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Histological Changes and Some Biochemical Analysis in Male Rats Exposed High Fat Diet

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Abstract
Fats enriched diets greatly raise the level cholesterol level in body tissues. The current study aims to studying the effects of dietary cholesterol on the histological changes in the testis of the male rats& physiological aspects of lipid profile in relation with cholesterol diet. Ten male of albino rats were subdivided into two groups (n=5): group 1 was given 1 ml of normal saline & considered as control group(G1), while the second group treated with enriched -cholesterol nutriment once at a dosage of 1.5 mg / k g body weight extent to 8 weeks & considered as treated group(G2) . The tissues of testes were obtained & stained with H & E stain. Blood samples were withdrawn after starvation of the animals at night after the end of the experiment to study the following parameters: measurement the intensification of (T C), (T A G), (H D L), (L D L) & (V L D L). Result showed the presence of a significant increase ($p < 0.05$) in the concentrations of TC, TAG, LDL, VLDL & significant decrease ($p < 0.05$) in the concentrations of HDL in processed group (G2) compared with the control group (G 1). Histological results from rats treated with cholesterol have showed histological abnormalities of testis structure represented as reduced sperm cells and thickened layers intertubular connective tissue, distorted Leydic cells, and thickened blood-vessel walls. is concluded from the current study that High-cholesterol diet causes defects in the in-testis tissue with alternation in the lipid profile in male albino rats.

Keywords: cholesterol, histology, physiology, testi

INTRODUCTION

Cholesterol is a high molecular weight sterol. Cholesterol is not a fat. (It is fat soluble). Cholesterol,found in all animal tissues.and it is a waxy material Chemically present and in blood plasma , Cholesterol is an organic compound of the steroid family; its molecular formula is C27H46O. Cholesterol is an organic compound of the steroid family. It is a white, crystalline material in its pure state which is tasteless and odourless (1).

Cholesterol is a central molecule in animal physiology owing to its importance in the maintenance of cell structure, bile salt metabolism cholesterol functions in a particular aspect of male rat. It is a major structural one constituent to Cell membrane permeability, and fluidity. Solving the balance between assembling And Cholesterol Catabolism. The diet is the principal source of cholesterol. The dietary cholesterol is first released into the liver from the small intestine and then belongs to the organs demanding **(2)**.

Administration of cholesterol diet can cause increase in body weight. The weight of the testes, epididymitis', seminal vesicle, ventral prostate and vas deferens were significantly decreased **(3)**.

As referenced by (4) the lipids cannot be transport in plasma or any kind of aqueous solutions, simply because it is a water insoluble molecule, that lead to lipids can transport in the plasma as macromolecular complexes as it called as lipoproteins. However, the free fatty acids able to transport but bounded to Albumin. In addition, it does not need to incorporation into lipoproteins to transport. Author has been used lipoproteins contain one and/or a variety of Apolipoproteins, and the metabolic functions were regular. Different physiological functions of lipoproteins were involved on Apolipoproteins such as lipids transport facilitations, structural integrity maintenance and enzyme activation. In other hand enzyme activation play a big role and it considers as a key for lipid metabolism. There is an enzyme locates in luminal surface of hydrolyzes triglycerides with lipoproteins as free fatty acids, mono and Di- glycerides, glycerol and capillary endothelial cells, this enzyme is called Lipoprotein lipase(5)

Cholesterol is the absorption of lipoproteins, the role of cholesterol in sperm maturation and male fertility, that the basic sperm development of vital cellular events that control its level during sperm formation and after sperm release. This is due to the extensive production of germ cells throughout the sperm In the physiology of mammals, sperm formation is particularly important for the reproductive system It is the beginning of the steroid hormone synthesis as well as cholesterol is important for sperm functions, not only at the level of gametogenesis **(6)**.Unbalanced cholesterol may affect post testicular sperm function, which is necessary for its maturation post testicular events **(7).**

The study's goal isto know the effect of cholesterol in the histological and physiological changes in male testicles in rats.

MATERIALS & METHODS

Experimental routine were authorized by the veterinary collage of Karbala University. Ten male rats with age surrounded between 12-16 weeks with weight (200-300) gm

were obtained from the house of laboratory animals at Biotechnology Research Center of collage. The animals were subdivided into two groups (five rats /group), the first group was given 1 ml of normal saline & considered as control group (G1), while the second group treated with high-fat diet once at a dose of 1.5mg/kg body weight & considered as treated group (G2). Blood samples were withdrawn after starvation of the animals at night, after the end of 8 weeks, when 5 ml of blood was withdrawn from the heart. The blood was then placed in clean plastic tubes, especially the non-containment of anticoagulants. At a speed of 3000 cycles / minute for 15 minutes to measure some of the following parameters: concentration of total cholesterol (T C), triglyceride (T A G), high density lipoprotein (H D L), low density lipoprotein (L D L) & very low density lipoprotein (V L D L).The concentration of cholesterol was measured according to method **(8),**the determination of the concentration of HDL was done by enzymatic method **(9).** While LDL was measured according to the Fried Ewald Equation. VLDL concentration can be calculated by dividing the value of TAG by 5**(10).** The concentration of triglycerides by enzymatic method was estimated according to the method **(11).** The histological sections of testis were stained with H & E stain, according to the method of Histological technique **(12).**

Statistical analysis

These studies was analyzed with using the A N O V A test, the variables shown as +/- S D & a value of (P < 0. 0 5) was considered as significant, the statistical analysis was done by using of the (S P S S Ver.19) **(13).**

RESULTS AND DISCUSSIONS

Histological changes study

There is general consensus that diet induced male obesity reduces sperm motility in rodent models, decreases sperm counts with increased epididymal sperm transit time and decreases the sperm count with normal morphology **(14, 15)**. The study rat group's main finding is The high-fat diet exposure leads to long term changes in the male reproductive system and the metabolism of rats which may include reproductive activity programming mechanisms and metabolism that affect many harmful testicular changes, showing few Sperm cells in the layers of dense intertubular connective tissue that distort Leydic Cells containing karyolobic Nuclei and Cytoplasm Degenerated, wider blood vessel walls, were accompanied by a decrease in Mature sperm count inside the tests (Figure 1,3 and 5), whereas the control rat group showed normal testis structure (Figure 2,4 and 6). This opinion is in line with those of the authors (**14, 15, and 20)**. In addition, our research reported an increase in testicular defects systems, Including Epithelial disturbance, in 6 Weeks old fat (20 basis points fat) feeding Prague-Dawdle rats (16). Generally speaking, they also previously demonstrated male Prague-Dawdle rats feeding of high feed HF (35 percent fat) from 21 to 90 days of life has not shown atypical coniferous tubule morphology (17). Suggesting that nutritional insult may influence fat intake reaction and practice differently based on the relative dietary fat material. A possible reason for these changes is shown by (18), which research showed a decrease in plasma concentrations.

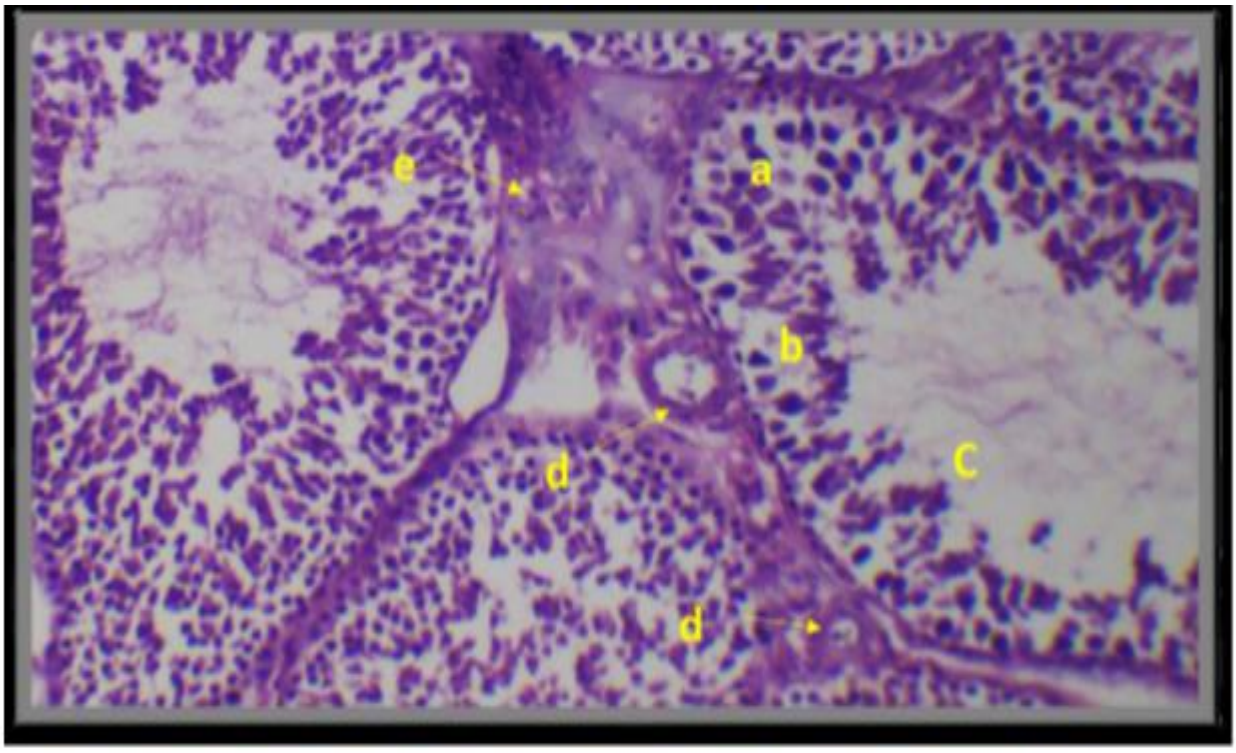


Figure (1). Showing photomicrographs of the testis treated group of chol. (H & E, x 20): Group of chol. (H & E, x 20): showing undeveloped spermatogenic layers (a) with odema in interstitial spaces (b) reduced sperm (c) and thickened blood vessel walls (d) with karyolobic nuclei in distorted Leydic cells showing regressed cytoplasm (e)

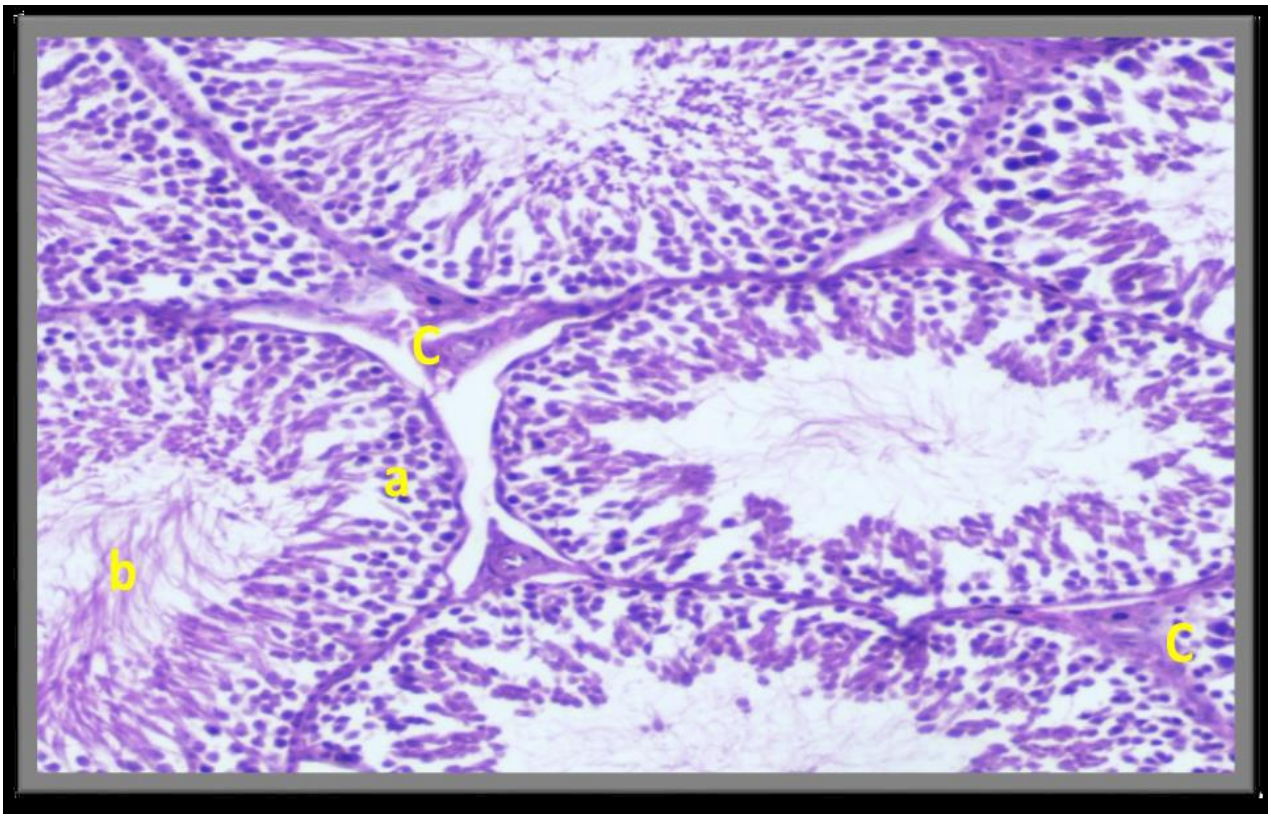
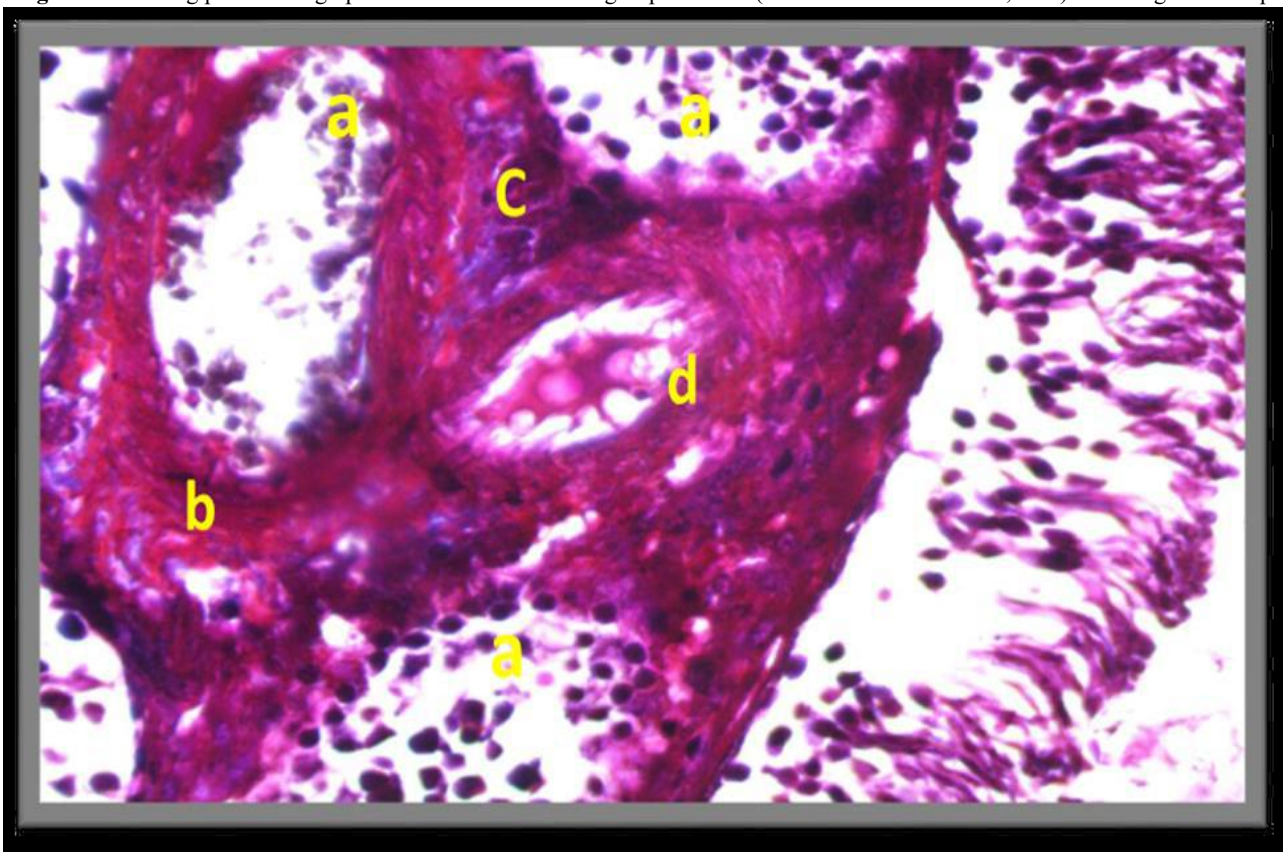


Figure 2. Showing photomicrographs of the testis group (cont.) (H&E stain, x 20): Showing the normal architecture of seminiferous tubules and structures of spermatogenic cells of it (a) normal mature spermatozoon within tubules (b) with normal organization of lydic cells and c.t inter tubular (c)

Figure 3. Showing photomicrographs of the rat testis treated groups of chol. (Masson´s trichrome stain, x 40): Showing undeveloped



spermatogenic layers with odema in the interstitial spaces (a) c.t inter tubular seminiferous is thick ened (b), with several nuclei in the karyolobic distorted of Leydic cells which revealed regressed cytoplasm (c) and thinking wall of the blood vessels (e)

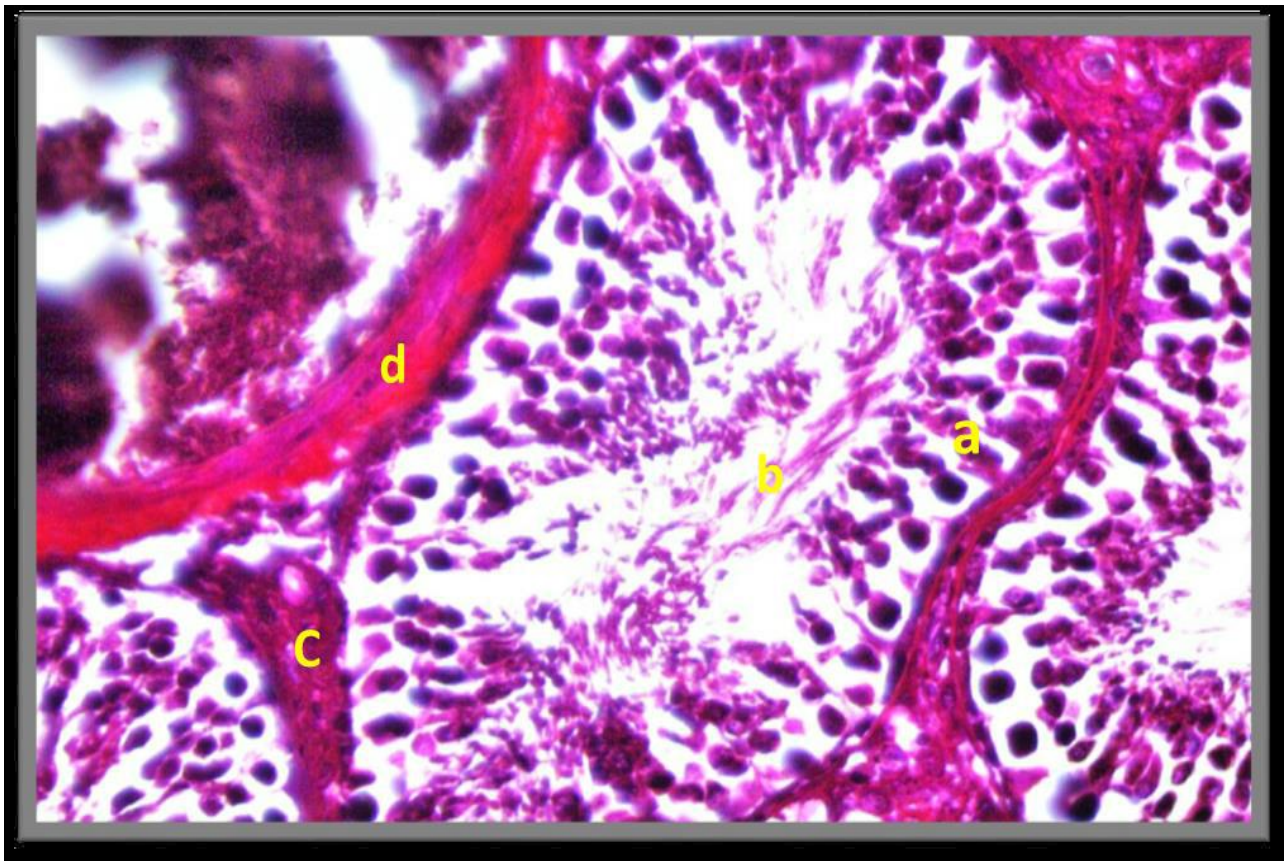


Figure 4. Showing photomicrographs of the testis group (cont.) (Masson´s trichrome stain., x 40): Showing the normal structures of spermatogenic cells of the seminiferous convoluted tubules (a) normal mature spermatozoon within tubules (b)with normal organization of lydic cells & c.t inter tubular (c) with certain normal blood vessles (d).

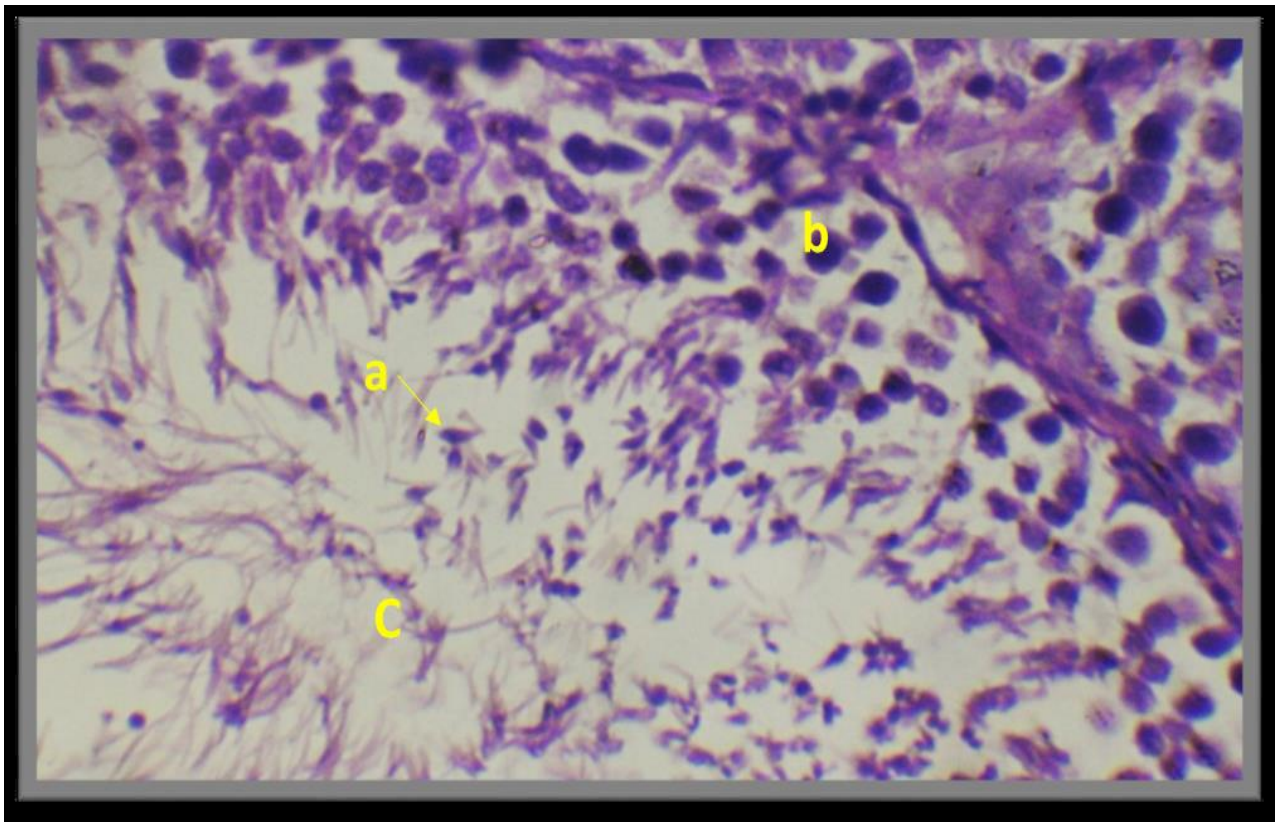


Figure 5. Showing photomicrographs of the testis of the treated group chol.(H & E, x 40).:Showing seminiferous tubules which contain immature spermatids with elongated head and very short tail (a), reduced cells of the spermatogenic layers (b)and presence of immature spermatozoa (c)

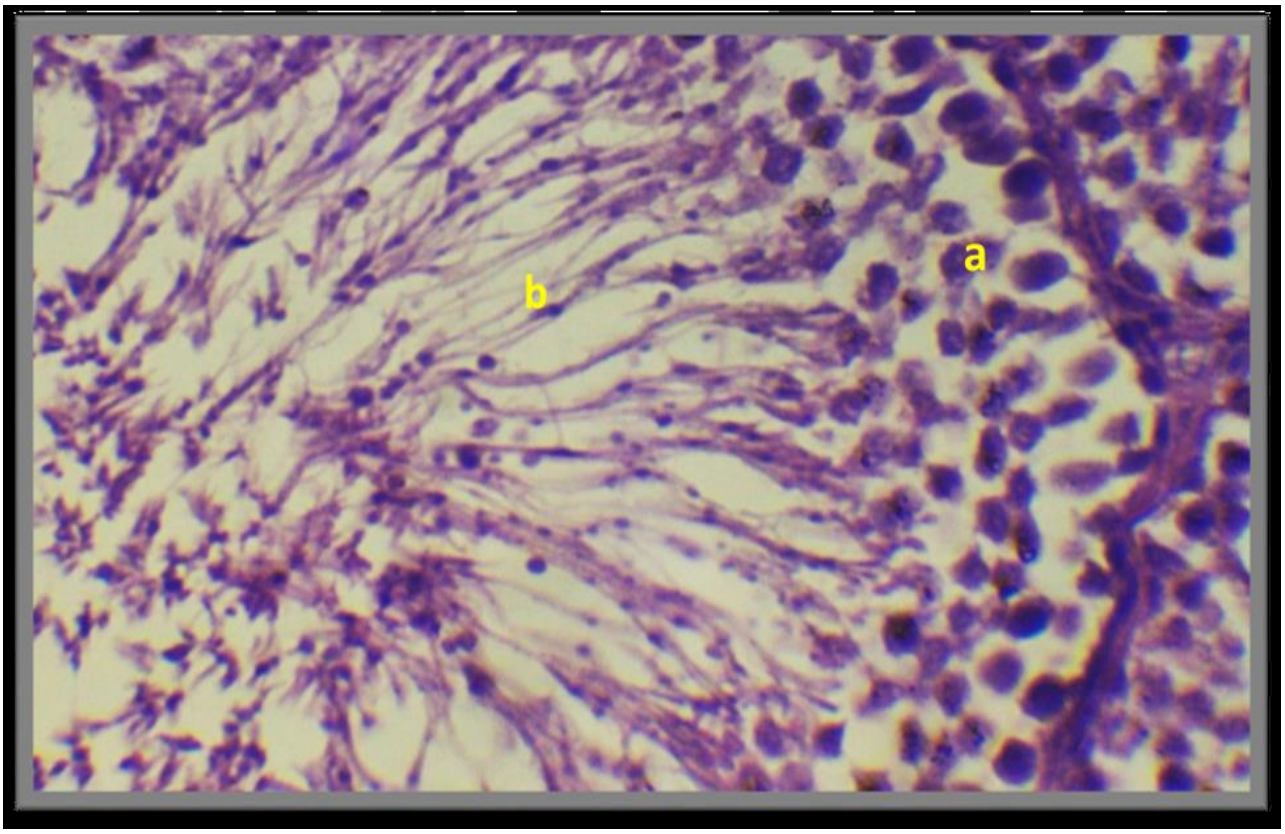


Figure 6. Showing photomicrographs of the testis group(cont.) (H & E, x 40). Showing the normal structures of spermatogenic cells of the seminiferous tubules (a) normal mature spermatozoa within tubules (b).

Physiological changes study

Measurement of Serum lipid profile

The Result of table 1 showed the presence of a major escalation (p <0.05) in the concentrations of T C , TAG , L D L , & V L D L ; & significant decrease (p <0.05) in the concentrations of H D L in treated group (G 2) compared with the control group (G1)**.**

Table 1. The Effect of high fat- diet on serum lipid profile in male albino rat.

VLDL	LDL	TAG	HDL	TC	Parameters\groups
0.02± 9.01	±0.15 29.11	0.13± 45.10	0.12 21.86 ±	±0.17 42.50	Control Group (G1)
* ±0.42 22.83	* ±2.19 124.16	* ± 0.30 112.20	* ± 0.30 11.94	* ±2.15 149.10	Rats treated with 1.5 mg/kg of cholesterol (G2)

Mean ± standard error, * = significant difference.

The diet interventions in an animal model (mouse) of male weight gain induced by a heavy-fat diet (HFD) recently showed that sperms function was closely linked with the metabolic health, to the serum glucose normalizing, total cholesterol, triglyceride levels, low-density lipoproteins (LDL) considered bad cholesterol, while (HDL) is called heavy-density lipoprotein good cholesterol, restoration is based on good cholesterol., restoration is associated with sperms motility, morphologically , oxidative stress, DNA damage and sperms binding **(19).** However, there is evidence about the alterations of fatty acid & histological abnormalities of in testis tissues after feeding with high cholesterol diet. Many studies showed that feeding of 2% cholesterol for 8 weeks might be associated with changed free fatty acid, proteins related to lipid metabolism, in parallel to histological alterations. The feeding of rats with high cholesterol-fed displayed considerably increased in serum cholesterol levels compared to controls. **(7,20)**.

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