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Protective Effect of Acetyl L-Carnitine on Rat Spermatogenesis Defects Induced by Lead Acetate

Abd UL-Rahman Muhammed Ibrahim and Enas Osama Husain*

Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

*Corresponding Author: enasosama982@uomosul.edu.iq

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Abstract

In this study the influences of the ameliorative effects of Acetyl-L-carnitine when exposed to lead acetate on functional aspects in spermatogenesis process in testes of albino male rats was studied, 24 male albino rats weight 178-250 g were randomly distributed into four groups, 6 rats in each, the control group received distilled water orally, Treatment 1 lead acetate group received 10mg\kg body weight orally, Treatment 2 acetyl L-carnitine(ALCAR) group received 100mg\kg body weight orally, and Treatment 3 lead acetate and acetyl L-carnitine group received 10mg\kg body weight and 100mg\kg body weight orally respectively. After the end of the experiment the weight of sexual organs were taken, in addition, the total sperm count number, and percentage of dead, live, with abnormal spermatozoa also have been calculated. Result showed that the total sperm count in lead acetate group was decreased in compared with control and ALCAR groups. The lead acetate with ALCAR showed increased in total sperm count and live sperm percentage in compared with control and ALCAR groups. Dead and abnormal sperm percentage increased in lead acetate group and in lead with ALCAR groups in

Keywards: Lead acetate acetyl L-carnitine, Spermatogenesis, Rats.

التأثير الوقائي للأستايل إل-كارنتين على أضرار عملية تكوين النطف المستحدثة بخلات الرصاص في الجرذان

الخلاصة

في هذه الدراسة، تم دراسة التاثيرات الوقائية للأسيتايل ل-كارنتين عند التعرض لخلات الرصاص على الجوانب الوظيفية لعملية تكوين الحيوانات المنوية في خصى ذكور الجرذان البيضاء ، تم توزيع 24 من ذكور الجرذان البيضاء بوزن 178-250 غرام عشوائيا على أربع مجاميع، 6 جرذان لكل منها ، اعطيت مجموعة السيطرة الماء المقطر عن طريق الفم، مجموعة المعاملة الاولى اعطيت خلات الرصاص 10 ملغم / كغم من وزن الجسم عن طريق الفم،مجموعة المعاملة الثانية اعطيت الأستايل ل-كارنيتين على 100 ملغم / كغم من وزن الجسم عن طريق الفم، ومجموعة المعاملة الثالثة اعطيت خلات الرصاص والأسيتايل 10ملغم / كغم من وزن الجسم و 100 ملغم / كغم من وزن الجسم عن طريق الفم على التوالي. أخذت اوزان الأعضاء التناسلية ،وحُسبت نسبة الحيوانات المنوية الميتة والحيّة والمشوهة. اظهرت النتائج انخفاض العدد في الكلي للحيوانات المنوية في مجموعة المعاملة بخلات الرصاص مقارنة مع مجموعة السيطرة ومجموعة المعاملة بالاستايل ل-كارنتين.اظهرت مجموعة خلات الرصاص مع ALCAR زيادة في العدد الكلي للنطف والنسبة المئوية للنطف الحية مقارنة مع مجموعتي السيطرة و ALCAR. النسبة المئوية للنطف الميتة والمشوهة ازدادت في مجموعتي خلات الرصاص وخلات الرصاص مع ALCAR بالمقارنة مع مجموعتي السيطرة وALCAR.

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Introduction

Spermatogenesis is The process of differentiation of a simple diploid spermatogonium into a spermatid ,occurs in seminiferous tubules during sexual life activity as the result of anterior pituitary gonadotropic hormones stimulation (1). Testis is the important organ of male reproduction with spermatogenesis and endocrine, That depend on the functions of normal spermatogonia and Leydig cell, respectively. Testosterone is the hormone secreted by Leydig cell, it maintains male fertility and spermatogenesis (2).Many studies reported that lead (Pb) damage the spermatogonia and Leydig cell and affecte the male's reproductive function (3) .The target of Pb is Leydig cell and testosterone synthesis also was suppressed during Pb exposure. (4). Lead considered as one of the most hazards that affect cumulative environmental pollutants of all biological systems through exposure to water, air, and food sources(5). There are evidences that Pb induces production of excessive reactive oxygen species (ROS), and ROS attacks system of antioxidant defense in cells, That eventually disturbs the balance of prooxidant/antioxidant in cells lead to causes oxidative stress(6). Acetyl-L-carnitine (ALCAR) is an ester of L-carnitine and plays a major role in function of normal mitochondria, being a transport free fatty acids molecule and an important acetyl-group donor in metabolism of high-energy and beta-oxidation of free fatty acid (7). L-carnitine and ALCAR play a key role in sperm metabolism which affects positively in sperm motility, the beneficial effect is mediated by the long chain fatty acids transport across inner membrane of mitochondria for utilization in metabolism through β-oxidation(8). It acts as an enzymatic antioxidant, that protects the cell, mitochondrial membrane, and integrity of DNA against free oxygen radicals(9). The aim of this study was to investigate functional features of the spermatogenesis process in male albino rats testes when exposed to lead acetate and to evaluate the underlying mechanism of the ameliorative effects of ALCAR to lead acetate exposed.

material and methods

All the steps of the experiment were performed in the laboratory animal house in the college of veterinary medicine/ University of Mosul from the period 26.10.2021 until 26.12.2021.

Animals study

In this study, 24 male rats weight 178-250 g obtained from University of Mosul were included, the rats maintained in cages with free access to water and food and with a 12/12 hours light –dark cycle and controlled temperature. The rats were divided randomly into four groups (6 animals per each) and treated as follows:

1- control group
Rats were given orally distilled water daily for 60 day .

2- Treatment 1:Lead acetate group treated 10mg/kg body weight orally for 60 day.

3- Treatment 2:Acetyl-L-carnitine group treated orally 100mg/kg body weight for 60 day.

4- treatment 3:lead acetate and Acetyl- l-carnitine group
Rats of this group daily administrated by lead acetate 10mg/kg body weight and Acetyl- l-carnitine orally 100 mg/kg body weight for 60 day.

The weight of the animal had been taken in 30 and 60 day.

At the end of the experiment, the weight of each individual animal has been taken and the animal was anesthetized and then killed by cervical dislocation. Performing of the anatomical character of the animal was done by making an opening in scrotum with scissor. The opening was done by cutting through the inguinal canal along the abdomen to the sternum and then the right and left testes were separated, as well as separated the part of epididymis form it (head, body and tail)and weighting separately with sensitive electrical scale, also the accessory glands (prostate and seminal vesicles) separated and weighting by the scale .

Calculating the number of sperms in the

head of the epididymis

Was done by using the method of Sakamoto and Hashimoto (10).They separated head of the right epididymis and then cut into small pieces in a petri dish which contain neutral formalin buffer solution(9.8 ml) after which a dye was added to it eosin (5%) 0.1ml, then a drop of the solution placed on the center of the blood cell counting device haemocytometer and then placed in the stage of light microscope and leave slide on stage for 5 minutes to ensure the stability of the sperms on the haemocytometer ,then the sperm were counted in 5 medium squares.

Measuring the percentage of sperm (live, dead and deformed)

It was done by cutting the right tail of epididymis And placed it in a Petri dish containing 2 ml of normal physiological salt solution (normal saline) at a temperature of 37° C then a drop was taken from the solution and placed on the a clean and dry glass slide and a drop of the prepared nigrocin-eosin dye was added to it simultaneously the two drops were gently mixed on the glass slide for half a minute using the edge of the glass. Then a part of the mixture was taken with one end of the slide and pull at an acute angle and gently on The first slide, and all used glass slides, after drying they were placed in the incubator At a temperature of 37 degrees to dry, and after the swab was completely dry, it was examined with an oil emersion lens 100x, The percentage of live, dead sperm and the percentage of sperm abnormalities in100 sperm from each segment.

Statistical analysis

The data analyzed by using one–way analysis of variance, followed by using Duncan's multiple range test (SPSS version 24, USA) to evaluate differences between groups. The resultswere expressed as mean ± standard error of the mean. Values considered significantly different at ($p \leq 0.05$).

Results and Discussion

Effect of lead acetate and ALCRA on total body weight.

Aperiode 30 day

The body weight in the lead acetate group was decreased significantly ($P \leq 0.05$) in compared with control group and the group treated with ALCAR alone and lead acetate with ALCAR group.(Tab.1). The body weight in the group treated with ALCAR alone and lead acetate with ALCAR increased significantly ($P \leq 0.05$)in compared with control group(Tab.1).

Aperiode 60 day

The body weight in the lead acetate group show no significant changes compared with control group(Tab.1),
lead acetate group is significantly ($P \leq 0.05$) increased in compared with ALCAR group and the group treated with both lead acetate ALCAR(Tab.1). The result of this experiment also showed that the group treated with ALCAR alone and the lead acetate with ALCAR group significantly($P \leq 0.05$) increased in compared with control group and the group treated with lead acetate(Tab.1). The group treated with ALCAR alone and the group with both lead acetate and ALCAR showed significant increase ($P \leq 0.05$) in the body weight during the 30 and 60 day of the experiment in compared zero time (Tab.1).

Table -1: Effect of lead acetate and ALCRA on total body weight(g)

time / groups	0 day	30 day	60 day
Control	202.00 ± 8.50 a A	222.0 ± 5.62 a B	243.33 ± 20.87 a A
Lead acetat	198.33 ± 6.85 A A	177.83 ± 9.47 b C	259.17 ± 3.12 B A
ALCAR	211.50 ± 1.05 c A	263.33 ± 3.04 b A	339.66 ± 12.83 a B
Lead acetat + ALCAR	210.16 ± 2.07 c A	288.0 ± 2.81 b A	359.66 ± 5.57 a B

Number of animals in each group =6,The different capital English letters within the column mean that there are a significant difference between the treatment at the level(P≤0.05). The different small English letters within the class mean that there are a significant difference between the periods at the level(P≤0.05).

Testicular oxidative stress is the main feature in male infertility(11). The lead acetate caused decrease in body weight in rat treated with 10 mg/kg B.W. this result may be because that ingestion of lead acetate reduced the content of hemoglobin because it decrease the appetite in the animal (12). This result is agreed with (13) that showed that the exposure of rat to 2000 ppm lead acetate in drinking water caused decreased in body weight of animals. The treatment of ALCAR 10 mg/kg of B.W. causes decrease in body weight of rats, this result is agreed with (14) that explained that three- month administration of ALCAR 100 mg/kg B.W. in drinking water to aged rat decreased the mean body weight in compared with control aged groups.

Effect of lead acetate and ALCRA on the weight of sexual organ.

Effect of lead acetate and ALCRA on the weight of testes.

Result of the experiment showed that a significant decrease (P≤0.05) in the weight of the testes in the group treated with lead acetate in compared with control group and the group treated with ALCAR alone (Tab.2). The group treated with ALCAR showed that this group did not differed significantly with control group (Tab.2). lead acetate and ALCAR group showed a significant decrease (P≤0.05) in compared with control group(Tab.2).

Effect of lead acetate and ALCRA on the weight of epididymis.

Weight of head of epididymis.

The weight of head of epididymis is increased significantly (P≤0.05)in the group treated with ALCAR alone in compared with control , lead acetate and lead acetate with ALCAR groups(Tab.2). furthermore the result of the weight of head of epididymis showed that the lead acetate group did not differ significantly in compared with control group and lead acetate group with ALCAR (Tab.2).

Weight of body of epididymis.

The weight of the body of epididymis in the group treated with ALCAR alone is significantly (P≤0.05) increased in compared

with control ,lead acetate and lead acetate with ALCAR groups (Tab.2). The lead acetate group and lead acetate with ALCAR groups did not differ significantly in compared with control group (Tab.2).

Weight of tail of epididymis.

Rats treated with ALCAR showed significant increase (P≤0.05) in the weight of tail of epididymis in compared with group treated with lead acetate alone and lead with ALCAR (Tab.2). The result showed that the rats treated with lead acetate did not significantly differ in compared with control and lead with ALCAR groups (Tab.2). Also the group treated with lead acetate and ALCAR did not differed significantly in compared with control group(tab.2).

Effect of lead acetate and ALCRA on weight of seminal vesicle gland.

Result of experiment showed that the groups treated with lead acetate ,ALCAR and lead acetate with ALCAR did not significantly differ in compared with control group and also did not differ significantly between all treated groups(Tab.2).

Effect of lead acetate and ALCRA on the weight prostate gland.

The ALCAR group showed increase significantly (P≤0.05) in the weight of prostate gland in compared with the control ,lead acetate and lead acetate with ALCAR groups(Tab.2). The lead acetate group did not differ significantly in the weight of prostate gland in compared with control and lead acetate with ALCAR groups (Tab.2), also the lead acetate with ALCAR group did not differed significantly in compared with control group in the weight of prostate gland(Tab.2).

Table -2: Effect of lead acetate and ALCRA on weight of the sexual organ (mg/100g B.WT)

organs / groups	testes	epididymis			Seminal vesicle gland	Prostate gland
		head	body	tail		
control	437 ± 0.06 a	90 ± 1. 47 b	14 ± 0.6 b	88 ± 4.9 ab	401 ± 10.46 a	254.33 ± 20.86 b
Lead acetate	352 ± 12 b	82 ± 5 b	19 ± 42 b	80 ± 6.3 b	440.00 ± 24.08 a	228.00 ± 21.91 b
ALCAR	443 ± 22 a	126 ± 6 a	52 ± 9.5 a	108 ± 8.4 a	517.83 ± 53.06 a	355.66 ± 38.02 a
Lead+ ALCAR	367 ± 32 b	70 ± 17 b	26 ± 3 b	71 ± 10 b	402.00 ± 47.59 a	213.33 ± 19.02 b

Number of animals in each group =6

The different English letters mean that there are a significant difference between the treatment at the level(P≤0.05).

Lead acetate and lead acetate with ALCAR led to decreased testes weight and epididymis compared with control groups in the rat ,this is may be caused by the lead acetate and ALCAR affected to the body weight . this result agreed

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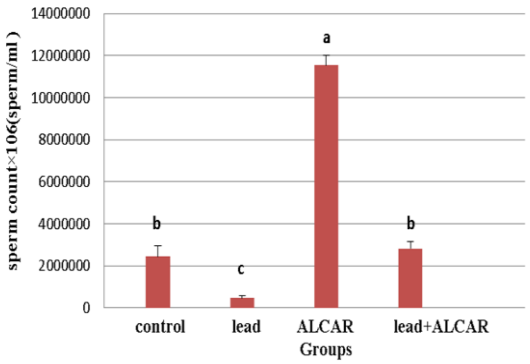
with (15). which referred that administration of lead acetate (30 mg/kg B.W.) lead to decreased in weight of testes and epididymis and accessory sex gland. Macroscopic changes in the accessory sex organs such as decreased in weight of testes, seminal vesicles, epididymis, and ventral prostate have been demonstrated in various studies by using the experimental animals(16).

Effect of lead acetate and ALCRA on Total sperm count

The effect of lead acetate on the total sperm number is significant (P≤0.05) decreased in compared with control group (Fig.4). Effect of ALCAR is increased significantly (P≤0.05) in the total number of sperm in compared with the control and lead acetate treated group (Fig.4). The treatment of the lead acetate with ALCAR group showed a significant increase (P ≤0.05) in total sperm number in compared with the group treated with lead acetate alone, The result also showed decrease significantly (P≤0.05)in total sperm number in compared with ALCAR alone and there are no significant difference in compared with control group (Fig.4).

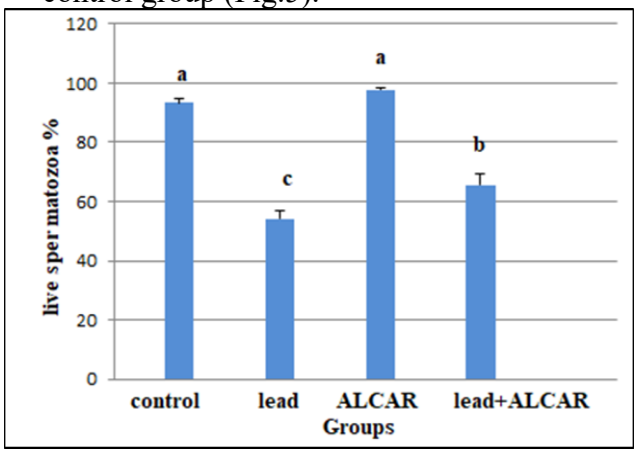


Number of animals in each group =6
The different English letters mean that there are a significant difference between the treatment at the level(P≤0.05).

Figure.1Effect the lead acetate and ALCRA on total sperm count(Total sperm count*10^6 sperm/ml)

Effect of lead acetate and ALCRA on percentage of live spermatozoa

The percentage of live sperm decreased significantly (P≤0.05) in the group treated with lead acetate in compared with control group (Fig.5).The group treated with ALCAR did not significantly differed in compared with control group in percentage of live sperm and significantly (P≤0.05) increased in compared with group of lead acetate (Fig.5). The percentage of live sperm in the group treated with lead and ALCAR showed increased significantly (P≤0.05) in compared with lead acetate group and significantly (P≤0.05) decreased in compared with with ALCAR and control group (Fig.5).

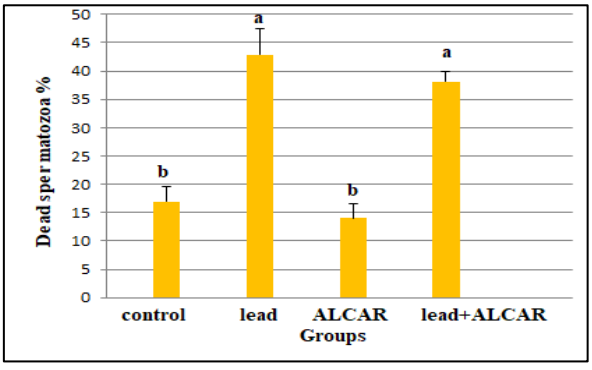


Number of animals in each group =6
The different English letters mean that there are a significant difference between the treatment at the level(P≤0.05).

Figure.2 Effect of lead acetate and ALCRA on percentage of live spermatozoa

Effect of lead acetate and ALCRA on percentage of dead spermatozoa

The percentage of dead spermatozoa showed that the lead acetate group and lead with ALCAR group are increased significantly (P≤0.05) in compared with control and ALCAR groups(Fig.6).The group treated with ALCAR showed that no significant differed in the percentage of dead spermatozoa in compared with control group(Fig.6).

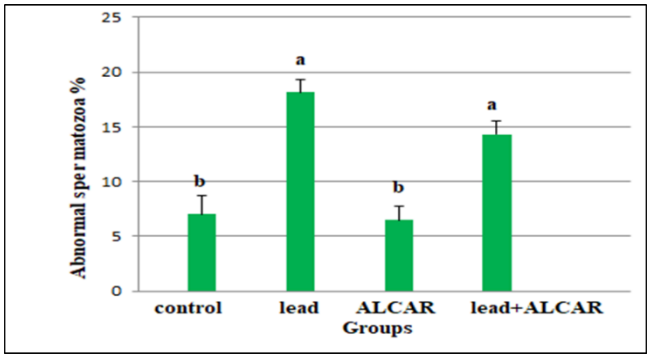


Number of animals in each group =6
The different English letters mean that there are a significant difference between the treatment at the level(P≤0.05).

Figure.3 Effect of lead acetate and ALCRA on percentage of dead spermatozoa

Effect of lead acetate and ALCRA on percentage of abnormal spermatozoa

The percentage of abnormal spermatozoa showed that the lead acetate group and lead acetate with ALCAR group are significantly increased (P≤0.05) in compared with control and ALCAR groups (Fig.7).The group treated with ALCAR showed no significant difference in percentage of abnormal spermatozoa in compared with control group(Fig.7).



Number of animals in each group =6
The different English letters mean that there are a significant difference between the treatment at the level(P≤0.05).

Figure.4 Effect of lead acetate and ALCRA on percentage of abnormal spermatozoa

Many studies on reproductive system of male animals have documented lead as a toxicant for testicular tissue and functions (17). Lead binds to glutathione(GSH) and like other divalent metals can leave the cell to circulate in serum or lymph. The subsequent precipitous deposition of lead give rise to tissue or organ damage (18).ROS have a detrimental effect on critical events on the steroidogenic pathway, elevated levels of ROS elicit lipid peroxidation and membrane damage which lead to loss of sperm(19). the result showed that lead acetate 10 mg/kg B.W. lead to decrease significantly in the total number of sperm and percentage of live sperm and increase percentage of the dead spermatozoa in compared with control group. This result is agreed with(20) who explained that significant reductions in number of the spermatozoa in the epididymis of wistar rats that administered lead acetate (6mg/Kg body weight) for 14 days(20). Many studies suggest spermatogenesis problems caused by lead, although, some researchers have failed to demonstrate correlations between lead and semen volume, pathologic sperm and sperm concentration among workers exposed to high lead levels(21). Or abnormalities in sperm count and/or the sperm morphology in rabbits(22). The result showed that ALCAR increased the total number of spermatozoa and increased the percentage of live and decreased percentage of dead spermatozoa in compared with control group .this result is agreed with (23) who explained that daily injection of L-carnitine (100 ,200mg/kg B.W.) in rat for 48 day lead to increase the count, motility, viability, maturity, and chromatin quality of spermatozoa and decreased abnormal morphology of the spermatozoa in comparison with control group.

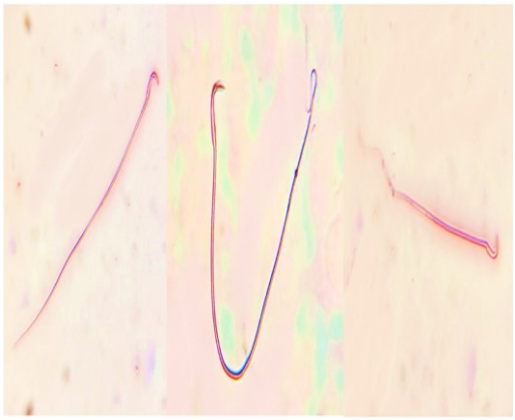


Figure.5 sperms in control group

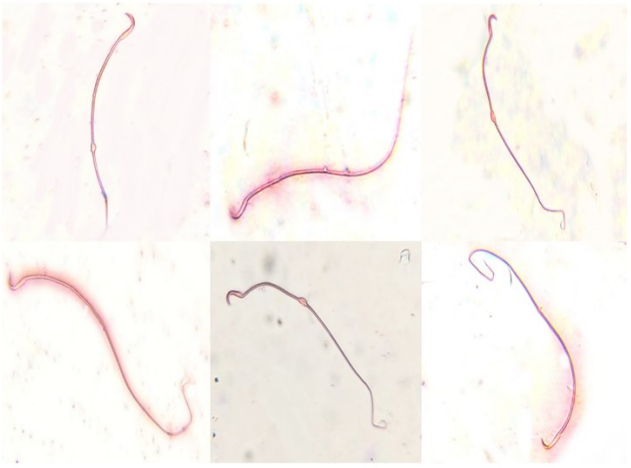


Figure.6 sperms in Lead acetate group

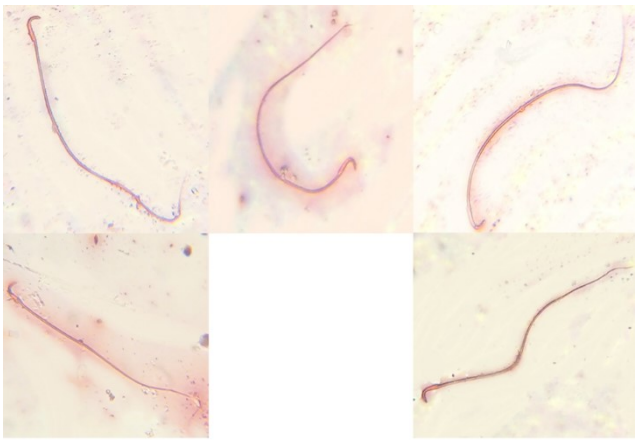


Figure.7 sperms in ALCAR group

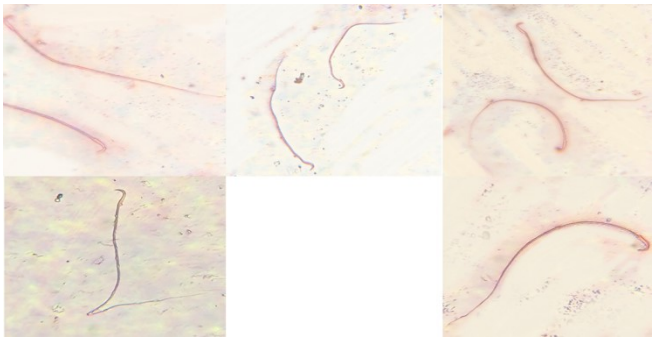


Figure.8 sperms in Lead acetate and ALCAR group

Conclusion

acetyl L-carnitine plays an important role in the reversing undesirable lead acetate effects and Administration of ALCAR improves the sperm parameters and particularly sperm counts.

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Conflict of Interest:

The author declares no conflict of interest.

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