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Research Article

A new HPLC method for selexipag analysis in pharmaceutical formulation and bulk form

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ABSTRACT

Selexipag is a new non-prostanoid prostacyclin receptor agonist used to treat pulmonary arterial hypertension. Selexipag is a long-acting IP receptor agonist with a shorter half-life than all other licensed drugs targeting the prostacyclin pathway, mostly administered intravenously or by subcutaneous infusion or inhalation. In this study, a new high performance liquid chromatography (HPLC) method was developed to analyze Selexipag in bulk and pharmaceutical formulations. The method used a column with Supelco Ascentis® Express (Sigma Aldrich, USA) model phenyl hexyl functional group (100×4.6 mm, ID, 2.7µm). Chromatographic separation was in isocratic elution mode, and the mobile phase mixture was acetonitrile containing 0.1% formic acid: water containing 0.1% formic acid (60:40, v/v) ratio. The method was linear in the concentration range of 15.7-117.6 µg/mL, and the LOD and LOQ were obtained as 2.4 and 3.1 µg/mL, respectively. Various method parameters have been tested according to the ICH Q2(R1) manual, and it is a method with high accuracy and precision. Therefore, the developed method is suitable for selexipag's bulk and pharmaceutical formulation analysis.

Keywords: Bulk, HPLC, Pharmaceutical formulation, Selexipag

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a medical condition characterised by the presence of chronic and infrequent cardiovascular complications that can have severe consequences, including mortality. Selexipag (SLP) is a chemical compound with the chemical name 2-{4-[(5,6-Diphenylpyrazin-2-yl) (isopropyl)amino]butoxy}-N-(methylsulfonyl) acetamide The compound in question is a pharmacological agent that can be administered orally and exhibits selectivity towards the prostacyclin receptor, acting as an agonist. The term "orphan prodrug" refers to a pharmaceutical compound that is designed to undergo a specific metabolic transformation within the body in order to produce an active drug. In the context of the given statement, it is being used to describe a compound that falls under this category. Furthermore, the compound is identified as a platelet aggregation inhibitor, which refers to its ability to prevent the clumping together of platelets in the blood, thereby inhibiting the formation of blood clots. Additionally, the In order to mitigate the advancement of disease and decrease the likelihood of hospitalisation, the Food and Drug Administration granted approval for the use of SLP in 2015 as a therapeutic intervention for pulmonary arterial hypertension (PAH) in patients classified as functional class II or III. The active metabolite of SLP, known as ACT-333679, is a prodrug that

exhibits a significantly higher selectivity for the IP receptor, as indicated by a 130-fold increase in selectivity compared to other receptors [1]. SLP is distinguished by its minimal adverse effects in comparison to Prostaglandin I2 (PGI2) analogues, primarily due to its heightened selectivity. The suggested initial dosage is 200 µg administered twice daily, with subsequent increments of 200 µg twice daily on a weekly basis until the maximum tolerated dosage of up to 1600 µg twice daily is achieved. The determination of the maintenance dose is based on the level of tolerability [2].

SLP with the molecular formula $C_{26}H_{32}N_4O_4S$ and a molecular weight of 496.63 g/mol is a pyrazine derivative bearing two additional phenyl substituents at the fifth and sixth positions. Its molecular structure was given in Figure 1. It is a monocarboxylic acid amide, an ether, a member of the pyrazines, an aromatic amine, a tertiary amine compound, and an *N*-(methylsulfonyl)acetamide. It is functionally related to an ACT-333679. SLP is a light yellow crystalline powder, almost insoluble in water. Solid SLP is very stable and has no hygroscopic and photosensitivity properties [3].

It has more advantages over other analytical techniques of high performance liquid chromatography (HPLC) analysis in pharmaceutical formulation and finished product analysis especially quality control laboratories. It is an automated system with fast, high accuracy, and precision results. Adequate chromatographic separation can be eliminated in some problems such as matrix interferences, allowing technical and biological analysis. In addition, thanks to the developing column technology, lower detection limits, faster analysis, and better chromatographic separation and peak shape can be obtained. However, the biggest advantage of drug analysis is its ease of automation in analysis and data processing. This advantage indicates that the HPLC method will retain its place long [4].

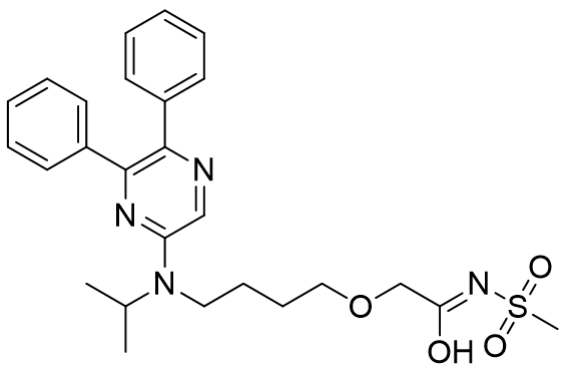

Figure 1. Structure of SLP

There are few studies on SLP analysis in the literature. These are HPLC analysis for SPL formulation and bulk analysis [5], stability indicating analysis with HPLC [6] and LC-MS/MS [7], and biological analysis with LC-MS/MS [3, 8-10], spectrophotometric method for determination of SLP in bulk and tablet formulation [9, 11]. Previous HPLC methods have disadvantages such as high flow, long column preference and more solvent and time consumption due to flour[5, 6]. It would also be better for them to make further improvements in method optimization and review system suitability parameters according to ICH (Q2) R1 [5]. This study proposes a fast, high-accuracy, and precision HPLC method for the analysis of SLP in bulk and pharmaceutical formulations.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

Analytical grade chemicals, formic acid, acetic acid, hydrochloric acid, sodium hydroxide, and HPLC grade solvents, water, acetonitrile, and methanol were purchased from Sigma-Aldrich (USA). SLP hydrochloride standard with 99.9% (*w/w*) purity was obtained from TRC Company (Canada).

2.2 Instruments

The HPLC device used in the study is Shimadzu (Japan) brand LC-Nexera-i 2040C model and is a 3D compact system. Apart from this, RK 100 H model ultrasonic bath from Bandelin (Germany), XSE 105 Dual Range model analytical balance and SevenMulti model pH meter from Mettler Toledo (Switzerland), Rotina 380 R centrifuge device from Hettich (Germany), 20 in the preparation of solutions. They are Research model pipettors from Eppendorf (Germany) that can operate in the range of -100 µL and 100-1000 µL.

Table 1. The properties of used stationary phase

Properties	Value
Particle size	2.7
Surface area (m^2/g)	135
Carbon load (%)	7.1
Pore volume/Diameter	90 Å
pH range	2.0-9.0
USP Code	L43

2.3 Stationary Phase

The stationary phase used and its properties are given in Table 1. The method used a column Supelco Ascentis® Express (Sigma-Aldrich, USA) model phenyl hexyl functional group (100×4.6 mm, ID, 2.7µm).

2.4 Experimental Parameters

During the analysis using HPLC, the flow rate of the mobile phase introduced into the system was set at 0.5 mL/min. Additionally, the temperature of the column furnace was maintained at 30.05 °C. The temperature of the autosampler thermostat was set at 15±0.1 °C in order to ensure the stability of both the sample and standard solutions. Additionally, the injection volume was determined to be 1 µL.

The wavelength at which the maximum absorbance of SLP was observed was determined to be 204 nm. Consequently, the photodiode array detector in the high-performance liquid chromatography (HPLC) system was adjusted to this specific wavelength. Furthermore, the spectra were observed in the detector within the wavelength range of 190 to 380 nm. The data sampling frequency was set at 1.5625 Hz, and a time constant of 0.640 seconds was applied.

2.5 Preparation of Solutions

1 mg of SLP was weighed and added to a 5 mL acetonitrile flask. Then the volume was completed with acetonitrile, and the stock solution concentration was calculated as 200 µg/mL. Working solutions were obtained by diluting this stock solution with acetonitrile.

In the recovery studies, while the solutions were prepared, they were kept in an ultrasonic bath for 30 min and then filtered with a PTFE (22/25 mm, 0.22 µm pore size, Isolab, Germany) type syringe.

In the experimental procedure, isocratic elution chromatography was employed as a technique for the separation of compounds. The mobile phase utilised in this study consisted of a mixture of acetonitrile and water, with the ratio of 60:40 (v/v). To enhance the chromatographic separation, both acetonitrile and water were supplemented with 0.1% formic acid. In this study, we employed non-sterile Sartorius cellulose acetate membrane filters sourced from Germany. These filters possessed a diameter of 47 mm and a pore size of 0.22 m. Following the dissolution of the solutions in an ultrasonic bath for a duration of 15 minutes, the aforementioned filters were utilised to filter the resultant solutions.

2.6 Method validation

The validity of the developed method has been tested as specified in the ICH guideline and has been shown to meet the analytical criteria. Linearity tests confirmed method validity, the lower limit of detection, specificity, precision, limit of detection, system suitability, and accuracy.

The prepared SLP stock solution was diluted with acetonitrile and kept at -20 °C for freeze-thaw cycles and different times and then analyzed for the stability of the mobile phase and solution, and the solution was stable.

3. RESULTS AND DISCUSSION

This study aimed to develop a method to distinguish SLP from other compounds in drugs used in the treatment of pulmonary arterial hypertension. The HPLC system can separate and detect each compound by the difference in the velocity of each compound in the column. In this way, it is possible to distinguish SLP from other compounds. For this reason, the HPLC method was seen as the most suitable method for this analysis.

First, studies were carried out for stationary phase selection. In the analyzes performed on acetonitrile and methanol, it was decided that more relevant results were obtained for our analysis of acetonitrile. Then, experiments were done with different ratios

Table 2. Calculated system suitability parameters

SST parameters	Calculated value	Accepted value (USP)
Retention time	4.5	-
Number of theoretical plate	12056	N>2000
Tailing factor	1.1	2≤T
Resolution	1.4	Rs>1.5
Peak asymmetry	1.1	0.95≤As≤2
Repeatability of the peak area	0.5	%BSS<1.5 General separation
		%BSS<5 Biological sample
		%BSS 5-15 Trace element analyzes

of the organic phase. In order to measure the effect of different temperatures, experiments were done with different mobile phase ratios at 30 °C, 35 °C, and 40 °C and it was determined that 40 °C was the most suitable. In addition, it was determined that the most relevant results were obtained with acetonitrile/water (60:40, *v/v*) as the mobile phase. System suitability parameters for the developed method are given in the Table 2. Previous HPLC methods have disadvantages such as high flow, long column preference and more solvent and time consumption due to flour. It would also be better for them to make further improvements in method optimization and review system suitability parameters according to ICH Q2 R1. Each parameter appears to comply with the ICH (Q2) R1 guideline. In this respect, the method outperforms the method of Damireddy et al. [5]. In addition, it is a faster, cheaper and greener method due to shorter columns and a lower flow rate. Calculations were made to determine method validity considering the result obtained from high-performance liquid chromatography and the prepared analyte concentration. A calibration chart was created by looking at the peak area corresponding to the analyte concentration. For the method, analyses were made considering all method validity parameters. The results of the precision and linearity studies for the method are given in Table 3. Also linearity of SLP was shown Figure 2. This method has the lowest linearity compared to its counterparts. In addition, lower LOD and LOQ were obtained. Accuracy studies for the HPLC method developed and optimized for the analysis of SLP were performed after precision and linearity studies. One of the samples collected from the market was selected for recovery, its solution was prepared, and

Table 3. Precision and linearity data for SLP

Parameter	Calculated value
Linearity (µg/mL)	15.7-117.6
Slope (n=7)	2955
Intercept (n=7)	7572
Regression coefficient (n=7)	0.9917
Standard deviation of slope	135
Standard deviation of the slope	10235
LOD (µg/mL)	2.4
LOQ (µg/mL)	3.1
ANOVA	F (2.13)=0.90
	P=0.35821(P>0.05)

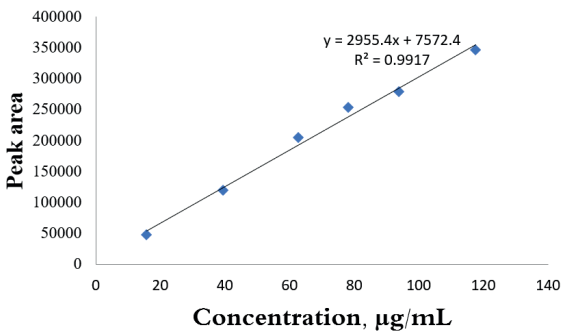

Figure 2. Linearity of SLP

SLP was added. The recovery studies were carried out with pharmaceutical formulation of SLP was Uptravi®. These analyzes were performed at three different concentrations and nine different analyzes. The obtained results were given in Table 4. Also recovery chromatogram for 25 µg/mL was given in Figure 3.

4. CONCLUSION

Table 4. Recovery studies for Uptravi® (n=3)

			Precision	Accuracy	
Added (µg/mL)	**Found (µg/mL)±CI***	**SD**	**RSD (%)**	**Recovery (%)**	**Error (%)**
20.00	20.01±0.11	0.21	1.05	100.1	+0.10
25.00	25.71±0.23	0.13	0.51	102.8	+2.80
30.00	30.30±0.41	0.22	0.73	101.0	+1.0

*95% confidence level

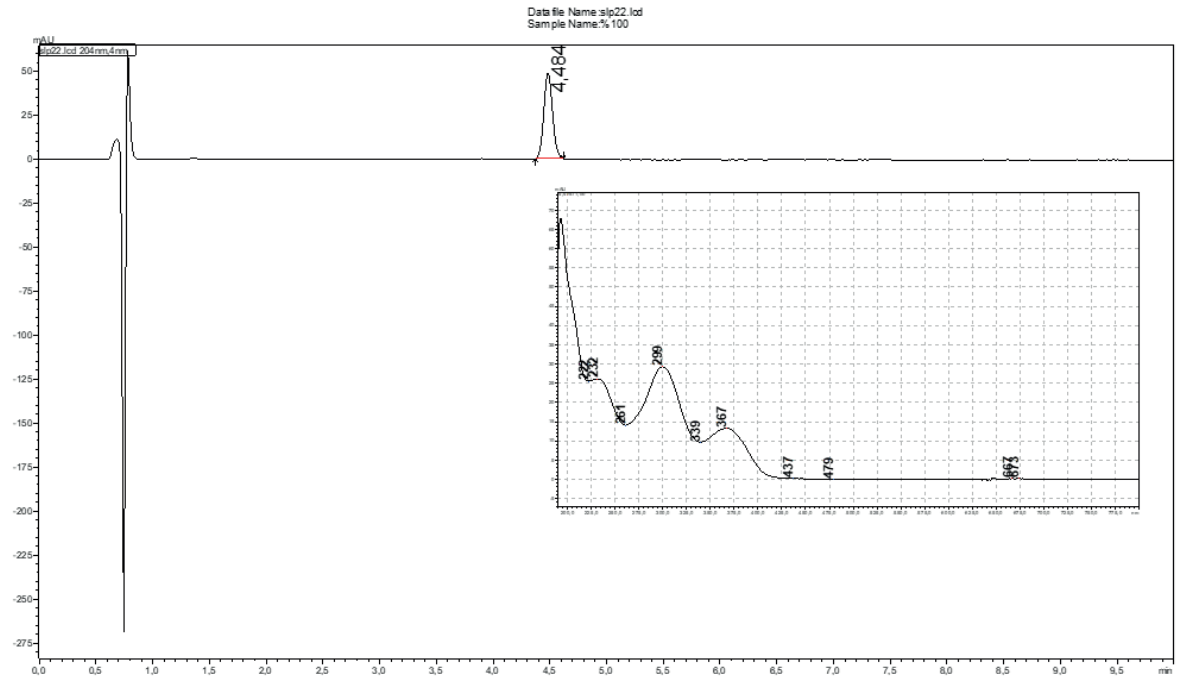

Figure 3. Chromatogram of 25 µg/mL SLP recovery solution

HPLC system is a chromatographic method that provides the opportunity to distinguish very well with the developing technology. It is the most widely used analytical instrument in analysis laboratories. HPLC separates compounds dissolved in a liquid sample and allows for qualitative and quantitative analysis of which components and how much of each component is present in the sample. In this study, a new HPLC method with high accuracy and reproducibility was developed to analyze SLP in bulk and pharmaceutical formulations. The developed method is a faster, less solvent-consuming, greener and reliable method when compared to similar studies in the literature. The current method is especially suitable for routine formulation and finished product analysis and will provide great convenience to analysts in quality control laboratories.

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Ethical approval

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

Author contribution

Concept: SÖ, NÖC; Design: SÖ, NÖC; Supervision: SL, NÖC; Materials: NÖC; Data Collection and/or Processing: SÖ, SL; Analysis and/or Interpretation: EGÖ; Literature Search: SÖ, SL; Writing: SÖ; Critical Reviews: SL, NÖC.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

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

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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

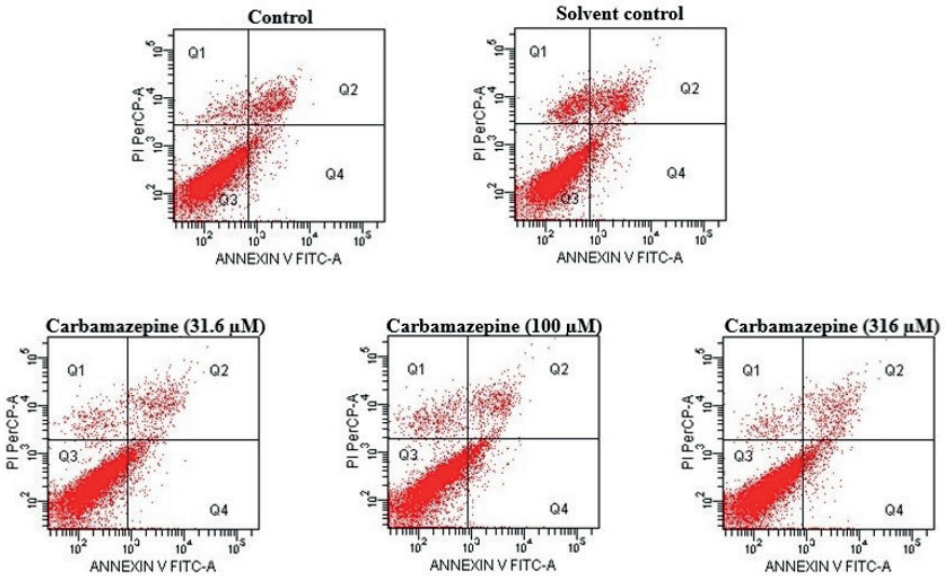

Figure 1. Flow cytometric analysis diagram of carbamazepine for HepG2 cell line.

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.).

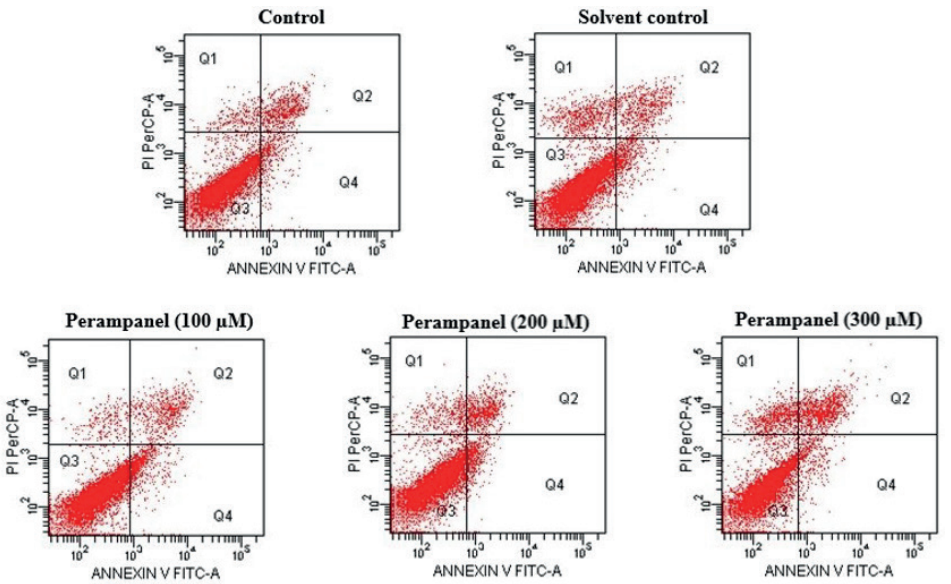

Figure 2. Flow cytometric analysis diagram of perampanel for HepG2 cell line.

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

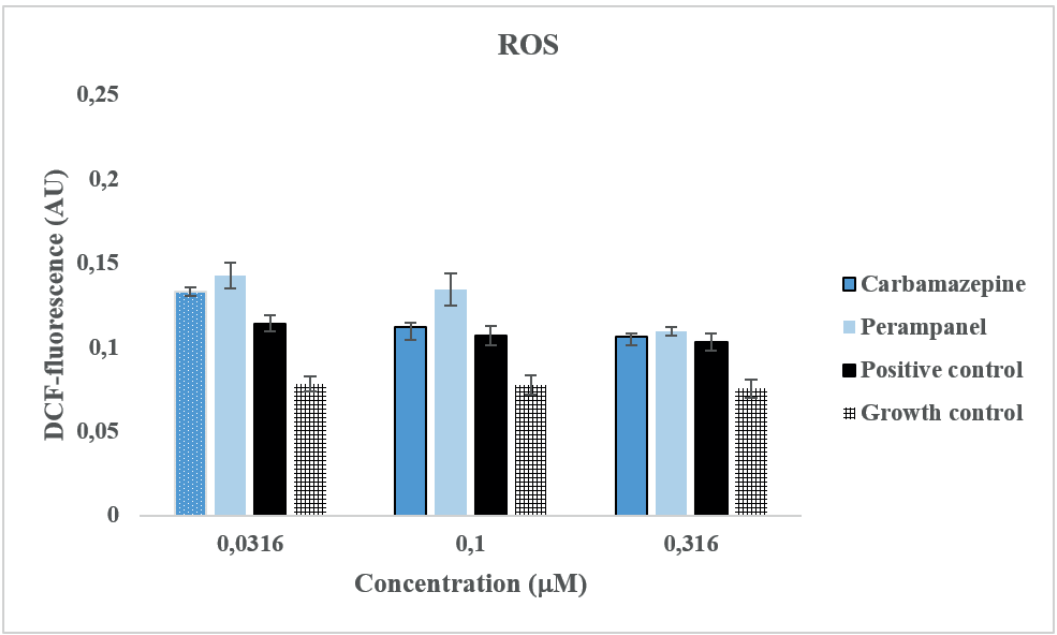

Figure 3. Levels of reactive oxygen species in cells treated with carbamazepine and perampanel at different concentrations.

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.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Screening of new morpholine dithiocarbamate derivatives bearing benzimidazole moiety for anticholinesterase potential

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ABSTRACT

Alzheimer's disease (AD) is basically associated with disturbances of cholinesterase metabolism which result in acetylcholine deficiency. Target of acetylcholinesterase (AChE) inhibitors used in symptomatic therapy of disease is to increase of ACh levels. Consequently, cholinesterase inhibitors were developed to increase acetylcholine is to inhibit AChE and butrylcholinesterase (BuChE). Studies demonstrate the clinical importance of dual inhibitors that inhibit not only the acetylcholinesterase enzyme but also the butyrylcholinesterase enzyme.

In recent years, benzimidazoles have attracted particular interest owing to their anticolinesterase activity. In this manner, we have synthesized benzimidazole and morpholine including compounds (**2a-i**). Final compounds were achieved with the reaction of (benzimidazol-2-yl) methyl morpholine-4-carbodithioate and α-bromoacetophenone derivatives in acetone at room temperature with stirring. Inhibition effects of novel morpholine dithiocarbamates (**2a-i**) were tested on AChE and BuChE. Compound **2d** demonstrated dual inhibitory activity on AChE and BuChE (78±1,56, 70,71±1,53, respectively), with the lowest cytotoxicity to normal cell line.

Keywords: Acetylcholinesterase, butrylcholinesterase, Alzheimer, benzimidazole

1. INTRODUCTION

Alzheimer's-type dementia (ATD) is characterized by behavioural disturbances and mood changes associated with progressive cognitive and memory loss. Extracellular deposition of the Aβ peptide in senile plaques is the main marker of the disease. Aβ can trigger neuronal cell death via oxidative stress [1]. Loss of neuron has been linked to a deficiency in brain, neuromediator acetylcholine. With less acetylcholine, it becomes more diffucult to maintain transmission of information and nerve signals between neurons. Consequently, AChE inhibitors (AChEIs) were developed to increase acetylcholine by inhibiting the enzyme AChE [2]. AChEIs including tacrin (hepatotoxic in 25% patients), donepezil, rivastigmine and galantamin has been approved for the treatment of moderate to severe Alzheimer's Disease (AD) [3]. Data obtained from clinical trials showed that rivastigmine 6-12 mg per day produces improvements in cognition, daily activities, and global evaluation rating in patients with mild to moderate AD. Effects of rivastigmine are dose dependent [4,5]. Rivastigmine is a pseudoirreversible, second-generation carbamate-based, noncompetitive AChE and BuChE inhibitor with equal potency. Both of enzymes are responsible for hydrolysis of ACh [6,7]. Two different cholinesterases exist in mammals: AChE, which can selectively hydrolyze ACh, and BuChE, which can hydrolyze both ACh and other

choline esters [2,8,9]. The peptidase or protease of the butyrylcholinesterase enzyme activity has a role in the pathological processes of AD. BuChE causes the production of amyloid protein and diffusion of the protein into ß-amyloid plaques in Alzheimer's disease [10]. Therefore, researchers working on this subject focused on the discovery of new dual cholinesterase inhibitor molecules to enhance cholinergic functions.

Benzimidazole scaffold is an important pharmacophore that has been extensively utilized as a drug in medicinal chemistry for years due to its high affinity towards various enzymes and receptors [11]. Among them, 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazoles have a particular interest as a result of studies which we have been reported before with satisfactory anticancer activity results [12,13]. Additionally, benzimidazoles have a broad range of pharmacological activities. In recent years, they have attracted particular interest due to their anticolinesterase activity [13-16]. Additionally, gefitinib, a recent morpholine carrying anticancer drug, is expected to play a significant role in designing and synthesizing new drugs [17].

In this present study, novel morpholine dithiocarbamate derivatives (**2a-i**) bearing 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazole moiety were synthesized and their potential anticholinesterase effects and cytotoxic properties against NIH/3T3 cells were investigated.

2. MATERIALS AND METHODS

2.1. Chemicals and Equipments

The chemicals and solvents used in the study were purchased from commercial suppliers. Electrothermal 9300 digital melting point apparatus (Essex, UK) Melting points (m.p.) was used for melting point detection. Spectroscopic analysis was realized with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan), NMR, Bruker 500 spectrometer (Billerica, MA, USA) in DMSO-d_6; M+1 peaks were detected by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA, USA) and Elemental analyses were worked on an elemental analyser (Perkin Elmer, Norwalk, CT, USA).

2.2. General procedure for the preparation of (1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (1)

2-(Chloromethyl)benzimidazole (0.05 mol) and potassium salt of morpholine dithiocarbamate (0.052 mol) were stirred in acetone for 5h. The reaction mixture was treated with excess water and precipitated raw intermediate product was filtered, later it was crystallised from ethanol [18].

2.3. General procedure for the synthesis of [1-(2-oxo-2-(4-substitutedphenyl)ethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate derivatives (2a-i)

The yellow intermediate product (**1**) was reacted with appropriate α-bromoacetophenone derivatives with the presence of potassium carbonate in acetone. After the reaction mixture was stirred at room temperature for 3 hours, it was collapsed with water. The products (**2a-i**) were given by filtration and then crytallisation from ethanol.

2.4.1. [1-(2-Oxo-2-phenylethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate (2a)

Yield : 67%. M.P. 167-169 °C. IR (cm^{-1}) : 1680 (C=O), 1595-1425 (C=C, C=N), 1269-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.57 (brs, 4H, NCH_2), 3.83 (brs, 2H, OCH_2), 4.11 (brs, 2H, OCH_2), 4.89 (s, 2H, SCH_2), 6.10 (s, 2H, $COCH_2$), 7.21-7.23 (m, 3H, Ar-H), 7.50-7.52 (m, 1H, Ar-H), 7.63-7.66 (m, 3H, Ar-H), 7.77 (t, 1H, J:7.5 Hz, Ar-H), 8.13 (d, 1H, J:7.0 Hz, Ar-H). $C_{21}H_{21}N_3O_2S_2$ calculated: (%) C 61.29, H 5.14, N 10.21; found: (%) C 61.35, H 5.18, N 10.35. MS $[M+1]^+$: *m/z* 412.

2.4.2. [1-(2-Oxo-2-(4-methylphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2b)

Yield : 62%. M.P. 105-108 °C. IR (cm^{-1}) : 1684 (C=O), 1604-1423 (C=C, C=N), 1230-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 2.82 (s, 3H, CH_3), 3.59 (brs, 4H, NCH_2), 3.84 (brs, 2H,

OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.11 (s, 2H, $COCH_2$), 7.24-7.25 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.68-7.71 (m, 3H, Ar-H), 7.95 (t, 1H, J:7.5 Hz, Ar-H), 8.12 (d, 1H, J:7.0 Hz, Ar-H). $C_{22}H_{23}N_3O_2S_2$ calculated: (%) C 62.09, H 5.45, N 9.87; found: (%) C 62.15, H 5.34, N 9.95. MS $[M+1]^+$: *m/z* 426.

2.4.3. [1-(2-Oxo-2-(4-methoxyphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2c)

Yield : 68%. M.P. 179-181 °C. IR (cm^{-1}) : 1674 (C=O), 1597-1420 (C=C, C=N), 1265-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 4.13 (brs, 2H, OCH_2), 4.86 (s, 2H, SCH_2), 6.02 (s, 2H, $COCH_2$), 7.16 (d, 2H, J: 9.0 Hz, Ar-H), 7.20-7.22 (m, 2H, Ar-H), 7.47-7.49 (m, 2H, Ar-H), 7.61-7.63 (m, 1H, Ar-H), 8.09 (d, 1H, J:9.0 Hz, Ar-H). $C_{22}H_{23}N_3O_3S_2$ calculated: (%) C 59.84, H 5.25, N 9.52; found: (%) C 59.89, H 5.33, N 9.47. MS $[M+1]^+$: *m/z* 442.

2.4.4. [1-(2-Oxo-2-(4-chlorophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2d)

Yield : 69%. M.P. 165-170 °C. IR (cm^{-1}) : 1683 (C=O), 1589-1424 (C=C, C=N), 1269-987 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.12 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.21-7.23 (m, 2H, Ar-H), 7.50-7.52 (m, 1H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.73 (d, 2H, J:8.5 Hz, Ar-H), 8.13 (d, 2H, J:8.5 Hz, Ar-H). $C_{21}H_{20}ClN_3O_2S_2$ calculated: (%) C 56.55, H 4.52, N 9.42; found: (%) C 56.61, H 4.39, N 9.46. MS $[M+1]^+$: *m/z* 446.

2.4.5. [1-(2-Oxo-2-(4-florophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2e)

Yield : 68%. M.P. 198-201 °C. IR (cm^{-1}) : 1681 (C=O), 1593-1426 (C=C, C=N), 1254-989 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.21-7.22 (m, 2H, Ar-H), 7.48-7.52 (m, 1H, Ar-H), 7.62-7.64 (m, 3H, Ar-H), 8.20-8.22 (m, 2H, Ar-H). $C_{21}H_{20}FN_3O_2S_2$ calculated: (%) C 58.72, H 4.69, N 9.78; found: (%) C 58.66, H 4.75, N 9.71. MS $[M+1]^+$: *m/z* 430.

2.4.6. [1-(2-Oxo-2-(4-nitrophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2f)

Yield : 63%. M.P. 135-140 °C. IR (cm^{-1}) : 1679 (C=O), 1599-1423 (C=C, C=N), 1267-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.88 (brs, 2H, OCH_2), 4.13 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.08 (s, 2H, $COCH_2$), 7.22-7.28 (m, 2H, Ar-H), 7.49-7.54 (m, 3H, Ar-H), 7.63-7.65 (m, 1H, Ar-H), 8.21-8.24 (m, 2H, Ar-H). $C_{21}H_{20}N_4O_4S_2$ calculated: (%) C 55.25, H 4.42, N 12.27; found: (%) C 55.32, H 4.56, N 12.35. MS $[M+1]^+$: *m/z* 457.

2.4.7. [1-(2-Oxo-2-(3-methoxyphenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2g)

Yield : 70%. M.P. 143-148 °C. IR (cm^{-1}) : 1677 (C=O), 1597-1422 (C=C, C=N), 1259-993 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.84 (brs, 2H, OCH_2), 3.97 (s, 3H, OCH_3), 4.13 (brs, 2H, OCH_2), 4.87 (s, 2H, SCH_2), 6.11 (s, 2H, $COCH_2$), 7.16 (d, 1H, J: 9.0 Hz, Ar-H), 7.22-7.24 (m, 1H, Ar-H), 7.53-7.55 (m, 1H, Ar-H), 7.58-7.60 (m, 1H, Ar-H), 7.72 (t, 1H, J:7.5 Hz, Ar-H), 7.87 (d, 1H, J:7.5 Hz, Ar-H), 8.06 (d, 1H, J:7.5 Hz, Ar-H), 8.14 (s, 1H, Ar-H). $C_{22}H_{23}N_3O_3S_2$ calculated: (%) C 59.84, H 5.25, N 9.52; found: (%) C 59.85, H 5.36, N 9.49. MS $[M+1]^+$: *m/z* 442.

2.4.8. [1-(2-Oxo-2-(3-chlorophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2h)

Yield : 75%. M.P. 102 °C (decomp). IR (cm^{-1}) : 1682 (C=O), 1572-1421 (C=C, C=N), 1255-991 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.59 (brs, 4H, NCH_2), 3.85 (brs, 2H, OCH_2), 4.12 (brs, 2H, OCH_2), 4.88 (s, 2H, SCH_2), 6.12 (s, 2H, $COCH_2$), 7.20-7.22 (m, 2H, Ar-H), 7.52-7.53 (m, 1H, Ar-H), 7.61-7.63 (m, 1H, Ar-H), 7.68 (t, 1H, J:7.5 Hz, Ar-H), 7.84 (d, 1H, J:7.5 Hz, Ar-H), 8.05 (d, 1H,

J:7.5 Hz, Ar-H), 8.15 (s, 1H, Ar-H). $C_{21}H_{20}ClN_3O_2S_2$ calculated: (%) C 56.55, H 4.52, N 9.42; found: (%) C 56.64, H 4.57, N 9.49. MS $[M+1]^+$: *m/z* 446.

2.4.9. [1-(2-Oxo-2-(3-florophenyl)ethyl]-1*H*-benzimidazol-2-yl)methyl morpholine-4-carbodithioate (2i)

Yield : 78%. M.P. 165-169 °C. IR (cm^{-1}) : 1680 (C=O), 1593-1423 (C=C, C=N), 1257-989 (C-O, C-N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 3.58 (brs, 4H, NCH_2), 3.87 (brs, 2H, OCH_2), 4.15 (brs, 2H, OCH_2), 4.89 (s, 2H, SCH_2), 6.14 (s, 2H, $COCH_2$), 7.24-7.27 (m, 2H, Ar-H), 7.54-7.56 (m, 1H, Ar-H), 7.63-7.68 (m, 1H, Ar-H), 7.74 (t, 1H, J:7.5 Hz, Ar-H), 7.92 (d, 1H, J:7.5 Hz, Ar-H), 8.10 (d, 1H, J:7.5 Hz, Ar-H), 8.22 (s, 1H, Ar-H). $C_{21}H_{20}FN_3O_2S_2$ calculated: (%) C 58.72, H 4.69, N 9.78; found: (%) C 58.62, H 4.76, N 9.76. MS $[M+1]^+$: *m/z* 430.

2.5. Biochemistry

2.5.1. Determination of AChE and BuChE inhibitory potency

A modified Ellman's assay was used for evaluation of their ChE inhibitory activities [19]. Experiment was done in triplicate.

The inhibition (percent) of AChE or BuChE was calculated using the following equation.

$$I\ (\%) = 100-(OD_{sample} / OD_{control}) \times 100$$

2.5.2. Determination of cytotoxicity

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used to determine the cytotoxic effects of compounds **2a-i** on NIH/3T3 cells [20, 21].

3. RESULTS AND DISCUSSION

3.1. Chemistry

Target molecules (**2a-i**) were synthesized in two steps. Two starting materials, 2-(chloromethyl) benzimidazole and potassium salt of morpholine *N*-dithiocarbamic acid, were synthesized according to the previously reported literature [13,22]. The obtained intermediate product (**1**) was reacted in acetone to give [1-(2-aryl-2-oxoethyl)-1*H*-benzimidazol-2-yl]methyl morpholine-4-carbodithioate (**2a-i**). IR, ^{1}H-NMR and MS spectroscopic analysis and elemental analysis were performed to confirm the structures of the compounds. In the IR spectra of the compounds, bands at 1684-1674, 1604-1420 and 1269-987 cm^{-1} were detected for C=O; C=C, C=N and C-O, C-N bonds, respectively. In the ^{1}H-NMR spectra of the compounds, peaks belong to piperazine ring were observed at 3.57-4.15 ppm range. Singlet peaks were determined for methylene protons at 4.86-4.89 and 6.02-6.14 ppm for S-CH_2 and $COCH_2$, respectively. All other aromatic protons were observed at estimated fields of the spectrum. MS spectroscopic data and elemental analysis data were confirmed the structures of the molecules with satisfactory results.

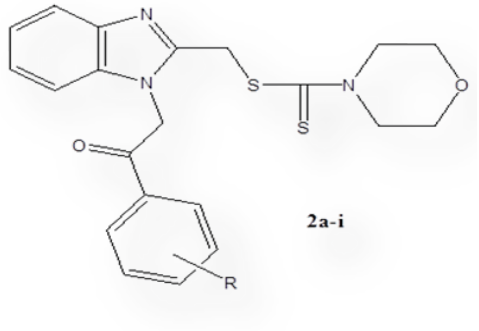
3.2. Evaluation of ChE inhibitory activity and cytotoxicity results

A colorimetric assay was employed to assess the inhibition effects of compounds **2a-i** on ChEs (Table 1). While compounds **2e** and **2d** displayed the highest inhibitory activity on AChE, compounds **2c** and **2d** showed the highest inhibition rates on BuChE, respectively In addition, compounds **2d** and **2e** exhibited the lowest cytotoxicity against normal (NIH/3T3) cells.

In recent years, results showing the anticholinesterase effect potential of various dithiocarbamate derivatives have been obtained and the importance of carbamate derivatives in the development of new drugs has been emphasized [23, 24].

When the structure-activity relationships of the compounds are examined, it is seen that the two derivatives containing 4-methyl (**2b**) and 4-chloro (**2d**) substituents exhibit high inhibition potential on both enzymes. In addition, compound **2e** bearing 4-fluoro substituent and compound **2c** including 4-methoxy substituent showed the highest inhibitory potential against AChE and BuChE, respectively. In the compounds, higher anticholinesterase activity was determined in the para-substituted derivatives compared to the meta-substituted derivatives.

Table 1. Cholinesterases inhibition (%) and cytotoxicity



Compound	AChE% inhibition (80 µg/mL)	BuChE %inhibition (80 µg/mL)	IC_{50}b
2a	33,93±0,11	29,69±1,01	69,0±5,29
2b	64,69±0,47	65,35±3,44	125,0±22,91
2c	15,06±2,30	80,51±1,58	125,67±8,14
2d	78±1,56	70,71±1,53	>200
2e	82,26±1,41	30,68±1,25	188,33±16,07
2f	33,9±4,06	49,95±1,12	128,33±20,21
2g	11,11±0,08	---	>200
2h	47,44±3,10	35,28±0,66	65,67±9,29
2i	57,76±1,08	27,50±2,60	68,33±15,28
Donepezil IC_{50}a	$3,76x10^{-3}$±$0,18x10^{-3}$	1,48±0,44	nt

---: not active; nt: non tested
a: The half maximal inhibitory concentration of the compounds to inhibit 50% of the indicated enzymes
b: The half maximal inhibitory concentration of the compounds to inhibit 50% of the mouse fibroblast cells (NIH/3T3)

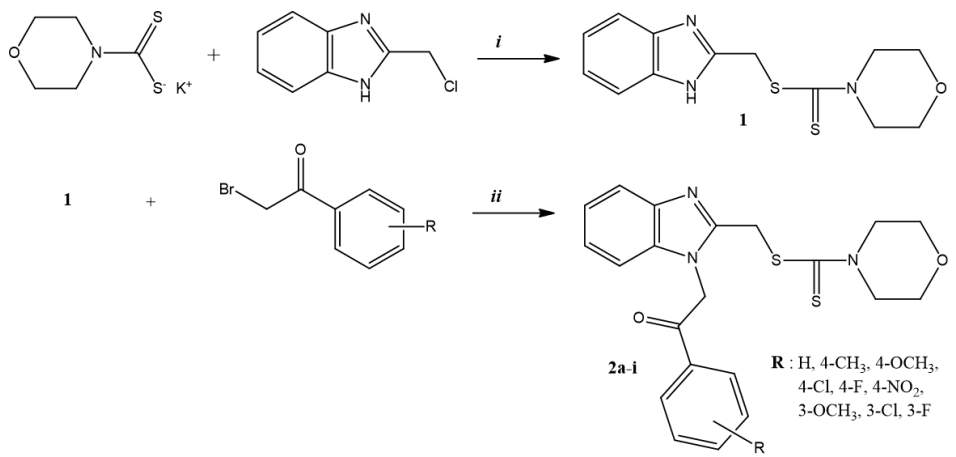


Figure 1. The synthesis of the compounds (**2a-2i**). Reagents and conditions : ***i*** : acetone, r.t., 5h; ***ii*** : K_2CO_3, acetone, 3h.

In addition, the lowest cytotoxicity against NIH/3T3 cell line was observed in the meta-substituted derivatives.

4. CONCLUSION

Compound **2d** may be a good drug candidate with either it's dual inhibitory effect on cholinesterases enzymes or with the lowest cytotoxicity to normal cell lines. This work could represent inhibition potential of morpholine dithiocarbamate derivatives bearing benzimidazole moiety on AChE and BuChE enzyme activity.

Ethical approval

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

Author contribution

Concept: HET, GAÇ, LY; Design: HET, GAÇ, LY; Supervision: HET, GAÇ, LY; Materials: HET, GAÇ, LY; Data Collection and/or Processing: HET, GAÇ, LY; Analysis and/or Interpretation: HET, GAÇ, LY; Literature Search: HET; Writing: HET; Critical Reviews: HET, GAÇ, LY.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Review Article

Etiopathogenesis of depression and experimental depression models used in preclinical studies*

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ABSTRACT

Depression is the most frequent psychiatric illness among mood disorders, affecting approximately 10% of adults. Especially recurrent and moderate/severe depression can become a serious public health problem by impairing people's life quality. The monoamine hypothesis is the most widely accepted hypothesis for clarifying the pathophysiology of depression. Depression's pathogenesis and etiology, however, are still poorly understood. Tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin or noradrenaline reuptake inhibitors, different atypical antidepressants, and electroconvulsive therapy are currently available therapies for depression. Although these treatment options are effective, a large number of patients do not respond to treatment or do not attain long-term remission. Furthermore, present antidepressants used in clinics have disadvantages such as delayed onset of effects, side effects, and patient compliance problems. Therefore, the discovery of new antidepressant medications is crucial. Animal models are critical in investigating the etiology of depression and developing novel treatments. Hence, in this review, the main mechanisms involved in the etiopathogenesis of depression and the experimental depression models used in preclinical studies have been demonstrated.

Keywords: Antidepressant, depression, depressive behavior, etiopathogenesis, experimental depression models

1. INTRODUCTION

Depression is a psychiatric disorder that ranks first among neurological and mental disorders [1,2]. Today, this disease, which can disrupt normal functioning in many societies worldwide, causes depressive thoughts, deterioration in cognitive and social functions, severely impair the patient's quality of life, and significantly increases morbidity and mortality [3-5]. Low socio-economic status, being divorced, unemployment, substance and alcohol addiction, anxiety disorders, history of depression, stress factors, childhood traumas, some drugs and diseases are the main risk factors for the development of depression [6,7].

Since depression is a heterogeneous and complicated disorder with multiple etiologies, the mechanisms involved in its pathophysiology are not fully understood [8]. Currently accepted mechanisms aiming to explain the etiopathogenesis of depression include the monoamine hypothesis, dysregulation of the hypothalamic-pituitary-adrenal (HPA) Axis, and involvement of environmental and genetic factors.

Other potential mechanisms involve impaired neurogenesis, enhanced inflammatory cytokine release, second messenger system abnormalities, and increased corticotropin-releasing factor (CRF) levels [8,9].

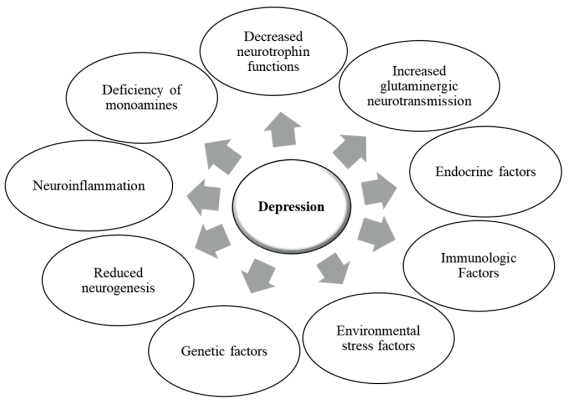

Figure 1. Pathophysiology of depression [8]

Animal models are crucial for understanding the pathogenesis of depression, as with most human diseases, and for developing new agents for its treatment. Although none of the models fully meets the symptoms of depression in human, numerous animal models resemble many of the symptoms observed in individuals with depression, and they are critical for investigating the disease's etiology, pathogenesis and treatment [10].

In this review, the main mechanisms included in the etiopathogenesis of depression and experimental depression models used in preclinical studies have been mentioned.

1.1. Etiopathogenesis of Depression

1.1.1. Biogenic Amines

The monoamine hypothesis is accepted as the most common hypothesis used to clarify the pathophysiology of major depressive disorder (MDD). The amount of monoamines such as noradrenaline, serotonin, and dopamine in the synaptic cleft are to be reduced during a depressive period [11]. Based on the monoaminergic deficiency theory, many antidepressants, such as tricyclic antidepressants, selective serotonin or noradrenaline reuptake inhibitors, and monoamine oxidase inhibitors, have been developed. Nevertheless, the specific mechanism of antidepressant efficacy and the molecular foundation of depression remain still unknown [12].

Serotonin

Serotonin is an essential neurotransmitter that regulates various physiological functions such as pain, sleep, appetite, and mood. Any abnormality in serotonin synthesis, metabolism or reuptake has been reported to be partly responsible for specific symptoms of depression, schizophrenia, learning problems, and compulsive disorders [13,14].

Several investigations have demonstrated that the serotonergic system plays an essential role in the pathophysiology of depression. Scientific research has shown that the function of serotonergic neurons decreases in depression. Postmortem and positron emission tomography imaging studies demonstrate that depressive people who do not use medications have lower presynaptic and postsynaptic serotonin levels and fewer serotonin transporter binding sites in the amygdala and midbrain. Furthermore, low levels of 5-hydroxyindole acetic acid, the major serotonin metabolite, were identified in the cerebrospinal fluid (CSF) of patients with suicidal depression [15]. Various antidepressant medications used in the clinic today are known to target serotonin receptors [14].

Noradrenaline

The noradrenergic system appears to be involved in a wide variety of brain activities, including stress response, arousal, attention, enhancement of memory, immunological response, endocrine functions, sleep/wake cycle, mood, and regulation of pain threshold [16-18].

Noradrenaline's role in depression and stress response is related to the neuroanatomical structure of the central noradrenergic system. Noradrenergic neurons are found in two main areas of the brain. These are the locus coeruleus (the region with the highest concentration of noradrenaline-producing neurons) in the brainstem and the lateral tegmental area. The locus coeruleus sends multiple projections to the brain's fear-related parts, including the

cortex, amygdala, thalamus, hippocampus, and hypothalamus. All of these regions are critical to comprehending the anatomical basis of stress-related disorders and depression [19].

In patients with MDD, a reduction in central noradrenaline level leads to depletion of positive emotional resources such as decreased enjoyment, happiness, alertness, interest, vitality, and loss of trust. Postmortem investigations in depressed patients have reported enhanced conformation of central α_2-adrenergic autoreceptors. It has been additionally found that the mRNA levels of α_2-adrenergic autoreceptors are elevated in the frontal cortices of patients with MDD who committed suicide. These findings have been associated with hypersensitive presynaptic α_2-adrenergic autoreceptors contributing to the decline in the release of noradrenaline and serotonin [20].

Dopamine

Dopamine is an essential neurotransmitter in the central nervous system. The mesolimbic pathway, one of the dopaminergic pathways in the brain, plays a vital role in emotional behavior. Anhedonia and altered reward systems in depressed patients are thought to be primarily caused by the hypoactivity of this dopaminergic pathway [21,22].

Postmortem studies in patients with severe depression have shown decreased levels of dopamine metabolites in both CSF and brain areas that regulate mood and motivation. The effectiveness of drugs that acts directly on dopaminergic neurons or receptors, such as pramipexole (D_2/D_3 receptor agonist) and monoamine oxidase inhibitors, suggests the existence of subtypes of depression caused by dopamine dysfunction [23-21].

The prevalence of depression in schizophrenia and Parkinson's disease, which are diseases caused by central dopaminergic system dysfunction, is another evidence of dopaminergic system alteration seen in depression. In addition, an increase in postsynaptic D_2/D_3 receptor density has been found in depressed patients in neuroimaging and postmortem studies. These data indicate that dopamine neurotransmission is reduced in depressed patients [15,24].

Numerous studies in neuroscience suggest that in addition to monoamines, other neurotransmitter systems involve the neurobiological features of mood disorders. The contribution of amino acid neurotransmitters like gamma-aminobutyric acid (GABA) and glutamate in depression is better-understood by recent preclinical and clinical investigations [25].

1.1.2. GABAergic system

GABA is abundant and widely distributed in the healthy human brain. About one-third of all synapses are estimated to be GABAergic. GABA, which is closely related to other neurotransmitter systems in terms of its function, is well known to interact with monoaminergic and cholinergic pathways. GABA modulates several behavioral and physiological mechanisms through its interactions with other neurotransmitter systems and its role as the major inhibitory neurotransmitter in the brain [25,26]. According to the GABAergic deficiency hypothesis of depression, decreased GABA concentration in the brain, dysfunction of GABAergic interneurons, changed expression and function of GABAA receptors, and alters in GABAergic transmission caused by disrupted chloride homeostasis may all contribute to the etiology of depression [25,27-29].

1.1.3. Glutamatergic system

Glutamate, the brain's principal excitatory neurotransmitter, contributes to learning and memory processes, brain development, neuronal life, neuronal differentiation, neuronal migration, and axon formation [30]. Changes in glutamatergic neurotransmission have been proposed to have an essential role in the pathophysiology of depression. Reduction of glutamate release or receptor function is a promising mechanism for developing more effective antidepressant therapies [31,32].

The rapid, potent and safe antidepressant effect of a single intravenous administration of the NMDA receptor antagonist ketamine in patients with treatment-resistant depression has led to the expansion of research into new glutamate-based therapeutic targets [33,34]. Intranasal esketamine spray, in addition to standard antidepressant

therapy, has recently been approved in the USA and Europe for the treatment of antidepressant-resistant depression [35,36].

1.1.4. Neuropeptides

Neuropeptides are short-chain proteins with neuromodulatory and neurohormonal functions as well as local neurotransmitter functions [37]. Neuropeptides have potential clinical importance in treating psychiatric disorders due to their neuromodulatory properties [38].

It has been reported that neuropeptides are changed in some brain regions as well as classical neurotransmitters in depression. In MDD, it has been shown that neuropeptides such as CRH, substance P, and thyrotropin-releasing hormone are hyperactive, while neuropeptides such as galanin and neuropeptide Y are hypoactive [39].

1.1.5. Neurotrophic factors

Neurotrophic factors, which nourish neurons and promote their development, survival, and regeneration [40], are known to act in the pathophysiology and treatment of depression [41,42].

Brain-derived neurotrophic factor (BDNF) levels have been reported to be decline in serum and brain areas such as the amygdala and hippocampus in depressed patients [43,44]. Tyrosine kinase (Trk) B mRNA levels have also been shown to be less in postmortem samples of depressive people, and genetic variations in the TrkB gene NTRK2 have been linked to suicide attempts [44]. Reduced levels of neurotrophic factors seen in depressed people are thought to lead to the atrophy of specific limbic tissues, such as the prefrontal cortex and hippocampus [45]. Other growth factors, including fibroblast growth factor-2, insulin-like growth factor-I, neurotrophin-3, glial cell line-derived neurotrophic factor, and artemin, may also affect neurogenesis, and there is evidence that these growth factors are diminished in depressed people [46,47].

1.1.6. Stress and neuroendocrine regulation

The HPA axis is a complicated system interacting with psychosocial, genetic, and developmental factors. This system assists humans in responding to acute stress and undergoes over time alterations in response to chronic stress exposures. These long-term changes may be significant in the etiology of depression [48].

In vulnerable people, stressful life experiences can trigger depressive episodes, and childhood trauma in the form of neglect or abuse raises the probability of depression later in life. In depressed patients, various abnormalities in the HPA axis related to stress response have been observed. These changes include excessive CRF secretion from the hypothalamic paraventricular nucleus, defective negative feedback mechanism of the HPA axis, hypertrophic adrenal glands and hypercortisolemia [49]. Chronic stress has been demonstrated to degenerate some prefrontal cortical layers, reduce pyramidal neuron dendritic density, and enhance the transcriptional function of GABA interneurons in the medial prefrontal cortex. Additionally, it has been hypothesized that higher cortisol levels disrupt the hippocampus's ability to adjust to a changing environment [49-51].

1.1.7. Inflammation and depression

Systematic reviews and meta-analyses demonstrate that depressed patients have higher concentrations of circulating C-reactive protein and other inflammatory indicators than healthy controls [52]. In major depressed patients, inflammatory markers in the peripheral blood have been indicated to be elevated. Inflammatory cytokines reaching the brain have been shown to interact with almost every pathophysiological event known to be associated with depression, involving neuroendocrine functions, neurotransmitter metabolism, and neural plasticity. Activation of inflammatory pathways in the brain has been shown to cause oxidative stress resulting in excitotoxicity and loss of glial elements, consistent with neuropathological findings in depressive disorders. Inflammation may also result in diminished neurotrophic support and alterations in glutamate release or reuptake [53].

It has been reported that there is positive feedback between inflammation and depression. While psychological stress increases cytokine production, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, and inflammation leads to depression and psychological stress. The

bidirectional association between inflammation and depression parallels the clinical link between inflammatory and depression diseases. Depression rates are higher in individuals suffering from an inflammatory disease. It has also been indicated that anti-inflammatory agents can be successfully used in addition to treatment with antidepressants [49].

1.1.8. Genetic, environmental and psychosocial factors

Family-based research has shown that the contribution of genetic factors to the risk of developing depressive disorders is significant. According to studies, family members of individuals with major depression have a 2-3 fold increased risk of developing depression [54,55]. Environmental factors are frequently stressful events such as bad childhood experiences, child sexual abuse, other lifelong traumas, a lack of social support, marital issues, and divorce [56]. Environmental factors (i.e., trauma, stressful life experience) raise the risk of depression by changing the brain's structure, chemistry and function [28].

Various genes and genetic polymorphisms have been linked to the development of MDD. Some of these genes are tryptophan hydroxylase 1 gene (TPH1), noradrenaline transporter gene (SLC6A2), dopamine transporter gene (SLC6A3), serotonin transporter gene (SLC6A4), serotonin receptor gene (HTR1A, HTR2A, HTR1B, HTR2C), dopamine receptor gene (DRD4) catechol-o-methyltransferase (KOMT), MAO-A and tyrosine hydroxylase (TH) gene. Apolipoprotein E (APOE ε2 and APOE ε4), guanine nucleotide binding protein (GND3), and the methylenetetrahydrofolate reductase gene (MTHFR 677T) are some of the other genes that have been investigated. Polymorphic variations related to point mutations or tandem repeat polymorphisms have been reported in depressed patients for each of these genes [8,54].

1.2. Experimental Models of Depression

Depression symptoms such as depressed mood, anhedonia, somnipathy, appetite/weight alterations, and psychomotor changes can be easily evaluated in animals [57].

Current depression models are based on manipulating the environment or biological functions of rodents [57]. Regardless of the used method, it has been suggested that a valid animal model must meet at least three essential criteria; appearance, structural and predictive validity. Etiological validity has been added to these criteria later [58-61].

Many experimental depression models are based on the implementation of various stressors. Some models also target other potential etiologies of depression (they directly target biological substrates that cause changes in various pathways, stress axis and immune system in the brain) [57]. Some of the experimental depression models are displayed in Table 1.

In this section, some of the most widely used experimental depression models are discussed.

Table 1. Experimental models of depression [10,57,62]

1. Adulthood stress models
• Learned helplessness
• Social isolation
• Chronic mild stress/Chronic unpredictable mild stress
• Repeated restraint stress
• Chronic social defeat stress
• Social instability stress
2. Early-life stress models
• Prenatal stress
• Maternal separation
• Post-weaning social isolation stress
3. Lesion-induced depression model
• Olfactory bulbectomy
4. Pharmacological models
• Reserpine-induced depression model
• Corticosterone-induced depression model
• Lipopolysaccharide-induced depression model
5. Genetic models
• Wistar Kyoto (WKY) model
• Genetically-selected Flinders Sensitive Line (FSL) rat model
• The Fawn-Hooded (FH/Wjd) rat
• Holtzman Albino rat model
• Transgenic model

1.2.1. Learned helplessness model

The "learned helplessness" model is one of the oldest models used to explore the consequences of uncontrollable stress in animals [63-65]. In the learned helplessness model, one of the well-validated animal models, uncontrollable and unexpected electrical foot shock stress leads to a depression-like condition in the experimental animal. Experimental animals exposed to unavoidable electric shocks develop "helplessness" behavior when exposed to the same shocks again. When animals are exposed to the same electric shock again in an environment where they can easily escape, it is observed that the animals delay their escape behavior or stop escaping completely this time [66]. The feeling of helplessness is among the main symptoms of MDD and is among the subject extensively researched in preclinical and clinical studies on depression [67].

With the learned helplessness model, in which animals are exposed to highly stressful and uncontrollable events, an animal model similar to human depression is obtained. Decreased body weight, appetite, locomotor activity, libido and grooming, as well as cognitive impairments and abnormalities, have been exhibited by helpless animals in this model [62,66,68]. The learned helplessness model has the advantages such as replicating the symptoms seen in severe depression patients, and most symptoms ameliorate with antidepressant medication. The learned helplessness model has high face validity and predictive validity, making it a reliable model for investigating the etiopathogenesis of depression. The model's major disadvantage is that most of the depression-like symptoms do not remain long enough after the shock stimulus is discontinued [66,69].

1.2.2. Early life stress models

Early life and adolescence are considered sensitive periods for depression and affective behaviors [70]. Difficult early life experiences are the main risk factors for developing psychiatric disorders like major depression. The early postnatal period is critical in the formation and plasticity of the nervous system. Therefore, the early postnatal environment is of great importance in terms of affecting adult behavior. Preclinical studies have shown that early life stress increases susceptibility to stress and causes permanent changes in the HPA axis [66].

As a type of neonatal stress, the separation stress model from the mother is frequently used in behavioral research to explore the impacts of early life stress and to model the pathology of some psychiatric disorders [71]. Rodents are highly dependent on maternal care after birth. The most common maternal separation protocol consists of a 3-hour separation per day from the second postnatal day to the 12th day. Biological and behavioral outcomes in animals are then evaluated in adulthood. This experimental manipulation leads to depression-like and anxiety-like behaviors and impaired memory and learning. Maternal separation is a traumatic occurrence that simulates early life neglect/parent loss in humans and can influence offspring's behavioral and biological phenotypes in adulthood. This model has been defined as a sensitive model for drug addiction, depression, anxiety, and stress-related illness [66]. Although maternal separation is a popular depression model used in the deterioration of the mother-offspring relationship, it has disadvantages, such as obtaining inconsistent findings about the investigated parameters and the different times of separation of the offspring from the mother in studies [67].

1.2.3. Social defeat stress model

Social defeat stress is a prolonged and recurrent arousal. In real-living conditions, most cases of depression are induced by high social pressure rather than direct neural circuit damage [72]. The social defeat model causes emotional and psychological stress by utilizing social disagreement as a source of stress. In this paradigm, another male rodent (test animal) is placed in the cage of an older, aggressive and dominating male rodent for 10 minutes per day. The test animal attacked and sometimes injured by the resident animal in the cage is defeated. This process is repeated every day for ten days with a new competitor. Then the animal is tested for different behavioral experiments. After ten days, these animals usually exhibit social withdrawal and anhedonia behavior. In addition, several physiological changes have been observed in animals, including decreased sexual behavior, increased defensive behavior and

anxiety, reduced locomotor or exploratory activity, changes in circadian rhythm, nutrition and body weight, sleep disturbances, and impaired immune functions. Similar to other depression models, the HPA axis has been demonstrated to be activated in defeated rodents. The social defeat also causes some neurobiological changes related to MDD, such as the release of proinflammatory cytokines, hypercortisolemia, and neurotrophin changes [57,62].

The advantage of the model is that it can be created in an average of 20 days with a simple method. It is accepted that female mice and rats have low aggression, and male-to-female attack is uncommon in both species. Therefore, the main disadvantages of this model based on regional aggression between males are that it cannot be studied in female subjects, and the subjects are limited to adult animals [62,72,73].

1.2.4. Chronic unpredictable mild stress model

The Chronic Unpredictable Mild Stress (CUMS) model is a widely used, well-validated, and realistic depression model [67,74,75].

The first chronic stress model based on the development of anhedonia has been created by Katz and Hersh (1981). The initial protocol, which has lasted for three weeks, used more severe stressors such as intense electric shock and prolonged food and water deprivation. In animals exposed to stress were reported to display increase in plasma corticosteroid levels, decrease in reward sensitivity, and decrease in sucrose preference which is indicative of the development of anhedonia [76,77]. Later, Willner updated this model by utilizing mild stressors that lasted longer and were more realistic, and the model was named CUMS [67,78].

The model is based on the unpredictable exposure of experimental animals to a range of mild stressors over several weeks or even months. Various stressors such as social isolation or crowded housing, water or food deprivation, changing the light/dark cycle, cage tilting, and wet bedding are chronically applied to experimental animals throughout the CUMS protocol [67]. Since repeated exposure to identical stressors may lead to adaptive behavior in animals, stressors are administered to experimental animals in an unpredictable order [66]. Experimental animals constantly exposed to mild stressors develop many behavioral changes, and "anhedonia" occurs, one of the main clinical symptoms of depression. Periodic tests based on the choice between a sweet solution and tap water are used in the model to assess reward sensitivity. Consumption or preference for the sweet reward has been reduced following weeks of stress exposure but can be restored to normal levels with chronic treatment with antidepressant medication [67,78].

In experimental animals for which a CUMS model has been created have been observed changes in various molecular parameters that are important in the neurobiology of depression. Some of these changes include an increase in HPA axis activity, a decrease in hippocampal neurogenesis, an increase in microglial activation, a decrease in serotonin neurotransmission in the forebrain, a decrease in neurotrophin levels, especially BDNF, reduced dendritic branching in the hippocampus and some frontal areas, increase in corticosterone levels and adrenal gland weight, reduction of antioxidant enzymes activity, and increase in proinflammatory cytokines [57,66].

Many alterations seen in animals exposed to stress procedures, confirming face validity and structural validity, are reversible after chronic administration of various clinically effective antidepressant class drugs. Thus it can be declared that this animal model also has predictive validity. The advantage of the model is that it causes long-term changes in behavioral, neuroimmune, neurochemical, and neuroendocrinological parameters similar to the abnormalities seen in depressive cases. However, the CUMS model has two essential disadvantages. Firstly, it is a labor-intensive procedure that requires a large experimental space in the laboratory, and it is physically and practically difficult to perform long-term CUMS experiments. Another problem is that it can be difficult to create the model in a new laboratory environment, and it can be challenging to maintain a consistent standard among laboratories [66,67,72].

1.2.5. Reserpine-induced depression model

According to the monoamine hypothesis, depression is induced by a decrease in the levels of noradrenaline, serotonin, and dopamine neurotransmitters. VMAT2 (vesicular monoamine transporter 2) is known to cause cytosolic monoamine accumulation in presynaptic vesicles. It has been proven that disruption of VMAT2 expression has been shown to limit active reuptake and storage of monoamines. Reserpine is an alkaloid that prevents serotonin and catecholamines from being stored in vesicles at the presynaptic terminal, resulting in monoamine depletion and depression-like symptoms in animals [79,80]. Pretreatment with antidepressants can reverse the depression caused by this model. This finding suggests that the reserpine-induced depression model can be used to assess the effectiveness of antidepressants. Despite being quickly developed, the model has disadvantages such as significant animal loss and an inability to fully explain depression pathophysiology [10,72].

1.2.6. Glucocorticoid/corticosterone-induced depression model

High levels of glucocorticoid administration produce effects similar to chronic stress in animals. Corticosterone can be administered to animals for weeks to months via subcutaneous injection, osmotic pump implantation, drinking water, or feeding [10]. Chronic corticosterone administration results in many behavioral abnormalities in rodents, including anhedonia, reduced grooming, increased immobility time in the forced swimming test, memory impairment in the Morris Water Maze and T maze tests, and anxiety-like behaviors in the open field test. Furthermore, long-term corticosteroid administration causes structural alterations in rodent brains, including a reduction in hippocampus volume [10,62]. On the other hand, chronic corticosterone administration has been demonstrated to generate several biochemical and metabolic abnormalities outside the brain, impacting animal behavior differently than human depression [10].

1.2.7. LPS-induced depression model

A single injection of the bacterial endotoxin lipopolysaccharide (LPS) at a dose of 0.5 to 0.83 mg/kg has created a model of inflammation-related depression [57]. LPS is a lipophilic compound that can pass to the brain via the blood-brain barrier (BBB) or circumventricular organs [81]. The secretion of proinflammatory cytokines in the blood reaches its peak about 2 hours after systemic LPS treatment, and illness behavior is noticed after 6 hours, followed by depression-like behavior (such as a decrease in sucrose preference and an increase in helplessness behavior) 24 hours later. LPS stimulates the immune system, leading to microglial activation and increased expression of proinflammatory cytokines such as IL-1 and TNF-α in the brain [57,82].

The LPS model has some limitations over the traditional animal model of depression in that it cannot more accurately mimic the depression phenotype [83]

1.2.8. Lesion-induced depression model- olfactory bulbectomy model

The olfactory bulbectomy model is a depression model that was developed surgically first by Leonard in 1984 by removing the bilateral olfactory bulb [84]. Removing the olfactory bulb in rats causes loss of smell (anosmia) and inhibits the perception of pheromones. Pheromones are chemical signals that carry information about an animal's behavioral and physiological state. Pheromones are crucial in reproductive behavior, sex recognition, aggressive behavior, male rodent social dominance, and avoidance behavior in rats. However, anosmia generated by bulbectomy is not the only mechanism contributing to behavioral problems [85].

Bilateral olfactory bulbectomy causes abnormalities in behavioral, immune, endocrine, and neurotransmitter systems similar to those in major depressed patients. The rat's olfactory system is part of the limbic area. The major mechanism underlying the behavioral alterations and other symptoms is bulbectomy-induced disruption of the cortical-hippocampal-amygdala circuit. These neuroanatomical areas have been reported to be dysfunctional in depressed persons [85]. Animals demonstrated hyperactivity in the open field test, poorer memory in the Morris Water Maze and 8-arm radial maze tests, higher open-arm entries in the elevated plus maze test, and alterations in food-conditional behavior after bilateral olfactory bulbectomy. Olfactory bulbectomy is also associated

with changes in the serotonergic, noradrenergic, cholinergic, glutamatergic, and GABAergic neurotransmitter systems. Following olfactory bulbectomy, various immunological alterations such as decreased lymphocyte count, increased leukocyte aggregation and neutrophil count, and changes in acute phase proteins are observed. Additionally, in bulbectomized rats has been reported an increase in nocturnal corticosterone production [86].

The limitations of this model are its low predictive validity and high morbidity rate [72].

1.2.9. Genetic models

Mutant methods provide a possibility to identify potential risk factors for depression. For instance, α_{2A} adrenergic receptor knock-out mice and mice with high cAMP response element binding protein expression may become more susceptible to developing depressive symptoms when exposed to stress [72]. Flinders Sensitive Line (FSL) rats with high muscarinic receptor densities in the striatum and hippocampus exhibit hypoactivity in the forced swim and open field tests. FSL rats exhibit a more pronounced decline in their sucrose preference when under acute or chronic stress. Holtzman Albino rats are especially preferred in investigations of learned helplessness. Wistar-Kyoto rats are the genetic models used in post-traumatic stress disorder, hyperactivity disorders, and anxiety research, in addition to being a good model of endogenous depression [10,62].

2. CONCLUSION

Depression is one of the primary disorders contributing to the global disease burden [87]. In this study, the general etiopathology of depression, experimental depression models that are widely used in research, and the advantages and disadvantages of these models are mentioned.

For many years researchers have focused on the monoamine hypothesis of depression, and they have conducted many studies to treat symptoms by increasing the concentration of monoamines. However, it is now recognized that depression is a considerably more complicated phenomenon. Inflammation, stress signaling pathways, growth factors, genetic and epigenetic regulation, environment, diet, other existing diseases and comorbidities have all been linked to depression's symptomatology and etiology [62].

Most of the available information on the pathogenesis of mood changes, impaired concentration, and neurovegetative symptoms observed in patients with major depression has been derived from animal models [88]. Animal models are very important because they allow researchers to examine brain circuits, molecular and cellular pathways in a controlled setting. In addition, manipulation and gene editing with pharmacological agents have been accepted methods to study depression in animal models [62].

Depression models can be categorized as genetic models, models caused by acute and chronic stressful situations, models caused by changes in brain neurotransmitters or specific brain injuries, and models induced by pharmacological agents [10]. A valid animal model must meet the face, structural, predictive, and etiological validity criteria [61,89].

The value of experimental animal models in studying the etiology of depression is well known. Once the underlying mechanisms of the depressive disorder are better understood, individualized treatment options can be planned [62].

Ethical approval

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

Author contribution

Concept: ÜK; Design: ÜK; Supervision: ÜK; Materials: ÜK; Data Collection and/or Processing: ÜK; Analysis and/or Interpretation: ÜK; Literature Search: ÜK; Writing: ÜK; Critical Reviews: ÜK.

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The authors declared that there is no conflict of interest.

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Case Report

Oral medicine administration errors in a patient with an enteral feeding tube

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ABSTRACT

Proper nutrition is vital for all patients with an expected lifespan. It is strongly recommended that patients whose oral intake is impaired or suspected to be impaired should be provided nutritional support. Concurrent medication administration during enteral nutrition may result in complications unless necessary precautions are taken. This study presents a case of a 94-year-old male patient with poor general health condition and being treated in a palliative care service. The patient was fed with an enteral feeding tube for seven drugs. There have been two tube occlusions causing the replacement of the tube since the enteral feeding tube was placed.The clinical pharmacist checked how the patient's drugs were given through a nasogastric tube and how the patient's relatives administered the drugs. Inappropriate dosage form selections and errors in administration through the nasogastric tube were identified. The interaction and incompatibility of the patient's medications with the nutritional formula were also investigated. The clinical pharmacist informed the person giving the medicine to the patient about the correct administration of the medicine from the tube. The proper method for administering medications from the tube was ensured accordingly. Following the clinical pharmacist's training, it was observed that the nasogastric tube was correctly used to administer the drug and the patient being monitored had no tube obstruction in the later phases of the treatment.It may be beneficial for a pharmacist to review drug dosage forms and applications in patients with a feeding tube in order to ensure correct administration and avoid undesired drug interactions.

Keywords: Clinical pharmacy, enteral feeding tube, nasogastric tube, palliative care

1. INTRODUCTION

Palliative care aims to improve and sustain patients' and their families' quality of life [1]. Nutrition is essential for patients to recover and considered a sign of good health [2]. Enteral nutrition must be given through an enteral feeding tube if the patient consumes a maximum of fifty percent of their daily nutritional needs, there are no contraindications or broncho-aspiration hazards and their life expectancy is shorter than six weeks [3]. Drug administration in enteral feeding patients is a complex and substantially critical issue during clinical practices. Moreover, the patients with enteral feeding tubes are more vulnerable to errors and challenges such as tube occlusion, incorrect administration techniques and inadequate dosage form selection when they have to take oral drugs [4]. This case study highlights

several medication errors that happened during drug administration via an enteral feeding tube.

2. CASE REPORT

A 94-year-old man was admitted to the palliative care service due to poor general condition, and he had chronic diseases such as diabetes, hypertension, Alzheimer's and decubitus ulcer. The patient had been fed by using an enteral feeding tube for seven months. Seven drugs (Table 1) and a specific product with 1kcal/ ml for diabetes treatment were given to the patient through the enteral feeding tube. A total of two tube occlusions leading to tube replacement occurred since the enteral feeding tube was inserted.

All the drugs were crushed by the patient's relatives, mixed with the enteral nutrition product and given to the patient. The tube was rarely flushed with water after this application.

3. FINDINGS

The errors observed in the administration of oral drugs are described below.

3.1. Inappropriate dosage form selection and oral medication preparation

Donepezil hydrochloride/ Metoprolol succinate/ Olanzapine / Quetiapine fumarate film tablet

The film coating can protect tablets containing active pharmaceutical components that are susceptible to light, moisture or oxidation, resulting in improved medical product stability throughout manufacturing and storage. Furthermore, film coating has the ability to control tablet drug release patterns in terms of rate, site and time. Film coating is also applicable to mask the taste and improve patient compliance. However, coated dosage forms should be administered with a special caution when they are given through an enteral feeding tube. Tablets coated only to improve the tablet's appearance and mask the unpleasant taste can be crushed when administered through an enteral feeding tube. However, when those coated to protect from moisture, light and air are crushed, the stability of the active drug substance may be impaired. In the case of crushing the tablets coated with polymer to provide a controlled release and protect the drug from gastric irritation, the release properties of the drug may change and obstruct the tube [5].

Donepezil hydrochloride is a film-coated tablet [6]. Micromedex® and Lexicomp® recommend the administration of this drug regardless of mealtimes [7,8]. Unfortunately, no specific data is available in these two databases regarding the enteral tube administration for this dosage form [9].

Metoprolol succinate tablets can be split into two parts or more whenever necessary; however, it is not recommended to crush or chew them as stated in the prospectus [6]. Although Micromedex® and Lexicomp® provide recommendations for metoprolol succinate capsule and some metoprolol tartrate forms, there is no information about metoprolol succinate film-coated tablet administration in the nasogastric tube [7,8]. Similarly, the handbook does not include any information or data regarding metoprolol succinate while only a limited amount of information is available about metoprolol tartrate in this handbook [9].

Table 1. Medicines administered through the patient's enteral feeding tube

Drug / Dose (tablets)	Dosage form	Time of use (hr)
Allopurinol 300mg	Tablet	18
Donepezil hydrochloride 5mg	F.C. Tablet	22
Metoprolol succinate 50mg	F.C Tablet	10-22
Nebivolol 5mg	Tablet	10
Olanzapine 5mg	F.C Tablet	10-22
Pantoprazole 40mg	E.C. Tablet	06
Quetiapine 25mg	F.C. Tablet	22

*F.C.: film-coated, E.C.: enteric coated

Olanzapine is a film-coated tablet and can also be administered regardless of meal times; however, there is no specific data on enteral tube administration as well [6-9].

Quetiapine fumarate is a film-coated tablet, too. It might be administered together with foods or not [6]. Despite the presence of some recommendations in Micromedex® and Lexicomp® regarding immediate and extended-release forms or suspensions, no information is provided for film-coated tablet dosage forms [7,8] neither in Micromedex® and Lexicomp® nor in the handbook [9].

Unfortunately, despite the presence of the above-mentioned information, all these drugs were used by this patient by crushing due to the lack of any other available alternative forms in the hospital.

Pantoprazol enteric-coated tablet

It is advised that enteric-coated (gastro-resistant tablet) should not be crushed or chewed [6]. Similarly, it is recommended that tablets containing pantoprazole should be swallowed whole, not chewed or crushed [10]. In Micromedex® and Lexicomp®, there are some recommendations about delayed release suspension forms but no information is provided for film-coated tablet dosage forms [7,8]. Based on the "Handbook of Drug Administration via Enteral Feeding Tubes," it is mentioned that Pantoprazole tablets can be crushed and dissolved in 10 mL of 8.4% sodium bicarbonate for administration through an enteral feeding tube. When kept at 5°C, this solution is stable for two weeks. Although the peak plasma concentration remains unchanged compared to orally administered tablets, the bioavailability is reduced to 75% of the oral equivalent [9]. Also, pantoprazole is sensitive to gastric acid and can deteriorate in acidic pH settings, just as other proton pump inhibitors (PPIs). Therefore, there is a danger of degradation when split tablets are given through an enteral feeding tube, which could lead to diminished pharmaceutical effectiveness [4]. In addition, crushing the enteric-coated tablets may obstruct the tube [11]. The physician was recommended by the clinical pharmacist that pantoprazole should be dissolved in sodium bicarbonate. However, the recommendation was not accepted, considering that it would be a problem to prepare an 8.4% sodium bicarbonate solution every day in the hospital. Therefore, pantoprazole tablets were taken by the patient by crushing.

All tablets were crushed together and mixed with an enteral nutrition product

In this case, the patient's all drugs were crushed together and mixed with the enteral nutrition formula. The American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines recommend not combining drugs for administration through an enteral feeding tube. Instead, each drug should be administered separately due to the potential for physical and chemical incompatibility, tube blockage, or alternations in pharmacodynamics [12]. The person administering the drug to the patient was informed about this recommendation by the clinical pharmacist. It was ensured that the drug was administered from the tube by using the correct method.

Inadequate rinse of the tube with water before and after drug administration

The patient's nasogastric tube was rarely flushed with water before or after drug administration. Practice guidelines recommend flushing feeding tubes with 30 mL of water every four hours or before and after intermittent feeding in adult patients. Before giving the medication, it is recommended to stop the feeding, flush the tube with at least 15 mL of water, and administer the medication and later flush the tube again with at least 15 mL of water by taking the patient's fluid volume status into account. According to the guidelines it is necessary to repeat with the following medication, and flush the tube again with at least 15 mL of water. In clinical practice, there can be differences in the amount, timing, and frequency of water flushes [12]. This recommendation was given by the clinical pharmacist and the tube was flushed correctly accordingly.

3.2. Drug interaction and incompatibility with nutrition formula

Pantoprazole: Food may reduce PPI's maximum plasma concentration although this does not have a significant impact on the AUC. However, if possible, it is recommended to administer PPI approximately

30 minutes before meals to improve absorption and maximize clinical effect. Also, it is advised that PPIs be issued with an acidic juice such as apple juice or orange juice rather than milk because PPIs may not absorb when used with non-acidic juices [10]. The clinical pharmacist made this recommendation and ensured that pantaprazole was given at the right time.

4. DISCUSSION

Malnutrition increases comorbidities and lowers physical performance and quality of life. As a result, nutritional support should be integrated into palliative care treatments and the implications for quality of life and life expectancy should be assessed. Enteral nutrition is frequently used as nutritional support [13]. When the oral route is insufficient or unsafe, the enteral feeding tube is essential to provide enteral nutrition [14]. Drug administration through enteral feeding tube is complex and critical in clinical practice. Concurrent drug administration during enteral nutrition may result in problems such as tube blockage (15%) [14], diarrhea (45%) and loss of therapeutic efficacy (26%) if adequate precautions are not implemented [15]. As can be seen in this case, mistakes made during the ordering and administration of the pharmaceuticals resulted in incorrect dosage administration.

There are many potential causes for these errors. The physician may lack knowledge of oral dosage forms, pharmaceutical knowledge or the proper dosage forms for administration through the feeding tube. Such errors may also be caused by inadequately qualified and inexperienced nurses caring for patients. Demirkan et al. suggested that around 40% of prescription drugs were not administered appropriately via a feeding tube, despite 98% of nurses and 86% of doctors stating that they paid special attention to drug suitability when administered through a feeding tube [16]. In addition, the absence of a computerized system that can warn health staff when inappropriate dosage form selection errors, drug incompatibility and drug interaction occur may increase medication errors [17]. Another factor contributing to medication errors is the lack of a multidisciplinary team comprised of various professionals, including a pharmacist. Oral medicine delivery mistakes in patients with enteral feeding tubes were reduced by 95% after intervention by a team of trained pharmacist, a quality manager, a pharmacy technician, a dietician and nursesa [18].

Appropriate drug administration via a feeding tube can be improved by following the Handbook of Drug Administration [9], ASPEN standards [19], drug information on Micromedex® IBM [7] and Lexicomp® programs [8].

In conclusion, due of changing drugs efficacy and safety profiles, as well as the possibility of tube occlusion, practitioners should be more cautious when selecting drugs to be supplied by feeding tube. A pharmacist's review of drug dose forms in patients with feeding tubes might be advantageous for appropriately administering and preventing drug interactions.

Ethical approval

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

Author contribution

Concept: ÖG; Design: KTY; Supervision: KTY; Materials: ÖG; Data Collection and/or Processing: ÖG; Analysis and/or Interpretation: UO; Literature Search: ÖG; Writing: ÖG, KTY; Critical Reviews: KTY, UO.

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Conflict of interest

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