**PHYTON-ANNALES REI BOTANICAE**
**ISSN: 0079-2047**
**Volume 66 No. 1 (2026)**

https://phyton-annales.com

# The Possible Ameliorating Role of L-Carnitine on Furan-Induced Testicular Damage in Adult Albino Rats

Rasha A. Alshali[1]
[1]Department of Clinical Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Received : 10-01-2025 Accepted : 05-02-2026 Published :04-05-2026

**Abstract**

**Introduction:** Furan is a chemical found in some industrial products and naturally occurs in heat-processed foods. This study investigated whether L-carnitine (LC) could mitigate testicular tissue damage caused by oral furan administration in male albino rats.

**Methodology:** Forty adult male albino rats were divided into four groups: Group I (negative control), Group II (L-carnitine-treated) received 350 mg/kg/day of LC orally, Group III (furan-exposed) received furan at 16 mg/kg daily for 35 days, and Group IV (furan+LC) received both LC and furan at the same doses as described. Biochemical, histopathological, and histomorphometric analyses were performed on testicular tissue.

**Results:** Furan caused vacuolations, reduced the thickness of the germinal epithelium, decreased the seminiferous tubule diameter. Degenerated and exfoliated spermatogenic cells were observed alongside degenerative changes in Sertoli cells. There was a significant decrease in serum testosterone and antioxidant markers, with a notable increase in the oxidant profile. However, LC alleviated furan-induced testicular toxicity by improving total antioxidant capacity (TCA) activities and reducing malondialdehyde (MDA) levels. It also increased the lowered levels of luteinizing hormone (LH) and testosterone. Additionally, LC treatment effectively alleviated the histopathological damage.

**Conclusion:** Pretreatment with L-Carnitine reduced the damaging impact of Furan on spermatogenesis parameters in adult albino rats.

**Keywords:** Rats, Furan, Testicular damage, L-carnitine, Sex hormones

## 1. Introduction

Nowadays, there is increasing public concern about reported high rates of human infertility, which affects about 190 million people globally [1,2]. Multiple factors can cause male infertility, but it is frequently attributed to unhealthy lifestyles, including nutrition. In connection with this, exposure to environmental and occupational hazards is also thought to be responsible for the increasing incidence of infertility [3].

Furan ($C_4H_4O$) is a colourless, highly volatile, aromatic heterocyclic organic chemical with lipophilic and aromatic properties [4]. Furan is a chemical agent that is used in industry

to produce organic compounds, herbicides, plastics, and pharmaceuticals. It is related to a group of dioxins (polychlorinated dibenzo-furans) that are formed within the thermal degradation of natural food products [5,6]. In a 2011 report, the European Food Safety Authority (EFSA) detected a high level of furan in coffee brands, including roasted bean coffee (3660 ng/g), unspecified coffee (2016 ng/g), roasted ground coffee (1936 ng/g), and instant coffee (394 ng/g). Also, low levels of furan were detected in food products such as soft drinks (0.8–1.2 ng/g), tea (1–1.7 ng/g), wine and liquors (1.3 ng/g), vegetable fats (1.5–1.7 ng/g), and fruit juice (2.2–4.6 ng/g)[7]. Moreover, according to the EFSA, the primary sources of furan exposure to children are milk-based products, fruit juices, and cereal-based products. At the same time, adults are primarily exposed to furans via brewed coffee [4,7].

Therefore, furan should be used with caution due to potential harmful effects on adipose tissue, liver, and kidneys [8–10]. Recent findings revealed that Furan exposure may cause male reproductive dysfunction and testicular injury. Furan can decrease daily sperm production and cause testicular atrophy [4,11,12].

A significant factor in the aetiology of male infertility is oxidative stress [13,14]. Oxidative stress is a mediator of Furan reproductive toxicity, causing a decrease in semen quantity and quality, which affects male infertility. Elevated levels of reactive oxygen species accelerate oxidative stress, which is dangerous to the process of spermatogenesis [15].

New strategies have been developed to prevent the toxic effects of furan by using antioxidants that protect against cell damage through neutralizing the harmful effects of free radicals [12].

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 lysine15. Through its ability to facilitate the transport of long-chain fatty acids into mitochondria, it makes a significant contribution to cellular energy metabolism. Research has demonstrated that it acts 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.

L-carnitine (LC) is found in the epididymis and sperm, with a concentration approximately 2000 times higher than in plasma. It is essential in lipid metabolism that facilitates the β-oxidation of long-chain fatty acids in mitochondria, which is necessary for energy production [16]. It acts as a substantial non-enzymatic antioxidant, protecting the cell's mitochondrial membrane and DNA integrity against free oxygen radicals [17]. Several studies have been performed to evaluate the effect of carnitine on infertile men, indicating the improvement of sperm fertility, count, and motility, so it is effective in improving fertility [18,19].

Therefore, the objective of the present study was to investigate the possible ameliorating role of L-carnitine (LC) on the testicular tissue damage induced by orally administered furan on the

testes in male albino rats for 35 days. In this respect, biochemical, histopathological, and histomorphometric examinations were performed.

## 2. Materials &methods

### 2.1. Experimental grouping

Forty male albino rats (10-12 weeks old), weighing around 250g were used in this study. Animals were housed in clean, stainless-steel cages under controlled conditions for two weeks prior to use, during which they adjusted to a 12-hour light/dark cycle, a relative humidity of 44–50%, and a temperature of 25 ± 2°C. The rats were acclimatised for seven days under natural daily photoperiod; fed and provided clean water *ad libitum*. Rats were divided randomly into four experimental groups. Each group consisted of 10 rats.

- **Group I (negative control group):** rats received saline and maize oil simultaneously at the same dose in Groups II and III for 35 days.
- **Group II (L-carnitine-treated group):** rats received LC at a dose of 350 mg/kg/day orally for 35 days. It was administered to the rats in Groups II and IV at this dosage as recommended by Ghanbarzadeh et al. [20]. Acetyl L-carnitine was obtained from Sigma-Aldrich Co., St. Louis, USA.
- **Group III (Furan-exposed group):** rats received oral administration of Furan at a dose of 16 mg/kg/day for 35 days as recommended by Alam et al. [21]. Furan was diluted with maize oil and subsequently stored in brown glass vials at 4 °C for exposures. New solutions were developed every week as needed. Furan ($C_4H_4O$, ≥ 99% purity, molecular weight 68.07, CAS number 110–00–9) was acquired from Sigma-Aldrich Co., St. Louis, USA.
- **Group IV (Furan + L-carnitine-treated group)**: rats received LC and were concurrently exposed to Furan at the previously described dose regimen.

Lastly, 24 hours after the last dose of both drugs, all animals were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 40 mg/kg [22]. Euthanasia was performed by decapitation.

### 2.2. Blood collection for Steroid hormone analysis

For steroid hormone analysis, blood samples were collected from the retro-orbital venous plexus of all rats and allowed to clot. Centrifugation of the samples at 1000 × g for 15 minutes was performed, and the sera were separated and stored at -20°C. Using commercial rat ELISA kits (MBS282195 and MBS2018978, respectively) and following the manufacturer's instructions (My BioSource), the concentrations of serum testosterone and luteinizing hormone (LH) were measured.

### 2.3. Biochemical analysis

Rats were sacrificed, and both testes from each animal were carefully dissected. The left testes were homogenized for analysis and assessment of lipid peroxidation levels and total antioxidant capacity. Testicular samples were treated with sodium phosphate solution, and the tissues were maintained at –80°C. Testis tissue samples were homogenised using a glass pestle

(glass homogenizer). The homogenate was centrifuged at 10,000 x g for 15 min at 4°C, and the supernatant was collected and stored at -80°C for further use for the determination of malondialdehyde (MDA) and total antioxidant levels. The MDA level was assessed utilizing the test described by Ohkawa et al.[23] and is expressed as nmol MDA/mg tissue. The total antioxidant capacity (TAC) of testicular tissue was measured using the ferric reducing antioxidant power (FRAP) assay method [24] and is expressed as Units per Gram of tissue.

### 2.4. Histological examination

The right testes were obtained and immediately fixed in Bouin's fixative, then manipulated through gradual dehydration, followed by clearing in xylol solution and embedding in paraffin. Sections were obtained at a thickness of 5–6 µm using a microtome. Testicular sections were processed for routine histological examination by staining using hematoxylin and eosin (H&E) stain and for the demonstration of collagen fibers using Masson Trichrome stain [25]. A light microscope (Olympus, Japan) with a built-in camera was used to examine and photograph all slides.

### 2.5. Morphometric analysis

Using ImageJ software (National Institute of Health, Bethesda, Maryland, USA), ten different non-overlapping randomly selected fields from each slide of each group were quantified for the mean diameter (mm) of the seminiferous tubules was measured in H&E-stained sections (× 400), and the mean thickness of germinal epithelium (µm) in H&E-stained sections (× 400) [26], and the mean area percentage of collagen fibers in Masson’s trichrome-stained sections (x 400) [27].

### 2.6. Statistical analysis

The means and standard deviations (SD) were used to express all the data. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was performed using GraphPad Prism version 10.6.0 for Windows at a P-value less than 0.05 (the level of significance).

## 3. Results

### 3.1. Biochemical findings

Mean serum testosterone and serum LH from group III (0.8450± 0.1153, 16.87± 3.596, respectively) demonstrated a significant drop (P<0.0001) regarding both groups I (1.803 ± 0.1109, 35.59 ± 1.513, respectively) and group II (1.793± 0.2657, 35.71 ± 1.523, respectively). However, group IV (1.510±0.3004, 32.56±2.586, respectively) exhibited a significant rise with respect to group III (P<0.0001), yet it was non-significantly different from the control group (P= 0.0523, P= 0.0888, respectively) (Figure 1 A&B).

Additionally, group III expressed a significant increase (P<0.0001) in mean tissue MDA (2.441± 0.3951) that was associated with a significant decrease (P<0.0001) in mean tissue TAC (0.8990± 0.07608) about both groups I (0.6760± 0.06275, 1.754± 0.05481, respectively) and group II (0.6820± 0.05329, 1.850± 0.07703, respectively). However, group IV demonstrated a significant decrease (P<0.0001) in mean tissue MDA (0.8980± 0.03393) that coupled with a significant increase (P<0.0001) in mean tissue TAC (1.558± 0.05203) with respect to group III. However, both parameters were still significantly different from those of the control group (P<0.0001, respectively) (Figure 2A&B).

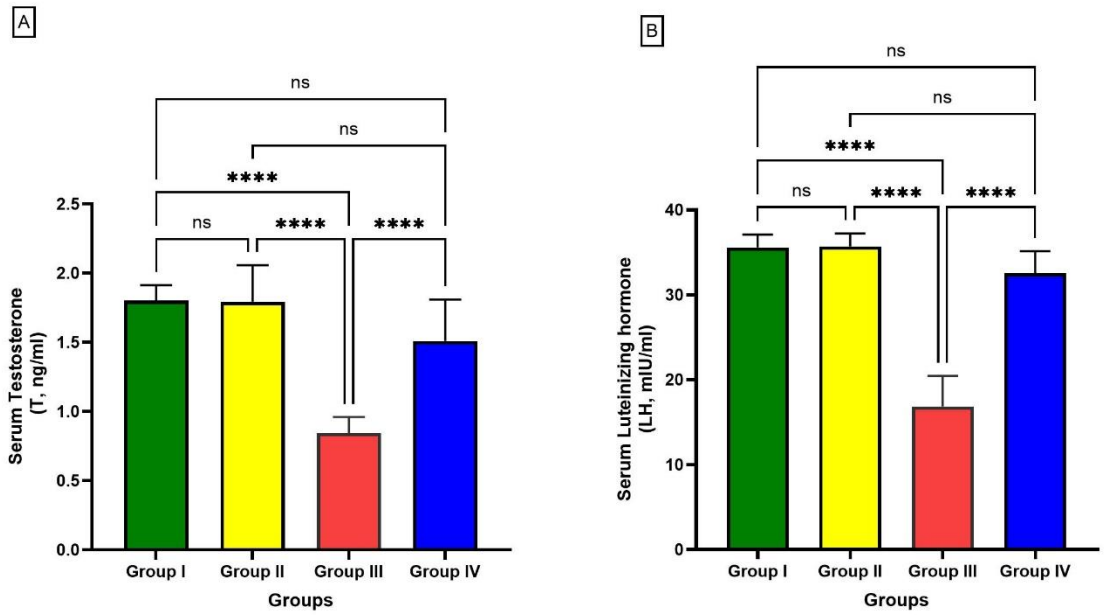



**Figure 1:** Effects of L-carnitine on the (A) serum level of Testosterone hormone and (B) serum level of luteinizing hormone (LH). Graphs are presented based on Mean ± standard deviation (SD) values. **** significant at P<0.0001, and ns means non-significant.

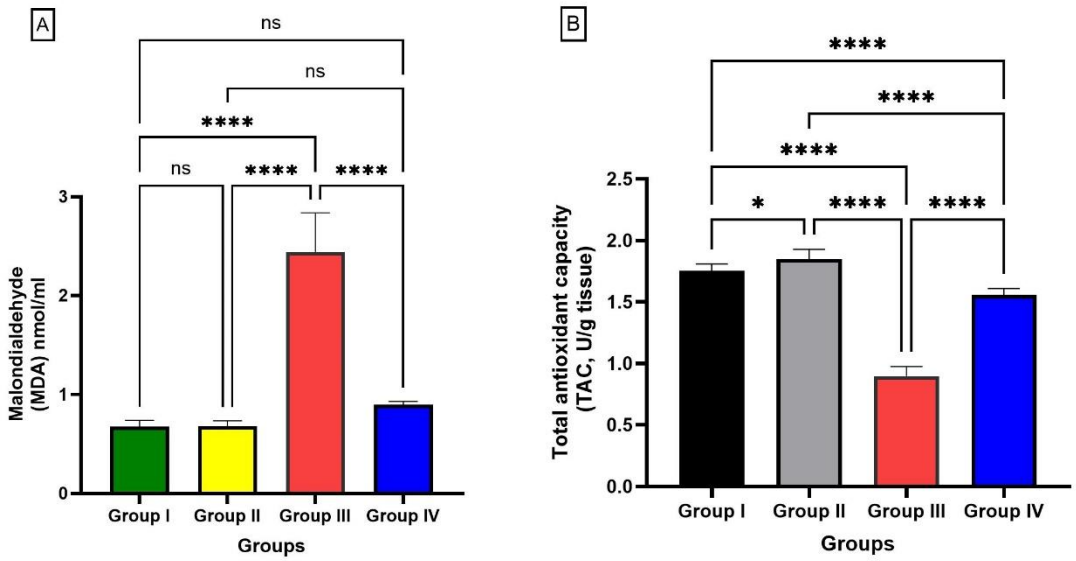



**Figure 2:** Effects of L-carnitine on the (A) malondialdehyde (MDA) and (B) total antioxidant capacity (TAC). Graphs are presented based on Mean ± standard deviation (SD) values.
* Significant at (P<0.05), **** Significant at P<0.0001, and ns means non-significant.

### 3.2. Histological and morphometric results:

#### 3.2.1. Hematoxylin and eosin-stained sections:

The **negative control group (Group I)** exhibited a regular histological pattern of the testis, characterized by a tunica albuginea surrounding it. Tunica albuginea is formed of connective tissue fibers and fibroblasts. The testicular tissue revealed seminiferous tubules separated by interstitial tissue, which contains blood vessels in addition to Leydig cells. The sections showed regular seminiferous tubules surrounded by a connective tissue layer called the basal lamina, which consisted mainly of flat myoid cells. These tubules were lined by Sertoli cells and germinal epithelium consisting of: spermatogonia, primary spermatocytes, rounded or elongated spermatids, and spermatozoa. The spermatogonia were observed resting on the basal lamina. The primary spermatocytes exhibited large, rounded nuclei, while the spermatids were located near the tubular lumen, where they were observed to have formed entirely sperms. Sertoli cells were found close to the basal lamina, having oval pale nuclei (Figure 3 A-C).

When testicular tissues from the **L-carnitine-treated group (Group II)** were histomorphologically evaluated, they were found to be similar to those of Group I (Figure 4 A-C).

Testes of all animals treated with 16 mg/kg b.w./day of Furan **(Furan-exposed group III)** showed marked distortion of the seminiferous tubules with thick basement membrane and thickening of tunica albuginea with congested subcapsular blood vessels. Several seminiferous tubules exhibited shrunken features, characterized by smaller diameters and obliterated lumina. The germinal epithelium presented marked disorganization. Many vacuoles appeared among

the spermatogenic cells, and the intercellular space widened. A depletion of the germinal cells was discerned, mainly at the spermatid and spermatocyte stages of spermatogenesis. The majority of the spermatogenic cells in some tubules became necrotic except for a few normal germ cells lining the seminiferous tubules. Notice that Multinucleated giant cells could be seen within the seminiferous tubule. Some Sertoli cells suffered degeneration. Sertoli cells revealed with cytoplasmic vacuolation and pyknotic nuclei. Likewise, the number of germ cells in some tubules was drastically reduced, and the spermatozoa, as well as spermatids, had partially disappeared. Blood vessels in the interstitium appeared congested with hemorrhage in the widened interstitial spaces, and homogenous acidophilic material. Interstitial cells of Leydig appeared with cytoplasmic vacuoles and dark nuclei (Figure 5 A-D).

**The Furan+ L-carnitine -treated rats (Group IV)** showed no detectable structural abnormalities and an apparently normal tunica albuginea. The seminiferous tubules were apparently resuming their normal architecture, and most tubules contained spermatozoa with normal morphological appearance. The interstitial tissue formed by Leydig cells and blood capillaries was normal (Figure 6 A-C).

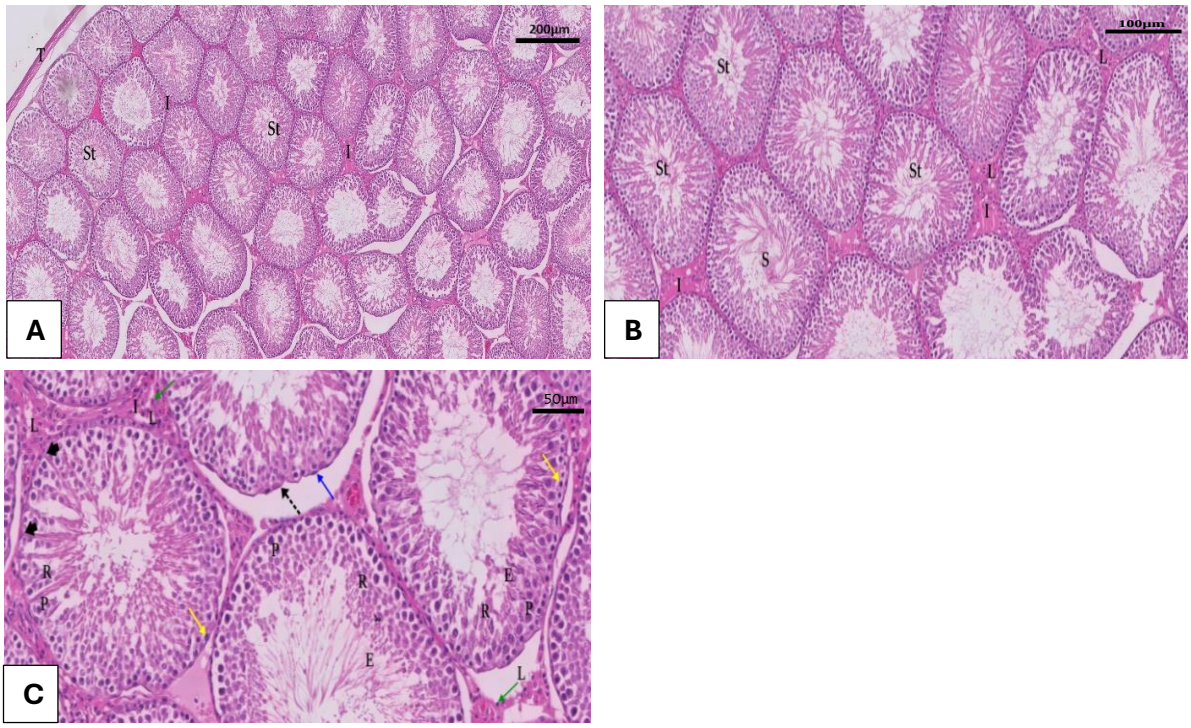



**Figure 3:** photomicrographs for testicular sections from **negative control rats (group I),** revealing a normal histological pattern of the testis with thin tunica albuginea (T) around it. Seminiferous tubules (St) are compactly packed together in a rounded or oval shape, having multiple layers of germinal epithelium. Regular seminiferous tubules (St) are surrounded by a connective tissue layer called basal lamina (dot arrow) that consists mainly of flat myoid cells (blue arrow). These tubules are lined by Sertoli cells (arrowhead) and germinal epithelium consisting of: spermatogonia (yellow arrow), primary spermatocytes (P), and rounded (R) or

elongated (E) spermatids. The lumen of the tubules shows the presence of sperm in a whirly appearance (S). Notice the presence of the interstitial tissue (I) between the tubules with Leydig (L) cells (green arrow) (H&E stain Ax 5, Bx10, Cx20).

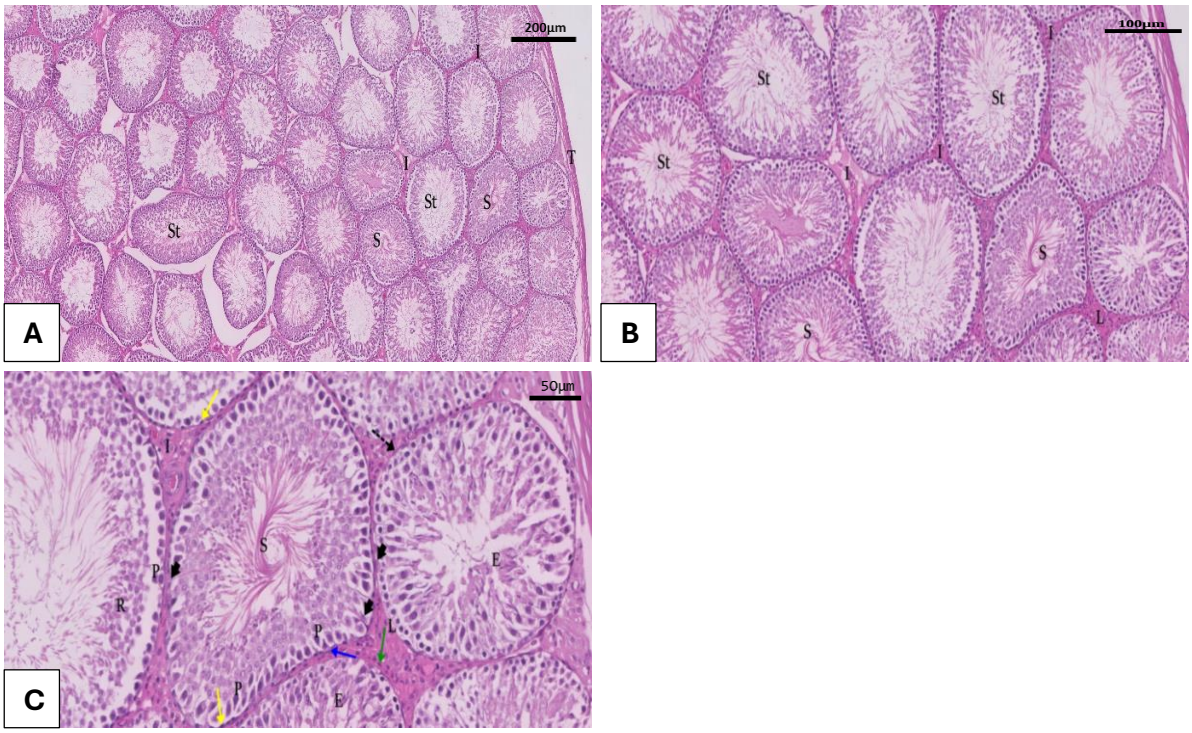



**Figure 4:** photomicrographs for testicular sections from the **L-carnitine-treated group (Group II)**, revealing a normal histological pattern of the testis with thin tunica albuginea (T) around it. Parts of adjacent seminiferous tubules (St) are separated by a narrow interstitium (I). Each tubule is lined by Sertoli cells (arrowhead), spermatogonia (yellow arrow), primary spermatocytes (P), and rounded (R) with elongated (E) spermatids. Multiple spermatozoa (S) are seen filling the lumen of seminiferous tubules (St). Peritubular myoid cells (blue arrow) and basal lamina (dot arrow) are also noticed. Notice the presence of the thin interstitial tissue (I) between the tubules with Leydig (L) cells (green arrow) (H&E stain Ax 5, Bx10, Cx20).

**PHYTON-ANNALES REI BOTANICAE**
**ISSN: 0079-2047**
**Volume 66 No. 1 (2026)**

https://phyton-annales.com

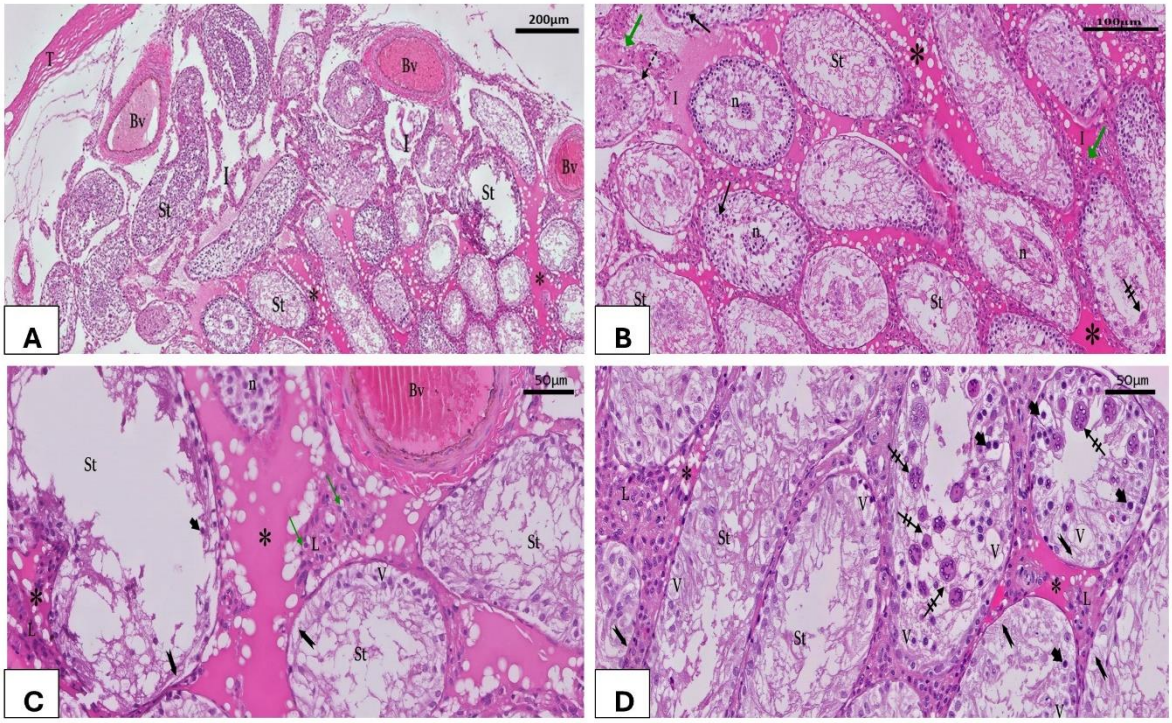



**Figure 5:** photomicrographs for testicular sections from **Furan-exposed rats (group III),** revealing thickening of tunica albuginea (T) and most of the seminiferous tubules (St) appear with irregular thickened basement membrane (dot arrow) and widened interstitial tissue (I). Some of the seminiferous tubules (St) are completely deteriorated and contain remnants of damaged spermatogenic cells (n). Some cells appear with dark, shrunken nuclei (black arrow). Sertoli cells (arrowhead) reveal cytoplasmic vacuolation and pyknotic nuclei. Notice large multinucleated giant cells are seen (double crossed arrow). Wide interstitial spaces (I) with darkly stained Leydig cell (L) nuclei (green arrow) and congested blood vessels (Bv) with and homogenous acidophilic material (*). Interstitial cells of Leydig (L) appear with cytoplasmic vacuoles and dark nuclei (green arrow). Many vacuoles (V) appear among the spermatogenic cells, and the intercellular space widens (bifid arrow) (H&E stain Ax 5, Bx10, C&Dx20).

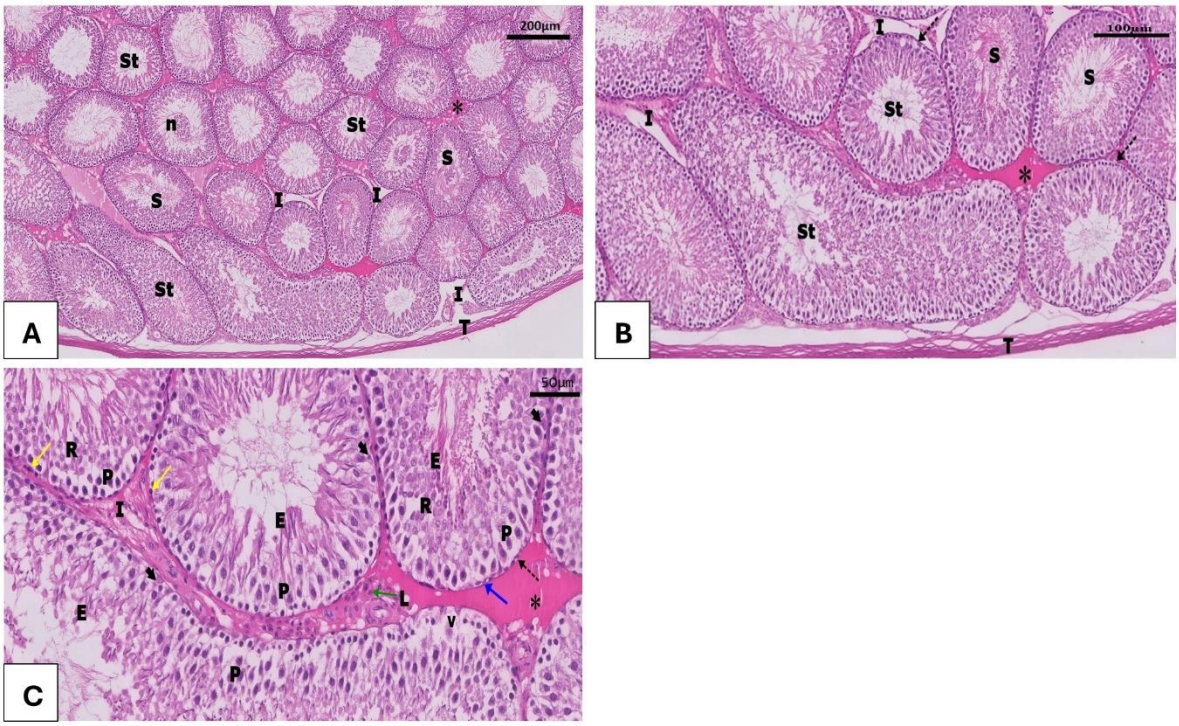



**Figure 6:** photomicrographs for testicular sections from **Furan+ L-carnitine-treated group (group IV),** revealing a normal histological pattern of the testis with thin tunica albuginea (T) around it, and most of the seminiferous tubules (St) appear with regular intact basal lamina (dot arrow). Most of the seminiferous tubules (St) appear normal with mostly intact germinal epithelium. Each tubule is lined by Sertoli cells (arrowhead), spermatogonia (yellow arrow), primary spermatocytes (P), and rounded (R) with elongated (E) spermatids. Abundant sperm are detected in the lumina (S). Few vacuoles (V) appear among the spermatogenic cells. Notice little interstitium (I) in between the tubules with some eosinophilic hyaline material (*) and apparently normal (green arrow) Leydig cells (L). Some of the seminiferous tubules (St) contain remnants of damaged spermatogenic cells (n) (H&E stain Ax 5, Bx10, Cx20).

### 3.2.2. Evaluation of the Seminiferous Tubules Diameter and the Thickness of Germinal Epithelium

The mean seminiferous tubular diameters (Figure 7A) were 1.169 ± 0.1139 mm in the negative control group (Group I); 1.089 ± 0.03834 mm in the LC-treated group (Group II); 0.6024 ± 0.07768 mm in the Furan-exposed group (Group III); and 1.012 ± 0.1833 mm in the FU+ LC-treated group (Group IV).

The mean values of germinal epithelium thicknesses (Figure 7B) were as follows: 157.0± 7.976 µm in the negative control group (Group I); 160.2± 6.397 µm in the LC-treated group (Group II); 55.28± 7.904 µm in the Furan-exposed group (Group III); 153.0± 4.341 µm in the FU+ LC-treated group (Group IV).

While no significant differences were observed between Groups I and II (P = 0.5244) regarding tubular diameter, the tubular diameters in Group III were significantly lower compared with

Group I (P<0.0001), Group II (P<0.0001), and Group IV (P<0.0001). Tubular diameters in Group IV were greater compared with Group III (P<0.0001) and lower compared with Group I (P =0.0547) and Group II (P = 0.5623).
Additionally, there were no significant differences between Group I and Group II in germinal epithelium thicknesses (P = 0.7236). The germinal epithelium thickness in Group III was significantly lower than in Group I (P<0.0001), Group II (P<0.0001), and Group IV (P<0.0001). The germinal epithelium thickness in Group IV was greater than in Group III (P<0.0001) and lower than in Group I (P =0.5590) and Group II (P =0.1029).

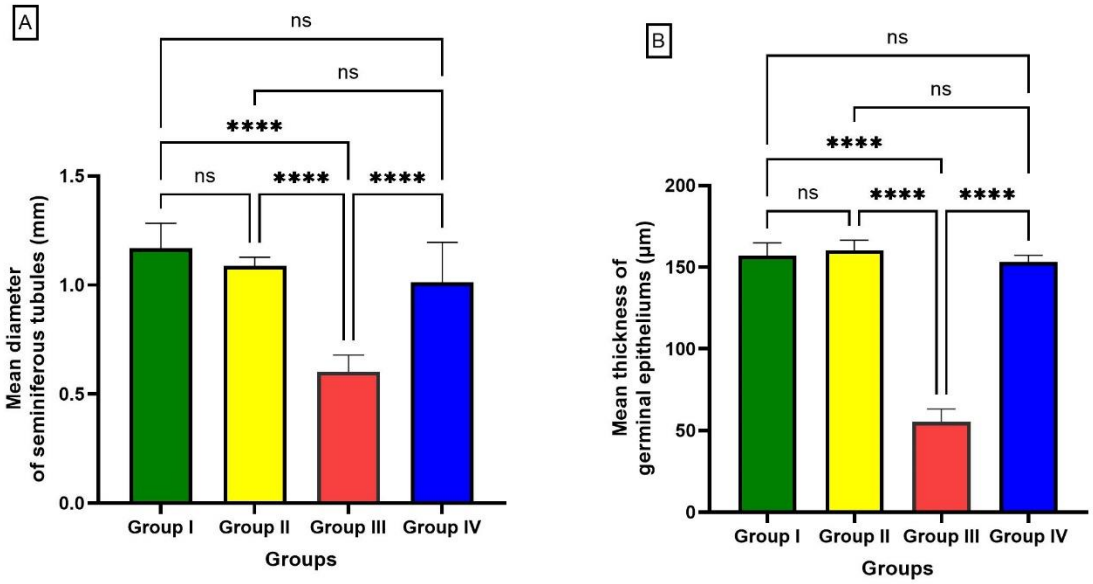


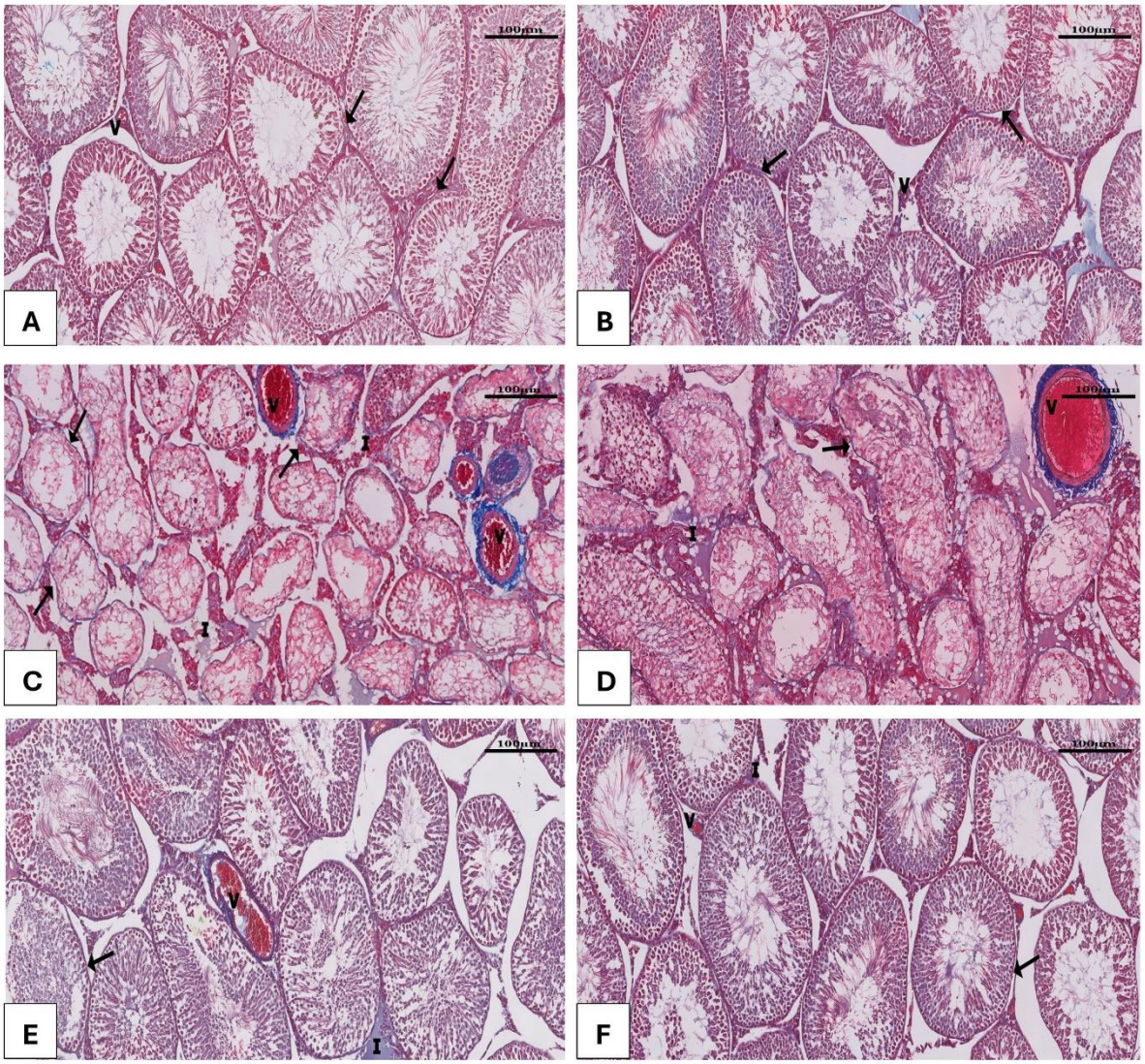

**Figure 7:** Effects of L-carnitine on the (A) mean seminiferous tubular diameters and (B) the mean germinal epithelium thicknesses. Graphs are presented based on Mean ± standard deviation (SD) values. **** Significant at P<0.0001, and ns means non-significant.

### 3.2.3. Masson's trichrome-stained sections:

Masson's trichrome-stained testicular sections from both groups I and II demonstrated a minimal amount of bluish-green-stained collagen fibers in the basal lamina of the seminiferous tubules and around the blood vessels (Figure 8 A&B). Group III sections showed an excessive accumulation of collagen fibers in the interstitium, basal lamina, and around the blood vessels (Figure 8 C&D). Meanwhile, group IV sections depicted a moderate accumulation of collagen fibers in the interstitium, basal lamina of some tubules, and around the blood vessels (Figure 8 E&F).
Morphometric analysis of the mean area percentage of collagen fiber content in group III demonstrated a significant increase (P<0.0001) with respect to both groups I and II, while group IV exhibited a substantial reduction in relation to group III (P<0.0001) but was not significantly different from the control group (P = 0.0971) (Figure 8 G).

PHYTON-ANNALES REI BOTANICAE
ISSN: 0079-2047
Volume 66 No. 1 (2026)

https://phyton-annales.com



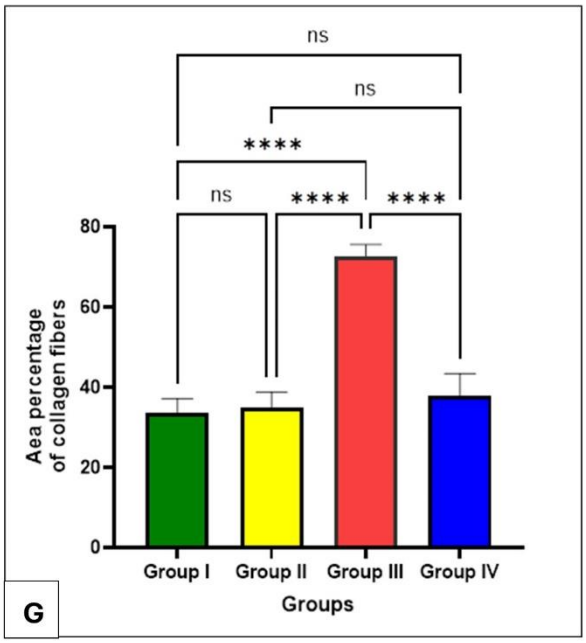



**Figure 8:** Photomicrographs for Masson's trichrome-stained testicular sections: (A) Group I and (B) Group II showing a minimal amount of bluish-green-stained collagen fibers in the basal lamina (↑) of the seminiferous tubules and around the blood vessels (v). (C&D) Group III sections show an excessive accumulation of collagen fibers in the interstitium (I), basal lamina (↑), and around the blood vessels (V). (E&F) Group IV sections depict a moderate accumulation of collagen fibers in the interstitium (I), basal lamina (↑) of some tubules, and around the blood vessels (V). (G) Effects of L-carnitine on the mean area percentage of collagen fibers. Graphs are presented based on Mean ± standard deviation (SD) values.
**** Significant at $P<0.0001$, and ns means non-significant.

## 4. Discussion

Male infertility is a complex and multifactorial condition impacting many individuals, caused by various factors such as oxidative stress disruptions and deficiencies in essential trace elements [28]. Current evidence connects oxidative stress (OS) to male infertility, decreased sperm motility, sperm DNA damage, and a higher risk of recurrent abortions and genetic diseases [29]. Furthermore, environmental pollutants and heat-related food contaminants, such as furan, have been shown to cause oxidative stress in the testes, highlighting how external factors negatively impact male reproductive health. [15].

The toxic properties of furan, which can be ingested through diet and air, result in its accumulation in mammalian fat tissues, where it can persist for several years. During stress or fasting, this stored furan is released into the bloodstream. Due to its low polarity, furan readily crosses biological membranes and interacts with the body's natural antioxidants, thereby disrupting their function and promoting the formation of free radicals [30]. Galimova et al. found that infertile males had higher levels of furans in their ejaculate compared to controls, indicating a potential association between furan exposure and reproductive health [31].

Some studies have reported using antioxidants and antioxidant-rich foods to limit oxidative damage [4]. Research has extensively examined the antioxidant properties of L-Carnitine and its role in energy metabolism, particularly regarding its potential therapeutic uses. Several studies have investigated its advantages in treating male infertility. Treatment with L-Carnitine has been linked to improvements in sperm motility and viability[32–35]. Male reproductive tissues, such as the epididymis and testis, exhibit higher levels of L-carnitine than plasma, highlighting its role in sperm maturation and motility. Free carnitine is transported through passive diffusion across the sperm plasma membrane and accumulates in the epididymis, especially in the caudal region. Ultimately, L-Carnitine is incorporated into spermatozoa, where it is acetylated during maturation [36]. Therefore, this study investigated the use of L-carnitine, which has potential antioxidant properties, to reduce furan-induced damage to rat testes.

Testosterone synthesis is essential for maintaining the structure and function of accessory sex organs. When testosterone is withdrawn, it leads to the premature detachment of spermatids from Sertoli cells, as this hormone is vital for the attachment of germ cells to Sertoli cells. This could explain our findings in the current study, where rats in Group III (Furan-exposed) showed lower testosterone levels, vacuolation in the germinal epithelium layers, reduced sperm in the lumen, and degenerated Leydig cells. These results align with El-Hamied et al., who reported that most pathological changes following Furan supplementation are linked to severe coagulative necrosis, congestion, disorganization, and degeneration of germ cells, leading to the leakage of cell contents. Furthermore, Furan’s composition affects germinal cell plasma membrane proteins, increasing cell permeability and leading to enzyme leakage. These effects may contribute to the observed lower testosterone levels, which likely resulted in decreased sperm count and quality [12]. The hypothalamic-pituitary-testicular system is tightly regulated, and male reproductive functions depend on maintaining gonadotropin-releasing hormone

(GnRH) levels, including LH. LH, a glycoprotein, stimulates Leydig cells in the testes to produce testosterone and regulate spermatogenesis. A decrease in LH levels negatively impacts spermatogenesis and testicular enzyme activity [40]. In our study, treating Furan-exposed rats with L-carnitine significantly increased serum levels of LH and testosterone. Similar results were reported in previous studies involving L-carnitine [41,42]. Moreover, El-Damarawi and Salama's infertility survey in obese men found that L-carnitine raises serum levels of LH and testosterone [43].

Moreover, Al-Daraji and Tahir reported that L-carnitine can increase serum levels of FSH and LH, which subsequently improve spermatogenesis and testosterone levels [44]. As an antioxidant, L-carnitine can inhibit free radicals and increase the expression of the antioxidant enzymes, leading to reduced oxidative stress [45]. Oxidative stress can decrease FSH, LH, and testosterone levels [42]. Additionally, L-carnitine can increase the release of luteinizing hormone-releasing hormone, which in turn activates the release of LH, leading to the release of testosterone [46].

Many studies have suggested that oxidative stress, a condition characterized by an imbalance between ROS production and antioxidant defense systems, is a new emerging factor in unexplained male infertility [14, 47, 48]. An excessive ROS production represents a significant cause of sperm injury. Indeed, due to the large amount of membrane unsaturated fatty acids and the lack of cytoplasmic antioxidant enzymes, spermatozoa are highly susceptible to oxidation, with consequent detrimental effects on sperm quality/functioning [49,50].

The administration of furan significantly reduced antioxidant enzyme activity in furan-treated rats, which primarily includes total antioxidant capacity (TAC) [51,52]. In the current study, MDA is an indicator of lipid peroxidation (LP). LP, as a result, increases the permeability of the membrane. The MDA level in the testes was considerably elevated following exposure to furan, accompanied by a concomitant reduction in testicular TAC level. These results align with prior studies indicating that exposure to furan elevated cellular levels of reactive oxygen species and lipid peroxidation [53–55]. Lipid peroxidation, which is a process that occurs because of a chain reaction between unsaturated fatty acids in cell membrane lipids and ROS, causes functional disorders by disrupting the cell membrane structure under stress conditions [56]. An in vivo investigation with albino rats found that exposure to furan substantially increased the levels of ROS and MDA [57]. According to a study on male rats, ROS activity increased significantly with increasing furan concentration [51].

However, the co-administration of L-carnitine upsurged antioxidant enzyme activity. Supplementation of L-carnitine in the Furan-treated group potentially alleviated the harmful impacts of furan via decreasing oxidative stress in the testicles. The present research has shown that L-carnitine considerably increased TAC activities, while lowering MDA, which may be attributed to its antioxidant properties. Our results align with those of Deliktaş et al. [58] and Mateus et al. [36].

In vivo studies have demonstrated that L-Carnitine can reduce the concentration of MDA, a marker of lipid peroxidation, ensuring efficient fatty acid oxidation and preventing the

accumulation of damaged lipids [59,60]. Additionally, L-carnitine is also involved in maintaining the cellular redox balance. It has been shown to enhance the activity of other antioxidants, such as glutathione, which plays a crucial role in cellular defense against oxidative stress, and promote the expression of enzymes with antioxidant properties when the testis is exposed to toxicants that induce oxidative stress [61]. On the other hand, L-Carnitine has been shown to have positive effects on improving sperm quality, motility, count, and morphology [62]. Additionally, it plays a role in protecting spermatozoa from DNA damage and preserving acrosome integrity, which is essential for successful fertilization [59]. These combined effects contribute to an overall increase in fertility rates [35,63]. By enhancing the antioxidant defense system, L-Carnitine improves the body's ability to combat oxidative stress-induced damage, which is known to impair sperm function and overall reproductive success. Its protective actions on spermatozoa can lead to improvements in key parameters related to male fertility, ultimately increasing the chances of successful conception.

Seminiferous tubules are responsible for about 80% of the total testicular volume. In the cross-section, the lumen of the seminiferous tubule is lined by Sertoli cells. It contains spermatozoa in various stages of development, from spermatogonia near the base of tubules to progressively mature forms (spermatocytes, spermatids, and spermatozoa) arranged towards the center of the lumen [64]. The most common pathological feature observed in relation to testicular toxicity is that the germinal epithelium is loosening [65,66]. Therefore, quantitative assessments of germinal epithelium thickness or seminiferous tubule diameter have been performed in various studies [64, 67, 68]. The results of the current study indicated that furan induced histopathological changes in the testes of the rats. These changes included a significant reduction in tubular diameter and germinal epithelium thickness, reflecting a decline in spermatogenic activity. In addition, seminiferous tubules showed maturation arrest, a reduction to an absence in the luminal sperm reserve, an exfoliation of the normal germinal epithelium in the lumen of seminiferous tubules, a formation of multinucleated giant cells, hypertrophied spermatocytes, hyalinization of luminal contents, spermatocytes with pyknotic nuclei, and vacuolations in Sertoli cells and interstitial connective tissue. Similar alterations in the rat testis were previously reported by El-Akabawy and El-Sherif [55] and Abd El-Hakim et al. [54].

The results of the current study confirmed that furan supplementation led to marked histological distortion of the seminiferous tubules and degeneration of Leydig cells. The germinal epithelium presented marked disorganization and depletion of the germinal cells. The majority of the spermatogenic cells in some tubules became necrotic. Some of the seminiferous tubules were completely deteriorated and contained remnants of damaged spermatogenic cells. Additionally, Sertoli cells exhibited degenerative changes. Several explanations can underpin the observed histopathological changes after furan treatment. First, the increased oxidative stress caused by furan may be responsible for these alterations [51]. Second, given that spermatogenesis is androgen-dependent, testosterone deprivation, resulting from Leydig cell apoptosis, may affect spermatogenic morphological parameters, such as tubular diameter and

germinal epithelium thickness [69]. Third, furan and/or its active toxic metabolites may have a direct toxic effect on germ cells [4,8,70] [55].

In the current study, multinucleated giant cells, a specific form of degenerating germ cells, were observed in the seminiferous tubules. These giant cells were suggested to be one of the evident features of testicular cell degeneration and atrophy. It resulted from the failure of spermatid separation due to damage in the intercellular bridges and impaired cytokinesis, or it could be a result of increased phagocytosis activity in apoptotic spermatogenic cells [54,55]. It was noted that sperm and spermatids were rarely observed. This might be explained by the ROS production, which is known to be cytotoxic and often causes tissue injury [11]. Wide interstitial spaces were observed in our study, and this finding was in line with Anuar et al., who accounted that the excess interstitial spaces could also be explained by the presence of acidophilic homogenous material in the interstitium. This material might be formed because of excess lymphatic exudate oozing from degenerated lymphatic vessels, as well as an increase in vascular permeability that may result from the accumulation of ROS and free radicals [71].

However, L-carnitine supplementation considerably alleviated the histopathological impairments instigated by furan. These results concur with other earlier studies [59,61,72,73]. These improvements may be attributed to the androgenic as well as anti-apoptotic and antioxidant nature of L-carnitine [74,75].

## 5. Conclusion

The current study's findings suggest that administering L-Carnitine alongside furan exposure might help rat testes recover from damage caused by furan. The combination of L-Carnitine and furan effectively reversed the testicular toxicity indicators induced by furan. The protective mechanism is likely related to reducing oxidative stress and lipid peroxidation. Additional research is needed to gain a clearer understanding of these mechanisms.

## 6. References

1 Faiad W, Soukkarieh C, Murphy DJ, et al. Effects of dioxins on animal spermatogenesis: A state-of-the-art review. *Front. Reprod. Heal.* 2022; **4**; 1009090.
2 Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. *Hum. Reprod. Update* 2015; **21**; 411-426.
3 Ma Y, He X, Qi K, et al. Effects of environmental contaminants on fertility and reproductive health. *J. Environ. Sci. (China)* 2019; **77**; 210-217.
4 Ijaz MU, Mustafa S, Ain QU, et al. Eriodictyol attenuates Furan induced testicular toxicity in Rats: Role of oxidative stress, steroidogenic enzymes and apoptosis. *Ecotoxicol. Environ. Saf.* 2023; **259**; 115003.
5 Batool Z, Xu D, Zhang X, et al. A review on furan: Formation, analysis, occurrence, carcinogenicity, genotoxicity and reduction methods. *Crit. Rev. Food Sci. Nutr.* 2021; **61**; 395-406.
6 Husøy T, Arukwe A, Bindrup M-L, et al. Risk Assessment of Furan Exposure in the Norwegian Population. *Eur. J. Nutr. Food Saf.* 2019; **11**; 44-46.
7 Food E, Authority S. Update on furan levels in food from monitoring years 2004–2010 and exposure assessment. *EFSA J.* 2011; **9**; 1-33.
8 Knutsen HK, Alexander J, Barregård L, et al. Risks for public health related to the presence of furan and methylfurans in food. *EFSA journal. Eur. Food Saf. Auth.* 2017; **15**; e05005.
9 Merheb C, Gerbal-Chaloin S, Casas F, et al. Omega-3 Fatty Acids, Furan Fatty Acids, and Hydroxy Fatty Acid Esters: Dietary Bioactive Lipids with Potential Benefits for MAFLD and Liver Health. *Nutrients* 2025; **17**.
10 Alshabeeb MA, Aithal GP, Daly AK. Investigation of Oxidative Stress-Related Candidate Genes as Risk Factors for Drug-Induced Liver Injury due to Co-Amoxiclav. *DNA Cell Biol.* 2020; **39**; 349-354.
11 Rehman H, Jahan S, Ullah I, et al. Effects of endocrine disruptor furan on reproductive physiology of Sprague Dawley rats: An F1 Extended One- Generation Reproductive Toxicity Study (EOGRTS). *Hum. Exp. Toxicol.* 2020; **39**; 1-16.
12 El-Hamied H, Elshenewany N, Meged samira. Evaluation of the effective role of natural antioxidants against the reproductive toxicity of furan in male albino rats. *J. Sci. Res. Sci.* 2024; **41**; 195-214.
13 Aitken RJ, Drevet JR, Moazamian A, et al. Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidants (Basel, Switzerland)* 2022; **11**.
14 Mannucci A, Argento FR, Fini E, et al. The Impact of Oxidative Stress in Male Infertility. *Front. Mol. Biosci.* 2021; **8**; 799294.
15 Aydin Y, Dikbasan YU, Orta-Yilmaz B. Furan impairs cell function by inducing oxidative stress, DNA damage and apoptosis in mouse Sertoli cells in vitro. *Drug Chem. Toxicol.* 2025; **48**; 1067-1079.
16 Nawaf M, Id S, Hatem L. Protective effects of L-Carnitine and kiwifruit extract against benzo[a]pyrene-induced testicular toxicity in Sprague-Dawley rats. *Babcock Univ. Med. J.* 2025; **8**; 366-378.
17 Abdel Aziz RL, Abdel-Wahab A, Abo El-Ela FI, et al. Dose- dependent ameliorative effects of quercetin and l-Carnitine against atrazine- induced reproductive toxicity in adult male Albino rats. *Biomed. Pharmacother.* 2018; **102**; 855-864.

18 Mongioi L, Calogero AE, Vicari E, et al. The role of carnitine in male infertility. *Andrology* 2016; **4**; 800-807.
19 Wang Y, Zhang R, Pan W, et al. Effects of L-carnitine combined with pancreatic kininogenase on thioredoxin 2, thioredoxin reductase 1, and sperm quality in patients with oligoasthenospermia. *Transl. Androl. Urol.* 2021; **10**; 3515-3523.
20 Ghanbarzadeh S, Garjani A, Ziaee M, et al. CoQ10 and L-carnitine attenuate the effect of high LDL and oxidized LDL on spermatogenesis in male rats. *Drug Res. (Stuttg).* 2014; **64**; 510-515.
21 Alam RT, Zeid EHA, Imam TS. Protective role of quercetin against hematotoxic and immunotoxic effects of furan in rats. *Environ. Sci. Pollut. Res. Int.* 2017; **24**; 3780-3789.
22 Abd El-Hay RI, Hamed WHE, Mostafa Omar N, et al. The impact of busulfan on the testicular structure in prepubertal rats: A histological, ultrastructural and immunohistochemical study. *Ultrastruct. Pathol.* 2023; **47**; 424-450.
23 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 1979; **95**; 351-358.
24 Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal. Biochem.* 1996; **239**; 70-76.
25 Layton C, Suvarna K. Bancroft's Theory and Practise of Histological Techniques (7th Edition) (Co-Author). 7th ed. (S.K., Suvarna, C. Layton, and J.D. Bancroft E, eds.). Churchill Livingstone, Elsevier. Philadelphia; 2013.
26 Abd-El-Hafez MA, El-Shafee MD, Omar SH, et al. The ameliorative effect of curcumin on cryptorchid and non-cryptorchid testes in induced unilateral cryptorchidism in albino rat: histological evaluation. *Folia Morphol. (Warsz).* 2021; **80**; 596-604.
27 Al-Gharabawi G, Emara T. Impact of royal jelly and L-Carnitine on improving the histological changes of Cyclophosphamide-induced Testicular cytotoxicity in adult male albino rats. *Al-Azhar Int. Med. J.* July 2022.
28 Ebaid H, Bashandy SAE, Hassan I, et al. The Preventive Effect of Zinc Sulfate against Olanzapine-Induced Testicular Toxicity in Male Rats. *Biol. Trace Elem. Res.* 2025; **203**; 3764-3778.
29 Alahmar AT. Role of Oxidative Stress in Male Infertility: An Updated Review. *J. Hum. Reprod. Sci.* 2019; **12**; 4-18.
30 Yilmaz V, Ari E. The effects of service quality, image, and customer satisfaction on customer complaints and loyalty in high-speed rail service in Turkey: a proposal of the structural equation model. *Transp. A Transp. Sci.* 2017; **13**; 67-90.
31 Galimova EF, Amirova ZK, Galimov SN. Dioxins in the semen of men with infertility. *Environ. Sci. Pollut. Res.* 2015; **22**; 14566-14569.
32 Chang D, Li F, Kang Y, et al. The effects of L-carnitine and fructose in improved Ham's F10 on sperm culture in idiopathic severe asthenospermia within 24h. *PLoS One* 2025; **20**; e0306235.
33 Banihani S, Agarwal A, Sharma R, et al. Cryoprotective effect of l-carnitine on motility, vitality and DNA oxidation of human spermatozoa. *Andrologia* 2014; **46**; 637-641.
34 EL-RAEY M, M.A. B, MM A, et al. L-CARNITINE ENHANCING ROLES ON BUFFALO SEMEN FREEZABILITY, ULTRA STRUCTURE AND FERTILIZING POTENTIALS. *Assiut Vet. Med. J.* 2016; **62**; 163-173.
35 Kopets R, Kuibida I, Chernyavska I, et al. Dietary supplementation with a novel l-carnitine multi-micronutrient in idiopathic male subfertility involving oligo-, astheno-,

teratozoospermia: A randomized clinical study. *Andrology* 2020; **8**; 1184-1193.
36 Mateus FG, Moreira S, Martins AD, et al. L-Carnitine and Male Fertility: Is Supplementation Beneficial? *J. Clin. Med.* 2023; **12**.
37 Li L, Lin W, Wang Z, et al. Hormone Regulation in Testicular Development and Function. *Int. J. Mol. Sci.* 2024; **25**.
38 Câmara ML, Almeida TB, de Santi F, et al. Fluoxetine-induced androgenic failure impairs the seminiferous tubules integrity and increases ubiquitin carboxyl-terminal hydrolase L1 (UCHL1): Possible androgenic control of UCHL1 in germ cell death? *Biomed. Pharmacother.* 2019; **109**; 1126-1139.
39 Mohamed A. Moringa oleifera attenuates testicular damage induced by high fructose diet in Albino rats: Histological study. *Egypt. J. Histol.* August 2023.
40 Owumi SE, Otunla MT, Arunsi UO, et al. 3-Indolepropionic acid upturned male reproductive function by reducing oxido-inflammatory responses and apoptosis along the hypothalamic-pituitary-gonadal axis of adult rats exposed to chlorpyrifos. *Toxicology* 2021; **463**; 152996.
41 Mady B, Ayoub M, Elshinety R, et al. PROTECTIVE EFFECT OF L-CARNITINE AND VITAMIN E ON CYCLOPHOSPHAMIDE INDUCED TOXICITY ON RAT TESTES. *Egypt. J. Anat.* 2019; **43**.
42 Rezaei N, Mardanshahi T, Shafaroudi MM, et al. Effects of l-Carnitine on the Follicle-Stimulating Hormone, Luteinizing Hormone, Testosterone, and Testicular Tissue Oxidative Stress Levels in Streptozotocin-Induced Diabetic Rats. *J. evidence-based Integr. Med.* 2018; **23**; 2515690X18796053.
43 El-Damarawi MA, Salama ME. Obestatin and L-carnitine as a defensive strategy against fertility disorders induced by obesity in male rats. *Tanta Med. J.* 2014; **42**.
44 Al-Daraji HJ, Tahir AO. Effect of L-carnitine supplementation on drake semen quality. *S. Afr. J. Anim. Sci.* 2014; **44**; 18-25.
45 Zambrano S, Blanca AJ, Ruiz-Armenta M V, et al. L-carnitine attenuates the development of kidney fibrosis in hypertensive rats by upregulating PPAR-γ. *Am. J. Hypertens.* 2014; **27**; 460-470.
46 Abo-Ghanema II, El-Nasharty MA, El-Far AH, et al. Effect of ginger and L-carnitine on the reproductive performance of male rats. *World Acad Sci Eng Technol* 2012; **64**; 980-986.
47 Cito G, Becatti M, Natali A, et al. Redox status assessment in infertile patients with non-obstructive azoospermia undergoing testicular sperm extraction: A prospective study. *Andrology* 2020; **8**; 364-371.
48 Agarwal A, Rana M, Qiu E, et al. Role of oxidative stress, infection and inflammation in male infertility. *Andrologia* 2018; **50**; e13126.
49 Agarwal A, Roychoudhury S, Sharma R, et al. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. *Reprod. Biomed. Online* 2017; **34**; 48-57.
50 Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell. Mol. Life Sci.* 2020; **77**; 93-113.
51 Rehman H, Jahan S, Ullah I, et al. Toxicological effects of furan on the reproductive system of male rats: An “in vitro” and “in vivo"-based endocrinological and spermatogonial study. *Chemosphere* 2019; **230**.
52 Zahran M, Alaryani FS, El Bohi KM, et al. Protective role of Lagenaria Siceraria seed

oil against furan-induced toxicity: Histopathological, biochemical, and molecular insights in male Albino rats. *PLoS One* 2025; **20**; e0322363.

53 Aydin Y, Yilmaz B, Dikbasan YU, et al. Assessment of the oxidative damage and apoptotic pathway related to furan cytotoxicity in cultured mouse Leydig cells. *Toxicol. Res. (Camb).* 2023; **12**; 400-407.

54 Abd El-Hakim YM, Mohamed WA, El-Metwally AE. Spirulina platensis attenuates furan reprotoxicity by regulating oxidative stress, inflammation, and apoptosis in testis of rats. *Ecotoxicol. Environ. Saf.* 2018; **161**; 25-33.

55 El-Akabawy G, El-Sherif NM. Protective role of garlic oil against oxidative damage induced by furan exposure from weaning through adulthood in adult rat testis. *Acta Histochem.* 2016; **118**; 456-463.

56 Albalawi A, Alhasani RHA, Biswas L, et al. Protective effect of carnosic acid against acrylamide-induced toxicity in RPE cells. *Food Chem. Toxicol. an Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 2017; **108**; 543-553.

57 Awad A, Khalil SR, Farag MR, et al. Differential susceptibility of kidneys and livers to proliferative processes and transcriptional level of the genes encoding desmin, vimentin, connexin 43, and nestin in rats exposed to furan. *Ecotoxicol. Environ. Saf.* 2018; **162**; 235-244.

58 Deliktaş H, Gedik A, Nergiz Y, et al. Reperfusion Injury due to Testicular Torsion in Rats. *Eur. J. Gen. Med.* 2012; **9**; 192-196.

59 Cabral REL, Mendes TB, Vendramini V, et al. Carnitine partially improves oxidative stress, acrosome integrity, and reproductive competence in doxorubicin-treated rats. *Andrology* 2018; **6**; 236-246.

60 Thangasamy T, Jeyakumar P, Sittadjody S, et al. L-carnitine mediates protection against DNA damage in lymphocytes of aged rats. *Biogerontology* 2009; **10**; 163-172.

61 Alharthi WA, Hamza RZ, Elmahdi MM, et al. Selenium and L-Carnitine Ameliorate Reproductive Toxicity Induced by Cadmium in Male Mice. *Biol. Trace Elem. Res.* 2020; **197**; 619-627.

62 Ma L, Sun Y. Comparison of L-Carnitine vs. Coq10 and Vitamin E for idiopathic male infertility: a randomized controlled trial. *Eur. Rev. Med. Pharmacol. Sci.* 2022; **26**; 4698-4704.

63 Wang Y, Yang S, Qu C, et al. [L-carnitine: safe and effective for asthenozoospermia]. *Zhonghua Nan Ke Xue* 2010; **16**; 420-422.

64 Mesbahzadeh B, Hassanzadeh-Taheri M, Aliparast M-S, et al. The protective effect of crocin on cisplatin-induced testicular impairment in rats. *BMC Urol.* 2021; **21**; 117.

65 Catlin NR, Willson CJ, Creasy DM, et al. Differentiating between Testicular Toxicity and Sexual Immaturity in Ortho-phthalaldehyde Inhalation Toxicity Studies in Rats and Mice. *Toxicol. Pathol.* 2018; **46**; 753-763.

66 Chen T, Zhang L, Yao L, et al. Zinc oxide nanoparticles-induced testis damage at single-cell resolution: Depletion of spermatogonia reservoir and disorder of Sertoli cell homeostasis. *Environ. Int.* 2023; **181**; 108292.

67 Coşkun N, Hatipoğlu MT, Ozoğul C, et al. The protective effects of acetyl L-carnitine on testis gonadotoxicity induced by Cisplatin in rats. *Balkan Med. J.* 2013; **30**; 235-241.

68 Jahan S, Munawar A, Razak S, et al. Ameliorative effects of rutin against cisplatin-induced reproductive toxicity in male rats. *BMC Urol.* 2018; **18**; 107.

69 Adamczewska D, Słowikowska-Hilczer J, Walczak-Jędrzejowska R. The Fate of Leydig Cells in Men with Spermatogenic Failure. *Life (Basel, Switzerland)* 2022; **12**.

70 Bakhiya N, Appel K. Toxicity and carcinogenity of furan in human diet. *Arch. Toxicol.* 2010; **84**; 563-578.
71 Anuar N, Shafie S, Maznan M, et al. Lauric acid improves hormonal profiles, antioxidant properties, sperm quality and histomorphometric changes in testis and epididymis of streptozotocin-induced diabetic infertility rats. *Toxicol. Appl. Pharmacol.* 2023; **470**; 116558.
72 Khushboo M, Murthy MK, Devi MS, et al. Testicular toxicity and sperm quality following copper exposure in Wistar albino rats: ameliorative potentials of L-carnitine. *Environ. Sci. Pollut. Res. Int.* 2018; **25**; 1837-1862.
73 Karam KM, Alebady AS, Al-Nailey KGC, et al. L-Carnitine effect on induced hyperlipidemia on premature rats: fertility profile. *J. Med. Life* 2022; **15**; 124-131.
74 Koohpeyma F, Gholizadeh F, Hafezi H, et al. The protective effect of L-carnitine on testosterone synthesis pathway, and spermatogenesis in monosodium glutamate-induced rats. *BMC Complement. Med. Ther.* 2022; **22**; 269.
75 Alharbi F. Ameliorative effects of L-carnitine as an antioxidant against testicular toxicity induced by AlCl 3 in male albino rats. *Eur. Rev. Med. Pharmacol. Sci.* 2024; **28**; 1680-1694.