# Alpha-lipoic acid protects against hyperlipidemia-induced testicular damage in premature rats

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**Abstract** This study was designed to investigate the effect of hypercholesterolemia on the reproductive performance of premature male rats and to evaluate the influence of alpha lipoic acid (ALA) in conserving their fecundity. Sixty rats were randomly assigned to one of three groups. The control group (CG n=20 rats), cholesterol feeding group 1 (CFG1 n=20 rats) were fed 1.5% cholesterol with diet for one month, and cholesterol feeding group 2 (CFG2 n= 20 rats) were fed 1.5% cholesterol with diet + ALA 100 mg/kg body weight supplied by water for one month. The results revealed that compared to CG and CFG2, CFG1's body weight increased significantly. After consuming cholesterol for one month, CFG1's lipid profile revealed a substantial rise in blood cholesterol and triglycerides when compared to CG and the group that consumed ALA CFG2. In comparison to CGF1 and CG, the results of the sperm parameters in CGF2 demonstrated a large rise in sperm count with sperm live percentage and a considerable decrease in sperm abnormality %. Rats from CFG1 had significantly lower serum testosterone levels than rats from CFG2 and CG, according to the hormonal profile. We conclude that the significance of alpha lipoic acid (ALA) in preserving fertility ALA is a strong antioxidant that may preserve the parameters of the sperm of hyperlipidemic preterm rats, which may improve the capacity of hyperlipidemic rats to conceive.

**Keywords:** antioxidants, cholesterol, hyperlipidemia, alpha lipoic acid, sperm parameters

## 1. Introduction

A high-fat diet can affect the reproductive efficiency of a male due to the accumulation of free radicals in testicular tissue. This causes damage in Sertoli and Leydig cells, which results in functional disorders of the hypothalamo-pituitary gonadal axis responsible for clear spermatogenesis processes (Awoniyi et al 2012; Nemzer et al 2014). Using antioxidants as a protective agent against the formation of free radicals is known scientifically as a strong tool in decreasing oxidative stress and maintaining the biological activities of cells. The use of antioxidants has a confirmed positive action on enhancing the fertile properties of semen because they scavenge free radicals that form during the metabolic activities of sperm and leukocytes, they decrease the development of premature sperm, and they prevent DNA fragmentation of sperm (Nikolovski et al 2014).

Alpha-lipoic acid **(**ALA) is a potent antioxidant that is used as a co-factor for several important mitochondrial enzymes (Agarwal et al 2004). The therapeutic activity of ALA depends on its action as a scavenger of ROS, its metal chelating abilities, its capability to repair oxidative damage and its capacity to regenerate endogenous antioxidant activity (Goraca et al 2011).

Hyperlipidemia decreases male fertility due to its harmful effect on spermatogenesis (Karam et al 2022). The main aim of this study was to investigate the effect of ALA in enhancing the reproductive performance of premature hyperlipidemic male rats.

## 2. Materials and Methods

The present study was performed on 60 premature Sprague Dawley (albino male rats); their ages were approximately 4 weeks with a body weight ranging between 125-150 grams. Rats were obtained from the animal house of the College of Veterinary Medicine/University of Al-Qadisiyah. They were fed and housed under standard nutrition and environment during the whole experimentation days (30 days), according to (Institute of Laboratory Animal Resources, 1985).

Sixty rats were divided randomly into three groups. The control group CG (20 rats) was fed a diet that did not contain cholesterol or ALA from day 30 to day 60 of age, cholesterol feeding group 1 CFG1 (20 rats) was fed a diet supplemented with 1.5% cholesterol from day 30 to day 60 of age (Elnaga 2012), and cholesterol feeding group 2 CFG2 (20 rats) was fed a diet supplemented with 1.5% cholesterol + ALA 100 mg/kg body weight given water (Selvakumar et el., 2005) from day 30 to day 60 of age.

One day after ceasing cholesterol supplementation (on day 61 of age), the weights of rats in all groups were checked, and blood samples were collected to measure serum total cholesterol and triglycerides. The rats were sacrificed, and specimens from the liver and testis of all groups were fixed in 10% formalin for histology (Hinting 1989). Semen samples were taken from the tail of the epididymis and checked for semen analysis (Al-Taii 1994).

*2.1. Statistical analysis*

The results are expressed as the mean ± standard deviation (SD) for an animal per group. Via SPSS 2016, one-way ANOVA of variance followed by Tukey's test was applied to determine the significant differences among means ($p < 0.05$), and the statistical analysis of data for physiological and histological parameters was performed by using Qi square (Machin et al 2007).

## 3. Results

The weights of rats in all groups on day 30 of age were 141.75±16.83 g, 139.93±12.45 g and 142.32±12.32 g for CFG1, CFG2 and CG, respectively, and after 30 days of cholesterol feeding (on day 61 of age), the weights for groups were 275.25±16.34 g, 244.33±15.32 g and 239.51±13.47 g for CGF1, CGF2 and CG, respectively. The weight of CGF1 was significantly higher than that of CGF2 and CG, as shown in Table 1.

**Table 1** Body weight (grams) of all groups before and after feeding cholesterol (Mean±SD).

| GROUPS | CFG1 | CFG2 | CG |
|---|---|---|---|
| B.W at 1 month of age (grams) | 141.75±16.83 | 139.93±12.45 | 142.32±12.32 |
| B.W after 2months of age (grams) | 275.25±16.34[a] | 244.33±15.32[c] | 239.51±13.47[c] |

*ac = significant difference in rows (P<0.01).

Table 2 presents the lipid profiles of CFG1 and CFG2 after one month of cholesterol feeding (on day 61 of age) and the lipid profile of CG. As shown for CFG1, the serum cholesterol was 133.15±4.91, and the serum triglyceride was 181.06±3.56. For CFG2, serum cholesterol was 103.34±2.32, and serum triglyceride was 109.77±3.63. Serum cholesterol for the control group was 95.32±3.85, and serum triglyceride was 87.51±2.96, with significant differences between all groups.

**Table 2** Lipid profile (cholesterol and triglyceride mg/dl) for all groups (M±SD).

| GROUPS | CFG1 | CFG2 | CG |
|---|---|---|---|
| Cholesterol | 133.15±4.91[a] | 103.34±2.32[b] | 95.32±3.85[c] |
| Triglyceride | 181.06±3.56[a] | 109.77±3.63[b] | 87.51±2.96[c] |

*ab = significant difference in rows (P<0.05). *bc = significant difference in rows (P<0.05). *ac = significant difference in rows (P<0.01).

Sperm parameters after 30 days of cholesterol feeding: Sperm counts in 1 ml of semen were $103.75 \pm 7.42 \times 10^6$, $123.75 \pm 8.32 \times 10^6$ and $116.27 \pm 6.92 \times 10^6$ for CFG1, CFG2 and CG, respectively, with significant differences between groups. The live sperm percentages for CFG1, CFG2 and CG were 76.33±3.28, 82.75±3.21 and 83.33±5.51, respectively, with a significant difference between CGF1 compared with CFG2 and CG. Abnormal sperm percentages were 5.75±1.22, 4.33±1.77 and 3.57±1.32 for CFG1, CFG2 and CG, respectively, with significant differences between groups, as shown in Table 3.

**Table 3** Sperms parameters of all groups on day 61 of age.

| GROUPS | Sperm count | Live % | Abnormal sperms % |
|---|---|---|---|
| CFG1 | $103.75 \pm 7.42 \times 10^{6c}$ | 76.33±3.28[a] | 5.75±1.22[a] |
| CFG2 | $123.75 \pm 8.32 \times 10^{6a}$ | 82.75±3.21[b] | 4.33±1.77[b] |
| CG | $116.27 \pm 6.92 \times 10^{6b}$ | 83.33±5.51[b] | 3.57±1.32[c] |

*ab = significant difference in columns (P<0.05). *bc = significant difference in columns (P<0.05). *ac = significant difference in columns (P<0.01).

The hormonal profile of all groups was recorded on day 61 of age, as shown in Table 4. Serum LH levels were 13.14±1.07, 14.03±1.98 and 5.34±0.87 mIU/ml in CFG1, CFG2 and CG, respectively, with a significant difference between CFG1 and CFG2 compared with CG. Serum FSH levels were 9.77±0.82, 10.29±0.78 and 7.23±1.46 mIU/ml for CFG1, CFG2 and CG, respectively, with a significant difference between CFG1 and CFG2 compared with CG. Serum testosterone levels were 3.62±1.32, 4.35±1.03 and 5.98±1.29 ng/ml for CFG1, CFG2 and CG, respectively, with a significant difference between CGF1 and CFG2 compared to CG.

**Table 4** Hormonal profile of all groups on day 61 of age (Mean±SD).

| Groups | Parameters | | |
|---|---|---|---|
| | LH (mIU/ml) | FSH (mIU/ml) | Testosterone (ng/ml) |
| CFG1 | 13.14±1.07[a] | 9.77±0.82[a] | 3.62±1.32[c] |
| CFG2 | 14.03±1.98[a] | 10.29±0.78[a] | 4.35±1.03[b] |
| CG | 5.34±0.87[c] | 7.23±1.46[b] | 5.98±1.29[a] |

*ab = significant difference in columns (P<0.05). *bc = significant difference in columns (P<0.05). *ac = significant difference in columns (P<0.01).

Histological findings of the liver in CG showed normal hepatic tissue, which was characterized by the presence of radially arranged hepatocytes around the normal central vein. At higher magnification (100X), the hepatocytes showed hexagonal and normal shapes, as shown in Figure 1 and Figure 2.
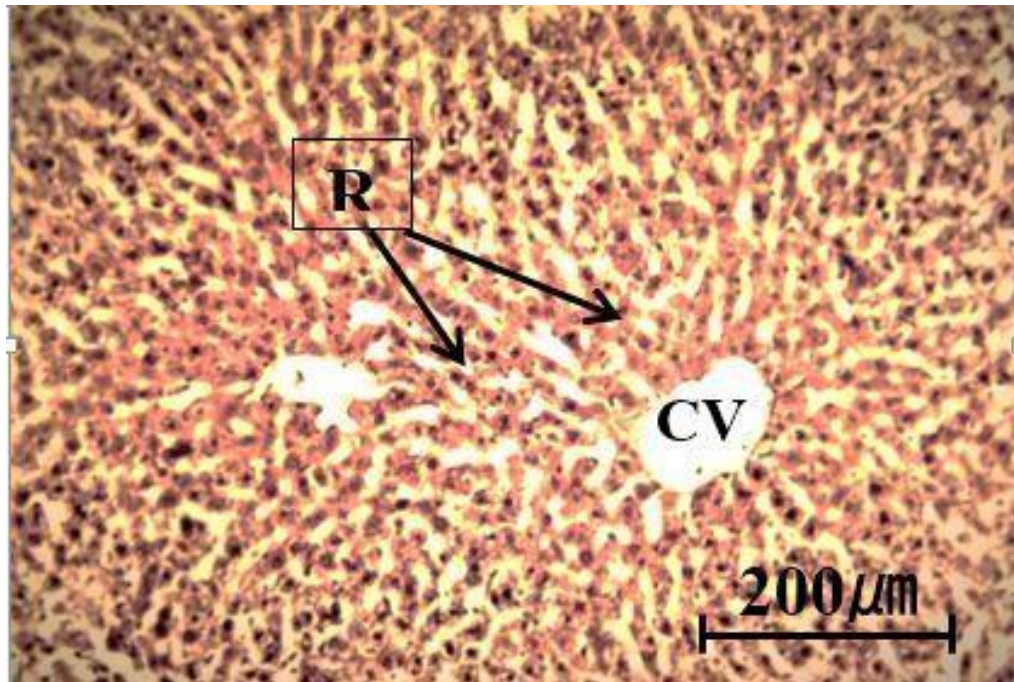


**Figure 1** Central vein (cv) with radially arranged cords of hepatocytes (R). The hepatocytes were normal in shape (H&E x40).
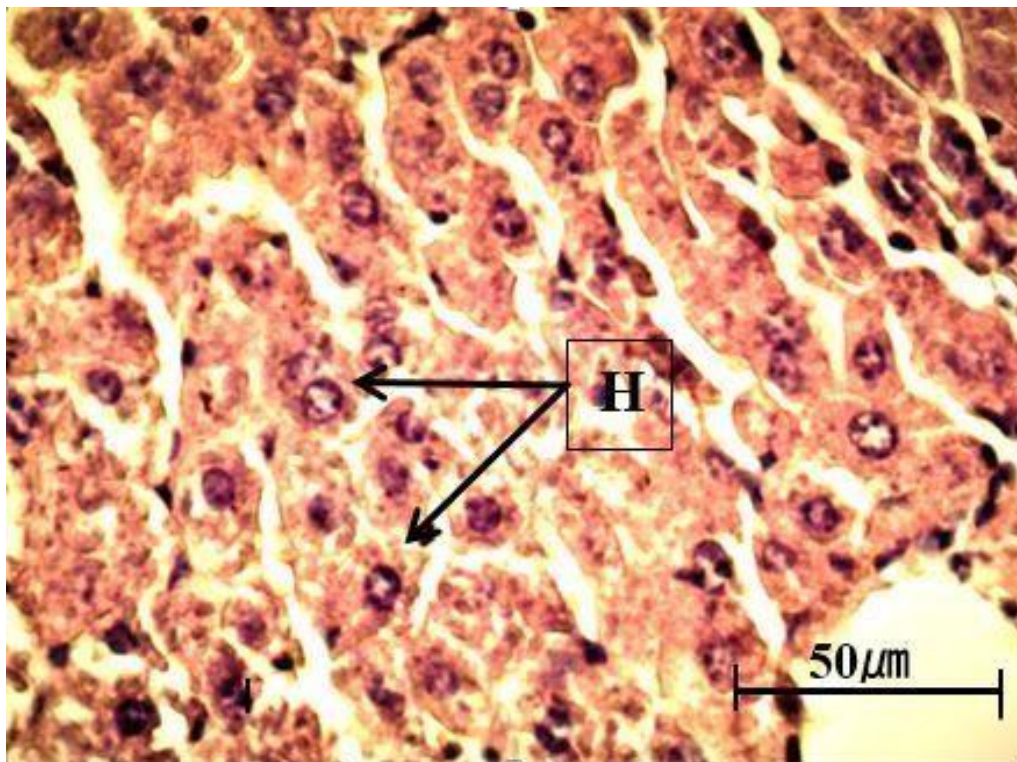


**Figure 2** Normal central vein with radially arranged cords of hepatocytes. The hepatocytes showed acidophilic cytoplasm with prominent nuclei (H). (H&E x100).

Histological findings of CFG1 livers after one month of cholesterol administration showed congestion of the central vein and loss of hepatic architecture. The bile duct showed congestion with hyperplasia, marked vacuolation of the hepatocyte, the presence of fatty changes within hepatocytes, hepatocytes that were binucleated, infiltration (aggregation) of inflammatory cells (mainly macrophages) within hepatic tissue, and hepatocytes, as shown in Figure 3 and Figure 4.
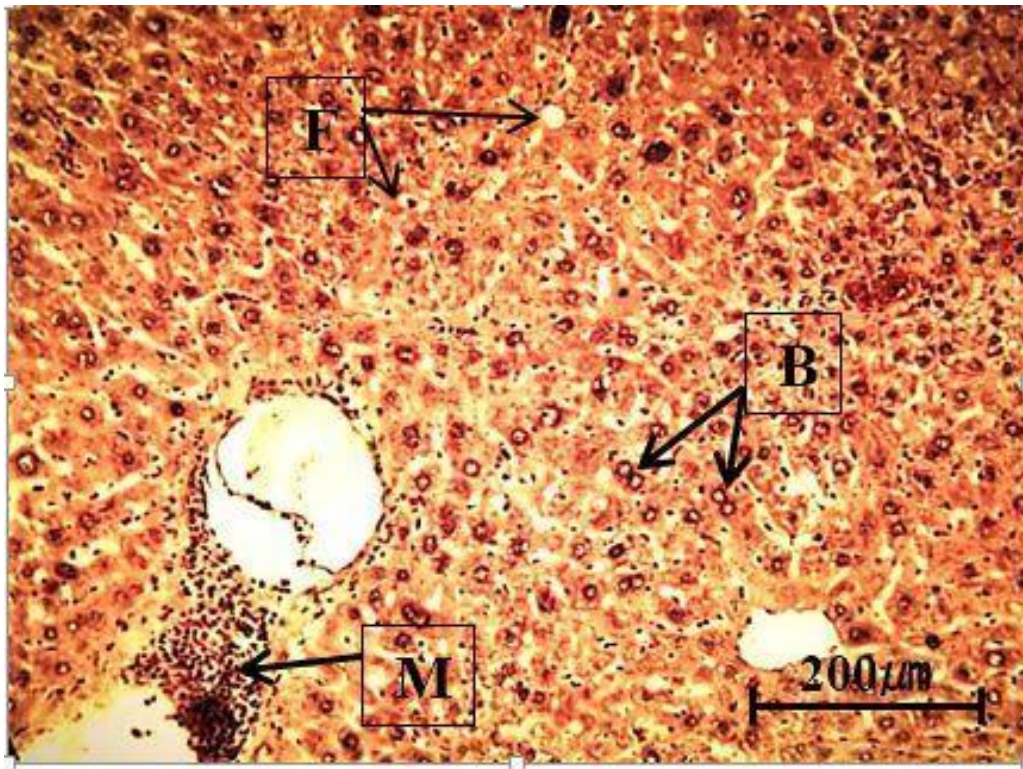
**Figure 3** Loss of hepatic cell architecture due to fatty changes (F). There was marked degeneration of hepatocytes, and most hepatocytes were binucleated (B). Additionally, there was infiltration of inflammatory cells in the hepatic tissue (M). (H&E x40).
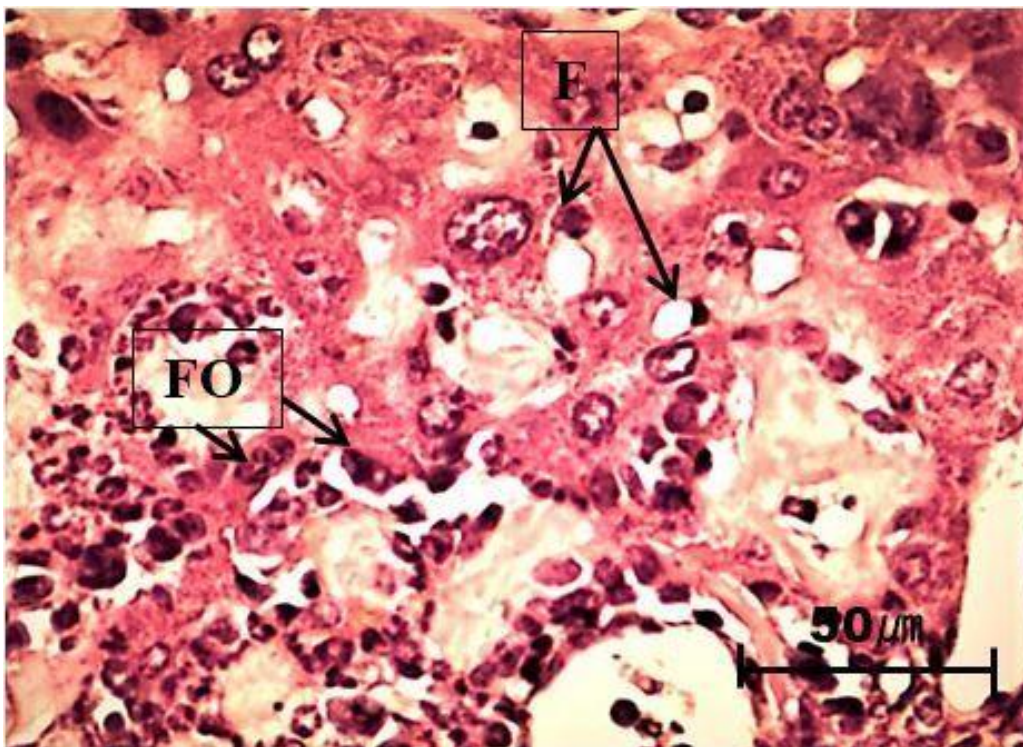


**Figure 4** Higher magnification. Note marked predominantly macrosvesicular steatosis (F) (the hepatocytes showed a signet-like shape). Additionally, there is infiltration of inflammatory cells, particularly foamy cells (FO). (H&E x100).

In the CFG2 (ALA) group, histological findings of the liver showed hepatic architecture (radial arrangement of hepatocytes around the normal central vein), dilation of sinusoids, mild proliferation of Kupffer cells, a normal hexagonal shape, congestion and mild hyperplasia of the bile duct (Figure 5 and Figure 6).
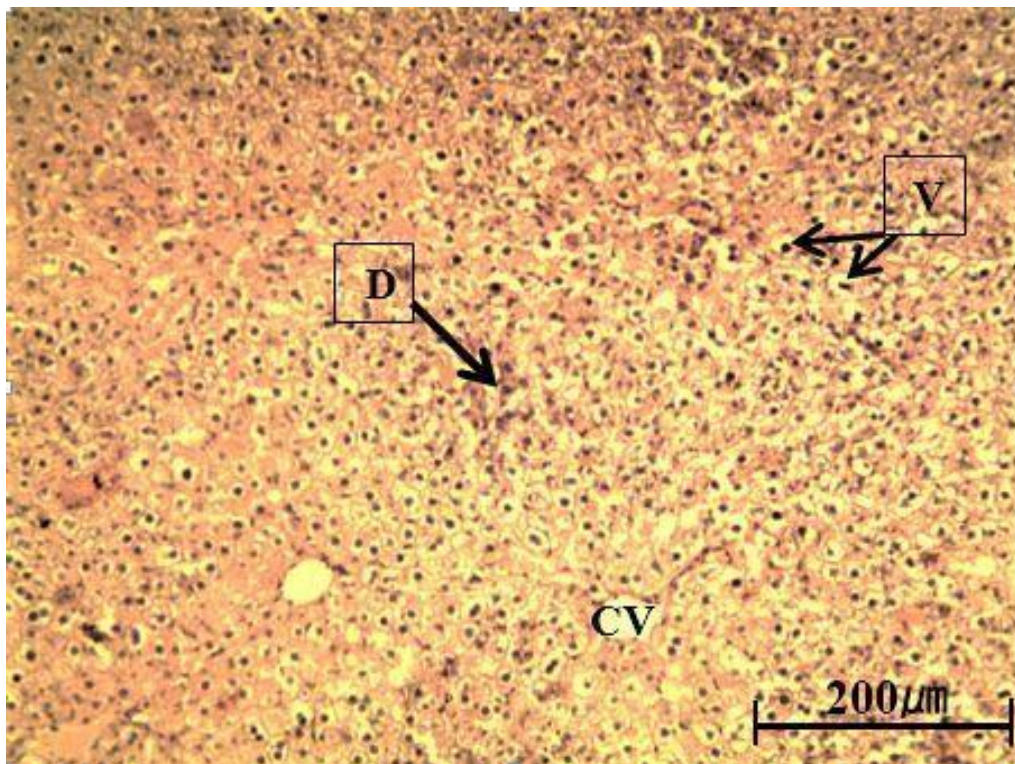
**Figure 5** There is a cytoarchitecture of hepatic lobules consisting of central veins (CV) and radially arranged hepatocytes (R). However, there was slight vacuolation (V) and mild degeneration (D) of some hepatocytes. (H&E x40).
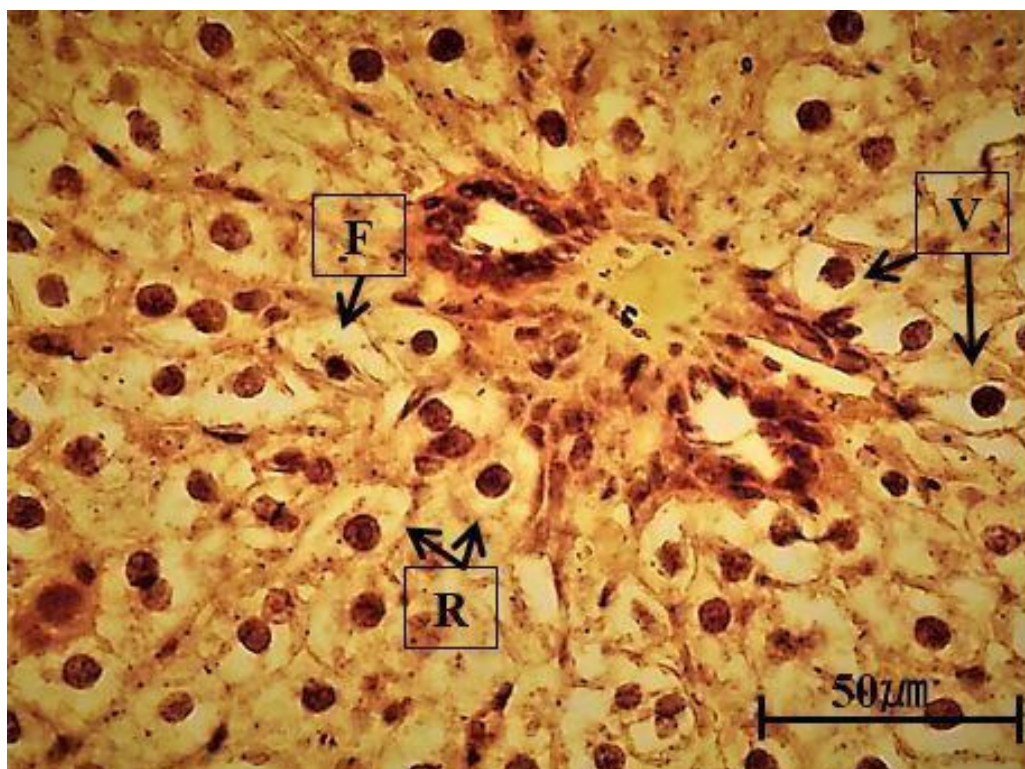


**Figure 6** There are normal radially arranged cords of hepatocytes (R). However, some hepatocytes showed slight fatty degeneration (F) and few vacuolation (H&E x100).

Histological findings of testicular tissue in CG characterized by the presence of complete spermatogenesis with compact seminiferous tubules that appear circular and normal in shape. Higher numbers of spermatogonia, primary and

secondary spermatocytes and spermatids can be seen in the lumen of seminiferous tubules, as represented in Figure 7 and Figure 8.
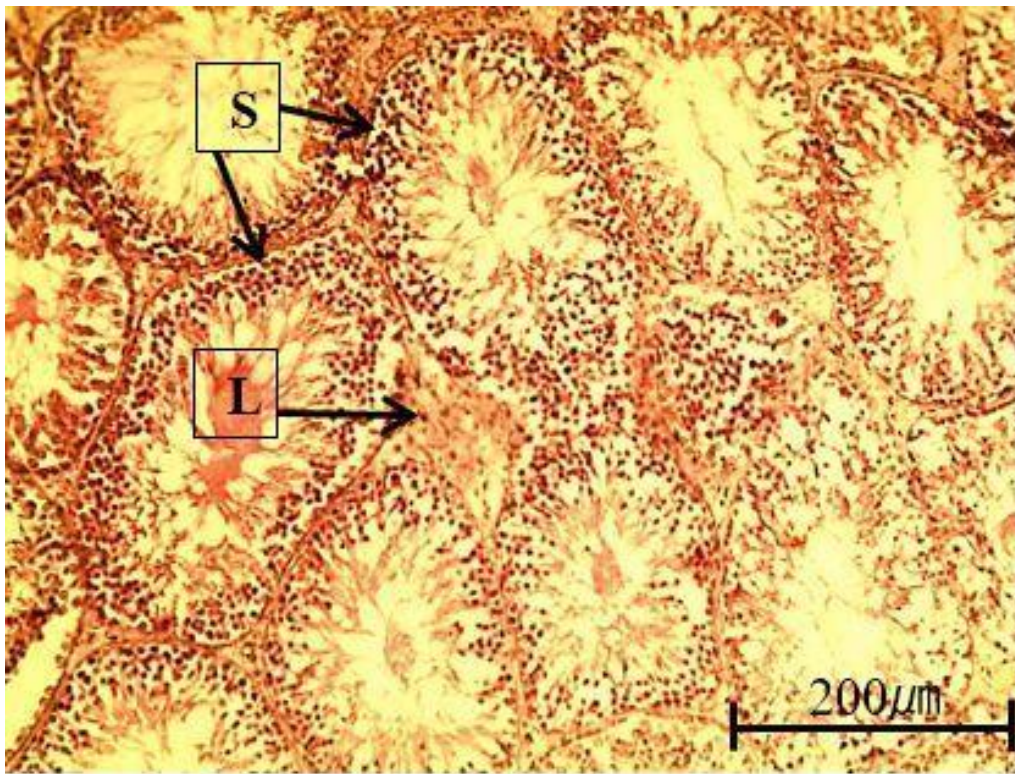


**Figure 7** Normally arranged seminiferous tubules that are circled and compact (S) with complete spermatogenesis. In the interstitium, there were Leydig (interstitial) cells (L). (H&E x40).
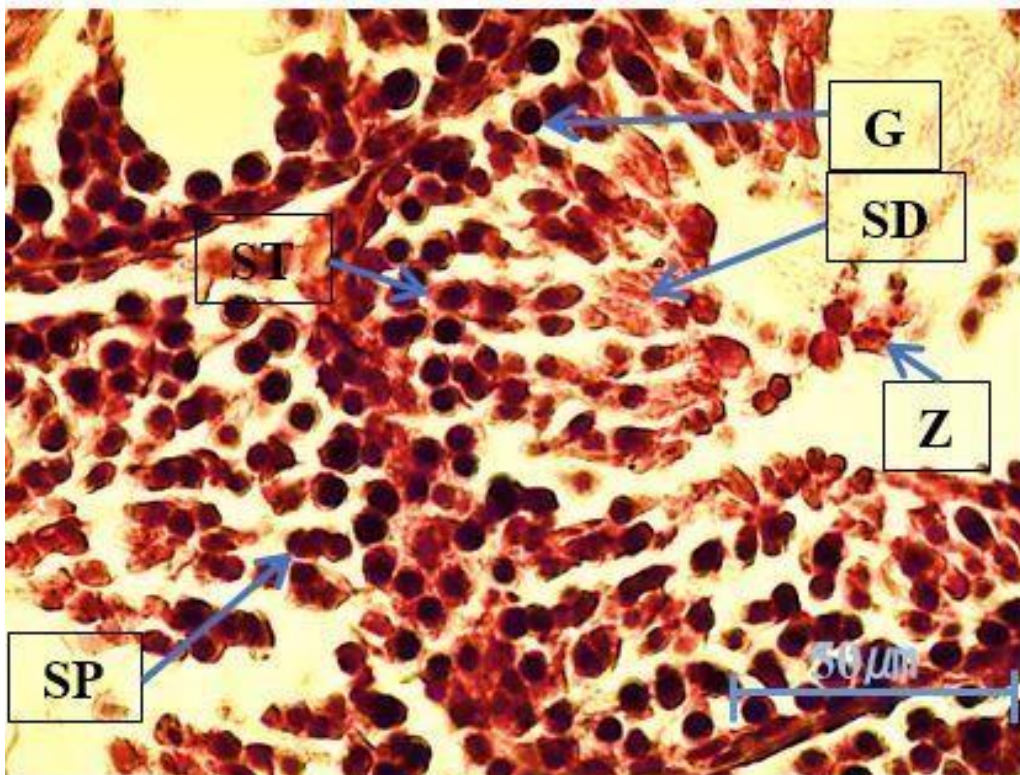


**Figure 8** Histological section of a seminiferous tubule that shows complete spermatogenesis with all testicular cells, such as spermatogonia (G), primary and secondary spermatocytes (SP), Sertoli cells (ST), spermatids (SD) and spermatozoa (Z) (H&E x100).

Histological findings of testes in the cholesterol feeding group (CFG1) showed suppression of spermatogenesis characterized by vacuolation of spermatogonia, with few numbers of primary and secondary spermatocytes, absence of sperm in the lumen of the seminiferous tubules, which were very wide, and few Leydig cells in the interstitial tissue with the presence of adipose tissue, as shown in Figures 9 and 10.
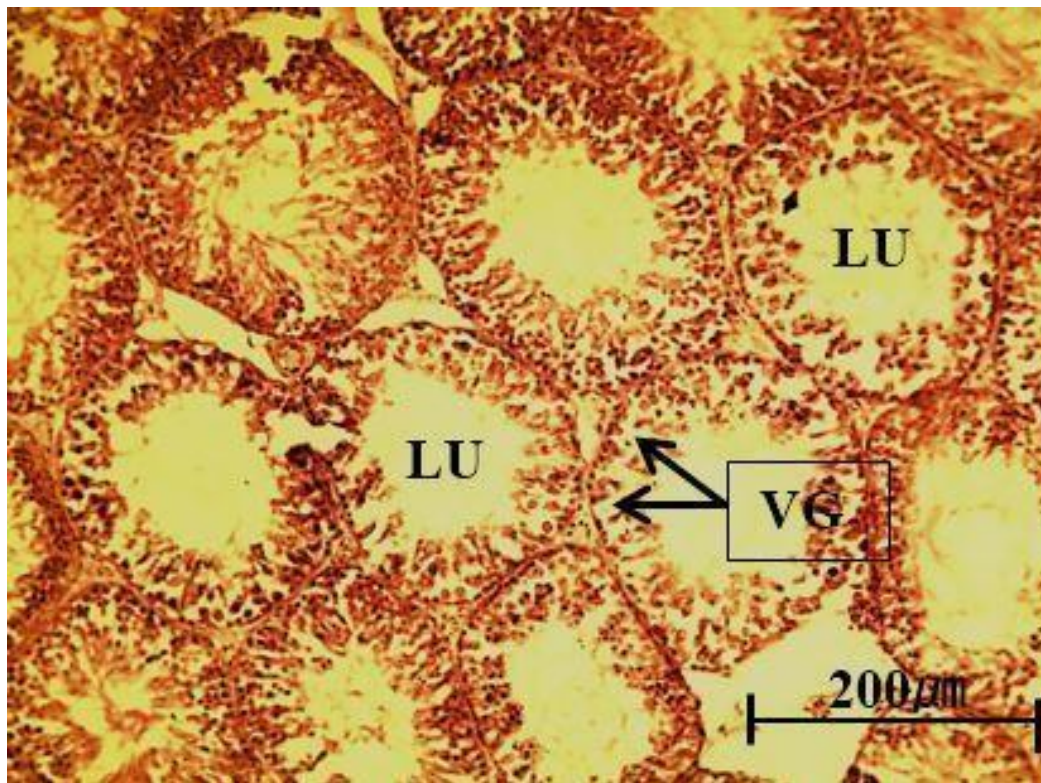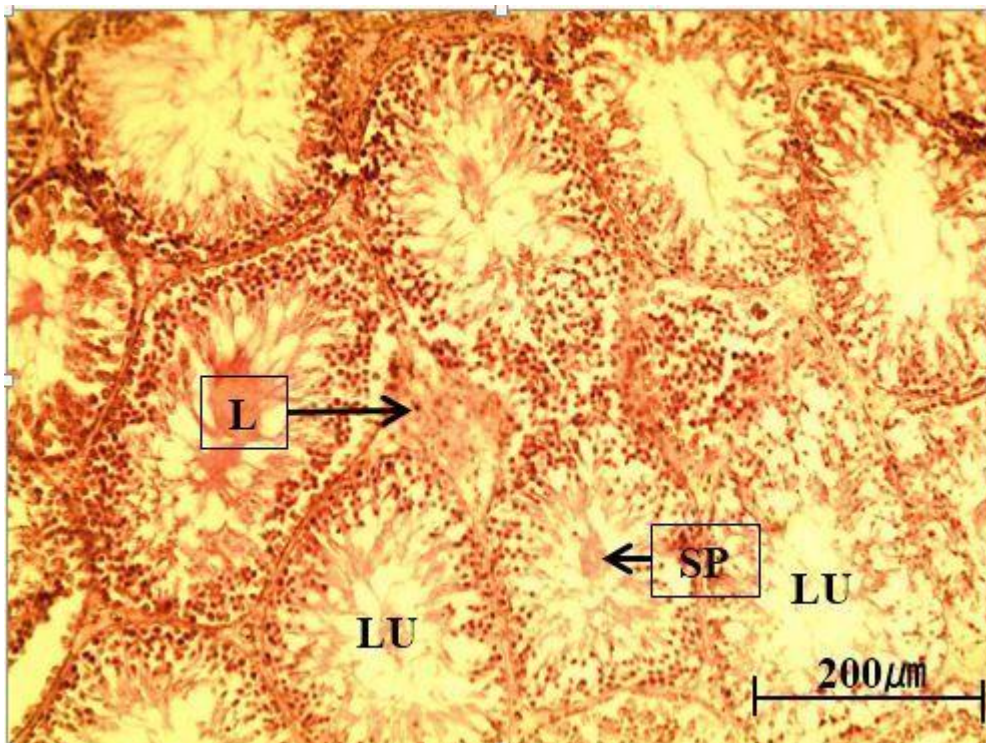


**Figure 9** Vacuolation of spermatogonia (VG) and the tubules are completely devoid of sperm with a wide lumen (LU) (H&E x40).
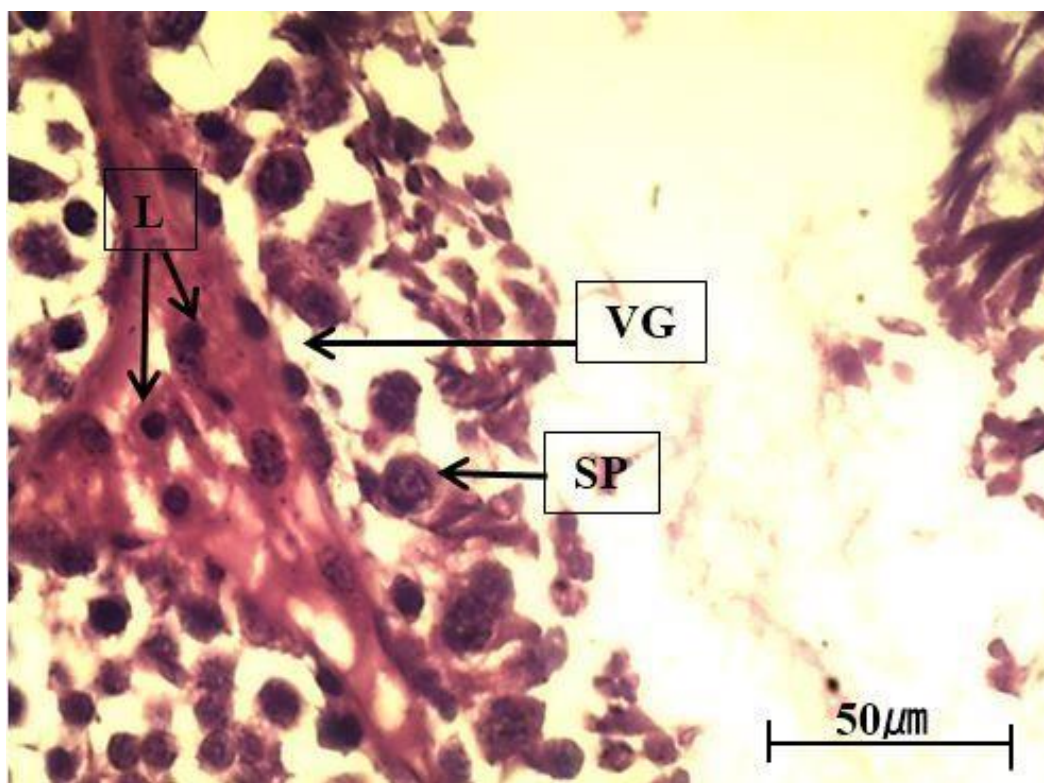


**Figure 10** Note vacuolation of spermatogonia (VG) and few spermatocytes inside the seminiferous tubule (SP) with few Leydig cells (L) (H&E x100).

In CFG2, complete spermatogenesis is characterized by the presence of spermatogonia and high numbers of spermatocytes and spermatids in the lumen of seminiferous tubules. There is mild vacuolation of spermatogonia in a few seminiferous tubules and proliferation of Leydig cells in the interstitial tissue, as shown in Figure 11 and Figure 12.



**Figure 11** Complete spermatogenesis without the vacuolation of spermatogonia (G). Presence of moderate amounts of sperm in the lumen of the tubules (SP), with decreased diameter of their lumen (LU) and proliferation of Leydig cells (L) (H&E x40)
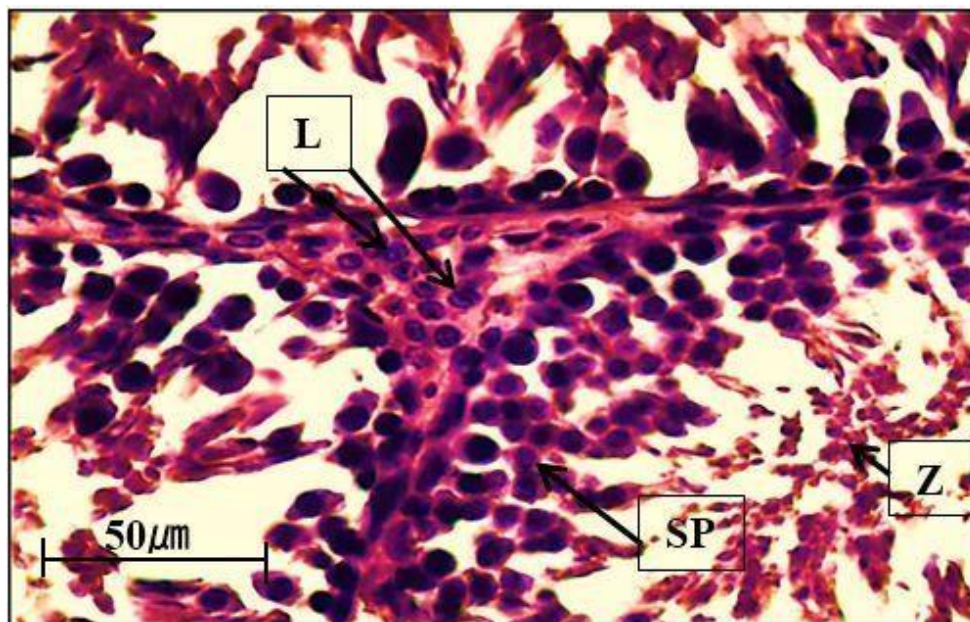


**Figure 12** Complete spermatogenesis (SP) with all testicular cells within the seminiferous tubules and the presence of spermatozoa in the lumen of the tubule (Z). In the interstitium, there is hyperplasia and proliferation of Leydig cells (L) (H&E x100).

## 4. Discussion

Table 1 shows that the body weights of CFG1 rats were increased significantly compared with those of CFG2 and CG rats after one month of cholesterol feeding due to high lipid intake, which causes the accumulation of lipids in the body and internal organs of rats, as represented by increasing body weight and inducing fatty changes in hepatocytes such as vacuolation and loss of architecture. Additionally, the blood levels of total cholesterol and triglycerides were increased

significantly in CFG1 and CFG2 rats compared with CG rats after one month of high lipid intake, as shown in Table 2. These results resemble the results of (Okazaki et al 1998; Ismail et al 1999; Ali et al 2000; Bennani et al 2000; Yu-Ming et al 2010; Karam et al 2022) who fed rats and mice 0.5-1% cholesterol for several weeks and gain body weight increase and hypercholesterolemia with hypertriglyceridemia.

This study was performed to induce partial infertility by feeding a high lipid content diet, which causes hyperlipidemia that affects the spermatogenesis process. Our results meet the goal of reducing rat fertility, as shown in Table 3. The results are similar to many previous data obtained from feeding a high cholesterol diet to lab animals (mice, rats, rabbits), which caused detrimental effects on testicular tissue and functions such as spermatogenesis, steroidogenesis, epididymal sperm maturation process, sperm quality parameters, sperm fertilizing capacity and fertility index (Lin and Ding 1996; Tanaka et al 2001; Shalaby et al 2004; Bataineh and Nusier 2005; Saes et al 2010; Ouvrier et al 2011; Ashrafi et al 2013). Several other studies (Diaz-Fontdevilla et al 1992; Diaz-Fontdevilla et al 1993; Yamamoto et al 1999) have suggested that feeding a high cholesterol diet for several weeks causes an adverse effect on the secretory functions of Leydig and Sertoli cells, which reduces sperm concentration and motility percentages and increases abnormal sperm morphology. Ouvrier et al. (2011) suggested that high-cholesterol diet intake can alter the epididymal epithelium structure due to the accumulation of cholesterol droplets in the smooth muscles that line the epididymal epithelium, weakening the peristaltic movement of the epididymis and delaying sperm maturation and progression. Table 4 shows that high lipid diet intake decreased serum levels of testosterone. Similar results were recorded by Martinez-Martos et al. (2011), who mentioned that hypercholesterolemia inhibited the steroidogenesis process in the testis by modulating the bioactive peptides of the rennin-angiotensin system that occur in testicles, which leads to decreased testosterone production. Tanaka et al. (2001) mentioned that hypercholesterolemia leads to reduced rat serum testosterone levels due to the reduction in testicular LH/HCG binding. Many studies on humans (Vignon et al 1989; Zmuda et al 1997; Ergün et al 2007; Alqubaty 2013) have reported a negative correlation between serum triglyceride levels and serum testosterone levels. They mentioned that hypercholesterolemia leads to elevation in serum triglyceride levels, which have deleterious effects on spermatogenesis, decrease sperm motility and decrease serum testosterone levels in infertile men. Our results showed the same data in a rat model. Hypercholesterolemia also causes damage to testicular tissue due to the excessive formation of free radicals that have cytotoxic effects on spermatozoa (Karam et al 2022). Previous studies have indicated that a diet supplemented with antioxidants and/or agents enhances lipid metabolism and can maintain the reproductive functions of the testis in hypercholesterolemic rats (Shalaby et al 2004; Bashandy 2007; Fang et al 2013). ALA is known to have antioxidant (Packer et al 1995) and anti-inflammatory (Shay et al 2009) properties. ALA can easily cross biological membranes because of its small size and high lipophilicity (Suzuki et al 1991). ALA can improve mitochondrial functions due to its ability to stimulate Sirtuin 1 and 3 (Valdecantos et al 2013), so it can quench free radicals and inhibit ROS generators. Furthermore, it downregulates the ALA-dependent pro-inflammatory NF-kB pathway (Shay et al 2009)). Our study showed significant testicular histological protection in the hyperlipidemic group of rats treated with ALA compared with the hyperlipidemic group that was not treated. This may be attributed to the ability of ALA to scavenge many free radicals, such as singlet oxygen, $H_2O_2$ and hydroxyl radicals, which form excessively during lipid peroxidation and metabolic processes, according to the interpretation of Moini et al. (2002). The results of this study showed good histological protection for spermatogenic layers in the ALA-treated group when compared with the non-ALA-treated group, which reflects the better concentration of sperm and the lower percentage of sperm abnormalities in the treated group.

## 5. Conclusions

According to our study's results and discussions, oral administration of ALA (100 mg/kg.B. W) with a diet established to lower ROS has a considerable favorable influence on male reproductive function by increasing sperm parameters. In addition to being a hepato-protective agent and having an anti-hyperlipidemic effect by lowering blood cholesterol and triglyceride levels, dealing with ALA in premature male rats resulted in the greatest reproductive profile.

## Ethical considerations

Approval for this study was obtained from the Ethics Committee of the College of Veterinary Medicine, University of Al-Qadisiyah under Ref. number 543/2018.

## Conflict of Interest

The authors declare no conflicts of interest.

## Funding

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