



# Assessment of the biomarkers of hepatotoxicity following carbamazepine, levetiracetam, and carbamazepine-levetiracetam adjunctive treatment in male Wistar rats

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## ABSTRACT

**Objective:** This study examined some of the biomarkers of hepatotoxicity following chronic treatment with carbamazepine (CBZ), levetiracetam (LEV), and CBZ + LEV adjunctive treatment in male rats.

**Method:** Twenty-four male Wistar rats (140–150 g) were randomized into four groups (n = 6) to receive oral dose of normal saline (0.1 mL), CBZ (25 mg/kg), LEV (50 mg/kg) or sub-therapeutic dose of CBZ (12.5 mg/kg) together with LEV (25 mg/kg) for 28 days. Activities of the liver enzymes and oxidative stress markers were determined while liver histomorphology was also carried out. Data were analyzed using descriptive and inferential statistics. The results were presented as mean ± SEM in graphs or tables, while the level of significance was taken at p < 0.05.

**Results:** The activities of alkaline-phosphatase and malondialdehyde concentrations increased significantly in all the drug treatment groups, while the activities of superoxide dismutase decreased significantly following CBZ, and CBZ + LEV treatment. Alanine-aminotransferase activities increased significantly in the CBZ and CBZ + LEV treated rats compared with control. The liver section of CBZ treated rats showed mild vascular congestion.

**Conclusion:** None of these AEDs treatment is devoid of hepatotoxicity. However, the adverse effects in CBZ were greater than LEV, or CBZ + LEV adjunctive treatment.

## 1. Introduction

Carbamazepine (C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O), a tricyclic compound sold under the trade name Tegretol®, is one of the first-generation antiepileptic drugs (AEDs). Its effectiveness is not only in the treatment of partial and generalized tonic-clonic seizures but also with proven efficacy in the management of neuropathic pain and bipolar disorder [1,2]. Most of the first-line AEDs like carbamazepine, phenytoin, valproic acid etc are potent liver enzyme inducers. Ahmed and Siddiqi [3,4] reported an elevated activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and Gamma-glutamyl transferase (GGT) following few weeks to one month of administration of these drugs, which may serve as a red flag for liver toxicity. Several reports from human studies have implicated many conventional AEDs as toxicants that increase lipid peroxidation at the expense of protective

antioxidants [5–7]. Glutathione, for example is an important biomolecule that protects the cell against chemical-induced cytotoxicity. This is often achieved by direct or enzymatic glutathione-S-transferase (GST) conjugation with electrophilic compounds and reactive oxygen species (ROS), such as lipid hydroperoxides and hydrogen peroxide, and is affected by anticonvulsant treatments [7,8].

The United States Food and Drug Administration (FDA) approved carbamazepine formulations include chewable tablets (Tegretol®), a suspension (Tegretol®), sustained release capsules (Carbatrol®), and sustained release tablets (Tegretol®-XR) since 1965 [9]. It is widely accepted that carbamazepine (CBZ) [C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O] achieves its anticonvulsant properties by its interaction with different types of channels and receptors, while the main target of CBZ are voltage-dependent sodium channels [10]. Studies have shown that the pharmacologically active component of CBZ, carbamazepine-epoxide reduced the frequency of

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sustained repetitive firing of action potentials in cultured mammalian central neurons by inhibiting high frequency but not low frequency firing [10,11]. This frequency-dependent block is attributable to a voltage-dependent inhibitory effect on voltage-gated sodium channels. [11,12]. Parada and his co-researcher [13] demonstrated that CBZ blocks [3 H]batrachotoxin or [3 H]batrachotoxinin A 20- $\alpha$ -benzoate binding to synaptosomes. Thus, this corroborates the electrophysiological observations of Elliott, and Macdonald and Kelly [14,15].

Carbamazepine possesses calcium antagonistic properties; a suggestive indication that the efficacy of CBZ in the treatment of seizures could be due to a frequency dependent block of sodium currents and a block of calcium currents [16]. This could be linked to the depressant action of CBZ and organic calcium antagonists on epileptic paroxysmal depolarizations [17,18]. CBZ and CBZ-10,11-epoxide inhibit the secretion of catecholamines by interfering with N-type voltage-sensitive calcium channels [19,20]. Schumacher et al. [21] also demonstrated that CBZ produces a reversible, concentration-dependent inhibition of high voltage-activated calcium currents, without affecting voltage-dependent activation, in human hippocampal granule cells. In another investigation, Ambrósio et al. reported that CBZ inhibits L-type calcium channels in cultured rat hippocampal neurons stimulated with glutamate receptor agonists [22]. However, it is important to mention that the effects of CBZ were not significant at therapeutic serum levels of CBZ (17–51 mM) in some studies [16,22,23].

Olpe et al. [24] found that the depressant effect of CBZ is attenuated by barium chloride and 4-aminopyridine, two potassium-channel blockers, suggesting that CBZ may interfere with potassium fluxes. Indeed, CBZ enhances outward, voltage-dependent K1 currents in rat neocortical cells [25]. However, CBZ also blocks delayed K1 currents [26] and calcium-activated K1 currents [27]. The therapeutic and prophylactic effects of CBZ in affective psychoses may, in part, be related to the potent interaction of CBZ with adenosine-binding sites in the brain. In fact, several reports have demonstrated that CBZ acts as an antagonist at adenosine A1 receptors [28–30]. Chronic treatment with CBZ induces up-regulation of adenosine A1 receptors in astrocytes [31] and rats [32], respectively. There are overwhelming evidences that serotonin has anticonvulsant properties in several seizure models. CBZ causes large increases in extracellular serotonin concentration and produces dose-related anticonvulsant effects in both genetically epilepsy-prone rats (GEPRs) and non-epileptic Sprague-Dawley rats [33–36]. The effects of CBZ were not prevented by tetrodotoxin and by removal of calcium, suggesting that the enhancement of serotonin release is not dependent on sodium channel function and does not take place by exocytosis (10, 33). Some data also suggest that CBZ may alter dopamine function (34). CBZ enhances dopamine release and turnover and causes differential alterations of monoamine levels in discrete brain regions [35–39]. Evidence has established that NMDA and non-NMDA receptors play a crucial role in seizure activity and are potential targets for AEDs [40]. Therefore, the inhibition of either glutamate release or ionotropic glutamate receptors might contribute to the efficacy of anticonvulsants against epileptic seizures [10].

Levetiracetam [C<sub>8</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub> also known as (S)- $\alpha$ -ethyl-2-oxo-1-pyrrolidine acetamide] is an established second-generation AED. It was approved by United State of America Food and Drug Agency under the trade name Keppra® as a treatment drug for myoclonic seizures associated with juvenile myoclonic epilepsy and primary generalized tonic-clonic seizures linked with generalized epilepsy [41]. LEV does not exhibit the classical actions of the conventional AEDs in that, it does not affect voltage-dependent Na<sup>+</sup> channels, GABAergic transmission, or affinity for either GABAergic or glutamatergic receptors [10]. Levetiracetam affects GABA turnover in the striatum and decrease levels of the amino acid taurine [42]. Besides, it removes the Zn<sup>2+</sup>-induced suppression of GABA<sub>A</sub>-mediated presynaptic inhibition, resulting in a presynaptic decrease in glutamate-mediated excitatory transmission [43]. It is also implicated in the modulation of the presynaptic P/Q-type voltage-dependent calcium (Ca<sup>2+</sup>) channel in a bid to reduce

excitatory glutamate release in the dentate gyrus [44]. Moreover, LEV treatment has also been implicated as an inhibitor of excitatory neurotransmitters through intracellular inhibition of presynaptic Ca<sup>2+</sup> channels [45]. Studies have shown that about 30 % of epilepsy patients are refractory to single AED treatment, despite chronic treatment [3,46] thus, it requires adjunctive treatment or polytherapy of AEDs of the varying mechanism of actions [47].

Common etiology for carbamazepine toxicity is coadministration of other medications. Patients are likely to take antiepileptic drugs (AEDs) are likely at the same time as carbamazepine. For example, lamotrigine is a common medication used in these patients, and symptoms of carbamazepine overdose are more likely when lamotrigine is added. A similar situation is also seen with levetiracetam. Any inhibitors of cytochrome P450, such as grapefruit juice, will cause elevated levels of carbamazepine. An intentional overdose of carbamazepine is less common and usually seen with a suicide attempt in a severely depressed patient during the initial administration of the medication [48]. The mortality rate due to carbamazepine toxicity is around 13 % [49]. Indeed, children in this study all showed an active attitude to life—their substance use was attributed to inadequate care or psychological immaturity without full recognition of the toxicity and side effects of drugs, as demonstrated by self-reported reasons for use of carbamazepine [50]. Our previous study showed that CBZ, and CBZ + LEV adjunctive treatments alter the pituitary–testicular axis with evidence of hormonal deregulation and alteration in the reproductive functions' indices, while LEV treatment remains the safest [51].

Since the liver remains one of the major organs exposed to drug-induced toxicity, it is expedient to study the hepatotoxicity potential of these drugs (whether as individual or adjuvant treatment) in experimental model in a bid to forestalling toxicity sequelae.

## 2. Methodology

### 2.1. Ethical approval and experimental procedure

Ethical approval to carry out this study was obtained from the Health Research Ethics Committee (HREC) of the College of Health Sciences, Osun State University, Osogbo, Nigeria with registration number UNI-OSUN HREC 2020/016 which is in line with the National Institute of Health (NIH) in the “Guild to the care and use of animals in Research and Teaching” (National Institute of Health, USA, 2011)

### 2.2. Animals

Twenty-four male Wistar rats (140–150 g) were obtained and kept in the animal holdings of the College of Health Science, Osun State University, Osogbo, Nigeria (Natural humidity, temperature, and a natural 12 h light/12 h dark cycle). The animals were acclimatized for seven days and were given access to feed on a standard pellet diet and water *ad-libitum*. After acclimatization, the animals were randomized into four groups (n = 6) to receive oral treatment of normal saline (0.1 mL), CBZ (25 mg/kg) [52], LEV (50 mg/kg) [53] or sub-therapeutic dose of CBZ (12.5 mg/kg) with LEV (25 mg/kg) for 28 days.

### 2.3. Feed intake and percentage body weight change

Pre-weighed feed was provided in standard stainless-steel hoppers on the 7th, 14th, 21st and 28th day of drug administration. After 24 h, rats were removed from their isolated cages and the weight of remaining feed including any crump on the bottom of the cages was determined. Intake was calculated as the weight (in grams) of feed provided less that recovered according to the method of Villar *et al.* [54] and Adefisayo *et al.* [55]. Moreover, the percentage bodyweight difference was determined for each rat twenty-four hours after the last dose as thus:

$$\frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

Thereafter, the rats were euthanized by cervical dislocation.

#### 2.4. Liver tissue homogenization

Some portion of the liver (1.5 g) was sectioned, washed in Ringer's solution, macerated and homogenized in ice-cold buffer (0.25 M sucrose, 1 mM EDTA, and 1 mM Tris-HCl, pH 7.4). The homogenate was spun in a cold centrifuge at 3000 rpm for ten minutes, while the supernatant was stored ( $-20^{\circ}\text{C}$ ) for the evaluation of the liver enzymes and markers of oxidative stress according to the method of Tsikas [56].

#### 2.5. Biochemical assessment

Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in the supernatant of the liver tissue with the aid of assay kits, while the markers of oxidative stress (superoxide dismutase [SOD], reduced glutathione and end-product of lipid peroxidation-malondialdehyde [MDA]) were assayed spectrophotometrically as described by Tsikas [57]. All measurements were done using commercially available diagnostic kits (Sigma- Aldrich, USA).

#### 2.6. Histomorphological analysis of the liver

The biopsies of the liver tissue from a representative rat of each group were fixed in 10 % neutral-buffered formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were then cut into 2- to 3-mm-thick sections by a microtome, fixed on the slides, and stained with hematoxylin-eosin (H & E). The slides were examined under a light microscope (Leica microscope GmbH, Germany). The photomicrographs were taken with a Leica DM 750 camera at 400 magnifications [58].

#### 2.7. Statistical analysis

The data were subjected to statistical analysis, while all statistical comparisons- 'T' test, one-way ANOVA and Student-Newman-Keuls post hoc tests were performed using graph pad prism (version 5.01). The results were presented as mean  $\pm$  SEM in graphs or tables; the level of significance was considered at  $p < 0.05$ .

### 3. Results

#### 3.1. Percentage weight change in male Wistar rat following CBZ, LEV, and CBZ + LEV adjunctive treatment

In this study, there was significant ( $p = 0.0025$ ) decrease in the percentage body weight following CBZ treatment, while LEV and CBZ + LEV adjunctive treatment had no significant ( $p = 0.6568$ ) effect compared with the control. However, the body weight gain increased significantly ( $p = 0.0021$ ) in the LEV, and CBZ + LEV adjunctive treatment groups compared with CBZ treated rats. Additionally, there was no significant body weight change ( $p = 0.4527$ ) between the LEV and CBZ + LEV adjunctive treatment groups (Fig. 1).

#### 3.2. Feed intake following CBZ, LEV, and CBZ + LEV adjunctive treatment in male Wistar rats

There was significant ( $p = 0.01$ ,  $0.001$ , and  $0.001$ ) decrease in the quantity of feed (g) consumed by CBZ treated rat on the 14th, 21st, and 28th day respectively compared with the control. Also, for LEV treatment, the quantity of feed intake decreased significantly ( $p = 0.01$ , and  $0.01$ ) on the 21st and 28th day of administration respectively relative to

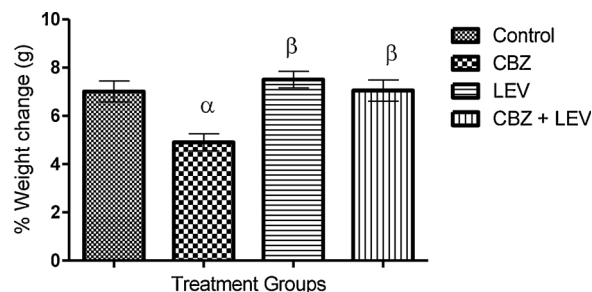


Fig. 1. Percentage weight change in male Wistar rat following CBZ, LEV, and CBZ + LEV adjunctive treatment.

$\alpha$ : Significant decrease compared with control ( $p = 0.0025$ ).

$\beta$ : Significant increase compared with control ( $p = 0.0021$ ).

Control: normal saline (0.1 mL); CBZ alone (25 mg/kg); LEV alone (50 mg/kg); CBZ -LEV adjunctive treatment [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.

the control. Additionally, the quantity of feed (g) consumed by the CBZ + LEV adjunctive treatment group decreased significantly ( $p = 0.05$ , and  $0.001$ ) at the end of the third and 4th week respectively compared with the control. There was significant increase ( $p = 0.05$ , and  $0.01$ ) in the quantity (g) of feed consumed by the LEV treated, and CBZ + LEV adjunctive treatment group compared with CBZ treated rats on the 21st and 28th day of administration respectively. However, there was no significant ( $p = 0.065$ ) change throughout the period of drug administration between the quantity of feed consumed by the LEV and CBZ + LEV treatment group (Fig. 2).

#### 3.3. Liver weight relative to the body weight following CBZ, LEV, and CBZ + LEV adjunctive treatment in male Wistar rat

There was significant ( $p = 0.0113$ ) decrease in the percentage liver weight relative to the body weight following CBZ treatment, while LEV, and CBZ + LEV had no significant ( $p = 0.0594$ ) weight change compared to the control. Additionally, there was no significant difference in the relative liver weight between the LEV and CBZ + LEV adjunctive treatment (Fig. 3).

#### 3.4. Effects of CBZ, LEV, and CBZ + LEV adjunctive treatment on the markers of oxidative stress in the liver of male Wistar rats

The activities of superoxide dismutase decreased significantly ( $p = 0.0145$ ) following CBZ treatment, while LEV and CBZ + LEV had no significant ( $p = 0.2082$ ) effect compared with the control. Additionally,

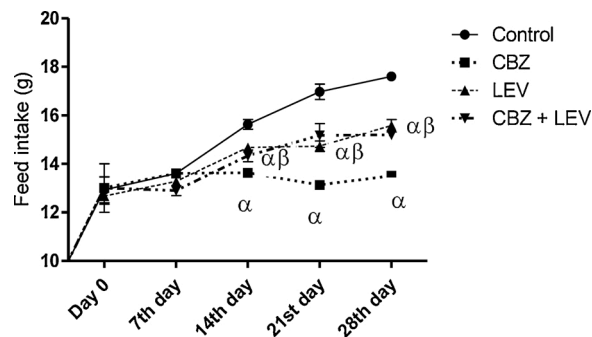
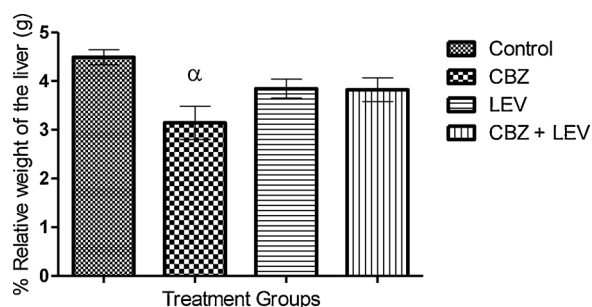


Fig. 2. Quantity of feed intake following CBZ, LEV, and CBZ + LEV adjunctive treatment in male Wistar rats.

$\alpha$ : Significant decrease compared with control ( $p = 0.05$ ).

$\beta$ : Significant increase compared with control ( $p = 0.01$ ).

Control: normal saline (0.1 mL); CBZ alone (25 mg/kg); LEV alone (50 mg/kg); CBZ -LEV adjunctive treatment [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.



**Fig. 3.** Liver weights relative to the body following CBZ, LEV, and CBZ + LEV adjunctive treatment in male Wistar rat.

$\alpha$ : Significant decrease compared with control ( $p = 0.0013$ ).

Control: normal saline (0.1 mL); CBZ alone (25 mg/kg); LEV alone (50 mg/kg); CBZ -LEV adjunctive treatment [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.

there was no significant difference in the activities of super oxide dismutase among the CBZ, LEV, and CBZ + LEV adjunctive treatment groups (Table 1).

There was significant ( $p = 0.0143$ ) decrease in the activities of GSH after CBZ treatment, while LEV and CBZ + LEV had no significant ( $p = 0.9427$ ) effect compared with the control. However, there was significant ( $p = 0.0282$ ) increase in the activities of GSH following LEV, and CBZ + LEV adjunctive treatment compared with the CBZ treated rat, while there was no significant ( $p = 0.9890$ ) change between the LEV and CBZ + LEV adjunctive treatment group (Table 1).

In this study, there was significant ( $p = 0.0001$ ) increase in the product of lipid peroxidation (malondialdehyde- MDA) after CBZ, LEV and CBZ + LEV adjunctive treatment compared with the control. Moreover, MDA concentration decreased significantly ( $p = 0.0059$ ) in the LEV treated rat, and CBZ + LEV adjunctive treatment group compared with the CBZ treated, while there was no significant ( $p = 0.0685$ ) difference between the LEV and CBZ + LEV adjunctive treatment group (Table 1).

### 3.5. Effects of carbamazepine, levetiracetam and carbamazepine-levetiracetam adjunctive treatment on the activities of liver enzymes in male Wistar rat

The activities of ALP decreased significantly ( $p = 0.0001$ ) following CBZ, LEV, and CBZ + LEV treatment compared with the control. However, there was a decrease ( $p = 0.0017$ ) in the ALP activities of LEV, and CBZ + LEV treated rats compared with the CBZ treatment group, while there was no significant change ( $p = 0.8069$ ) between the LEV and CBZ + LEV adjunctive treatment group (Table 2).

There was significant ( $p = 0.0062$ ) increase in the activities of ALT after CBZ, and CBZ + LEV treatments, while LEV treatment had no significant ( $p = 0.3446$ ) effect compared with control. The activities of

**Table 1**

Effects of CBZ, LEV, and CBZ + LEV adjunctive treatment on the markers of oxidative stress in the liver of male Wistar rats.

Treatment group	SOD (mM)	GSH (mM)	MDA (mM)
Control	0.47 ± 0.02	2.96 ± 0.30	0.59 ± 0.05
CBZ	0.29 ± 0.03 <sup>α</sup>	0.30 ± 0.07 <sup>α</sup>	3.65 ± 0.28 <sup>β</sup>
LEV	0.42 ± 0.05	2.73 ± 0.37 <sup>β</sup>	2.56 ± 0.32 <sup>β δ</sup>
CBZ + LEV	0.38 ± 0.01	2.72 ± 0.84 <sup>β</sup>	1.53 ± 0.26 <sup>β δ</sup>

$\alpha$ : Significant decrease compared with control ( $p = (0.0145)$ ).

$\beta$ : Significant increase compared with control ( $p = 0.0282$ ).

$\delta$ : Significant decrease compared with CBZ ( $p = 0.0059$ ).

Control: normal saline (0.1 mL); CBZ alone (25 mg/kg); LEV alone (50 mg/kg); CBZ -LEV adjunctive treatment [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.

**Table 2**

Effects of carbamazepine, levetiracetam and carbamazepine-levetiracetam adjunctive treatment on the activities of liver enzymes in male Wistar rat.

Treatment group	ALP (U/L)	ALT (U/L)	AST (U/L)
Control	15.70 ± 0.88	27.70 ± 3.28	87.60 ± 17.8
CBZ	47.30 ± 1.76 <sup>β</sup>	79.00 ± 9.29 <sup>β</sup>	157.00 ± 6.77 <sup>β</sup>
LEV	27.00 ± 3.21 <sup>β<math>\alpha</math></sup>	38.70 ± 9.74 <sup>α</sup>	92.7 ± 4.00 <sup>α</sup>
CBZ + LEV	28.00 ± 2.08 <sup>β<math>\alpha</math></sup>	82.30 ± 12.4 <sup>β</sup>	95.3 ± 3.59 <sup>α</sup>

$\beta$ : Significant increase compared with control ( $p = 0.0062$ ).

$\alpha$ : Significant decrease compared with CBZ ( $p = 0.0401$ ).

Control: normal saline (0.1 mL); CBZ alone (25 mg/kg); LEV alone (50 mg/kg); CBZ -LEV adjunctive treatment [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.

ALT decreased significantly ( $p = 0.0401$ ) in the LEV treatment group relative to the CBZ treated rats, while there was no significant difference ( $p = 0.8405$ ) CBZ and CBZ + LEV treatment groups (Table 2).

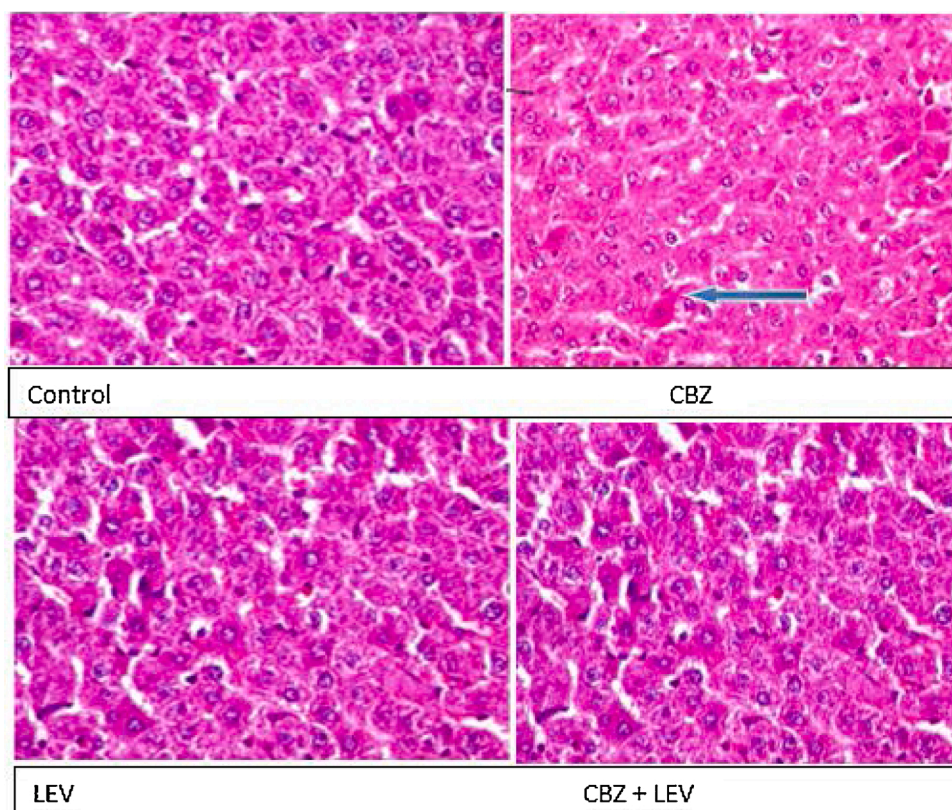
There was also observed, a significant ( $p = 0.0034$ ) increase in the activities of AST following CBZ, while LEV, and CBZ + LEV adjunctive treatment had no significant ( $p = 0.8764$ ) effect compared with the control. However, AST activities decreased significantly ( $p = 0.0002$ ) in the LEV, and CBZ + LEV treatment groups relative to CBZ treated group, while there was no significant ( $p = 0.6539$ ) difference between LEV and CBZ + LEV adjunctive treatment group (Table 2).

### 3.6. Effects of carbamazepine, levetiracetam and carbamazepine-levetiracetam adjunctive treatment on the histomorphological profile of the liver in male Wistar rat

The section of the liver of normal saline, LEV, and CBZ + LEV treated rats showed the appearance of normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appear normal and not infiltrated, no pathological lesion seen. However, the section of the CBZ treated representative rat showed central venules with mild congestion (blue arrow). The sinusoids of the CBZ was dilated and mildly infiltrated by inflammatory cells (slender arrow) (Fig. 4)

## 4. Discussion

The liver remains one of the vital organs that deserves special consideration when deciding the choice of drugs for the management of epileptic seizures. This is important because of the induction of liver enzymes and toxicity exerted by most of the first line AEDs [59,60]. A significant decrease in the percentage weight following CBZ treatment recorded in this study is consistent with our previous findings [61,62]. The pathophysiology of this significant decline in body weight is not fully understood. It will be recalled that there are two areas of the hypothalamus responsible for the integration of the afferent signals to regulate feeding patterns: the lateral hypothalamus and the medial hypothalamic nuclei [62], while carbamazepine has been reported to have influence on the hypothalamic neurons [62]. Therefore, it could be deduced that decrease in body weight is attributable to a decline in the quantity of feed consumed by the CBZ treated rats. Finding in this study is in agreement with the report of Greenwood [63] that caloric balance may be altered, with ventromedial lesions causing hyperphagia and lateral hypothalamic lesions producing a syndrome of aphagia and weight loss in experimental animals. This finding is in contrast with the report of De-Gaspari, and Guerreiro [64] that CBZ and LEV treatment increased body weights. The disparity may be attributable to variation in the study design. In this study, healthy, non-epileptic male Wistar rats were used, while De-Gaspari and Guerreiro used epileptic male and female individuals. However, an increase in the body weight recorded in the LEV and CBZ + LEV treated groups relative to CBZ treated rats is a piece of suggestive evidence that LEV, and CBZ + LEV adjunctive treatment may be less toxic relative to CBZ. A significant decrease in the relative weight of the liver following chronic administration of CBZ,



**Fig. 4.** Effects of carbamazepine, levetiracetam and carbamazepine-levetiracetam adjunctive treatment on the histomorphological profile of the liver in male Wistar rat.

**Stain:** Haematoxylin and eosin; **Magnification:** X 400; **Blue arrow:** Mild congestion.

**Control:** normal saline (0.1 mL); **CBZ alone** (25 mg/kg); **LEV alone** (50 mg/kg); **CBZ -LEV adjunctive treatment** [sub-therapeutic dose of CBZ (12.5 mg/kg) in concomitant with LEV (25 mg/kg)] for 28 days.

LEV, and CBZ + LEV treatment is a pointer to their potential toxicity. This finding is in agreement with the previous report of Simmons et al. [65] that reduction in weight or a change in either absolute or relative organ weight after administration of a chemical is an indication of the toxic effect of such chemical.

A significant increase in the activities of ALP, ALT, and AST in all the treatment groups, especially the CBZ treated group is an indication of liver failure and the assertion of their potential toxicity. This is in consonance with the previous findings of Hussein et al. [66] and Jayashankar et al. [67], who reported an acute liver failure following epilepsy treatment with LEV. However, it is worthy of note that LEV treatment whose effect was considered toxic by Jayashankar and co-worker is safer than CBZ treatment in this study. This finding is consistent with the report from a clinical study that patients on CBZ treatment have significantly higher ALP than the phenytoin treated [68]. An elevated level of ALP in this study is an assertion that chronic administration of CBZ may induce obstruction in the bile flow cholestasis. Pavlidis et al. [68] reported the consequential effects of this biliary obstruction, stemming from hepatic dysfunction, renal failure, cardiovascular impairment, nutritional deficiencies, bleeding problems, infection, and death.

Oxidative stress is said to be oxygen radical-mediated damage to biological material by either increased generation of oxygen radicals or due to diminished removal or inadequate protection against these oxygen radicals [69]. In this study, a significant increase in the concentration of malondialdehyde across the drug treatment groups suggests the membrane peroxidation of the phospholipid's bilayer by the AEDs. This can also be linked to a significant decrease in the activities of the superoxide dismutase (SOD) observed in this study. Mao et al. [70] demonstrated an inverse correlation between the activities of the SOD and the level of malondialdehyde. Based on this finding, it could, therefore, be inferred that an abrupt increase in the concentration of the hepatic malondialdehyde in this study is dependent on the depleting activities of SOD sequel to CBZ and CBZ + LEV chronic treatment.

Surprisingly, elevated activities of GSH in the CBZ, and CBZ + LEV treatment groups could not attenuate the product of lipid peroxidation in the liver as this was evident in the accumulation of malondialdehyde. This finding is in line with the conclusion from a clinical study by Tutanc et al. [71] that increased oxidative stress induced by CBZ could be the cause of CBZ-induced seizures.

This study concludes that none of these drugs and even their adjunctive treatment is devoid of hepatotoxicity if used chronically. However, LEV may pose the least adverse effect while CBZ treatment tends to have the worse. Therefore, physicians and their clients need to be careful when administering these AEDs, while further experimental and clinical investigations on the effects of co-administration of LEV with CBZ are recommended.

#### CRediT authorship contribution statement

**Opeyemi Samson Osuntokun:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Ademola Adeniyi Babatunde:** Investigation, Data curation. **Gbola Olayiwola:** Methodology, Validation, Writing - review & editing, Visualization. **Tope Gafar Atere:** Investigation, Writing - review & editing. **Olayemi Olutobi Oladokun:** Funding acquisition. **Kabiru Isola Adedokun:** Investigation, Funding acquisition.

#### Declaration of Competing Interest

The authors report no declarations of interest.

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