European Review for Medical and Pharmacological Sciences 2024; 28: 1680-1694

Ameliorative effects of L-carnitine as an antioxidant against testicular toxicity induced by $AlCl_3$ in male albino rats

F. ALHARBI

Department of Biology, College of Science, Qassim University, Buraydah, Saudi Arabia

Abstract. – OBJECTIVE: The goal of this study was to investigate the potential protective effect of L-carnitine (20 mg/kg bw, 1/20 LD 50) against aluminum chloride ($AlCl_3$) on the quality of the male rats' testicles and sperm, as well as to determine whether or not the effects of $AlCl_3$ could be counteracted by using L-carnitine as an antioxidant.

MATERIALS AND METHODS: Six groups of 36 adult male albino rats (n=6) were randomly formed. In Group I (Gp I), saline injection was given orally as a control. Group II (Gp II) was injected orally with 75 mg/kg body weight of L-carnitine. Group III (Gp III) was given a high dose of L-carnitine (150 mg/kg body weight) orally, while Group IV (G IV) was given a low dose of $AlCl_3$ (20 mg/kg body weight). Group V (Gp V) was given an oral injection of $AlCl_3$ (20 mg/kg) and L-carnitine (75 mg/kg body weight). Group VI (Gp VI) was given $AlCl_3$ at a dose of 20 mg/kg and L-carnitine at a dose of 150 mg/kg body weight for 60 days. The reproductive capacity of each group was assessed. Thus, in addition to histopathological analysis and the comet assay to evaluate sperm DNA deterioration, final body weight, testicular weight change, and sperm analysis were carried out.

RESULTS: The findings revealed that $AlCl_3$ caused a significant decrease in final body weight, relative weight of sex organs, sperm concentration, motility and viability, serum testosterone concentration, and a significant increase in sperm abnormalities. Furthermore, $AlCl_3$ caused visible changes in the histological structure of the testis.

CONCLUSIONS: L-carnitine treatment alleviated the harmful effects of $AlCl_3$, as evidenced histopathologically by a noticeable improvement in testis tissues. When it comes to treating $AlCl_3$-induced reproductive toxicity in male rat testes, L-carnitine shows promise.

Key Words:
L-carnitine, $AlCl_3$, Male rats, Testis dysfunction.

Introduction

Over the last few decades, there has been a global decline in the quality of human sperm. Many factors can affect male fertility; one theory for the downward trend in fertility is that sperm quality is declining due to lifestyle choices and occupational and/or environmental factors[1]. It has been shown[1] that several factors can impede the regular progression of spermatogenesis, leading to a significant reduction in the quantity and/or quality of spermatozoa, which can result in infertility. Oxidative stress, which dramatically impairs sperm function and germ cell development, is one of the most harmful elements and a primary cause of male infertility. In humans, drinking water, consuming fruit juices, or consuming citric acid can all lead to aluminum absorption and accumulation. These approaches dramatically raise urinary excretion and gastrointestinal absorption of aluminum in healthy individuals[2]. Due to its widespread distribution in nature, aluminum (Al) poses a risk of toxic harm to human tissues and organs[3].

Food additives, toothpaste, water-purifying agents, phosphatic binders, antidiarrhea, and antacids are a few examples of pharmaceutical aluminum compounds in use[4,5]. It has been demonstrated[6,7] that the bloodstream, seminal plasma, testes, liver, kidneys, lungs, and brain all produce reactive oxygen species (ROS) and inhibit antioxidant enzymes when exposed to aluminum chloride ($AlCl_3$). Moreover, ROS participate in lipid peroxidation (LPO), which modifies the function of mitochondria and increases their permeability[8]. $AlCl_3$ consumption disrupts the oxidative/antioxidative equilibrium, induces LPO, and decreases the antioxidant enzyme activities, leading

Corresponding Author: F. Alharbi, Ph.D; e-mail: hrbief@qu.edu.sa

to oxidative damage, cell death, and toxicity[9,10]. Oxidative stress in sperm is caused by an excess of reactive oxygen species (ROS) and is the cause of infertility[11]. Additionally, $AlCl_3$ was found[12] to have an impact on the mice's ventral prostate, seminal vesicles, deferential canal, and epididymis. Moreover, testicular development and testosterone synthesis were observed[13] to be altered in laboratory animals exposed to $AlCl_3$. The amino acid L-carnitine (LC), also referred to as β-hydroxy-γ-trimethylamino-butyric acid, is produced when methionine and lysine are combined. LC helps to stabilize cell membranes by promoting the oxidation of long-chain fatty acids and participating in the metabolism of branched-chain amino acids[14]. Humans get it primarily from exogenous sources found in animal diets, with smaller amounts coming from the brain, kidney, and liver for methionine or lysine[15]. Through its ability to facilitate the transport of long-chain fatty acids into mitochondria, it makes a significant contribution to the metabolism of cellular energy[16]. Research[17,18] has demonstrated that it functions as an antioxidant by scavenging reactive oxygen species (ROS). It might also stabilize damaged cell membranes and stop mitochondrial oxidative stress caused by apoptosis and mitochondrial damage in various cell types. High amounts of L-carnitine, which is necessary for metabolism, sperm development, and maturation, are found in the epididymis[18]. It might be protective against cardiotoxicity because of its antiapoptotic and antioxidant qualities[19]. According to its profile of activities, this molecule might be able to lessen the harmful side effects of some pesticides[20]. Reactive oxygen species (ROS) are produced when endogenous processes or exogenous agents react with cells; the endogenous cellular antioxidant defense system prevents this from happening. When the cellular endogenous antioxidant defense system's capacity is surpassed by the production of ROS, oxidative stress occurs[21]. ROS can harm macromolecules found in biochemistry, such as proteins, lipids, and nucleic acids. These ROS actions will disrupt the normal motility, viability, and count of the sperm as well as their ability to fertilize the oocyte, thereby damaging the DNA and sperm membrane's structure and function[22,23].

Spermatogenesis and steroidogenesis are highly susceptible to damage even in the presence of low oxygen tension and poor intratesticular vascularization, owing to the elevated levels of reactive oxygen species (ROS) produced in the testis[24]. Finally, it should be noted that the testis is extremely susceptible to exogenous agents, including a variety of pharmaceutical treatments, which may harm spermatogenesis, sperm parameters, and reproduction[25]. The purpose of this study is to examine the possible protective effect of L-carnitine (20 mg/kg bw, 1/20 LD 50) against aluminum chloride ($AlCl_3$) on the quality of the testicles and sperm of male rats. Additionally, the study aims to determine whether or not L-carnitine can be used as an antioxidant to mitigate the effects of $AlCl_3$.

Materials and Methods

Experimental Animals

A total of 36 male albino rats in good health, weighing between 150 and 155 g, were collected. All the animals were given two weeks to acclimatize. The animals were housed in plastic cages with a 12-hour light/dark cycle and a temperature of 25°C. Every day, the health of the animals was examined, and they were provided with conventional food and water without limits. Every attempt has been made to minimize animal suffering and to use as few animals as possible to generate trustworthy scientific data. This study was approved by the Committee of Health Research Ethics, Deanship of Scientific Research, Qassim University, with Ethical Committee approval IP (IRP No. 23-41-14).

The source of aluminum chloride ($AlCl_3$)[26] was provided by Sigma Chemical Co. (St. Louis, MO, USA). The Arab company for pharmaceutical and medicinal plants (MEPACO), located in Cairo, Egypt, provided the L-carnitine. Each capsule contains 350 mg of L-carnitine. The L-carnitine powder was dissolved in distilled water and then given orally *via* a stomach tube every day at doses of 75 and 150 mg/kg of body weight per day. L-carnitine (75 and 150 mg/kg) was given orally to rats on a daily basis, as reported by Kim et al[27].

Experimental Protocol

The following six groups of thirty-six rats were created: Group I consisted of rats receiving saline; Group II consisted of rats receiving 75 mg/kg of L-carnitine; and Group III consisted of rats receiving 150 mg/kg of L-carnitine. Group IV received an oral injection of $AlCl_3$ (20 mg/kg), while Group V received an oral injection of 75 mg/kg L-carnitine and 20 mg/kg $AlCl_3$. Additionally, rats in Group VI were given 150 mg/kg of L-car-

nitine and 20 mg/kg of $AlCl_3$. For two months[28,29], $AlCl_3$ and L-carnitine were given to all groups once a day.

Sample Collection and Testosterone Analysis

Blood samples were taken, rats were decapitated, and they were given anesthesia. The samples were stored at 4°C for the entire night to allow them to clot. The serum was extracted from the samples the following day and stored at -20°C after they had been centrifuged for 15 minutes at 9,000 g. The testosterone-coated-tube radioimmunoassay kits used to measure serum testosterone had a lower limit of detection of 0.04 ng/ml.

Testosterone assay determination

The Ismail method[30] was utilized to estimate the serum testosterone level.

Measurement of Body and Testicular Weight and Gonadosomatic Index

Each rat's weight was determined at the beginning of the study, and throughout the experiment, the rat's body weight was noted once a week. Once the experiment's sixty days were up, the weights of the right and left testicles were recorded. From there, the gonadosomatic index (GSI), which is determined by dividing the testes' weight by the animal's body weight, was determined.

Sperm Analysis

The epididymis was then taken out and put in a preheated petri dish with 0.2 ml of calcium and magnesium-free Hank's solution for 15 minutes at 37°C. The sperm motility, viability, count, and anomalies were assessed in the epididymis.

Sperm Motility

Sperm motility was measured by covering a microscope slide with a cover slip and adding two drops of sperm solution, as per Ekaluo et al[31]. In order to calculate the percentage, the total number of spermatozoa counted under lenses (40 X) was divided by the number of progressively motile cells.

Sperm Viability

The viability of the sperm was assessed using Eosin-Nigrosin staining[32]. A portion of the sperm suspension was combined with an equal volume of Eosin-Nigrosin to create each sample, which was then air-dried on a glass slide before being stained. The slides' viability was expressed as a percentage. Normal, healthy sperm cells appeared whitish, while dead sperm cells took up satin and looked reddish. The percentage viability was calculated as the number of viable sperm cells out of the total number of cells found.

Sperm Count

The epididymal sperm count was obtained using the upgraded Neubauer hemocytometer, and the heads were manually counted under a light microscope. The data was represented by the total number of sperm/ml[33].

Sperm count = (No. of spermatozoa × Dilution factor × Depth factor) ÷ No. of areas counted.

Sperm Abnormality

Inside the glass sliding privies, the proportion of sperm abnormalities (head, mid-piece, and tail) in every 200 spermatozoa seen on a slide for a given sample was calculated. The proportion of abnormal sperm heads was calculated according to Ekaluo et al[34].

Histological Examination

The entire left testis and samples of the right testis were preserved in 10% neutral buffer formalin and then processed using a rotary microtome to create paraffin sections that were 4-6 μm thick. These sections were then stained with hematoxylin and eosin and examined under a light microscope[35].

Comet Assay

With a few minor adjustments, the comet assay was used to assess DNA damage as defined by Singh et al[36]. The comet assay protocol was applied to all samples, albeit with a few small adjustments from the method described by Enciso et al[37]. A fraction of the total semen was defrosted and washed three times in saline buffered with phosphate. Then, 25 μl of this mixture was mixed with 50 μl of 1% low-melting-point agarose (Sigma Aldrich, St. Louis, MO, USA) in distilled water. Sperm cells were diluted to contain 10 x 10^6 spermatozoa/ml. To encourage gel adhesion, two separate slides were pre-treated with 1% low-melting-point agarose and then quickly filled with 15 μl of the mixture. After that, coverslips were placed over the slides, and they were placed on a cold plate to gel for five minutes at 4°C. The slides were carefully stripped of their coverslips and then submerged in comet lysis solutions (Halotech, Madrid, Spain) for half an hour. Subsequently, they were thoroughly cleaned in Tris bo-

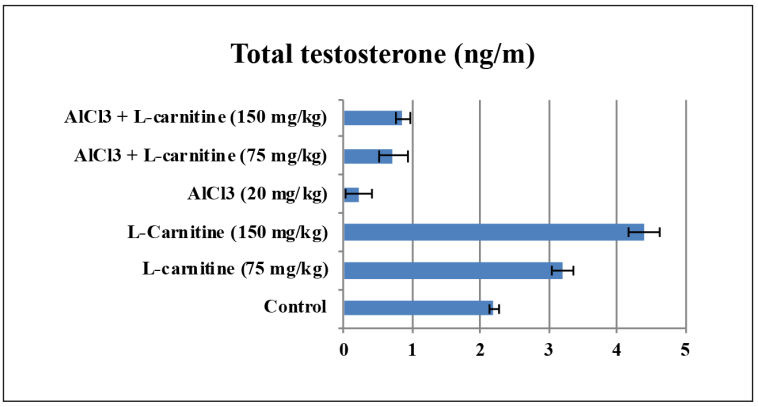

Figure 1. Photographs of total free testosterone hormone (ng/m) in different treated groups. All values were expressed as mean ±SE (n=6).

rate EDTA (TBE), which contains 0.01 M EDTA, 0.445 M Boric acid, and 0.445 M Tris-HCL. After 12 minutes and 30 seconds of electrophoresis in the TBE buffer at 20 V (1 V/cm), a 2-minute wash in 0.9% NaCl was performed. The slides were incubated for 5 minutes in the neutralizing solution (0.4 M Tris-HCL, pH 7.5), for 2 minutes in TBE, and for 2 minutes in a series of ethanol concentrations of 70, 90, and 100% to dehydrate them. For every comet assay sample, at least 400 spermatozoa were counted. The samples were then stained with DAPI SlowFade® Gold antifade (Invitrogen, Eugene, OR, USA) and examined under an Olympus AX70 fluorescence microscope. There were two types of sperm cells: fragmented and non-fragmented. Different levels of DNA damage were depicted in sperm cells. An automated image analysis technique collected images, computed integrated intensity profiles for every cell, estimated the comet cell's constituent parts, and evaluated the range of generated parameters. Subsequently, the software calculated the tail moment (TM). The amount of DNA damage was determined by measuring the distance between the comet's genetic material in the nucleus (the comet head) and its tail. The amount of DNA damage was determined by measuring the tail length (TL), tail DNA%, and tail moment (TM). The length of DNA migration, or TL, is directly correlated with the size of the DNA fragment and is measured in micrometers. It was calculated from the center of the cell out.

Statistical Analysis

All data from this study were collected and statistically analyzed using the IBM SPSS Statistics software version 27.0 (IBM Corp., Armonk, NY, USA) for Windows. The Shapiro-Wilk test was used for the test of data normality. For normally distributed data, the one-way ANOVA test was used to compare more than 2 independent groups, and the Duncan test was used to detect pair-wise comparison. For non-normally distributed data, the paired sample *t*-test was used to compare 2 paired groups and the Kruskal-Wallis test to compare more than 2 groups. Mann-Whitney test was used to detect pair-wise comparison. The significance of the results was done using a 0.05 level of significance. Results are presented as mean ± SEM or mean rank. After conducting an ANOVA test, significant letters ranging from "a" to "f" represented the significant mean values obtained from the Post Hoc Tests (Duncan).

Results

Total Testosterone Analysis

The results in Figure 1 show a highly significant decrease in total testosterone in the $AlCl_3$ (20 mg/kg) group for 60 days when compared to the control and L-carnitine (75 and 150 mg/kg/day) groups, which showed a significant increase. However, when compared to the $AlCl_3$ group, the animals' total testosterone showed a significant improvement following an injection of $AlCl_3$ and treatment with L-carnitine (75 and 150 mg/kg/day).

Body and Testicular Weight and Gonado-Somatic Index (GSI)

As indicated in Table I, the body weights were measured on the first day of the experiment and 60 days later. When compared to the control and L-carnitine (75 and 150 mg/kg/day) groups, which demonstrated significant increases in their body weight gain percent by 27.91%, 27.51, and

Table III. Maternal-neonatal outcomes in various research groups.

	HEV group (n=116)	HBV group (n=198)	HEV-HBV group (n=86)	Healthy group (n=117)	*p*
Delivery mode, n (%)					
Eutocia	29 (25.0)	91 (46.0)	16 (18.6)	64 (54.7)	< 0.001
Cesarean	76 (65.5)	107 (54.0)	68 (79.1)	53 (45.3)	< 0.001
Infant body weight (g)	3,200 (2,880-3,475)	3,265 (3,000-3,545)	3,202 (2,900-3,552)	3,230 (2,990-3,530)	0.068
Breech delivery	2 (1.7)	6 (3.0)	9 (10.5)	2 (1.7)	< 0.001
Fetal macrosomia	5 (4.3)	6 (3.0)	6 (7.0)	4 (3.4)	0.466
Obstetric complications, n (%)					
Oligohydramnios	25 (21.6)	22 (11.1)	19 (22.1)	10 (8.6)	< 0.001
Postpartum hemorrhage	11 (9.5)	10 (5.1)	7 (8.1)	1 (0.9)	< 0.05
Premature rupture of membranes	23 (19.8)	20 (10.1)	18 (20.9)	12 (12.3)	< 0.05
Meconium contamination	17 (14.7)	35 (17.7)	19 (22.1)	5 (4.3)	< 0.01
Adverse perinatal outcomes, n (%)					
Spontaneous abortions	10 (8.6)	0 (0)	1 (1.2)	0 (0)	< 0.001
Low birth weight infant	14 (12.1)	8 (4.0)	14 (16.3)	2 (1.7)	< 0.001
Premature delivery	24(20.7)	13 (6.6)	9 (10.5)	3 (2.6)	< 0.001
Fetal distress	22 (19.0)	20 (10.1)	17 (19.8)	3 (2.6)	< 0.001
Neonatal asphyxia	13 (11.2)	10 (5.1)	10 (11.6)	0 (0)	< 0.01
Admitted to NICU	10 (8.6)	2 (1.0)	3 (3.5)	0 (0)	< 0.001
Stillbirth	1 (0.9)	0 (0)	2 (2.3)	0 (0)	0.098
Maternal death	0	0	0	0	/

HEV, hepatitis E virus; HBV, hepatitis B virus; NICU, neonatal intensive care unit.

29.55, male rats injected with $AlCl_3$ (20 mg/kg) for 60 days showed a slightly non-significant increase in their body weight gain% by 18.46%. However, there was a noticeable improvement in the percentage of body weight gain following $AlCl_3$ injection. Animals treated with 75 and 150 mg/kg/day of L-carnitine showed a significant increase in the percentage of body weight gain by

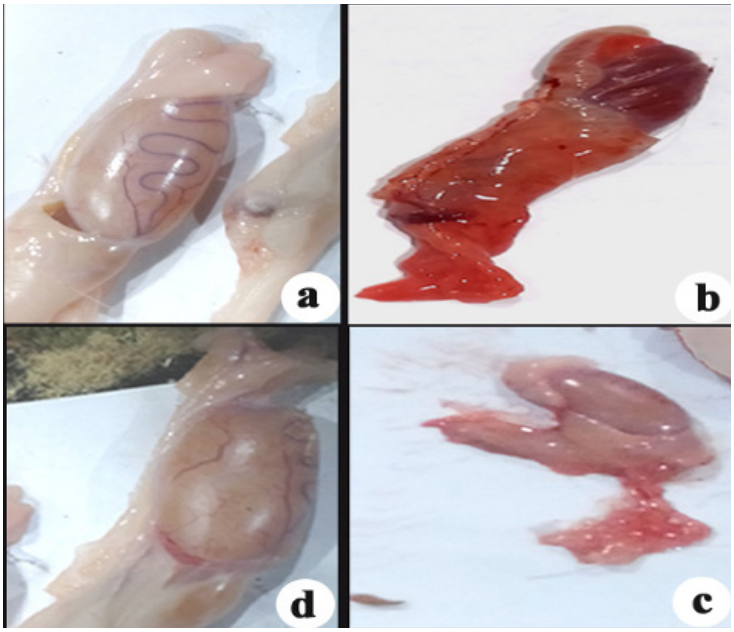

Figure 2. Whole testis in rats. **a**, Control and L-carnitine (75 and 150 mg/kg) groups, (**b**) $AlCl_3$, (**c**) $AlCl_3$ and treated with L-carnitine (75 mg/kg), (**d**) $AlCl_3$ (20 mg/kg) then treated with L-carnitine (150 mg/kg) for 60 days.

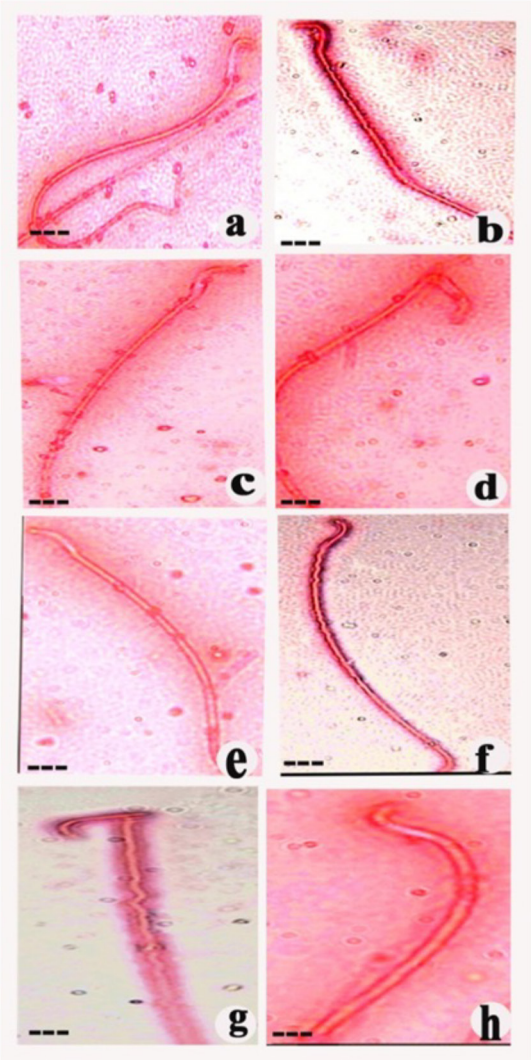

Figure 3. Photographs of sperm abnormalities. **a**, Unstained live sperm, (**b**) stained dead sperm, (**c**) hookless sperm, (**d**) hammer head, (**e**) short and with banana shape head, (**f**) amorphous head, (**g**) hammer and abnormal mid-piece, (**h**) hookless and bent mid piece, 20 μm.

20.82% and 24.61% when compared to the $AlCl_3$ injected group.

Rat tests revealed a significant drop in weight in the $AlCl_3$-injected group (5.066) when compared to the L-carnitine and control groups (6.56, 6.44, and 6.56), respectively. Rats treated with 75 and 150 mg/kg/day of L-carnitine after receiving $AlCl_3$ injections, on the other hand, showed increases in testis weight of 5.43 and 5.70, respectively, which were similar to those of the control group (Table II and Figure 2). Rats administered L-carnitine following $AlCl_3$ injection exhibited a noteworthy reduction in the percentage of gonad somatic index (GSI) (0.028) in contrast to the control group (0.033) and the L-carnitine groups (0.033 and 0.033), respectively. The $AlCl_3$ group also demonstrated an ameliorative effect by increasing GSI (0.029) and (0.030), respectively (Table II).

Sperm Analysis

Sperm count

In comparison to groups treated with L-carnitine (75 and 150 mg/kg) and the control (61, 67.5, and 68.1 x 10^6/ml), it was found that rats given an injection of $AlCl_3$ (20 mg/kg) had significantly lower epididymal sperm counts (49.6 x 10^6/ml). When compared to the $AlCl_3$-treated group, rats given L-carnitine at 75 and 150 mg/kg had signi-

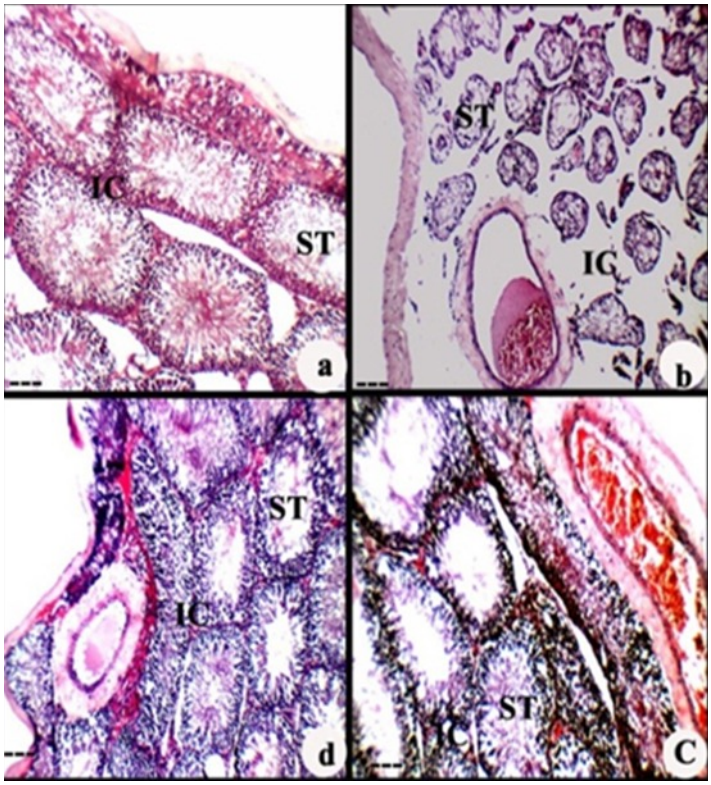

Figure 4. Transverse sections of the testis. **a**, Control, (**b**) $AlCl_3$ injected group, (**c**) L-carnitine (75 mg/kg) treated group after $AlCl_3$ injection, (**d**) L-carnitine (150 mg/kg) treated group after $AlCl_3$ injection. Seminiferous tubule (ST), Interstitial connective tissue (IC). H&E 20 µm.

ficantly higher sperm counts, reaching 58.4 and 61.1 x 10^6/ml, respectively (Table III).

Sperm motility

The outcomes showed that rats given 75 and 150 mg/kg of L-carnitine following an $AlCl_3$ injection had higher percentages of motile sperm (68.1 and 68, respectively). Rats injected with $AlCl_3$ only had a significantly lower percentage of motile sperms (40.6%) than the control group (70) and the L-carnitine group (71.2 and 71) (Table III).

Sperm viability

Sperm smear analysis revealed that while dead sperm were obtained and appeared pinkish, normal live sperm appeared whitish and unstained (Figures 3a and b). The sperm viability of the mice was toxic to the injection of $AlCl_3$, as Table III demonstrates. This was in contrast to the control group's 71.6% and the L-carnitine-treated group's 70.8% sperm viability. However, after receiving an $AlCl_3$ injection, treatment with L-carnitine (75 and 150 mg/kg) together improved sperm viability to 69.1 and 68.7%.

Sperm abnormalities

The sperm tail, mid-piece, and head differences between the abnormal sperm in the $AlCl_3$-injected group and the normal sperm are shown in Figure 3 and Table IV. The percentages and table of total sperm abnormalities revealed a highly significant increase ($p<0.01$) in the $AlCl_3$ (20 mg/kg) group (25.64%) compared to the L-carnitine (75 and 150 mg/kg) (6.04% and 5.2) and control (8.3%) groups. Nevertheless, rats administered 75 and 150 mg/kg of L-carnitine following 20 mg/kg/day of $AlCl_3$ demonstrated a noteworthy rise in the quantity of normal sperm and the incidence of sperm abnormality (20.23% and 18.6%).

Histological Examination

Transverse sections through the testicles of male rats given varying doses of L-carnitine (75 and 150 mg/kg) for 60 days revealed a histological

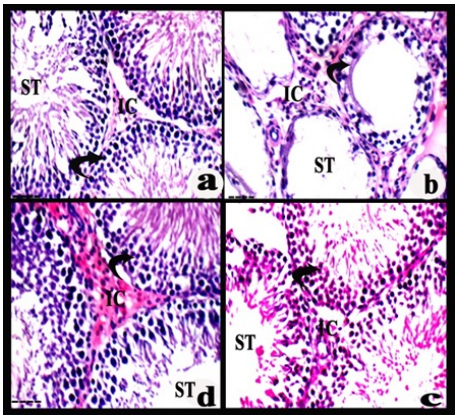

Figure 5. Photomicrographs of transverse sections in the testis. **a**, Control, (**b**) $AlCl_3$ injected group, (**c**) L-carnitine (75 mg/kg) treated group after $AlCl_3$ injection, (**d**) L-carnitine (150 mg/kg) treated group after $AlCl_3$ injection. Seminiferous tubule; (ST), Interstitial connective tissue (IC). H&E 10 µm.

architecture resembling that of untreated control mice (Figures 4a and 5a). The testis of the rats given L-carnitine treatment and the rats given no treatment are comprised of many seminiferous tubules, round, oval, or elongated, and have multiple layers of spermatocytes, secondary spermatocytes, spermatids, and spermatozoa, as well as an outer membrane (tunica albuginea) composed of connective tissue, including blood vessels. The interstitial cells (Leydig cells) in the intertubular connective tissue are representative of the endocrine portion of the testis. These cells have a dark stain and are tiny and polygonal in shape (Figure 4). The outermost layer of seminiferous tubule cells is called spermatogonia, and they are tiny, spherical cells with spherical nuclei. There are pyramid-shaped Sertoli cells located in between these cells (Figures 4 and 5).

When the testes of the $AlCl_3$-injected group were examined under a microscope, many degenerated and deformed seminiferous tubules filled with primary spermatogonia alone were visible. These tubules were devoid of sperm formation because the spermatogenic cells had shed their pyknotic nuclei. In the center of the seminiferous tubules, there was an accumulation of the shed cells. Additionally, in certain locations, the germinal epithelium was displaced and positioned erratically on the irregular basement membrane. Furthermore, compared to the normal control rats, the interstitial tissue displayed an edematous stroma containing small groups of Leydig cells. In addition, there was widespread bleeding and dilated, clogged blood vessels in the interstitial tissue. Each of these dose-dependent lesions is shown in a figure based on how frequently they occur (Figures 4b and 5b).

The germinal epithelium of the seminiferous tubules and interstitial tissue, as well as most of the previously mentioned lesions, had improved when the testis of rats given L-carnitine for 60 days following an $AlCl_3$ injection was examined under a microscope. With almost normal histological architecture and spermatogenic cells

Table I. Mean ± standard deviation (SD) values of initial and final weight, and the percent of body weight changes of male rats..

Treatments	Initial body weight (g)	Final body weight (g)	Body weight change (g)	Body weight change %
Control	151.2±7.09	193.4±8.08	42.2±2.52	27.91±1.04
L-carnitine (75 mg/kg)	151.2±5.00	192.8±5.13	41.6±1.15	27.51±0.55
L-carnitine (150 mg/kg)	151.6±1.53	196.4±3.06	44.8±4.36	29.55±1.79
$AlCl_3$ (20 mg/kg)	151.6±14.89	179.83±16.32	28±1.71	18.46±0.41
$AlCl_3$+L-carnitine (75 mg/kg)	151±11.67	182.44±19.60	31.44±12.78	20.82±4.78
$AlCl_3$+L-carnitine (150 mg/kg)	151.2±6.22	188.4±6.14	37.2±1.87	24.60±0.87
Sig.	0.999	0.748	0.033	0.046

Mean values were significantly different at $p \leq 0.05$.

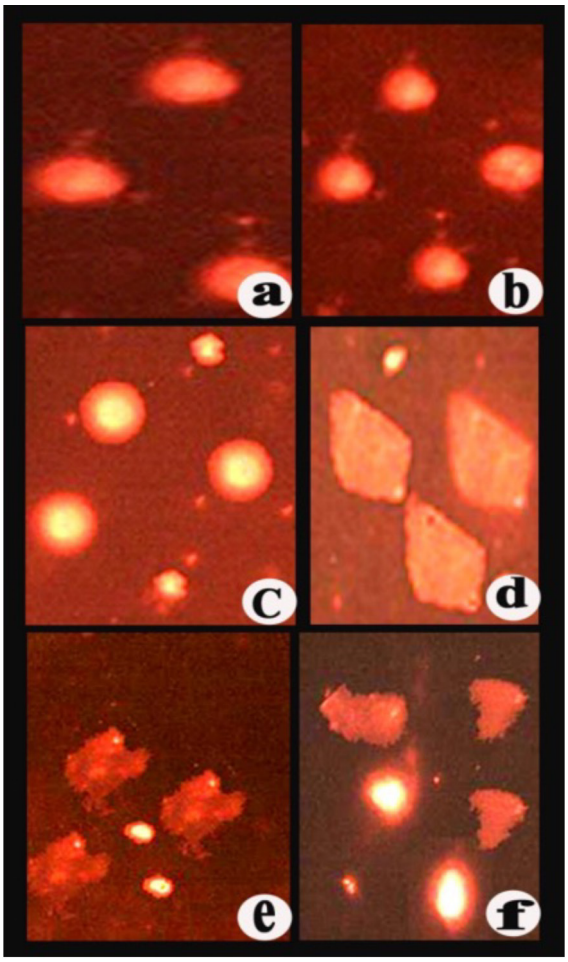

Figure 6. Photographs of comet assay of testis showing DNA damage induced in male rats' testes in different treated groups. **a**, Control, (**b**) L-carnitine 75 mg/kg/day, (**c**) L-carnitine (150 mg/kg/day), (**d**) $AlCl_3$ injected group (20 mg/kg/day), (**e**) $AlCl_3$ and L-carnitine (75 mg/kg/day) treated group, and (**f**) $AlCl_3$ and L-carnitine (150 mg/kg/day).

Table II. Mean ± SD values of left, right, total testis weights, final body weights and Gonadosomatic index % of male rats.

Treatments	Left testes weight (g)	Right testes weight (g)	Total testes weight (g)	Final body weight (g)	Gonadosomatic index %
Control	3.3±0.16	3.33±0.16	6.56±0.16	193.4±8.08	0.034±0.001
L-carnitine (75 mg/kg)	3.2±0.06	3.23±0.16	6.4±0.15	192.8±5.13	0.033±0.02
L-carnitine (150 mg/kg)	3.3±0.10	3.33±0.06	6.56±0.09	196.4±3.06	0.033±0.03
$AlCl_3$ (20 mg/kg)	2.5±0.66	2.56±0.53	5.07±0.59	179.83±16.32	0.028±0.02
$AlCl_3$+L-carnitine (75 mg/kg)	2.63±0.25	2.80±0.08	5.43±0.15	182.44±19.6	0.029±0.08
$AlCl_3$+L-carnitine (150 mg/kg)	2.67±0.26	2.83±0.3	5.71±.18	188.4±6.14	0.031±0.07
Sig.	0.030	0.027	0.010	0.748	0.434

Mean values were significantly different at $p \leq 0.05$.

(spermatogonia, primary and secondary spermatocytes) arranged normally between Sertoli cells, the majority of seminiferous tubules appeared. They also had mature spermatozoa filled in their lumina. Although some cells remain detached and atrophied, L-carnitine's repairing effects against $AlCl_3$ are noteworthy (Figures 4c-d and 5c-d).

Table III. Mean ± SD values of sperm count (X 10^6/mm^3) and motility of male rats of traits as affected by $AlCl_3$ individually or co-administered with L-carnitine.

Treatments	Count×10^6	Progressive Motility %	Viability %
Control	66.1±1.53[a]	70.0±2.08[b]	71.6±6.66[a]
L-carnitine (75 mg/kg)	67.5±2.08[a]	71.2±1.53[a]	71.7±2.52[a]
L-carnitine (150 mg/kg)	68.1±1.0[a]	71.0±1.53[a]	70.8±1.53[b]
$AlCl_3$ (20 mg/kg)	49.6±8.02[c]	40.6±4.349[d]	41.6±3.87[d]
$AlCl_3$+ L-carnitine (75 mg/kg)	58.4±4.40[c]	68.1±4.113[c]	69.1±4.65[b]
$AlCl_3$+ L-carnitine (150 mg/kg)	61.1±6.76[b]	68.0±3.834[b]	68.7±4.32[c]
Sig.	0.0001	0.0001	0.0001

Mean values with superscript letters in the same column were significantly different at $p \leq 0.05$. The letters ([a]: the highest to [d]: the lowest) represent the significant mean values obtained from the Post Hoc Tests (Duncan).

Table IV. Mean ± SD values of sperm abnormality types in male rats.

Treatments	Head abnormality %				Mid-piece abnormality %	Tail abnormality %	Total abnormality sperm %
	Hookless	Haammer	Banana	Amorphous			
Control	1.1±0.577	1.1±0.58	1.0±1.0	1.0±0.577	2.10±0.100[d]	2.1±0.58	8.4±2.08
L-carnitine (75 mg/kg)	2.5±0.577	0.0±0.0	1.0±0.58	0.0±0.0	1.20±0.10[e]	2.1±0.58	6.8±0.58
L-carnitine (150 mg/kg)	1.1±0.000	0.0±0.0	0.0±0.0	1.0±0.58	1.00±0.10[f]	2.8±0.58	5.9±0.58
$AlCl_3$ (20 mg/kg)	4.6±2.160	3.1±0.58	4.0±0.82	3.2±1.50	5.60±0.08[a]	4.6±0.58	25.14±2.63
$AlCl_3$+ L-carnitine (75 mg/kg)	3.4±1.155	3.5±0.58	2.0±0.58	3.1±0.82	4.10±0.08[c]	4.1±0.50	20.23±2.22
$AlCl_3$+ L-carnitine (150 mg/kg)	3.0±1.095	3.0±0.55	2.0±0.71	3.1±0.84	4.26±0.11[b]	2.7±0.45	18.1±2.39
Sig.	0.002	0.002	0.005	0.005	0.0001	0.145	0.002

Mean values with superscript letters in the same column were significantly different at $p \leq 0.05$. The letters ([a]: the highest to [f]: the lowest) represent the significant mean values obtained from the Post Hoc Tests (Duncan).

Table V. Mean ± SD values of comet assay in the sperm of male albino rats.

Treatments	Comet %	Tail length	Tail moment
Control	13.20±0.20[d]	7.60±0.21[b,c]	1.77±0.07[b]
L-carnitine (75 mg/kg)	13.10±0.21[d]	7.20±1.36[b,c]	1.07±0.27[b]
L-Carnitine (150 mg/kg)	11.60±0.30[e]	7.0±2.82[c]	0.97±0.01[b]
$AlCl_3$ (20 mg/kg)	22.0±0.64[a]	9.30±0.10[a]	2.92±0.12[a]
$AlCl_3$+ L-carnitine (75 mg/kg)	19.50±0.55[b]	8.50±0.61[a,b]	1.90±0.14[b]
$AlCl_3$+ L-carnitine (150 mg/kg)	17.40±0.26[c]	7.90±0.68[b,c]	1.88±0.19[b]
Sig.	0.001	0.010	0.021

Mean values with small superscripts in the same column were significantly different at $p \leq 0.05$. The letters ([a]: the highest to [e]: the lowest) represent the significant mean values obtained from the Post Hoc Tests (Duncan).

Comet Assay

The length of DNA migration and the percentage of migrated DNA in the testicles of rats in each group were used to quantify and qualitatively assess the degree of DNA damage in the cells. Three comet parameters were the tail moment, the olive tail moment, and the percentage of tail DNA used to quantify each comet. $AlCl_3$ caused a statistically significant ($p<0.05$) and highly significant ($p<0.01$) increase in the average comet percent in the control and L-carnitine groups, from 13.2 and 13.1 and 11.6, respectively, to 22.1, according to the comet assay.

In contrast, groups that received an $AlCl_3$ injection (20 mg/kg) and then L-carnitine treatment (75 and 150 mg/kg) demonstrated improvements in comet percent (19.5 and 17.4). While the co-administered groups L-carnitine (75 and 150 mg/kg) after $AlCl_3$ injection showed significant change in comparison with the control (8.5 and 7.9), the tail length of the $AlCl_3$ injected group increased significantly (9.3) compared to the control

and L-carnitine groups (7.6, 7.2, and 7.0). In contrast to the control group (1.77) and the L-carnitine groups (1.07 and 0.97), the percentage of DNA in the comet tail increased significantly in the $AlCl_3$ injected group (2.92); however, the L-carnitine (75 and 150 mg/kg) treated groups after $AlCl_3$ injection demonstrated a significant change (1.9 and 1.88) in comparison with the $AlCl_3$ group (Table V and Figure 6).

Discussion

Excessive consumption of aluminum causes accumulation in target organs and has been linked to harming the tissues of the testicles in both humans and animals[38]. Lately, with L-carnitine and its derivatives have been proposed as a candidate for treating male infertility, and several published human and animal studies[39] suggest that carnitine may have some utility. L-carnitine is widely found in nature, and people are becoming aware of its possible health advantages. It was discovered that only L-carnitine was bioactive, that it could speed up lipid metabolism, and that it shuttles activated long-chain fatty acids into the mitochondria. It is a tiny, water-soluble molecule crucial to the metabolism of fat in mammals[40]. L-carnitine and its derivatives exhibit a wide range of biological activities, including an anti-peroxidative effect on several tissues and antioxidant and anti-inflammatory effects on various pathophysiological conditions[41,42]. L-carnitine has been demonstrated to be effective in treating varicocele, a major cause of male infertility, and in preventing oxidative damage to sperm, thereby improving sperm quality[43,44]. In this study, rats administered varying dosages of L-carnitine alone exhibited a noteworthy rise in their body weight gain when juxtaposed with the control cohort. These findings are consistent with research conducted by Coşkun et al[45], which showed that male albino rats given a cisplatin injection and then given acetyl L-carnitine treatment experienced a decrease in body weight.

When compared to the control group in the current study, the $AlCl_3$-injected group did not exhibit a significant increase in weight gain. These findings are consistent with the findings of Entissar et al[46], who used 80 mg/kg of $AlCl_3$ for 60 days. Previous studies[47] have reported a significant decrease in serum testosterone levels in rats treated with $AlCl_3$, which is in line with our investigation. FSH and LH levels significantly decreased after oral administration of $AlCl_3$ (100 mg/kg wt) for eight weeks in male rats, as reported by Ige and Akhigbe[48], indicating that male rats exposed to $AlCl_3$ were rendered infertile. The findings demonstrated that aluminum chloride significantly decreased the average body weight. In the current study, rats given $AlCl_3$ injections had significantly lower testis weights and gonadosomatic indices. The findings of Entissar et al[46] are consistent with this one. They discovered that rats treated with aluminum chloride at a dose of 80 mg/kg experienced a significant decrease in testicular size and weight, as well as a significant decrease in sperm counts and the percentage of live sperm, along with a significant increase in the percentage of morphologically abnormal sperm. Additionally, male rats exposed to $AlCl_3$ experienced a marked decrease in body weight along with a significant reduction in the weight of their relative genital organs, such as the testis and epididymis[38,49]. Rats treated with $AlCl_3$ showed decreased sperm count and motility, which is consistent with earlier findings[38,50,51]. Furthermore, as several studies[47,52,53] have shown in the past, giving rats $AlCl_3$ causes the spermatogenic cells to degenerate and the seminiferous tubules to atrophy.

Conversely, the current findings demonstrated that after receiving an $AlCl_3$ injection, animals treated with 75 and 150 mg/kg of L-carnitine exhibited significant improvements in all sperm parameters. Similarly, L-carnitine was found to significantly suppress changes in sperm properties observed in mice exposed to cisplatin and gamma irradiation, indicating a decrease in sperm abnormalities and an increase in sperm motility and count[54,55]. Additionally, rats were administered 500 mg/kg b.w. L-carnitine intraperitoneally for 16 days to prevent cadmium-induced reproductive toxicity demonstrated an increase in caudal epididymis sperm viability and quantity[56]. Additionally, Yaman and Topcu-Tarladacalisir[57] observed that rats treated with L-carnitine prior to prepubertal cisplatin injection had increased epididymal sperm count and viability during the adult period. Similar to this, Khushboo et al[58] employed oral administration of 50 and 100 mg/kg L-carnitine dissolved in 0.9% saline water for 30 days in male albino rats. This prevented the detrimental effects of prolonged copper consumption on sperm quality, including concentration, viability, motility, and morphology. Indeed, it has been proposed[59] that low carnitine levels are a contributing factor to sperm disorders like asthenospermia and azoospermia. Similar to this, numerous studies[45] have shown that in rats treated with ci-

splatin and sacrificed after 72 hours, the amount, motility, and maturity of spermatozoa are influenced by the free carnitine levels in the epididymis.

For example, during sperm metabolism, the spermatozoa utilize the energy supplied by carnitine and acetylcarnitine, which has a beneficial effect on the entire spermatogenic process. In line with these findings, Adewoyin et al[60] reported that regular carnitine and acetylcarnitine consumption improves sperm quality and function. Furthermore, a prior investigation conducted by Banihani et al[61] demonstrated that human spermatozoa motility was significantly enhanced by an L-carnitine dosage of 0.5 mg/mL ($5x10^6$ cells/mL). Actually, the capacity of high doses of L-carnitine to bind to Ca_2^+, an essential ion required for sperm motility, may be the primary cause of their deleterious effects[61]. From what has been said thus far, it is evident that L-carnitine and its esters, acetyl-L-carnitine and propionyl-L-carnitine, are useful in improving sperm parameters, especially total and progressive motility, lowering ROS levels in seminal fluid, and potentially enhancing semen quality. Therefore, the use of these molecules, either separately or in combination, is a sensible and successful therapeutic approach for treating male infertility. Nevertheless, given the evidence of L-carnitine's calcium chelator activity, which may determine cell damage and lower serum calcium levels, clinical benefits should not be obtained at high doses[62]. The testis from rats given an $AlCl_3$ injection underwent microscopical analysis, which revealed that the treatment resulted in testicular damage in the form of atrophied seminiferous tubules, focal disorganization of seminiferous tubules, and a notable reduction in the populations of spermatogenic cells. Several of the seminiferous tubules showed signs of tubular luminal exfoliation of the damaged spermatocytes and spermatids. Additionally, the intertubular connective tissue became clogged and displayed a relative decrease in Leydig cells, which are interstitial cells. When compared to the $AlCl_3$-treated group in our study, the testicular histoarchitecture of the animals treated with L-carnitine and $AlCl_3$ improved. This is in line with the findings of Coşkun et al[45], who discovered that administering L-carnitine showed protective effects against radiation-induced damage and mitigated the reduction in germ cell numbers and morphological damage in testicular tissues. This apoptosis may also be facilitated by the anti-apoptotic effect(s) of carnitine in the testis[45]. The advantages of the comet assay include its sensitivity in identifying even minute amounts of DNA damage, its small cell count requirement, its flexibility, affordability, ease of use, and quick experiment completion[63]. Since a higher percentage of tail DNA was seen in the treated L-Carnitine (75 and 150 mg/kg) after $AlCl_3$ injection groups in the current study than in the control and L-Carnitine groups, there was evident DNA degradation in the nuclei. Conversely, animals that received $AlCl_3$ and L-carnitine at the same time had better DNA integrity. Similarly, Lewis et al[64] reported that L-carnitine may protect spermatozoa against oxidative DNA and membrane damage by reducing singlet oxygen and the detrimental effects of lipid peroxidation on sperm. It has been shown[65] that L-carnitine improves antioxidant status, lowers oxidant production during stress, and protects cells against free radicals and mitochondria-induced nuclear DNA damage.

Carnitines protect the sperm and cell membrane from ROS-induced DNA fragmentation and apoptosis in this way[60]. Acetyl L-carnitine has been demonstrated[65-67] to improve mitochondrial functions by reducing stress-mediated DNA damage through reducing the production of oxidants and enhancing antioxidant status, as well as protecting cells against nuclear DNA damage caused by free radicals and mitochondria. Numerous investigations[68,69] have shown that the amount, motility, and maturity of spermatozoa are influenced by the free carnitine levels in the epididymis.

Conclusions

The current study's findings unequivocally show that male rats exposed to $AlCl_3$ experienced reproductive toxicity. Treatment with L-carnitine alleviated these symptoms, suggesting that L-carnitine is an antioxidant that guards against $AlCl_3$-induced male reproductive toxicity. Therefore, a thorough investigation of the molecular and metabolic mechanisms connected to $AlCl_3$ is necessary. To assess the protective or preventive effects of various antioxidants against the reproductive toxic effect of $AlCl_3$, more research utilizing different parameters, such as antioxidant measurements, is advised. Consuming foods high in L-carnitine is necessary to lessen the negative effects of $AlCl_3$.

Conflict of Interest

The author declares that there is no conflict of interest.

Informed Consent
Not applicable..

Ethics Approval
This study involving animal participants was approved by the Committee of Health Research Ethics, Deanship of Scientific Research, Qassim University, with ethical committee approval IP (IRP No. 23-41-14).

ORCID ID
Fawiziah Alharbi: 0009-0004-3048-201X.

Authors' Contributions
FA– designed study protocol, writing the manuscript. The author approved the final version of the article to be published.

Funding
There are no funds for this study.

Acknowledgment
Researchers would like to thank the Deanship of Scientific Research, Qassim University, for funding the publication of this project.

Availability of Data and Materials
All data generated or analyzed during this study are included in this published article.

References

1) Durairajanayagam D. Lifestyle causes of male infertility. Arab J Urol 2018; 16: 10-20.
2) Venturini-Soriano M, Berthon G. Aluminum speciation studies in biological fluids: Part 7. A quantitative investigation of aluminum(III)–malate complex equilibria and their potential implications for aluminum metabolism and toxicity. J Inorg Biochem 2001; 85: 143-154.
3) Shaw CA. Aluminum as a CNS and immune system toxin across the life span. Adv Exp Med Biol 2018; 1091: 53-83.
4) Igbokwe IO, Igwenagu E, Igbokwe NA. Aluminium toxicosis: a review of toxic actions and effects. Interdiscip Toxicol 2019; 12: 45.
5) Kalaiselvi A, Suganthy OM, Govindassamy P, Vasantharaja D, Gowri B, Ramalingam V. Influence of aluminium chloride on antioxidant system in the testis and epididymis of rats. Iran J Toxicol 2014; 8: 991-997.
6) Al-Otaibi SS, Arafah MM, Sharma B, Alhomida AS, Siddiqi NJ. Synergistic effect of quercetin and α-lipoic acid on aluminium chloride induced neurotoxicity in rats. J Toxicol 2018; 2018: 2817036.
7) Güvenç M, Cellat M, Gökçek İ, Arkalı G, Uyar A, Tekeli İO, Yavaş İ. Tyrosol prevents AlCl 3 induced male reproductive damage by suppressing apoptosis and activating the Nrf-2/HO-1 pathway. Andrologia 2020; 52: e13499.
8) Sumida Y, Niki E, Naito Y, Yoshikawa T. Involvement of free radicals and oxidative stress in NAFLD/NASH. Free Radic Res 2013; 47: 869-880.
9) Hasona NA, Ahmed MQ. Antioxidant and ameliorative effects of Zingiber officinale against aluminum chloride toxicity. Int J Chin Med 2017; 1: 124-131.
10) Zahedi-Amiri Z, Taravati A, Hejazian LB. Protective effect of Rosa damascena against aluminum chloride-induced oxidative stress. Biol Trace Elem Res 2019; 187: 120-127.
11) Agarwal A, Sengupta P. Oxidative stress and its association with male infertility. Male infertility: contemporary clinical approaches, andrology, ART and antioxidants. Springer Nature 2020; 57-68.
12) ALMurshidi MMH, Raheem SA, Razaq RA. Some histological and physiological effects of aluminum chloride on some reproductive organs of male albino mice (Musmusculus). Ann Romanian Soc Cell Biol 2021; 25: 5906-5918.
13) Lokman M, Ashraf E, Kassab RB, Abdel Moneim AE, El-Yamany NA. Aluminum chloride–induced reproductive toxicity in rats: the protective role of zinc oxide nanoparticles. Biol Trace Elem Res 2022; 200: 4035-4044.
14) Flanagan JL, Simmons PA, Vehige J. Willcox MD, Garrett Q. Role of carnitine in disease Nutr Metab (Lond) 2010; 7: 30.
15) Cave MC, Hurt RT, Frazier TH, Matheson PJ, Garrison RN, McClain CJ, McClave SA. Obesity, inflammation, and the potential application of pharmaconutrition. Nutr Clin Pract 2008; 23: 16-34.
16) Inazu M, Matsumiya T. Physiological functions of carnitine and carnitine transporters in the central nervous system. Nihon Shinkei Seishin Yakurigaku Zasshi 2008; 28: 113-120.
17) Ghonem MM, Lashin HI, Hodeib AA, Soliman NA. L-carnitine as an adjuvant treatment in acute organophosphorus pesticides poisoning: a randomized clinical trial. Mansoura J Forensic Med Clin Toxicol 2018; 26: 37-52.
18) Sallam AO, Rizk HA, Emam MA, Fadl SE, Abdelhiee EY, Khater H, Elkomy A, Aboubakr M. The ameliorative effects of L-carnitine against cisplatin-induced gonadal toxicity in rats. Pak Vet J 2021; 41: 147-151.
19) Aboubakr M, Elsayd F, Soliman A, Fadl SE, El-Shafey A, Abdelhiee EY. L-Carnitine and vitamin E ameliorate cardiotoxicity induced by tilmicosin in rats. Environ Sci Pollut Res 2020; 27: 23026-23034.
20) Correia S, Vaz CV, Silva AM, Cavaco JE, Socorro S. Regucalcin counteracts tert-butyl hydroperoxi-

de and cadmium-induced oxidative stress in rat testis. J Appl Toxicol 2017; 37: 159-166.

21) Cocuzza M, Athayde KS, Agarwal A, Pagani R, Sikka SC, Lucon AM, Srougi M, Hallak J. Impact of clinical varicocele and testis size on seminal reactive oxygen species levels in a fertile population: a prospective controlled study. Fertil Steril 2008; 90: 1103-1108.

22) Fatemi N, Sanati MH, Jamali Zavarehei M, Ayat H, Esmaeili V, Golkar-Narenji A, Zarabi M, Gourabi H. Effect of tertiary-butyl hydroperoxide (TBHP)-induced oxidative stress on mice sperm quality and testis histopathology. Andrologia 2013; 45: 232-239.

23) Kaur P, Kaur G, Bansal MP. Tertiary-butyl hydroperoxide induced oxidative stress and male reproductive activity in mice: role of transcription factor NF-κB and testicular antioxidant enzymes. Reprod Toxicol 2006; 22: 479-484.

24) Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev 2008: 154-171.

25) Semet M, Paci M, Saïas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H, Perrin J. The impact of drugs on male fertility: a review. Andrology 2017; 5: 640-663.

26) Krasovskiĭ GN, Vasukovich LY, Chariev OG. Experimental study of biological effects of leads and aluminum following oral administration. Environ Health Perspect 1979; 30: 47-51.

27) Kim JH, Pan JH, Lee ES, Kim YJ. L-Carnitine enhances exercise endurance capacity by promoting muscle oxidative metabolism in mice. Biochem Biophys Res Commun 2015; 464: 568-573.

28) Mosbah WST. Supplementation Against Reproductive Toxicity in Male Albino Mice Administered Monosodium Glutamate. PhD diss Faculty of Science Fayoum University 2019.

29) Mohamed B, Fares NH, Ashaat NA, Abozeid F. Biochemical, histological, and immunohistochemical changes associated with AlCl3-induced hepatic injury in rats: protective effects of L-carnitine. Egypt J Histol 2022; 45: 90-100.

30) Ismail AAA, Astley P, Burr WA, Wood MCA, Short F, Wakelin K, Wheeler MJ. The role of testosterone measurement in the investigation of androgen disorders. Ann Clin Biochem 1986; 23: 113-134.

31) Ekaluo U, Ikpeme E, Ibiang Y, Amaechina O. Attenuating role of vitamin C on sperm toxicity induced by. J Biol Sci 2013; 13: 298-301.

32) Björndahl L, Söderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum Reprod 2003; 18: 813-816.

33) Ekaluo UB, Udokpoh AE, Ikpeme EV, Peter EU. Effect of chloroquine treatments on sperm count and weight of testes in male rats. Global J Pure Appl Sci 2008; 14: 175-177.

34) Ekaluo UB, Ikpeme EV, Udokpoh AE. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. Internet J Toxicol 2009; 7: 1-9.

35) Sheehan DC, Hrapchak BB. Theory and practice of histotechnology. 2nd ed. Columbus, Ohio: Battelle Press; 1987.

36) Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184-191.

37) Enciso M, Sarasa J, Agarwal A, Fernández JL, Gosálvez J. A two-tailed comet assay for assessing DNA damage in spermatozoa. Reprod Biomed Online 2009; 18: 609-616.

38) Yousef MI, Salama AF. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem Toxicol 2009; 47: 1168-1175.

39) Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, Santulli M, Agarwal A, Gandini L. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. Fertil Steril 2004; 81: 1578-1584.

40) Kanter M, Topcu-Tarladacalisir Y, Parlar S. Antiapoptotic effect of L-carnitine on testicular irradiation in rats. J Mol Histol 2010; 41: 121-128.

41) Onem G, Aral E, Enli Y, Oguz EO, Coskun E, Aybek H, Ozcan AV, Sacar M, Bir LS, Baltalarli A. Neuroprotective effects of L-carnitine and vitamin E alone or in combination against ischemia-reperfusion injury in rats. J Surg Res 2006; 131: 124-130.

42) Pignatelli P, Lenti L, Sanguigni V, Frati G, Simeoni I, Gazzaniga PP, Pulcinelli FM, Violi F. Carnitine inhibits arachidonic acid turnover, platelet function, and oxidative stress. Am J Physiol Heart Circ 2003; 284: H41-H48.

43) Marin-Guzman J, Mahan DC, Whitmoyer R. Effect of dietary selenium and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. Anim Sci J 2000; 78: 1544-1550.

44) Mateus FG, Moreira S, Martins AD, Oliveira PF, Alves MG, Pereira MD. L-carnitine and male fertility: is supplementation beneficial? J Clin Med 2023; 12: 5796.

45) Coşkun N, Hatipoğlu MT, Özoğul C, Korkmaz C, Akyol SN, Mıcılı SC, Arık GS, Erdoğan D. The protective effects of acetyl L-carnitine on testis gonadotoxicity induced by cisplatin in rats. Balkan Med J 2013; 30: 235.

46) Entissar M, Nathem AH, Karam H. Effect of aluminium chloride on sexual efficiency in adult male rats. J Educ Sci 2009; 22: 27-44.

47) Moselhy W, Helmy N, Abdel-Halim B, Nabil T, Abdel-Hamid M. Role of ginger against the reproductive toxicity of aluminium chloride in albino male rats. Reprod Domest Anim 2012; 47: 335-343.

48) Ige SF, Akhigbe RE. The role of Allium cepa on aluminum-induced reproductive dysfunction in experimental male rat models. J Hum Reprod Sci 2012; 5: 200.

49) Mouro VGS, Menezes TP, Lima GDA, Domingues RR, Souza ACF, Oliveira JA, Matta SLP, Machado-Neves M. How bad is aluminum exposure to reproductive parameters in rats? Biol Trace Elem Res 2018; 183: 314-324.

50) Güvenç M, Cellat M, Gökçek İ, Arkalı G, Uyar A, Tekeli İO, Yavaş İ. Tyrosol prevents AlCl3 induced male reproductive damage by suppressing apoptosis and activating the Nrf-2/HO-1 pathway. Andrologia 2020; 52: e13499.

51) Mahmoud AA, Elfiky AM, Elreedy HA, Salaheldin K. Quercetin attenuates testicular dysfunction induced by aluminum chloride in male Wistar rats. Egypt J Chem 2022; 65: 665-676.

52) Abdel-Moneim AM. Effects of taurine against histomorphological and ultrastructural changes in the testes of mice exposed to aluminium chloride. Arh Hig Rada Toksikol 2013; 64: 405-414.

53) Sun X, Sun H, Yu K, Wang Z, Liu Y, Liu K, Zhu Y, Li Y. Aluminum chloride causes the cysfunction of cestes through inhibiting the ATPase enzyme activities and gonadotropin receptor expression in rats. Biol Trace Elem Res 2018; 183: 296-304.

54) Ahmed MM, Ibrahim ZS, Alkafafy M, El-Shazly SA. L-carnitine protects against testicular dysfunction caused by gamma irradiation in mice. Acta Histochem 2014; 116: 1046-1055.

55) Eid A. Protective effect of L-carnitine against cisplatin-induced testicular toxicity in rats. Al-Azhar J Pharm Sci 2016; 53: 123-142.

56) Yari A, Asadi MH, Bahadoran H, Dashtnavard H, Imani H, Naghii MR. Cadmium toxicity in spermatogenesis and protective effects of L-Carnitine in adult male rats. Biol Trace Elem Res 2010; 137: 216-225.

57) Yaman O, Topcu-Tarladacalisir Y. L-carnitine counteracts prepubertal exposure to cisplatin induced impaired sperm in adult rats by preventing germ cell apoptosis. Biotech Histochem 2018; 93: 157-167.

58) Khushboo M, Murthy MK, Devi MS, Sanjeev S, Ibrahim KS, Kumar NS, Roy VK, Gurusubramanian G. Testicular toxicity and sperm quality following copper exposure in Wistar albino rats: ameliorative potentials of L-carnitine. Environ Sci Pollut Res 2018; 25: 1837-1862.

59) Agarwal A, Said TM. Carnitines and male infertility. Reprod Biomed Online 2004; 8: 376-384.

60) Adewoyin M, Ibrahim M, Roszaman R, Md Isa ML, Mat Alewi NA, Abdul Rafa AA, Anuar MNN. Male infertility: the effect of natural antioxidants and phytocompounds on seminal oxidative stress. Diseases 2017; 5: 9.

61) Banihani SA, Bayachou M, Alzoubi K. L-Carnitine is a calcium chelator: a reason for its useful and toxic effects in biological systems. J Basic Clin Physiol Pharmacol 2015; 26: 141-145.

62) Mongioi L, Calogero AE, Vicari E, Condorelli RA, Russo GI, Privitera S, Morgia G, La Vignera S. The role of carnitine in male infertility. Andrology 2016; 4: 800-807.

63) Glei M, Schneider T, Schlörmann W. Comet assay: an essential tool in toxicological research. Arch Toxicol 2016; 90: 2315-2336.

64) Lewis SE, Sterling ESL, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil Steril 1997; 67: 142-147.

65) Cetinkaya A, Bulbuloglu E, Kantarceken B, Ciralik H, Kurutas EB, Buyukbese MA, Gumusalan Y. Effects of L-Carnitine on Oxidant/Antioxidant Status in Acetic Acid-Induced Colitis. Dig Dis Sci 2006; 51: 488-494.

66) Calò LA, Pagnin E, Davis PA, Semplicini A, Nicolai R, Calvani M, Pessina AC. Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. Int J Cardiol 2006; 107: 54-60.

67) Rani PJA, Panneerselvam C. Carnitine as a free radical scavenger in aging. Exp Gerontol 2001; 36: 1713-1726.

68) Jeulin C, Lewin LM. Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. Hum Reprod Update 1996; 2: 87-102.

69) Ng CM, Blackman MR, Wang C, Swerdloff RS. The Role of Carnitine in the Male Reproductive System. Ann N Y Acad Sci 2004; 1033: 177-188.