Tegretol (Carbamazepine) Effect on the Morphometric Assay of Liver in Female White Mice (*Mus musculus)*

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Article history: Received 15 September 2018, Accepted 6 November 2018, Published December 2018

Abstract

Carbamazepine (CBZ) is one of many anticonvulsants used to treat trigeminal neuralgia and epilepsy. Antiepileptic drugs (AED`s) are the second most important class of medications that lead to hepatotoxicity and induced liver injury, this study was conducted to evaluate the effects of CBZ on the liver. A total of 40 female mice were taken and divided into four groups (A/treated for 14 days, B/ control, C/ treated for 30 days, D/ control), the drug was given as an oral suspension formula 100mg/5ml at dose 20 mg/kg/mouse via gastric gavage daily for 14 and 30 days. Statistical analysis revealed that there were no significant differences in the white female mice body weight ($P>0.05$) in the treated group for 14 days as well as the treated group for 30 days, but there were significant differences between the treated groups studied. Statistical analyses result of liver weight, hepatocytes diameter, central vein and portal vein diameters showed significant differences in the treated group for 14 days ($P<0.05$) as well as the treated group for 30 days, and there were significant differences between all treated groups studied. The study concluded that carbamazepine (CBZ) can had no effect on body weight but it can induce several hepatic changes if it used on a short or long terms, therefore, it was advised to take cautions when describing this drug.

Key words: Carbamazepine, Liver, Morphometric assay

1. Introduction

The uses of antiepileptic drugs (AED`s) these days are not limited to epilepsy treatment only, they can be used to treat variety of neurological and psychiatric disorders [1], those drugs are the second most important class of medications that lead to hepatotoxicity [2], and can also have teratogenic effects on many organs such as kidneys [3, 4, 5], the cerebral cortex [6], ovary [7], and even spermatogenesis [8]. Drug induced liver injury is one of the complications of numbers of treatments specially AED`s [9], it can simulate any liver disease and have no specific features [10], Drug induced liver injury mechanism can either be hypersensitivity reaction or an idiopathic drug reaction, the hepatotoxicity is connected to the amount of dose and most reactions appear in the very first weeks of therapy [11].

After the skin, the liver is considered the second largest organ in the body [12]. With more than 200 functions it counts as the most versatile organ and have a major role in the regulation of metabolism in the body [13]. In addition, exocrine and endocrine functions that are

performed by the main functional cell, the hepatocyte [14] the liver performs detoxification of many drugs and toxins that cannot be easily removed by the kidney [15]. Also, the liver is a target for many xenobiotics that can cause organ dysfunction or even failure by direct effects that lead to a change in liver blood flow [16].

Carbamazepine (CBZ) is an enzyme inducer drug [17] that discovered in the 50`s by the Swiss chemist Walter Schindler, an anticonvulsant that was approved in (1964) by food and drug administration (FDA). As a medication for trigeminal neuralgia and in (1974) it was approved as an AED [18]. This drug showed its effectiveness among many AED`s in epilepsy treatment such as the treatment of partial epilepsy [19], bipolar disorder beside complex partial seizers [20]. Although, it has its own downsides, studies showed that there is a link between using carbamazepine (CBZ) and HLA allele in patients of Asian ethnicity. Using this drug can cause morbid side effects such as Stevens-Johnson syndrome (SJS) and Toxic epidermal necrolysis (TEN), therefore screening test is needed when using this drug specially on specific populations [21].

Carbamazepine (CBZ) works by blocking Na^+ channels. It reduces the abnormal impulses in the brain by blocking these channels and inhibiting the repetitive regeneration of the seizer preventing it from propagation [22]. It metabolizes in the liver by CYP450 [23], converts to many metabolites by the epoxide-diol pathway [24]. The most known metabolite of this drug is carbamazepine 10, 11 epoxides (CBZ-E) [25]. These metabolites can form a covalent bond with proteins that change its location or inhibit the protein and works as a Neoantigen (hapten) that induces an immune response and cause liver disease [26], not only it can injure the liver but it also can affect the embryos by its teratogenic effects like affecting the formation of the spinal cord [27], kidneys [28] and brain development [29].

2. Materials and Methods

2.1. Experimental Animals

Female white mice *Mus musculus* were obtained from the National Center for Drug Control and Research in Baghdad. The age (7-12) weeks and weighted (22-29) gm, they were brought to the animal house in College of Education for Pure Science / Ibn Al-Haitham. All the animals were kept under controlled environmental condition from air, light and temperature.

2.2. Experimental Design

This study was conducted on (40) white female mice. They were divided in to four groups. Group A as an experimental group with 10 female mice were administered carbamazepine drug as an oral suspension formula 100mg/5ml at dose 20 mg/kg/mouse via gastric gavage daily for 14 days. Group B as a control group with 10 female white mice were administered in drinking tap water for 14 days. Group C as an experimental group with 10 white female white mice were administered carbamazepine drug as an oral suspension formula 100mg/5ml at dose 20 mg/kg/mouse via gastric gavage daily for 30 days, and group D as a control group with 10 female white mice were administered in drinking tap water for 30 days.

2.3. Treatment

Tegretol® carbamazepine (CBZ) drug was procured from Novartis pharma AG, Basle, Switzerland.

2.4 .Samples Collection

After 14th and 30th day mice were killed after euthanizing and weighed by using a sensitive balance, the liver was removed and weighted as well, then fixed in formalin (10%) for 72 hours. It dehydrated with ascending grades of ethanol (70%-80%-90% and 100%), followed by clearing in two changes of xylene, then infiltrated with paraffin wax and embedded in plastic cassettes. Afterwards, blocked out, sections (6) µm thin were stained with (H&E) stain, followed by examination under compound light microscope at 10x, 40x, 100x magnifications.

2.5. Morphometric Parameters

Using the ocular micrometer lens and Image J image analyzing program, the hepatocytes diameter was measured along with the central vein and portal vein diameters at magnification 40x.

3. Results

In the present study body weight of white female mice after 14 days of administration showed no significant differences (P>0.05) in mean of body weight in comparison with the control group as well as between the treated group for 30 days when compared to their control group, but there were significant differences between the treated groups, as shown in **Figure 1.** and **Table 1.**

With respect to the statistical analysis results of liver weight, the differences in the treated group for 14 days were statistically significant (P<0.05) in comparison with the control group. As well as between the treated group for 30 days when compared with control group, and there were significant differences between the treated groups (**Figure 2.**) and (**Table1**.).

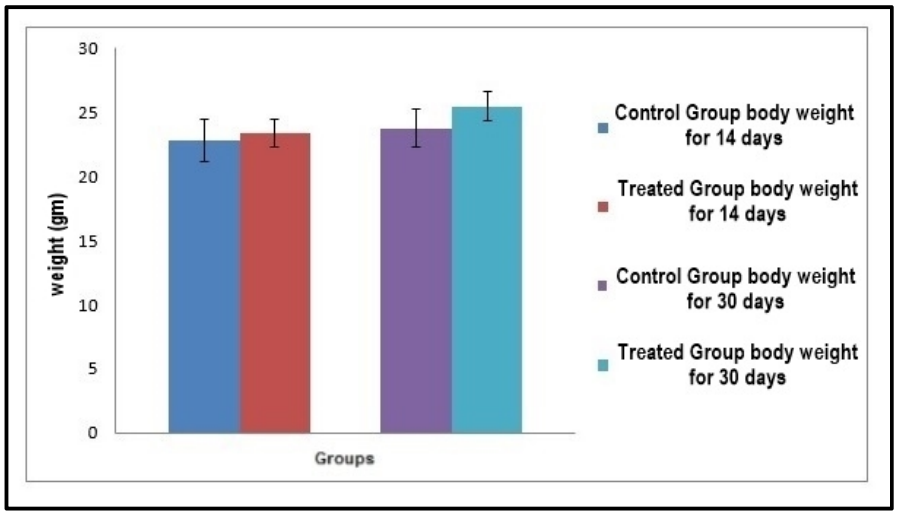

Figure 1. The effect of carbamazepine drug at dose 20 mg/kg/day on female white mice body weight treated for 14 and 30 days with the control groups.

Table 1. Comparison of mice body and liver weight between the control groups and the treated groups.

Day	Body weight (gm) Mean ± S.E		Liver weight (gm) Mean ± S.E	
	Control group	Treated group	Control group	Treated group
Day 14	22.820±1.619	23.330±1.079*	1.387±0.036	2.832±0.162*
Day 30	23.749±1.453	25.456±1.157*	1.411±0.044	2.389±0.172*

In this table the 14 days' group showed no significant differences (P>0.05) in mean of body weight in comparison with the control group as well as between the treated group for 30 days when compared to their control group, significant differences were found between treated groups.

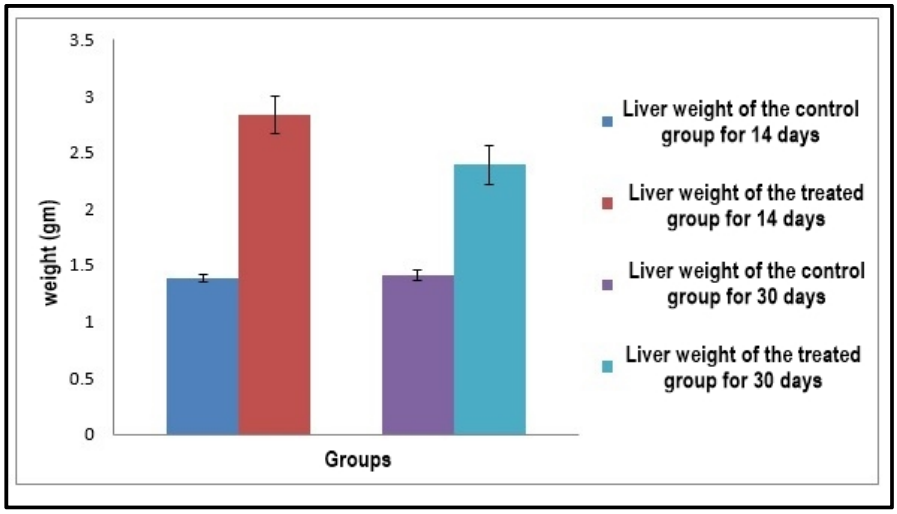

Figure 2. The effect of carbamazepine drug at dose 20 mg/kg/day on female white mice liver weight treated for 14 and 30 days with the control groups.

While the statistical analysis results of the hepatocytes diameter in the 14 days' group were significantly different (P<0.05) in comparison to their control group as well as between the treated group for 30 days in comparison to control group and between the treated groups as in **Figure 3.** and **Table 2.**

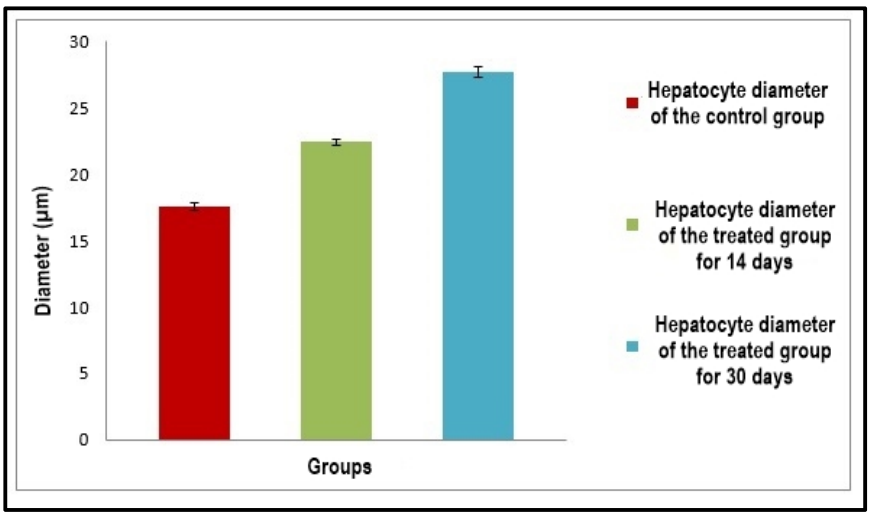

Figure 3. The effect of carbamazepine drug at dose 20 mg/kg/day in the mean diameter of hepatocytes of female white mice liver treated for 14 and 30 days with the control groups.

Table 2. Comparison of hepatocyte diameter between the control groups and the treated groups.

Day	Hepatocytes diameter (µm) Mean ± S.E	
	Control group	Treated group
Day 14	17.5400±0.29766	22.4200±0.24372*
Day 30	17.5400±0.29766	27.7400±0.41304*

The statistical analysis results of the central vein and portal vein diameters were significantly increased (P<0.05) in the treated group for 14 and 30 days in comparison with their control groups. There were significant differences between the treated groups, (**Figures 4. & 5.** and **Table 3)**.

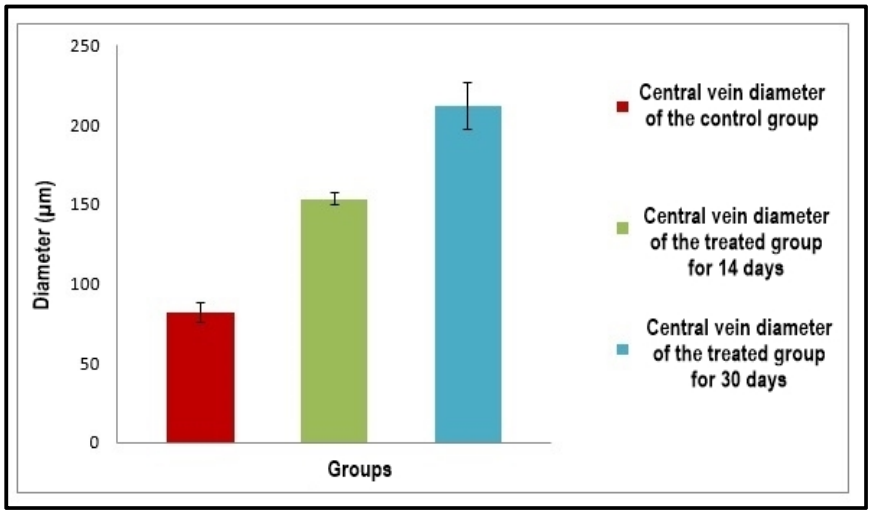

Figure 4. The effect of carbamazepine drug at dose 20 mg/kg/day in the mean diameter of the central vein of female white mice liver treated for 14 and 30 days with the control groups.

Table 3. Comparison of central vein and portal vein diameters between the control groups and the treated groups.

Day	Central vein diameter (µm) Mean ± S.E		Portal vein diameter (µm) Mean ± S.E	
	Control group	Treated group	Control group	Treated group
Day 14	81.5400±6.14773	153.3600±3.50423*	130.1400±4.46930	172.8000±9.95304*
Day 30	81.5400±6.14773	211.5000±14.63158*	130.1400±4.46930	229.8600±13.02235*

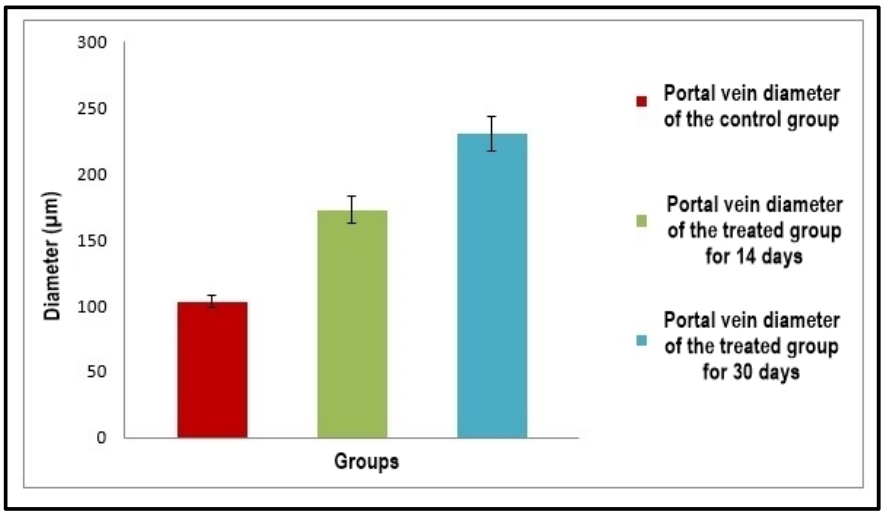

Figure 5. The effect of carbamazepine drug at dose 20 mg/kg/day in the mean diameter of the portal vein of female white mice liver treated for 14 and 30 days with the control groups.

4. Conclusion

One of the classic drugs or first generation AED`s that is effective in treating epilepsy seizers and neurological pain is carbamazepine (CBZ) [30], but it seems that carbamazepine (CBZ) treatment can generate liver injury and toxicity [31].

14 and 30 days later of treatment, white female's mice weight did not show significant differences when compared with control groups. It appeared that carbamazepine (CBZ) have no effect on body weight [32, 33]. Yet, some first generation drugs like Phenytoin (PHT) can induce weight gain [34]. Liver weight of white mice females after 14 and 30 days of treatment showed significant differences when compared with control groups. This can be explained by the induction that happened due to congestion, inflammation and fibrosis that lead to liver enlargement [35]. Also, hepatocyte hypertrophy and enzymic induction induced by chemical substance can cause the same results [36, 37]. Hepatocyte diameter showed significant differences in the treated female white mice liver after 14 and 30 days of treatment when compared to control groups. It appears that different toxins and drugs can engender hypertrophy due to enzymic induction that increases the number of smooth endoplasmic reticulum, peroxisomes and mitochondria [38]. Central vein diameter also showed significant differences in the treated white female mice liver after 14 and 30 days of treatment when compared to control groups. Its denoted that congestion in this blood vessel due to

impairment in venous outflow can be the reason [39]. Significant differences in portal vein diameter of the treated female white mice liver after 14 and 30 days of treatment were noticed when compared to control groups. It has been found that the accumulation of the fibrous tissue in the portal areas can lead to this result, and this fibrosis is in fact secondary to the congestion in the portal vein [40, 41].

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