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Impact of Carbamazepine Treatment on Testicular Tissues in Albino Mice (*Mus Musculus*)

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Abstract

Carbamazepine (CBZ) is an antiepileptic medication (AED) intended to treat epilepsy, although it is also used to treat psychiatric problems and neuropathic pain. CBZ use has been linked to male reproduction problems such as hormonal changes, sexual problems, and sperm quality loss in addition to its effect on the hypothalamic-pituitary-gonadal (HPG) axis. The present study aimed to check the potential consequences of carbamazepine on the histology of the testis. In this experiment, 24 adult males of Swiss albino mice were divided into four groups. The control group was gavaged with distilled water, and the others were treated with carbamazepine orally administered with 0.1 ml of a daily dose of concentrations (2.5, 5 and 10) mg/kg bw for 60 consecutive days. Several histological alterations were caused by the therapy in the testis, which included congestion, necrosis, edema, hemorrhage, shrinkage tubule, germ cell shedding, and amyloid.

Keywords: Carbamazepine, testis, histological changes, mice.

1. Introduction

Carbamazepine ($C_{15}H_{12}N_2O$), a tricyclic compound sold under the trade name Tegretol, is one of the first-generation antiepileptic drugs AEDs [1]. Its mechanisms of action include stabilizing the inactivated state of voltage-gated sodium channels, acting as a GABA agonist, and exhibiting serotonin-releasing activity. Carbamazepine (CBZ) is also used to treat psychiatric disorders, bipolar disorder, and neuropathic pain, but it is not effective in coma bouts. Carbamazepine is used in the treatment of schizophrenia in combination with other drugs [2, 3].

Most of the anti-epileptic drugs available act on the central nervous system by exerting a modulatory effect on the bioelectric activity of the cell membrane. Carbamazepine increased the metabolism of sex hormones and caused a decrease in the free androgen index as a consequence

of increased serum sex hormone-binding globulin (SHBG) concentrations [4]. The adverse effects of AEDs, particularly on testicular dysfunction, deregulation of sex hormones, and reproductive toxicity [5; 6], AEDs are toxicants that increase lipid peroxidation at the expense of protective antioxidants [7]. Oxidative stress linked to reproductive damage from CBZ has been observed in disruptions of reproduction. Due to the oxidative stress caused by long-term CBZ therapy, men's serum concentrations of sex hormone-binding globulin are increased. The decreased bioactivity of serum androgens caused by the elevated blood concentration of sex hormone-binding globulin may be interpreted as a reduction in sexual activity [8]. This study aimed to investigate the effect of carbamazepine on the testicular tissues of albino male mice.

2. Materials and Methods

2.1. Experiment animals

The study included 24 adult male albino mice whose weights ranged from (25-30) gm. The animals were divided randomly into four groups: a treatment group, which was treated orally with 0.1 ml of carbamazepine with concentrations of 2.5, 5, and 10 mg/kg, as well as a control group, which was given distilled water.

2.2. Histological Study

The testes were preserved for 24 hours in a 10% formalin solution, then moved to a 70% alcoholic solution for storage after being washed with tap water. The slides were then stained with hematoxylin and eosin after the preserved testes underwent standard laboratory histology processes [9].

2.2.1. Histological Preparations [10]

2.2.1.1. Washing

Washing the tissue is to eliminate the bits and pieces of the fixative. The washing was made via numerous changes of water when the tissue was fixed with formalin.

2.2.1.2. Dehydration

The tissue was passed during an ethanol alcohol series descent, (50%, 60%, 70%, 95%, and 100%) for 45 minutes to eliminate the water from the tissue.

2.2.1.3. Clearing

The tissue was moved to xylene for 45 minutes. Clearing reagents make the tissue more crystal-clear.

2.2.1.4. Infiltration

The sample was placed in the mix of xylene and molten paraffin wax at a ratio of 1:1 in an oven at a temperature of 56–58 for 15 minutes, and then the wax was replaced three times each time. The specimens remain in the wax for an hour.

2.2.1.5. Embedding and Sectioning

The tissue was embedded inside pure wax, the molten paraffin wax was decanted into an appropriate template, and the tissue was moved to the mold, which was kept in the refrigerator

until sectioning. The Paraffin sections were trimmed using a sharp blade as well as put into a rotary microtome to be ready for histological sectioning at 7 µm thickness. Histological sections were moved to the slide, including Mayer's albumin, and then the slide was placed on a hot plate at 40 °C for the final drying.

2.2.1.6. Staining

The slide is sited in xylene in two stages, each for 30 minutes, for the removal of paraffin wax from histological sectioning. The xylene is then removed by passing the slide in a sequential descending concentration of ethanol alcohol (100%, 95%, 90%, 80%, 70%) for two minutes in each concentration. The slide is transferred to distilled water for two minutes, then to hematoxylin stain for 37 seconds, and then washed via tap water for five minutes. Next, the slide is moved to eosin stain, and then to a series of ascending concentrations of ethanol alcohol (70%, 80%, 90%, 95%, 100%) for a short period. The slide is finally moved to xylene in two periods, each lasting 30 minutes.

2.2.1.7. Mounting

The Distrene Dibutyl Phthalate Xylene (DPX) material is used in slide mounting via the fitting of an amount of DPX to the slide, and then the slide is covered through the cover slide. The slide is placed on a hot plate at 40 °C until dripping**.**

3. Results

Based on the examination of the histological section of the testes in mice treated with carbamazepine It was possible to observe the various effects caused by this drug on the testicular tissue, represented by necrosis, edema, shrinkage tubules, shedding germ cells, hemorrhage, and amyloid. The histological section from the control group showed a normal shape and size of the seminiferous tubules and Leydig cells (**Figure 1**).

Histological sections of the treated group with a concentration of 2.5 mg/kg showed necrosis, shedding germ cells in seminiferous tubules, edema, necrosis in Leydig cells, and shrinking seminiferous tubules **(Figures 2; 3**). Moreover, the histological examination revealed other types in the treated groups at the same concentration (5 mg/kg); these included the occurrence of edema, congestion, amyloid, rupture tubule, segregation of germ cells, and atrophy tubule, as clearly demonstrated by **Figure (4; 5)**. On the other hand, the histological section of the treated group with a concentration of 10 mg/kg showed edema, congestion, hemorrhage, and amyloid; this is clearly demonstrated by **Figures 6, 7,** and **8**.

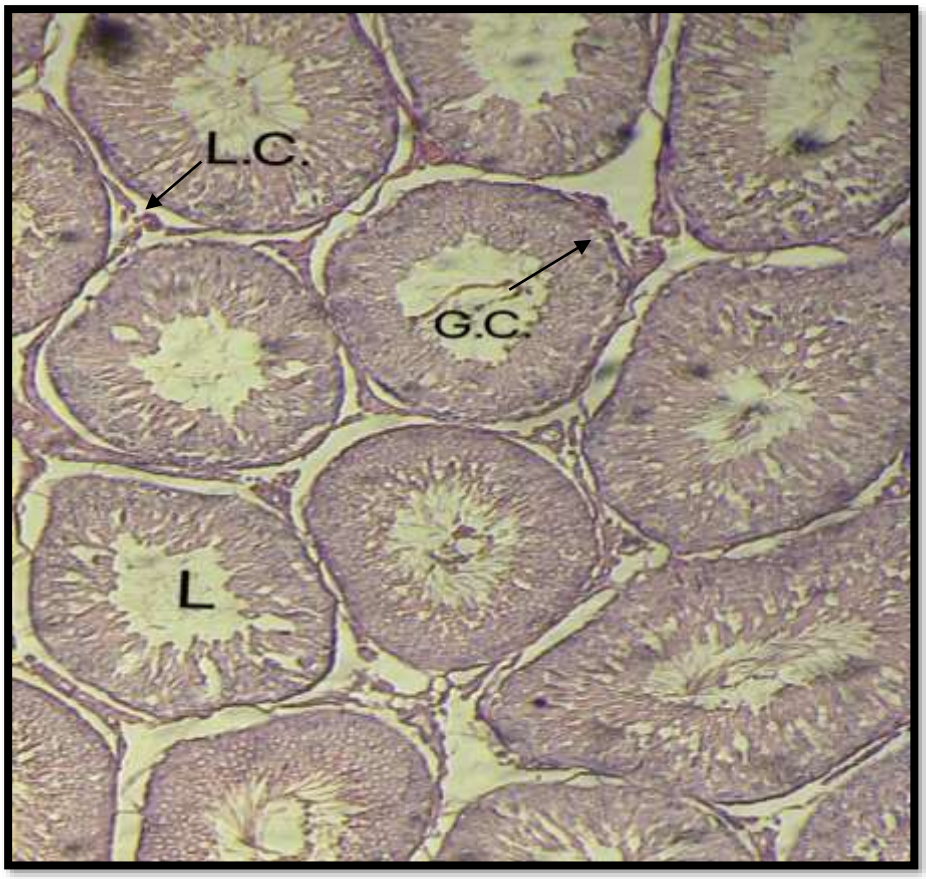

Figure1. Cross section of testicular tissue of control group Shows: Germ cell (G.C.) , Lumen (L), Leydig cell (L.C.). 100X (H&E).

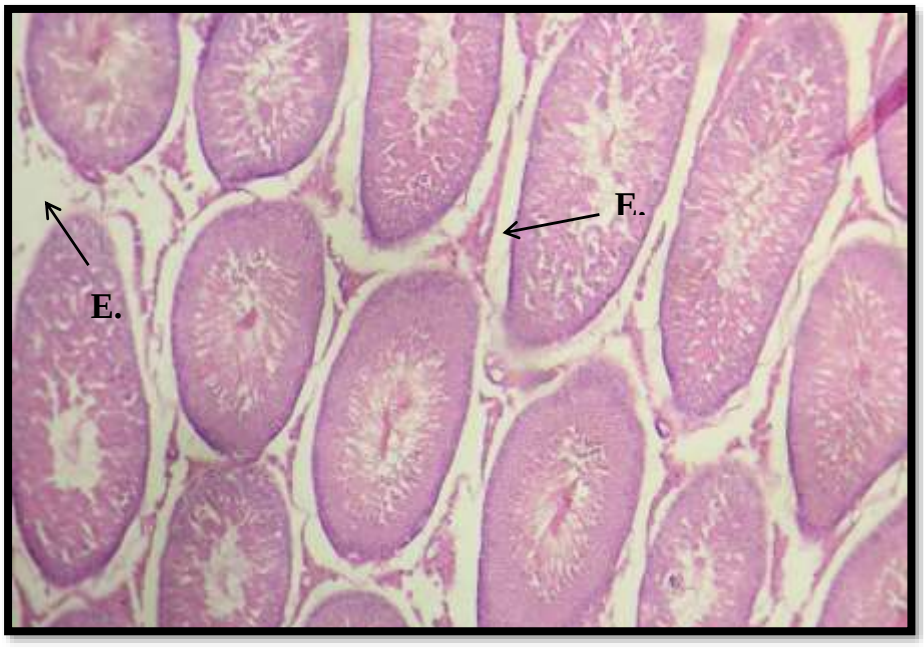

Figure2. Cross section of testicular tissue of treated group with Carbamazepine Concentration (2.5)mg/kg, Shows : Edema (E). 100 X, H&E.

Figure3. Cross section of testicular tissue of treated group with Carbamazepine Concentration (2.5) mg/kg, Shows: Necrosis (N), Edema (E). 100X, H&E.

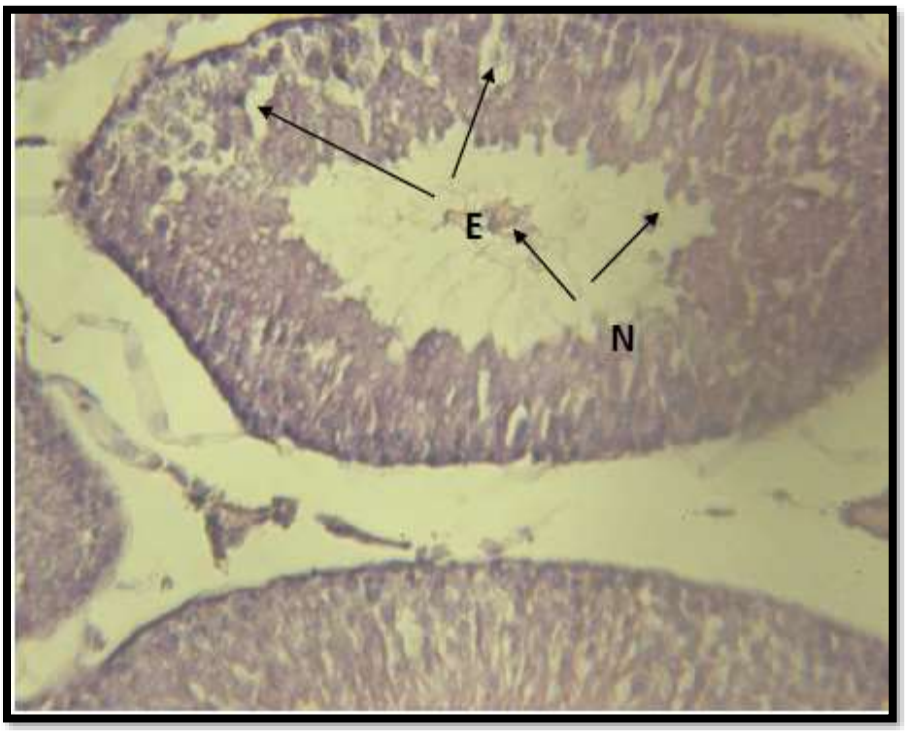

Figure4. Cross section of the testicular tissue of treated group with CBZ Concentration (2.5) mg/gk, Shows: Necrosis (N), Edema (E), 400X, H&E.

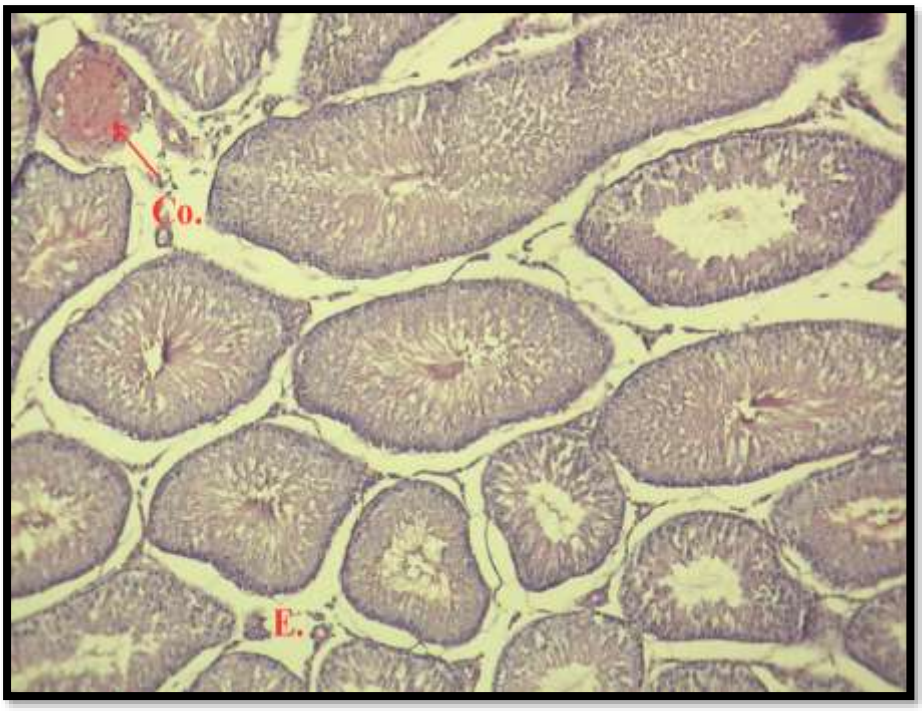

Figure5. Cross section of testicular tissue of treated group with CBZ Concentration (5) mg/kg, Shows: Congestion (Co.), Edema (E), 100X, H&E.

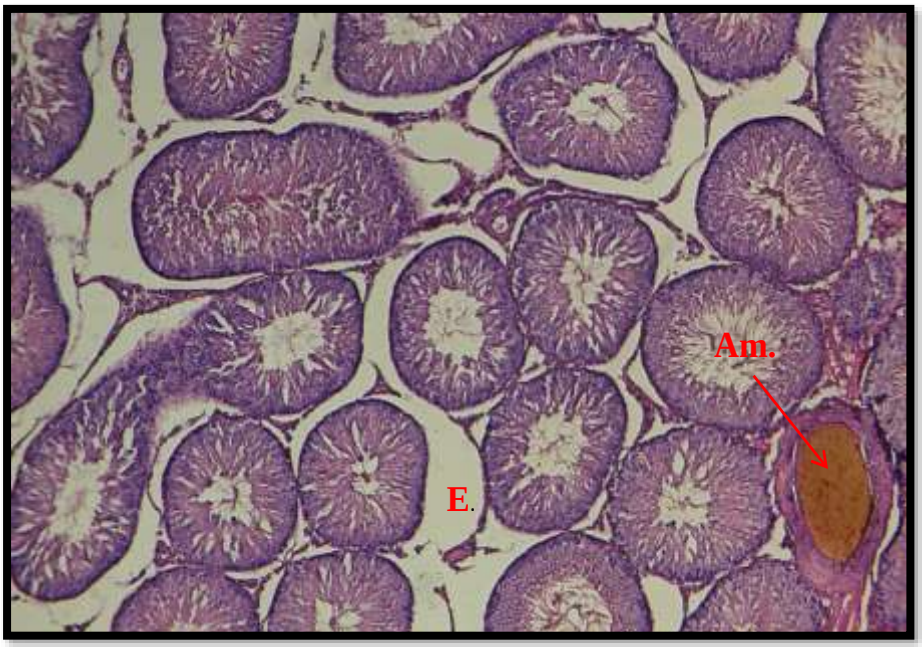

Figure6. Cross section of testicular tissue of treated group shows with CBZ Concentration (5) mg/kg. Shows: Amyloid (Am.), Edema (E), 100X, H&E.

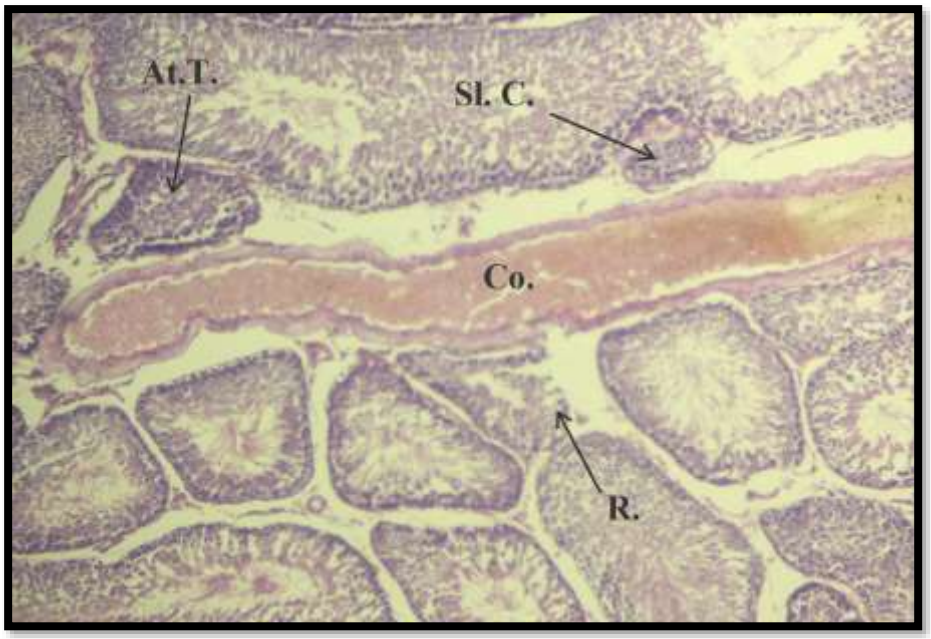

Figure7. Cross section of testicular tissue of treated group with CBZ concentration (5) mg/kg Shows: Congestion (Co.), Rupture of Tubule (R), Sloughing cells (Sl.C), Atrophy of Tubule (At.T). 100X, H&E

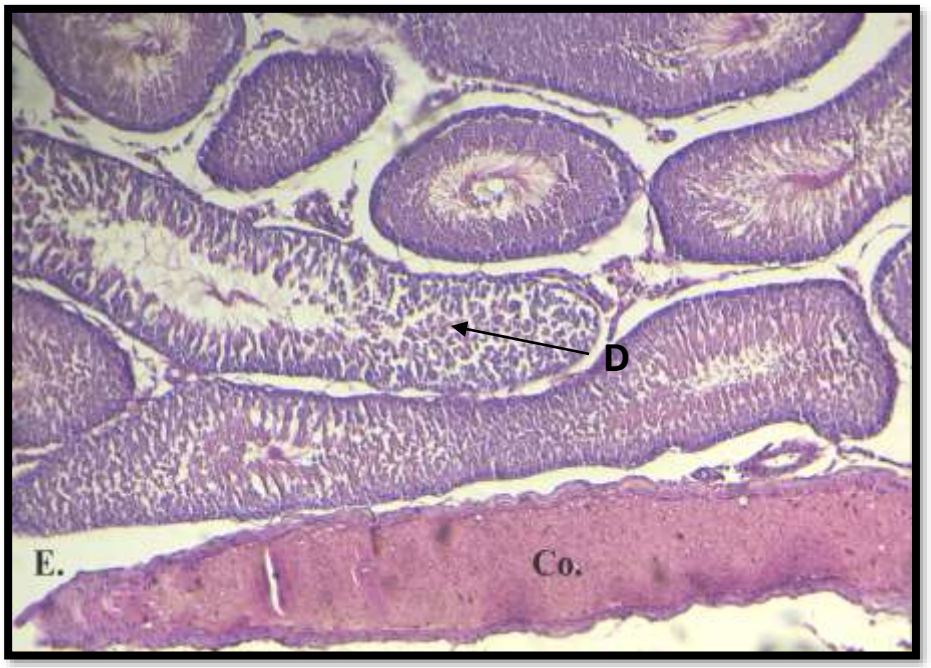

Figure8. Cross section of testicular tissue of treated group with CBZ Concentration (10) mg/kg Shows: Congestion (Co.), Edema (E), Degeneration(D). 100X, H&E

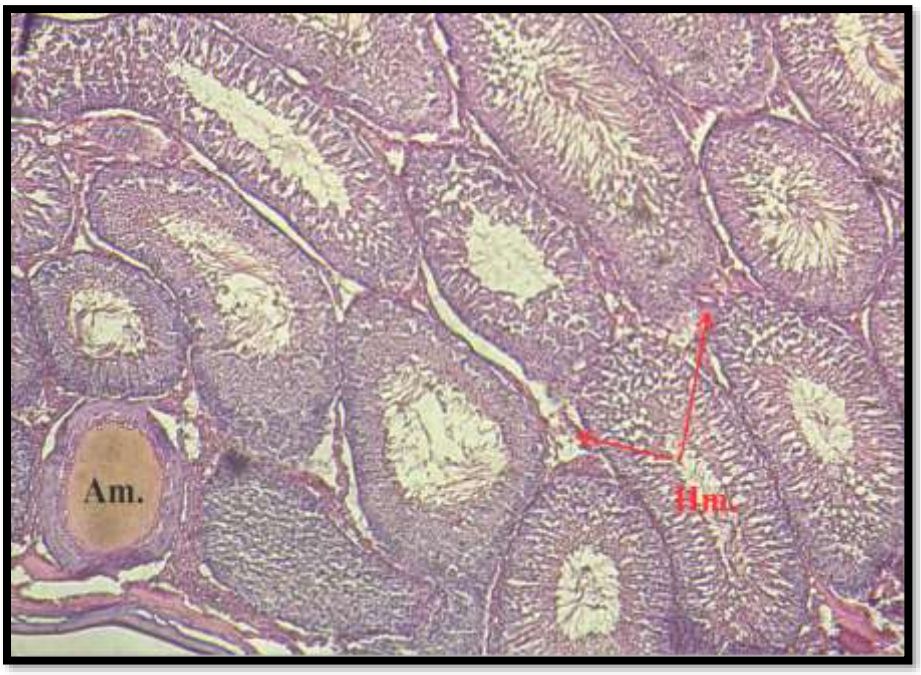

Figure9. Cross section of testicular tissue of treated group with CBZ concentration (10)mg/kg. Shows: Hemorrhage (Hm), Amyloid (Am) .100X, H&E

Figure10. Cross section of testicular tissue of treated group with CBZ concentration (10) mg/kg. Shows: Amyloid (Am), Hemorrhage (Hm). 100X .H&E

4. Discussion

The findings of the current investigation demonstrated that all CBZ-treated groups had considerably more histological abnormalities than the control group.

Low androgen levels, one of the hormonal imbalance diseases that carbamazepine can cause, can lead to defects in reproduction [11]. FSH coordinates the development of seminiferous tubules and maintains Sertoli cell activity, both of which are essential for the development of spermatogenesis and sperm [12]. Hypogonadism can be brought on by a reduction in LH or FSH output. LH increases the testosterone release Leydig cells produce on Sertoli and peritubular

cells of the seminiferous tubules, which then prompts the onset of spermatogenesis in males, who typically exhibit this condition as a drop in sperm production [8, 12, 13]. Testicular and epididymal damage is caused by the evident variations in testosterone plasma levels [11]. AEDs are thought to have the ability to penetrate the blood-testis barrier and reach the seminiferous tubules because of the direct impact they have on spermatozoa and germinal lineage cells. [14,15] . Intercellular bridges connect germ cells at the same stage of differentiation during normal spermatogenesis. These bridges remain intact until later spermatids are released into the tubular lumen [16, 17]. However, in the CBZ-treated group, intercellular bridge disintegration was seen [18].

Carbamazepine affects the hormonal neuro sexual organ, specifically androgens and testosterone hormone, and blocks the action of nerve receptors. This results in the testicle not stimulating sperm production and maturation [19], as well as the drug's influence on the immaturity and differentiation of Sertoli cells and cell damage (20). On the other hand, it affects the stages of sperm formation and lowers pituitary activity [21, 22; 23].

Extreme levels of free radical synthesis could be brought on by carbamazepine in the biosystem [24]. Changes in antioxidant status indicate that CBZ caused oxidative damage to the testes, and It is possible that enhanced lipoperoxidation led to an adverse environment for the Leydig cells' ability to make testosterone. Actually, the CBZ group's Leydig cells had histological changes that made the environment unfavorable for the production of testosterone even worse. Therefore, the testes and pituitary glands may suffer oxidative damage as a result of the reduced serum testosterone levels in the CBZ group [25]. The presence of testosterone and epididymal proteins is necessary for spermatozoa to mature and develop their increasing motility and ability to fertilize [26]. Therefore, the decreased progressive motility in the CBZ group may be caused by the reduced serum testosterone concentration. Furthermore, CBZ-evoked oxidative damage to spermatozoa may be caused by an overabundance of proteins in the testis and epididymis as a result of androgen deficiency, which leads to an abnormally high production of sperm [27]. Since elevated ROS (Reactive Oxygen Species) generation has been linked to decreased motility parameters, which have a detrimental influence on fertility [28, 29], this may have played a role in the CBZ group's decreased sperm motility. CBZ and certain medications change the quality of the sperm, decrease sperm mobility, and increase the number of sperm cells with defects [30.31].

5. Conclusion

According to biochemical, hormonal, and histological characteristics, the current study demonstrated that chronic CBZ administration causes unfavorable reproductive outcomes, particularly oxidative injury and hormonal abnormalities.

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