

Physiological and histological effect of aqueous and alcoholic extract of Garlic (*Allium sativum*) on testicular function of albino male mice treated with lead acetate

التأثير الفسيولوجي والنسجي للمستخلص المائي والكحولي لنبات الثوم على الوظائف الخصوية لذكور الفئران البيض المعاملة بخلات الرصاص

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Abstract

This study was carried out to investigate the effect of oral administration of aqueous and alcoholic extract of *Allium sativum* (Garlic) on testicular function in male mice exposed to lead acetate. Sixty adult (7-8) weeks old male mice were divided into six groups (10 mice/group), first and second group were administered with 150mg/kg body weight BW/day of aqueous and alcoholic extract of plant respectively. However, the third group was treated with 100 mg/kgBW/day of lead acetate. While the fourth and fifth groups were administered with 150 mg/kgBW/day. Of aqueous and alcoholic extract combination with 100 gm/kg. BW of lead acetate. The sixth group treated with distilled water and served as control group throughout five weeks. At the end of experiment, treated animals were sacrificed and sperm were collected from caudal epididymis to use for following tests: sperm concentration, motility, dead/alive sperm and morphological abnormalities. Serum was isolated to assay for the analysis of, FSH, LH and testosterone level. The results showed a significant increase in sperm concentration, motility and decrease in dead and abnormal sperm in the group treated with aqueous and alcoholic extract of *Allium sativum*. However, the results showed decrease in sperm concentration, motility and increase in dead and abnormal sperm in the group treated with lead acetate. The other groups of animals groups treated with aqueous and alcoholic extract combination with lead acetate showed improvement in sperm concentration and motility compared to that treated with lead acetate. The FSH, LH and testosterone level significantly increased in treated groups with aqueous and alcoholic extract of plant compared to group treated with lead acetate. It's concluded that aqueous and alcoholic extract of *Allium sativum* were significantly improved fertility in treated medicine compared to animals treated with lead acetate.

Key words: Fertility, lead acetate, *Allium sativum*

المستخلص

صممت هذه التجربة لدراسة تأثير التجريع الفموي للمستخلص المائي والكحولي لنبات الثوم على بعض الوظائف الخصوية لذكور الفئران البيض المعاملة بخلات الرصاص . قسمت حيوانات التجربة بعمر (6-7) اسابيع الى ست مجاميع (10 فأر مجموعته) . جرعت المجموعتين الاولى والثانية بالمستخلص المائي والكحولي وبتركيز 150ملغم\كلغم من وزن الجسم\يوم على التوالي وجرعت المجموعة الثالثة بخلات الرصاص وبتركيز 100ملغم\كلغم من وزن الجسم\يوم . اما المجموعتين الرابعة والخامسة فتم تجريعهما بالمستخلص المائي والكحولي وبتركيز 150ملغم\كلغم من وزن الجسم\يوم والمعاملة بخلات الرصاص وبتركيز 100ملغم\كلغم من وزن الجسم\يوم ، واعتبرت المجموعة السادسة مجموعة سيطرة واعطيت ماء مقطر . استمر التجريع لمدة خمس اسابيع . اظهرت النتائج وجود زيادة معنوية بتركيز النطف وحركتها وانخفاض النطف غير السوية والميته في الحيوانات المعاملة بالمستخلص المائي والكحولي بينما اوضحت النتائج وجود انخفاض معنوي بتركيز النطف وحركتها ووزيادة في النطف غير السوية والميته في الحيوانات المعاملة بخلات الرصاص . فيما اظهرت النتائج تحسن معنوي بتركيز النطف وحركتها وكذلك انخفاض النطف غير السوية والميته في الحيوانات المعاملة بالمستخلص المائي والكحولي وخلات الرصاص مع مقارنة مع الحيوانات المعاملة بخلات الرصاص فقط . كذلك اظهرت النتائج حدوث تحسن معنوي في مستوى الهرمون المحفز للجريبات واللوتيني والتستوستيرون في الحيوانات المعاملة بالمستخلص المائي والكحولي وخلات الرصاص مع مقارنة مع الحيوانات المعاملة بخلات الرصاص فقط . نستنتج من الدراسات الحالية ان المستخلص المائي والكحولي لنبات الثوم له تأثير ايجابي على الخصوبة في الفئران البيض .

الكلمات المفتاحية: الخصوبة ، خلات الرصاص ، الثوم

Introduction

Lead is one of the known and most widely studied occupational and environmental toxicant, lead is mineral which has been associated with human activities from the past 6000 years. Today, lead is still used in batteries, some insecticides and found in cigarette smoke. Sources of lead may be natural, as it is found in the earth's crust and thus enters the food and water supply. Lead compounds are known to adversely affect the various mammalian systems. Reproductive toxicity, lead being one that can affect the gonad structure and function, can cause alteration in fertility and impaired gamete function, the toxicant can also lead to induction of malformation in children, reduce chance of conception, may be embryocidal and cause still birth, the inability to sustain pregnancy and reduced pregnancy outcome [1,2]. Lead remains a significant occupational and public health problem. Men exposed to lead not only at work places, but also from other sources like lead paints in older housing and from soil. Lead is known to cause a number of adverse consequences in both men and women. Effect in women induces infertility, miscarriage, premature membrane rupture, pregnancy hypertension [3]. Lead poisoning can cause variety of symptoms and signs which vary dependability on the individual and the duration of lead exposure [4]. A number of heavy metal are still widely used in industry and lead, in particular, is generally considered as one of the most toxic metal to human as well as animals [4,5]. Symptom from organic lead, which is probably more toxic than inorganic lead due to its lipid solubility, occur rapidly. Lead is highly toxic with the injurious effects on the hemapoitic, nervous and reproductive system. Lead crosses the placenta during pregnancy [6] headache abdominal pain, memory loss, kidney failure male reproductive problem [7]. Markets and changes occur in sperm concentration, their motility and their morphology [8]. One of the main causes for the pathology of lead is that interferes with activity of an essential enzyme called delta- amino levulinic acid dehydretase (ALAD).

Medicinal plants

The contribution of medicinal plants in the traditional system of medicine for curing diseases has been documented. Nowadays increased scientific interest and consumer demand have promoted the development of herbal products as dietary supplements oriental herbal medicines have a prominent role to play in the pharmaceutical and health markets. It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals, both the deficiency and excess of essential micronutrients and trace toxic metals may cause serious effects on human health [9].

Common name:

Allium sativum – Garlic family\Liliaceae, Garlic is a bulbous perennial herb, closely related to the onion. It has a tall, erect flowering stem that reaches 2-3 feet in height. The plant has pink or purple flowers that bloom in mid to late summer. The part used medicinally is the bulb [10].

Active chemical constitutes

Sulfur compounds:

Aliin, Allicin, Ajoene, Allylpropylsulfide, Diallyltrisulfide, Sallcysteine, Vinylthiines, S-allyl mercaptocystei and others. Enzymes such as Allinase, Peroxidase, Myrosinase and others amino acids and glycosidase: Arginine, Selenium, Germanium, Tellurium and other trace minerals [11,12]. The components of garlic are carbohydrates, proteins and essential oils and sulfur compounds and 17 amino acid include alanin, arginine and serine and proline. The garlic plants rich balgulwadat and alsabonyat dragons and filavonat add it to contain many vitamins, and minerals is a good source of vitamin A, B and C also contains a percentage of phosphorus, potassium, calcium and sulfur [13,14].

Extraction of plant

Aqueous extract

50 gram of garlic powder were put in conical flask, then 500 ml of distilled water were added in percentage 1:5w.v. During extraction the mixture was shaken continuously for two hours, the suspension was filtered by Whatman filter paper no.1 and filtrates concentrated by using vacuum rotary evaporator. The crud extract was stored at 4C [15].

Alcoholic extract

Fifty grams of garlic powder were put in conical flask, then 500 ml of absolute methanol 96% were added under continuous stirring for 10hr.the suspension was filtered by Whatman filter paper no.1 and filtrates concentrated by using vacuum rotary evaporator. The crud extract was stored at 4C[16].

Experimental Design

Sixty healthy albino male mice with average body weight (25-30) gram were used for this study. They were obtained from animal house of the Biotechnology Research Center \AL-Nahriane University. The animals were kept in an air-conditioned room at(25±2)C with light period of 12±2 hours. They were divided into six groups (10 mice\group) each one involved ten animals. Aqueous and alcoholic extract of *Allium sativum* in a dose of 150 mg\kg BW\day were orally administrated to the male in group one and two respectively for five weeks. The third group was orally administrated with 100mg\kg BW\day of lead acetate. While the fourth and fifth groups were orally administrated in a dose of 150 mg\kg BW\day combination with100mg\kg BW\day of lead acetate. The plant extract and lead acetate were given orally by stomach tube as a dose of 0.1 ml\day for five weeks. The sixth group allowed drinking distilled water only as control group. The epididymis was put in small Petri dish containing worm RPMI-1640 medium, organ was minced into tiny pieces with micro surgical scissors until getting homogenized solution ,which contain the sperm suspension then subjected to sperm function test, including microscopic examination to record the concentration of sperm(sperm\ml), sperm motility, abnormal sperm morphology and sperm viability. The portion of blood samples were collected and allowed to coagulate at room temperature, the blood was centrifuged at 3000 r.p.m for 10 minutes, and clear non-haemolysed supernatant serum were quickly removed and stored at -20°C for subsequent analysis, for measurement of FSH, LH and testosterone (aBiomerieux Italia S.P a vidia Campigliano,58 50015-point AEMACF, Italia, mini-VIDAS. The testes tissue samples were collected and fixed in 10% neutral buffered formalin and processed for histopathology using haematoxylin and eosin staining method according to [17]. Semen sample were collected from the caudal epididymis, and the samples were analyzed immediately after collection. Method for estimation of sperm concentration was used by [18]. A drop of sperm suspension was placed on slid and covered with cover slip. Concentration of sperm (sperm\ml) was calculated from the mean number of sperm in five high powers microscopically field. This number was multiplied by a factor of one million($\times 10^6$ sperm\ml). Sperm motility was recorded to the [18]. Sperm suspension 50µl was placed over slid and covered by slid cover, using light microscope several field were examined to estimate the percentage of individual motility of sperm. A total of 100 spermatozoa from each mice were examined for morphological change and viability. These were determined from a total count of 100 sperm in smear obtained with stains(0.2 g Eosin and 6g of fast green dissolved in distilled water and ethanol in ratio 2:1.Live\dead ratio was determined using 1% Eosin and 5% Nigrosin in 3% sodium citrate dehydrate solution according to the method described by[19].

Statistical analysis

The program was used SPSS 10.01 \2001 in the statistical analysis of the results extracted the arithmetic average and standard error (mean ±SE) and use T-test to Analysis the differences between the groups in the level of probability $p < 0.05$ [36].

Results

Effect of aqueous and alcoholic extract of *Allium sativum* in this study was presented in Table (1), the result showed a significant increase in sperm concentration in animals treated with aqueous and alcoholic extract (150 mg\kg WB\day)(42.5±67.53,43.45±7.11)) respectively compared to animals which treated with 100mg\kg WB\day. of lead acetate(28.05±5.09)and animals treated with aqueous and alcoholic extract of plant combination with lead acetate(34.41±5.06,36.0±26.24). The results of statistical analysis indicated a significant increase in sperm motility in all groups compared with animals treated with lead acetate Table (1). It has been found that the lead exposed animals were shown in Table (1) increase in dead and abnormal sperm significantly differed from those which treated with aqueous and alcoholic extract combination with lead acetate. Also the result showed a significant increase in the level of FSH, LH and Testosterone in animals which treated with aqueous and alcoholic extract (150 mg\kg WB\day) compared with animals treated100mg\kgBW\day of lead

acetate, while the results showed improvement in the level of FSH, LH and Testosterone compared with animals treated 100mg/kgBW/day of lead acetate Table (2).

Histological study

The Histological study of testes of mice treated with lead acetate, showed decrease diameter of seminiferous tubules and Leydig cells and increase interstitial space fig.(1,4) compared with control group notes that normal diameter of seminiferous tubules and Leydig cells fig.(3,5) and testes of mice treated with alcoholic extract and lead acetate fig.(2,6)

Table (1): Effect of aqueous alcoholic extract of *Allium sativum* and Lead acetate on sperm parameter in albino male mice.

Group	Sperm concentration 10 ⁶ sperm/ml	Motility%	Viability%	Morphologically abnormal%
Control	40.72± 8.43	87.05± 10.33	12.084± 0.54	8.26± 5.40
	A	A	A	A
Aqueous extract	42.56± 0.35	86.12± 9.24	16.13± 5.62	6.36± 4.12
	A	A	B	A
Alcoholic extract	43.65± 7.11	85.23± 9.25	15.19± 4.72	7.435± .19
	A	A	B	A
Lead acetate	28.65± 8.09	67.63± 6.33	26.32± 4.30	19.32± 4.62
	B	B	C	B
Aqueous extract t and Lead acetate	34.41± 7.06	82.67± 8.40	14.27± 5.62	15.32± 4.83
	C	C	B	C
Alcoholic extract and Lead acetate	36.02± 6.24	80.71± 9.08	15.22 ± 0.82	14.04± 5.33
	C	C	B	C

Values are means ± standard error.

Different letters refer to significant differences (p<0.05) compared between columns groups

Table(2):Effect of aqueous alcoholic extract of *Allium sativum* and Lead acetate on FSH, LH and testosterone in albino male mice

Group	FSH mIU/ml (means±SE)	LH mIU/ml (means±SE)	Testosterone ng/ml (means±SE)
Control	1.58± 0.04	1.60± 0.05	1.56± 0.09
	A	A	A
Aqueous extract	1.40± 0.03	1.70. ± 0.07	1.48± 0.07
	A	A	A
Alcoholic extract	1.48.± 0.09	1.62± 0.12	1.15± 0.04
	A	A	B
Lead acetate	0.93± 0.03	0.96± 0.04	0.92± 0.06
	B	B	C
Aqueous extract and Lead acetate	1.29± 0.06	1.30± 0.03	1.1 3± 0.03
	C	C	B
Alcoholic extract and Lead acetate	1.19± 0.07	1.20± 0.26	1.16± 0.05
	C	C	B

Values are means ± standard error.

Different letters refer to significant differences (p<0.05) compared between columns groups.



Fig (1): Section in testes of mice treated with lead acetate, showing decreased diameter of seminiferous tubules and Leydig cells and increase interstitial space stain 10X(H and E.)



Fig (2): Section in testes of mice treated with alcoholic extract and lead acetate, showing normal diameter of seminiferous tubules and Leydig cells and decrease interstitial space stain 10X(H and E.)



Fig (3): Section in testes of mice (control group), showing normal structure of seminiferous tubules and Leydig cells and interstitial space (I.S.) stain 10X(H and E.)

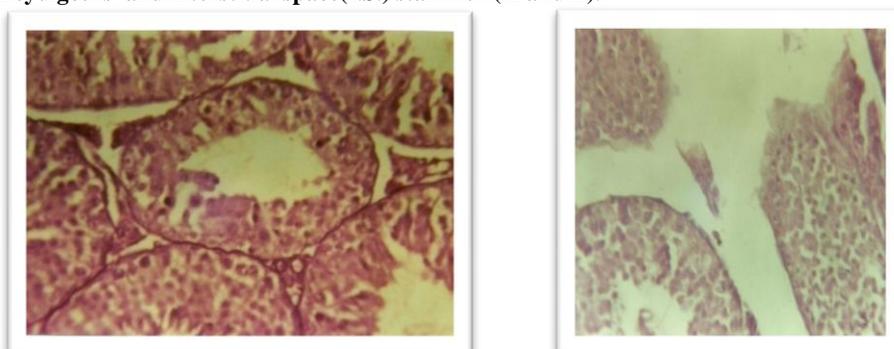


Fig (4): Section in testes of mice treated with lead acetate, showing decreased diameter of seminiferous tubules and Leydig cells and increase interstitial space stain 40X(H and E.)

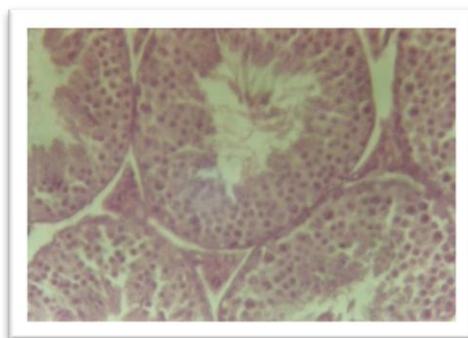


Fig (5): Section in testes of mice (control group), showing normal structure of seminiferous tubules and Leydig cells and interstitial space(I.S.) stain 40X(H and E).

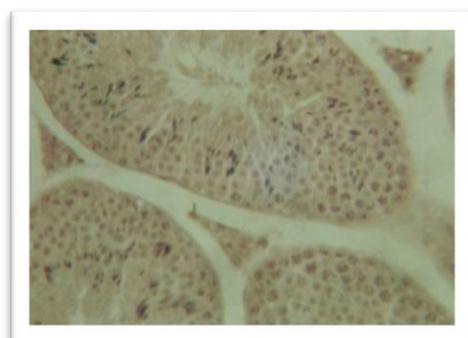


Fig (6): Section in testes of mice treated with alcoholic extract and lead acetate, showing normal structure of seminiferous tubules and Leydig cells and interstitial space(I.S.) stain 40X(H and E).

Discussion

The mechanism of lead induce oxidative stress and imbalance between generation and removal of reactive oxygen species (ROS) in tissue and cellular component causing damage to membranes, DNA and proteins. The presence of double bond in the fatty acid on cell membrane weakens the C-HA bonds on the carbon atom adjacent to the double bonds and makes removal easier, therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than polyunsaturated fatty acid with more than two double bonds [21], the intrinsic mechanism underlying lead induced oxidative damage membrane associated with change in its fatty acid composition [22].The fatty acid chain length and unsaturation are the determinant for membrane susceptibility to peroxidation and lead induce archidonic acid elongation might be responsible for the enhanced lipid peroxidation of membrane [23].Thus, lead affects membrane-related processes such as the activity of membrane enzymes, endo and exocytosis transport of solute across the bilayer and single transduction processes by causing lateral phase separation[24].Lead accumulation in tissue causes oxidative and damage including strand break [25].

Functional deficiencies may develop in the organelles due to lipid per oxidation caused by free oxygen radicals. Cellular death and increased synthesis of collagen follow increased fragility of lysosome and change in microsomal enzymes' lipid peroxidation products easily create reactive carbon compounds[26].Lead has been reported to cause damage in vital organs such as liver, kidney, testes and brain. One of the first materials to be demonstrated as detrimental to fertility was leadan increase in the level of lead in blood testes was linked to an amplified risk. Analysis of sperm count in lead workers showed a decrease in sperm count as well as decrease in motility and lifespan of sperm[27,28]. On the other hand the significant reduction in sperm concentration recorded, may be due to reduction in sperm production in testes since lead could disturb mitosis and alter Sertoli cells proliferation which produce a significant decrease of sperm count the reduction of motility may be due to the direct effect of lead on sperm cells, or it may affect the epididymis since Parsed and Raja [29] showed that the sperm motility dependent on the phospholipids that found in caput epididymis as a source of energy. In the present study there was a significant increase in abnormal sperm

morphology similar results were also reported by [30] in mice semen collected after acute exposure to lead acetate. The lead was accumulate in all male reproductive organ with specially high concentration in epididymis where it have caused alternation in the function which lead to the abnormal morphology of sperm [31]. Presence lead in the seminal plasma exerts toxic effect on sperm parameter such as motility, morphology and viability, therefore, adverse change in testicular sperm structure lead to non-viable sperm production [32]. As the lead opposite effect to the action of FSH and LH hormones a big role in the spermatogenesis and its effect on the Lydeig cells responsible for the secretion of testosterone addition to the effect of lead in the composition and reduce the effectiveness of testosterone hormone through influence on the hypothalamus - pituitary axis and thus its impact on the FSH and LH hormones attributed the cause of this decline in fertility to the effect of lead in the organism's ability to reproduce to get through chromosomal defect or lethal mutation in both human and animal as possible exposure to lead can affect gene expression or affect the DNA in mice The oxidative stress which caused by heavy elements such as lead, produces free radicals affecting the activity of the cell and in particular the plasma membrane function as it is poisonous and harmful materials for animal cell will naturally protect itself from free radicals by antioxidants complex studies showed rats exposure to lead cause change in the level of calcium within the cell and alters the permeability of the cell membrane of liquids as well as the lead change of the components of cellular membranes which unsaturated fatty acids [33,34]. The results show the positive effects of garlic extract on increasing the number of sperm and their movement the other hand decrease the numbers of dead and abnormal sperm compared to animals that were treated with lead acetate was due in to the ability of Garlic extract on removing toxic affectivity of heavy metals and pollutants through its association with and destroy[35]. In addition to its role in increasing the secretion of testosterone through its effects on the pituitary and hypothalamus and stimulate secretion the FSH and LH hormones is selenium, germanium, vitamin C and flavoniods present in garlic and known characteristics antioxidant and detoxification resulting from heavy elements and therefore maintaining the Sertoli AND Lydeig cells from negative effects of the oxidative effect of lead acetate and increased free radicals. Whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum level of two antioxidant enzymes, catalase and glutothion peroxidase [36].

Conclusion

Most pathological conditions arise as a result of the exposure of body tissues to injurious substances including heavy metals. Lead exposure even at low level can affect male and female fertility in human being and animals. The primary targets of lead exposure are tissues of testes and ovaries organs responsible for reproduction. Lead alter the normal histology band intern the physiology of the testes. The adenohipophysical-hypothalamic gonadal axis is also affected by lead which play the key role in controlling different factors accountable for reproduction.

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