REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Evaluating the Protective Potential of Nano L-Carnitine on the Gonadal Pathway in Lead Acetate-Exposed Male Rats

Krishana Kumar Sharma[1], Manashree Avinash Mane[2], Dr. Jayabhaye Mahesh Shrirnag[3]

[1]Professor, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id: drkk108@gmail.com

[2]Assistant Professor, Department of Forensic Science, School of Sciences, JAIN (Deemed-to-be University), Karnataka, India, Email id: m.manashree@jainuniversity.ac.in

[3]Professor, Department of Ayurveda, Sanskriti University, Mathura, Uttar Pradesh, India, Email id: satyapir.samch@sanskriti.edu.in

Abstract

The goal of the research was to find out whether L-Carnitine (LC) and Nano L-Carnitine (NLC) could protect against the testicular toxicity brought on by lead acetate (PbAc). By encasing LC in Chitosan (CS) - tripolyphosphate (TPP) - based NPs, they created LC nanoparticles (NPs). Each of the experiment has divided into six Teams; there are ten adult male Wistar albino rats. The first Team functioned as the controlling Team and received distilled water that was provided orally. The second Team was given 30 mg/kg of lead acetate (LA) orally once daily for 30 days. In the third Team, oral doses of LC (100 mg/kg body mass/day for two months) and LA (30 mg/kg body mass for 30 days) were given. The fourth Team was given oral doses of LA (for 30 days at 30 mg/kg body mass) and NLC (for 2 months at 100 mg/kg body mass/day). LC was administered orally to the fifth Team twice daily for 2 months an amount of 100 mg/kg body mass. The sixth Team ingested 100 mg/kg of LC -NPs twice each day for two months. The finding indicated that the levels of testosterone hormones were noticeably greater in the LA+ NLC Team compared to the LA Team. The LA+ NLC Team also showed lower follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations in comparison to the LA Team. Additionally, when compared to Team two (LA), Team three (LA+ LC) and four (LA+ NLC) both significantly increased the percentage of Sperm motility and count. The Sperm motility and count gains in Team four were more pronounced. Additionally, compared to Team two (lead acetate), there was a significantly lower incidence of stale sperm and aberrant morphology in Teams 3 and 4. The testes' histopathology revealed significant alterations, including the germinal epithelium's severe degeneration and sloughing, the basement membrane's disorganization, the seminiferous tubule lumens were empty of spermatids, and there was vacuolization with few spermatogonia in the lumens, along with atrophy and irregularity. However, in the treated Team, these harmful alterations decreased or vanished. Overall, the research showed that giving male rats LC and NLC prevented the harmful effects of LA on their testicles. The treated Team demonstrated improvements in testicular histopathological damage, sperm count, motility, and morphology, as well as testosterone hormone levels.

Keywords: lead acetate, sperm characteristics, chitosan, L-carnitine, nanoparticles, and reproductive hormones.

Introduction

The development of all areas of the life sciences and biomedical research, including diagnosis, therapy, and pharmaceutical administration, is now thought to be based on nanobiotechnology. The effectiveness of many medicines is constrained by a variety of issues, for many of these medicines, surpass the safe dose concentrations, as well as minimal bioavailability, low in vivo stability, solubility, and intestinal absorption issues. Most of the time, just a small portion of the necessary dosage reaches the desired target. Most of the medicine is transported throughout the remainder of the human body, based on its kinetics

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

and biological characteristics. The use of NPs delivery methods has several benefits, including high real-life strength, a long-time payload release, and passage through minute capillaries and cellular compartments (1). Additionally, NPs can control a drug's pharmacokinetics and bio-distribution, increase its therapeutic index, and help develop long-term drug reservoirs. A few more requirements include focused optimization to the particular area that minimizes absorption by neighboring tissues, smaller NPs (50-200 nm), a high loading capability, compound dissolution delay in real life, and so on. For the development of efficient delivery systems, formulations with these characteristics that are also cost and complexity-effective must be created (2). Chitosan is a Chitin a linear polysaccharide composed of acetylated sugars. Chitin's N-deacetylated derivative N-acetyl-D-glucosamine and deacetylated units of (1-4)-linked D-glucosamine. Chitin, which is taken out of the shells of shrimp and other crustaceans, is processed with the alkali sodium hydroxide to produce chitosan. Given that so many natural resources were employed to create the polymer-drug carrier material, Studies have been done on chitosan to determine its biocompatibility, low toxicity, biodegradability, and reasonably low manufacturing costs (3).

Levo Carnitine, often known as L-Carnitine, is a kind of amino acid that is present in the body by nature. It can also be ingested by people as an oral supplement or in their food. Because it converts fat into energy, LC is crucial for producing energy. LC is high in antioxidants and has fewer negative health effects. As a result, oxidative stress and associated health problems are prevented and treated using it. Long-chain fatty acids can be used for energy thanks to the antioxidant LC. Among the many biological roles it possesses are anti-inflammatory, anti-apoptotic, cardioprotective, and gastroprotective qualities. By providing energy, LC promotes sperm metabolism, maturation, motility, and spermatogenesis. It also stops apoptosis in testes that have received radiation. In testes impacted by IR, corticosteroids have been shown to positively affect spermatogenesis. These anti-inflammatory medications protect the cell wall's integrity and stop the generation of free radicals. They also prevent vessels from being porous (4). One of the most prevalent and harmful heavy metals is lead, which is widely distributed in nearby industrial areas' soil and aquatic environments. Lead metal is notable for its typical silver hue, faintly bluish undertone, and brilliance under dry conditions. The main causes of exposure to lead in daily life are gasoline, food, cigarettes, common objects, manufacturing processes, piping containers, gun slugs, batteries, toys for kids, and water taps. Industrial processes and automobile emissions release lead into the environment, where it gets into water sources and soil and is taken up by plants. After then, lead exposure happens through food and water consumption. The liver, testicles, brain, kidneys, and bones of mammals are frequently the focus of studies on wildlife toxicology since lead accumulates in these tissues (5).

In the research, the efficiency of biosynthesized LC-NPs on male rat fertility was examined to evaluate the role of LC and NLC in protecting lead acetate (PbAc)-induced testicular damage in male rats.

The order in which the remaining details are given is listed below. The relevant literature is discussed in Section 2, whereas Section 3 explores methodologies. Section 4 of the report examines the findings, and Section 5 of the report presents the conclusions.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Related works

In the study (6), the anti-inflammatory, antioxidant, and anti-apoptotic properties of natural curcumin (CUR) and liposomal nano-curcumin (N-CUR) against oxidative injury, inflammation, abnormal steroidogenesis, and Nrf2/HO-1 signaling in male rat testicles were compared. By measuring the levels of testosterone, FSH, and LH in the serum, the researchers evaluated the histological alterations in the testicular tissue. Male rats exposed to the reproductive toxicity of CuSO4 showed protective effects from N-CUR and CUR. The aim of the research (7) was to emphasize the physiological and pathological effects of oxidative stress on the reproductive performance of male and female laboratory animals, as well as how the use of various antioxidants can help to treat oxidative stress in various reproductive anomalies. Alpha-interferon, lopinavir/ritonavir, and an RNA polymerase inhibitor that has in-vitro efficacy against some RNA viruses, including Ebola, may be helpful for both preventing and treating human CoV infections.

A rat model of azoospermia was used in the study (8) to explore the possible therapeutic advantages of exosomes and other extracellular vessels made from amniotic liquid (AF-Exos) affecting the ability to produce sperm returning. Busulfan was injected intratesticular to cause non-obstructive azoospermia (NOA) in rats. Based on their studies, AF-Exos improved sperm quality and restored spermatogenesis in NOA rats. The study's (9) objective was to find any possible modulatory impact of methamphetamine (MTX) on the oxidative stress, energy deficiency, and spermatogenic condition of rat testicles. Semen analysis was done to ascertain the concentration of sperm cells, and the increased motility of the sperm. These results suggest that EGb 761 may be used therapeutically to decrease the reproductive harm caused by MTX.

A study (10) employed the aquatic invertebrate Mactara dendriform (clams) as a model to investigate any possible adverse effects of the progestin nongestural (NGT). According to histological analysis of the tissues from the digestive gland, exposure to NGT caused the basement membrane to become less distinct and epithelial cells to expand. The growing concern over progestins' potential ecological consequences on non-target animals in aquatic settings is exacerbated by these findings. The study (11) sought to determine whether the algae Chlorella vulgaris and Spirulina platensis could protect male rats from testicular dysfunction brought on by lead acetate. As seen by a decline in the weights of the reproductive organs and the gonadosomatic index (GSI), LA caused testicular damage. LA injection caused testicular damage, as shown by decreased weights of the reproductive organs and GSI.

The purpose of the study (12) was to look at how lead affected the rat Leydig cell's ability to synthesize steroid hormones.Ex vivo and in vitro tests were performed in the study to determine how lead affected Leydig cell function. These findings imply that lead directly inhibits steroidogenesis, which has a negative impact on Leydig cell function. In the study (13), testicular antioxidant enzyme activity, sperm parameters, and the hypothalamic-pituitary-testicular axis were evaluated in male Wistar rats that were exposed to LA (Pb)-induced reproduction harm. In the context of reproductive damage brought on by lead

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

acetate via luteinizing hormone, clomiphene citrate may promote testicular testosterone production, sperm motility, and viability.

The goal of the research (14) was to determine when C. asiatica could aid male rats suffering from lead-induced oxidative damage and impaired ovarian function. The production of steroid hormone-producing enzymes was likewise markedly reduced in lead-exposed rats. A few reproductive indices have improved in C. asiatica-treated male rats compared to lead-exposed animals, demonstrating the plant's advantages in lowering lead-induced oxidative damage and restoring male rats' decreased fertility. Rats were used as a mammalian model in the study (15) examined any potential protective properties of LC against UVA-induced skin tissue damage. Pro-liberating cell nuclear antigen (PCNA), a DNA repair protein, was interestingly enhanced by LC. LC likely controls oxidative stress and inflammatory responses to reduce UVA-induced skin tissue damage in rats.

Materials and Methods

These are the methods of evaluating the Protective Potential of NLC on the Gonadal Pathway in Lead Acetate-Exposed Male Rats.

The study's animal

The current investigation was carried out in the physiology department's animal house at Basrah University's College of veterinary medicine. 60 male adult albino rats (Rattus Rattus), all of which before the trial, were housed for two weeks, to allow for acclimatization, weighed an average of 21210g, and were between the ages of 8 and 10 weeks. Each of the ten animals was kept in a 15 x 35 x 50 cm separate plastic cage. The exact temperature (22-25) oC and a fourteen-hour day and ten-hour night schedule for lighting were maintained for them and given the meal of regular diet pellets as they pleased. Water for drinking was also freely available to them.

The layout of the experiments

Six Teams of animals were used in the experiment. Ten male rats in each Team, utilized for the following design studies, are in each Team.

- Control Team: oral consumption of distilled water
- Team 2: (the LA Team) received LA orally once daily for 30 days at a dose of 30 mg/kg of body mass. depend on LD50
- Team 3: LA (30 mg/kg body mass for 30 days) and LC (100 mg/kg body mass in everyday for two mouths)
- Team 4: NLC (100 mg/kg body mass in everyday for two mouths) and LA (30 mg/kg body mass for 30 days)
- Team 5 (LC) received LC orally twice daily for two months at a dosage of 100 mg/kg body mass.
- Team 6 (NLC) received 100 mg/kg body mass of LC-NPs orally every day for two months.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Collection of samples and blood

Blood sampling

The approach was used to get the blood sample using a heart puncture. Before being sacrificed, the managed and treated animals underwent anesthesia. The heart was punctured to obtain a blood sample using a disposable 5ml syringe. To get the serum, 5ml of the blood sample had been centrifuged at 3000 revolutions per minute for 15 minutes in a non-heparinized plane tube. The serum was then put into Androff tubes and stored at -4C until all measurements were taken. The sample used in the test was the one that was taken simultaneously for the histopathology and sperm viability testing.

Enzyme-Linked Immunosorbent Assay ("ELISA") for hormones

Testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH). An enzyme is used in an enzyme-linked immunosorbent to distinguish between the antigen (Ag) and antibody binding (Ab). Abs binding is present because the enzyme converts a non-colored substrate into a colored product.

Chitosan and carnitine were used to make NPs

These are the procedures to create a Carnitine-1-Chitosan adduct.

- The ionic gelation method was used to create Cs-Ca NPs by using the Cs-Ca adduct and TPP.
- TPP solution and Cs-Ca (1 mg/ml) for six hours were combined.
- In a solution of acetic acid (1% w/v), at the ambient temperature, stirring continuously at a ratio of 1: 2.5 (w/w %),
- The ionic gelation of Cs-Ca/TPP NPs was started by TPP.

This was the initial stage of the procedure. These NPs were separated, cleaned, and dried; causing the precipitate to be red closed in water, and dried.

L-carnitine-chitosan NPs' characterization

In the material research labs, these characterization tests were carried out by , the director of environmental and water research and technology (EWRTD) at the Ministry of Sciences and Technology in Baghdad.

Sperm Analysis

Preparation of sperm in suspension in the epididymis's tail:

Epididymis on the left tail was removed and inserted into an untouched watch glass with a millimeter of warm physiologic normal saline after being removed. To complete the sperm examination, the tail was then opened and sliced into extremely little bits using microsurgical scissors**.**

Dead and life sperm (viability) %

The nigrosin stain was employed in the Eosin-nigrosin viability sperm procedure to increase the distinction between the sperm heads and the background**.**

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Motility of sperm (%)

The method described below was used to calculate the percentage of motility.

Proportion of Motility= Number of motile spermatozoa * 100 spermatozoa in total

B. Sperm abnormal morphology (%)

By averaging the results of two slide smears, the percentage was computed using the formula below.

Sperm morphological abnormality (%) = (number of sperms with aberrant morphology)/(Total number of sperms) * 100

Sperm concentration in 107/ml

For the sperm count, Mohan et al.'s (1980) methodology was employed. 0.1 milliliters of sperm suspension were pipette-collected and diluted with 19.9 milliliters of diluent using a hemocytometer (Neubauer type). A drop of a dilute suspension of sperm was added to the hemocytometer's sides, and the slide was placed on top. In the chamber's five medium squares, sperm were counted. To calculate the quantity of sperm, the formula shown below was used:

Sperm concentration = (Sperm number in five medium-sized squares)/80 * 400*200*10

Here, the five medium squares contain a total of 80 smaller squares. There are 400 smaller squares altogether. 200 is the dilution factor. 10 is the volume coefficient.

Histological research

All Teams under study had their testicles removed to prepare slides for a light microscope histological.

Analytical statistics

Microsoft Office Excel 2016 and the statistical software program SPSS used to analyze the data. A significant difference between the Team mean values was found utilizing a one-way ANOVA and a post hoc LSD test. To compare standards, paired t-tests were applied whenever practical. The results were regarded as significant when the p-value was less than 0.06.

Result

Carnitine-loaded Chitosan nanoparticles (LC-NPs)

Ionic gelatin or the interaction of The NPs was produced when the positive-charged chitosan combined with the phosphate groups that were negatively charged in TPP.

Characterization of LC- NPs

The synthesized LC-NPs' shape, elemental information, crystalline structure, functional Team, and stability were evaluated using a variety of spectrophotometric approaches, including UV-visible and FTIR.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

The ultraviolet-visible spectroscope

Using a UV-visible spectrophotometer to scan the prepared solution from (200 to 800 nm), produced CNP and functionalized Chitosan NPs with LC were subjected to an ultraviolet examination. As can be seen in (Figures 1 and 2), the CS-LC solution's absorption peak is visible at a 733 nm wavelength, while the LC-NP peak was acquired at a wavelength of 257 nm**.**

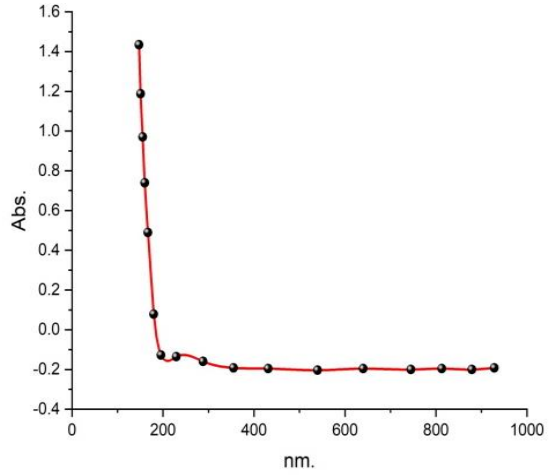


Figure 1: UV-visible spectroscope view of NLC-NPs

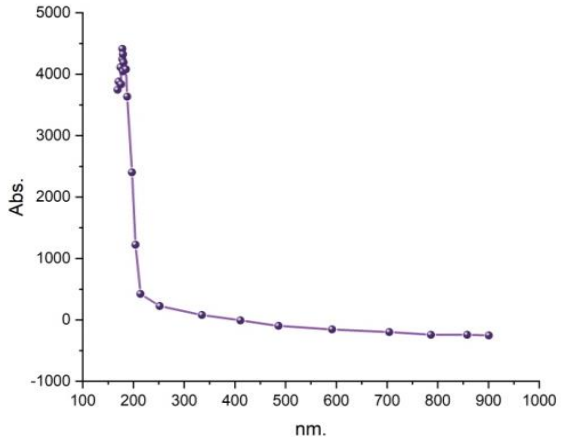


Figure 2: UV-visible spectroscope view of Chitosan**.**

Analysis using Fourier transforms in infrared spectroscopy

To determine chitosan's molecular composition, its solution was captured for a Fourier Transform Infrared (FTIR) analysis using wavelength ranges of LC-CNPs between (400 to 4000 cm-1). This can be shown in (Figure 3). When chitosan's chemical makeup was examined using FTIR measurements, distinct bands generated by the inclusion of amine and methylene glycol are observed.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

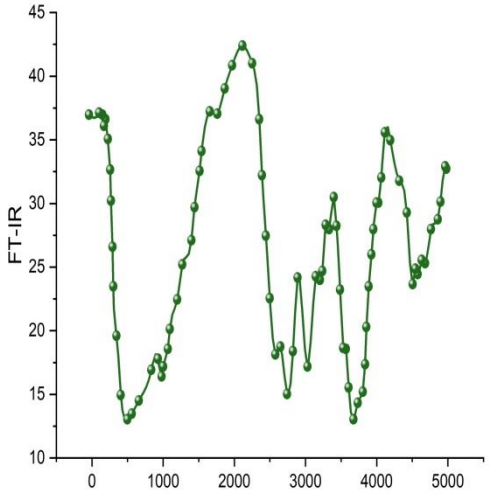


Figure 3: FT-IR spectrum of Chitosan displaying functional Team

Theoretically, the CH2 anti-symmetric stretching modes are what cause the bands at 2925 cm-1. Due to the inclusion of the amines Team, two bands—at 1076-1325 cm-1 and 1649 cm-1—are assigned to C-N stretching modes. Additionally, FTIR spectra were used to locate the functional Team of the compounds. It proved the molecule contained the expected functional Team. The stretching vibrations of C=O and NH were visible in the FTIR spectrum of chitosan at 1560 cm-1 and 2926 cm-1, respectively. An NH Team attached to an NH Team in the -NH-CO- (acetylated amine Team) of chitosan exhibits a unique stretching vibration. Chitosan's amine Team vibrated at 3417 cm-1, which is consistent with an OH vibration because amines have a weaker and less polar hydrogen bond. Compared to the N-H bond, the OH bond vibrates more actively as a result. This reveals that LCNPs loaded by ionic gelation interact with the chitosan functional Team (Figure 4).

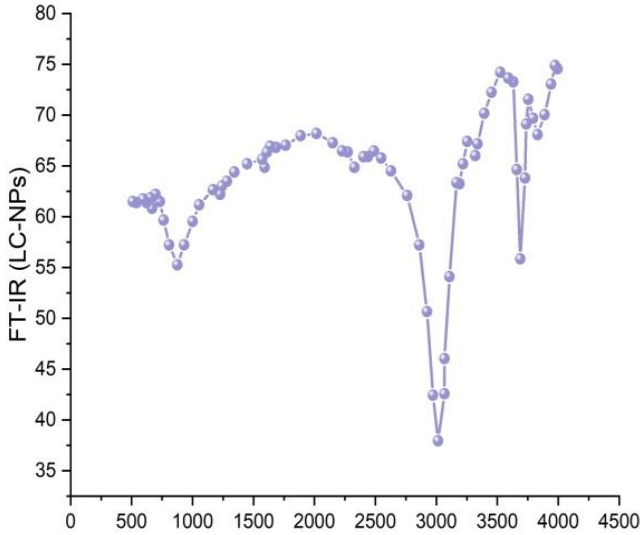


Figure 4: Functional LC-NPs are shown in the FT-IR spectra.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

The FTIR spectrum bands at 3755 cm-1, 3437 cm-1, 2924 cm-1, 1654 cm-1, and 1136 cm-1, which correspond to OH stretching and NH2 C=C stretch, CH2 Team, and C-O-C linkage, C=O Team, respectively, are increased in Chitosan-LCNPs films with LC inclusion. These findings indicate that there is a significant coordinating connection between the functional Teames of chitosan and L-carboxylic acid, which has been attributed to the interaction of the phosphoric and ammonium ions. As a result, we believe that the Tri polyphosphoric Team in TPP and the ammonium Teames in chitosan are connected. Chitosan NPs improve intra- and intermolecular activity.

Parameters of sperm

(Table 1(a) and (b)) provides examples of the characteristics of the epididymal sperm of the experimental Teams rat. According to the findings, there was a significant (P<0.06) decline in sperm motility and count in the LA Team compared with the control Team. According to the data, LA increased the quantity of dying sperm and aberrant morphology in comparison to the controlling Team in a way that was statistically significant (P<0.06).

The percentages of count and motility in Team three (LA+LC) and four (LA+NLC) were noticeably higher (P<0.06) than in Team two (lead acetate). Better percentage improvements should be shown for Team four (LA+ NLC). Additionally, Team three and Team four demonstrated a substantial (P<0.06) less dead sperm percentage and abnormal morphology in comparison to Team two (lead acetate).

The percentages of sperm count and motility did not differ significantly between the LC Team and the NLC Team (P<0.06). The data showed that the proportion of dead sperm and aberrant morphology were considerably reduced in the NLC Team compared to the controlling Team (P<0.06).

Table 1 (a): Result of sperm parameters in mean

Groups	Parameters (Mean)			
	Dead (%)	Motility	Abnormal (%)	Count *10
Control	11.0	94.4	13.8	20.2
LA (U/L)	28.3	51.7	30.6	10.4
LA+LC (U/L)	19.3	77.3	21.4	14.4
LA+NLC(U/L)	14.2	85.9	16.6	18.9
LC (U/L)	9.5	94.6	13.4	20.9
NLC (U/L)	8.4	95.2	10.6	21.8
LSD	1.73	2.86	2.09	1.62

Table 1 (b): Result of sperm parameters in SD

Groups	Parameters (SD)			
	Dead (%)	Motility	Abnormal (%)	Count *10
Control	2.2	2.08	1.78	1.39
LA (U/L)	1.89	5.87	3.12	3.79
LA + LC (U/L)	1.33	2.48	1.48	2.67

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

LA+NLC (U/L)	1.96	3.44	1.84	1.59
LC (U/L)	1.89	1.54	1.34	2.06
NLC (U/L)	1.66	1.26	1.33	1.52
LSD	1.62	2.86	1.73	2.09

Effects of LC, NLC, and LA on male adult rat hormone Levels

The characteristics of various male reproductive hormones are shown in (Table 2 (a) and (b)) to significantly lower rate of exposure (P<0.06) to LA (Thirty mg/kg) compared to other research teams. In contrast to other research teams and demonstrates a significantly higher (P<0.06) level of (LH) and (FSH) in the rats treated with LA (Thirty mg/kg).

The LA + NLC Team had significantly higher levels of the hormone testosterone than the LA Team, according to the data (P<0.06). Contrasted with the LA Team, the LA + NLC Team displayed lower levels of FSH and LH.

FSH and LH levels were considerably (P<0.06) lower in the NLC and LC Teams than in the lead-treated Team. Contrarily, testosterone levels increased substantially (P<0.06) compared to the lead-treated Team.

Table 2 (a): Results of adult male rat hormones in mean

Groups	Parameters (Mean)		
	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)
Control	5.26	2.76	3.19
LA (U/L)	3.96	3.69	4.15
LA+LC (U/L)	4.12	3.39	3.79
LA+NLC (U/L)	4.77	3.00	3.62
LC (U/L)	5.52	2.87	3.08
NLC (U/L)	5.74	2.63	2.98
LSD	0.28	0.31	0.34

Table 2 (b): Result of hormones of adult male rats in SD

	Parameters (SD)		
Groups	**Testosterone (ng/ml)**	**FSH (mIU/ml)**	**LH (mIU/ml)**
Control	0.37	0.45	0.39
LA (U/L)	0.34	0.29	0.36
LA+LC (U/L)	0.14	0.27	0.48
LA+NLC(U/L)	0.29	0.33	0.36
LC (U/L)	0.33	0.36	0.34
NLC (U/L)	0.35	0.34	0.32
LSD	0.28	0.31	0.34

According to the findings, Team three (LA + LC) and Team four (LA with NLC) had significantly higher percentages of count and motility than Team two (lead acetate). Better percentage increases should be shown for Team 4. Additionally, Team three and Team four exhibited a much lower rate of dead sperm and aberrant morphology when compared to Team two (lead acetate).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Effects of LC and NLC on the histological findings of the testicles and the effects of LA (Pb)

Testes

Histological analysis of the rat tests from the control Team revealed that spermatogenic and Sertoli cells were present in many layers in the seminiferous tubules, extending from the membrane at the bottom to the tubule lumen. The interstitial cells had been discovered in (figure 5).

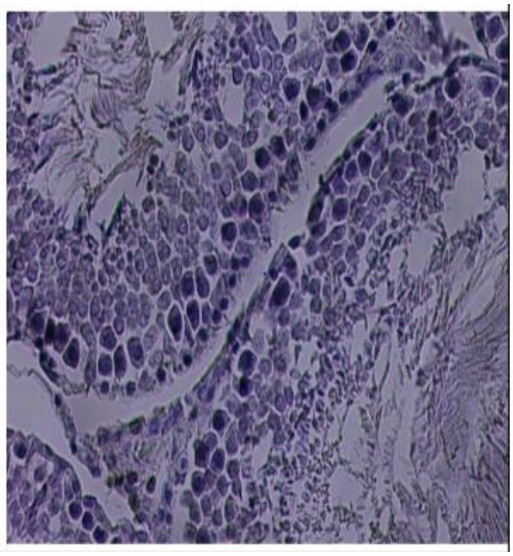


Figure 5: In control group, display the seminiferous tubules of the rat testis.

Interstitial edema and seminiferous tubules that are vascularized, unusual mitosis shows a reduction in the LA seminiferous tubules' diameter and an expansion of the interstitial space. Vascular narrowing in the interstitial, Edema, and interstitial hemorrhage displays apoptotic cells in the lead Team and a necrotic spermatid in the center of the ST. The tubules on display have dispersed Sertoli cells, an empty lumen, and a degraded interstitial space are shown in (Figures 6, 7, and 8).

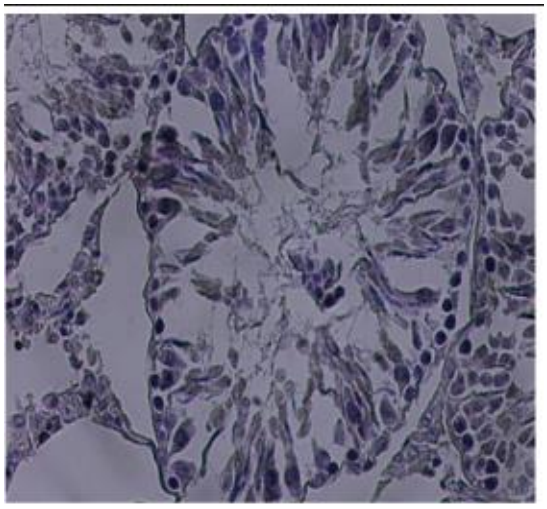


Figure 6: Rats treated with LA had testicular sections

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

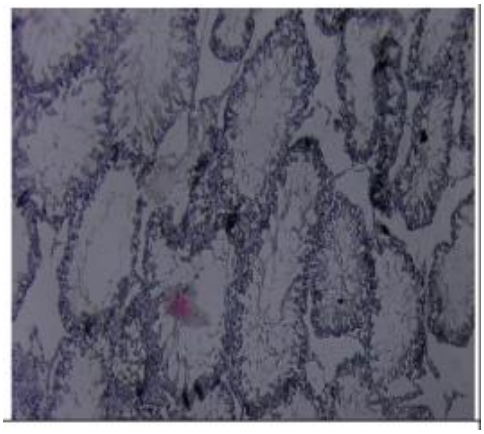


Figure 7: Testicular portions from the team that received LA treatment.

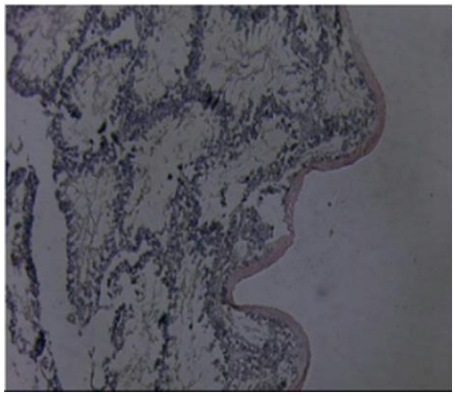
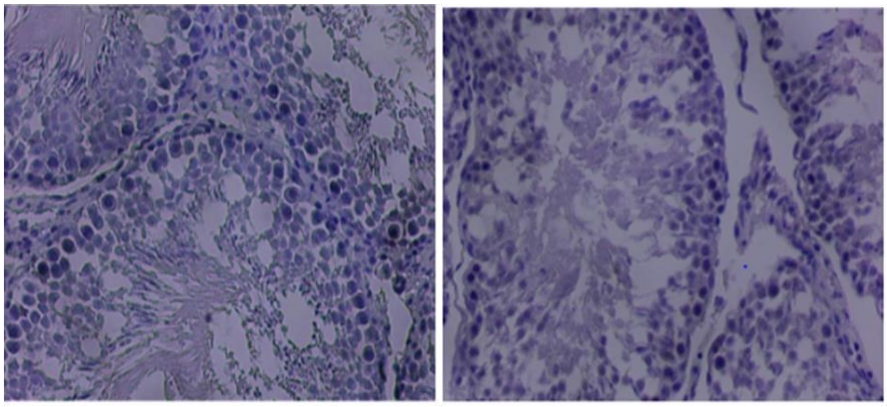


Figure 8: Testicular portions from the team that received LA treatment.

The rats in groups three and four received LA along with LC and Nano NLC. LC's protective role as an antioxidant allowed lead's potentially harmful effects to be reduced. It was also discovered that most seminiferous tubules contained multiple layers of germ cells, but some of these tubules had incomplete spermatogenesis and larger luminal sizes than others. It will be discovered in (Figures 9 and 10).



Figures 9 and 10: Rat testicular sections received LC and NLC for 60 days after receiving LA for 30 days.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 01 April 2023; Revised: 29 May 2023; Accepted: 12 June 2023

Rats were provided with LC and NLC, which promoted the spermatogenesis process, to demonstrate the importance of LC for fertility and how it improves germ cells in finishing the spermatogenic cycle. The spermatogenic cells in this Team, which includes spermatogonia, primary as well as secondary spermatocytes, spermatozoa, and sperm cells were unchanged Leydig cells that were going through mitosis and mitotic division are displayed in (Figures 11 and 12).

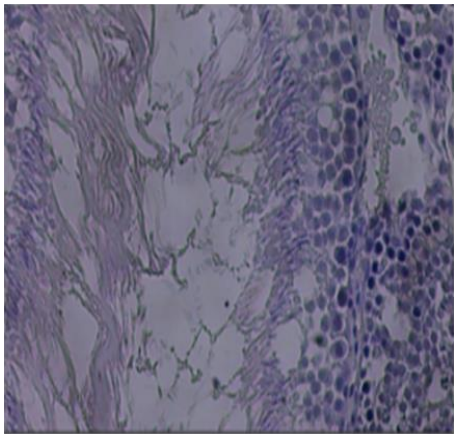


Figure 11: LC was administered to rat testicular parts.

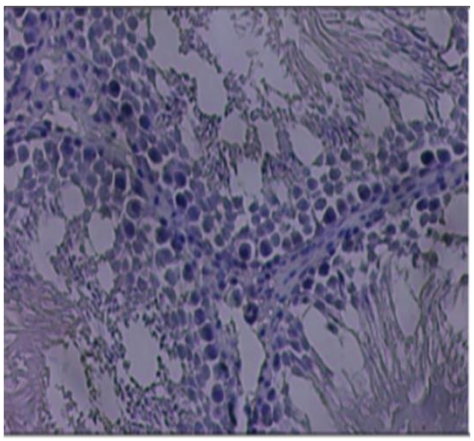


Figure 12: NLC was administered to rat testicular tissue.

L-Carnitine-chitosan NPs Characterization

In the CSNPs that were ready for study, UV spectroscopy showed a peak at 257 nm. This could be a result of the amido Team found in chitosan. The peak of chitosan NPs was discovered to be at 310 nm. The peak chitosan wavelength is 201 nm. Chitosan and LC-NPs both include a range of functional Teams; FT-IR data are shown in (Figures 1 and 2). They are present in phenols, alcohols, alkanes, alkynes, and aldehydes.

Conclusion

The study shows that LC and NLC protect male rats' testicles from lead acetate-induced testicular damage. The rats' reproductive status was significantly improved by the usage of biosynthesized LC-NPs that contained LC in Chitosan-tripolyphosphate-based NPs.

According to the findings, testosterone hormone levels significantly increased in the LA+ NLC Team compared to the LA Team. The following are some possible restrictions: Only male rats were used in the study, thus it's possible that neither humans nor other species can directly apply the findings. The underlying mechanisms by which LC and NLC exert their protective benefits were not examined in the study. Further research into the mechanism is needed to fully comprehend how LC and NLC protect against the testicular toxicity brought on by LA. This can entail researching the relevant metabolic pathways, the interaction between LC-NPs and LA, and the particular cellular targets that LC-NPs influence.

References

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

[2] Wu, L., Zhou, W., Lin, L., Chen, A., Feng, J., Qu, X., Zhang, H. and Yue, J., 2022. Delivery of therapeutic oligonucleotides in nanoscale. Bioactive materials, 7, pp.292-323.

[3] Zaboon, M.H., Saleh, A.A. and 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), pp.178-188.

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

[5] Bassi, E., Facoetti, R., Ferloni, M., Pastorino, A., Bianchi, A., Fedrizzi, G., Bertoletti, I. and Andreotti, A., 2021. Lead contamination in tissues of large avian scavengers in south-central Europe. Science of The Total Environment, 778, p.146130.

[6] Sarawi, W.S., Alhusaini, A.M., Fadda, L.M., Alomar, H.A., Albaker, A.B., Alghibiwi, H.K., Aljrboa, A.S., Alotaibi, A.M., Hasan, I.H. and Mahmoud, A.M., 2022. Nano-curcumin prevents copper reproductive toxicity by attenuating oxidative stress and inflammation and improving Nrf2/HO-1 signaling and pituitary-gonadal axis in male rats. Toxins, 10(7), p.356.

[7] NASEER, Z., RAZA, S., AHMAD, E. and AKSOY, M., 2022. USE OF ANTIOXIDANTS TO IMPROVE THE REPRODUCTIVE PERFORMANCE IN MODEL LABORATORY ANIMALS. The Role of Exogenous Antioxidants in Enhancing Reproductive Function and Performance, p.1.

[8] Mobarak, H., Heidarpour, M., Rahbarghazi, R., Nouri, M. and Mahdipour, M., 2021. Amniotic fluid-derived exosomes improved spermatogenesis in a rat model of azoospermia. Life Sciences, 274, p.119336.

[9] Mansour, D.F., Saleh, D.O., Ahmed-Farid, O.A., Rady, M., Bakeer, R.M. and Hashad, I.M., 2021. Ginkgo biloba extract (EGb 761) mitigates methotrexate-induced testicular insult in rats: Targeting oxidative stress, energy deficit, and spermatogenesis. Biomedicine & Pharmacotherapy, 143, p.112201.

[10] Zhao, X., Wang, Q., Li, X., Xu, H., Ren, C., Yang, Y., Xu, S., Wei, G., Duan, Y., Tan, Z. and Fang, Y., 2023. Norgestrel causes digestive gland injury in the clam Mactra dendriform's: An integrated histological, transcriptomics, and metabolomics study. Science of The Total Environment, 871, p.162110.

[11] Ibrahim, I.A., Shalaby, A.A., Abd Elaziz, R.T. and Bahr, H.I., 2021. Chlorella vulgaris or Spirulina platensis mitigate lead acetate-induced testicular oxidative stress and apoptosis with

regard to androgen receptor expression in rats. Environmental Science and Pollution Research, 28, pp.39126-39138.

[12] Thoreux-Manlay, A., Le Goascogne, C., Segretain, D., Jégou, B. and Pinon-Lataillade, G., 1995. Lead affects steroidogenesis in rat Leydig cells in vivo and in vitro. Toxicology, 103(1), pp.53-62.

[13] Wahab, O.A., Princely, A.C., Oluwadamilare, A.A., Oore-oluwapo, D.O., Blessing, A.O. and Alfred, E.F., 2019. Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. JBRA assisted reproduction, 23(4), p.336.

[14] Sainath, S.B., Meena, R., Supriya, C.H., Reddy, K.P. and Reddy, P.S., 2011. Protective role of Centella asiatica on lead-induced oxidative stress and suppressed reproductive health in male rats. environmental toxicology and pharmacology, 32(2), pp.146-154.

[15] Salama, S.A., Arab, H.H., Omar, H.A., Gad, H.S., Abd-Allah, G.M. and Maghrabi, I.A., 2018. L-carnitine mitigates UVA-induced skin tissue injury in rats through the downregulation of oxidative stress, p38/c-Fos signaling, and proinflammatory cytokines. Chemico-Biological Interactions, 285, pp.40-47.