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Effect of different doses of L-carnitine on fertility of the male reproductive system of albino rats

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ABSTRACT

In the current study, different doses of L-carnitine were tested on 20 healthy albino male rats to determine the effect of L-carnitine on the physiological and histological parameters of the reproductive system. The animals were distributed into four groups, the first group was the control group. In this group the animals were not given any dose of L-carnitine, The second group was the treatment group and in this group the animals were dosed with L-carnitine at a dose of 28 mg / kg, and the third group was the double dose group, as the animals dosed 56 mg / kg of carnitine, while the fourth group was dosed with an overdose when they were dosed with 112 mg / kg of L- carnitine .

The results of the hormonal study showed that the double dose and the double dose group led to a significant decrease in the level of ICSH, SSH and testosterone, while the treatment group had no significant differences compared to the control group. The treatment group did not notice significant differences in the level of male hormones compared with the control group.

The results of the study showed that the sperm parameters of the treated group were normal, while the sperms in the double and excessive group of L-carnitine showed the presence of twisted tailed sperm and the occurrence of deformation in the sperm head. The testicular tissue sections in the L-carnitine treatment group showed normal spermatogenesis during spermatogenesis, spermatogenesis and Leydig cells, while the L-carnitine double group showed a low number of mature sperms. Also, in the testicular sections of the doubled L-carnitine group, the occurrence of histological necrosis of the testicles was found. Additionally, the stages of spermatogenesis are not clear.

Keywords: Albino systems; ICSH; SSH; L-carnitine

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INTRODUCTION

L-carnitine (LC) is an essential amino acid that perform a significant role as a cofactor in production of cellular energy in the mitochondrial matrix, it is transport of activated acyl groups across the mitochondrial inner membrane and needed for the oxidation of long-chain fatty acids in the mitochondria of all cells [1]. carnitine is mostly taken in diet and then stored in skeletal muscle [2, 3]. Carnitine is a compound which has D and L-form ,the last one form is metabolically active form that only endogenously synthesized in tissues [4] such as brain, kidneys and liver via conversion from lysine and methionine [5, 6]. L-carnitine increases synthesis of phospholipids, which are necessary for cell membranes, ensures re-acylation of phospholipids, resulting with integrity of membrane structure or repair of damages [7]. L-carnitine plays a protective role as a lipid peroxide preservative (LPO) by antagonizing H2O2 and its ability to remove the superoxide radical [8,9]. In addition, there are many important biological activities of carnitine, including the protective role of the heart and stomach, anti-inflammatory, and anti-ovulation [10,11]. L-Carnitine plays a preventive role against anemia and thrombocytopenia and reduces oxidative stress in the heart of mice. [12]. Through several experimental and clinical studies, it was found that the total carnitine level in the seminal plasma was low in patients with idiopathic oligospermia, while oral administration of L-carnitine improved sperm motility [13]. It was also found that adding L-carnitine to diets, fodder and food rations, as an antioxidant substance, had positive effects on overall metabolic activity and increased mitochondrial function without any increase associated with oxidative stress. [14].

There is L-Carnitine In the epididymis and sperms its concentration is about 2000 times higher than that o Blood plasma: L-carnitine is important for sperm It facilitates the metabolism of fats into long fatty acids and is necessary for energy production . Acts as a great non-enzymatic antioxidant that protects mitochondria and cell membranes, as well as DNA integrity against free oxygen radicals. [15] . L-Carnitine works to stabilize the membrane and also play an important role in the process of sperm maturation and development. In addition, L-carnitine protect sperm DNA and cell membranes from reactive oxygen species (ROS) that promote damage and programmed cell death, and L-carnitine works to restore the phosphorylation of mitochondrial membranes by reducing fatty acid oxidation. [16].

MATERIAL AND METHODS

2-1-Experimental Design:

The animals were randomly assigned to four groups as follows:

Group 1 – Assign this group as a control group as the animals were given distilled water.

Group 2 – Therapeutic group: L-carnitine animals were given (28 mg / kg of b.w) for 30 days.[17].

Group 3- The Double group: was given a double dose of L-Carnitine (56 mg / kg b.w) for 30 days

Group 4 – Overdose: An overdose of (112 mg / kg b.w) of L-Carnitine has been administered for 30 days.

2-3-Blood Collection

At the end of the experiment, the animals were starved for 24 hours and sacrificed after chloroform anesthesia. Blood samples were taken by clot and allowed to clot. Serum was separated by centrifugation at 2500 rpm for 15 min using a centrifuge. The serum was stored at freezing point. (-45C°).

2-4-Sperm Preparation and Staining

After dissecting the animal, the right epididymis was taken, cut and placed in physiological salt (0.9). A drop of semen solution was taken on a clean, dry glass slide. Then, a similar amount of eosin-necrosin dye was added, then mixed well and spread on the slide to make a small smear to observe the shape and vitality of the sperms by light microscopy at a magnification power of 400X.[18]

2-5-Assays for Serum FSH, LH and Testosterone

Serum hormone levels were measured from SSH, ICSH and testosterone using VIDAS technique using ELFA (enzyme bound fluorescent assay). The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) by using a Biomerieux kit for all of the three hormones. [19]

2-6-Histological examination

After dissection of animals, the required histological examination tissues were taken and fixed in 10% formalin for 24 hours, and then were dried by ethyl alcohol in increasing concentrations (70), (80), (95), (100) and (100)%, then added to Paraffin. All required tissues were sectioned with paraffin at 5 µm and stained with hematoxylin and eosin. These samples were examined under an optical microscope at 400X magnification. [20]

RESULTS

Table 1. Effect of L-Carnitine on Testosterone, SSH, ICSH, in Serum (Mean ± SD) in different Rat Groups During 4 Weeks of Study.

Standers / Groups	Testosterone ng /ml	ICSH(LH) mIU/ ml	SSH(FSH) mIU/ ml
Control group	7±0.4 a	0.22±0.2 c	0.56±0.4 b
Therapeutic L- carnitine group	6±0.2 a	0.26±0.4 c	0.64±0.3 b
Double dose L- carnitine group	3±0.7 b	0.89±0.4 a	0.76±0.4 a
Overdose L- carnitine group	4±0.5 b	0.64±0.6 b	0.32±0.2 c

- The values represent the arithmetic mean of the standard error.
- Vertically different letters mean there is a significant difference at the level of significance ($P \leq 0.05$).
- Number of animals 5 in each group.

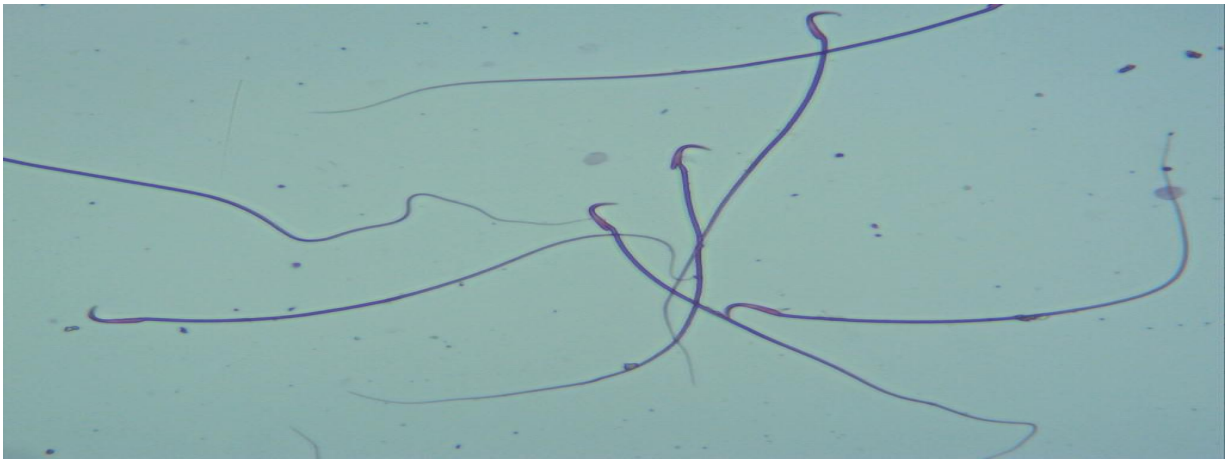

Image (1). The sperm in the control group showed that the sperms were in their normal shape .

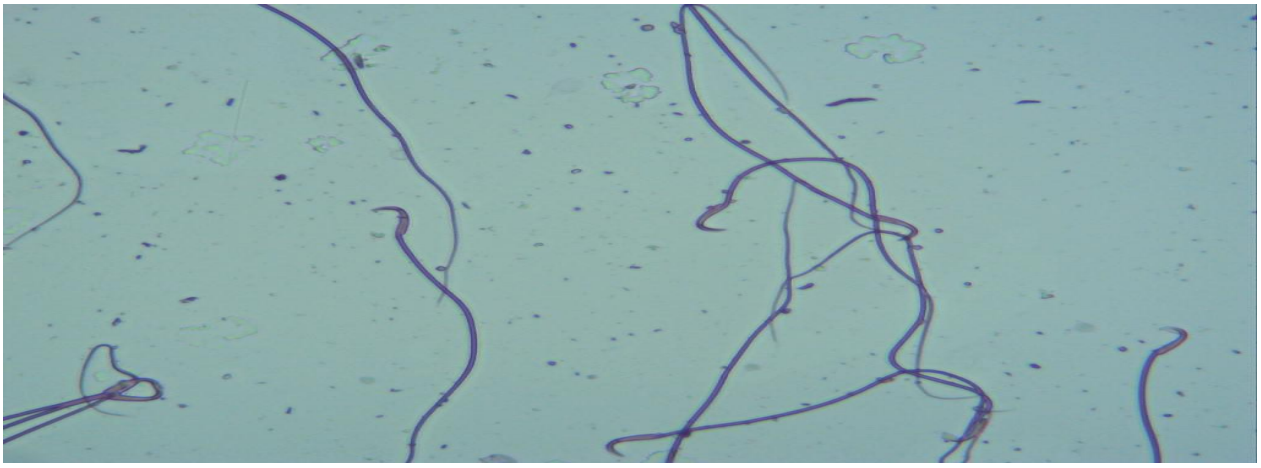

Image (2). The L-carnitine treatment dose group (28 mg / kg body weight) showed sperms of normal shape .

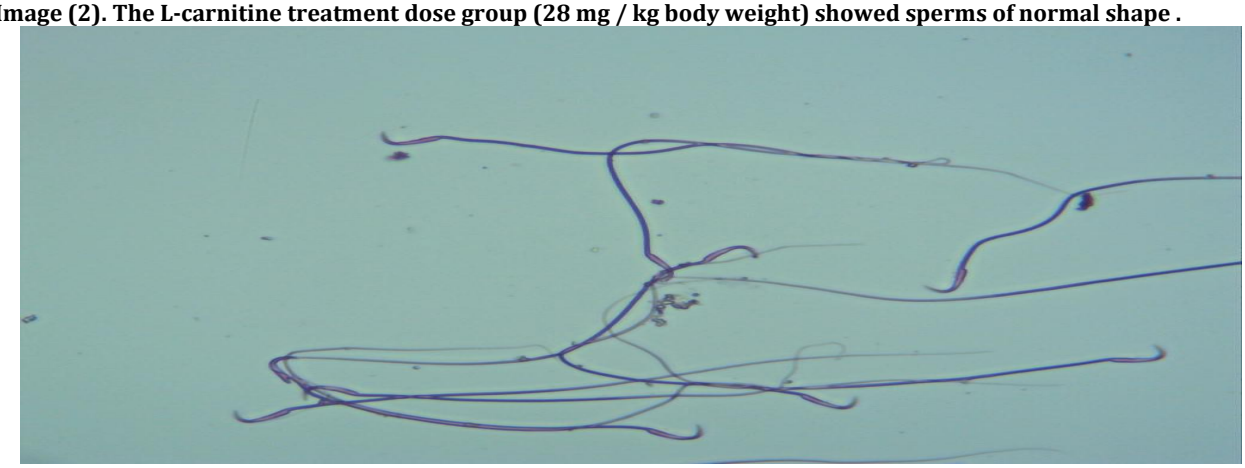

Image 3. The L-carnitine treatment dose group (28 mg / kg b. w) showed sperms of normal shape .

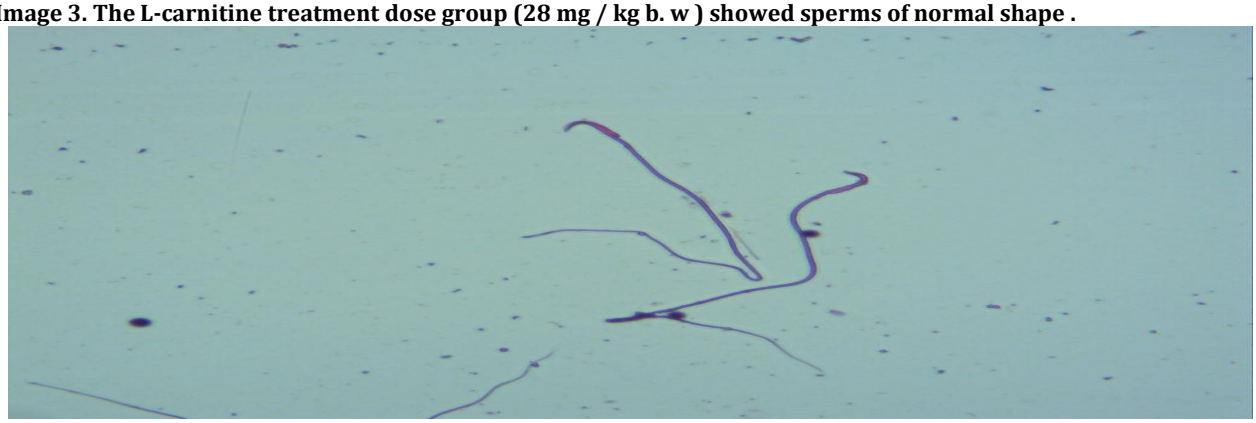

Image 4. The effect of a double L-carnitine dose in the double L-carnitine group (56 mg / kg of b. w) on the sperm showed that it took an abnormal shape, as the tail wrap and the abnormal head shape were also observed..

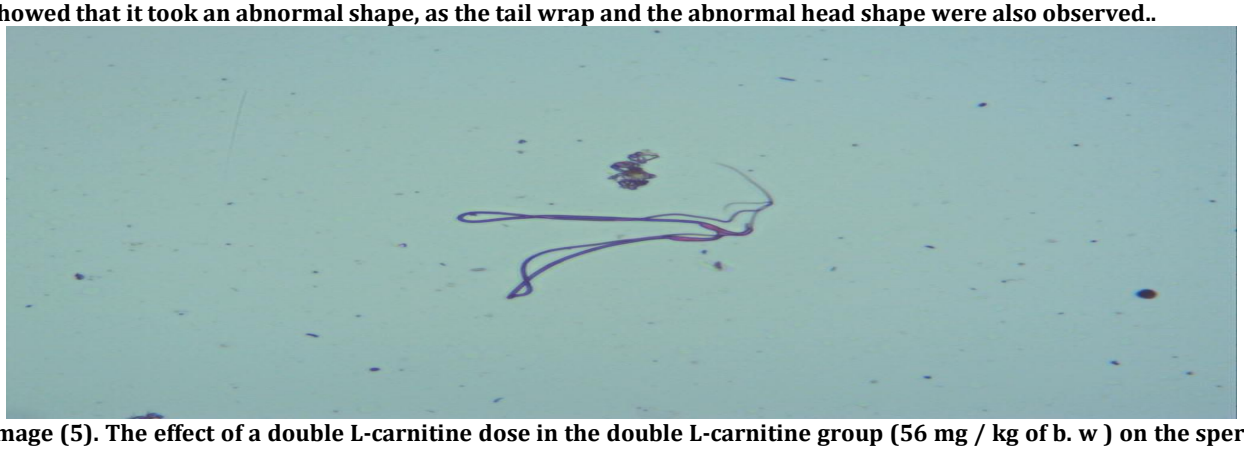

Image (5). The effect of a double L-carnitine dose in the double L-carnitine group (56 mg / kg of b. w) on the sperm showed that it took an abnormal shape, as the tail wrap .

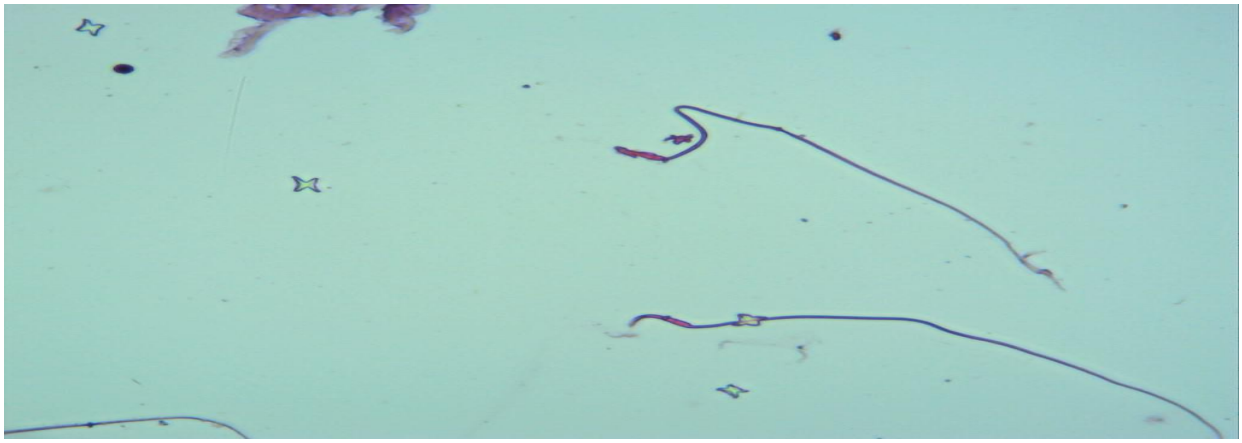

Image (6). The effect of L-carnitine overdose (112 mg / kg b.w) on sperm showed deformation of the head and midsection..

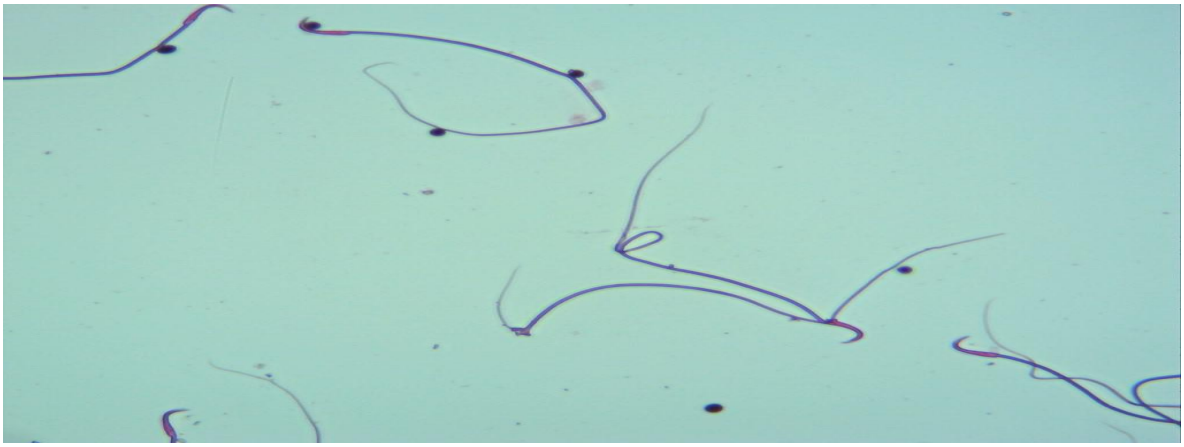

Image 7. Showed the effect of L_carnitine overdose (112 mg / kg of b. w) deformed sperm in the head and tail .

Histological Results

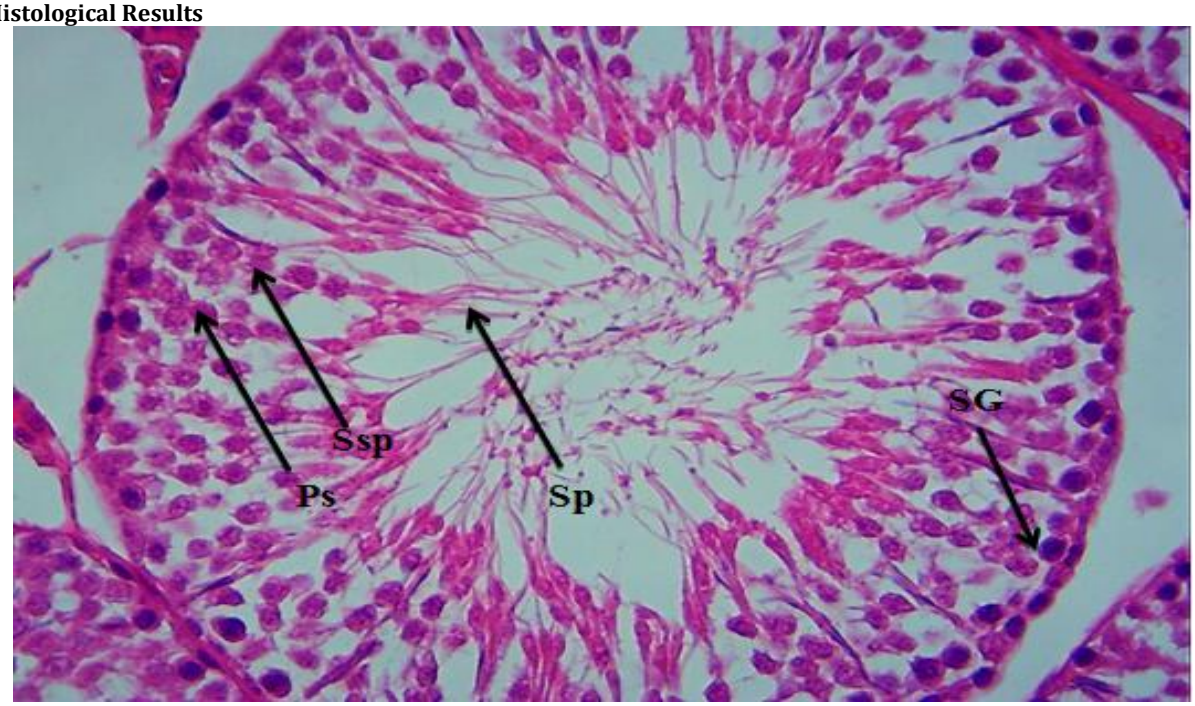

Image (8) Section in Rat testis of control group showed the normal shape of Spermatogonia (SG), Primary spermatocytes(Ps), Secondary spermatocytes (Ssp) and Spermatids (SP) , H&E 400X.

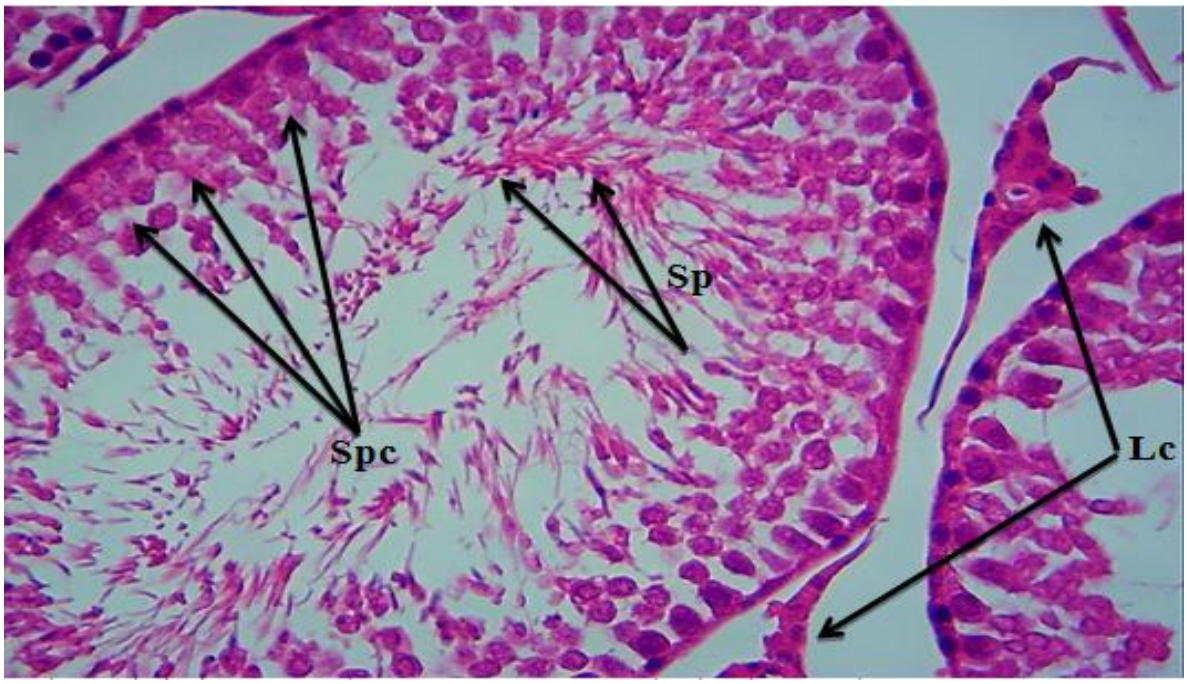

Image (9) Section in Rat testis of Therapeutic L- carnitine group (28mg/kg B.W) showed the normal shaped of sperms during Spermatogenesis(spC), Spermatids (SP) , leydig cells, H&E 400X .

Image (10) Section in rat testis of Therapeutic L- carnitine group (28mg/kg B.W) showed the normal shape for sperm stages (SPc) and mature sperms (sp) H&E 400X .

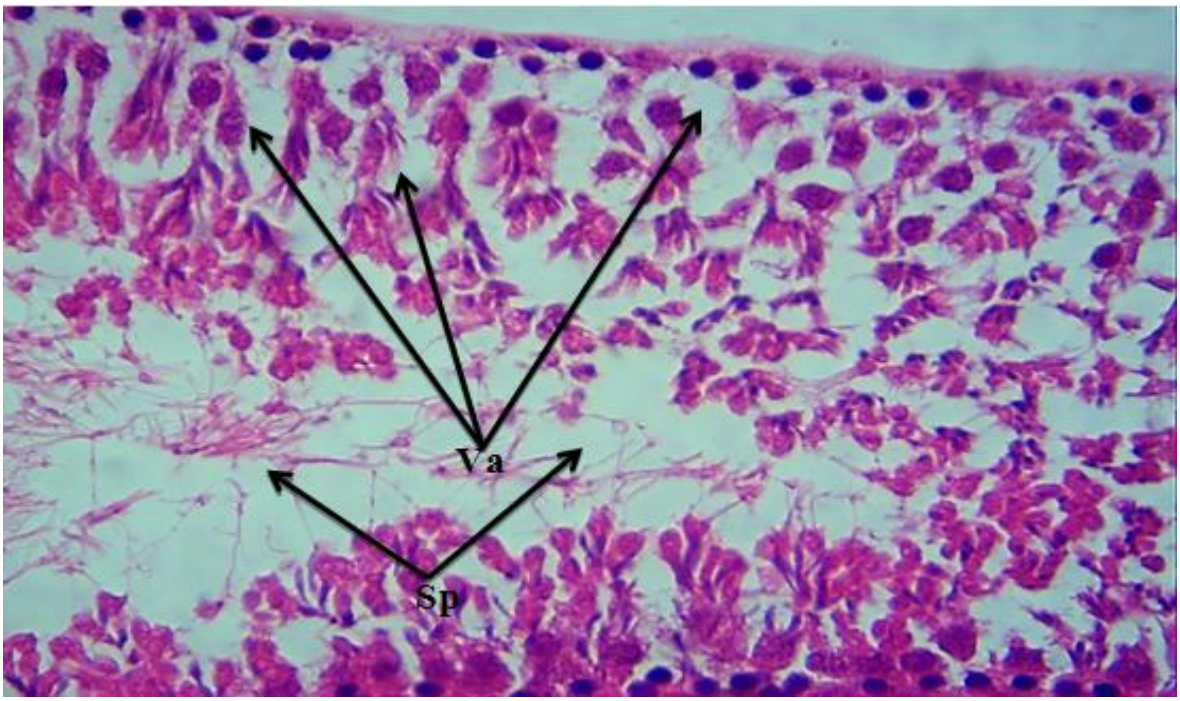

Image (11) Section in rat testis of Double L-carnitine group (56mg/kg B.W) showed vasodilation (va) in cells that forming sperms and decreased the number of mature sperms (sp) H&E 400X .

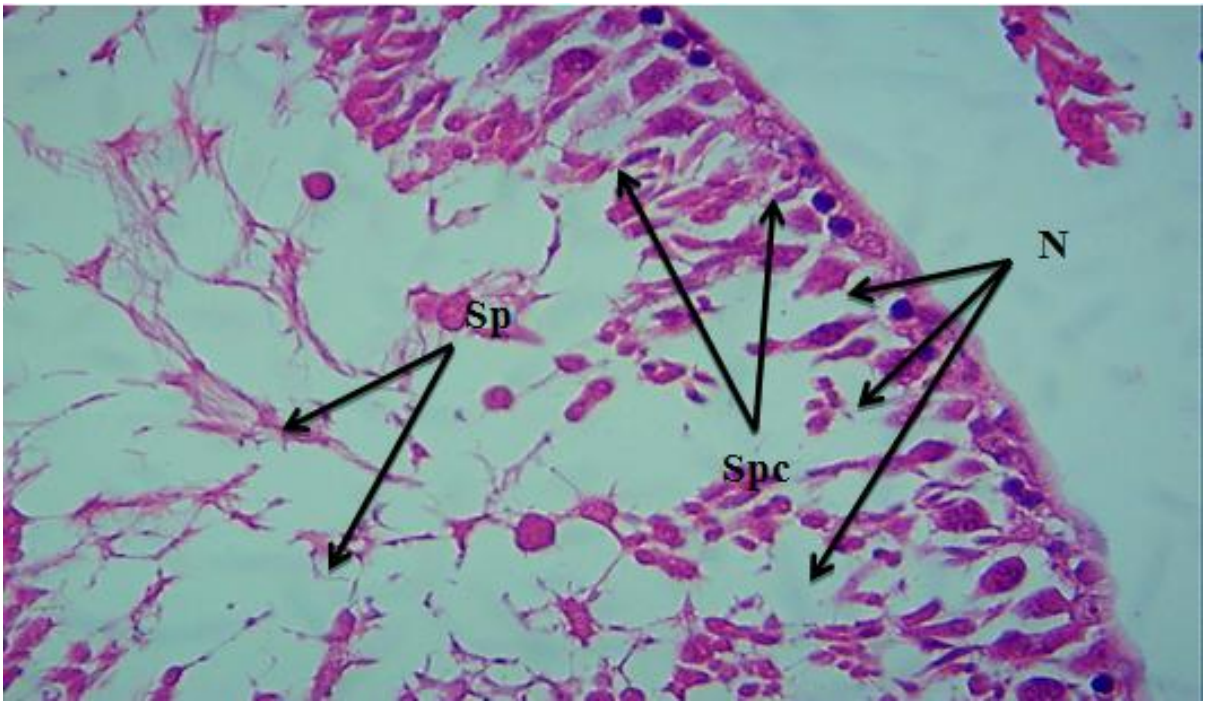

Image (12) Section in rat testis of Over L-carnitine group (112mg/kg B.W) showed tissue necrosis of testis and unclear spermatogenesis stages (spc) and hypo in mature sperms number, H&E 400X .

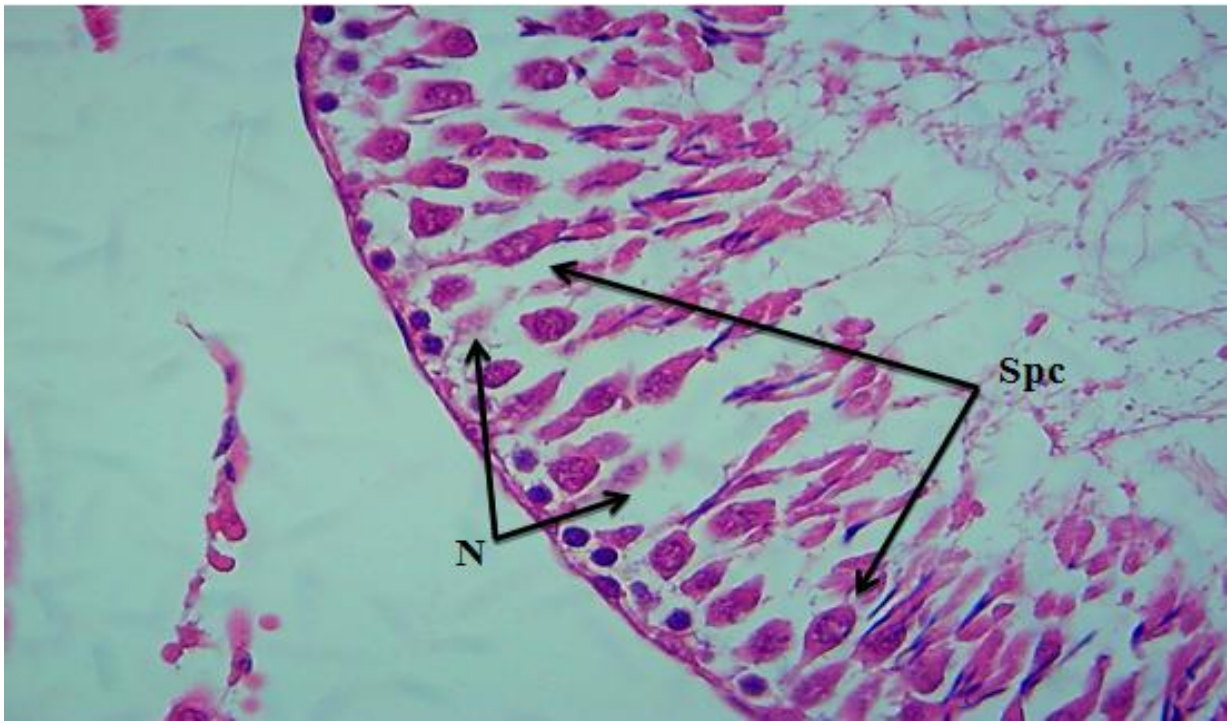

Image (13) Section in rat testis of Over L-carnitine group (112mg/kg B.W) showed unclear in spermatogenesis stages (spc) and tissue necrosis (N) in testis tissue, H&E 400X .

DISCUSSION

As shown in Table 1, the double dose and overdose of L-carnitine in the group of rats dosed significantly (P≤0.05) decreased testosterone levels compared to the control group, while the L-carnitine treatment group had no statistically significant differences compared to the control group. As for the interstitial cell stimulating hormone (ICSH), double and excessive doses of L-carnitine resulted in a significant increase compared to the control group. Whereas, L-carnitine doses in double doses and overdose from mice significantly lowered the level of sperm stimulating hormone compared to the control group .

These results are in agreement with the [21,22] study of the effect of treatment with L-carnitine, which resulted in increased FSH levels and decreased LH levels. This study also agrees with results that show that supplementation of L-carnitine leads to stimulation of GnRH hormone from the hypothalamus, which increases the stimulation of SSH, ICSH, SSH and LH homologs, and increases the secretion of estrogen and progesterone. In the blood plasma [23]. The free passage of L-carnitine from the blood plasma, then passes into the epididymal plasma, is controlled by androgens. [24].

It was observed through the results of the sperm parameters in the doubled L-carnitine group (56 mg / kg b. w) and the group of overdosed animals with L-carnitine (112 mg / kg b. w) that the sperm morphology changed and took an abnormal shape, and these results were consistent with the Baharara et al study. . , 2014 who found that abnormalities in sperm morphology are caused by low levels of testosterone from Leydig cells [25].

Histopathological examination of the testis in the double treatment and L-carnitine groups showed that treatment with L-carnitine had an effect on the diameter of the seminiferous tubules, and the thickness of the germinal epithelium, compared to the control group. Regarding the stimulating effect of L-carnitine on the diameter of the seminiferous tubules, it can be said that it has an antioxidant role. And a role against free radicals that reduce oxidative stress, accelerate spermatogenesis cell differentiation, and increase sperm release from the luminal surface of the seminal tubules [26] .

The role of carnitine treatment is by preventing testicular tissue from changing, by preserving the normal structure of the testicle, and improving spermatogenesis. [27].

The apoptosis depends on several factors, the first is to reduce the levels of hormones essential for the formation of sperms such as FSH, LH and testosterone, and the second reduces the level of antioxidants such as glutathione (GSH) in the mitochondria in sperm, and the last factor increases the formation of free radicals. Because mammalian sperm cells due to their high fat content contain high levels of unsaturated fatty acids, it is a good site for free radical synthesis due to lipid peroxidation. [28,29].

Among the properties of L-carnitine it has an antioxidant role, it protects cells and testicular tissues from necrosis and the harmful effect of ROS released from oxidative stress. [30].

CONCLUSIONS

Through the results, it was noted that the double dose and the overdose had a negative effect on the male reproductive system in rats through their effect on the level of male hormones and causing negative changes in testicular tissue, and then the effect was clearly shown on the sperm .

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