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Determination of Antimicrobial and Antioxidative Properties of Several Anesthetic Drugs

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Abstract: For various reasons, the balance between oxidative stress and the antioxidative defence system is disturbed during general anesthesia. On the other hand, thanks to their antioxidant effect, certain anesthetics have been suggested to protect from oxidative stress caused due to pathological states. In this study, potential antimicrobial and antioxidative activities of commonly used anesthetic drugs were evaluated to reveal possible effects after surgery. The antimicrobial activities of commercially purchased anesthetic drugs diluted with sterile physiological saline were investigated according to the Kirby-Bauer disc diffusion method. Furthermore, minimum inhibitory concentration and minimum bactericidal concentrations were determined. Antioxidative potentials of the drugs were screened according to 2,2 diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl ($OH^{\bullet}$) radical scavenging assays. One of the tested drugs, Ketalar, containing ketamine hydrochloride was found to have an inhibition effect on all tested pathogenic microorganisms. At the same time, Mivacron and Pental Sodium formed the most significant inhibition zones on *Micrococcus luteus*. As expected, Propofol had no antimicrobial activity on most tested organisms. However, its antioxidant activity was the highest among the other drugs. Calculated SC_{50} values for DPPH-free and hydroxyl radical scavenging activities of Ultiva, Blok-L, and Zolamid were very close to Propofol. It can be concluded that using these drugs for anesthesia may suppress the risk of contamination and oxidative stress that may occur during use in many cases.

Keywords: DPPH, Kirby-Bauer disc diffusion method, MIC, Propofol.

Çeşitli Anestezik İlaçların Antimikrobiyal ve Antioksidatif Özelliklerinin Belirlenmesi

Öz: Genel anestezi sırasında çeşitli nedenlerle oksidatif stres ile antioksidan savunma sistemi arasındaki denge bozulur. Öte yandan, antioksidan etkileri sayesinde bazı anestetiklerin patolojik durumların neden olduğu oksidatif stresten korunmaları önerilmiştir. Bu çalışmada ameliyat sonrası olası etkileri ortaya çıkarmak için yaygın olarak kullanılan anestezik ilaçların potansiyel antimikrobiyal ve antioksidatif aktiviteleri değerlendirildi. Kirby-Bauer disk difüzyon yöntemine göre steril fizyolojik tuzlu su ile seyreltilmiş ticari olarak satın alınan anestezik ilaçların antimikrobiyal aktiviteleri araştırıldı. Ayrıca minimum inhibitör konsantrasyon ve minimum bakterisidal konsantrasyonlar belirlendi. İlaçların antioksidan potansiyelleri 2,2 difenil-1-pikrilhidrazil (DPPH) ve hidroksil ($OH^{\bullet}$) radikal süpürücü yöntemleri ile tarandı. Ana bileşen olarak ketamin hidroklorür içeren test edilen ilaçlardan biri olan Ketalar'ın, test edilen tüm patojenik mikroorganizmalar üzerinde inhibisyon etkisi olduğu bulundu. Aynı zamanda, Mivacron ve Pental Sodium, *Micrococcus luteus* üzerinde en büyük inhibisyon bölgelerini oluşturmuştur. Beklendiği gibi, Propofol, test edilen organizmaların çoğunda antimikrobiyal aktivite göstermemiş olup, antioksidan aktivitesi diğer ilaçlar arasında en yüksek değere sahiptir. Ultiva, Blok-L ve Zolamid'in DPPH serbest radikali ve hidroksil radikal süpürücü aktiviteleri için hesaplanan SC50 değerleri Propofol'e çok yakın olduğu belirlendi. Bu ilaçların birçok durumda anestezi amaçlı kullanımı sırasında oluşabilecek kontaminasyon ve oksidatif stres riskini baskılayabileceği sonucuna varılabilir.

Anahtar kelimeler: DPPH, Kirby-Bauer disk difüzyon metodu, MİK, Propofol.

1. Introduction

Anesthetics are drugs that slow down or stop the biological functions of cells, especially nervous system cells. Although the definition and use of anesthetics coincided within the first half of the eighteenth century, it was only in the twentieth century that its use became widespread. After ether and chloroform, the anesthetic properties of which were first discovered, studies on this subject intensified and the discovery of muscle relaxants paved the way for surgical developments (Bilgin, 2013).

Today, different aspects of anesthetic drugs are also the subject of research. For example, some studies on anesthetic drugs' antioxidant and antimicrobial properties are available in the literature. (Razavi & Bazzaz, 2019; Kesici et al., 2021; Volti et al., 2008). Still, most of them focus on Propofol, a widely used sedative-hypnotic drug (Tulgar et al., 2018). One reason for this is the similar chemical structure of Propofol to some free radical scavengers such as tocopherol and butylated hydroxene toluene (Ozkan et al., 2012). The other is that it is suitable for microbial growth (Tulgar et al., 2018). It is known that contamination may occur during anesthetic drug application because of production or preparation. On the contrary, evidence that anesthetics have antimicrobial activity is also involved. Despite all these, investigations focusing on other anesthetic drugs' antimicrobial effects and antioxidative capacities are scarce (Bostan et al., 2014).

This experimental study investigated the

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antimicrobial characters and antioxidative activities of nine different drugs (Fentanyl, Zolamid, Ultiva, Propofol-Lipuro 1%, Mivacron, Blok-L, Esmeron, Ketalar, and Pental Sodium) used as general anesthetics (Dantas et al., 2000; Johnson et al., 2008; Bostan et al., 2014; Ozkan et al., 2012). Rocuronium bromide, the significant component of Esmeron, has a role as a muscle relaxant (Büyükkoçak et al., 2011). Muscle relaxants are used during an operation as part of a general anesthetic. Esmeron can also be used in Intensive Care Units to keep your muscles relaxed. Fentanyl is used as a part of anesthesia to avoid pain after surgery or for other medical procedures (Kesici et al., 2020). Fentanyl, which has come to the fore with the death of the famous singer Prince in recent years, is a member of the powerful painkiller drug group called opioids. The narcotic analgesic, Fentanyl citrate, gives analgesia in low doses during short surgical procedures. The same narcotic agent in high doses is used as a respiratory/analgesic depressant in patients who need assisted ventilation. It is also used as part of the neuroleptanalgesia technique when combined with a neuroleptic drug. Fentanyl is also used to treat severe pain such as cancer or myocardial infarction. Remifentanil, which is the main component of Ultiva, is an opioid medication that is called a narcotic drug from time to time. Remifentanil is used to prevent or treat pain after surgery or for other medical procedures to cure the illness (Apan et al., 2007).

One of the most critical problems of our age is nosocomial infections. Most hospital-acquired infectious agents are bacteria that can maintain viability despite exposure to antiseptic/disinfectant substances in routine practices and bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* are frequently reported (Nouri et al., 2020). Long-term treatments, surgeries, and intensive care units are important critical control points to be considered in nosocomial infections. We preferred to select pathogens from bacteria, yeast, and fungi reported in previous studies by researchers.

On the other hand, *Saccharomyces cerevisiae* has been noted as a safe microorganism for nutritional use, regardless of its undesirable aspects. Nevertheless, this opinion is now changing due to the increased incidence of infections associated with these yeast strains. S. cerevisiae fungemia in humans occurs mainly in immunocompromised patients. Although uncommon, cases of fungemia by *S. cerevisiae* have also been reported in healthy hosts; thus, the presence of *S. cerevisiae* with inherent virulent potential cannot be excluded. A limited number of recent studies have been conducted on this subject (Pérez-Torrado & Querol, 2016; Fadhel et al., 2019). Therefore, we consider the antimicrobial activity of *S. cerevisiae* to be an essential part of our study.

Do some drugs used for anesthetic purposes in this study have any antimicrobial effect and reduce the oxidative stress in the cell due to the active ingredients? These questions are tried to be answered.

2. Material and Methods

2.1. Drugs

Commercially available anesthetic drugs were diluted with sterile physiological saline taking into account the final active substance concentrations. Investigated drugs and ingredients are listed in Table 1.

Table 1. Investigated drugs and their ingredients

Drugs	Ingredients
Fentanyl	*1mL Ampoule:* • Fentanyl Citrate 0.05 mg • NaCl • H_2O for injection
Zolamid	*In 5 mL Ampoule:* • Midazolam 5 mg • NaCl 45 mg • HCl • NaOH • H_2O for injection
Ultiva	*In 2 mg:* • Remifentanil hydrochloride 1mg/mL • Glycine 15 mg • HCl
Propofol-Lipuro 1% (BRAUN)	*In 1mL:* • Propofol 10 mg • Soybean oil 50 mg • Medium-chain triglycerides 50 mg • Sodium oleate 0.3 mg • The egg lecithin 12 mg • Glycerol 25 mg • H_2O for Injection 850 mg
Mivacron	*In 10 mg Ampoule for IV Injection:* • Mivacurium chloride 10 mg • HCl • H_2O for injection
Blok-L	*In 4 mg Containing lyophilized powder ampoule:* • Vecuronium bromide 4 mg • Citric acid anhydrous 8.3 mg • Dibasic sodium phosphate anhydrous 6.5 mg • Mannitol 24.5 mg • H_2O for injection 1mL
Esmeron	*In 50 mg/5mL vial containing a solution for injection:* • Rocuronium bromide • Sodium acetate • NaCl • Acetic acid • H_2O for injection • 1.72 mg sodium (in 1mL)
Ketalar	*In 500 mg injectable flacon:* • Ketamine hydrochloride 500 mg • Benzethonium chloride • H_2O for injection
Pental Sodyum	*In 0.5 mg Pental sodium injection flacon*: • Thiopental sodium 0.5 g

2.2. Microorganisms and culture media

Antimicrobial activities of anesthetics were assayed against Gram negative bacteria (*Escherichia coli* ATCC 25922™, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 13883™, *Proteus vulgaris* NRRL B-123, *Pseudomonas aeruginosa* NRRL B-2679, *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610™), Gram-positive bacteria (*Staphylococcus aureus* subsp. *aureus* CRM-6538™, *Micrococcus luteus* NRRL B-1018, *Bacillus subtilis* NRRL B-209), two yeasts (*Candida albicans* ATCC 10231™, *Saccharomyces cerevisiae* ATCC 9763)), and a fungus (*Aspergillus brasiliensis* ATCC 9642™). All bacterial strains were grown in Mueller Hinton Agar (MHA; Merck) for 24 h at 37°C and yeast-fungal strains were grown in Sabouraud Dextrose Agar (SDA; Difco) at 30°C 48 h. Pathogen bacteria concentrations were adjusted to 10^7-10^8 cells/mL and the concentration of yeast suspensions to 10^6 cells/mL and the concentration of fungal suspensions to 10^4.

2.3. Antimicrobial activity tests

Antimicrobial activity of the local anesthetics was investigated by The Kirby-Bauer disc diffusion method, the broth microdilution minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods (Hudzicki, 2009; Clinical and Laboratory Standards Institute, 2018; 2021).

For The Kirby-Bauer disc diffusion method, 0.5 McFarland bacterial and 1.0 McFarland fungal standard suspensions were prepared. Test tubes containing sterile ringer solution containing glass beads were used to form spore suspensions for fungal pathogens such as *Aspergillus brasiliensis*. The spores, scraped under aseptic conditions, were transferred to these test tubes, vortexed for about 1 minute to separate the spores from the hyphae, and then turbidity was checked immediately. This process was repeated until the desired concentration was obtained during the McFarland turbidity adjustment. Fresh pathogen microorganism suspension was placed over 20 mg/mL MHA media and dispersed. Then, 6 mm diameter sterile blank discs (Oxoid) were placed on MHA to load 50 µl of each drug solution. After the appropriate incubation temperature and time for each pathogen mentioned above, the inhibition zone diameters were determined. Inhibition zones of the different organisms by different samples were measured by the digital caliper to estimate the potency of antibacterial and antifungal substances and tabulated. The study was conducted in three replicates. The obtained results were the mean of three measurements.

The minimum inhibition concentration (MIC) was determined following the liquid dilution method according to the Clinical and Laboratory Standards Institute standard procedures (CLSI, M7, 2018; CLSI, M100, 2021), observed for antimicrobial screening using 96-well plates (Corning). Dilution ratios of 1, 0.75, 0.5, and 0.25 of drugs were used for MIC. Dilutions of anesthetic drugs were performed with sterile saline. Positive control (growth medium with only bacteria/yeast/fungus), sterility control (only growth medium), and standard antibiotic (Gentamicin 30 µg) were used for each test. All assays were performed in triplicate.

In addition, the minimum bactericidal/fungicide concentration (MBC/MFC) was determined. To determine the MBC/MFC value, 50 µL of the suspension in each well that did not show any visible growth (viability) in the MIC test after 24 hours was inoculated onto nutrient agar/SDA medium. To observe the colony growth, bacterial plates were incubated at 37°C for 24 hours and fungal plates for 48 hours at 30°C. The colonies that developed on the medium were counted. Petri dishes with less than 300 colonies were evaluated (Andrews, 2001).

2.4. Antioxidative tests

2.4.1. DPPH free radical scavenging activity

As the standard radical, the scavenging activity of the DPPH free radical was assayed using 2,2 diphenyl-1-picrylhydrazyl (DPPH). 0.1 mM solution of DPPH in methanol was prepared and 0.75 mL of this solution was added to 0.75 mL of each anesthetic sample at different concentrations. After incubation in the dark for 30 min, absorbance was measured at 517 nm. The percentage scavenging activity values were calculated as follows:

DPPH scavenging effect (%) = $((A_{cont}-A_{test})/A_{cont}) \times 100$

where A_{cont} was the absorbance of the control reaction (the solvent used to dilute the drugs + DPPH solution) and Atest was the absorbance in the presence of the drugs. Values of SC_{50}, the extract concentration scavenging half of the radicals, were calculated from the graph of extract concentrations against the scavenging ratios (Blois 1958).

2.4.2. OH radical scavenging activity

Hydroxyl radical scavenging activities of the investigated drugs were screened according to the deoxyribose method modified by Hagerman et al. (1998). Reactions were performed in 10 mM phosphate buffer (pH 7.4) containing deoxyribose (2.8 mM), H_2O_2 (2.8 mM), $FeCl_3$ (25 µM), EDTA (100 µM), and the drug. The reactions were initiated with the addition of ascorbic acid (100 µM final concentration) and the mixtures were incubated at 37°C for one h. After adding thiobarbituric acid (TBA, 1%), and trichloroacetic acid (TCA, 2.8%), the resultant mixtures were incubated in a water bath for 20 min to yield color formation. Mixtures were transferred to n-butanol after cooling, and the absorbance of each tube was measured against n-butanol at 532 nm. The reaction mixture did not contain a sample that was used as a blank. Scavenging activity for hydroxyl radical was calculated using the equation for DPPH scavenging activity. SC_{50} values were also calculated for all samples followed in the DPPH test.

2.5. Statistical analysis

The results were presented as mean and standard deviations. The data were analyzed on SPSS PASW Statistic 18.

3. Results

3.1. Antimicrobial activity

The mean diameter of inhibition zones related to the antimicrobial activity of investigated drugs is presented in Table 2. The Kirby-Bauer disk diffusion results of the anesthetic drugs used in the study were analyzed using the SPSS package program. Accordingly, it was determined that the disc diffusion results did not show normal distribution and nonparametric tests were started. Disk diffusion results of the anesthetic drug groups were analyzed with the Kruskal-Walllis test and a statistically significant difference was reached between at least two of the anesthetic drugs compared ($X^2(7, 108) = 19.011$ $p=0.008$). Two or more independent samples tests, one of the nonparametric tests, were used to determine which anesthetic drug/drugs caused the difference. According to the results of the analysis, a statistical difference was found between esmeron-pental sodium, esmeron-fentanyl, esmeron-zolamide, esmeron-mivacron, ketalar-pental sodium, ketalar-fentanyl, and ketalar-mivacron ($p < 0.05$).

Propofol, Mivacron, Esmeron, Fentanyl, and Blok-L showed only antibacterial effects but Ketalar, Zolamid, Ultiva, and Pental Sodium had both antibacterial and

antifungal activity. Ketalar only showed antimicrobial activity at varying degrees on all the tested pathogens among the tested drugs. All anesthetic drugs showed different inhibition zone diameters on *M. luteus,* but the most effective was Mivacron (38.26 mm/50 µL). The highest microbial activity was observed on *P. vulgaris* in the presence of Zolamide. Except for Zolamide, the most effective drug was Ketalar (Fig. 1).

On the other hand, except for *P. vulgaris, M. luteus* was the most affected microorganism from drugs. According to the numerical results, *E. coli* and *C. albicans* were the most resistant bacteria and yeast, respectively.

Table 2. Mean diameter (mm) of inhibition zones by anesthetics drug samples as a result of antimicrobial activity according to the Kirby-Bauer disc diffusion method

DRUGS (Concentration of drug solution mg/mL)	*BS*	*ML*	*SA*	*EC*	*KP*	*PA*	*PV*	*YE*	*AB*	*CA*	*SC*
Propofol (10 mg/mL)	10.1±0.03	12.18±0.13	-	-	-	-	-	-	-	-	-
Mivacron (2 mg/mL)	-	38.26±0.08	15.6±0.47	-	-	-	-	13.28±0.13	-	-	-
Ketalar (50 mg/mL)	15±0.1	11.74±0.08	20.14±0.04	7.99±0.1	14.29±0.06	27.73±0.08	12.02±0.51	22.86±0.04	10.35±0.38	8.35±0.03	7.5±0.04
Esmeron (10 mg/mL)	10.95±0.1	10.42±0.12	-	-	-	-	13.28±0.26	9.9±0.35	-	-	-
Zolamid (1 mg/mL)	19.71±0.08	15.16±0.25	-	-	7.14±0.14	7.33±0.2	8.5±1.16	-	-	-	8.54±0.27
Fentanyl (50 mg/mL)	10.22±0.04	18.87±0.47	29.64±0.34	-	-	-	-	-	-	-	-
Blok L (1 mg/mL)	-	21.81±0.65	-	-	-	-	-	-	-	-	-
Ultiva (0.5 mg/mL)	-	7.5±1.1	-	-	-	-	-	-	8.9±0.15	-	-
PentalSodium (0.05 mg/mL)	12.05±0.4	26.86±0.48	15.48±0.27	15.26±0.33	17.37±0.22	-	-	-	8.7±0.14	-	9.2±0.2
Gentamicin (30 µg)	42.79±0.08	51.02±0.25	42.13±0.35	37.85±0.14	20.54±0.47	27.12±0.1	40.38±0.13	27.25±0.35	30.52±0.14	38.48±0.13	-

BS: Bacillus subtilis NRRL B-209; **ML**: Micrococcus luteus NRRL B-1018; **SA:** Staphylococcus aureus subsp. aureus CRM-6538™; **EC**: Escherichia coli ATCC 25922™; **KP**: Klebsiella pneumoniae subsp. pneumoniae ATCC 13883™; **PV**: Proteus vulgaris NRRL B-123; **PA**: Pseudomonas aeruginosa NRRL B-2679; **YE:** Yersinia enterocolitica subsp. enterocolitica ATCC 9610™; **AB:** Aspergillus brasiliensis ATCC 9642™; **CA**: Candida albicans ATCC 10231™; **SC**: Saccharomyces cerevisiae; ± standard deviation; - no inhibition zone.

Table 3. MIC and MBC/MFC values for anesthetic drugs

DRUGS (Concentration of drug solution mg/mL)	MIC and MBC/MFC values (mg/mL)	*BS*	*ML*	*SA*	*EC*	*KP*	*PA*	*PV*	*YE*	*AB*	*CA*	*SC*
Propofol (10 mg/mL)	MIC	5±0	5±0	>>	>>	>>	>>	>>	>>	>>	>>	>>
Mivacron (2 mg/mL)	MBC/MFC	10±0	10±0	>>	>>	>>	>>	>>	>>	>>	>>	>>
Ketalar (50 mg/mL)	MIC	1±0.7	1±0.15	1±5.9	>>	>>	>>	>>	1±0.15	>>	>>	>>
Esmeron (10 mg/mL)	MBC/MFC	1±0.7	2±0.15	>>	>>	>>	>>	>>	2±0.15	>>	>>	>>
Zolamid (1 mg/mL)	MIC	25±5.8	12.5±2.7	12.5±3.4	25±5.8	12.5±0	12.5±3.4	12.5±5.8	12.5±2.7	12.5±2.7	12.5±3.4	25±0
Fentanyl (50 mg/mL)	MBC/MFC	50±5.8	50±2.7	37.5±3.4	25±2.7	12.5±0	37.5±3.4	50±5.8	25±2.7	25±2.7	37.5±3.4	25±0
Blok L (1 mg/mL)	MIC	5±0	5±0	>>	>>	>>	5±0	5±0	>>	>>	>>	>>
Ultiva (0.5 mg/mL)	MBC/MFC	10±0	10±0	>>	>>	>>	10±0	10±0	>>	>>	>>	>>
PentalSodium (0.05 mg/mL)	MIC	0.5±0	0.5±0	>>	>>	0.5±4	0.75±2.7	0.37±4	>>	>>	>>	0.75±2.7

MIC= Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration; MFC= Minimum Fungicidal Concentration; ± standart deviation; The symbol >> indicates that a MIC was not detected within the range tested. ***BS****: Bacillus subtilis* NRRL B-209; ***ML***: *Micrococcus luteus* NRRL B-1018; ***SA:*** *Staphylococcus aureus* subsp. *aureus* CRM-6538™; ***EC***: *Escherichia coli* ATCC 25922™; ***KP***: *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 13883™; ***PV***: *Proteus vulgaris* NRRL B-123; ***PA***: *Pseudomonas aeruginosa* NRRL B-2679; ***YE:*** *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610™; ***AB:*** *Aspergillus brasiliensis* ATCC 9642™; ***CA****: Candida albicans* ATCC 10231™; ***SC***: *Saccharomyces cerevisiae*

The lowest antimicrobial concentration that can inhibit a microorganism's visible growth after overnight incubation is defined as MIC (Andrews, 2001). MIC values of the investigated drugs on tested pathogens

were measured and listed in Table 3. Propofol only showed antibacterial activity against *B. subtilis* and *M. luteus*. Ketalar caused an effective inhibition on all tested organisms; although, the MIC values were not as low as other drugs (Table 3). Ketalar MIC values on *B. subtilis*, *E. coli*, and *S. cerevisiae* were 25 mg/mL and on the different test strains, they were 12.5 mg/mL. It is the only drug determined to be effective on *C. albicans* with a MIC value of 12.5 mg/mL ≤. Zolamid was the most effective drug on *P. aeruginosa* and *P. vulgaris*, with MIC values of 0.75 mg/mL and 0.37 mg/mL, respectively. It was already the most effective drug after pental sodium, considering the effect on other organisms. 25 mg/mL of Fentanyl showed an antibacterial effect only on Gram-positive bacteria among the tested species. Blok-L at 0.5 mg/mL showed an antibacterial effect only on *M. luteus* but no antimicrobial effect on other pathogens. Ultiva at 0.5 mg/mL showed an antibacterial effect on *M. luteus* and 1.5 mg/mL showed antifungal activity on *A. brasiliensis*. Pental sodium was effective on Gram-positive bacteria and *E. coli*, *K. pneumoniae* subsp. *pneumoniae* at 0.025 mg/mL concentration. Pental sodium showed an antifungal effect on *A. brasiliensis* and *S. cereviseae* but did not affect *C. albicans* at 0.025 mg/mL. It can be seen from Table 3 that the most effective drug was pental sodium. Because it had an inhibition effect on most of the tested pathogens and the effective concentration of it was the lowestSeveral dilutions were performed and the concentrations between 0.025 mg/mL and 50 mg/mL for every drug were experienced against all tested microorganisms to determine MBC/MFC Table 3). The highest dilution that yielded no bacterial/fungal growth (Fig. 1A-B) or less than 300 single colonies (Fig. 1C) on a solid medium was taken as MBC/MFC. The drug doses in the petri dishes, in which the number of single colonies over 300 were determined, were recorded as ineffective (Fig. 1D-F). MBC is the lowest antimicrobial concentration that prevents an organism's growth after subculturing it onto antibiotic-free media (Andrews, 2001). Table 3 also contains MBC/MFC values for tested microorganisms in the presence of the studied drugs. Pental sodium was the most effective drug on bacteria and fungus with the MBC/MFC value of 0.025≤. In the case of the other drugs, MBC/MFC values were generally higher than the MIC values, as expected.

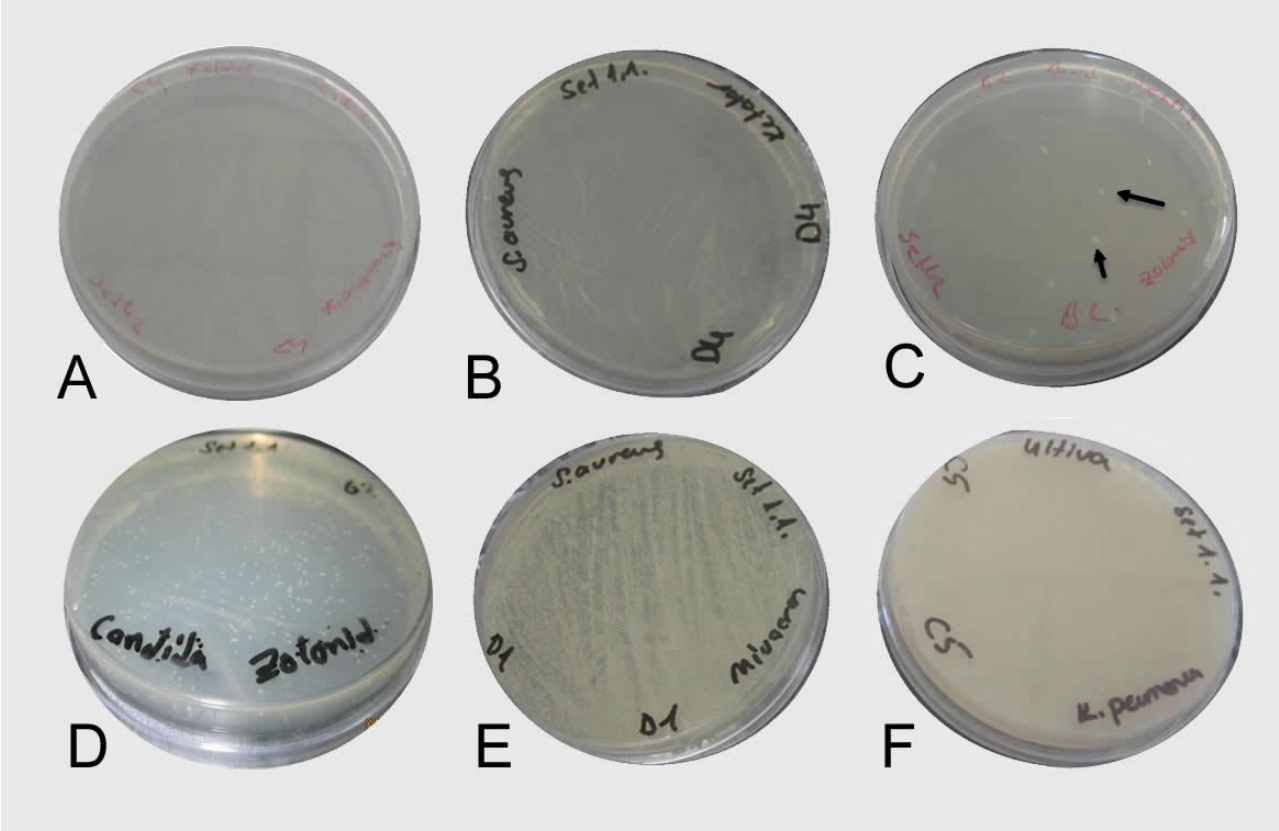

Figure 1. MBC/MFC plate samples. A: 25 mg/mL Ketalar effective on *K. pneumoniae* subsp. *pneumoniae* (no bacterial growth), B: 50 mg/mL Ketalar effective on *S. aureus* subsp. *aureus* (no bacterial growth), C: 0.5 mg/mL Zolamid effective on *B. subtilis* (single colonies are indicated by the blue arrow), D: 1 mg/mL Zolamid was not effective on *C. albicans* (single colony number above 300), E: 2 mg/mL Mivacron was not effective on *S. aureus* subsp. *aureus*, F: 0.5 mg/mL Ultiva was not effective *K. pneumonae* subsp. *pneumoniae* (good bacterial growth)

3.2. Antioxidative activity

SC_{50} values were calculated according to the scavenging powers of the drugs for DPPH and hydroxyl radicals. Table 4 shows the results for each drug. As expected, it can be easily seen from Table 4 that Propofol is the most effective drug in terms of its DPPH and hydroxyl radicals scavenging abilities. Among the tested drugs, Ultiva was almost as effective as Propofol. There was a conspicuous correlation (R^2=0.94) between the obtained SC_{50} values for DPPH and hydroxyl radicals, except for the calculated values for Fentanyl and Pental Sodium. The SC_{50} values obtained for DPPH free radical scavenging activities of Fentanyl (531.82 mg/mL) and Pental Sodium (534 mg/mL) were not also reflected in the graph because they were too high. The possibility of the drug contents

that has interfered with may be the reason for this case. Unfortunately, the results obtained for the antioxidative test are inconsistent with the results of the antimicrobial activity test. Adequate amounts of the tested drugs to scavenge equal amounts of radicals vary over a wide range. However, only a few are particularly important as antioxidants.

Table 4. Scavenging activities (SC_{50}, mg/mL) for DPPH and hydroxyl radicals of anesthetic drugs

DRUGS	Scavenging activities (SC_{50}, mg/mL)	
	DPPH	OH•
Propofol	0.0214±0.0012	0.089±0.003
Mivacron	31±0.57	0.58±0.02
Ketalar	186±1.93	4.29±1.05
Esmeron	26.594±0.257	1.58±0.07
Zolamid	4.34±0.02	0.2479±0.0089
Fentanyl	531.82±1.67	55.66±2.33
Blok L	5.49±0.03	0.12±0.01
Ultiva	2.36±0.05	0.12±0.15
Pental Sodium	534±2.33	4.47±0.37
Ascorbic acid	0.029±0.002	-
BHA	-	0.00378 ±0.0011
Gallic acid		0.514 ±0.104

DPPH: 2,2 diphenyl-1-picrylhydrazyl; BHA: Butylated hydroxy anisole; SC_{50}: the anesthesic drug concentration scavenging the half of the radicals; ± standart deviation

4. Discussion

Since the introduction of cocaine in 1884, anesthetic drugs have been used to withstand pain (Jaan et al., 2020). Oxidative stress may increase and pathogenic processes may develop due to complications that may occur during the preparation and use of these drugs. However, nowadays, we know that some local anesthetics also show antimicrobial and antioxidant effects. Findings and positive results of different anesthetics except Propofol (bupivacaine, lidocaine and ropivacaine, sevoflurane, dopamine) are available in the literature (Sıvacı et al., 2006; Erbaş et al., 2015; Bostan et al., 2014, Johnson et al., 2008) Yet the antioxidant property of Propofol is based on its similar chemical structure to known antioxidant substances. That's why, although there is no structural similarity, it is essential to identify and encourage the use of several anesthetic drugs with antioxidant and antimicrobial effects. For this reason, this study aimed to investigate the antimicrobial characters and antioxidative activities of nine different drugs (Fentanyl, Zolamid, Ultiva, Propofol-Lipuro 1%, Mivacron, Blok-L, Esmeron, Ketalar and Pental Sodium) used for general anesthetics.

Gargiulo et al. (2016) asked the anesthesiologists to apply anesthetic drugs other than propofol, which they use, through a sterile filter with 0.2 mm pore diameter, with the experimental setup they prepared, and then isolated *Bacillus*, *Staphylococcus* species and *M. luteus* from these filters and the remaining anesthetic drugs. Inspired by this report, common pathogens and less common opportunistic pathogens were included in the study. For this reason, our study is also the first in vitro study showing the direct antibacterial effect of anesthetic drugs on *M. luteus* obtained from the type culture collection.

Propofol is a 1% w/v aqueous emulsion, which contains 10% w/v soya bean oil (as a solubilizing agent), 1.2% egg lecithin (as an emulsifying agent), 2.5% glycerol (to make the preparation isotonic), sealed under nitrogen (Damitz, 2015). Previous studies have shown that Propofol supports the growth of many organisms like *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Apan et al., 2007; Altan et al., 2019). There are also published reports of systemic bacteremia due to Propofol (Zorrilla-Vaca et al., 2016; Cole et al., 2015). Our data were consistent with the findings of these studies. Propofol did not show any antibacterial effect on the aforementioned species but was effective only on *B. subtilis* and *M. luteus*. Rocuronium bromide is a relatively new non-depolarizing muscle relaxant widely used in procedures conducted under general anesthesia. Many anesthetic drugs like Rocuronium bromide are known to interact with antibiotics (Srivastava et al., 2014). Büyükkoçak et al. (2011) investigated the antibacterial effect of rocuronium bromide against six different bacteria (*S. epidermidis, S. aureus, Streptococcus pyogenes, P. aeruginosa, Enterococcus faecalis,* and *E. coli*) and no antibacterial effect could be reported. According to our study, Esmeron including Rocuronium bromide as an active ingredient was identified as an effective antibacterial drug against *B. subtilis, M. luteus, P. aeruginosa*, and *P. vulgaris*. This activity may be due to the synergistic effects of other components such as sodium acetate, sodium chloride, sodium, and acetic acid in drug ampoules. According to a study by Hanci et al. (2011), Mivacurium has antimicrobial properties on *P. aeruginosa, E. faecalis, S. aureus,* and *E. coli*. According to this study, when used regularly, Rocuronium, Atracurium, and Mivacurium do not increase a systemic antibacterial effect. However, their antibacterial effects may be sufficient to inhibit contamination during the preparation of the drugs. According to another study *E. coli* grows in Mivacurium, Atracurium, Pancuronium, Cisatracurium, and Vecuronium at room temperature; therefore, they may bring about nosocomial infection if contaminated (Memiş et al., 2009). Our results showed that *E. coli* grows in Fentanyl, Zolamid, Ultiva, Propofol, Mivacron, Blok-L, and Esmeron but not in Ketalar and Pental Sodium. Blok-L, including Vecuronium bromide, only inhibited the *M. luteus*' bacterial growth among the test strains.

According to the results of the present study, Gram-positive bacteria were more sensitive than Gram-negative ones to the tested drugs. Fentanyl is an effective bactericidal anesthetic drug against Gram-positive bacteria. This data is also supported by previous anesthetic solution and preservative studies (Dantas et al., 2000; Kesici et al., 2020). Only Ketalar showed antifungal activity against *C. albicans* and no other anesthetic drugs showed antifungal activity against *C. albicans*. In our opinion, the antimicrobial effects of all tested drugs, especially Ketalar, may be advantageous for inhibiting the spread of microbial contamination during the preparation of the infusion solutions.

Furthermore, tested drugs can scavenge free radicals and especially SC_{50} values for hydroxyl radicals were shallow and close to the value calculated for Propofol. Thus, it can be said that these anesthetic drugs' antioxidant properties depend not only on the chemical structure but also on the synergistic effect of all their components. This is seen for both standards tested for hydroxyl radical scavenging activity. While the value

obtained for BHA is very small, the value obtained for gallic acid is higher than most of the drugs. This reveals that drugs such as Blok-L, Ultiva and Zolamide are more effective in scavenging hydroxyl radicals than gallic acid. In addition, the DPPH radical scavenging efficiency of Propofol is even more effective than the standard tested ascorbic acid.

In conclusion, this study, which includes a broader list of drugs in addition to such studies carried out with certain anesthesia drugs in the literature, provides essential data and reminds us once again that aseptic conditions should be taken into consideration during the preparation and administration of anesthetic drugs. Authors recommend using ingredients with antimicrobial-antioxidant effects in the composition of newly developed anesthetic drugs.

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