ANADOLU UNIVERSITY

European Journal of Life Sciences 2023; 2: 59-70

Research Article

Evaluation of *in vitro* hepatotoxicity of perampanel in comparison with carbamazepine: old versus new

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https://doi.org/10.55971/EJLS.1324501

Received: 10.07.2023
Accepted: 21.07.2023
Available online: 30.10.2023

ABSTRACT

Since the liver metabolizes many drugs, including antiepileptics, this organ is the main target of drug-induced damage. There is very little data on hepatotoxicity due to carbamazepine and perampanel metabolized in the liver. The available data are based solely on published case reports. For this reason, this study aims to evaluate the hepatotoxicity of carbamazepine and perampanel, which are frequently used in treating epilepsy and which do not have a detailed investigation, although they are suspected of hepatotoxicity. Hepatotoxicity in the HepG2 cell line, IC_{50} values were calculated by MTT cytotoxicity test, followed by determination of apoptosis/necrosis, various biochemical analyzes (ALT, AST, urea), which is currently a biomarker for liver injury, and hepatotoxicity by ROS and GSH determination. Both drugs increased liver biomarkers, oxidative stress, and cytotoxicity in HepG2 cells. The investigation found that the drugs triggered liver apoptosis, not necrosis. In conclusion, Perampanel may have hepatotoxicity similar to carbamazepine.

Keywords: Antiepileptic drugs, epilepsy, hepatotoxicity, HepG2, liver

1. INTRODUCTION

Epilepsy is characterized by epileptic seizures caused by abnormal and excessive electrical discharge in cortical neurons, resulting in sudden, repetitive, uncontrolled tremors [1,2]. The term "epilepsy" encompasses a range of clinical features that manifest not only during seizure occurrence but also in relation to comparable seizure types, age at which seizures commence, electroencephalogram (EEG) results, and factors that can provoke seizures like heredity, and response to antiepileptic drugs (AEDs). There are numerous causes for the development of this disorder in people with cerebral dysfunction [3]. Epilepsy constitutes around 0.5% of the global disease burden, with a significant majority of approximately 80% of those affected by epilepsy residing in low- and middle-income countries. Epilepsy is typically treated with pharmaceuticals. The therapeutic efficacy can be improved by combining the proper drugs [4].

An ideal epilepsy treatment should consider the type of seizure, the epilepsy syndrome, the patient's probable attitudes and behaviors, living conditions, and psychosocial status. The primary objectives in the treatment of epilepsy are to entirely eradicate seizures without triggering adverse reactions, decrease the incidence to as little as possible, reduce the adverse reactions related to chronic therapy, and assist the patient in keeping or recovering standard psychosocial and work balance [5].

The tricyclic compound carbamazepine ($C_{15}H_{12}N_2O$) is an antiepileptic drug from the first generation. Not only has it been shown to be beneficial in treating partial and generalized tonic-clonic seizures, but

it has also been shown to be effective in treating neuropathic pain and bipolar illness [6,7]. Perampanel is a new-generation antiepileptic drug, a non-competitive, selective AMPA-receptor antagonist. This medication holds the distinction of being the initial orally administered AMPA antagonist, and it is prescribed for either monotherapy or adjunctive therapy in the management of primary generalized tonic–clonic or focal seizures [8,9]. Perampanel has gained significant attention and investigation in several neurological disorders, including epilepsy, Parkinson's disease, and amyotrophic lateral sclerosis, due to its potential broad-spectrum features, minimal interaction with other antiepileptic drugs, and favorable clinical and economic outcomes [4]. On the other hand, Perampanel is a new-generation antiepileptic utilized in the adjunctive treatment of partial onset, primary generalized tonic-clonic seizures in patients aged 12 and older [10].

Several drugs can induce significant hepatotoxicity [11]. Due to clinical and fundamental studies conducted by experts on the avoidance and control of drug-induced liver damage, it has been determined that some commonly used antiepileptic drugs cause liver damage of idiosyncratic origin. Very little is known about the pathophysiological mechanisms underlying the hepatotoxicity of these agents [9,11,12]. The clinical manifestations of drug-induced liver injury range from asymptomatic laboratory abnormalities to acute hepatitis with jaundice to fulminant liver failure [9]. Assessment of hepatotoxicity in vivo and *in vitro* studies can inform pharmacists and chemists about safe drug design and expedite drug development [13].

According to previous studies, carbamazepine, phenobarbital, oxcarbazepine, phenytoin, valproate, lamotrigine, and clobazam were all associated with hepatotoxicity. On the other hand, zonisamide, perampanel, pregabalin, felbamate, or primidone did not enhance the risk of hepatotoxicity [9]. However, given that it is the first AMPA receptor antagonist licensed for use in the treatment of epilepsy, its safety should be emphasized. Perampanel safety research is based primarily on clinical trial data and post hoc analysis, pharmacokinetic research, and system evaluation. The research mainly concentrated on the adverse effects of Perampanel on mental reactions, alterations in sleep structure, movement function, and cognitive function. Because clinical trials have limitations such as (1) small sample size; (2) short observation time; (3) exclusion of special populations; (4) strict control of the patient's condition or medication regimens; and (5) limitations of observed indicators, post-marketing safety research is critical. However, only some Perampanel safety studies are based on large-scale post-market real-world data [4].

This study aimed to determine the *in vitro* hepatotoxicity of carbamazepine and perampanel using the HepG2 cell line. The inhibitory concentration 50 (IC_{50}) values were calculated using the MTT method to determine hepatotoxicity. Based on these values, the apoptotic/necrotic cell death mechanisms in cell lines were determined, and the changes in ALT, AST, urea, and GSH levels were measured as biomarkers of hepatic damage. Additionally, the levels of reactive oxygen species (ROS) in cell lines were determined to clarify oxidative stress's function in hepatotoxicity.

With the results to be obtained from this study, both the hepatotoxicity monitoring of this critical drug class and a new monitoring method for drug-induced hepatotoxicity, which is difficult to detect in the preclinical period but can have devastating effects, were implemented.

2. MATERIALS AND METHODS

2.1. Cell Culture

The HepG2 (ATCC® HB-8065™) human hepatocellular carcinoma cell line was used to investigate the hepatotoxicity of antiepileptic medications to be studied in this thesis. The medium was Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100 IU/mL-100 mg/mL) antibiotic solution [14]. The HepG2 cells were regularly passaged at intervals of 2 to 3 days for growth and preparation purposes. The cell culture bottle, from which the incubator had been removed, was subjected to gentle agitation to facilitate the incorporation of dead cells into the medium solution. Subsequently, the medium was

extracted. The cells underwent a washing process by introducing 5 mL of phosphate buffer into the cell culture vial and subsequently eliminating the washing solution from the medium. Following the addition of a 1X trypsin EDTA solution to the culture vial, the vial was subsequently placed in an incubator set at 5% CO2, 95% humidity, and a temperature of 37 °C for an approximate duration of 5 minutes. Following the conclusion of the experimental timeframe, the cells were suspended by introducing medium into the culture bottles from which the incubator had been withdrawn. Subsequently, the cells were divided in ratios of 1:2 and 1:3, and subsequently transferred to newly prepared culture bottles. The culture flasks were placed inside the incubator and subjected to incubation.

2.2. Application of MTT Cytotoxicity Test to Cells

MTT measures cell metabolism. In intact metabolic and respiratory chains, mitochondrial succinate dehydrogenase converts tetrazolium salts to formazan. Mitochondrial succinate dehydrogenase transforms yellow tetrazolium salt to soluble orange-colored formazan in an electron-coupled reagent (Altntop et al., 2018; Mosmann, 1983) [15]. The incubator was emptied of media. Cultured cells received 1X trypsin-EDTA solution (3-5 mL for 75 cm^2 flasks and 1-3 mL for 25 cm^2). After five minutes in the incubator, the cells' separation was examined under a microscope. After gently tapping the cells off the surface, a solution with twice as much trypsin-EDTA was added. It was pipetted into a centrifuge tube, spun at 1200 rpm at +4°C for five minutes, and the supernatant removed. The cell pellet was gently suspended in media using a pipette. 10 L of cell suspension was stained with 10 L of Trypan blue. and counted automatically. 1 x 10^4 HepG2 cells per 100 L were planted in 96-well plates for 24.hours. Inverted cell culture dishes were removed after incubation. After washing the cells with phosphate buffer and removing the washing solution from the medium, different concentrations of carbamazepine and perampanel, 1 to 0.000316 mM, were applied to each well of the cell culture plate eight times and incubated for 24 hours. Inverting the cell culture plate removed the solutions after 24 hours. The wells were incubated for 3 hours with 100 µL of MTT. solution (0.5 mg/mL) in.PBS. MTT solution was withdrawn from wells after 3 hours of incubation. The well-formed formazan salts were dissolved in ethanol for carbamazepine and 100 L/well of DMSO for perampanel. OD was measured at 540 nm. Non-linear regression analysis computed the half-inhibitory concentration (IC_{50}) of carbamazepine and perampanel and assessed their cytotoxicity. Three seperate MTT cytotoxicity assays were performed for Carbamazepine and perampanel.

2.3. Apoptosis measurement (Flow Cytometric Method)

If the cell receives an apoptosis stimulus, phosphatidylserine from the lipid row on the cytoplasmic surface of the cell membrane migrates to the exterior lipid layer. The phenomenon of displacement is observed during the initial phases of apoptosis, as indicated by previous studies [16,17]. Annexin protein V is a protein capable of binding to phosphatidylserine. In order to render the apoptotic cell detectable, V is conjugated with a fluorescent material, specifically FITC. This binding rate can also be measured using a device for flow cytometry. Since annexin binding can also be observed in necrotic cells, the vital dye propidium iodide (PI) is also used. Since living cell membranes are intact, they are not stained with PI.dye. Living cells are distinguished as.FITC (-) / PI (-), early apoptotic cells as.FITC (+) / PI (–), and necrotic cells as.FITC (+) / PI (+) [17-18].

After determining the number of cells in the cell suspension prepared as described above, HepG2 cells were seeded into 6-well plates at 1x10^6/well and incubated for 24 hours.

After the incubation period, the medium was discarded, and 3 separate concentrations of carbamazepine and perampanel, prepared as 0.3-0.2-0.1 mM based on the MTT cytotoxicity results, were applied to each well of the cell culture plate in duplicate and incubated for 24 hours. After the period, the medium was collected, the cells were rinsed with phosphate buffer (PBS), and 1X trypsin EDTA solution was added to the wells and incubated for 5 minutes. The medium was added to each well of the cell culture plate, and a pipette was used to prepare

the cell suspension. Each well's cell suspension was transferred to the corresponding centrifuge tube and centrifuged for 5 minutes at 1200 rpm. The medium was drained of its supernatant, and 100 L of "Binding buffer" was added to the cell particle. Then, 5 L of Annexin-5, FITC, and 10 L of PI were added to the tube, which was incubated for 15 minutes at ambient temperature in the dark. At the end of the period, 400 L of "Binding buffer" was added to the tube to resuspend the cells. After completing the experimental procedures, the Anadolu University Plant, Medicine, and Scientific Research Center, Cell Culture Laboratory, analyzed the samples within 60 minutes using a Flow Cytometry device. The flow cytometry assay for carbamazepine and perampanel was repeated three times.

2.4. Biochemical Analysis

Following the quantification of cells in the produced cell suspension, a total of $5x10^5$ HepG2 cells were seeded into a 25 cm^2 cell culture vial and subsequently cultured for a duration of 24 hours. Following the incubation time, the media was removed. Subsequently, utilizing the MTT cytotoxicity outcomes, three discrete concentrations of carbamazepine and perampanel were administered to unique cell culture vessels and subjected to a 72-hour incubation period. The media in the cell culture flask was collected subsequent to the designated time interval and subjected to centrifugation at a temperature of +4 °C, with a force of 1200 g, for a duration of 5 minutes. The amounts of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and total bilirubin were quantified in the supernatant using the enzyme-linked immunosorbent assay (ELISA) kit protocol.

2.5. Determination of Reactive Oxygen Species

After determining the number of cells in the cell suspension prepared, 5 x 104 HepG2 cells were inoculated into each well of a 96-well cell culture plate and incubated for 24 hours. After the period of incubation, the medium was withdrawn. The medium was then incubated for 24 hours with three distinct concentrations of carbamazepine and perampanel, prepared as 0.316-0.1-0.0316 mM. After the period, a 20 M DCFH-DA solution was added to each well of the cell culture plate and incubated for 30 minutes. The medium was removed at the end of the period, and the cells were washed with a lukewarm phosphate buffer. After the rinsing solution was removed from the medium, the fluorescence of the cell culture plate was measured at 485 nm excitation and 530 nm emission wavelengths. A 0.5 mM t-BOOH solution was used as a positive control in the experiments.

2.6. Statistical analysis

The findings are presented in the form of the mean and standard deviation. The statistical analyses will be performed using GraphPad Prism 5 software. A one-way analysis of variance (ANOVA) was conducted, followed by the Tukey multiple comparison test. A significance level of $P<0.05$ is commonly regarded as indicating statistical significance.

3. RESULTS AND DISCUSSION

Prior research indicates that carbamazepine, phenobarbital, oxcarbazepine, phenytoin, valproate, lamotrigine, and levetiracetam are associated with a relatively high risk of hepatotoxicity [9]. A significant proportion of drug-induced hepatotoxicity case reports involve AEDs. It is known that carbamazepine, a potent anticonvulsant used to treat partial and tonic-clonic seizures, can induce granuloma formation and cholestatic and hepatocellular damage in the liver [11-19]. In addition, the FDA approved the AMPA antagonist antiepileptic drug perampanel in 2012 [4-20]. Even though it undergoes hepatic biotransformation, few studies assess its hepatotoxicity [21]. Using HepG2 cells, this study aimed to compare the potential hepatotoxic effects of perampanel and carbamazepine. Although it is known that the specific cytochrome (CYP) P450 enzyme levels, which play a role in phase I and II reactions of biotransformation of HepG2 cells compared to primary hepatocytes, are minimal, this cell line continues to be utilized in numerous toxicology studies. In addition, it is known that HepG2 cells contain a variety of phase II enzymes, excluding UDP-glucuronosyl-transferases [22,23]. Researchers have demonstrated that ROS

formation, glutathione depletion, and membrane integrity can be measured using HepG2 cells, which can be used for sensitive cytotoxicity screening. ROS formation and glutathione depletion are efficacious mechanisms of drug-induced hepatotoxicity in cellular organelles [22]. In a study conducted by Brien et al., hepatotoxicity of HepG2 cells was evaluated with 80% sensitivity and 90% specificity [24]. It is also anticipated that *in vitro* transcriptomic analysis of HepG2 cells will be able to detect drug-induced liver toxicity at an early stage [22]. Important biomarkers of hepatotoxicity, including cytotoxicity determination with MTT, examination of apoptosis/necrosis and its effects on living cells, determination of ROT levels, and determination of ALT, AST, urea, and total bilirubin levels, were performed to determine the hepatotoxic effect.

3.1. Cytotoxicity Results of Compounds by MTT Method

HepG2 cells exposed to carbamazepine experienced a decrease in cell viability of 4.83 % at 0.000316 mM, 5.78 % at 0.001 mM, and 40.17% at 0.00316 mM. HepG2 cells exposed to perampanel exhibited a decrease in viability of 5.33 % at 0.00316 mM, 5.56 % at 0.01 mM, 25.55 % at 0.00316 mM, 25.84 % at 0.01 mM, 23.70 % at 0.00316 mM, 39.60 % at 0.001 mM, and 66.34 % at 0.000316 mM. The MTT test, a cell viability assay, is frequently used to determine cytotoxicity after toxic substance exposure [25]. The colorimetric.3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) microplate test, which is one of the most used methods to measure the metabolic activity of live cells, was developed by Mosmann in 1983. MTT is a tetrazolium salt [15]. Mitochondrial succinate dehydrogenase in the mitochondria of living cells converts MTT to violet-colored formazan [26]. Since only living cells can convert MTT to formazan, coloration indicates only the presence of viable cells [27].

The calculated IC_{50} value for perampanel was 0.50± 0.09 mM, whereas the maximum concentration of carbamazepine failed to inhibit cell viability by 50% at the concentrations tested. A study determined that perampanel inhibits glioblastoma cell line differentiation and proliferation [28]. Based on the results, it can be said that the cytotoxic effect of perampanel is higher on HepG2 cells than carbamazepine.

3.2. Apoptotic effect results of compounds by annexin V/PI method

Apoptosis is the cellular death pathway stimulated by carbamazepine and perampanel, according to flow cytometry studies. Figures 1 and 2 depict the flow cytometric analysis diagrams for carbamazepine and perampanel. Table 1 displays the percentages of viable/apoptotic/necrotic cells for the three concentrations analyzed.

Annexin V method and flow cytometry are extensively utilized in determining cell death types, apoptosis, and necrosis, one of the indicators of drug-induced liver toxicity [16,17]. Annexin V binds to the phosphatidylserine released by the inner plasma membranes and stains the membranes with a fluorescent substance (e.g., FITC), rendering apoptotic cells visible. Flow cytometry can then be used to ascertain the changes in the cell surface that occur during apoptosis [17]. To differentiate between apoptotic and necrotic cells, propidium iodide is applied as an additional stain [17,18].

It was determined that the agents used in our study

Table 1. Percentage of viable/necrotic/apoptotic cells at different concentrations for perampanel and carbamazepine

Drugs	µM	Q1	Q2	Q3	Q4
	100	2.3	5.9	85.4	6.4
Perampanel	200	4.0	6.6	84.4	5.1
	300	6.6	10.0	78.9	4 .5
	31.6	2.6	4.8	87.6	5.0
Carbamazepine	100	4.1	4.6	86.6	4.6
	316	3.2	4.3	84.7	7.8

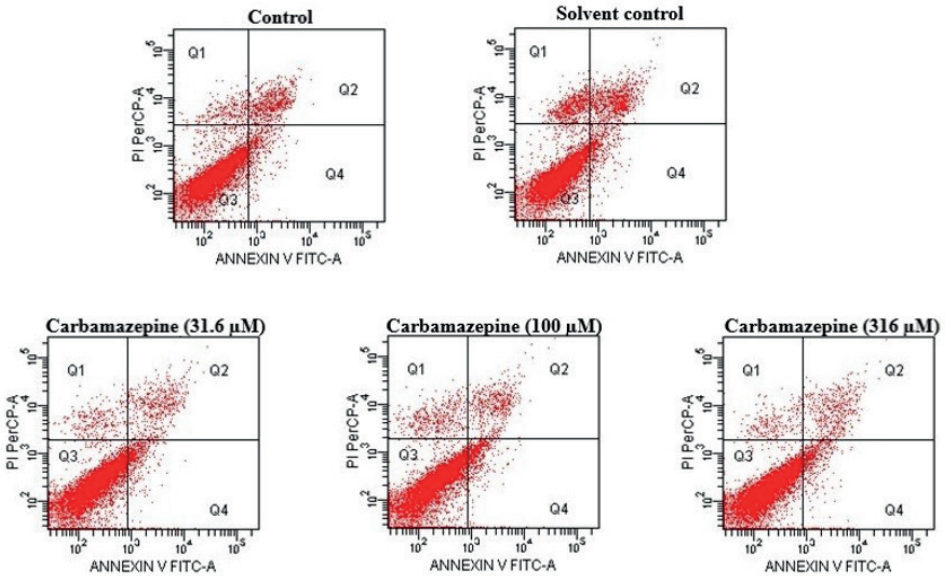

Figure 1. Flow cytometric analysis diagram of carbamazepine for HepG2 cell line.

Upper left quadrant necrotic (Q1; Annexin V-negative/PI-positive.); upper right quadrant late apoptotic cells (Q2.; Annexin V-positive/PI-positive.); lower left quadrant viable cells (Q3; Annexin V-negative/PI-negative) and lower right quadrant apoptotic cells.(Q4.; Annexin V-positive/PI-negative.).

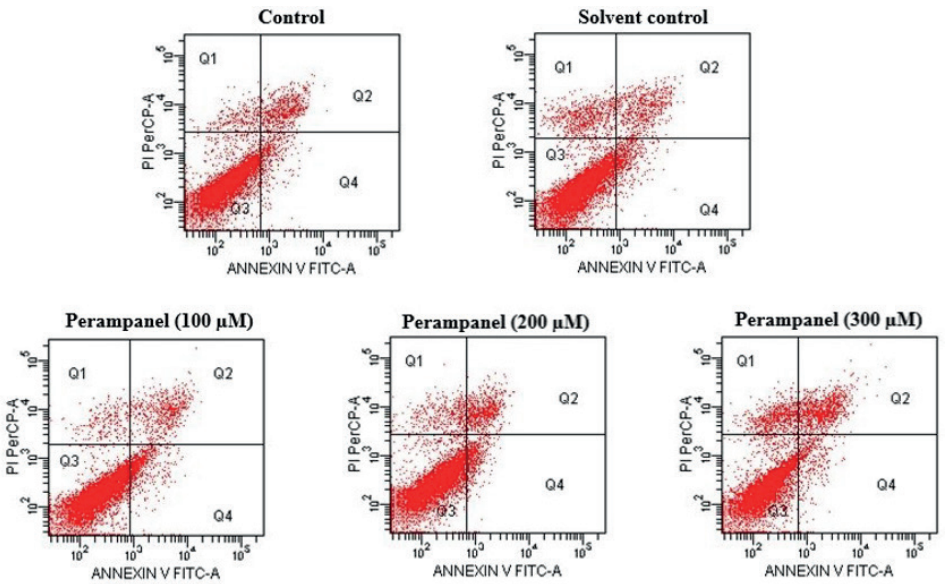

Figure 2. Flow cytometric analysis diagram of perampanel for HepG2 cell line.

Upper left quadrant necrotic ((Q1; Annexin V-negative/PI-positive.); upper right quadrant late apoptotic cells (Q2.; Annexin V-positive/PI-positive.); lower left quadrant viable cells (Q3; Annexin V-negative/PI-negative) and lower right quadrant apoptotic cells.(Q4.; Annexin V-positive/PI-negative.).

Table 2. Biochemical analysis results

Biochemical markers	Growth control	Solvent control	C316	C100	C31.6	P300	P200	P100
Urea	3.29± 0.09	3.31± 0,09	4.137± 0.05 (*)	3.507± 0.09(*)	3.81± 0.09 (*)	3.807± 0.099	3.8± 0.09 (*)	2.52± 0.08(*)
TB	0.012 ± 0.001	0.011± 0.007	0.01± 0.001	0.01± 0.005	0.02± 0.010 (*)	0.02± 0.010 (*)	0.01± 0.002	0.03± 0.009 (*)
AST	9.1± 0.26	9.09± 0.072	16.3± 1.47 (*)	11.167± 1.259	6,1± 0.854	20.3± 1.47(*)	17.2± 1.31(*)	6.03± 1.00
ALT	5.9± 0.78	6.51± 0.79	8.17± 1.04 (*)	5.17± 0.96	3.13± 0.96(*)	11.267± 1.17(*)	8±1(*)	2.03± 0.96(*)

induced apoptosis more than necrosis. It has been observed that carbamazepine's ability to induce cell mortality is related to its function as an HDAC inhibitor [29]. Inhibition of HDAC enhances cell differentiation and demise. Carbamazepine is known to induce apoptosis through various mechanisms [30]. On the other hand, according to Babu and Gupta [31], perampanel contributes to cell viability by assuring the expected continuation of calcium influx. Perampanel's ability to induce apoptosis rather than necrosis is not genuinely outstanding.

3.3. Determination of the levels of biochemical markers

Patients with symptomatic and asymptomatic liver disease are frequently evaluated using biochemical liver assays [32]. Serum ALT and AST levels can effectively detect liver injuries [33,34]. Although urea and total bilirubin measurements are typically used to assess kidney function, they can also be a biomarker of hepatotoxicity caused by certain drugs [35,36]. The results of the biochemical analysis are shown in Table 2.

Induction of hepatic enzymes is a prevalent side effect of carbamazepine treatment, with 5% to 10% of patients experiencing asymptomatic liver enzyme elevations. There are two types of carbamazepine-induced hepatotoxicity: hypersensitivity-induced granulomatous hepatitis with cholestasis and acute hepatitis without cholestasis and hepatocellular necrosis [37]. Several studies have demonstrated that carbamazepine can increase hepatotoxic potential and hepatic enzymes (ALT, GGT) [38,39]. Significant increases in ALT and AST levels with the maximum dose of carbamazepine are highlighted in this study. Except at modest doses, there are significant increases in the amount of urea, which is an indicator of hepatic synthesis. Numerous investigations have demonstrated that carbamazepine currently disrupts the urea cycle. In conclusion, biological markers demonstrated the anticipated hepatotoxicity of carbamazepine in this study.

On the other hand, Perampanel significantly increased ALT, AST, and urea levels, except at modest doses, and caused significant increases in total bilirubin levels at doses of 0.3 and 0.1 mM. Perampanel can induce liver enzymes in some instances, according to a study [40]. The evaluation of the pharmacokinetics of perampanel in patients with mild to moderate hepatic impairment revealed that the dose should not exceed 8 mg, and the drug should not be administered to patients with severe hepatic impairment (HTTP-5). A study evaluating the adverse effects of perampanel in patients with treatment-resistant status epilepticus showed modest cholestatic liver injury not requiring specific treatment. (Beretta et al., 2017) They defined ALT as 5 upper limits of normal (UNS), ALP as 2 UNL or ALT as 3ULN, and bilirubin concentration as >2UNS based on an examination of alterations in hepatic blood tests in these patients. Our findings are consistent with previous research. Perampanel generates signals in aberrant hepatic function and hyperammonemia. It suggests that perampanel is associated with a risk of hepatotoxicity, and physicians and patients should pay close attention to routine liver function testing [4]. Recent data suggested that with the extensive use of perampanel, however, some clinical reports of hepatotoxicity are associated with an elevation in

-glutamyl transpeptidase (GTP) and AST/ALT. The mechanisms behind the documented liver damage are unknown.

Perampanel, for which there are limited data, exhibited a similar hepatotoxicity risk as carbamazepine, with significant increases in biological indicators. The biological indicators affecting hepatocellular, hepatobiliary, and hepatic synthesis capacity may be compromised.

3.4. Determining the levels of ROS

Oxidative stress is caused by a decrease in antioxidant defense and the development of some biomolecular modifications due to intense ROS production [42,43]. The DCFDA method with 2,7-dichlorofluorescein (H2DCF) dye is the most commonly used technique for determining ROS and oxidative stress.

The liver is a vital organ that is susceptible to ROS attack. Reactive oxygen species (ROS) induce hepatocellular damage, apoptosis, and liver fibrosis by various mechanisms, including alterations in mitochondrial function, manipulation of cytokine expression, modification of immunological response, and activation of signaling cascades. There exists a considerable body of research indicating that the extent of oxidative protein and lipid alteration resulting from heightened levels of reactive oxygen species (ROS) is correlated with the severity and progression of various liver disorders [44].

In cells treated with carbamazepine, the DCF fluorescence intensity was 0.133 for 0.316mM t-BOOH, 0.112 for 0.1mM, and 0.106 for 0.316mM; In cells treated with perampanel, the DCF fluorescence intensity was 0.142 for 0.316mM, 0.134 for 0.1mM, and 0.109 for 0.316mM. Compared to the positive control t-BOOH, carbamazepine and perampanel increased DCF levels and H_2O_2 formation. Several investigations have indicated that the occurrence of liver damage generated by carbamazepine is linked to the creation of reactive metabolites through hepatic drug-metabolizing enzymes, specifically P450 enzymes, and their subsequent interaction with endogenous proteins [45,46]. The precise mechanisms underlying the relationship between carbamazepine metabolism and the occurrence of liver injury remain incompletely elucidated [45], despite the recognition of its potential to generate infrequent yet severe hepatotoxicity in human populations. The rat model investigation demonstrated that the development of liver injury was attributed to the presence of 2-hydroxy carbamazepine and 3-hydroxy carbamazepine

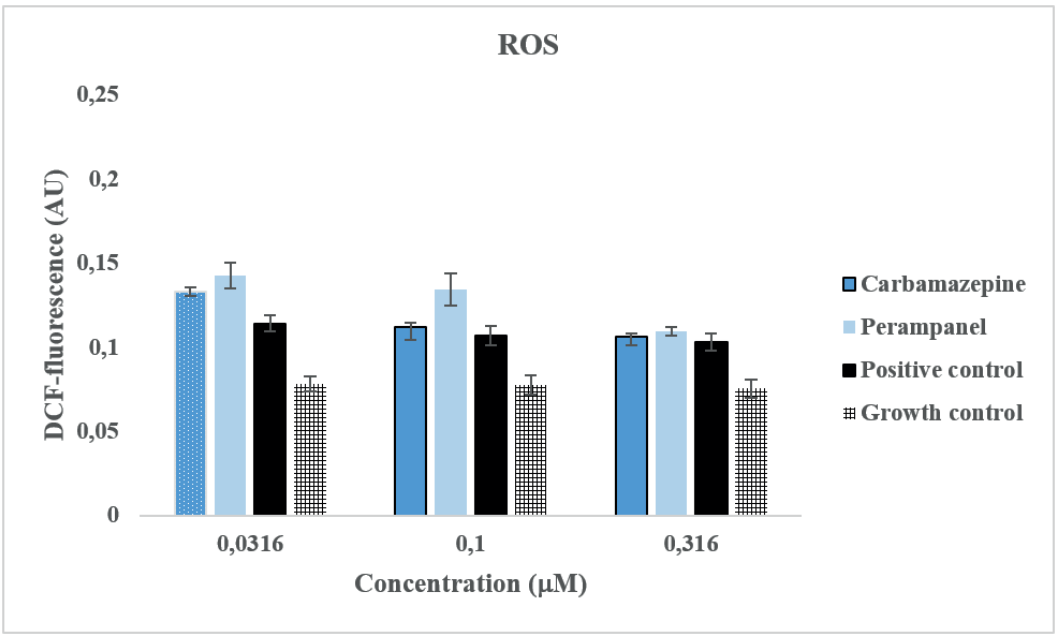

Figure 3. Levels of reactive oxygen species in cells treated with carbamazepine and perampanel at different concentrations.

metabolites [47]. In a study conducted by Eghbal et al., it was observed that carbamazepine administration led to the induction of oxidative stress, resulting in an elevation in reactive oxygen species (ROS) production and lipid peroxidation products. The impact of carbamazepine on mitochondria, which are crucial organelles responsible for energy production in hepatocytes, was assessed. It was observed that the toxicity of carbamazepine in rat hepatocytes is contingent upon its concentration. Specifically, it was reported that exposure to 400 M carbamazepine resulted in the death of 50% of hepatocytes within a span of 2 hours (LC_{50} = 400 M) [48]. Our results also indicate carbamazepine-induced oxidative stress. Notable at this juncture is that perampanel-induced oxidative stress is comparable to carbamazepine. Perampanel is 90% metabolized by the liver. It is extensively metabolized in the liver by oxidation and subsequent glucuronidation, forming 13 inactive metabolites. CYP3A4 may also play a role in the metabolism of perampanel [49,50]. Perampanel is also a known inducer of hepatic cytochrome P450 enzymes. As with carbamazepine and a few other AEDs, this causes serum concentrations to decrease [51]. Perampanel undergoes oxidative metabolism, mediated by CYP3A4 or CYP3A5 isoenzymes, according to *in vitro* investigations utilizing recombinant human CYP enzymes and human liver microsomes [50,52]. Lim et al. suggested that perampanel was converted to epoxide intermediates that were reactive to GSH and NAC. In vitro and in vivo, CYP1A2 was primarily responsible for PRP metabolic activation. The identified reactive metabolites may explain the liver damage and cytotoxicity generated by perampanel [8]. It is feasible to associate oxidative stress induced by perampanel with all these conditions.

4. CONCLUSION

Perampanel investigated for this study is extensively metabolized by the liver; remarkably, fewer hepatotoxicity studies have been conducted. On the human hepatocellular carcinoma cell line HepG2, which is used in the *in vitro* evaluation of liver toxicity, the cytotoxic effects of perampanel, biomarkers of hepatic injury, and reactive oxygen species were compared, point by point, to the hepatotoxic drug carbamazepine. These results determined that both agents have a cytotoxic effect on the HepG2 cell line, increase hepatic biomarkers, and induce oxidative stress. These factors suggest that perampanel may pose a risk of hepatotoxicity comparable to carbamazepine and the potential for significant adverse effects. Based on the results obtained, it was determined that the agents studied for the study induced apoptosis, which is programmed cell death in the liver, rather than necrosis, which is the form of cell death. This circumstance is more beneficial to the organism than necrosis. In this dissertation, hepatotoxicity surveillance was conducted on both carbamazepine and perampanel, which are commonly used to treat epilepsy, as well as perampanel, for which there are insufficient data.

Regarding other drugs, a practical monitoring method for drug-induced hepatotoxicity, which is challenging to detect in the preclinical phase, was also presented. Nonetheless, hepatotoxicity marker enrichment studies, data collection from people with liver disorders, and more extensive epidemiological studies are necessary. Future research should investigate the potential hepatotoxicity of perampanel using both human-based studies and other models, and clinicians should be aware of this and inform patients about therapeutic follow-up procedures, concomitant drug use, and special conditions.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: ÖAE, SI, BE; Design: ÖAE, SI; Supervision: ÖAE; Materials: SI, BE, MB, ABK, ÖAE; Data Collection and/or Processing: GF, SI, BE, MB, ABK, ÖAE; Analysis and/or Interpretation: GF, SI, BE, MB, ABK, ÖAE; Literature Search: GF, ÖAE; Writing: GF, ÖAE; Critical Reviews: ÖAE, SI, BE.

Source of funding

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1905S075).

Conflict of interest

The authors declared that there is no conflict of interest.

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