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Effect of Carbamazepine (Tegretol) on Some Functional Testicular Parameters in Adult Male Mice

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Abstract

Carbamazepine as a drug used in the treatment of epilepsy and neuropathic pain has been shown to cause reproductive disorders. The present study was aimed to determine the effects of different doses of Carbamazepine (CBZ) (Tegretol) on the sex hormones and some testes parameters of adult albino Swiss mice. The study sample included 24 male mice divided into 4 groups. 1st, 2nd and 3rd groups were orally administrated with a daily dose 2.5, 5 and 10 mg/kg bw carbamazepine respectively. While group four served as control and administrated with 0.1 mL distilled water for 60 days. The present study investigates effects of carbamazepine medication on serum sex hormones (folicall stimulating hormone FSH, prolactin hormone, lutinizing hormone LH and testosterone hormone) as well as sperm functional parameters. It can be concluded that acute exposure to carbamazepine exerts negative effects on testes parameters of treated mice.

Key words: Carbamazepine, sex hormones, and sperm parameters.

1. Introduction

Epilepsy is a chronic disease characterized by frequent bouts caused by a defect in the transmission of electrical signals in the brain, which affects about 50 million people worldwide [1, 2].

Carbamazepine ($C_{15}H_{12}N_2O$) is a tricyclic compound and commonly known as tegretol and one of the most famed drugs used to treat epilepsy. Carbamazepine is an anticonvulsant and mood-stabilizing drug used mainly in the management of bipolar disorder (trigeminal neuralgia). Attention-deficit hyperactivity disorder ADHD, schizophrenia, phantom limb syndrome, complex regional pain syndrome, paroxysmal extreme pain disorder.

Moreover, neuromyotonic, and post-traumatic stress disorder [3,4].

Carbamazepine is a sodium channel blocker. Carbamazepine quandaries preferentially to voltage-gated sodium channels in their inactive conformation, which prevents repetitive and sustained firing of an action potential. Carbamazepine has effects on serotonin systems but the relevance to its antiseizure effects is uncertain [5].

Other studies showed that reproductive hormonal and sexual dysfunction occur in men with epilepsy (28-70 %). Also showed as decreased libido, infertility, and erectile difficulties [6,7]. CBZ induce the metabolism of sex steroid hormones and causes of reproductive and sexual dysfunction [6, 8].

2. Materials and Methods

2.1 Preparation of carbamazepine of required dosage

One dose of carbamazepine was selected 200 mg/kg to test its toxic effect. While weights of mice used in the experiments was ranged between 25-30 g. The required dose of the drug was dosed orally for all mice once per day for 60 days for three concentrations (2.5, 5, 10 mg/kg).

2.2 Experiment animals

Adult white male mice used in this study were 24 animals with an average weight of about (25-30 g). mice were selected from the animal house of the Iraqi centre for cancer research. The animals were placed in plastic cages, and the cages floors were covered with sawdust. Cages were cleaned in terms of replacing sawdust twice during the week and were sterilized with disinfectants to avoid diseases.

During the experiment stages, all animals were subjected to similar laboratory conditions in terms of continuous ventilation and lighting (12 hours of light and 12 hours of darkness), and the temperature ranged between (22- 25 °C), with humidity between (40-60 %). Furthermore, water was given towards continuous duration of the study. This study is aimed at evaluating the effects of CBZ on sperm function parameters and some sex hormones in adult male mice.

2.3 Experiment groups

Animals were divided randomly into four groups each group contains six animals. Animals were given carbamazepine with concentrations of (2.5, 5 ,10 mg/kg). Administrated as aqueous solution of the substance orally. For the fourth group were give distilled water.

2.4 Blood collection

Blood was collected in one stage from the animals by a heart stab method while they were alive. The blood was kept

in anticoagulant tubes (EDTA tube). Then placed in a cooled centrifuge at a mouse of 3000 rpm at a temperature of 25 ºC for a period of 10 minutes. To obtain the serum, after isolating the serum in sterile tubes, tests were performed to see Levels of the following hormones (testosterone, prolactin, LH, and FSH) were done at the biotechnology research centre at Al-Nahrain University.

2.5 Total sperms count

For studying specifications of the sperms, the tail of epididymis was taken and embedded in one drop of phosphate buffer solution at 37 ºC in a watch glass. Then the tail was cut in to at least 200 sections by microsurgical scissors, to calculate sperm-specific function parameters including count, motility and viability. Moreover, added one drop of Eosin-Nigrosine stain and mixed. Random fields of the sperm preparation were microscopically examined to assess the percentage of sperms with abnormalities such as changes in head and tail, through the equation below.

Percentage of abnormal sperms = Number of abnormal sperms / total number × 100

2.6 Statical analysis

Statistical Analysis System (SAS) (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference–LSD test (Analysis of Variation-ANOVA) was used to

significant compare between means in this study [10].

3. Results

Results showed a significant decrease ($P \leq 0.05$) in concentration FSH in groups treated with a drug for concentrations (2.5, 5, 10) mg/kg of body weight (265.34 ± 30.47), (244.59 ± 26.76), (131.55 ± 12.38) international unite respectively compared with the control group (432,38 ± 75.88) IU.

As for the hormone LH, the results showed a significant decrease ($P \leq 0.05$) in the levels of the hormone in the tree groups treated with drug for concentrations (2.5, 5, 10) mg/kg of body weight (47.34 ± 4.23), (47.85 ± 5.17), (34.80 ± 5.10) IU compared with the control group (139.93 ± 18.87) IU.

The obtained results found significant decrease ($P \leq 0.05$) in the levels of testosterone hormone in the three groups treated with CBZ for concentrations (2.5, 5, 10) mg/kg of body weight. Also (1.85 ± 0.32), (2.12 ± 0.37), (0.83 ± 0.12) ng/ml compared with the control group (3.49 ± 0.45) ng/ml. Results also showed a significant decrease ($P \leq 0.05$) in the levels prolactin hormone in groups treated with CBZ for concentrations (2.5, 5, 10) mg/kg

of body weight (9.09 ± 0.92), (8.95 ± 0.63), (4.55 ± 0.49) IU (4.55 ± 0.49) IU.

The comparison with the control group (14.39 ± 1.19) IU as shown in (table 1). Results showed a high significant decrease ($P \leq 0.05$) in total sperm count in group treated with drug for concentrations (2.5, 5 and 10) mg/kg of body weight (20.25 ± 2.01), (13.50 ± 1.04), and (11.00 ± 0.91) sperm X106 compared with control group (28.50 ± 1.84) sperm X106.

As for the motility of sperms, Results showed a significant decrease ($P \leq 0.05$) in sperm motility in group treated for concentrations (2.5, 5 and 10) mg/kg of body weight (85.00 ± 2.04) (73.75 ± 4.26), (58.75 ± 4.26) % compared with control group (91.75 ± 1.37) %. Obtained results presence of abnormal forms of sperm in group treated for concentrations (2.5, 5, 10) mg/kg of body weight. Also (13.50 ± 1.55), (14.50 ± 1.32), (22.25 ± 2.01) % in comparison with control group (8.75 ± 1.49) %.

The results showed a significant increase ($P \leq 0.05$) in the percentage of dead sperm in the groups treated for concentrations (2.5, 5, 10) mg/kg of body weight with (12.2 ± 1.25), (19.25 ± 1.11), and (25.50 ± 1.84) % compared with the control group (8.0 ± 1.29) % in (table 2).

Table 1: The effect of different concentrations of the carbamazepine on the levels of prolactin, FSH, LH and testosterone.

Groups	Mean ± SE			
	FSH (m IU/ml)	LH (m IU/ml)	Testosterone (Ng/ml)	Prolactin (m IU/ml)
Control	432.38 ± 75.88 a	139.93 ± 18.87 a	3.49 ± 0.45 a	14.39 ± 1.19 a
CBZ 2.5 mg/kg	265.34 ± 30.47 b	47.34 ± 4.23 b	1.85 ± 0.32 bc	9.09 ± 0.92 b
CBZ 5 mg/kg	244.59 ± 26.76 b	47.85 ± 5.17 b	2.12 ± 0.37 b	8.95 ± 0.63 b
CBZ 10 mg/kg	131.55 ± 12.38 b	34.80 ± 5.10 b	0.83 ± 0.12 c	4.55 ± 0.49 c
LSD value	133.93 *	31.83 *	1.053 *	2.64 *
Means having the different letters in same column differed significantly. * ($P \leq 0.05$).				

Table 2: The effect of different concentrations of the carbamazepine on total count sperm, motility, abnormality and viability 10^6.

Group	Mean ± SE			
	Total count sperm $\times 10^6$	Motility %	Abnormality %	Viability %
Control	28.50 ± 1.84 a	91.75 ± 1.37 a	8.75 ± 1.49 c	8.00 ± 1.29 c
CBZ 2.5 mg/kg	20.25 ± 2.01 b	85.00 ± 2.04 a	13.50 ± 1.55 bc	12.25 ± 1.25 c
CBZ 5 mg/kg	13.50 ± 1.04 c	73.75 ± 4.26 b	14.50 ± 1.32 b	19.25 ± 1.11 b
CBZ 10 mg/kg	11.00 ± 0.91 c	58.75 ± 4.26 c	22.25 ± 2.01 a	25.50 ± 1.84 a
LSD value	4.722 *	10.046 *	4.982 *	4.323 *
Means having the different letters in same column differed significantly, * ($P \leq 0.05$) .				

Results showed appearance of deformed sperms, including cytoplasmic droplet (figure 2), amorphous head with hook tail (figure 3), wavy tail (figure 4), folded tail (figure 5), atrophy head and folded tail (figure 6), headless sperm (figure

7), bended tail (figure 8), compare with the normal shape of sperm (figure 1).

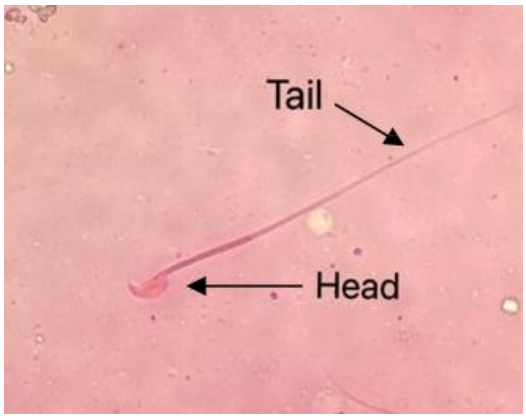

Figure 1: Normal sperm of mice.

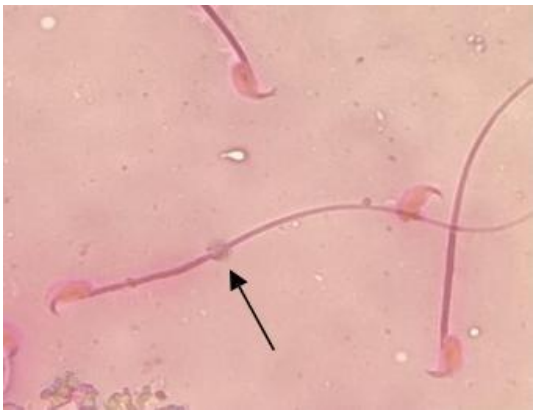

Figure 2: Cytoplasmic droplet of mice sperm after treated by carbamazepine 2.5 mg/kg.

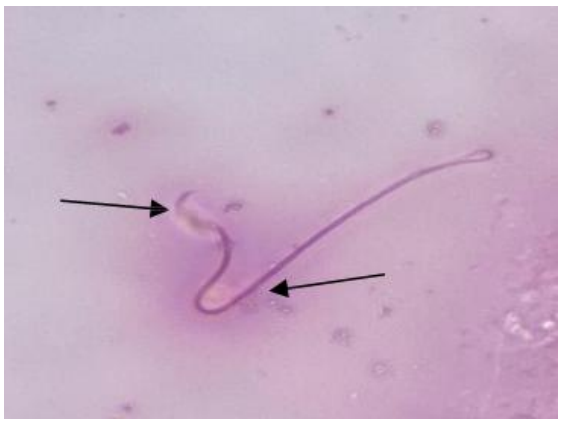

Figure 3: Amorphous head with hook tail.

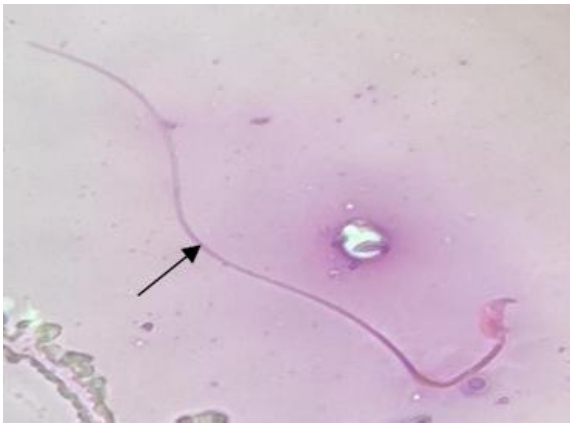

Figure 4: Wavy tail of mice sperm after treated by carbamazepine for concentration 5 mg/kg.

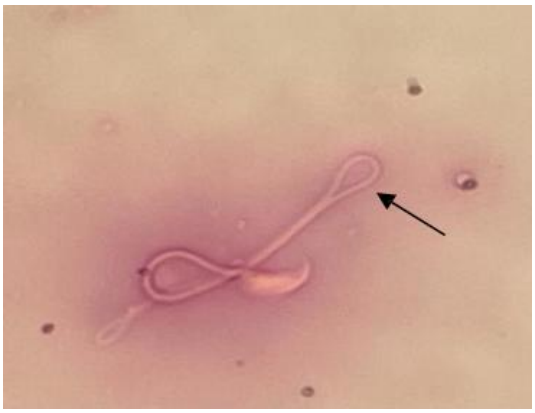

Figure 5: Folded tail.

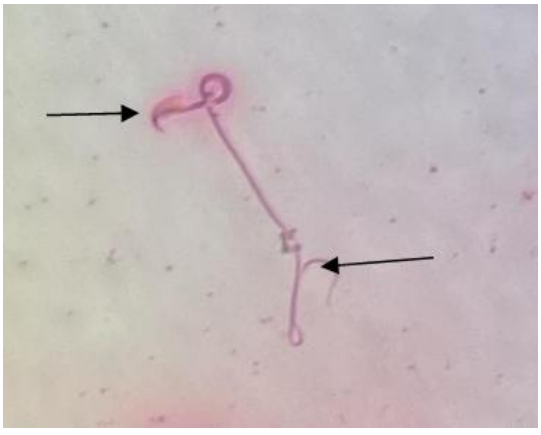

Figure 6: Atrophy head with folded tail of mice sperm after treated by carbamazepine for concentration 10 mg/kg.

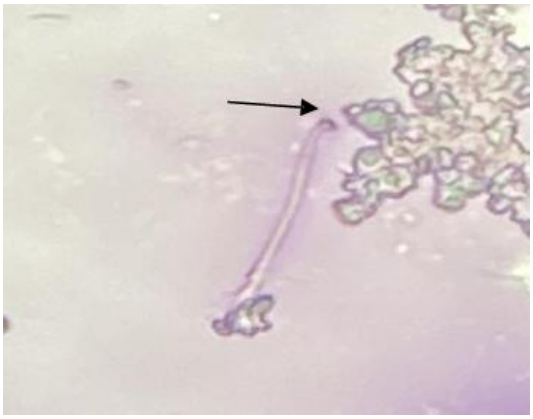

Figure 7: Headless sperm.

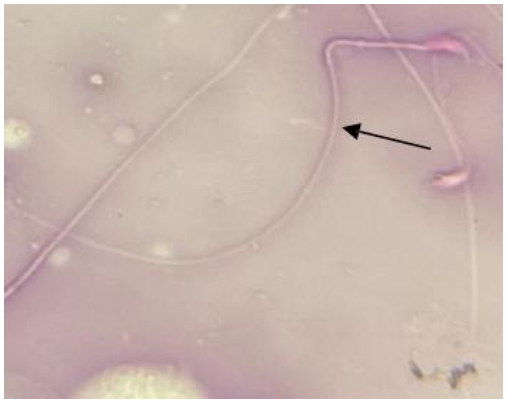

Figure 8: Bended tail of mice sperm after treated by carbamazepine for concentration 10 mg/kg.

4. Discussions

Results of the present study indicated that a change occurred in serum sex hormones balance and significant decrease were showed in sperm count, sperm motility during CBZ. CBZ and some chemicals induce the metabolism of sex steroid hormones resulting in reduced blood concentration of bioactive testosterone, which results in diminished potency [8,11].

The plasma concentration of free testosterone is decreased in male epileptic patients and same trend was observed in rats treated with anti-epileptic drugs and used androgen [12-14). This suggests that CBZ curb fertility in rats, not only by interfering with testicular functions, but maybe by affecting the hypothalamus-pituitary-testicular (gonadal) axis.

Thus, decreasing the amount of testosterone in circulation. Testicular functions and male reproductive system are almost completely controlled by testosterone [15]. The anti-epileptic drugs act on the pituitary gland and decline the secretion of FSH and LH responsible for spermatogenesis.

The process of spermatogenesis and accessory reproductive organs are androgen dependent. Produced androgen production will cause a decrease in the number of mature Leydig cells and their functional cases [16,17]. The present study found lower in prolactin hormone levels during CBZ-treated male mice than control group. The evidence shows that single AEDs can alter the responsiveness of the anterior pituitary to various stimuli [18].

Norepinephrine (NE) may stimulate LH release and serotonin (5-HT) may have an inhibitory and/or a stimulatory effect. The release of PRL from the anterior pituitary is primarily controlled by an inhibitory influence mediated by the tuberoinfundibular dopaminergic pathway [19].

Results of the present study indicated that a decrease in sperm count and showed decrease in sperm motility during CBZ medication. Also, the study showed the presence of abnormal forms of sperm and obtained increase in the percentage of dead sperm. AED can directly act on the HPG axis or on peripheral hormones and induce various reproductive or sex hormonal abnormalities [20]. AED has been shown to decrease sperm quantity and quality.

Also affect spermatogenesis, resulting in infertility [21,22]. Disturbances in reproductive hormones and gonadal morphology have been observed in patients with epilepsy and in non-epileptic animals on AED [23,24]. Studies on AED including CBZ indicated drug-related side effects which include abnormal sperm morphology, decreased motility, lower sperm count [25,26].

Those results suggested that the semen quality may be harmfully affected because of direct toxic effect of AED on the gonads [27]. A side effect of AEDs on the HPG axis with abnormalities of sex hormones (such as testosterone) which are essential for normal sperm [28]. A result of carnitine deficiency associated with some AED including CBZ [29-31].

L-carnitine is produced by hepatocytes, concentrated in the blood stream, secreted by epididymal epithelium and taken up by the spermatozoa. L-carnitine and acetyl-carnitine play a crucial role in sperm metabolism, maturation and motility. Low levels of carnitine decline fatty acid concentrations within the mitochondria leading to reduce energy production and decrease sperm motility [32].

CBZ was found to result in germ cell mutagens and an increase in the number of sperm with head defects in experimental studies [33]. The severity in the decreasing of sperm counts and motility is drug dependent. That is because to direct effect CBZ on spermatogenesis, specifically its interference with acetylcholine-esterase and glucose, both of which are required for the process of spermatogenesis. Because sperm motility, in addition to being under the control of acetylcholine also relies on glucose availability [34].

Enzyme induction can also contribute to the development of chronic harmful effects, such as decreased sexual dysfunction, and potentially deleterious changes in cholesterol concentrations and other markers of vascular risk [35]. CBZ-evoked oxidative harm to the spermatozoa could be because of excessive proteins in the epididymis and testis, androgen decreased causing sperm abnormality [36, 37].

The elevated reactive oxygen species (ROS) that may harm sperm membrane causing changing morphology [38]. Also directs effect of AEDs and some drugs on spermatozoa germinal lineage cells because

these drugs are highly lipid-soluble and cross the blood-testis barrier into the seminiferous tubules [39, 40]. The reduction in the percentage viability of sperm could be interconnected with the reduction in sperm motility, since non-motile or slow spermatozoa were usually considered dead and could not be counted as live spermatozoa [41].

5. Conclusion

The present study has shown that administration of CBZ (2.5, 5 and 10) mg/kg to male mice, reduced serum follicle stimulating hormone, lutinizing hormone, testosterone hormone and prolactin hormone. That is associated with decrease in sperm count, motility, abnormal forms of sperm and increase of percentage of sperm. This may be affected by a Hypothalamus-Pituitary-Testicular axis as compared to treated mice by CBZ.

6. References

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