

Some Studies On The Effect of Lead On Fertility, Some Hormone And Trace Elements In Male Rabbits

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ABSTRACT

This study was designed to determine the most adverse effect of underground well water contain high level of lead on fertility, leukogram and some biochemical parameters in mature balady male rabbits. Sample from underground well water from 10th Ramadan City area and fresh tap water was examined for detection of lead level. Analysis of underground well water sample revealed presence of 0.84 ppm lead but fresh tap water had 0.12 ppm.

A total of 10 mature male balady rabbits were divided into 2 equal groups (5 of each). First group mature male rabbits drink fresh tap water that contains 0.12 ppm lead (control group) and second group mature male rabbits drink underground well water contain that 0.84 ppm lead for 60 successive days. At 61th day all rabbits were slaughtered and two blood and semen samples were taken for determination of leukogram and some biochemical parameters beside evaluate semen characters.

Mature male balady rabbits drink underground well water high in lead show significant leukocytosis, neutrophilia, monocytosis, lymphocytosis beside to significant elevation in serum lead, AST ALT ALP, GGT, total sperm abnormalities % beside a significant reduction in testosterone, T3, T4 level, iron, zinc copper, total protein, albumin, sperm number, sperm motility alive sperm, weight of (testis epididymis, seminal vesicle gland and prostate gland) and insignificant decrease in eosinophils, basophils and globulin

It could be concluded that high lead underground well water induce infertility in mature male balady rabbits beside alterations in leukogram, biochemical parameters and some hormones. So, It is recommended not use underground water high level of lead in rabbits farms.

INTRODUCTION

Male fertility includes processes which require strict and rigid conditions to produce mature and healthy sperm (1). One of these requirements is chemical nutrients which are important for semen quality and for Sertoli and Leydig cell development (2). Semen quality varies with age, illness and diet of organisms (3).

Heavy metals have low elimination rates from body have a toxic effect (4). They affect male fertility by direct effect on testicular function (5). Among these heavy metals; lead which is

non-essential toxic metal (6). Lead is considered as one of major pollutants causing poisoning in domestic animals more than any other substance (7). Lead is a harmful metal even in low levels and it has both age and dose dependent (8). Lead toxicity induces reproductive failure and adverse semen quality (9) beside hemato-biochemical and hormonal changes (10).

Pure water does not exist in nature (11). The contamination of water is directly related to the degree of contamination of our environment. Rainwater collects impurities while passing

through the air, dangerous products from industry and agriculture reaches the rivers, lakes, and underground water (12).

The objective of this study is to determine the effect of underground well water with high lead level on male fertility, leukogram and blood biochemistry in mature male balady rabbits.

MATERIAL AND METHODS

Water samples and Chemical analysis

One underground well water sample from 10th Ramadan City area and one fresh tap water samples were collected in glass bottle for detection of lead level by using Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (13).

Animals and Experimental design

The present study was carried out on ten mature male balady rabbits 8 months old about 2-2.5 kg body weight. Rabbit were divided into 2 equal groups (5 each). First group drink fresh tap water contains 0.12 ppm lead (control group) and the second group drink underground well water contains 0.84 ppm lead for 60 successive days (tested group). Both groups feed same ration. At 61 day all rabbits were slaughtered and two blood and semen samples were collected.

Blood samples

First blood sample was collected on tube contain EDTA as anticoagulant for determination of leukogram (14) and second sample was collected in centrifuge tube to obtain clear serum for determination trace elements (lead, copper, iron and zinc) using atomic absorption

spectro-photometer (15) testosterone and thyroid (T4&T3) hormones were determined by radioimmunoassay (16,17) respectively, (AST &ALT) (18) GGT (19) alkaline phosphates ALP (20) and total protein (21).

Examination of epididymal sperm

Post rabbits slaughtering epididymal content of each rabbit was collected and squeezed gently in sterile watch glass contain 1ml sodium citrate solution 2.9% to estimate sperm character (22).

Sex organs weight

After slaughtering of rabbits testis, epididymis, seminal vesicle gland and prostate gland were dissected out and weighted.

Statistical analysis

The obtained results were statistically analyzed using (T test) according (23).

RESULTS AND DISCUSSION

Analysis of underground well water ample from 10th Ramadan City area revealed presence of lead in a level of 0.84 ppm meanwhile fresh tap water from 10th Ramadan City area revealed presence of lead in level 0.12 ppm (Table 1). Lead in fresh tap water was slightly exceeded standard permissible limit (0.1 ppm) (24). Increased concentration of lead in underground water well is correlated to high industrial and agriculture wastes (11). Similar lead levels were recorded by (25) in drainage ponds water of 10th Ramadan City. High level of lead was reported in underground water in El-Salam areas 0.90 ppm (12).

Table 1. Lead concentration in the examined water samples (n=1)

Water sample	fresh tap water	Underground water (tested)	Permissible limit of lead in water according (WHO, 1984).
Lead (ppm)	0.12	0.84	0.1

Results of the present study indicate that lead caused significant leukocytosis, neutrophilia, monocytosis, lymphocytosis beside insignificant decrease in eosinophils and basophile (table, 2). Similar findings were reported by (26) in rabbit exposed to lead. Leukocytosis in our study may be due to inflammatory effect of lead (27). Our data is

agreed with (28) who found that increase in serum lead induce significant leukocytosis neutrophilia and monocytosis. Also leads induce lymphocytosis in dogs and cats (29). Insignificant decrease in eosinophil and basophil in rats exposed to lead was recorded previously by (30).

Table 2. Effect of lead on leukogram ($10^3/\text{cmm}$) in mature male balady rabbits (N=5)

Groups	Parameter T leukocytic Count	Differential count (103/cmm)				
		Neutrophils	Lymphocytes	Eosinophils	Basophilis	Monocytes
G1	11.64±0.13	4.44±0.03	4.18±0.06	0.94±0.06	0.81±0.01	0.83±0.05
G2	12.03±0.16**	4.83±0.07**	4.61±0.08**	0.81±0.05	0.79±0.03	1.00±0.04*

The obtained results for serum trace elements were summerized in table (3). The results revealed a significant decrease in iron zinc and copper in mature male rabbits received underground water high in lead. Similar result was recorded (31) in dogs and cats. High level of serum lead was associated with high lead of

underground water (32). Chronic lead toxicity induces decrease of serum zinc in rats (33). Reduction in trace elements in this study may be due to lead stimulates urinary excretion of trace elements interfering with their reabsorption in kidney (34).

Table 3. Effect of lead in serum trace elements in mature male balady rabbits (n=5)

Groups	Parameter Lead (ppm)	Copper (ng/dL)	Iron (ng/dL)	Zinc (ng/dL)
G1	0.14±0.035	271.07±4.33	168.41±3.73	90.21±0.21
G2	0.35 ± 0.075*	252.75±4.50*	152.93±2.99**	83.58±2.34*

*Significant at $P < 0.05$

** Significant at $P < 0.01$

In the current work, it has been found that mature male balady rabbits drink underground water high in lead show significant decrease in testosterone, T3 & T4 levels beside insignificant effect in T3/T4 and T4/T3 (Table 4). Similar results were recorded previously by (35) in rabbits. Lead induces dose dependent decrease serum testosterone (36). Reduction in testosterone may be due to cytotoxic effect of lead on interstitial leydig cells (37) and inhibit

enzyme involved steroid hormones biosynthesis (38). Lead induces significant decrease in T3&T4 (39) in rats. Thyroid gland is a target organ of lead (40). Reduction in thyroid hormone may be due to lead inhibits synthesis and release of thyroid hormone through affecting thyroid iodine uptak or TSH release from pituitary or TRH release from hypothalamus (41)

Table 4. Effect of lead on testosterone and thyroid hormone in mature male balady rabbits (n=5)

Groups	Parameter Testosterone (ng/mL)	Thyroid hormone			
		T3ng/ ml	T4 (µg/ dl)	T3/T4	T4/T3
G1	3.92±0.09	1.07± 0.19	4.04±0.16	0.26±0.009	3.90±0.16
G2	2.95±0.26**	0.89±0.03**	3.55±0.12*	0.25±0.008	4.003±0.14

*Significant at $P < 0.05$

** Significant at $P < 0.01$

Statistical analysis of obtained data revealed that rabbit drink underground water high lead show significant elevation in AST, ALT, ALP and GGT beside to significant reduction in total protein, albumin and insignificant reduction in globulin (Table 5). same change in liver enzymes and protein profile were recorded by

(42, 43) in rabbits exposed to high level of lead. Elevation in liver enzyme is due to liver injury (44). Reduction in total protein may be due to impaired albumin synthesis by liver (45) or due to toxic effect of lead on sulfhydrylcarboxy and imidazole containing protein (46).

Table 5. Effect of lead on liver enzymes and total protein in mature male balady rabbits (n=5)

Parameter Groups	Liver enzymes (U/L)				Protein picture (gm/dl)			
	AST	ALT	Alk.ph	GGT	T.protein	Albumin	Globulin	A/G ratio
G1	63.31± 0.72	48.19± 1.39	53.40± 1.32	32.21± 1.21	5.87± 0.86	3.08± 0.10	2.36± 0.21	1.35± 0.13
G2	68.61± 1.40**	56.10± 1.61**	55.45± 0.98**	36.73± 0.63**	4.56± 0.09**	2.38± 0.11**	2.04± 0.15	1.22± 0.19

*Significant at P < 0.05

** Significant at P < 0.01

The obtained results for some sperm analysis were summerized in table (6). The results revealed a significant decrease in sperm number, motility, alive sperm beside significant increase in total sperm abnormalities % in male rabbits provided with underground water high in lead. Same result was recorded by (47,48) in rabbits and rats respectively. This may be explained on the bases of a fact reported by (49) stated that lead induces testicular degeneration. Furthermore, lead toxicity induce dose depended decrease in sperm number and motility in white mouse (50). Also, lead acts as spermicidal agent in rats (51). Our results came in agreement with (52) stated that lead has

direct testicular toxicity by inhibiting activities of testicular steroidogenic enzyme or indirect effects through targeting endocrine control of reproduction or both. Same result was recorded by (53) who mentioned that lead alter testes and reproductive tract function in rats. Change in sperm picture may be due to lead inhibited spermatogenesis by reducing length of the stages related to spermiation and onset of mitosis (54) and lead enhances intracellular reactive oxygen species production and lipid peroxidation, which led to tissue damage (55). Lead induces reduction in male fertility due to decrease in testosterone (56).

Table 6. Effect of lead on spermatozoal picture in mature male balady rabbits (n=5)

Parameter Groups	Sperm cell concentration x106 spz/ml,	Sperm Motility (%)	Live Sperm %	Total abnormality %
G1	2.28±0.10	81.20±1.54	80.20±0.66	14.40 ±1.03
G2	1.99±0.06*	68.60±2.65**	77.60±0.75**	20.40 ±0.74**

*Significant at P < 0.05

** Significant at P < 0.01

Our study revealed that lead induced significant decrease in weight of testis, epididymis, seminal vesicle and prostate gland (Table, 7). The same results were recorded (57) in rabbits (58,59) in swiss mice and rats

respectively. Significant decrease in weight of accessory sex organs of rabbit exposed to lead could be due to decrease in testosterone hormone level (60).

Table 7. Effect of lead on weight of testis and accessory sex organ in male balady rabbits (n=5)

Groups	Parameter	Testis (gm)	Epididymis (gm)	Seminal vesicle gland (gm)	Prostate gland (gm)
G1		2.48±0.08	1.62±0.11	1.45±0.04	0.99±0.05
G2		2.20±0.09*	1.30±0.07*	1.29±0.05*	0.79±0.07*

*Significant at P < 0.05

** Significant at P< 0.01

From the previously mentioned results it can be concluded that, underground water high in lead level induce infertility in mature male rabbits beside alterations in leukogram and biochemical parameters as well as some hormones. So, recommended not use underground water contains high level of lead in rabbits farms.

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الملخص العربي
بعض الدراسات عن تأثير الرصاص على الخصوبه, بعض الهرمونات والمعادن النادره
فى ذكور الارانب

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الهدف من هذه الدراسة هو معرفه تاثير زياده نسبه الرصاص فى مياه الابار الجوفيه على الخصوبه الذكريه وبعض القياسات الهرمونييه, الدمويه والبيوكيميائية فى ذكور الارانب البلدى البالغه. تم تجميع عينه واحده من كلا من مياه الابار الجوفيه ومياه من الصنبور فى منطقه العاشر من رمضان. وبتحليل عيني المياه فى منطقه العاشر من رمضان وجد ان مياه الابار الجوفيه تحتوى على نسبه ٠,٨٤ جزء فى المليون رصاص بينما مياه الصنبور تحتوى على ٠,١٢ جزء فى المليون رصاص. فى هذه الدراسة تم أستخدم عدد ١٠ ذكور ارانب بالغه عمرها حوالى ٨ شهور ووزنها ٢,٥-٢ كجم. تم تقسيمهم إلى مجموعتين متساويتين يحتوى كلا منها على ٥ ارنب. المجموعه الاولى ذكور ارانب بالغه تشرب مياه الصنبور بها مستوى الرصاص ٠,١٢ جزء فى المليون (مجموعه ضابطة) بينما المجموعه الثانيه ذكور ارانب بالغه تشرب مياه جوفيه بها نسبه الرصاص (٠,٨٤ جزء فى المليون) لمدة ٦٠ يوم متتاليه. عند اليوم ٦١ من التجربه تم ذبح الارانب كلها وتم أخذ عينتين دم وعينه سائل منوى من كل ارنب لدراسة تاثير زياده نسبه الرصاص على صورة الدم, بعض الهرمونات وبعض الوظائف البيوكيميائية وصورة الحيوانات المنويه.

وأظهرت النتائج بعد تحليلها إحصائيا أن الرصاص أدى إلى حدوث زياده معنويه فى العدد الكلى لكرات الدم البيضاء، الخلايا المتعادله الخلايا الليمفاويه، الخلايا الملنهمه الكبيره, نشاط إنزيمات الكبد (AST- GGT-ALP-ALT), نسبة العيوب الشكلية فى الحيوانات المنوية الحية. بالاضافه الى وجود نقص معنوى فى الخلايا الحامضيه, هرمون التستستيرون, هرمونات الغده الدرقيه (T3 & T4), الحديد, النحاس, الزنك, البروتين الكلى, الزلال, عدد الحيوانات المنوية, معدل الحركة المتقدمه للحيوانات المنويه, نسبه الحيوانات المنوية الحية ونقص فى وزن الخصيه, البربخ, الغده المنويه وغده البروستاتا, بجانب نقص غير معنوى فى الخلايا الحامضيه, الخلايا القاعديه والجلوبولين بالمقارنه بالمجموعه الضابطة.

وخلاصة هذه الدراسة أن استعمال المياه الجوفيه التى تحتوى على نسبه مرتفعه من الرصاص فى مزارع الارانب تؤدى الى تقليل الخصوبه الذكريه وتغيير فى صورته الدم البيضاء وبعض الوظائف الحيويه. لذلك من المهم عدم استخدام المياه الجوفيه فى مزارع الارانب الا بعد قياس نسبه الرصاص بها وتكون فى الحدود المسموح بها حتى لا تؤثر على خصوبه الذكور بالمزرعه وتقليل الانتاج.