RESEARCH ARTICLE

F.U. Med.J.Health.Sci.
2026; 40 (2): 132 - 136
http://www.fusabil.org

Investigation of the Effect of Bupivacaine on Lung Tissue in Rats with Experimental Renal Failure

Fadime TOSUN [1, a]
Elfide Gizem BAKIRHAN [2, b]

[1] Adıyaman University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Adıyaman, TÜRKİYE

[2] Adıyaman University, Faculty of Medicine, Department of Histology and Embryology, Adıyaman, TÜRKİYE

[a] ORCID: 0000-0003-4660-5497
[b] ORCID: 0000-0002-7401-2317

Objective: Although local anesthetics are widely used in many clinical applications, their metabolism and elimination processes can be affected in cases of renal failure, leading to changes in their pharmacokinetic properties. In such cases, the potential for systemic toxicity may increase, causing alterations not only in the heart and central nervous system but also in the lungs. This study aimed to histopathologically examine the effects of clinically used bupivacaine on lung tissue in rats with experimentally induced acute renal failure.

Materials and Methods: A total of 28 adult male Wistar-Albino rats were divided into four groups: the Control group received intraperitoneal 1 mL/kg saline; the Glycerol group received intramuscular 10 mL/kg glycerol; the Glycerol+LA group received intramuscular 10 mL/kg glycerol and intraperitoneal 4 mg/kg bupivacaine; the LA group received intraperitoneal 4 mg/kg bupivacaine. All animals were sacrificed 24 hours after a single-dose injection. After sacrifice, lung tissues were collected from all rats for histopathological analysis.

Results: Histopathological changes were observed in both the Glycerol and Glycerol+LA groups, with more pronounced alterations in the latter. In the Glycerol+LA group, significant increases were observed in epithelial degeneration compared with the LA and Control groups, alveolar septal thickening and vascular congestion.

Conclusion: Bupivacaine administered at clinical doses in renal failure may lead to histopathological changes in lung tissue.

Key Words: *Bupivacaine, lung damage, renal failure, glycerol*

Deneysel Böbrek Yetmezliği Oluşturulan Ratlarda Bupivakainin Akciğer Dokusu Üzerine Etkisinin İncelenmesi

Amaç: Lokal anestezikler birçok klinik uygulamada yaygın olarak kullanılmalarına rağmen, böbrek yetmezliğinde metabolizma ve eliminasyon süreçleri etkilenebilir, farmakokinetik özellikleri değişebilir. Bu durumda sistemik toksisite potansiyeli artabilir, kalp ve merkezi sinir sisteminin yanı sıra akciğerlerde de değişikliklere neden olabilir. Bu çalışmada deneysel akut böbrek yetmezliği oluşturulan ratlarda, klinik dozlarda kullanılan bupivakainin akciğer dokusuna etkilerinin histopatolojik olarak incelenmesi amaçlandı.

Gereç ve Yöntem: Çalışmada toplam 28 yetişkin erkek Wistar-Albino rat dört gruba ayrıldı. Kontrol grubuna intraperitoneal 1 mL/kg serum fizyolojik; Gliserol grubuna intramusküler 10 mL/kg gliserol; Gliserol+LA grubuna intramusküler 10 mL/kg gliserol+intraperitoneal 4 mg/kg bupivakain; LA grubuna intraperitoneal 4 mg/kg bupivakain verildi. Tek doz enjeksiyondan 24 saat sonra tüm hayvanlar sakrifiye edilmiştir. Sakrifikasyon sonrası tüm ratlarda histopatolojik analizler için akciğer dokusu alındı.

Bulgular: Gliserol+LA grubunda daha belirgin olmak üzere gliserol grubunda da histopatolojik değişiklikler görüldü. Gliserol+LA grubunda; hem LA hem de Kontrol grubuna göre epitelyal dejenerasyon, alveolar septal kalınlaşma, vasküler konjesyon bakımından istatistiksel olarak anlamlı bir artış olduğu gözlendi.

Sonuç: Böbrek yetmezliğinde klinik dozlarda uygulanan bupivakain akciğer dokusunda histopatolojik değişikliklere yol açabilir.

Anahtar Kelimeler: *Bupivakain, akciğer hasarı, böbrek yetmezliği, gliserol*

Received : 14.01.2025
Accepted : 12.02.2026

Correspondence

Fadime TOSUN
Adıyaman University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Adıyaman, TÜRKİYE

fadimetosun@gmail.com

Introduction

Local anesthetics are indispensable in modern medicine and are used in various areas of healthcare. Bupivacaine, a long-acting amide local anesthetic, is commonly preferred in regional anesthesia applications. Bupivacaine temporarily binds to sodium channels in neuronal cell membranes, blocking the propagation of nerve action potentials in the targeted area. This interaction results in sensory and motor blockade (1). The absorption rate and metabolism of local anesthetics are significantly influenced by factors such as the type, dose, and volume of the anesthetic, the injection site, and the patient's overall health status (2, 3). In patients with renal failure, the metabolism and elimination of local anesthetics may be affected (1). This condition can lead to pharmacokinetic changes that increase the risk of systemic toxicity, even with low doses (4, 5). The resulting toxicity can cause alterations not only in the heart and central nervous system but also in the lungs.

Glycerol, a substance that mimics rhabdomyolysis by inducing inflammatory cellular infiltration and acute cortical tubular necrosis, is one of the agents commonly used to induce experimental acute renal failure (6). The aim of this study was to histopathologically investigate the effects of clinically used bupivacaine on lung tissue in rats with experimentally induced acute renal failure.

Materials and Methods

Research and Publication Ethics: This study was conducted at the Animal Laboratory Facility of Adıyaman University after obtaining approval from the Adıyaman University Animal Studies Ethics Committee (Adıyaman-HADYEK: 30.06.2022- 2022/040).

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twenty-eight adult male Wistar-Albino rats, aged 4-6 months and weighing between 300-350 grams, were used in the study. All animals were fed a standard diet and provided with ad libitum access to water. Room temperature (22-25°C) and humidity (50-55%) were monitored daily. Lighting was provided using cool white fluorescent lamps with a 12-hour light-dark cycle (06:00-18:00). For anesthesia, rats were injected intramuscularly with ketamine hydrochloride (50 mg/kg Ketalar; Eczacıbaşı, Istanbul, Türkiye) and xylazine hydrochloride (20 mg/kg Rompun; Bayer Türk İlaç Ltd. Şti.) under the supervision of a veterinarian. The 28 rats were randomly divided into 4 groups, each consisting of 7 rats.

Control Group: Each rat was administered a single intraperitoneal injection of 1 mL physiological saline.

Glycerol Group: Each rat was administered a single 10 mL/kg glycerol intramuscularly.

Glycerol+ Local Anesthesia (Glycerol+LA) Group: Each rat received a single intramuscular injection of 10 mL/kg glycerol, followed by 4 mg/kg (1 mL) bupivacaine intraperitoneally.

Local Anesthesia (LA) Group: Each rat received a single intraperitoneal injection of 4 mg/kg (1 mL) bupivacaine.

Rats were not given water for 12 hours before the injection of 10 mL/kg glycerol (7). After 24 hours, all rats were sacrificed under anesthesia, and their tissues were collected for histopathological examination.

Histopathological Procedures: Lung tissues obtained at the end of the experiment were post-fixed with 10% formaldehyde for one week. Routine histological processing (alcohol, xylene, and paraffin series) was carried out using an automatic tissue processor (Leica TP1020, Nussloch, Germany). After tissue processing, the samples were embedded in paraffin blocks. Thin sections (7 µm) were cut from the paraffin blocks using a Thermo Shandon Finesse ME microtome (Thermo Fisher Scientific, Cheshire, UK). The sections were stained with the hematoxylin-eosin method for histopathological examination. The histopathological assessments were performed by photographing the tissue samples using a Carl Zeiss Axiocam ERc5 model digital camera attachment mounted on a microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Histopathological Scoring: Histological scoring for lung tissue was performed using a semi-quantitative method (8). The following scoring system was applied: 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The findings of epithelial degeneration, cellular infiltration, alveolar septal thickening, and vascular congestion were evaluated. Each parameter was assessed by an independent, blinded histologist with expertise in the field.

Statistical Analysis: The sample size was determined through power analysis using the G*Power software, which calculated that a minimum of 7 rats per group would be required to achieve a power of 0.80 and a Type I error rate of 0.05, with an effect size of 1.2 for histological parameters. Thus, 28 rats were divided into 4 groups, each consisting of 7 rats. Statistical analyses were performed using SPSS software (SPSS version 21.0; SPSS Inc., Chicago, IL, USA). The data are presented as mean ± standard deviation. Normality (Shapiro-Wilk) and homogeneity tests confirmed that all groups followed a normal distribution. Independent-Samples Kruskal-Wallis H test was used to assess intergroup differences. *p*-values <0.05 were considered statistically significant.

Results

Histopathological Results: The lung tissue sections stained with hematoxylin-eosin from all groups were examined (Figure 1). The lung tissues from the Control group exhibited a healthy appearance. The alveoli, bronchioles, and vascular structures appeared intact, and all structures were clearly distinguishable (Figures A, E). In the Glycerol group, the lung tissue showed areas of thickening in the alveolar walls, along with minimal epithelial degeneration in the bronchiolar walls. Additionally, a slight infiltration of leukocytes and vascular congestion were observed in the peribronchiolar-perivascular regions (Figures B, F). In the Glycerol+LA group, significant leukocyte infiltration was detected in the peribronchiolar-perivascular regions and alveolar parenchyma. The bronchiolar walls showed degenerating epithelial cells, and intense vascular congestion was noted. Furthermore, prominent alveolar septal thickening and areas of collapse were observed (Figures C, G). In the LA group, the lung tissue sections closely resembled normal lung architecture. However, mild vascular congestion and alveolar septal thickening were observed in some areas. The alveolar, bronchiolar, and vascular structures appeared healthy, and all layers were clearly distinguishable (Figures D, H).

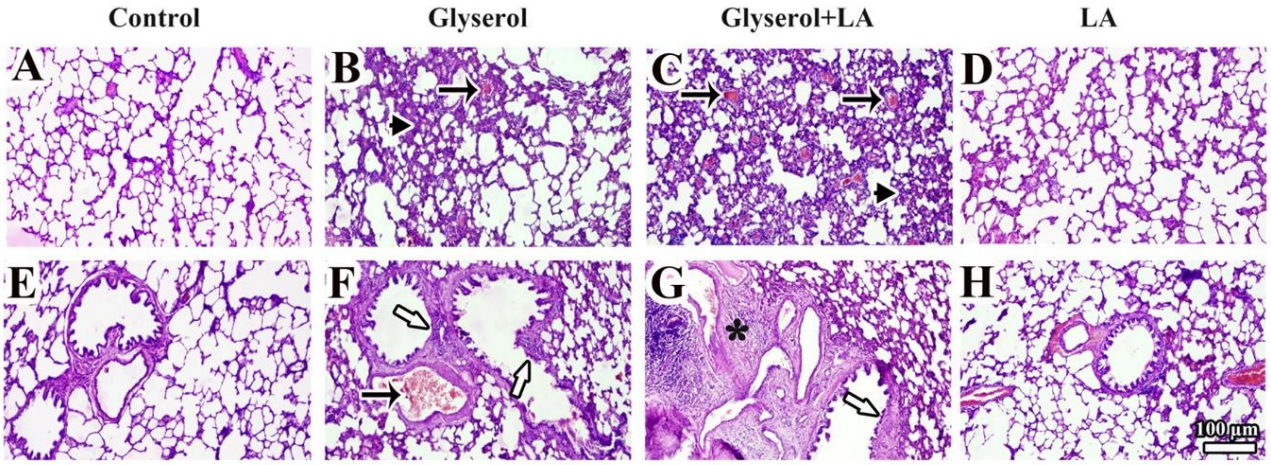

Figure 1. Representative light microscopic images of lung tissue sections from all experimental groups stained with Hematoxylin and Eosin. Two representative images are shown for each group, and all micrographs were obtained from comparable bronchiolar regions to ensure anatomical consistency. Panels A and E represent the Control group and show normal bronchiolar, vascular, and alveolar architecture. Panels B and F represent the Glycerol group and demonstrate minimal epithelial degeneration in the bronchiolar wall (white arrow). Panels C and G represent the Glycerol+LA group and show prominent epithelial degeneration in the bronchiolar wall (white arrow). Panels D and H represent the LA group. All images were obtained from similar bronchiolar regions to allow appropriate intergroup comparison.

Table 1. Histopathological scoring results of lung tissue samples

Histopathological changes	Control	Glycerol	Glycerol+LA	LA	Statistic
Epithelial degeneration	0.2±0.447*	0.6±0.547	1.6±0.894	0.4±0.547*	X^2:8.681 *p*: 0.034
Cellular infiltration	0.2±0.447	0.80±1.095	2.0±1.000	0.6±0.894	X^2:7.746 *p*: 0.052
Alveolar septal thickening	0.1±0.223*	0.6±0.547	1.8±1.095	0.4±0.547*	X^2:9.995 *p*: 0.019
Vascular congestion	0.3±0.670*	1.2±1.303	2.2±0.836	0.4±0.547*	X^2:8.987 *p*: 0.029

* It indicates a significant difference between the Glycerol+LA group and the Control and LA groups ($p<0.05$).

X^2: Independent-Samples Kruskal-Wallis H Test.

Histopathological Scoring Findings: The histological scoring results evaluated epithelial degeneration in the bronchiolar walls, cellular infiltration, alveolar septal thickening, and vascular congestion.

When epithelial degeneration in the bronchiolar walls was compared among the groups, a statistically significant increase was observed in the Glycerol+LA group compared with the Control group ($p=0.006$). Similarly, epithelial degeneration scores were significantly higher in the Glycerol+LA group than in the LA group ($p=0.025$, Table 1). No other pairwise comparisons showed a statistically significant difference ($p>0.05$).

No statistically significant difference was observed among the groups regarding cellular infiltration ($p>0.05$, Table 1).

For alveolar septal thickening, the Glycerol+LA group demonstrated significantly higher scores than the Control group ($p=0.002$). Likewise, alveolar septal thickening was significantly increased in the Glycerol+LA group compared with the LA group ($p=0.025$). No statistically significant differences were detected between the Glycerol group and the other groups ($p>0.05$, Table 1).

Regarding vascular congestion, the Glycerol+LA group showed significantly greater vascular congestion than both the Control group ($p=0.007$) and the LA group ($p=0.014$). No significant difference was found between the Glycerol group and the Control group or between the Glycerol group and the Glycerol+LA group ($p>0.05$, Table 1).

Overall, the Glycerol+LA group exhibited the most pronounced histopathological alterations, characterized by increased bronchiolar epithelial degeneration, alveolar septal thickening, and vascular congestion compared with the Control and LA groups, whereas cellular infiltration did not differ significantly among groups.

Discussion

Local anesthetics are frequently preferred agents due to their properties and are widely used in pain management for various surgical procedures. For these anesthetics to be effective, the injection needs to be made close to the target nerve structure. After the injection, a large portion of local anesthetics is absorbed into the systemic circulation and distributed throughout the body. According to research, only 2-3% of the administered dose reaches the target nerve, while more than 90% is absorbed by the systemic circulation within 30 minutes after the injection (9). Once in the systemic circulation, the free fraction of the drug, which binds to plasma proteins, significantly decreases. This is clinically important because only the free or unbound fraction has the potential to exert a bioactive effect (9). Organ effects are generally related to the transition of local anesthetics into the systemic circulation. Therefore, the aim of this study was to investigate the histopathological effects of local anesthetics on distant organs, specifically the lungs, in rats with induced renal failure.

Lung damage is a condition that can be triggered by various factors and can have clinically severe consequences. Diagnosis and grading of this type of damage are possible through histopathological examinations (10). In lung injury, findings such as inflammation, alveolar structural abnormalities, fibrosis, epithelial cell changes, and vascular alterations are key indicators (11). Therefore, this study focused on changes in the lungs, including epithelial degeneration, cellular infiltration, alveolar septal thickening, and vascular congestion, which reflect cellular damage in the lung tissue.

Renal failure is a clinical condition characterized by the loss of renal function, and due to its systemic effects, it can lead to multiple organ dysfunction (12). The effects of acute kidney injury on the lungs have been supported by data from both patient and animal models, confirming that this condition negatively impacts lung function (13, 14). Additionally, renal failure leads to the increased production and release of inflammatory mediators, which induce a strong inflammatory response that also plays a significant role in lung injury (15). Changes observed in the lung histopathology during renal failure include alveolar damage, interstitial edema, inflammatory and vascular alterations (14). In our study, the Glycerol group, which had renal failure induced by glycerol, exhibited thickening of the alveolar walls in some areas, mild epithelial degeneration in the bronchiolar walls, and slight leukocyte infiltration and vascular congestion in the peribronchiolar and perivascular areas. Consistent with the literature, our study suggests that lung damage can occur in renal failure, representing a significant consideration for clinical practice.

The use of local anesthetics in patients with renal failure should be approached with caution (4). In these patients, the decreased clearance of local anesthetics and their increased absorption lead to significant elevations in plasma concentrations (16). Furthermore, since the kidneys cannot excrete acid load, metabolic acidosis is frequently observed (17, 18). As a result, the binding of local anesthetics to plasma proteins decreases, and the amount of free local anesthetic in the blood increases. This is particularly common with bupivacaine (19). All these pharmacological considerations suggest that the risk of systemic toxicity may increase in renal failure. If a significant amount of local anesthetic accumulates in the systemic circulation, its distribution to organs is determined by the vascular perfusion of those organs. This explains why well-perfused organs, such as the brain, heart, lungs, liver, and kidneys, are exposed to the highest concentrations of un-metabolized local anesthetic (9, 20). Indeed, studies have shown that high levels of bupivacaine in the lungs are associated with increased blood perfusion (20). Our study, consistent with the literature, found significant degenerated epithelial cells, intense vascular congestion, marked alveolar septal thickening, and collapsed alveolar areas in the Glycerol+LA group, suggesting that bupivacaine passed in high amounts to the lungs, exacerbating lung damage.

Although local anesthetics have anti-inflammatory properties that can help reduce lung inflammation (21-24), their systemic toxicity poses a risk to lung health. A review of the literature reveals limited information on the effects of local anesthetics on cellular and molecular changes in the lungs. Barroso-Moguel et al. demonstrated that intraperitoneal administration of cocaine, inducing toxicity, caused severe lung damage with notable thickening of alveolar septa, interstitial hemorrhages, progressive thrombosis, and diffuse fibrosis of reticular-elastic fibers (25). In line with the literature, our study found a statistically significant increase in epithelial degeneration, alveolar septal thickening, and vascular congestion in the Glycerol+LA group.

Despite the clear histopathological evidence obtained in the present study, some limitations should be acknowledged. Although our findings demonstrate significant structural alterations in lung tissue, the underlying molecular and cellular mechanisms could not be explored in detail. The absence of molecular and immunohistochemical analyses, such as the evaluation of inflammatory cytokines, oxidative stress markers, apoptosis pathways, or fibrosis-related signaling molecules, limits the mechanistic interpretation of the observed tissue injury. In our research group, renal histopathology and renal biochemical parameters (such as serum creatinine and BUN) were evaluated in a separate, previously conducted study using the same glycerol-induced acute renal failure model (26). Therefore, the present study should be regarded primarily as a histopathological proof-of-concept investigation demonstrating that bupivacaine exacerbates lung injury in the presence of experimental renal failure. Future studies incorporating molecular, immunohistochemical, and biochemical approaches (e.g., TNF-α, IL-6, TGF-β, oxidative stress and apoptosis markers) are warranted to clarify the precise pathophysiological pathways involved. In addition, the lack of pulmonary functional assessments and renal

biochemical parameters represents another limitation of the present study.

In conclusion, this study demonstrates that in the presence of renal failure, the pharmacokinetic properties of local anesthetics can change, leading to an increased transition of these substances into systemic circulation and potentially increasing the risk of toxic effects. Additionally, as the lungs are organs with high perfusion rates, toxic effects in this area may become more pronounced. Therefore, we suggest that local anesthetics be used with caution in patients with renal failure. Considering this in clinical practice will enhance patient safety and contribute to the prevention of potential side effects.

References

1. Uppal NN, Jhaveri M, Hong S, et al. Local anesthetics for the nephrologist. Clinical Kidney Journal 2022; 15: 186-193.
2. Gitman M, Fettiplace MR, Weinberg GL, Neal JM, Barrington MJ. Local anesthetic systemic toxicity: A narrative literature review and clinical update on prevention, diagnosis, and management. Plast Reconstr Surg 2019; 144: 783-795.
3. De Valle MK, Adkison M, Maredia R, Stevenson C, Murugan S. Local anesthetic systemic toxicity in pregnancy: A retrospective cohort analysis. Cureus 2024; 16: e69215.
4. Ishida T, Tanaka S, Sakamoto A, Hirabayashi T, Kawamata M. Plasma ropivacaine concentration after TAP block in a patient with cardiac and renal failure. Local Reg Anesth 2018; 11: 57-60.
5. Gheisari R, Saatchi M, Estakhri F, et al. Effect of local anesthetics on renal function: An animal study in Iran. Dent Res J (Isfahan) 2023; 20: 106.
6. Ahmed RF, Okasha AM, Hafiz SHI, et al. Guanosine protects against glycerol-induced acute kidney injury via up-regulation of the klotho gene. Iran J Basic Med Sci 2022; 25: 399-404.
7. Li YF, Xu BY, An R, et al. Protective effect of anisodamine in rats with glycerol-induced acute kidney injury. BMC Nephrol 2019; 20: 223.
8. Tüfekci KK, Bakirhan EG, Terzi F. A maternal high-fat diet causes anxiety-related behaviors by altering neuropeptide Y1 receptor and hippocampal volumes in rat offspring: The potential effect of N-acetylcysteine. Mol Neurobiol 2023; 60: 1499-1514.
9. Dillane D, Finucane BT. Local anesthetic systemic toxicity. Can J Anaesth 2010; 57: 368-380.
10. Hsieh PC, Wu YK, Yang MC, et al. Deciphering the role of damage-associated molecular patterns and inflammatory responses in acute lung injury. Life Sci 2022; 305: 120782.
11. Matute-Bello G, Downey G, Moore BB, et al. Acute lung injury in animals study group. An Official American Thoracic Society workshop report: Features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol 2011; 44: 725-738.
12. Anees M, Akbar H, Ibrahim M, Saeed MS, Ismail M. Pulmonary functions and factors affecting them in patients with chronic kidney disease. J Coll Physicians Surg Pak 2020; 30: 1082-1085.
13. Domenech P, Perez T, Saldarini A, Uad P, Musso CG. Kidney-lung pathophysiological crosstalk: Its characteristics and importance. Int Urol Nephrol 2017; 49: 1211-1215.
14. Faubel S, Edelstein CL. Mechanisms and mediators of lung injury after acute kidney injury. Nat Rev Nephrol 2016; 12: 48-60.
15. Bollenbecker S, Czaya B, Gutiérrez OM, Krick S. Lung-kidney interactions and their role in chronic kidney disease-associated pulmonary diseases. Am J Physiol Lung Cell Mol Physiol 2022; 322: L625-L640.
16. Park KK, Sharon VR. A review of local anesthetics: Minimizing risk and side effects in cutaneous surgery. Dermatol Surg 2017; 43: 173-187.
17. Kuhn C, Mohebbi N, Ritter A. Metabolic acidosis in chronic kidney disease: Mere consequence or also culprit? Pflugers Arch 2024; 476: 579-592.
18. Raphael KL. Metabolic Acidosis in CKD: Core curriculum 2019. Am J Kidney Dis 2019; 74: 263-275.
19. Gurajala I, Nikhar SA, Jayaram K, Gopinath R. Regional anesthesia is safe and effective for lower limb orthopedic surgery in patient with renal tubular acidosis and hypokalemia. J Anaesthesiol Clin Pharmacol 2018; 34: 117-119.
20. Shi K, Xia Y, Wang Q, et al. The effect of lipid emulsion on pharmacokinetics and tissue distribution of bupivacaine in rats. Anesth Analg 2013; 116: 804-809.
21. Krishnamoorthy V, Chung L. Bench-to-bedside: The use of local anesthetics to attenuate inflammation in acute respiratory distress syndrome. Int J Crit Illn Inj Sci 2014; 4: 98-100.
22. Stawicki SP, Swaroop M, Galwankar SC, Papadimos TJ. What's new in critical illness and injury science? State of the art in management of ARDS. Int J Crit Illn Inj Sci 2014; 4: 95-97.
23. Garutti I, Rancan L, Simón C, et al. Intravenous lidocaine decreases tumor necrosis factor alpha expression both locally and systemically in pigs undergoing lung resection surgery. Anesth Analg 2014; 119: 815-828.
24. Weinschenk S, Weiss C, Benrath J, et al. Anti-inflammatory characteristics of local anesthetics: Inhibition of TNF-α secretion of lipopolysaccharide-stimulated leucocytes in human blood samples. Int J Mol Sci 2022; 23: 3283.
25. Barroso-Moguel R, Villeda-Hernández J, Méndez-Armenta M, Santamaría A, Galván-Arzate S. Alveolar lesions induced by systemic administration of cocaine to rats. Toxicol Lett 1999; 110: 113-118.
26. Turk A, Metin TO, Duran M. Evaluation of the effect of bupivacaine on the heart tissue in rats with glycerol-induced acute kidney injury. Pathol Res Pract 2025; 275: 156193.