REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Physiological Effects of Nano L-carnitine on the Gonadal Pathway in Adult Male Rats Exposed to Lead Acetate

Hayder Talib Mahdi[1,2] , Dr. Rashad Fadhil Ghadhban[1]
1-Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq
2- Department of Physiology, College of Medicine, Al-Ameed University, Karbala, Iraq
Email : hayder@alameed.edu.iq

Abstract:

This study was conducted to determine the role of L-Carnitine (LC) and Nano L-Carnitine protected male rats from testicular toxicity caused by lead acetate (PbAc) by investigating the effectiveness of biosynthesized L-Carnitine nanoparticles (LC-NPs) on the fertility status of male rats. LC-NPs were synthesized, which could be by encapsulating l-carnitine in Chitosan (CS)-tripolyphosphate (TPP)-based nanoparticles (NP);
Materials and methods: six groups of adult Wistar albino rats (10 per group) were divided as follows: The first group (control group): received orally Distilled water. the second group: received orally lead Acetate at a dose of 30 mg/kg of body weight daily for 30 days. third group: received (lead acetate 30mg/kg b.w.for 30 days + L-carnitine 100mg/ kg b.w. /day for two months). fouth group received lead acetate 30mg/kg b.w.for 30 days +Nano L-carnitine 100mg/ kg b.w./day for two months). fifth group: received L-Carnitine orally at a dose of 100mg/ kg b.w./day for two months. sixth group: received L-Carnitine-NPs orally at 100mg/ kg b.w./ day for two months.
Results: a significant increase ($P<0.05$) in the testosterone hormone levels in the lead acetate+ Nano L-Carnitine group compared with the lead acetate group. While showed decreased levels of FSH and LH in the lead acetate+ Nano L-Carnitine group compared with the lead acetate group.
On the other hand, The results showed a significant increase ($P<0.05$) in the percentage of count and motility in group three(lead acetate+l-carnitine) and group four (lead acetate + nano l-carnitine) compared with group two (lead acetate). Show better improvements in the percentage in group four. Also showed a significant ($P<0.05$) decrease in the rate of dead sperm and abnormal morphology in group three and group four compared with group two (lead acetate). Histopathological changes were marked in testes; these changes included damage. Severe degeneration and sloughing of germinal epithelium with basement membrane disarrangement; no spermatid in seminiferous tubule lumens accompanied with atrophy and irregularity, vacuolization with few spermatogonia in seminiferous tubule lumens those changes reduced or disappeared in the treated groups.

Keywords: nanoparticles, chitosan, L-carnitine, lead acetate, sperm parameters, testes, reproductive hormones.

Introduction:

Nanobiotechnology is today regarded as the foundation for advancing all life sciences and biomedical research sectors, including diagnostics, treatment, and medication delivery (1). Many factors restrict the efficiency of many therapies, including low bioavailability, in vivo low stability, solubility, intestinal absorption difficulties and therapeutic effectiveness, side effects, and exceeding the safe dose concentrations of many of these therapies (2). In most cases, only a fraction of the required dosage reaches the intended target (3). According to its kinetics and biological features, the majority of the drug is distributed throughout the

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

rest of the human body. (4, 5). Among the numerous benefits of using nanoparticle delivery methods are high in vivo stability, long-term payload capacity release, and passing through narrow capillaries and cellular compartments. (6).

Nanoparticles might also improve the medication's therapeutic index, regulate its pharmacokinetics and biodistribution, and help form long-term drug reservoirs. (7). Smaller nanoparticles (50–200 nm), with high loading capacity, delayed complex dissociation in vivo, and target optimization to the desired region with little absorption by surrounding tissues are among the additional criteria. The design of formulations that incorporate these properties while also being cost- and complexity-effective is necessary for effective delivery systems (8).

Chitosan, a linear polysaccharide of deacetylated units of -(1-4)-linked D-glucosamine and acetylated N-acetyl-D-glucosamine, is an N-deacetylated derivative of chitin. Chitosan is made by treating chitin extracted from shrimp and other crustacean shells with sodium hydroxide, an alkali (9). Chitosan has been investigated for biocompatibility, low toxicity, biodegradability, and relatively inexpensive manufacturing costs owing to the abundance of natural materials used in the creation of the polymeric drug carrier material (10,11).

L-carnitine, commonly known as levocarnitine, is an amino acid molecule that occurs naturally in the body (12). People may also receive it via their food or an oral supplement. L-carnitine is essential for energy generation because it turns fat into energy (13). L-carnitine has low health impacts and is abundant in antioxidants. As a result, it is utilized to prevent and treat oxidative stress and associated health concerns. (14). When long-chain fatty acids are used for energy, L-carnitine is an antioxidant that aids in the process. Anti-inflammatory, anti-apoptotic, cardioprotective, and gastroprotective properties are only a few of the numerous biological functions it possesses (15). It also contributes to sperm metabolism, maturation, motility, and spermatogenesis by supplying energy, in irradiated testes, L-carnitine inhibits apoptosis. Furthermore, there is evidence that corticosteroids positively impact spermatogenesis in IR-affected testes. (16). These anti-inflammatory medicines inhibit the generation of free radicals and preserve the cell wall. They also prevent vessels from becoming more permeable (17,18).

Lead is one of the most common and dangerous heavy metals, and it is abundant in aquatic habitats and soil near industrial regions(19). Lead metal is distinguished by its popular silver, a little bluish color, and brightness in dry weather (20). The primary sources of lead exposure in our everyday lives include drinking water, food, cigarettes, residential sources, and manufacturing operations like fuel, home gilding, plumbing tubes, buckshot slugs, batteries, children's toys, and water taps (21). Lead is emitted into the atmosphere via industrial operations and car exhausts, and it travels to soil and water bodies, where plants absorb it, then people are exposed to lead by drinking water and eating (22). Lead accumulates in mammalian tissues such as the liver, testes, brain, kidneys, and bones, and these organs are often examined in wildlife toxicology research. (23, 24, 25).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Materials and Methods:

Synthesis and Characterization of LC-NPs:

Chitosan-carnitine acid adduct (Cs-Ca) synthesis:

According to references, these steps were used to make chitosan-l-carnitine adduct (26,27,28)

Nanoparticles made of Chitosan and carnitine were synthesized.

Synthesis of Cs-Ca NPs, by ionic gelation method, was used using TPP and Cs-Ca adduct. For six hours, Cs-Ca (1 mg/ml) and TPP solution were mixed at ambient temperature with constant stirring at a ratio of 1: 2.5 (w/w %) in an acetic acid solution (1 percent w/v). TPP triggered ionic gelation of Cs-Ca/TPP nanoparticles. This was the first step in the process. Separation, washing, and drying of these nanoparticles resulted in the precipitate being resuspended in water and drying.

Characterization of L-carnitine-chitosan nanoparticles:

These characterization tests were done in the material research laboratories at the ministry of sciences and technology environment and water research and technology director (EWRTD) in Baghdad city.

UV-VIS spectrophotometer

The stability and production of L-carnitine nanoparticles were studied using a double beam (labored). Spectrophotometers with wavelengths ranging from (200 – to 800) nm. The solution was measured at the wavelength (of 480) nm (29).

Fourier Transform Infrared (FTIR Analysis):

Fourier-transform infrared (FTIR) spectroscopy is the most practical method for identifying chemicals based on the presence of functional groups or interactions between them. Organic and inorganic chemicals in an unknown combination were analyzed using FTIR (Shimadzu) in solid, liquid, and gaseous forms. The fact that most molecules absorb light in the infrared region of the electromagnetic spectrum is the basis for FTIR. It turns data from the interference pattern into a spectrum (30) by measuring the frequency as wave numbers, which are generally in the range of 4000–400 cm. For the characterization of unknown compounds, FTIR is a useful instrument.

Animal of the study:

The present study was conducted in the college of Veterinary medicine –at Basrah University, in the animal house of the department of physiology. A total number of 60 male adult albino rats(*RattusRattus*), with an average weight between (212±10g) and the ages (8 - 10)weeks, were housed for two weeks for an adaptation before the experiment. Every ten animals were housed in an individual plastic cage measured as 15x35x50cm. They were fed *ad libitum* with the meal of standard pellet of diet, they had free access to water to drink, and

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

they were kept under the exact condition of temperature (22-25) C° and light, the regime of 14hours of light, and 10 hours, of darkness.

The Experiments design :

Animals in the study were divided into six groups. Each group consists of 10 male rats used for the design experiments as the following:

1- Group-1(control Group): received orally Distilled water
2- Group -2 (lead acetate Group): received orally lead Acetate at a dose of 30 mg/kg of body weight daily for 30 days. Depended on LD50.
3- Group 3 received (lead acetate 30mg/kg b.w.for 30 days + L-carnitine (100mg/ kg b.w. daily for two mouths)
4- Group 4: lead acetate 30mg/kg b.w.for 30 days +Nano L-carnitine (100mg/ kg b.w. daily for two mouths)
5- Group 5 (L-carnitine): received LC orally at a dose 100mg/ kg b.w. daily for two mouths
6- Group 6 (Nano L-carnitine): received LC-NPs orally at 100mg/ kg b.w daily for two months.

Note: - all experiments proceed for two months.

Blood and sample collection:

Blood collection:

The blood sample was collected via cardiac puncture according to (Hoff and Ralatg's, 2000) method. The controlled and treated animals, before sacrificing, were first anesthetized. The blood sample was collected directly from the heart by using a 5ml disposable syringe, and about (5)ml of the blood collection were put in a non-heparinized plane tube to be centrifuged at (3000 rpm for 15 minutes) to obtain the serum, which was then transferred to epndroffe tubes and stored at (-4C) until analyzed for all measurement. At the same time, the sample collecting was the testis for the sperm viability and histological examination.

Hormones Assay (Enzyme-Linked Immunosorbent Assay "ELISA")

Testosterone, follicular stimulating hormone (FSH), and Luteinizing Hormone (LH). An enzyme-linked immunosorbent works on the principle of using an enzyme to detect antigen (Ag) antibody binding (Ab). The enzyme produces a colorful product from a colorless substrate, indicating the presence of Ag: Ab binding (31).

Sperm evaluation

Preparation of suspended sperm in the tail of the epididymis.

The tail of the left epididymis was excised and placed in a clean watch glass containing one millimeter of warm physiological normal saline. Then the tail was opened and

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

cut by microsurgical scissors into very miner fragments for performing sperm examination **(32)**.

Sperm concentration (107/ml).

The sperm count was performed using the technique given by (Mohan et al.,1980). Using Hemocytometer (Neubauer Type), 0.1 ml from sperm suspension by pipette was taken and diluted by 19.9 ml from diluting fluid. The Hemocytometer sides were filled with the drop from sperm suspension diluted and covered by a slide; the sperm were counted in five medium squares of the chamber. The estimation of sperm was measured according to the following formula:

$$\text{Sperm conc.} = \frac{\text{No. of sperm counted in five medium square} \times 400 \times 200 \times 10}{80}$$

80 = No. of small squares counted in five medium squares. 400= The total number of small squares. 200= Dilution factor. 10= Volume coefficient

3.3.7 B. Sperm abnormal morphology (%).

The percentage was calculated by taking the average of two slide smears according to the following equation **(33)**.

$$\text{Abnormal sperm morphology (\%)} = \frac{\text{No. of morphologically abnormal sperms}}{\text{Total sperms No.}} \times 100.$$

Sperm motility (%).

The percentage of motility was estimated using the method below (34).

$$\text{Percentage of motility} = \frac{\text{No. of motile spermatozoa} \times 100}{\text{Total spermatozoa}}$$

Dead and life sperm (viability) %.

Eosin-nigrosin viability sperm technique used nigrosin stain to increase the contrast between the background and the sperm heads **(35)**.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Histological studies:

the testis was collected from all studied groups to prepare slides for a histological examination according to (36) with the aid of the light microscope.

Statistical analysis:

For data analysis, I used the statistical software package Statistical Package for Social Scientists (SPSS version 18.0) and Microsoft Office Excel 2016. A one-way ANOVA with an LSD post hoc test revealed a significant difference in group mean values. Whenever possible, paired t-tests were used to compare standards. When the p-value was less than $p<0.05$., the results were considered significant. (37)

3. Results:

Carnitine-loaded Chitosan nanoparticles (LC-NPs).

The nanoparticles were produced by the ionic gelation process by interacting between Chitosan's positively charged and TPP's negatively charged phosphate groups.

Characterization of L-Carnitine nanoparticles (LC-NPs);

Different spectrophotometric techniques like UV-visible, and FTIR, analysis were used to investigate the morphology, elemental composition, crystalline nature, functional group, and stability of synthesized LC-NPs.

UV-visible spectroscope:

The Ultra violate analysis of prepared CNP and functionalized Chitosan nanoparticles with L-carnitine was carried out by scanning the ready solution with a UV-visible spectrophotometer from (200- to 800 nm). As evident in figure (1), the absorption peak for the solution of CS-LC is observed at a wavelength of 733 nm, whereas the peak of the LC-NP was obtained at 257 nm wavelength.

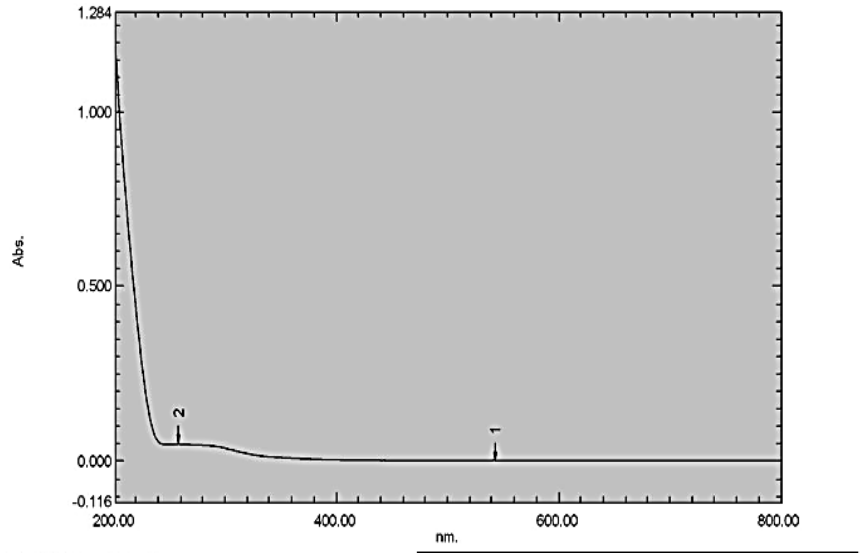

Figure (1) UV-visible spectroscope in Nano LCNPs

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

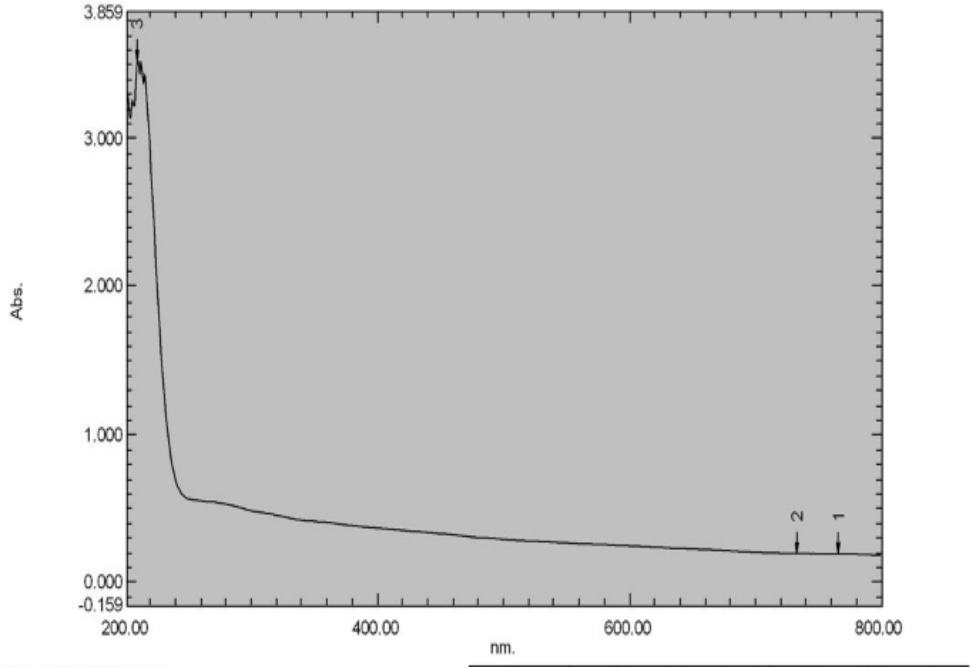

Figure (2) UV-visible spectroscope chitosan.

4.2.2. Fourier transforms infrared spectroscopy analysis (FTIR):

To determine the molecular structure of Chitosan, a Fourier Transform Infrared (FTIR) analysis was carried out by scanning the prepared solution of L-Carnitine (LC-CNPs) with wavelength ranges from (400- 4000 cm-1) to indicate the bonding of substance, as shown in figures (3), The chemical structure of Chitosan was investigated using FTIR measurements; various distinctive bands deriving from the presence of amine and methylene groups on Chitosan were discovered. The CH2 antisymmetric stretching modes are attributed to bands at 2926 cm-1. A band at 1649 cm-1 is ascribed to the N-H bending mode, while another at 1076-1325 cm-1 is assigned to C-N stretching modes due to the presence of the amine group. Also, the analysis by the functional groups of the products was identified using FTIR spectra. It revealed that the molecule had the anticipated functional group. Chitosan's FTIR spectra revealed various vibration patterns, including 2926 cm-1 for NH, 3417 cm-1 for -OH, 1560 cm-1 for C=O stretching vibration, and 1325 cm-1 for NH stretching vibration. Chitosan has a distinctive stretching vibration of NH related to an NH group in -NH-CO- (acetylated amine group). Because the hydrogen bond in amine is weak and less polar, the vibration of the amine group of Chitosan in the 3417 cm-1 corresponded with OH vibration. It causes the N-H bond to vibrate less intensely than the OH bond. The appearance of an absorption band at 653-1076 cm-1 indicated the presence of a C-O vibration. As predicted, the absorption pattern indicates that the residue is Chitosan.

This demonstrates that chitosan functional groups interact with LCNPs loaded via ionic gelation (Fig. 4). The incorporation of L-Carnitine results in an increase in the intensities of FTIR spectral bands in Chitosan-LCNPs films at 3755 cm-1, 3437 cm-1, 2924 cm-1, 1654 cm-

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

1, and 1136 cm-1, corresponding to NH2 and OH stretching, CH2 group, C=C stretch, and C=O group C-O-C linkage, respectively. implying the presence of extensive coordination bonding between functional groups of Chitosan and L-Carnitine, leading to the conclusion that CS-LCNPs should be located between chitosan chains connecting via its functional groups The relationship between phosphoric and ammonium ions has been blamed for these outcomes. As a result, we believe that TPP's tripolyphosphoric groups are connected to Chitosan's ammonium groups. In chitosan nanoparticles, inter-and intramolecular activities are improved.

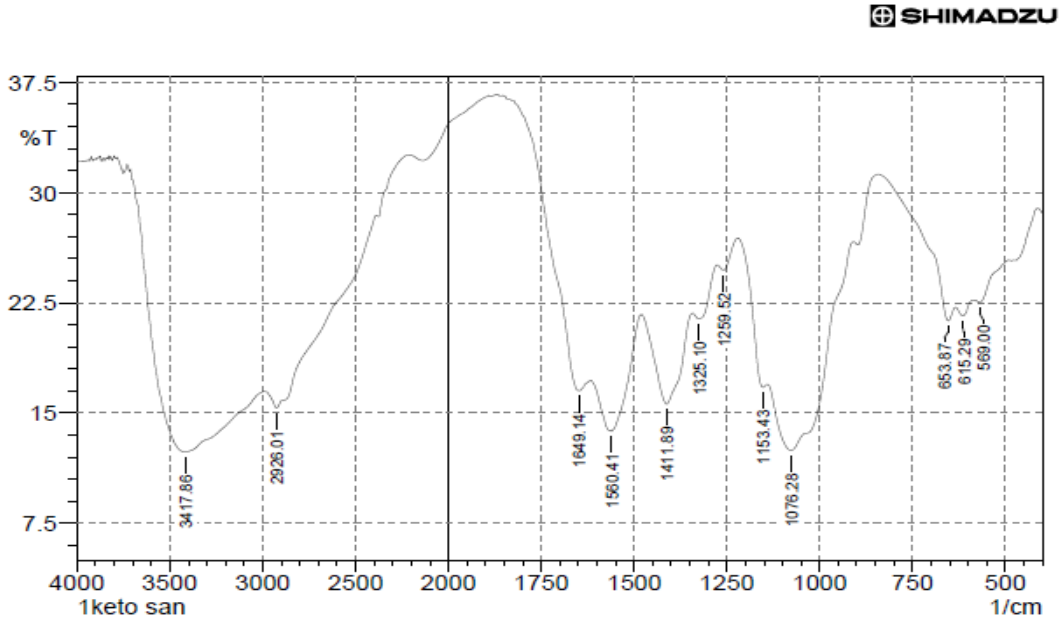

Figure (3) FT-IR spectrum showing functional groups of Chitosan.

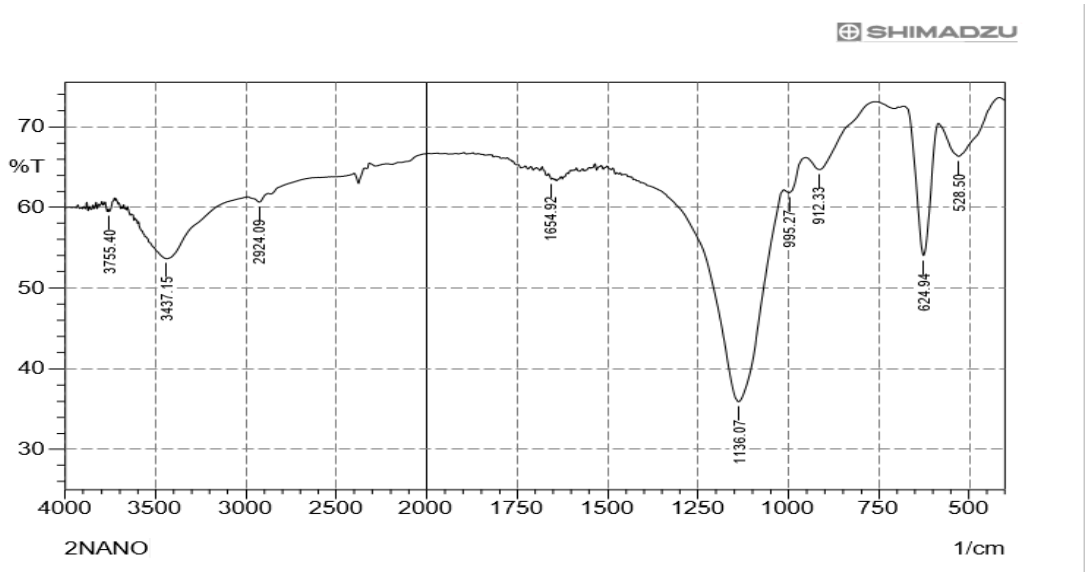

Figure (4). FT-IR spectrum showing functional groups of biogenic L-carnitine nanoparticles (LC-NPs)

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

sperm parameters:

Characteristic features of rat epididymal sperm in experimental groups are illustrated in Table (1). The results showed a significant (P<0.05) decrease in the percentage of the count and motility of sperm in the Lead Acetate group compared to the control group. At the same time, the results showed a significant (P<0.05) increase in the percentage of dead sperm and abnormal morphology with Lead Acetate compared to the control group.

The results showed a significant increase (P<0.05) in the percentage of count and motility in group three (lead acetate + l-carnitine) and group four (lead acetate + nano l-carnitine) compared with group two (lead acetate). Show better improvements in the percentage in group four(lead acetate + nano l-carnitine). Also showed a significant (P<0.05) decrease in the percentage of dead sperm and abnormal morphology in group three and group four compared with group two (lead acetate).

Non-significant differences (P<0.05) in the percentage of the count, and motility, of sperm between the L-carnitine group and Nano L-Carnitine, compared to the control group.

The results showed a significant (P<0.05) decrease in the percentage of dead sperm and abnormal morphology in Nano L-carnitine compared to the control group.

Table (1): physiological effect of lead acetate, L-Carnitine, and Nano L-Carnitine or combination (Pb+LC) on sperm parameters.

Groups	**parameters (Mean ± SD)**			
	Count *10^7	**Motility**	**Dead** (%)	**Abnormal** (%)
Control	20.0 ± 2.0b^{a}	94.2 ± 2.09^{a}	11.0± 1.76^{d}	13.9 ± 1.37^{dc}
Lead acetate (U/L)	10.2 ± 1.87^{d}	51.5 ± 5.85^{d}	28.1 ± 3.10^{a}	30.4 ± 3.77^{a}
Lead + L.Carnitin (U/L)	14.2 ± 1.31^{c}	77.0 ± 2.49^{c}	19.0 ± 1.49^{b}	21.2 ± 2.65^{b}
Lead+NanoL.Carnitin (U/L)	18.8 ± 1.93^{b}	85.8 ± 3.42^{b}	14.0 ± 1.82^{c}	16.4 ± 1.57^{c}
L.Carnitin (U/L)	20.8 ± 1.87^{ba}	94.9 ± 1.52^{a}	9.3 ± 1.33^{ed}	13.2 ± 2.04^{ed}
L.Carnitin Nano (U/L)	21.6 ± 1.64^{a}	95.0 ± 1.24^{a}	8.2 ± 1.31^{e}	10.4 ± 1.50^{e}
LSD	**1.60**	**2.84**	**1.71**	**2.07**

*The different small letters de not show significant differences at (P<0.05)

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Effect of lead acetate, l-carnitine, and Nano l-carnitine on reproductive hormones of adult male rats.

In Table (2), the Characteristic features of some male reproductive hormones show a significant decrease (P<0.05) in the (TESTO) in the rats exposed to Lead Acetate (Pb) (30 mg/kg) in comparison with other experimental groups. And shows a significant increase (P<0.05) in the (FSH) and (LH) in the rats exposed to Lead Acetate (30 mg/kg) in comparison with other experimental groups.

The results showed a significant increase (P<0.05) in the testosterone hormone levels in the lead acetate+ Nano L-Carnitine group compared with the lead acetate group. While showed decreased levels of FSH and LH in the lead acetate+ Nano L-Carnitine group compared with the lead acetate group.

While the Nano L carnitine and L Carnitine only groups showed a significant (P<0.05) decrease in the concentration of FSH and LH compared to the lead treated group. In contrast, a significant (P<0.05) increase in the concentration of Testosterone in comparison with lead treated group.

Table (2): physiological effect of lead acetate, L-Carnitine, and Nano L-Carnitine or combination (Pb+LC) on reproductive hormones of adult male rats.

Groups	**parameters (Mean ± SD)**		
	Testosterone(ng/ml	**FSH(mIU/ml)**	**LH (mIU/ml)**
Control	5.24 ± 0.35^{b}	2.74 ± 0.43^{c}	3.17 ± 0.38^{cd}
Lead acetate (U/L)	3.98 ± 0.32^{d}	3.68 ± 0.27^{a}	4.13 ± 0.34^{a}
Lead + L.Carnitin (U/L)	4.10 ± 0.12^{d}	3.38 ± 0.25^{ab}	3.77 ± 0.46^{ab}
Lead + L.Carnitin Nano (U/L)	4.75 ± 0.29^{c}	3.00 ± 0.31^{bc}	3.60 ± 0.34^{bc}
L.Carnitin (U/L)	5.50 ± 0.31^{ab}	2.85 ± 0.34^{c}	3.06 ± 0.32^{d}
L.Carnitin Nano (U/L)	5.72 ± 0.33^{a}	2.61 ± 0.32^{c}	2.97 ± 0.30^{d}
LSD	**0.26**	**0.29**	**0.32**

*The different small letters de not show significant differences at (P<0.05)

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Effect of Lead acetate (Pb) on the histological finding of the testes and the protective effects of L-carnitine and Nano L-carnitine.

Testes

The histological examination of rat tests in the control group consists of seminiferous tubules; it contains multilayers of spermatogenic and Sertoli cells, which extend from the basement membrane to the lumen of the tubule. The interstitial cells were located (fig 5).

A histological section taken from testes of the rats after administration of lead acetate showed severe histopathological changes, including Damage, degeneration, and sloughing of the germinal epithelium with disarrangement of the basement membrane; no spermatid in the lumens of seminiferous tubules accompanied with atrophy and irregularity; vacuolization with small numbers of spermatogonia in the lumen of seminiferous tubules. Interstitial edema and seminiferous tubules vacuole. Abnormal mitosis. Shows a reduction in diameter of seminiferous tubules of lead acetate and increased interstitial space. Vascular congestion of interstitial space. Interstitial hemorrhage with edema. Shows apoptotic cells in the lead group with necrotic spermatid in the center of ST. There is an empty lumen and dispersed Sertoli cells, as well as a degraded interstitial space in the tubules shown. Figures (6), (7), and (8).

In groups three and four, the rats administrated lead Acetate with L- carnitine and Nano L-carnitine. The hazardous effect of lead was decreased due to the protective role of L- carnitine as an antioxidant; it was revealed that most of the seminiferous tubules have consisted of multilayers of germ cells, but the spermatogenesis process was incomplete in some seminiferous tubules, as well as increase the luminal size of tubules figure (9- 10).

In group l-carnitine with lead acetate, shows a typical architecture of ST with narrow lumen and prominence Leydig cells with spermatids, showing mature spermatozoa in the lumen of ST, the spermatogenesis was incomplete. Increase the luminal size of seminiferous tubules due to the effect of the spermatogenic process by lead acetate fig (9).

In Nano l-carnitine with lead, Acetate shows Seminiferous tubules in the epithelial layer of cells exhibit decreased sloughing and elongation when exposed to nano l-carnitine, showing restoration of spermatogenesis in most of the seminiferous tubules and expansion of intratubular space and mild improvement in the seminiferous tubules structure with narrow intestinal space and restoration of spermatogenesis. fig (10)

Were revealed that when rats were administrated with L-carnitine and Nano l- carnitine, which led to activation of the spermatogenesis process, L-carnitine is necessary for fertility and improves the germ cells to complete the spermatogenic cycle. This group showed the normal leydig' s cells, and mitotic and mitosis division was apparent in the spermatogenic cells (spermatogonia, primary and secondary spermatocyte, spermatid, and spermatozoa). Figures (11) and (12)

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

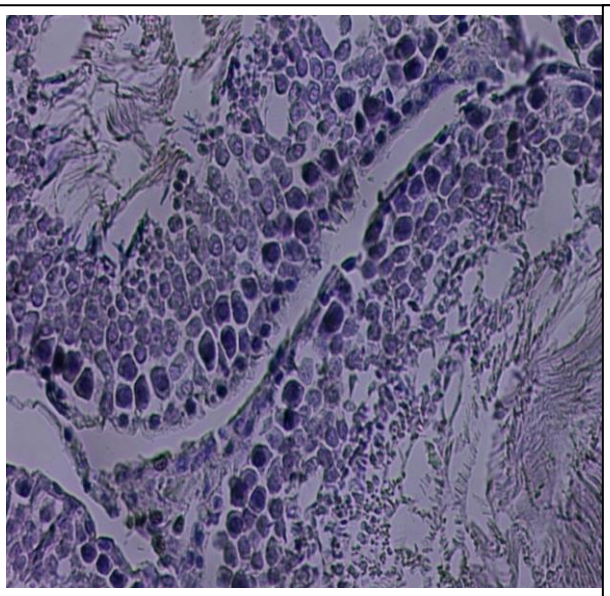

Figure (5). Show seminiferous tubules of rat testis in the control group(hematoxylin and Eosin .40x).

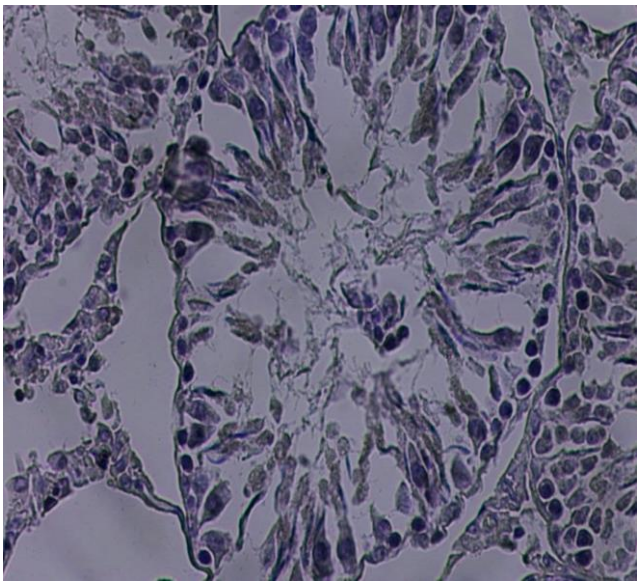

Figure (6).testicular sections of lead acetate treated rats.the treated rats were administrated 30mg/kg body weight of lead acetate. (hematoxylin and Eosin .40x).

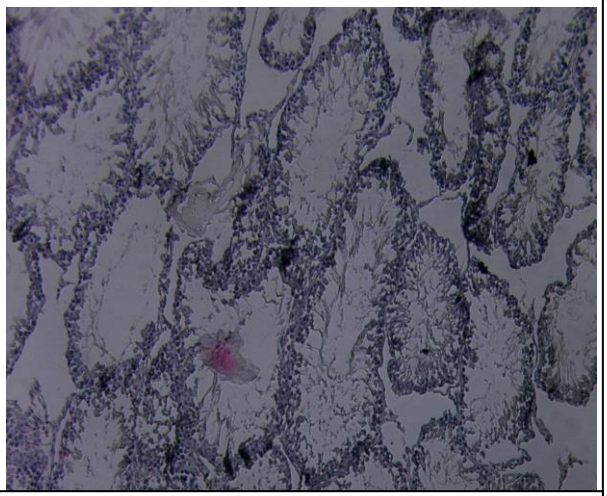

Figure (7). Testicular sections of Lead Acetate treated group. (hematoxylin and Eosin .10x).

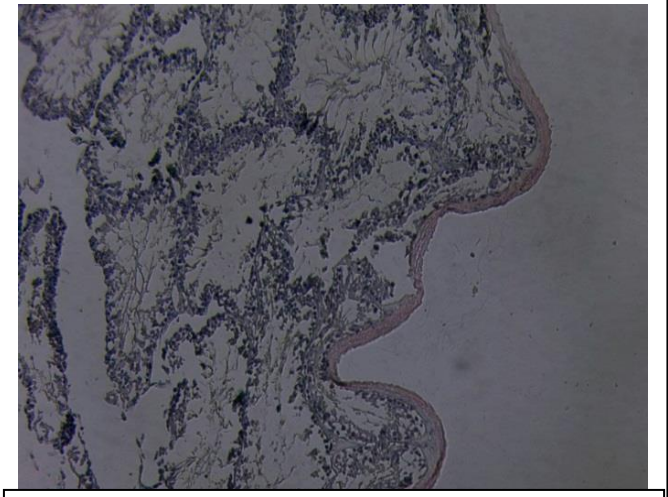

Figure (8). Testicular sections of Lead Acetate treated group. (hematoxylin and Eosin .10x).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

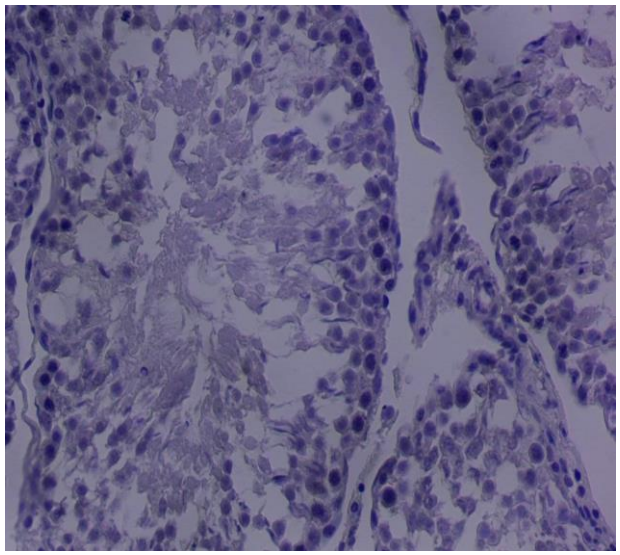

Figure (9). Testicular sections of rats administrated lead acetate for 30 days with L-carnitine for 60 days. (hematoxylin and Eosin .40x).

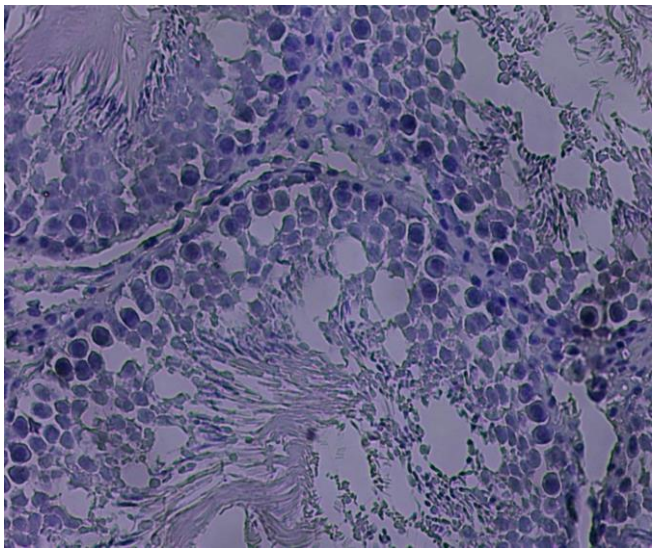

Figure (10). Testicular sections of administrated lead acetate for 30 days with Nano L-carnitine for 60 days. (hematoxylin and Eosin .40x).

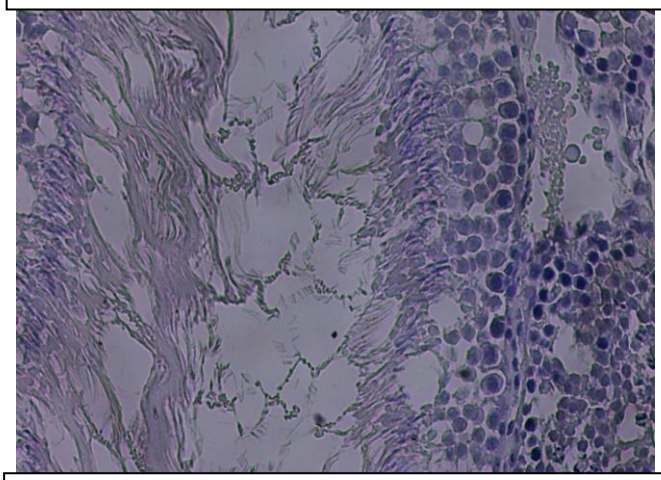

Figure (11). Testicular sections of rat administrated L-Carnitine. (hematoxylin and Eosin .40x).

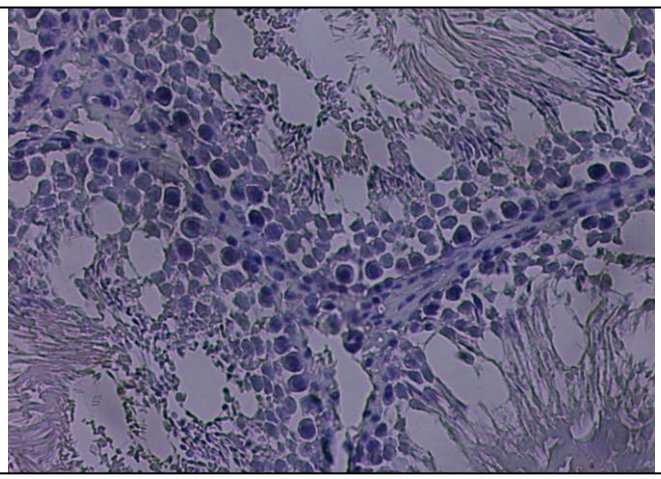

Figure (12). Testicular sections of rat administrated Nano L-Carnitine. (hematoxylin and Eosin .40x).

Characterization of L-Carnitine-chitosan Nanoparticles.

UV spectroscopy revealed a peak at 257 nm in the CSNPs prepared for analysis. This might be because Chitosan contains an amido group. As agreed with (38) of has a peak at 226 nm in his spectra. Chitosan nanoparticles were found to have a peak at 310 nm by (39). Chitosan peaks at 201 nm, according to (40). According to the FT-IR data (Figure 1) and (2), chitosan and L-

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Carnitine Nanoparticles (NPs) both contain a wide variety of functional groups. Phenols, alcohols, alkanes, alkynes, and aldehydes have these functional groups. (41) made similar results in According to (42).

Effect of lead acetate, L-Carnitine, and L-Carnitine loaded chitosan-nanoparticles on sperm parameters, reproductive hormones, and histopathological changes in adult male rats.

The results showed a significant increase in the percentage of count and motility in group three (lead acetate + l-carnitine) and group four (lead acetate + Nano L-carnitine) compared with group two (lead acetate). Show better improvements in the percentage in group four. Also showed a significant decrease in the rate of dead sperm and abnormal morphology in group three and group four compared with group two (lead acetate).

The detrimental effect of lead acetate as an oxidative agent affects the testes (43, 44, 45). Furthermore, in the lead acetate group, a drop in serum testosterone, as well as an increase in MDA concentrations, might be attributable to caused changes in spermatogenic processes and Leydig's cells steroid biosynthesis, which impaired sperm quality and male infertility, resulting in azoospermia (46, 47). Chronic lead exposure inhibits spermatogenesis, causing sperm counts to drop (48). Lead exposure also results in many defective sperm and immature spermatozoa (49). Another research found that high lead levels in the blood and sperm harmed sperm count, morphology, viability, and motility in battery industry employees. According to (50) this was related to significant lipid peroxidation and decreased seminal plasma ascorbic acid.

Furthermore, Lead in drinking water has been shown to diminish sperm motility, density, and viability, as well as produce major structural flaws in spermatozoa, according to recent research by (51). In exposed rats, chronic lead exposure resulted in a significant loss in sperm vitality, motility, density, and morphology and a significant decrease in zinc content in testicular homogenates (52). Spermiogenesis, sperm capacitation, acrosome response, and sperm–oocyte contact need low ROS generation by spermatozoa at physiological levels. In spermatozoa, superoxide is primarily produced by intracellular oxidases/peroxidases and leakage of mitochondrial electrons from the electron transport chain. Spermatozoa defects, exceptionally high cytoplasmic residue, or activation of the intrinsic apoptotic pathway, may significantly enhance ROS generation, resulting in OS. As stated before (53), plasma lipid peroxidation levels can be used to indicate seminal oxidative stress (54). Unbalanced ROS and an increase of SO2• or H2O2 can cause male infertility. They are associated with the destruction of the sperm plasma membrane, losing potential in the mitochondrial membrane, germ cell programming death, and sperm DNA damage, leading to several abnormal morphological changes during sperm morphogenesis, simultaneously reducing the inability of fertilization (55, 56).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Administration of lead acetate in concomitant with L-carnitine and Nano L-Carnitine significantly improved sperm parameters compared with lead acetate values, which may be due to the antioxidant properties of L-Carnitine.

Antioxidant enzymes are protected from oxidative stress by l-carnitine, a free radical scavenger that prevents the buildup of oxidative stress byproducts, according to the study (57).

Lead-treated rats received L-carnitine supplementation, which resulted in considerable improvements in sperm parameters. A significant improvement in testicular tissue oxidant and antioxidant status was also observed. Lysine and methionine amino acids are the building blocks of L-carnitine, a bioactive molecule that may improve lipid metabolism. Cellular energy generation is facilitated by its participation in mitochondrial beta-oxidation. DNA and cell membranes are also protected from free oxygen radical damage by L-carnitine (58; 59). Sperm motility is greatly influenced by large amounts of L-carnitine in the epididymal fluid and spermatozoa as opposed to blood (52). L-carnitine generated a considerable increase in sperm motility in testes, according to (60). Sperm motility improved statistically significantly when L-carnitine was administered to patients with idiopathic oligoasthenozoospermia. Remarkably, the levels of progressive motility returned to their pre-treatment levels after stopping L-carnitine (61). Carnitine has been shown to enhance sperm motility by increasing mitochondrial fatty acid oxidation and ATP synthesis in the mitochondria (62). L- carnitine's antioxidant capability may enhance sperm motility and count (63). Previous research has shown that L-carnitine improves sperm parameters. Sperm counts, viability, and morphology all improved directly (52,64). L- carnitine antioxidant capabilities may be contributed to the improvement in sperm parameters, it stops the semen from producing free radicals (65). Carnitine's ability to scavenge free radicals directly; chelate catalytic metals-promoters of ROS, such as Fe and Cu; maintain mitochondrial integrity and prevent ROS formation; inhibit ROS-generating enzymes, such as xanthine oxidase and NADPH oxidase, with an additional synthesis of antioxidant enzymes, such as L-carnitine, has been proposed as an antioxidant mechanism (66).

Toxic levels of free radicals cause oxidative stress, and when the system's ability to remove them from the body is impaired, it quickly leads to cellular damage (52). In the present investigation, the blood testosterone levels were significantly reduced by using lead acetate. Lead exposure has also been linked to a decreased testosterone level (67). Chronic lead exposure impairs spermatogenesis because of hormonal imbalance caused by a rise in FSH, LH, and gonadotropin-releasing hormone caused by disturbance of the hypothalamic-pituitary axis (68). Testicular spermatogenesis and epididymal sperm are directly affected by lead, which causes reproductive damage (69).

Workers exposed to Pb for an extended period exhibited a drop in testosterone levels, which may be due to the inadequacy of Sertoli and Leydig cells (70). Due to the direct toxic action of Pb on the testicles, these data show that Pb exposure lowers testosterone production, which in turn increases FSH and LH levels (71). This enzyme is essential for converting androstenedione into Testosterone, and it is mainly found in the testicles. Rats treated with lead

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

acetate had lower amounts of 17-HSD in their testicles, correlated with lower testosterone levels in the bloodstream. Testicular 17-HSD activity was decreased in rats treated with lead acetate for 30 days by (72).

The 17-HSD level in the testicles was elevated when L-carnitine and lead Acetate were administered together (73). In our experiment, we found that administering L-carnitine to rats exposed to high amounts of lead elevated their testosterone levels significantly. Similar outcomes have been seen in prior investigations using L-carnitine (74,52). Aside from lowering FSH and LH blood levels, L-carnitine has also been shown to increase testosterone levels in males. L-carnitine, an antioxidant, may reduce oxidative stress by inhibiting free radicals and increasing the production of antioxidant enzymes (75).

The cross-sections of testes in rats exposed to lead acetate showed histopathological alterations in testicular architecture. There are no spermatogonia in the seminiferous tubule lumen due to atrophy of the seminiferous tubules and irregularity, and vacuolization with a small number of spermatogonia can occur as a result of Lead Acetate harming the male reproductive system through degenerative testes and the loosing of germinal epithelial cells that disrupt the basement membrane. Seminiferous tubules vacuole and interstitial edema are both present. The present study's findings on the effects of lead were consistent with those of previous investigations. (76, 77).

Administration of L-Carnitine and Nano L-Carnitine concurrently with lead acetate caused significantly improved histopathological alterations of testes, near to normal and protected them from induced oxidative injury, and this might be due to the free radical scavenging activity of L-Carnitine, manifested by rearrangement of cells of seminiferous tubules and restored their activity, treatment with LC would reduce the levels of ROS, which would avoid the lipoperoxidation and its harmful effects at the cellular level. (78; 79). In mice, pretreatment with L-carnitine 1 hour before being exposed to a magnetic field protected sperm motility and count against the effects of a high magnetic field (80). Aside from that, the outcomes of According to the results of the previous investigation, pretreatment with Reversing histopathological alterations with L-carnitine was the most effective treatment Magnetic field-induced tubular dilation, sloughing of the lining, and absence of spermatogonia and spermatogenesis (80). Studies have shown that carnitine acts directly on Sertoli cells' intermediate metabolism, pointing to the Sertoli cell as a potential target for carnitine activity in the testis (81). Rat Sertoli cells in primary culture were shown to have increased fatty acid oxidation, glucose uptake, and lactate/pyruvate production in response to carnitine (81).

Administration of L-Carnitine and Nano L-Carnitine, which improved hormonal profile and maintained Leydig's cells integrity, attributed to its antioxidant and androgenic properties against lead acetate properties.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Conclusion: Lead acetate poisoning in rats might benefit from L-carnitine administration to protect the testis, reproductive hormones, and sperm parameters, as well as improving this harm with nano l-carnitine.

Acknowledgment

We want to express our gratitude and appreciation to Dr. Ali Majeed omara, who is invaluable in helping to do statistics, Dr. Labib in helping with nanomaterial work and characterization, and Dr. Walaa Fadil in aiding histopathologic slide preparation.

Conflict of Interest: The authors state that there is no conflict of interest.

Reference:

1-Alwan, S. H., & Al-Saeed, M. H. (2021). Biosynthesized silver nanoparticles (using Cinnamomum zeylanicum bark extract) improve the fertility status of rats with polycystic ovarian syndrome. *Biocatalysis and Agricultural Biotechnology*, *38*, 102217.

2-Al-MUDHAFAR, S. M., & Al-HAMDANI, M. A. (2021). EVALUATION FOR ANTIBACTERIAL AND ANTIPROLIFERATIVE ACTIVITY AGAINST CANCER CELL LINES OF BIOSYNTHESIZED SILVER NANOPARTICLES FROM VITEX AGNUS-CASTUS AND UNCARIA TOMENTOSA EXTRACTS. *Journal of Basrah Researches ((Sciences))*, *47*(1).

3-Bachii, S. A., & Abd-Al Sahib, W. H. (2021). Preparation and Characterization of Silver Nanoparticles Biosynthesis by Pseudomonas stutzeri Environmental Bacteria Isolated from Oil Fields and Their Antimicrobial Activity.

4- Patra, N., Kar, D., Pal, A., & Behera, A. (2018). Antibacterial, anticancer, anti-diabetic and catalytic activity of bio-conjugated metal nanoparticles. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *9*(3), 035001.

5- Asgher, M., Qamar, S. A., & Iqbal, H. (2021). Microbial exopolysaccharide-based nano-carriers with unique multi-functionalities for biomedical sectors. *Biologia*, *76*(2), 673-685.

6- Ameeduzzafar, Ali, J., Fazil, M., Qumbar, M., Khan, N., & Ali, A. (2016). Colloidal drug delivery system: amplify the ocular delivery. Drug delivery, 23(3), 700-716.

7- Hauser-Kawaguchi, A., & Luyt, L. G. (2015). Nanomedicine—nanoparticles in cancer imaging and therapy. In Genomic Instability and Cancer Metastasis (pp. 205-244). Springer, Cham.

8- Wu, L., Zhou, W., Lin, L., Chen, A., Feng, J., Qu, X., ... & Yue, J. (2022). Delivery of therapeutic oligonucleotides in nanoscale. Bioactive materials, 7, 292-323.

9-Sabu, S., Sasidharan, A., & Venugopal, V. (2022). Influence of Isolation Conditions on the Physicochemical and Biological Properties of Chitosan and Chitosan Oligosaccharides from Marine Crustacean Shell Wastes. In Chitooligosaccharides (pp. 333-352). Springer, Cham.

10-Martău, G. A., Mihai, M., & Vodnar, D. C. (2019). The use of Chitosan, alginate, and pectin in the biomedical and food sector—biocompatibility, bioadhesiveness, and biodegradability. Polymers, 11(11), 1837.

11-Zaboon, M. H., Saleh, A. A., & Al-Lami, H. S. (2021). Synthesis, characterization and cytotoxicity investigation of chitosan-amino acid derivatives nanoparticles in human breast cancer cell lines. *Journal of the Mexican Chemical Society*, *65*(2), 178-188.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

12- Mosa, R. K., Jassim, J. M., & Al-Sereah, B. A. H. EFFECT OF INDIVIDUAL AND COMBINATION ADDITION OF L–CARNITINE AND DL–METHIONINE TO DIET ON PRODUCTIVE PERFORMANCE FOR QUAIL (COTURNIX).

13-D'souza, P., & Selvam, R. (2022). Evaluation of polyherbal formulation in broilers fed high energy diet: Implications on zootechnical parameters, fat accretion, and serum L-carnitine levels. Journal of Advanced Veterinary and Animal Research, 9(1), 166.

14-El-Sherbini, E. S., El-Sayed, G., El Shotory, R., Gheith, N., Abou-Alsoud, M., Harakeh, S. M., & Karrouf, G. I. (2017). Ameliorative effects of L-carnitine on rats raised on a diet supplemented with lead acetate. Saudi Journal of Biological Sciences, 24(6), 1410-1417.

15-Li, X. F., Shi, Z. D., Song, H., Wang, Y. L., Li, Q. C., Diao, X. H., ... & Liu, H. Y. (2021). Protective effects of L-carnitine on reproductive capacity in rats with diabetes. *Journal of Physiology and Pharmacology*, *72*(1).

16-Kazemi-Darabadi, S., Asadpour, R., Shahbazfar, A. A., & Alizadeh, S. (2019). Effects of L-carnitine and betamethasone on ischemia-reperfusion injuries and sperm parameters following testicular torsion in a rat model. In Veterinary Research Forum (Vol. 10, No. 2, p. 125). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

17-Moghaddas, A., & Dashti-Khavidaki, S. (2018). L-carnitine and potential protective effects against ischemia-reperfusion injury in noncardiac organs: from experimental data to potential clinical applications. Journal of Dietary Supplements, 15(5), 740-756.

18-Mardanshahi, T., Rezaei, N., Zare, Z., Shafaroudi, M. M., & Mohammadi, H. (2019). Effects of L-Carnitine on the sperm parameters disorders, apoptosis of spermatogenic cells and testis histopathology in diabetic Rats. *International Journal of Reproductive BioMedicine*, *17*(5), 325.

19- Adnan, M., Xiao, B., Xiao, P., Zhao, P., Li, R., & Bibi, S. (2022). Research Progress on Heavy Metals Pollution in the Soil of Smelting Sites in China. *Toxics*, *10*(5), 231.

20-Proshad, R., Kormoker, T., Mursheed, N., Islam, M. M., Bhuyan, M. I., Islam, M. S., & Mithu, T. N. (2018). Heavy metal toxicity in agricultural soil due to rapid industrialization in Bangladesh: a review. International Journal of Advanced Geosciences, 6(1), 83-88.

21-El-Belbasy, H., Hussein, M., & El-Ghitany, M. (2021). The Ameliorative Role of Cranberry Extract Use on Hematological Changes Induced by Lead Acetate in Rats. Zagazig Veterinary Journal, 49(1), 102-113.

22-Ikpesu, J. E., Sunny, E., Daramola, M., Okewale, A. O., Farouq, A. U., Ali, P., ... & Nwaezeapu, A. (2021). Investigation of selected heavy metal and physio-chemical properties of Ekpan River in Delta State, Nigeria.

23-Mumtaz, S., Ali, S., Khan, R., Shakir, H. A., Tahir, H. M., Mumtaz, S., & Andleeb, S. (2020). Therapeutic role of garlic and vitamins C and E against toxicity induced by lead on various organs. Environmental Science and Pollution Research, 27(9), 8953-8964.

24-Rahimi, G., Ebrahimi, E., Shali, S. Y., & Zanjani, R. S. (2022). Investigation of temporal changes in the uptake of heavy metals in strawberries by different organs of rat.

25-Bassi, E., Facoetti, R., Ferloni, M., Pastorino, A., Bianchi, A., Fedrizzi, G., ... & Andreotti, A. (2021). Lead contamination in tissues of large avian scavengers in south-central Europe. Science of The Total Environment, 778, 146130.

26-El-Ghaffar, M. A., & Hashem, M. S. (2009). Immobilisation of α-amylase onto chitosan and its amino acid condensation adducts. *Journal of applied polymer science*, *112*(2), 805-814.

27-El-Ghaffar, M. A., Atia, K. S., & Hashem, M. S. (2010). Synthesis and characterisation of binary copolymers of methyl methacrylate with glycidyl methacrylate and 2-hydroxy ethyl methacrylate as carriers for cellulase. *Journal of Applied Polymer Science*, *117*(2), 629-638.

28- Zaboon, M. H., Saleh, A. A., & Al-Lami, H. S. (2021). Synthesis, characterization and cytotoxicity investigation of chitosan-amino acid derivatives nanoparticles in human breast cancer cell lines. *Journal of the Mexican Chemical Society*, *65*(2), 178-188.

29- Olteanu, R. L., Nicolescu, C. M., & Bumbac, M. (2017). Influence of phytochemical reductive capacity on ultraviolet–visible spectroscopic behavior of silver nanoparticles. *Analytical Letters*, *50*(17), 2786-2801.

30- Choudhary, R. C., Kumari, S., Kumaraswamy, R. V., Pal, A., Raliya, R., Biswas, P., & Saharan, V. (2019). Characterization Methods for Chitosan-Based Nanomaterials. In *Plant Nanobionics* (pp. 103-116). Springer, Cham.

31- Ma, H., & Shieh, K. J. (2006). ELISA Technique. *Nat Sci*, *4*(2), 36-37.

32- Sakamoto, J., & Hashimoto, K. (1986). Reproductive toxicity of acrylamide and related compounds in mice—effects on fertility and sperm morphology. *Archives of toxicology*, *59*(4), 201-205.

33- Vashisht, A., & Gahlay, G. K. (2020). Using miRNAs as diagnostic biomarkers for male infertility: Opportunities and challenges. *Molecular Human Reproduction*, *26*(4), 199-214.

34- Salisbury, G. W., VanDemark, N. L., & Lodge, J. R. (1978). Physiology of reproduction and artificial insemination of cattle-2.

35- Björndahl, L., Söderlund, I., & Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Human reproduction*, *18*(4), 813-816.

36- Mescher AL and Jungueira LC (2010). Jungueira's Basic Histology: Text & Atlas. McGraw-Hill medical New York. 12th Edition. pp 20-21.

37-Dugard, P. (2014). Randomisation tests: A new gold standard. *Journal of Contextual Behavioral Science*, *3*(1), 65-68.

38-Agarwal, M., Agarwal, M. K., Shrivastav, N., Pandey, S., Das, R., & Gaur, P. (2018). Preparation of chitosan nanoparticles and their in-vitro characterization. *Int J Life Sci Scienti Res*, *4*(2), 1713-1720.

39- Krishnaveni, B., & Priya, P. (2014). Biosynthesis and antimicrobial activity of silver nanoparticles from Murraya koenigii, Ocimum teniflorum, Chitin and Chitosan. *International Journal of Pharmaceutical & Biological Archives*, *5*(1), 49-55.

40- Liu, C. G., Chen, X. G., & Park, H. J. (2005). Self-assembled nanoparticles based on linoleic-acid modified chitosan: Stability and adsorption of trypsin. *Carbohydrate Polymers*, *62*(3), 293-298.

41- Tan, W., Zhang, J., Zhao, X., Li, Q., Dong, F., & Guo, Z. (2020). Preparation and physicochemical properties of antioxidant chitosan ascorbate/methylcellulose composite films. *International journal of biological macromolecules*, *146*, 53-61.

42- Thamilarasan, V., Sethuraman, V., Gopinath, K., Balalakshmi, C., Govindarajan, M., Mothana, R. A., ... & Benelli, G. (2018). Single step fabrication of chitosan nanocrystals using Penaeus semisulcatus: potential as new insecticides, antimicrobials and plant growth promoters. *Journal of Cluster Science*, *29*(2), 375-384.

43- Elgawish, R. A. R., & Abdelrazek, H. M. (2014). Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicology Reports*, *1*, 795-801.

44- Al-Okaily, B. N., & Al-Shammari, Z. M. (2016). The Impact of Eruca sativa Seeds on Leydig's Cells Number and Hormonal Profile In Cadmium Exposed Rats. *مجلة الكوفة للعلوم الطبية البيطرية| Kufa Journal For Veterinary Medical Sciences*, *7*(2).

45- Zhang, J., Huang, X., Chen, Y., Luo, B., Luo, J., Zhang, W., ... & Yang, F. (2019). Characterization of lead-containing atmospheric particles in a typical basin city of China: Seasonal variations, potential source areas, and responses to fireworks. *Science of the Total Environment*, *661*, 354-363.

46- Du Plessis, S. S., Cabler, S., McAlister, D. A., Sabanegh, E., & Agarwal, A. (2010). The effect of obesity on sperm disorders and male infertility. *Nature Reviews Urology*, *7*(3), 153-161.

47- Soliman, G. A., Ganaie, M. A., Althurwi, H. N., Albaqami, F. F., Salkini, M. A., & Abdel-Kader, M. S. (2017). Extract of Salvadora persica roots attenuates lead acetate-induced testicular oxidative stress in rats. *Journal of Pharmacy & Pharmacognosy Research*, *5*(4), 238-250.

48- Kašuba, V., Rozgaj, R., Milić, M., Želježić, D., Kopjar, N., Pizent, A., ... & Jazbec, A. (2012). Evaluation of genotoxic effects of lead in pottery-glaze workers using micronucleus assay, alkaline comet assay and DNA diffusion assay. *International archives of occupational and environmental health*, *85*(7), 807-818.

49- Hsu, P. C., Chang, H. Y., Guo, Y. L., Liu, Y. C., & Shih, T. S. (2009). Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage. *Fertility and sterility*, *91*(4), 1096-1103.

50- Naha, N., Bhar, R. B., Mukherjee, A., & Chowdhury, A. R. (2005). Structural alteration of spermatozoa in the persons employed in lead acid battery factory. *Indian journal of physiology and pharmacology*, *49*(2), 153.

51- Li, C., Zhao, K., Zhang, H., Liu, L., Xiong, F., Wang, K., & Chen, B. (2018). Lead exposure reduces sperm quality and DNA integrity in mice. *Environmental Toxicology*, *33*(5), 594-602.

52- Abdel-Emam, R. A., & Ahmed, E. A. (2021). Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. *Veterinary Medicine and Science*, *7*(4), 1426-1435.

53- Toor, J. S., & Sikka, S. C. (2019). Human spermatozoa and interactions with oxidative stress. In *Oxidants, Antioxidants and Impact of the Oxidative Status in Male Reproduction* (pp. 45-53). Academic Press.

54- Wolkmer, P., Stumm, A. M., Borges, L. F., Ferreira, E. P., Favaretto, B., & Siqueira, L. C. (2019). Plasma lipid peroxidation as a marker for seminal oxidative stress in stallion. *Journal of Agricultural Science*, *11*(401), 10-5539.

55- Agarwal, A., Parekh, N., Selvam, M. K. P., Henkel, R., Shah, R., Homa, S. T., ... & Harlev, A. (2019). Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *The world journal of men's health*, *37*(3), 296-312.

56- Bora, A. J., & Dutta, R. K. (2019). Removal of metals (Pb, Cd, Cu, Cr, Ni, and Co) from drinking water by oxidation-coagulation-absorption at optimized pH. *Journal of Water Process Engineering*, *31*, 100839.

57- Martínez-Holguín, E., Lledó-García, E., Rebollo-Román, Á., González-García, J., Jara-Rascón, J., & Hernández-Fernández, C. (2020). Antioxidants to Improve Sperm Quality. *Male and Sperm Factors that Maximize IVF Success*, *106*.

58-Kim, J. (2019). *Non-invasive Urine Markers of Interstitial Cystitis*. Cedars-Sinai Medical Center.

59-Kidd, P. M. (2005). Neurodegeneration from mitochondrial insufficiency: nutrients, stem cells, growth factors, and prospects for brain rebuilding using integrative management. *Alternative Medicine Review*, *10*(4), 268.

60- Aliabadi, E., Karimi, F., & Talaei-Khozani, T. (2013). Effects of L-carnitine and pentoxifylline on carbohydrate distribution of mouse testicular sperm membrane. *Iranian Journal of Medical Sciences*, *38*(2), 107.

61- Kopets, R., Kuibida, I., Chernyavska, I., Cherepanyn, V., Mazo, R., Fedevych, V., & Gerasymov, S. (2020). Dietary supplementation with a novel l-carnitine multi-micronutrient in idiopathic male subfertility involving oligo-, astheno-, teratozoospermia: A randomized clinical study. *Andrology*, *8*(5), 1184-1193.

62- Zhu, Z., Li, R., Feng, C., Liu, R., Zheng, Y., Hoque, S. A., ... & Zeng, W. (2020). Exogenous oleic acid and palmitic acid improve boar sperm motility via enhancing mitochondrial β-oxidation for ATP generation. *Animals*, *10*(4), 591.

63- Fattah, A., Sharafi, M., Masoudi, R., Shahverdi, A., Esmaeili, V., & Najafi, A. (2017). L-Carnitine in rooster semen cryopreservation: Flow cytometric, biochemical and motion findings for frozen-thawed sperm. *Cryobiology*, *74*, 148-153.

64- Abd-Elrazek, A. M., & Ahmed-Farid, O. A. H. (2018). Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Andrologia*, *50*(1), e12806.

65- Busetto, G. M., Agarwal, A., Virmani, A., Antonini, G., Ragonesi, G., Del Giudice, F., ... & De Berardinis, E. (2018). Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study. *Andrologia*, *50*(3), e12927.

66- Surai, P. F. (2015). Antioxidant action of carnitine: molecular mechanisms and practical applications. *EC Veterinary Science*, *2*(1), 66-84.

67- Vallverdú-Coll, N., Mougeot, F., Ortiz-Santaliestra, M. E., Rodriguez-Estival, J., López-Antia, A., & Mateo, R. (2016). Lead exposure reduces carotenoid-based coloration and constitutive immunity in wild mallards. *Environmental toxicology and chemistry*, *35*(6), 1516-1525.

68- Ozawa, H. (2022). Kisspeptin neurons as an integration center of reproductive regulation: Observation of reproductive function based on a new concept of reproductive regulatory nervous system. *Reproductive Medicine and Biology*, *21*(1), e12419.

69- Gandhi, J., Hernandez, R. J., Chen, A., Smith, N. L., Sheynkin, Y. R., Joshi, G., & Khan, S. A. (2017). Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. *Zygote*, *25*(2), 103-110.

70- HAMADOUCHE, N. A., NESRINE, S., & ABDELKEDER, A. (2013). Lead toxicity and the hypothalamic-pituitary-testicular axis. *Notulae Scientia Biologicae*, *5*(1), 1-6.

71- Anjum, M. R., Madhu, P., Reddy, K. P., & Reddy, P. S. (2017). The protective effects of zinc in lead-induced testicular and epididymal toxicity in Wistar rats. *Toxicology and industrial health*, *33*(3), 265-276.

72- Roychoudhury, S., Chakraborty, S., Choudhury, A. P., Das, A., Jha, N. K., Slama, P., ... & Kesari, K. K. (2021). Environmental factors-induced oxidative stress: Hormonal and molecular pathway disruptions in hypogonadism and erectile dysfunction. *Antioxidants*, *10*(6), 837.

73- M Brzóska, M., Borowska, S., & Tomczyk, M. (2016). Antioxidants as a potential preventive and therapeutic strategy for cadmium. *Current drug targets*, *17*(12), 1350-1384.

74- Aziz, R. L. A., Abdel-Wahab, A., El-Ela, F. I. A., Hassan, N. E. H. Y., El-Nahass, E. S., Ibrahim, M. A., & Khalil, A. T. A. (2018). Dose-dependent ameliorative effects of quercetin and l-Carnitine against atrazine-induced reproductive toxicity in adult male Albino rats. *Biomedicine & pharmacotherapy*, *102*, 855-864.

75- Zambrano, S., Blanca, A. J., Ruiz-Armenta, M. V., Miguel-Carrasco, J. L., Arévalo, M., Mate, A., & Vázquez, C. M. (2014). L-carnitine attenuates the development of kidney fibrosis in hypertensive rats by upregulating PPAR-γ. *American journal of hypertension*, *27*(3), 460-470.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

76- Balachandar, R., Bagepally, B. S., Kalahasthi, R., & Haridoss, M. (2020). Blood lead levels and male reproductive hormones: A systematic review and meta-analysis. *Toxicology*, *443*, 152574.

77- Abdelhamid, F. M., Mahgoub, H. A., & Ateya, A. I. (2020). Ameliorative effect of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environmental Science and Pollution Research*, *27*(10), 10950-10965.

78- Mardanshahi, T., Rezaei, N., Zare, Z., Shafaroudi, M. M., & Mohammadi, H. (2019). Effects of L-Carnitine on the sperm parameters disorders, apoptosis of spermatogenic cells and testis histopathology in diabetic Rats. *International Journal of Reproductive BioMedicine*, *17*(5), 325.

79- Lee, Y. C. G., Chou, H. C., Chen, Y. T., Tung, S. Y., Ko, T. L., Buyandelger, B., ... & Juan, S. H. (2022). l-Carnitine reduces reactive oxygen species/endoplasmic reticulum stress and maintains mitochondrial function during autophagy-mediated cell apoptosis in perfluorooctanesulfonate-treated renal tubular cells. *Scientific reports*, *12*(1), 1-14.

80- Ramadan, L. A., Abd-Allah, A. R., Aly, H. A., & Saad-El-Din, A. A. (2002). Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice. *Pharmacological research*, *46*(4), 363-370.

81- Topcu-Tarladacalisir, Y., Kanter, M., & Uzal, M. C. (2009). Role of L-carnitine in the prevention of seminiferous tubules damage induced by gamma radiation: a light and electron microscopic study. *Archives of toxicology*, *83*(8), 735-746.