04
2ndWeak post-treatment	0.743±0.008	c 38.75±2.39	c 26.25±1.25	c 102.5±2.5	c 23.5±2.39
3rd Weak post-treatment	0.730±0.009	c 56.25±2.39	b 18.75±2.39	b 127.5±11.09	c 22.5±1.44
4th Weak post-treatment	0.715±0.012	d 67.5±3.22	d 10.5±1.44	d 162.5±10.5	d 13.25±2.39

Within the same column values having the same letter differ significantly at a : (p(0.05), b: at (p (0.01) and c :at (p (0.001) Values of the fourth week post treatment are significantly different from that of the six week of the treatment at p (0.001 in case of d).

*** The total volume ** Primary abnormalities.**

Table 4: Semen characters of lead 2 treated animals (10 ppm)

Characters / Control	* Volume (ml)	Motility (%)	Dead (%)	Concentration X 10^6 (ml)	** Abnormalities (%)
Control	0. 72±0.006	abc 74.5±1.84	abc 8.4±0.27	abc 188.±3.59	ac 9.9±0.67
1stWeak	0.73±0.01	71.25±1.25	9.0±0.58	173.0±16.0	10.0±2.86
2ndWeak	0.75±.02	a 67.5±1.44	b 13.75±1.25	a 150.0±11.5	c 12.75±2.29
3rdWeak	0.71±0.014	c 61.25±1.25	c 17.5±1.44	c 120.5±9.74	c 18.75±1.25
4thWeak	0.74±0.03	c 53.75±1.25	c 21.25±1.25	c 95.0±2.89	c 23.75±1.25
5thWeak	0.73±0.014	c 46.25±2.39	c 28.75±1.25	c 72.5±8.54	c 31.25±3.75
6thWeak	0.68±0.013	cd 28.75±3.15	cd 41.25±1.25	cd 67.5±6.29	cd 40.0±2.02
1stWeak post-treatment	0.70. ±0.01	c 22.5±2.5	c 28.75±1.45	c 80. ±07.12	c 37. 5±1.44
2ndWeak post-treatment	0.72(0.014	c 28.75±3.15	b 22.5±2.5	c 102.5±7.5	c 28.5±3.75
3rdWeak post-treatment	0.71±0.035	c 47.5±4.79	b 18.75±2.25	c 122.5±5.79	c 21.25±2.39
4th Week post-	0.68±0.043	bd 63.9±3.7	ad 15.5±1.99	bd 145.5±8.87.	ad 16.0±1.94

Within the same column values having the same letter differ significantly at a: (p (0.05), b: at (p (0.01) and c :at (p (0.001) Values of the fourth week post treatment are significantly different from that of the six week of the treatment at p (0.001 in case of d) . * The total volume ** Primary abnormalities.

Table 5: The results of semen characters of male rabbits of both, control, (pretreatment) and during treatment with low and high levels of Cadmium chloride.

Semen characters of Cadmium 1 treated animals (2 ppm)

Treatments /characters	* Volume (ml)	Motility (%)	Dead (%)	Concentration X 10^6 (ml)	** Abnormalities (%)
Control	0.742±0.009	75.0±1.29 c	8.8±0.33 bc	204.0±3.16 bc	9.8±0.36 abc
1st Week	0.731±0.033	75.0±2.04	9.25±1.11	202.5±8.54	10.5±2.4
2nd Week	0.729±0.021	75.75±2.17	9.75±0.85	202.5±6.61	10.75±1.11
3rd Week	0713±0.015	67.5±3.23	13.0±1.22	189.3±1.49	12.5±1.04
4th Week	0.710±0.018	56.75±2.69	17.5±1.99	150.0±4.08	23.75±1.25
5th Week	0.725±0.01	42.5±3.23	23.25±2.69	111.3±6.57	32.25±1.03
6th Week	0.70±0.022	no sperms c	no sperms c	no sperms c	no sperms c
1st Week post-treatment	0.712±0.022	no sperms c	no sperms c	no sperms c	no sperms c
2nd Week post-treatment	0.715±0.024	no sperms c	no sperms c	no sperms c	no sperms c
3rd Week post-treatment	0.731±0.017	40.5±3.23 c	31.75±1.18 c	72.5±7.5 c	26.25±3.15 b
4th Week post-treatment	0.735±0.014	72.0±3.17 d	13.0±2.04 d	190.0±8.57 d	12.5±3.23 d

Within the same column values having the same letter differ significantly at a: (p(0.05), b: at (p (0.01) and c: at (p (0.00) Values of the fourth week post treatment are significantly different from that of the six week of the treatment at p (0.001 in case of d).

*** The total volume ** Primary abnormalities.**

Table 6: semen characters of cadmium 2 treated animals (4 ppm)

/characters treatments	* Volume (ml)	Motility (%)	Dead (%)	Concentration X 10^6 (ml)	** Abnormalities (%)
Control	0.738±0.01	bc 72.5±1.34	abc 9.2±0.44	ac 194.0±4.0	abc 10.4±0.5
1st Week	0.720±0.027	71.25±1.25	11.25±1.49	190.75±5.45	10.25±1.65
2nd Week	0.718±0.012	67.5±3.23	c 17.0±1.22	c 170.0±11.5	11.75±1.18
3rd Week	0.730±0.007	c 45.0±2.04	c 25.0±2.04	c 130.0±3.39	b 20.75±2.53
4th Week	0.743±0.022	c 32.5±3.23	c 41.25±3.15	c 90.25±5.54	c 31.25±2.39
5th Week	07513±0.022	c 17.5±2.23	c 42.5±3.23	c 58.75±7.74	c 47.0±3.39
6th Week	0.725±0.01	cd no sperms	cd no sperms	cd no sperms	cd no sperms
1st Weak post-treatment	0.725±0.036	c no sperms	c no sperms	c no sperms	c no sperms
2nd Weak post-treatment	0.735±0.009	c no sperms	c no sperms	c no sperms	c no sperms
3rd Weak post-treatment	0.743±0.022	b 43.75±6.25	b 20.25±2.75	c 57.5±7.22	b 33.5±5.15
4th Weak post-treatment	0.725±0.014	d 65.0±3.54	ad 15.5±2.5	ad 137.0±15	ad 16.5±2.22

Within the same column values having the same letter differ significantly at a: (p(0.05), b: at (p (0.01) and c: at (p (0.001) Values of the fourth week post treatment are significantly different from that of the six week of the treatment at p (0.001 in case of d . * The total volume ** Primary abnormalities.