Teratogenic Effect of Carbamazepine Drug on the Histological Structure of Testes in the Albino Mouse (*Mus musculus*)

Thekra Atta Ibrahim[1], Mohammed Ali Mohammed[2], Ibtisam Khalaf Abd ali[3], Mohammed Nsaif Abbs[4], Suaad Abbas Hussien[1]

[1]Assit prof. Department of Biology, College of Education for Pure Science, Diyala University, Iraq, [2]Prof. College of Dentistry, University of Al-Iraqia, Iraq, [3]Prof. Departement of Basic Science, College of Nursing, University of Baghdad, Iraq, [4]Prof.Environmental Engineering Department, Collage of Engineering, Mustansiriyah University, Iraq

Abstract

The aim of this study was to evaluate the side effect of Carbamazepine on the histological structure of the testis in white mouse males.

A total of 24 male of Swiss white mice were divided into three groups, treated with Carbamazepine Drug for (30) days. Within each treatment period, animals were assigned into three subgroups each of eight mice; control treated with vehicle and the others treated with 200, and 300mg/kg of Carbamazepine, The required dose of the drug was dosed orally for all mice once per day for one month (30 days) for two concentrations. At the end of treatments, all mice were sacrificed; Testes was removed and weighed then kept in buffered formalin solution for microscopic examination.

The results of this study showed that animals that were injected with Carbamazepine caused a change in the shape and thickness of the wall of the somniferous tubules, where the appearance of the irregular, and also observed the occurrence of an embolism in some germ cells and collected in the center of the cavity of the somniferous tubules in addition to the observation of Vaculation and necrosis in most areas of somniferous tubules.

The results of the current study suggested the possible time and dose-dependent disrupting potential of Carbamazepine Drug on the Histological Structure of Testes in the Albino Mouse.

Keywords: *testis, Sertoli cells, Carbamazepine, seminiferous tubules, germ cells*

Introduction

Epilepsy is a chronic disease characterized by frequent bouts caused by a defect in the transmission of electrical signals in the brain. This may be happened due to genetic factors or due to various brain damage such as stroke [(1,2)]. Defects and disorders of the reproductive system are more prevalent among men (males) who suffer from epilepsy compared to other healthy people [(3)]. Both epilepsy and anti-epileptic drugs containing aromatic compounds like phenol[(4)] may play an important role in creating these disorders, so it is difficult to distinguish between the direct negative effects of epilepsy and anti-epileptic drugs on the reproductive system[(5)].

One of the most famed drugs used for treating epilepsy is Carbamazepine (CBZ), commonly known as Tegretol, which is a major drug used to treat epilepsy and nerve pain, but it is not effective in coma bouts. Carbamazepine is used in the treatment of schizophrenia in combination with other drugs; and it is considered as the first generation of drugs with appropriate

Corresponding author:
Thekra Atta Ibrahim
thekra.atta@gmail.com

pharmacological properties and appears to be effective in improving control of nerve attacks[6].

There are some studies that refer to changes occur in the form of testis and the occurrence of infertility and deformities in the sperms in people who treated with Valparvat 3,3-5 carbamazepine (CBZ) drug [7].

The aim of this study was to evaluate the side effect of Carbamazepine on the histological structure of the testis in white mouse males.

Materials and Methods

Preparation of Carbamazepine Required Dosage

he dose of carbamazepine was prepared depending on the half lethal dose (LD_{50}) of the drug which was 529 mg/kg b.w. [8]. Two doses of Carbamazepine were selected 300 and 200 mg/kg to test its toxic effect, while the weights of mice used in the experiments was ranged between 20-30 g. The required dose of the drug was dosed orally for all mice once per day for one month (30 days) for two concentrations. It was possible to calculate the amount of drug used for the mice in this study based on the following equation:

$$\frac{x}{D} = \frac{W_{mouse}}{1000}$$

Where: x: The required amount of drug to be dosed for mice in the experiment, (g)

W_{mouse}: The body weight of the mouse, ranging among 20-30 (g)

D: The selected dose of carbamazepine drug which were 200 or 300 mg/kg

Animals used in the laboratory experiments and histological study

In this study, 20 male of Swiss white mice (*Mus musculus*) was used to achieve the practical experiments part. The mice obtained from animal house in biological department-college of science/Baghdad University. The average weights of used mice were ranged between 20-30 g and its age were between 8-10 weeks. These mice were divided randomly to two groups of the following details: the 1st group was the control group which has 4 mice. The 2nd group was tested group which contains 16 mice, and this group was sub-divided into two equal groups, 8 mice was belonged for each sub-group. The mice in one sub-groups was treated with the carbamazepine dose of 200 mg/kg while the other sub-groups dosed its animals with the second carbamazepine dose which was 300 mg/kg. Each tested doses (i.e. 200 and 300mg/kg) were subjected for mice for 30 days. When the experiments were finished after one month, all mice were anesthetized with chloroform, then the animals were excised and the testes were removed from its position. The samples were fixed with formalin solution for 24 hours, and transferred to 70% alcoholic solution after washing with tap water for storing. The histological sections of samples were prepared according to the method used in Bancroft and Gamble [9].

Results and Discussion

There are many different parameters that influence on the formation of sperms, among that factors are chemical agents such as drugs and toxic chemicals that are contaminated the environment [10]. The results of the present study showed that the mice that were treated with a dose of 200 mg/kg of carbamazepine drug had histological variations represented by shrinking and increasing the thickness of seminiferous tubule, which resulted with irregular appearance. In addition, it could observe atrophy in some Seminiferous tubule, as shown in Figure (1). These results are agreed with the results obtained by [11] which reported that the basal plate has a vital role in maintaining the transfer of materials between the interstitial tissues and germs Spermatogonic and also maintaining the composition and function of these tissues, from another hand. While [12] noted that the increased in the wall thickness of Seminiferous tubule reduces the relationship between it and the interstitial tissue and thus cause the emergence of several disease disorders inside the testis, which will affect the differentiation of germ cells and inhibition of sperm formation. The reason for thickening of Seminiferous tubule walls, as illustrated by [13] was collagen fibers IV fiber which secreted by the Sertoli cells, thus lead to weak the formation of sperms. One of the results of the current study also was the absence of sperm Seminiferous tubule in some seminiferous tubule as well as the presence of vaculation in the testicular areas, in addition to the widening distance between germ cells, dissociation of

the epithelial tissue and collected in the cavity of sperm. Also there was a degeneration occurrence in the cells of Sertoli and increase the area between the cells of the nearby Sertoli cells. This is clearly demonstrated by Figure (2). On the other hand, Figure (3) shows the appearance of large Macrophage cells within the seminal vesicle cavity. disorder happening in the Sertoli cells will inevitably affect the germ cells and eventually lead to a defect in testicular tissue and this is what the results reached by [14,15] were explained the essential role of the Sertoli cell in the development of germ cells through the formation of a testicular blood barrier that protects germ cells and transfer of the nutrients and hormones to germ cells.

It believes that all these signs of disease are occurring due to a malfunction in the structure and function of Sertoli cells. The results of the current study revealed that the apparent effect of the drug on the Leydig cells and interstitial tissue via emergence of decay and necrosis and also the emergence of Vaculation in the interstitial tissue as shown in Figure (2). This result is identical with the result of [16] that the Leydig cells are the center of fertility regulation through the production of testosterone hormone, while [17] pointed to the Leydig cells are promoted by the hormone LH Luteinizing hormone, arachidonic acid and hormone testosterone. The results of current study also showed that the shrinkage was increased of sperm tubes and the depletion of some germ layers of these tubes in mice treated with a concentration of 300 mg/kg of the drug in addition to the occurrence of degeneration and explorations, apoptosis in the Spermatogonia, primary sperm cells and Spermatid and mature sperm the return of Spermatid and mature sperm into of Seminiferous tubules while many of the of Seminiferous tubules were empty of germ cells and as shown in Figures (4 and 5). These results are getting along with the results of [18] in their study on sperm characteristics and the precise composition of testes in rats after long-term with methanol treatment. They attributed the cause of the disorder in the Sertoli cells, which will be affected by the basic proteins required in the process of synthesis and necessary for the differentiation of bacterial cells, which produce these proteins at the highest level during the stage of differentiation of sperm. Also, the result of this study is agreed with [19] which show that the regressive movement of Spermatid and sperm within the wall of somniferous tubules may be due to an alert to testicular toxicity resulted by the drug.

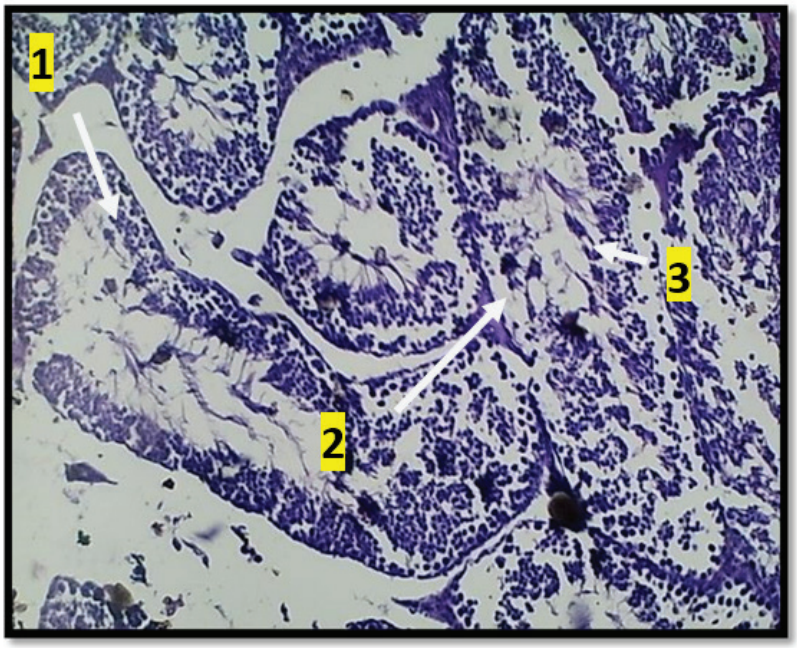

Figure 1: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine drug for one month showing 1) irregularity of the semeniferous epithelium, 2) vaculation, 3) necrosis of the semeniferous epithelium. H&E, 40×

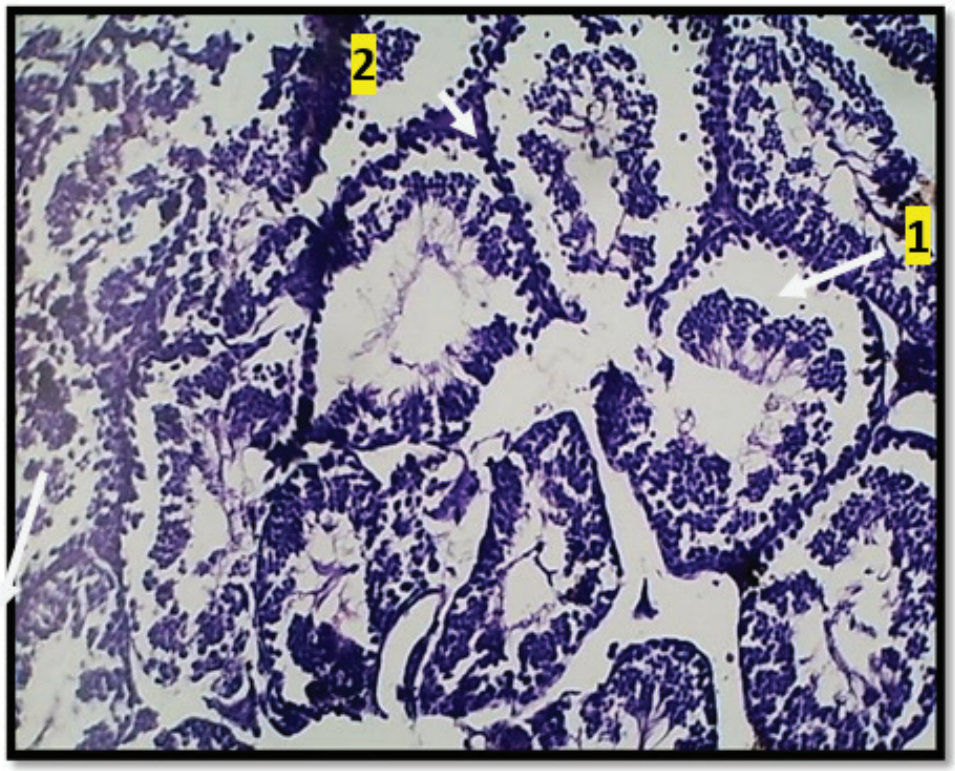

Figure 2: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine for one month showing 1) aggregate the germinal cells in the lumen of Seminiferous tubules, 2) necrosis of the semeniferous epithelium. H&E, 40×

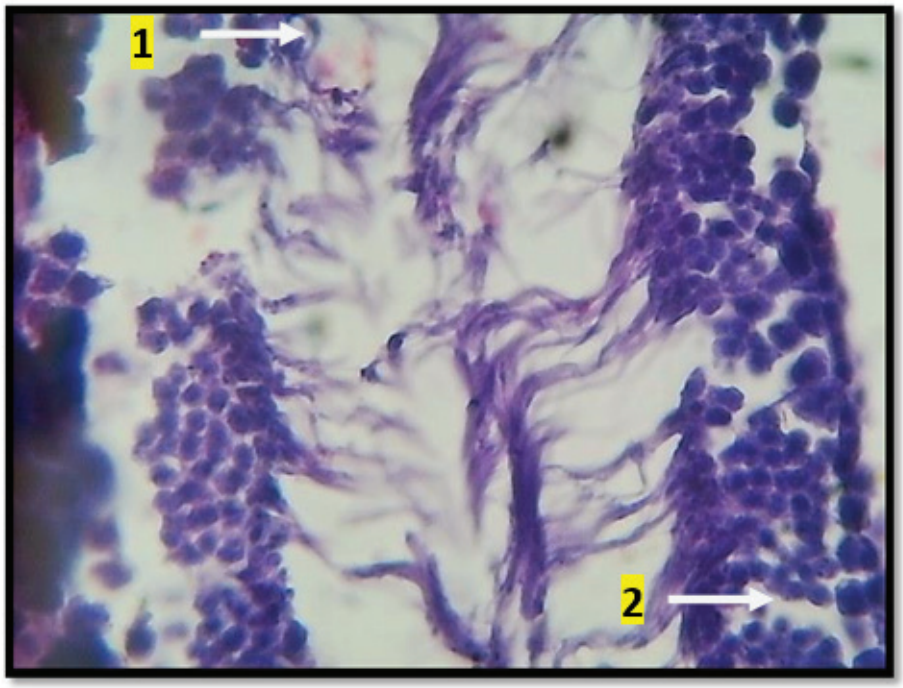

Figure 3: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine for one month showing, 1) The emergence of large cells phagocyte within the cavity semeniferous, 2) degeneration in Sertoli cells. H&E, 40×.

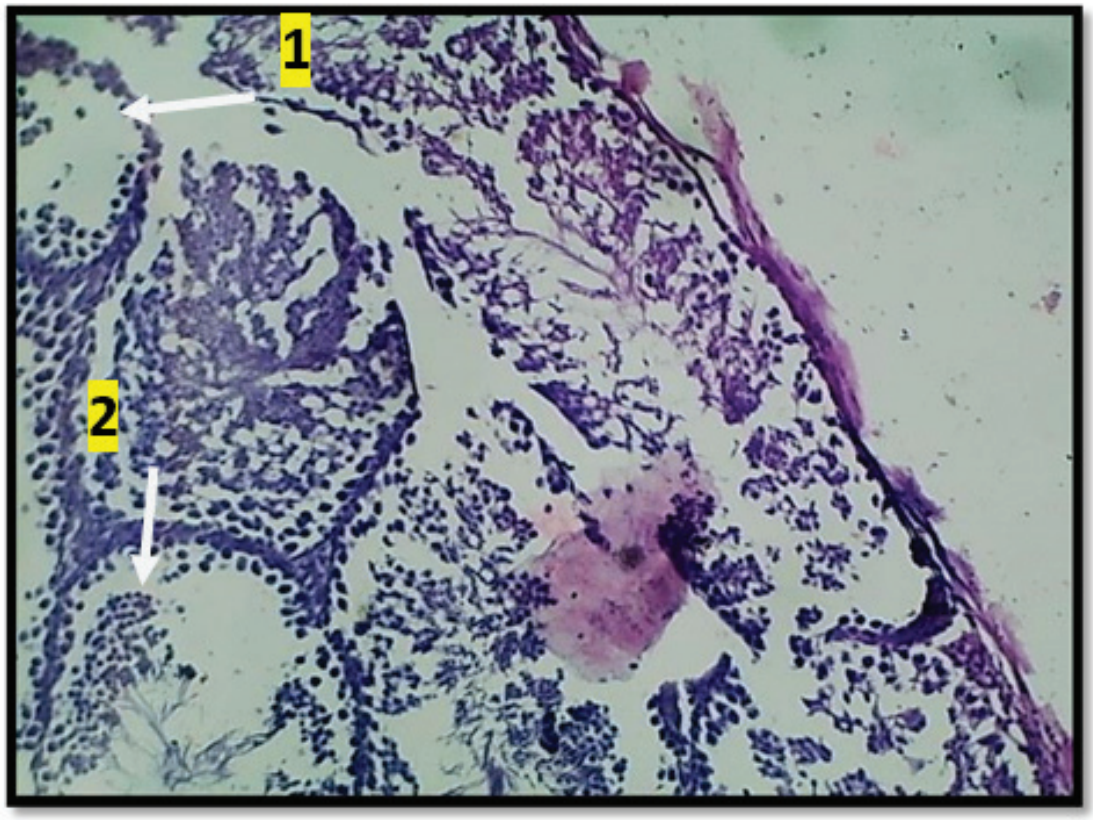

Figure 4: Parasagittal section of the Testis of mice receiving with 300 mg/kg carbamazepine for one month showing 1) Empty the Seminiferous tubules from germinal cells, 2) necrosis of the semeniferous epithelium). H&E, 40×.

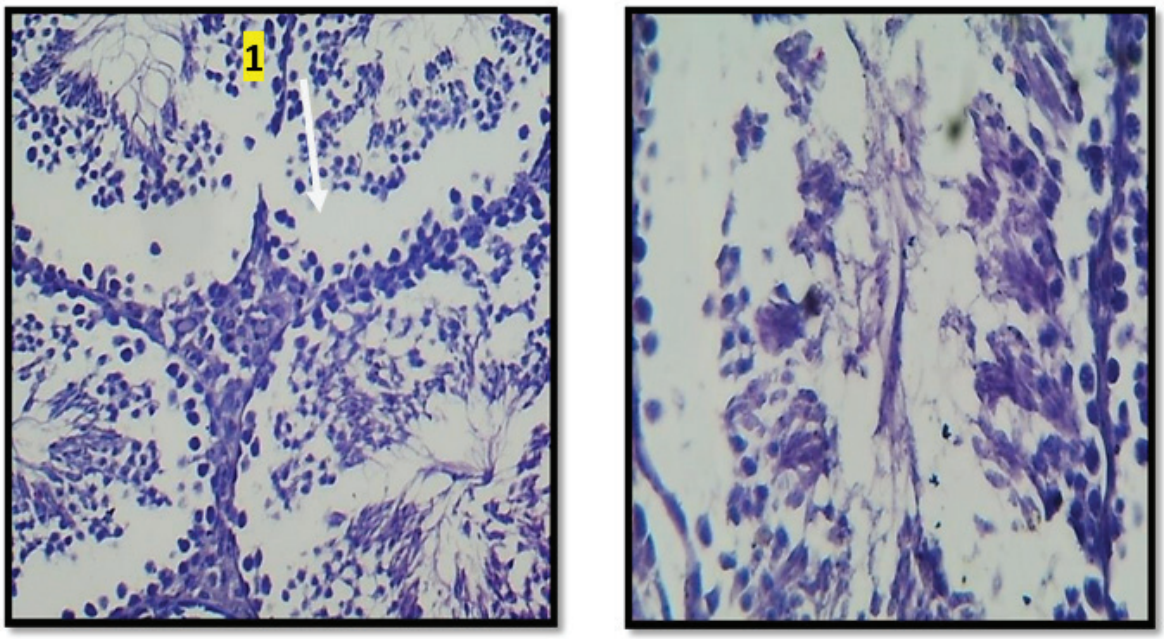

Figure 5: Parasagittal section of the Testis of mice receiving with 300 mg/ carbamazepine for one month showing 1) Empty the Seminiferous tubules from germinal cells and, degeneration in Sertoli cells. H&E, 40×.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Engel J Jr. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia, 2001; 42:96–803.
2. El-Alameey IR and Ahmed HH.Serum brain derived neurotrophic factor as biomarker for Epilepsy diagnosis In Egyptian Children with Epilepsy; Relationship to Disease Severity and Cognitive Function. Asian Journal of Pharmaceutical and Clinical Research, 2018; 11(1): 306-309.
3. Chen SS, Shen MR, Chen TJ, Lai SL. Effects of antiepileptic drugs on sperm motility of normal controls and epileptic patients with long-term therapy. Epilepsia, 1992; 33(1):149-53.
4. Abbas MN, Al-Hermizy SMM, Abudi ZN, and Ibrahim TA. Phenol biosorption from polluted aqueous solutions by Ulva lactuca alga using batch mode unit. J. Ecol. Eng., 2019; 20(6):225–235
5. Gates JR. Epilepsy versus antiepileptic drugs and gonadal function in men. Neurology, 2004; 62(2): 174-5
6. Erdem SA, Nabavi SF, Orhan IE, Daglia M, Izadi M, Nabavi SM. Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus Eryngium. DARU J. Pharm. Sci., 2015; 23(53):1–22.
7. Carbamazepine. The American Society of Health-System Pharmacists. Archived from the original on 2015-02-27. Retrieved 28 Mar.
8. Abd ali IK, Ibrahim TA, Farhan AD, Abbas MN. Study of the effect of pesticide 2,4-D on the histological structure of the lungs in the albino mice (Mus musculus). J. Phrm. Sci. & Res. 2018; 10(6): 1418-1421.
9. Suvarna SK, Layton C and Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. Churchill Livingstone, Elsevier, 2013.
10. Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Levallet J. Reproductive system: aromatase and estrogens. Molecular and Cellular Endocrinology. 2002; 193(1–2):137–143.
11. Richardson LL, Kleinman HK and Dym M.Altered basement membrane synthesis in the testis after tissue injury. Journal of Andrology, 1998; 19(2): 145-155.
12. Zheng Y, Zhang X, Zhon J, Cheng F, Rao T and Yao Y. Expression of extracellular matrix proteins and vimentin in testes of azoospermic man: an immunohistochemical and morphometric study. Asian Journal of Andrology, 2002; 4(1):55-60.
13. Winters SJ. Male Hypogonadism: Basic, Clinical, and Therapeutic Principles. Humana Press Inc., Totowa, NJ. Chapter 2: Leydig Cell Function in Man by Dong Q. and Hardy M. P. 2004; 23-43.
14. Monsees TK, Franz M, Gebhardt S, Winterstein U, Schill WB and Hayatpour J. Sertoli cells as a target for reproductive hazards. Androlgia, 2000; 32(4-5): 239–246.
15. Reis MM, Moreira AC, Sousa M, Mathur PP, Oliveira PF and Alves MG..Sertoli cell as a model in male reproductive toxicology: Advantages and disadvantages. Journal of Applied Toxicology, 2015; 35(8): 870–883.
16. Kaur G, Thompson LA and Dufour JM. Sertoli cells--immunological sentinels of spermatogenesis. Seminars in Cell & Developmental Biology. 2014; 30: 36-44.
17. Papaioannou M D, Pitetti J, Ro S, Park C, Aubry F, Schaad O, Vejnar CE, Descombes P, Zdobnov EM, McManus MT, Guillou F, Harfe BD, Yan W, Jégou B and Nef S. Sertoli cell Dicer is essential for spermatogenesis in mice. Developmental Biology. 2009; 326, (1): 250–259.
18. Shaikh NH, Deshmukh VM and Walvekar MV. Altered basement membrane synthesis in the testis after tissue injury. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(3): 72-77.
19. Foley GL. Mechanisms of Male Reproductive Organ Toxicity. Toxicologic Pathology. 2001; 29(1):49 –63.