European Review for Medical and Pharmacological Sciences 2023; 27: 1336-1345

Comparison of cytotoxic, reactive oxygen species (ROS) and apoptotic effects of propofol, thiopental and dexmedetomidine on liver cells at accumulative doses (AML12)

B. PEHLIVAN[1], V.F. PEHLIVAN[1], İ. KOYUNCU[2], E. DURAN[1], H. ERDOGDU[3]

[1]Department of Anesthesiology and Reanimation, [2]Department of Medicinal Biochemistry, [3]Department of Biostatistics, Faculty of Medicine, Harran University, Sanliurfa, Turkey

Abstract. – OBJECTIVE: Propofol, thiopental and dexmedetomidine are hypnotic, sedative, antiepileptic and analgesic agents used in general anesthesia and intensive care. There are many known and yet unknown side effects.

Our aim in this study was to examine and compare the cytotoxic, reactive oxygen species (ROS) and apoptotic effects of propofol, thiopental and dexmedetomidine drugs, which are widely used in anesthesia, on liver cells (AML12) *in vitro*.

***MATERIALS AND METHODS*: The half-maximum inhibitory concentration (IC50) doses of the three drugs on AML12 cells were determined using the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) method. Then at two different doses of each of the three drugs, apoptotic effects were determined by the Annexin-V method, morphological examinations were determined by acridine orange ethidium bromide method and intracellular reactive oxygen species (ROS) levels were determined by flow cytometry.**

***RESULTS*: The IC_{50} thiopental, propofol and dexmedetomidine doses were found to be 255.008, 254.904 and 34.501 μgr/mL, respectively ($p<0.001$). The highest cytotoxic effect on liver cells was found in the lowest dose of dexmedetomidine (34.501 μgr/mL) compared to the control group. This was followed by thiopental and propofol, respectively.**

***CONCLUSIONS*: In this study, propofol, thiopental and dexmedetomidine drugs on AML12 cells were found to have toxic effects by increasing intracellular ROS at two different concentrations higher than clinical doses. It was determined that cytotoxic doses caused an increase in ROS and induced apoptosis in cells. We believe that the toxic effects of these drugs can be prevented by examining the values obtained from this study and the results of future studies.**

Key Words:
Anesthesia, Apoptosis, Cytotoxicity, Dexmedetomidine, Liver cells (AML12), Propofol, Reactive oxygen species (ROS), Thiopental.

Introduction

Propofol[1], thiopental[2] and dexmedetomidine[3] are the most commonly used sedative and hypnotic drugs in clinical practice. The administration of anesthetic agents to patients in clinical doses is considered safe. However, in recent years, findings[4,5] regarding the toxicity of anesthetic drugs have emerged. Specifically, studies have shown[4,5] that general anesthetics can impair the development of systems such as neurodevelopment in animals exposed to these agents. This aroused concern for all patient groups, especially young children.

The drugs and techniques used in anesthesia are also widely accepted to contribute to patients' post-operative results. For example, it has been reported[6] that patients who received volatile anesthetics had a shorter life expectancy compared to those who received intravenous anesthetic propofol during their surgical treatment for cancer. Despite their widespread use, general anesthetics (although their specific mechanisms of action remain unclear[7]) sometimes cause undesirable side effects, leading to various complications. Since there are no alternatives to these drugs, they continue to be used, nevertheless. Today, studies are being carried out to elucidate the mechanism of action of such complications. Since the liver is the main junction point at which most of these drugs are metabolized, their effects on the liver are being investigated. Many pharmaceutical agents, including anesthetic drugs, are fully or partially metabolized in the liver; therefore, it would be useful to know well the cellular mechanisms in order to prevent liver damage. The knowledge of these mechanisms would be advantageous for the development and use of new and more "liver-friendly" anesthetic drugs[8].

Corresponding Author: Basak Pehlivan, MD; e-mail: bpehlivan@harran.edu.tr

ROS occurs as an intermediate product in the detoxification mechanism of drugs in liver cells. The ROSs formed are neutralized by the antioxidant system in the mitochondria. However, the detoxification of some drugs causes the production of ROS that exceeds the antioxidant mechanism's capacity, leading to oxidative stress and thus liver damage. This antioxidant process may be insufficient in an unhealthy liver or when the liver is exposed to an undesirable toxin or drug load[9,10]. Oxidative damage disrupts many parts of the cell structure in hepatocytes. Apoptosis and subsequent necrosis, particularly due to anesthetic drugs or viral injury, are two of the main mechanisms of liver injury[10,11]. Apoptosis in different cells can be induced in two ways: intrinsically or exogenously. Although both pathways lead to similar results (elimination of stressed cells), the mechanisms of initiation are different and not yet clearly known[12-15]. Therefore, in our study, the cytotoxic, genotoxic and apoptotic effects of propofol, thiopental and dexmedetomidine on liver cells was investigated and the effects of these drugs on liver toxicity mechanism was studied for the first time.

Among hypnotic agents, gamma-aminobutyric acid (GABA) receptor agonists, such as propofol or thiopental, and Alpha-2 adrenergic receptor agonists, such as dexmedetomidine, are frequently used in anesthetic practice[2,3,16].

Propofol is a fast-acting intravenous (IV) anesthetic agent whose mechanism of action is not fully understood[16]. There are studies[12,13,17,18] showing that propofol has antioxidant properties or causes apoptosis with prooxidant damage at high and cumulative doses. Propofol has been found[19] to have various uses other than anesthesia, such as anti-inflammatory effects. Recently, studies[12,13,17-19] have shown that propofol may be associated with anti-inflammatory and antioxidant effects occurring in multiple organs.

Thiopental is a barbiturate derivative that is frequently used in anesthesia due to its GABA-A agonistic effect[2]. To the best of our knowledge, no studies in the literature have investigated the antioxidant effects of thiopental on humans. However, although the effect of thiopental was observed to be less than that of propofol, animal studies and *in vitro* studies have suggested that thiopental has antioxidant capacities[2]. Barbiturates are also hepatic enzyme inducers; thus, they induce the metabolism of other drugs, including other barbiturates[20]. While these three drugs have an oxidant effect at normal doses, they can have a pro-oxidant effect at high doses[3,12,13,15,21-24].

Dexmedetomidine[3] is a synthetic Alpha-2 adrenergic receptor agonist, sedative, and hypnotic agent. Studies[3] have claimed that dexmedetomidine can prevent isoflurane-induced apoptosis in the brain and other organs, while other studies[25,26] have claimed that does not. Tufek et al[23] reported the protective potential of dexmedetomidine against hepatic lipid peroxidation and histological damage in an animal model of sepsis and ischemia-reperfusion. However, the mechanism of the protective effect is still unclear. In one study[27], 10 μM dexmedetomidine was found to cause a significant decrease in cell viability.

In our study, we investigated the effects of propofol, thiopental and dexmedetomdine on cytotoxicity, apoptosis and intracellular free radicals in embryonic liver AML12 cells *in vitro* and examined their possible mechanisms of action. The most distinctive feature of this study is that it is the first study to examine the effects of propofol, thiopental and dexmedetomdine at different doses on *in vitro* cytotoxicity, apoptosis and intracellular free radicals in embryonic liver AML12 cells and compare their possible mechanisms of action.

Materials and Methods

Cells and Culture Conditions

We obtained the liver fibroblast (AML12) cells[28,29] from the American Type Culture Collection (ATCC). The cell lines were grown in Dulbecco's Modified Eagle Medium: F12 (DMEM) containing 1% P/S, 10% Fetal Bovine Serum (FBS), and 1% glutamine. All the cells were incubated at 37°C in an atmosphere of 5% CO_2. The cells were removed with a mixture of 0.25% trypsin and 0.03% ethylenediamine tetraacetic acid (EDTA) and passaged 1:2 or 1:3 as recommended by the ATCC. The unused cells were stored in a cell freezing solution prepared with 95% nutrient medium and 5% dimethylsulfoxide (DMSO), in a -80°C deep freezer, for the short- or long-term in liquid nitrogen.

Administration of Drugs to the Cells

Cytotoxicity analysis with MTT assay

The medium was refreshed 24 hours after the AML12 cells were seeded in 96 sterile plates at 10^4 cells per well. Ten doses (0-2.5-5-10-25-50-100-200-250-400-500 μg/mL) were selected as the drug concentrations to be applied to the cells. Propofol and thiopental in 10 doses, dexmedetomidine in 7 doses were administered in three rep-

etitions. No drug was administered to the control group. The cell lines were placed in an incubator for 24 hours after the drugs were applied. After the drug administration, the cell medium was removed from the medium. The cytotoxic effects of drugs were evaluated using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric test. Measurements were read at absorbances of 570 nm to 690 nm using a plate reader (Thermo Multiskan Go, Waltham, MA, USA). Graphics have been created. IC_{50} values were then calculated. According to IC_{50} values found in cytotoxicity tests, 100-200 µg/mL for propofol and thiopental, and 50 and 100 µg/mL for dexmedotomidine were studied.

Cell morphology and acridine orange/ethidium bromide (AO/EB) analysis

Cell morphology images were taken with an inverted fluorescent microscope (Olympus CKX, DP73, Shinjuku, Tokyo, Japan). According to the cell nucleus morphology, the apoptosis fluorescence in the cells was examined using the AO/EB staining method. The medium was removed 24 hours after the administration of the drugs, then 50 µL of AO/EB dye (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was added and images were taken with the Olympus CKX 51, DP73 microscope.

Determination of apoptosis using the annexin V/propidium iodide (PI) method

This analysis was performed using the commercially available Fluorescein (FITC) Annexin-V Apoptosis Detection Kit I (Cat No./ID:556.547, BD Biosciences, Franklin Lakes, NJ, USA) method. Shortly after administering the drugs, cells were harvested and immediately stained according to the kit protocol. The stained cells were analyzed by flow cytometry (FACS Via, BD Biosciences, Franklin Lakes, NJ, USA). Annexin V is displayed in green, and propidium iodide (PI) is displayed in red. Viable cells [(FITC-)/(PI-)] were differentiated as early and moderately apoptotic [(FITC+) /(PI-)], late apoptotic, and necrotic cells [(FITC+) /(PI+)].

Determination of intracellular ROS

Intracellular free radical exchange was performed according to the protocol for the commercially available kit (MHC100111, Millipore-Merck, Burlington, MA, USA). The Muse® Oxidative Stress Kit provides quantitative (number and percentage of cells) measurements of Reactive Oxygen Species (ROS), i.e., superoxide radicals, in cells exposed to oxidative stress. After administering the drugs, the cells were harvested using trypsin enzymes and washed with cold phosphate buffered saline (PBS). After adding 100 µl of ROS working solution, the cells were incubated at 37°C for 30 minutes. After incubation, the cells were analyzed by flow cytometry (FACS Via, BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

The data were analyzed using SPSS v. 26.0 software (IBM Corp., Armonk, NY, USA). All analyzes were performed in triplicate and presented as mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test. Normally distributed data in all experiments were analyzed using One-Way Analysis of Variance (ANOVA). Post-hoc analysis was performed to find out which group means differ from one another. A p-value <0.05 was considered statistically significant.

Results

Cytotoxic Effect of Propofol, Thiopental, and Dexmedetomidine on AML12 Cells

The effects of propofol, thiopental, and dexmedetomidine on the viability of AML12 cells were determined by MTT assay (Figure 1). Based on the analysis results, the cytotoxic effects of all three drugs increased as the drug dose increased. The IC_{50} values for AML12 cells were 255.008 µg/mL, 254.804 µg/mL and 34.501 µg/mL, respectively. The cytotoxic effects for propofol and thiopental at doses of 100 µg/mL and 400 µg/mL were similar compared to the control group, but significant compared to the control group (p<0.001). However, the cytotoxic effect of propofol was found to be significantly lower than that of thiopental at doses of 200, 250 and 500 µg/mL compared to the control group. These decreases in cell viability were statistically significant compared to the control group (p<0.001)

The Effects of Propofol, Thiopental, and Dexmedetomidine on AML12 Cell Morphology

When the effects of the drugs on cell morphology were compared with the control group, it was observed that the number of cells decreased, and the number of apoptotic cells increased depending on the dose increase in all three drugs (Figure 2a). The apoptotic effect of

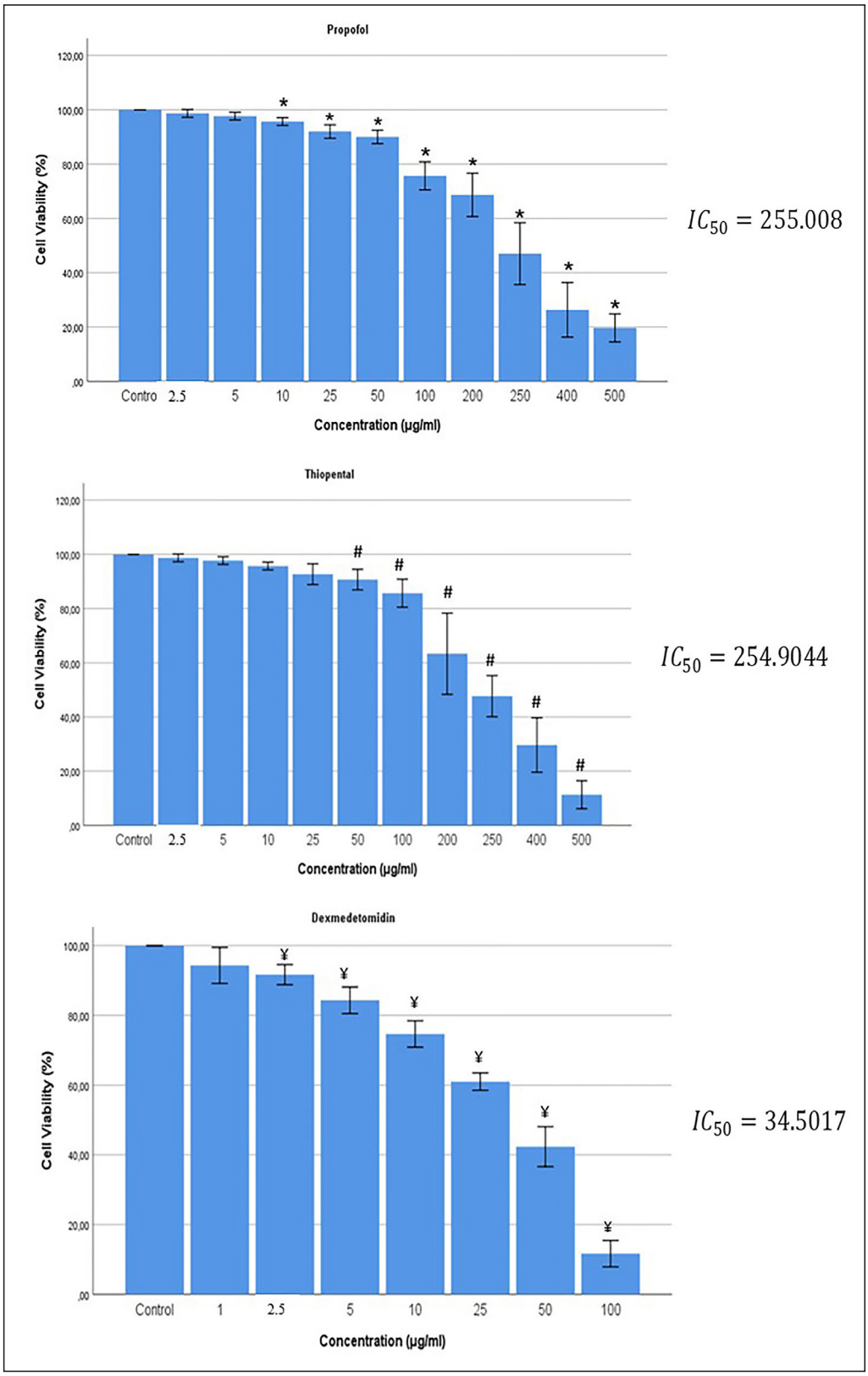

Figure 1. % Changes in viability of AML12 liver cells treated with different concentrations of propofol, thiopental and dexmedetomidine for 24 hours. The data obtained are shown as mean ± standard deviation. *p<0.001 *vs.* control group for propofol. $^{\#}p$<0.001 *vs.* control group for thiopental. $^{¥}p$<0.001 *vs.* control group for dexmedetomidin.

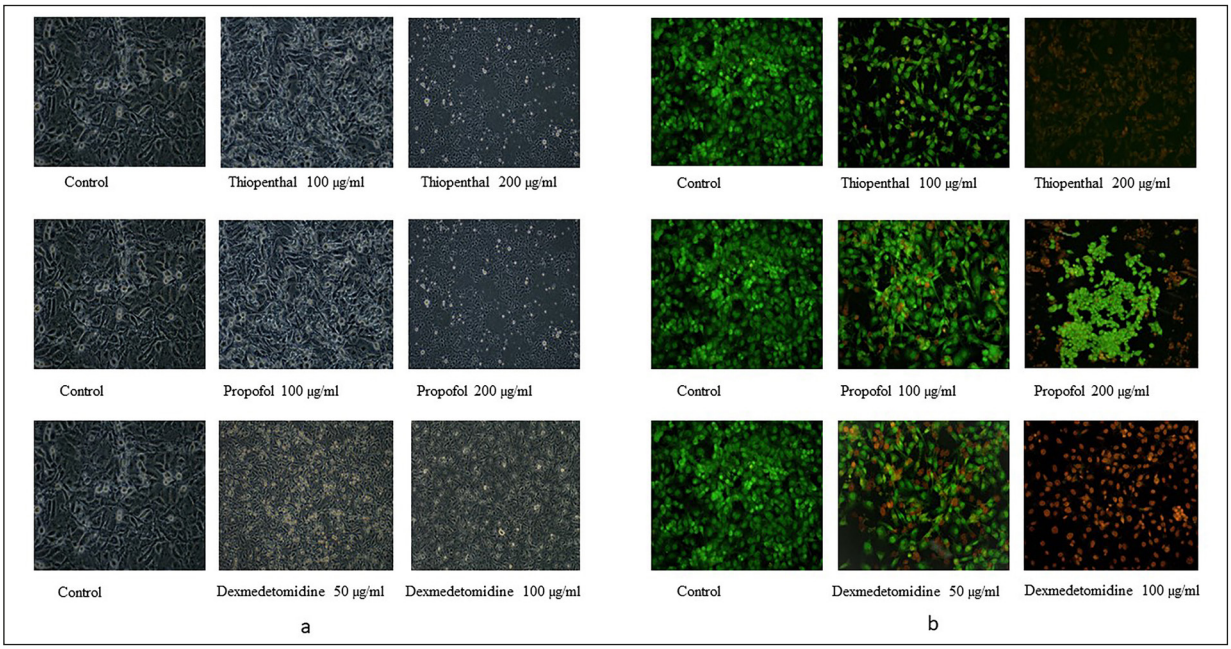

Figure 2. Image of morphological and apoptotic effects of drugs on AML12 cells. **a**, Morphological imaging; (**b**), Fluorescent imaging.

the drugs on AML12 cells was analyzed by AO/ET fluorescent staining (Figure 2b). As shown in Figure 2b, the viable cells are green, the apoptotic cells are orange, and the necrotic cells are red. When the apoptotic effects of the three drugs were compared, the following result was observed: dexmetomidine>thiopental>propofol. Thus, the highest apoptotic and cytotoxic effect was observed in dexmedetomidine.

Flow Cytometric Annexin-V Analysis of the Drugs' AML12 Cell Apoptotic Effect

While 88.30±2.06% and 82.96±2.41% of AML12 cells treated with propofol were viable at 100 µg/mL and 200 µg/mL doses, 2.60 ± 0.52 and 2.10±0.79 were observed to be early apoptotic (Table I, Figure 3). While 16.80±1.15% and 16.16±1.95% of AML12 cells treated with thiopental were viable at 100 µg/mL and 200 µg/mL doses, it was observed that 59.80±1.47% and 48.36±0.60% of these cells underwent early apoptosis (Table I, Figure 3). While 32.33±2.60% and 4.33±2.51% of dexmedetomidine-treated AML12 cells could survive at 50 µg/mL and 100 µg/mL doses, 58.90±1.40% of these cells underwent early apoptosis and 88.43±2.25% had late apoptosis. There was a statistically significant difference between all drugs (p<0.001), (Table I, Figure 3).

Table I. Flow cytometric Annexin-V analysis of the AML12 cell apoptotic effect of drugs.

	Control	Doses (µg/mL)	Propofol	Thiopental	Doses (µg/mL)	Dexmedetomidin	p
Live	97.10 ± 1.86	100 µg/mL	88.30 ± 2.06	16.80 ± 1.15	50 µg/mL	32.33 ± 2.60	*
		200 µg/mL	82.96 ± 2.41	16.16 ± 1.95	100 µg/mL	4.33 ± 2.51	*
Early Apoptotic	0.63 ± 0.41	100 µg/mL	2.60 ± 0.52	59.80 ± 1.47	50 µg/mL	58.90 ± 1.40	#
		200 µg/mL	2.10 ± 0.79	48.36 ± 0.60	100 µg/mL	5.33 ± 0.66	#
Late Apoptotic	0.26 ± 0.20	100 µg/mL	0.73 ± 0.41	19.96 ± 1.53	50 µg/mL	7.86 ± 1.07	¥
		200 µg/mL	0.53 ± 0.35	28.90 ± 0.75	100 µg/mL	88.43 ± 2.25	¥
Necrotic	1.93 ± 1.88	100 µg/mL	8.40 ± 1.24	3.53 ± 1.73	50 µg/mL	0.90 ± 1.13	&
		200 µg/mL	14.43 ± 1.26	6.60 ± 1.04	100 µg/mL	1.90 ± 0.95	&

(Values are given as mean±standard deviation. For all drugs: *p<0.001 *vs.* control group live, #p<0.001 *vs.* control group early apoptotic, ¥p <0.001 *vs.* control group late apoptotic, &p<0.001 *vs.* control group necrotic).

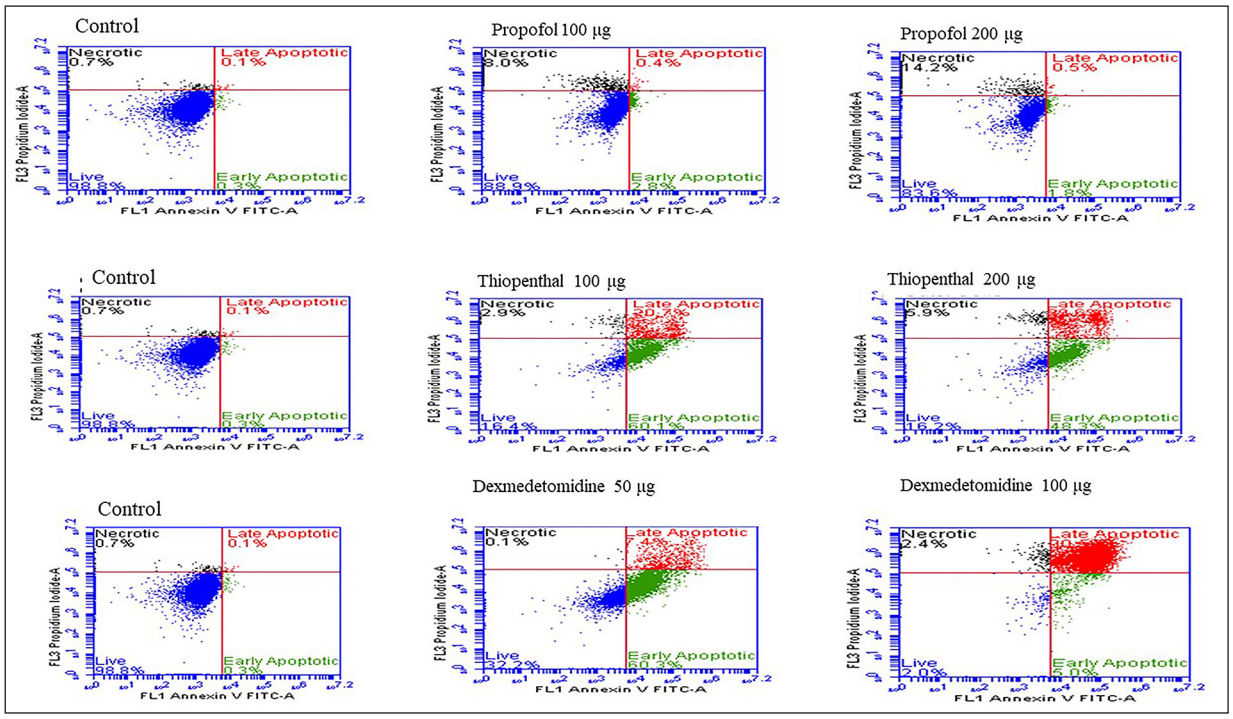

Figure 3. Flow cytometric Annexin-V analysis of the AML12 cell apoptotic effect of drugs.

Flow Cytometric Investigation of the Effects of Propofol, Thiopental, and Dexmedetomidine on Intracellular Free Radical Formation of AML12 Cells

In all drugs, intracellular cytosolic ROS levels were found to increase in a dose-dependent manner (p<0.001), (Table II, Figure 4). The drugs we used had intracellular free radical levels at the first dose of 100 µg/mL for propfol and thiopental and 50 µg/mL for dexmedetomidine. At this dose, intracellular free radical levels were 6.23±1.70% in propofol, 16.13±2.57% in thiopental, and 27.03±2.68% in dexmedetomidine (p<0.001). The ROS level in propofol and thiopental at a dose of 200 µg/mL was observed to be lower than in dexmedetomidine at a dose of 100 µg/mL (p<0.001). Lower doses of dexmedetomidine have been found to cause ROS generation and cell death compared to propofol and thiopental. In conclusion, we found that as the dose of dexmedetomidine, propofol, and thiopental increased, the viability of cells decreased, resulting in an increase in ROS formation (Table II and Figure 4).

Discussion

Use of drug doses higher than clinical-level doses may occur due to conditions such as re-administration of anesthesia in clinical anesthesia, total intravenous anesthesia (TIVA), and continuous infusion for sedation in ICUs. When we compared

Table II. ROS effect of different doses of drugs on AML12 cells.

	Doses	Control	Propofol	Thiopental	Doses	Dexmedetomidin	p
ROS (-)	100 µg/mL	98.30 ± 1.25	93.70 ± 1.70	82.70 ± 0.36	50 µg/mL	71.73 ± 2.77	*
	200 µg/mL	95.93 ± 1.90	83.83 ± 2.08	76.00 ± 2.22	100 µg/mL	67.50 ± 2.50	*
ROS (+)	100 µg/mL	4.03 ± 1.89	6.23 ± 1.70	16.13 ± 2.57	50 µg/mL	27.03 ± 2.68	#
	200 µg/mL	4.03 ± 1.89	5.50 ± 1.41	22.90 ± 0.75	100 µg/ml	31.66 ± 2.83	#

Values were given as the mean±standard deviation. *p<0.001, statistically different from negative control. #p<0.001, statistically different from positive control.

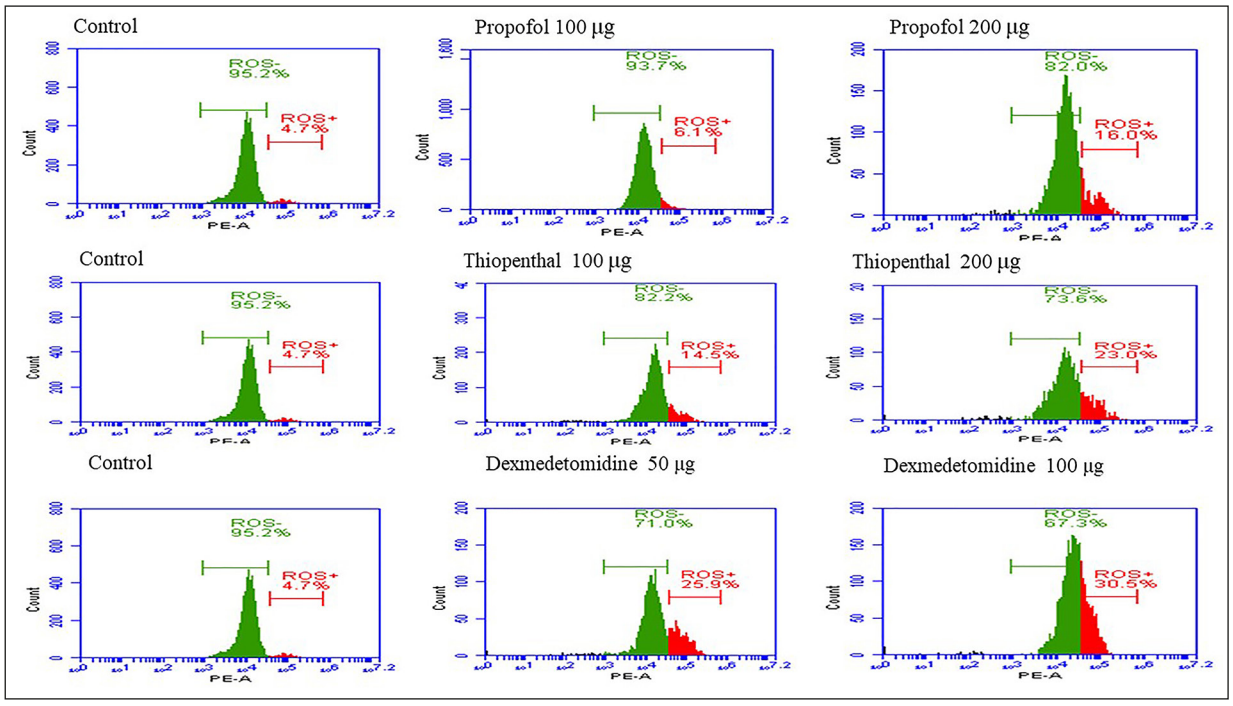

Figure 4. Flow cytometric investigation of the effects of propofol, thiopental and dexmedetomidine on intracellular free radical (ROS) formation of AML12 cells.

the toxic effects of propofol, thiopental and dexmedetomidine in liver fibroblast cells (AML12) at two different high doses, we found that they undergo apoptosis due to increased intracellular ROS. However, these three drugs did not show any cytotoxic effects at clinically relevant doses.

Although propofol[1], thiopental[2], and dexmedetomidine[3] are used for sedation, anesthesia, and analgesia in anesthesia practice, they have various side effects. In propofol, those side effects include propofol infusion syndrome (PRIS)[17,30], electrocardiogram (ECG) QT prolongation, local muscle pain, hypotension, and myoclonus[31]. It is known[21] that thiopental, which is a barbiturate, has a toxic effect on hepatic cytochrome P450. Hypotension and bradycardia may develop due to dexmedetomidine[22]. Apart from these known side effects, various unknown complications can occur, depending on the dose or the person.

The exact cause of complications from the administration of these drugs is still unknown. *In vitro* studies[28,29] are frequently used to determine the effects of such drugs on organisms. In these studies, researchers often select the type of cell in which drugs are metabolized or in which they show activity. The liver is the main junction in the metabolism of most drugs, especially anesthetic drugs. Therefore, AML12 cells are a good choice for an *in vitro* model as they enable researchers to study the physiological functions of liver cells[28,29]. We used AML12 cells to examine the effects of propofol, thiopental and dexmedetomidine at clinical and high doses.

Cell death due to propofol, thiopental and dexmedetomidine has been reported[30,32]. Although it does not have any toxic effect at clinical doses, it has been determined that in long-term use, if clinical doses are exceeded or repeated doses of these drugs are used for sedation, a cumulative effect may occur and may have a cytotoxic effect on many cells. Similarly, Ludbrook at al[32] showed that plasma concentrations of propofol can range between 2 (11 µM) and 5 µg/mL (27.5 µM). Vanlander et al[33] found that the concentration of propofol in the tissues of rats treated with 20 mg/kg/hr of this drug could reach 200 µM under certain conditions. Therefore, although toxic effects of these three drugs at clinical doses were not observed in AML12 cells in our study, the toxic effects of higher doses were examined to model their cumulative effects.

When the cytotoxic effects of these drugs on AML12 cells were examined, it is consistent with the findings reported by Ohno et al[34]. Moreover, we observed that propofol and thiopental had a moderate cytotoxicity range, while dexmedetomidine had a greater cytotoxic effect at lower doses ($p<0.001$). Peng et al[35] found that dexmedetomidine had a protective effect on cardiomyocytes in hypoxia/reox-

ygenation injury at clinical doses. However, only dexmedetomidine showed a cytotoxic effect at high doses, especially at doses of 25 μg/mL and above, in our study, which is consistent with the results reported by Peng at al[35], Zhu et al[27], the latters found that 10 μM dexmedetomidine on liver L-02 cells caused a significant reduction in cell viability. In addition, they found that it was protective in liver cells damaged by oxygen-glucose deprivation at clinical doses. In our study, we found that dexmedetomidine had a statistically significantly higher cytotoxic effect than propofol and thiopental (p<0.001, Figure 1). Propofol and thiopental showed cytotoxic effects at higher doses than dexmedetomidine. In clinical use, doses for dexmedetomidine are lower than for propofol and thiopental. However, the clinical doses of propofol and thiopental are similar[1,2]. Considering the cytotoxic effects of propofol and thiopental, we found that propofol at doses of 200, 250 and 500 μg/mL showed lower levels of cytotoxicity than thiopental at the same doses. (Figure 1).

It is a known[36] fact that various drugs increase intracellular ROS. Deceleration of their detoxification due to overproduction of ROS or insufficiency of antioxidant systems leads to accumulation of these radicals and toxic effects on lipid and protein molecules in cells and DNA. In our study, depending on the increase in ROS, we observed that the number of viable cells decreased, and the number of apoptotic cells increased at two different doses of propofol and thiopental and dexmedetomidine compared to the control group (p<0.001, Figure 4).

Increasing the level of free radicals in the cell for different reasons causes oxidative stress. When the antioxidant system is also deficient, cells are prone to apoptosis or necrosis. In our study, we used the Annex-V method to determine whether an apoptotic event or a necrotic event occurred due to an increase in ROS. Consistent with our results, Keel et al[37] found that thiopental at doses of 200 μg/mL and higher increased necrosis and apoptosis. Hao et al[38] found that ROS levels in brain endothelial cells increased significantly with propofol stimulation. In our study, the increase in ROS with propofol and thiopental was consistent with the findings reported by Hao et al[38]. However, the increase in ROS at two different doses was statistically higher with dexmedetomidine than with propofol and thiopental at lower doses was high (p<0.001).

In recent years, intravenous anesthetic agents (such as propofol, thiopental and dexmedetomidine) have been reported to have apoptotic properties in animal studies[24,39-42]. These drugs exerted their effects in a dose-dependent manner, especially in animal livers, and induced apoptosis. Although some studies[24,39-42] claim that these agents are hepatoprotective and protect liver tissue from undesirable side effects such as apoptosis, degeneration, inflammation and energy deficiency, it has also been reported[24,39-42] to cause apoptosis. The cell toxicity of propofol and thiopental was similar, but we found that dexmedetomidine caused cell toxicity in all cell lines tested in the low concentration range. However, the possible role of anesthetic agents in liver injury is not yet clear.

Apoptosis is a tightly regulated process involving the activation of specific proteases responsible for the organized removal of damaged cells[30]. In our study, propofol, thiopental, and especially dexmedetomidine, induced apoptosis in AML12 cells by increasing the formation of ROS at doses higher than those used in a clinical setting.

Limitations

Our study has some limitations. We tested the toxicity of three drugs using cultured cells. We primarily used established cell lines obtained from primary cultured cells, not from various tissue origins. Studies involving animal models are warranted to confirm our findings. Although we performed our tests using 10% FBS, the free fraction of all three drugs was not determined. Another limitation of our study is that results from a cell model cannot simply be translated/transported into a clinical setting.

Conclusions

In our study propofol, thiopental and dexmedetomidine drugs on AML12 cells were found to have toxic effects by increasing intracellular ROS at two different concentrations higher than clinical doses. It was observed that this cytotoxic effect was due to the increase in intracellular ROS that triggered apoptosis. With the examination of the data obtained as a result of this study and the new studies to be done, we believe that complications associated with the use of these three drugs can be clarified and prevented.

Conflict of Interests

The authors declare that they have no conflict of interests.

Funding

This research was supported by Harran University Scientific Research Projects Unit (HUBAK: 31/12/2019 date and project number: 19342)

Ethics Approval
Since this study was a cell study, ethical approval was not obtained.

Informed Consent
Informed consent was not required due to the nature of the study.

ORCID ID
Başak Pehlivan: 0000-0001-6985-343X
Veli Fahri Pehlivan: 0000-0001-5661-4499
İsmail Koyuncu: 0000-0002-9469-4757
Erdogan Duran: 0000-0002-9606-8266
Hamza Erdogdu: 0000-0002-5025-2367

Availability of Data and Materials
The data supporting this article are available from the corresponding author upon reasonable request.

Acknowledgements
We thank Associate Prof. Evren Buyukfirat (Medical University of Harran, Turkey) and Associate Prof. Ataman Gonel (Medical University of Hasan Kalyoncu, Turkey) for their support.

Authors' Contribution
Başak Pehlivan designed the research, analyzed and interpreted the data and conducted the research, prepared the article; Veli Fahri Pehlivan designed the research, accessed the data, analyzed and interpreted the data, provided financial support, prepared the article, İsmail Koyuncu did the laboratory studies, analyzed the data, interpreted them; Erdoğan Duran reached the data, analyzed and interpreted the data; Hamza Erdogdu analyzed the data, interpreted them, did the statistical work. All authors have read and approved the final version of the article.

References

1) Heim M, Draheim R, Krupp A, Breihan P, O'Rourke A, Wells J, Fish J. Evaluation of a Multidisciplinary Pain, Agitation, and Delirium Guideline in Mechanically Ventilated Critically Ill Adults. Hosp Pharm 2019; 54: 119-124.
2) Senoner T, Velik-Salchner C, Luckner G, Tauber H. Anesthesia-Induced Oxidative Stress: Are There Differences between Intravenous and Inhaled Anesthetics? Drevet JR, ed. Oxid Med Cell Longev 2021; 2021: 1-11.
3) Lee S. Dexmedetomidine: present and future directions. Korean J Anesthesiol 2019; 72: 323-330.
4) Vutskits L, Davidson A. Update on developmental anesthesia neurotoxicity. Curr Opin Anaesthesiol 2017; 30: 337-342.
5) Walters JL, Paule MG. Review of preclinical studies on pediatric general anesthesia-induced developmental neurotoxicity. Neurotoxicol Teratol 2017; 60: 2-23.
6) Wigmore TJ, Mohammed K, Jhanji S. Long-term Survival for Patients Undergoing Volatile versus IV Anesthesia for Cancer Surgery. Anesthesiology 2016; 124: 69-79.
7) Armstrong R, Riaz S, Hasan S, Iqbal F, Rice T, Syed N. Mechanisms of Anesthetic Action and Neurotoxicity: Lessons from Molluscs. Front Physiol 2018; 8: 1138.
8) Dabbagh A, Rajaei S. The Role of Anesthetic Drugs in Liver Apoptosis. Hepat Mon 2013; 13: e13162.
9) Gottschalk S, Zwingmann C, Raymond V-A, Hohnholt MC, Chan TS, Bilodeau M. Hepatocellular apoptosis in mice is associated with early upregulation of mitochondrial glucose metabolism. Apoptosis 2012; 17: 143-153.
10) Yano A, Higuchi S, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. Involvement of immune-related factors in diclofenac-induced acute liver injury in mice. Toxicology 2012; 293: 107-114.
11) Sitia G, Iannacone M, Aiolfi R, Isogawa M, van Rooijen N, Scozzesi C, Bianchi ME, von Andrian UH, Chisari FV, Guidotti LG. Kupffer Cells Hasten Resolution of Liver Immunopathology in Mouse Models of Viral Hepatitis. PLoS Pathog 2011; 7: e1002061.
12) Senoner T, Schindler S, Stättner S, Öfner D, Troppmair J, Primavesi F. Associations of Oxidative Stress and Postoperative Outcome in Liver Surgery with an Outlook to Future Potential Therapeutic Options. Oxid Med Cell Longev 2019; 2019: 1-18.
13) Malsy M, Graf B, Hofer V, Bitzinger D, Bundscherer A. Effect of Propofol and Etomidate on the Proliferation, Cell-cycle Distribution, Apoptosis and Necrosis of Pancreatic Tumour Cells. In Vivo (Brooklyn) 2022; 36: 2722-2729.
14) Hsing CH, Chen YH, Chen CL, Huang WC, Lin MC, Tseng PC, Wang CY, Tsai CC, Choi PC, Lin CF. Anesthetic Propofol Causes Glycogen Synthase Kinase-3β-regulated Lysosomal/Mitochondrial Apoptosis in Macrophages. Anesthesiology 2012; 116: 868-881.
15) Straiko MMW, Young C, Cattano D, Creeley CE, Wang H, Smith DJ, Johnson SA, Li ES, Olney JW. Lithium Protects against Anesthesia-induced Developmental Neuroapoptosis. Anesthesiology 2009; 110: 862-868.
16) Antkowiak B, Rammes G. GABA(A) receptor-targeted drug development -New perspectives in perioperative anesthesia. Expert Opin Drug Discov 2019; 14: 683-699.
17) Condello I, Santarpino G, Fiore F, Di Bari N, Speziale G, Moscarelli M, Nasso G. Propofol pharmacokinetics and pharmacodynamics—a perspective in minimally invasive extracorporeal circulation. Interact Cardiovasc Thorac Surg 2021; 33: 625-627.
18) Folino TB, Muco E, Safadi AO, Parks LJ. Propofol. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022

19) Zhou P, Liu H, Wu Y, Chen D. Propofol Promotes Ankle Fracture Healing in Children by Inhibiting Inflammatory Response. Med Sci Monit 2018; 24: 4379-4385.
20) Suddock JT, Cain MD. Barbiturate Toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
21) Dumps C, Halbeck E, Bolkenius D. Medikamente zur intravenösen Narkoseinduktion: Barbiturate. Anaesthesist 2018; 67: 535-552.
22) Page RL, O'Bryant CL, Cheng D, Dow TJ, Ky B, Stein CM, Spencer AP, Trupp RJ, Lindenfeld J. Drugs That May Cause or Exacerbate Heart Failure. Circulation 2016; 134: e32-69.
23) Tüfek A, Tokgöz O, Aliosmanoglu İ, Alabalik U, Evliyaoglu O, Çiftçi T, Güzel A, Yıldırım ZB. The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. Int J Surg 2013; 11: 96-100.
24) Bedir Z, Ozkaloglu Erdem KT, Ates I, Olmezturk Karakurt TC, Gursul C, Onk D, Kurt N, Suleyman Z, Suleyman H. Effects of ketamine, thiopental and their combination on the rat liver: A biochemical evaluation. Adv Clin Exp Med 2021; 31: 285-292.
25) Hwang L, Cho IY, Kim SE, KO IG, Shin MS, Kim CJ, SH K, Jin JJ, Chung JY, Yi JW. Dexmedetomidine ameliorates intracerebral hemorrhage-induced memory impairment by inhibiting apoptosis and enhancing brain-derived neurotrophic factor expression in the rat hippocampus. Int J Mol Med 2013; 31: 1047-1056.
26) Koca U, Olguner ÇG, Ergür BU, Altekin E, Taşdöğen A, Duru S, Girgin P, Gündüz K, Cilaker Mıcılı S, Güzeldağ S, Akkuş M. The Effects of Dexmedetomidine on Secondary Acute Lung and Kidney Injuries in the Rat Model of Intra-Abdominal Sepsis. Sci World J 2013; 2013: 1-11.
27) Zhu YX, Zhou JH, Li GW, Zhou WY, Ou SS, Xiao XY. Dexmedetomidine protects liver cell line L-02 from oxygen-glucose deprivation-induced injury by down-regulation of microRNA-711. Eur Rev Med Pharmacol Sci 2018; 22: 6507-6516.
28) Si F, Hu X, Wang C, Chen B, Wang R, Dong S, Yu R, Li Z. Porcine Epidemic Diarrhea Virus (PEDV) ORF3 Enhances Viral Proliferation by Inhibiting Apoptosis of Infected Cells. Viruses 2020; 12: 214.
29) Verheije MH, Raaben M, Mari M, te Lintelo EG, Reggiori F, van Kuppeveld FJM, Rottier PJM, de Haan CAM. Mouse Hepatitis Coronavirus RNA Replication Depends on GBF1-Mediated ARF1 Activation. PLoS Pathog 2008; 4: e1000088.
30) Sumi C, Okamoto A, Tanaka H, Nishi K, Kusunoki M, Shoji T, Uba T, Matsuo Y, Adachi T, Hayashi JI, Takenaga K, Hirota K. Propofol induces a metabolic switch to glycolysis and cell death in a mitochondrial electron transport chain-dependent manner. PLoS One 2018; 13: e0192796.
31) Garza MG, Najera RIS, Ramírez EN, Quintero MTP, Dominguez SLA, Ramirez EL, Paz MR, Soto JMS. Sedation in pediatric dentistry, an overview and current update. Int J Appl Dent Sci 2022; 8: 176-180.
32) Ludbrook GL, Visco E, Lam AM. Propofol. Anesthesiology 2002; 97: 1363-1370.
33) Vanlander AV, Okun JG, de Jaeger A, Smet J, De Latter E, De Paepe B, Dacremont G, Wuyts B, Vanheel B, De Paepe P, Jorens PG, Van Regenmortel N, Van Coster R. Possible pathogenic mechanism of propofol infusion syndrome involves coenzyme q. Anesthesiology 2015; 122: 343-352.
34) Ohno S, Kobayashi K, Uchida S, Amano O, Sakagami H, Nagasaka H. Cytotoxicity and type of cell death induced by midazolam in human oral normal and tumor cells. Anticancer Res 2012; 32: 4737-4747.
35) Peng K, Qiu Y, Li J, Zhang ZC, Ji FH. Dexmedetomidine attenuates hypoxia/reoxygenation injury in primary neonatal rat cardiomyocytes. Exp Ther Med 2017; 14: 689-695.
36) Özcan O, Erdal H, Çakırca G, Yönden Z. Oxidative stress and its impacts on intracellular lipids, proteins and DNA. J Clin Exp Investig 2015; 6: 331-336.
37) Keel M, Mica L, Stover J, Stocker R, Trentz O, Härter L. Thiopental-induced Apoptosis in Lymphocytes Is Independent of CD95 Activation. Anesthesiology 2005; 103: 576-584.
38) Hao J, Zhang W, Tong R, Huang Z. Febuxostat Prevents the Cytotoxicity of Propofol in Brain Endothelial Cells. ACS Omega 2021; 6: 5471-5478.
39) Delen LA, Dişli ZK, Taş HG, Kuyrukluyildiz U, Yazici GN, Süleyman B, Kuzucu M, Gürsu C, Suleyman H. The effects of thiamine pyrophosphate on propofol-induced oxidative liver injury and effect on dysfunction. Gen Physiol Biophys 2022; 41: 63-70.
40) Campos S, Félix L, Venâncio C, de Lurdes Pinto M, Peixoto F, de Pinho PG, Antunes L. In vivo study of hepatic oxidative stress and mitochondrial function in rabbits with severe hypotension after propofol prolonged infusion. Springerplus 2016; 5: 1349.
41) Romuk E, Szczurek W, Nowak P, Hudziec E, Chwalińska E, Birkner E. Effects of Propofol on the Liver Oxidative-Antioxidant Balance in a Rat Model of Parkinson's Disease. Adv Clin Exp Med 2016; 25: 815-820.
42) Su ZY, Ye Q, Liu XB, Chen YZ, Zhan H, Xu SY. Dexmedetomidine mitigates isoflurane-induced neurodegeneration in fetal rats during the second trimester of pregnancy. Neural Regen Res 2017; 12: 1329-1337.