The Effect of Pregabalin (Lyrica) on the Spermatogenic Cells in Rat

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Abstract

Background:

Pregabalin is an anti-convulsent drug that is indicated in epilepsy, neuropathic pain and anxiety. This study evaluates its effect on spermatogenesis in male rats through histological and physiological examination of the seminiferous tubules.

Two doses of Pregabalin were employed, 300, 600 mg/Kg B.Wt. given orally and daily to groups of 30 male albino rats (*Rattaus rattus*) for 35 days. The changes in the testes were evaluated by paraffin sections stained with Hematoxylin and Eosin.

The results showed relative preponderance of primitive germ cells over other stages of more mature spermatogenic cells. There was reduction in the diameter of the seminiferous tubule. These findings were more prominent with increase of the dose of Pregabalin.

The above results were discussed in dose related context indicating that the current therapeutic dose of Pregabalin seems to not be within the range of dosage of Pregabalin that adversely affects spermatogenesis.

Key words: Pregabalin, Seminiferous tubule, Histopathology, certain sperm function parameters.

Introduction:

Pregabalin is a potent anti-convulsent agent. It is used as adjunctive therapy for epilepsy, neuropathic pain and anxiety [(1)]. It was first marketed by Pfizer under the trade name Lyrica, and approved by the Food & Drug Administration (FDA) in 2004.

Pregabalin is an analogue to the neurotransmitter gamma amino butyric acid (GABA). Its main site of action is in the peripheral and central nervous systems, where it appears to block voltage-dependent calcium channels. Pregabalin partially reduces pre-synaptic neurotransmitter release as glutamate, norepinephrene and substance P [(2)].

Treatment of men for convulsive disorders during their reproductive life, involves the exposure of the spermatogenic process to the influence of anti-convulsent drugs.

During spermatogenic cycle, number of developmental phases (mitosis, meiosis, differentiation and maturation) of various durations takes place [(3)].

In pre-clinical studies of Pregabalin, it was found that spermatogenesis in experimental rat was affected leading to some degree of impairment of male rat fertility. In fertility studies, in which male rats were orally administered Pregabalin (50-2500mg/Kg B. Wt.) was given prior to and during mating with untreated females, a number of adverse reproductive effects were observed. These included decrease sperm counts and sperm motility, increased sperm abnormalities, reduced fertility and increased pre-implantation embryo loss [(4)].

The aim of this study is to evaluate the influence of exposure to Pregabalin on the testis of the rat, and to detect any histopathological alterations in the seminiferous tubules and detecting possible adverse effects of this drug on spermatogenesis.

Materials and Methods:

This study was carried on sexually mature laboratory breed of male albino rats (*Rattus rattus norvegicus albinos*) , their body weight was 200-300 gm and average age is 6-8 weeks. The animals were obtained from the animal house of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies.

Pregabalin ((Lyrica®), Pfizer) was given to two groups of rats, each comprises of 30 males, in two doses. One group was given 300mg/Kg. B.Wt, the other 600mg/Kg.B.Wt.. The drug was given with appropriate dilution in one milliliter of distilled water administered orally using polyethylene tube connected to hypodermic syringe.

The dose of Pregabalin was given daily at approximately the same time. A control group of similar 15 rats received the same volume of distilled water.

The duration of drug administration was for 35 continuous days. This duration covers at least one spermatogenic cycle in rat.

At the end of treatment period, the animals were sacrificed and the testes were retrieved. Histological preparations were performed in the standard methods (5), and sections were stained with hematoxylin and eosin, and then subjected for histopathological observation and evaluation. The semen analyses was done to examination the certain sperm function parameter.

Results:

In histological sections, the seminiferous tubules were shown to be composed of seminiferous epithelium (germinal epithelium), surrounded by thin connective tissue, the tunica propria (Figure (1)).In the seminiferous epithelium of the Pregabalin treated group, the spermatogonia were observed as rounded cells located at the basal part of the seminiferous epithelium, they over-populate most of the other spermatogenic cells. Thus, there is relative preponderance of primitive germ cells on the expense of more mature ones (Figure (2), Figure (3)). These cells with well defined rounded nuclei can not be cytologically segregated into dark or pale spermatogonia.

The lumen of the seminiferous tubules are clear and empty, in Pregabalin treated group, compared to the crowded seminiferous tubules, filled by spermatozoa in the control group.

Generally, in overview sections, there is some decrease in the seminiferous tubules caliber.The above findings are more pronounced with increasing dose of Pregabalin i.e. more in group receiving 600 mg/KgB.Wt. than that receiving 300mg/Kg B. Wt. of Pregabalin.

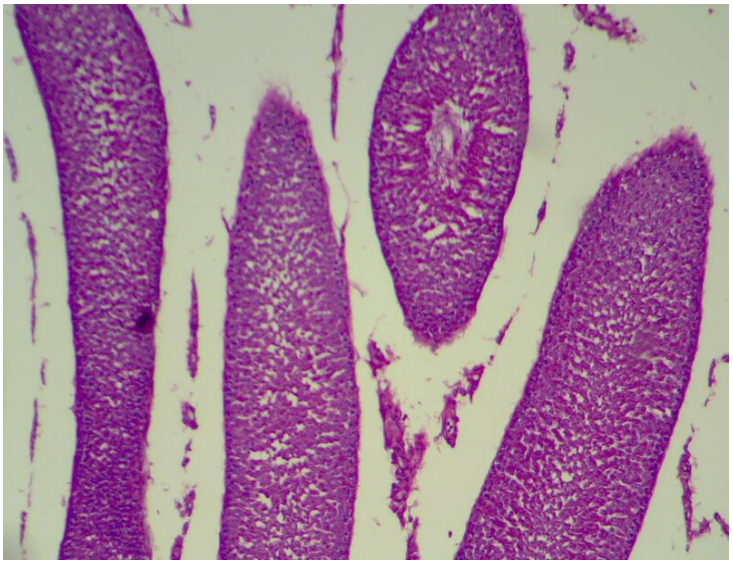

Figure (1): Section in the seminiferous tubules of the control group. H&E stain.

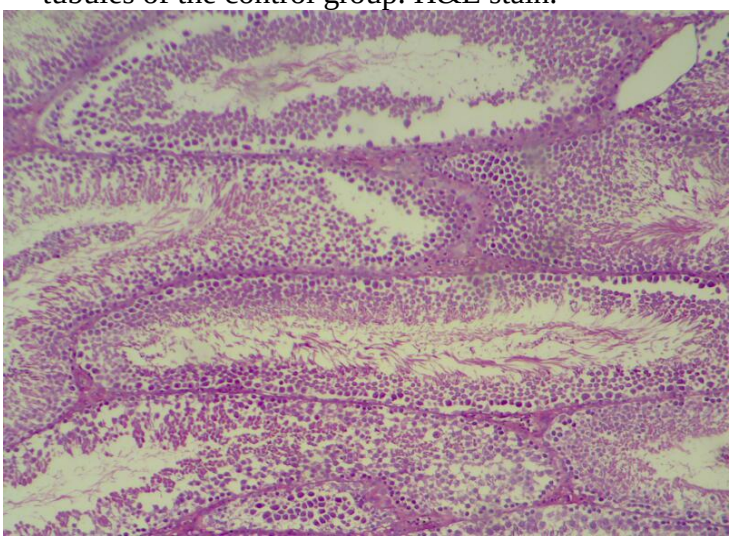

Figure (3): Seminiferous tubule of rat treated with 600 mg/Kg B.Wt. Pregabalin. H&E stain.

Figure (2): Seminiferous tubule of rat treated with 300 mg/Kg B. Wt. Pregabalin. H&E stain.

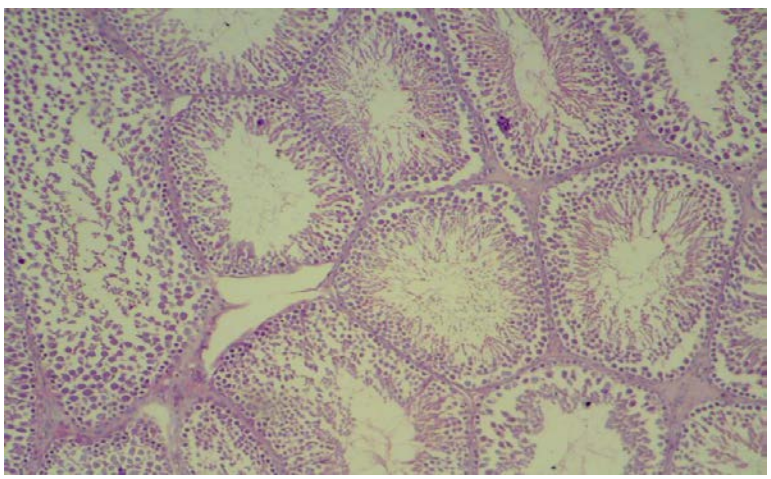

Table 1: Comparison of certain sperm function parameter between the treated and control groups.

Certain Sperm function Parameters	Treatments			P-value
	Control	Low	High	
Sperm Concentration millions/mL	63.250 a ±0.62	53.400 b ±0.45	40.917 c ±1.71	0.000**
Sperm Motility (%)	72.813 a ±2.46	66.000 b ±0.74	65.967 b ±2.06	0.046 *
Progressive Sperm Motility (%)	40.720 a ±2.88	32.500 b ±0.48	30.625 b ±3.44	0.038*
Non Progressive Sperm Motility (%)	42.188 a ±3.56	33.083 b ±0.48	25.333 c ±1.78	0.000**
Immotile Sperm (%)	24.688 b ±2.16	34.000 a ±0.74	34.033 a ±2.06	0.006**
Sperm Agglutination (%)	7.500 b ±0.57	13.938 a ±0.54	14.067 a ±0.35	0.000**
Morphologically Normal Sperm (%)	86.833 a ±0.53	78.500 b ±0.47	78.233 b ±0.36	0.000**

Discussion:

The period of spermatogenesis is considered as a period of intense cellular transformation which is regarded to be highly susceptible to external insults. Various germ cell stages are affected differentially by exposure to injurious agents. Spermatogonia have been recognized as target cells for ionizing radiation, while chemical agents are more considered as spermatocytes toxins [6]. In this study, the population of primitive germ cells i.e. spermatogonia, showed a relative prominence in the seminiferous tubules compared to other stages of more mature cells. It seems that spermatocytes kinetics are more affected by Pregabalin, in particular their division by meiosis, leading to a decrease in the formation of spermatocytes in the lineage of spermatogenesis [7]. On the other hand, mitosis of spermatogonia seems not to be influenced, and this may explain the relative abundance of primitive cells in the seminiferous tubules of Pregabalin treated rats. This discrepancy between primitive spermatogonia cell population and more mature spermatocytes is more evident in the group that received higher dose of Pregabalin. The relative clearance of the lumen of the seminiferous tubules in Pregabalin treated group as compared with spermatozoa-crowded lumens of control group may reflect the hindrance of the process of production of spermatozoa through spermatogenesis. This is in agreement with the findings of reduced fertility parameters produced by Pregabalin reported previously [4]. These interpretations of the histopathological picture require more conclusive evidences that may be obtained by seminal analysis. It is evident from this study that there is a decrease in the caliber of seminiferous tubules, although morphometric measurements will be needed to precisely quantify our results. We can postulate that a decrease in the maturation rate that is more pronounced at higher doses of Pregabalin is the outcome of the effect on this drug on spermatogenesis. In this work, the histopathological alterations observed in the seminiferous tubules were detected in rats exposed to Pregabalin 300 mg/Kg Body Wt and are more clear at the dose of Pregabalin 600 mg/Kg. B. Wt. Previous toxicological studies of Pregabalin for 28 days of Pregabalin exposure, observed changes in male rats in doses of 50-1250 mg/Kg. B. Wt. [4].It was also stated that the "no effect" dose on male reproductive organs histopathology in rats was 250 mg/Kg. B. Wt. This is associated with Plasma exposure approximately eight times human exposure at therapeutic dose

[8]. This makes Pregabalin rather safe drug for human usage regarding its effect on spermatogenesis. Even more, these findings make its role in male-mediated teratogenesis a questionable one. To reinforce the above conclusion, it was found that in controlled trials to assess the effect of Pregabalin on sperm motility in healthy men whom were exposed to Pregabalin 600 mg/Kg. B.Wt. for three months (one complete sperm cycle in human), Pregabalin did not exhibit significant detrimental effects on the reproductive functions of healthy male subjects, as measured by semen analysis [9].

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