J CLIN PRACT RES

Original Article

Official Journal of Erciyes University Faculty of Medicine

DOI: 10.14744/cpr.2023.29392
J Clin Pract Res 2023;45(5):511–6

Rat Model: Intrathecal Lipid for Total Spinal Block-Induced Hemodynamic Instability

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ABSTRACT

Objective: This study aimed to assess the potential of intrathecal administration of lipid emulsions as a targeted therapeutic strategy to mitigate the hemodynamic consequences of high or total spinal anesthesia induced by bupivacaine in a rat model.

Materials and Methods: After receiving approval from Koç University's Local Ethics Board of Animal Experiments, 14 adult female Wistar Albino rats were randomized into two groups: Lipid and Saline. After inducing a high spinal block, each group was treated with either intrathecal lipid emulsions or isotonic saline solutions, respectively. Hemodynamic parameters such as systolic, diastolic, and mean arterial blood pressures, as well as heart rate, were continuously monitored. A histological analysis was conducted upon completion of the study.

Results: The Lipid group exhibited a significant increase in mean systolic (p=0.002), diastolic (p=0.002), and arterial pressures (p=0.002) compared to the Saline group. The mean pulse rate also differed significantly between the groups (p=0.009). Notably, while all rats in the Saline group succumbed, only two fatalities occurred in the Lipid group, with the other five rats surviving (p=0.021).

Conclusion: This study suggests that intrathecal administration of lipid emulsions can serve as a rapid and effective intervention to counteract the hypotension and bradycardia induced by high or total spinal anesthesia with bupivacaine in rats. While these findings are promising, further experimentation and clinical trials are required to confirm this method's potential, focusing on its long-term safety, efficacy, and the establishment of an optimal administration protoco.

Keywords: Intrathecal lipid emulsion, bupivacaine, spinal anesthesia, hypotension, rat model, bradycardia.

*Dr. Kamil Darçın and Dr. Muhammet Ahmet Karakaya have contributed similarly to the study and share the position as first author. Both are equal first authors.

Cite this article as:
Darçın K, Karakaya MA, Yenigün Y, Kulaç İ, Çetin S, Ünlükaplan A, Erçelen Ö. Rat Model: Intrathecal Lipid for Total Spinal Block-Induced Hemodynamic Instability. J Clin Pract Res 2023; 45(5): 511–6.

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Submitted: 18.08.2023
Revised: 14.09.2023
Accepted: 22.09.2023
Available Online: 27.09.2023

Erciyes University Faculty of Medicine Publications - Available online at www.jcpres.com

INTRODUCTION

High or total spinal anesthesia is a rare yet profoundly serious complication associated with epidural analgesia. This complication can arise from the inadvertent administration of excessive local anesthetic into the intrathecal space, possibly due to accidental intrathecal or subdural insertion of the epidural catheter or the catheter's migration after proper placement. When this complication occurs, patients may experience severe consequences such as respiratory failure and cardiovascular collapse stemming from sympathectomy.[1–4]

Bupivacaine is a widely used local anesthetic in various clinical settings, including nerve blocks, epidural, and intrathecal anesthesia. Despite its widespread use as a potent pain management agent, bupivacaine carries significant risks. It is reported to be the most myotoxic local anesthetic available and is associated with notable cardiotoxic and neurotoxic effects.[5–7]

Existing medical literature has explored intravenous lipid emulsions and cerebrospinal fluid (CSF) exchange as potential strategies to counteract the undesirable effects of a high or total spinal block. Although the symptoms of sympathectomy manifest rapidly, current treatment approaches tend to be limited in their application and often exhibit a delayed onset of action. To date, there is no fast-acting, targeted therapeutic strategy for this condition.[8,9] Notably, our literature review did not find any documented use of intrathecal lipid emulsion as a treatment method.

With this study, we aim to investigate and demonstrate that administering lipid emulsion directly into the intrathecal space is an effective and promising method to counteract the hemodynamic consequences of a high or total spinal block. This block is experimentally induced using high-dose intrathecal bupivacaine in a rat model.

MATERIALS AND METHODS

This study was conducted with approval from Koç University's Local Ethics Board of Animal Experiments under protocol number 2017.HADYEK.001. All procedures strictly adhered to both the protocol approved by the Institutional Animal Care and Use Committee and the Guide for the Care and Use of Laboratory Animals.

Animal Preparation and Monitoring

At the Koç University Experimental Animals Center, 14 adult female Wistar Albino rats – weighing between 300 and 350 grams and kept at a consistent ambient temperature with a 12-hour day/night cycle – were equally divided into two groups: Lipid and Saline. These groups were designated to receive either intrathecal lipid or intrathecal isotonic 0.9% saline solutions as treatment after inducing a high spinal block.

Initially, the rats were housed in a chamber and positioned on heating pads throughout the experiment to maintain stable body temperatures. Anesthesia was induced using an isoflurane-O2 mixture. Once immobilized, the rats were removed from the chamber and placed in supine positions. Spontaneous breathing was preserved using a face mask connected to an anesthesia machine.

Hemodynamic Monitoring

After skin antisepsis, an incision was made in the inguinal region to access the femoral artery. Femoral artery catheterization was performed under a microscope using a 26-gauge catheter (BD Neoflon, Helsingborg, Sweden) to facilitate continuous blood pressure monitoring. This catheter was securely affixed with 2/0 silk sutures. The arterial catheter was then connected to a pressure transducer (Sensor Plus, Pressure Transducer, SCW Medicath Ltd, Guangzhou, China) using standard pressure tubing. Calibration ensued, and the transducer was zeroed at the mid-axillary level. The natural frequency and damping coefficient of each system were identified using the flush method, which accounts for errors from damping and frequency alterations. Comprehensive monitoring, including invasive blood pressure and heart rate, was performed (GE Health Care, B40 Patient Monitor, St. Louis, USA). Only subjects with blood pressures and heart rates within the normal range were included in the study.

Intrathecal Procedures

Under face mask ventilation with 100% oxygen and 2% isoflurane, rats were positioned prone and secured using adhesive tape on all four extremities. After shaving the lumbar region, the L3-L4 intervertebral space was identified. A 29-gauge needle (Anhul Hongyu Wuzhou MM Co. Ltd., Anhui, China) was introduced into this spinal interval, and the correct intrathecal positioning was verified by the tail-flick sign. Subsequently, a 0.5 µl/g body weight dose of 0.5% bupivacaine (Marcaine 0.5%, Sanofi Drug, Lüleburgaz, Kırklareli) was administered intrathecally.

Treatment Intervention

A 20% decrease in mean arterial pressure (MAP) was set as the Intervention Threshold Value (ITV) for administering the predetermined treatment solution to each group. The lipid solution used in this study was a 20% lipid solution (SMOF Lipid 20%, Fresenius Kabi, İstanbul). Both groups received their respective treatments—either the 20% lipid solution or isotonic saline (0.9%)—at a dose of 0.5 µl/g, based on each animal's body weight.

Animals reaching the ITV were given the respective treatment solution. If stabilization of blood pressure—defined as a MAP above 80% of the ITV—was not achieved within one minute following the treatment administration, additional treatment doses were administered without a predefined limit of doses.

Table 1. Demographic data, initial heart rate, and blood pressures – Student's T-test

	Weight (gr)	Basal SBP (mmHg)	Basal DBP (mmHg)	Basal MAP (mmHg)	Basal HR (beats per minute)
Lipid	316.9 (18.4)	129.3 (6.5)	97.3 (5)	107.7 (3.9)	330.9 (1.6)
Saline	304 (11.4)	126.4 (8.9)	95.7 (9.6)	105.6 (9)	330.7 (1.4)
	p=0.140	p=0.505	p=0.708	p=0.576	p=0.860

Values are summarized as means and standard deviations; p-values are two-tailed. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate.

Table 2. Hemodynamic parameters – Mann-Whitney U test

	Mean SBP (mmHg)	Mean DBP (mmHg)	Mean MAP (mmHg)	Mean HR (beats per minute)
Lipid	101 (76;101)	64 (46;66)	76 (56;77)	272 (214;282)
Saline	43 (42;44)	26 (25;28)	32 (31;32)	155 (154;157)
	p=0.002	p=0.002	p=0.002	p=0.009

Values are summarized as medians with the 25th and 75th percentiles; p-values are two-tailed. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate.

Hemodynamic parameters, including Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP), and Heart Rate (HR), were recorded every minute up to 15 minutes post-bupivacaine injection or until the animal's demise.

Histological Analysis

At the study's conclusion, rats were humanely euthanized using a high isoflurane dose. Subsequently, the brain and spinal cord were extracted en bloc and fixed in 10% formalin for 24 hours. Tissues were then processed with a standard alcohol-xylene-paraffin method, embedded in paraffin, sectioned at 4 µm thickness, placed onto glass slides, and stained with hematoxylin and eosin.

Outcomes

The primary outcome of this study was the mean of the MAP values, recorded at one-minute intervals, continuing either until the 15-minute mark or the occurrence of death in the experimental subjects. Secondary outcomes included the survival rates of the rats up to 15 minutes, treatment doses required to stabilize blood pressure, time to a 20% MAP decrease, and evaluation of hematoxylin/eosin sections.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (Version 24.0, SPSS Inc., Chicago, IL, USA). The normality of continuous variables was assessed with the Kolmogorov-Smirnov and Shapiro-Wilk tests and by visually inspecting histograms. Normally distributed variables were summarized as means (with standard deviations) and analyzed using the Student's T-test. Non-normally distributed data were represented as medians (25th percentile; 75th percentile) and analyzed using the Mann-Whitney U test. Categorical variables were analyzed using the Chi-squared test. A p-value of <0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the rats in the two study groups, Lipid and Isotonic, are summarized in Table 1. A Student's T-test revealed that no statistically significant differences between the groups.

To evaluate the differences in hemodynamic parameters between the groups from the time of bupivacaine injection until either death or the 15-minute mark, a Mann-Whitney U test was conducted (Table 2). Mean SBP, DBP, and MAP significantly differed between the Lipid and Saline groups (all with p=0.002). The Mean Pulse Rate also significantly differed between the two groups, with a two-tailed p-value of 0.009 (Fig. 1).

All rats in the Saline group succumbed. Conversely, only two rats in the Lipid group did not survive, with the remaining five rats still alive. A Chi-Square test revealed a statistically significant difference in survival rates between the Lipid and Saline groups (two-tailed p=0.021) (Fig. 2).

In the Lipid group, a total of 43 doses of intrathecal lipid were administered. Interestingly, 25 of these doses were administered to the two animals that ultimately succumbed. The median number of doses administered before achieving stable blood pressure or death was 4, with a 25th percentile of 3.5 and a 75th percentile of 8.5. When data from the deceased animals are excluded, the median number of doses remains 4, with an interquartile range of 3 to 4 (Fig. 3).

Table 3. Time until treatment solution application – Mann-Whitney U test

	Lipid (sec)	Saline (sec)	
Mean (SD)	183 (8.9)	177 (12.9)	p=0.332

SEC: Second; SD: Standard deviation.

In contrast, despite all the rats in the Saline group succumbing, they received a higher total number of administered doses, totaling 61. The median number of doses in this group was 8, with an Interquartile Range (IQR) of 8 to 9.5.

The average time required to observe the Intervention Threshold Value for all animals was 180 seconds, with a standard deviation of 11.12 seconds (Table 3). A Student's T-test revealed no statistically significant difference between the groups in this parameter, with a two-tailed p-value of 0.332. In the Saline group, all rats died between the 8th and 10th minutes following the bupivacaine injection. In contrast, the two fatalities in the Lipid group occurred after the 14th minute post-injection.

We performed histopathological examination to determine whether the intrathecal lipid application caused any damage to the medulla spinalis. The histopathological report revealed no specific morphological abnormalities, and no differences were found between groups.

DISCUSSION

In this study, we demonstrated that intrathecal administration of a lipid solution effectively reverses the hemodynamic effects of high or total spinal anesthesia generated by intrathecal bupivacaine in rats.

Unintentional insertion or displacement of an epidural catheter into the intrathecal space can have clinical repercussions depending on the volume of local anesthetic or other drugs administered into the CSF. Smaller volumes can cause numbness in the lower extremities or a higher but acceptable level of spinal blockade. In contrast, larger doses can lead to a potentially dangerous high spinal or total spinal block. This situation can rapidly develop into severe bradycardia and hypotension, which may subsequently escalate into cardiac collapse.

Bupivacaine is a commonly used amide local anesthetic for regional anesthesia.[10] As reported by Yahalom et al.,[11] intrathecal bupivacaine at a dose of 7.5 mg/kg leads to insensitivity to painful stimuli on the forearm, respiratory depression, and symptoms of high spinal anesthesia resulting in death in approximately 30 seconds. However, spinal anesthesia performed with an intrathecal bupivacaine dose of 3.75 mg/kg maintains motor and sensory functions in the forearm while establishing spinal anesthesia with a normal respiratory pattern. In our study, we determined the bupivacaine dose causing hypotension and bradycardia in rats to be 5 mg/kg.

Currently, total spinal block resulting in bradycardia and hypotension is managed through the administration of vasopressors, intravenous (IV) fluid support, CSF exchange, and intra-

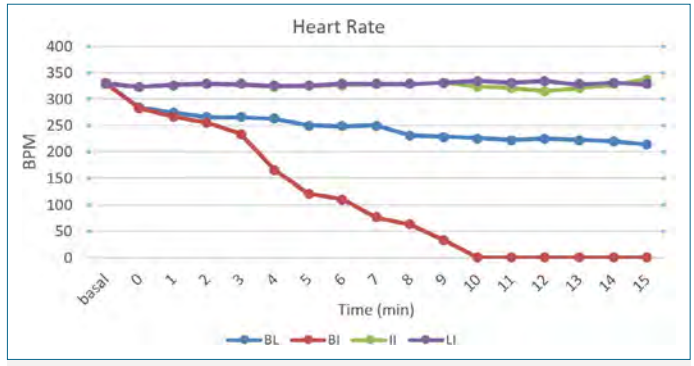

Figure 1. Mean arterial pressures of all rats after Bupivacaine injection.

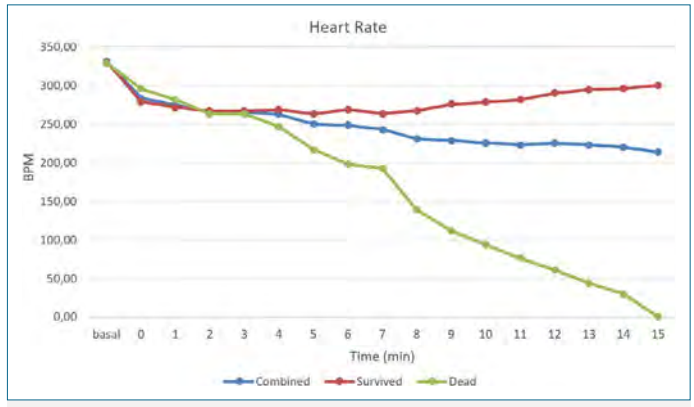

Figure 2. Heart rates of the Lipid group: survived, dead and combined.

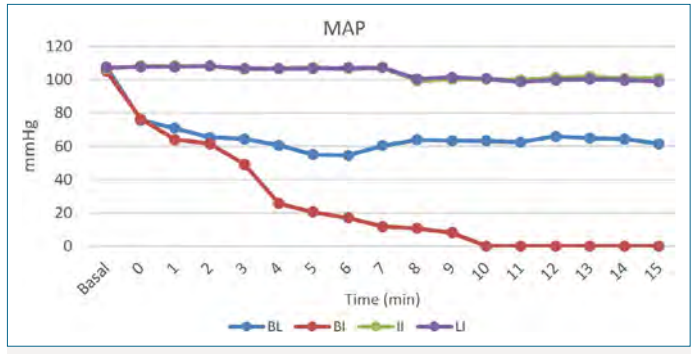

Figure 3. Mean arterial pressure (MAP) of groups.

venous lipid injection. While these symptomatic approaches have rapid onset, their short duration of action and potential cardiac complications present significant disadvantages.[8,9]

The precise mechanism of action of intralipid emulsions remains unclear. According to the initial "lipid sink" theory proposed by Weinberg et al.,[12] the lipophilic bupivacaine may dissolve into a lipid compartment formed in the blood, thereby removing it from the aqueous plasma circulation. The "lipid sink" binds to bupivacaine, reducing the free concentration accessible to organs, like the heart and brain, that are vulnerable to the effects of local anesthetics.

Studies on rats have shown that the amount of local anesthetic (bupivacaine) used to induce spinal anesthesia ranges from 0.03–0.06 µL/g.[13,14] For our study's purpose, a total spinal block may occur when a local anesthetic, intended for the epidural space, is accidentally administered to the spinal space. We aimed to examine the effectiveness of intrathecal lipid usage in the management of hemodynamic difficulties related to total spinal block. Clinically, the dosage used for epidural analgesia is roughly 8–10 times the dose used for spinal anesthesia. We administered bupivacaine at a dosage of 0.5 µl/g, roughly 8–10 times the 0.03–0.06 µl/kg bupivacaine dose used for spinal anesthesia in rat studies.[15,16] In the literature, we were unable to find any information on intrathecal lipid administration. The 20% lipid treatments referenced in studies equated to the local anesthetic amount according to the lipid sink theory. We believed it appropriate to compare the amount of local anesthetic we used with the amount of lipid capable of binding local anesthetics in the intrathecal region, in line with the lipid sink theory.

Lipid emulsions have been employed to antagonize the effects of local anesthetics due to the steroid-like nature of these anesthetics. The efficacy of lipid emulsion therapy in treating local anesthetic systemic toxicity has been established. Intravenous lipid emulsions have also been utilized in the treatment of total spinal block.[8,9,12] To our knowledge, intrathecal lipid administration as a therapeutic intervention has not been previously studied.

In vitro experiments suggest that a significant percentage of bupivacaine uptake by lipid emulsions occurs within one minute of mixing.[17–20] This time frame is comparable to that required for lipid distribution throughout the bloodstream. This supports the assumption of rapid lipid distribution in the body and quick equilibration within the vascular compartment.

In this study, the hemodynamic effects of spinal anesthesia induced by 5% bupivacaine at a dose of 0.5 µl/g commenced, on average, 180 seconds post-administration. When the baseline value of mean arterial pressure decreased by more than 20%, the first dose of intrathecal lipid was administered. This procedure was repeated as needed until stable blood pressure was achieved. The repetition times lengthened progressively. Hemodynamic parameters improved within seconds following lipid administration. Unlike the Saline group, bradycardia and hypotension resulting from spinal anesthesia were halted in five rats in the Lipid group, and their hemodynamic values stabilized upon lipid injection. The two rats in the intrathecal Lipid group that did not survive are believed to have experienced a secondary wave of respiratory depression due to elevated spinal anesthesia.

Our study has some limitations. We chose femoral artery cannulation for hemodynamic monitoring. As rats cannot survive after femoral artery decannulation, they were euthanized with high-dose anesthesia. This means the long-term neurological, physiological, and pathological effects of intrathecal lipid administration could not be studied. Additionally, technical constraints limited the prospects for performing a tracheostomy or mask ventilation in rats.

CONCLUSION

Lipid emulsions are routinely used to treat local anesthetic toxicity. Our study suggests that intrathecal of lipid emulsion administration in rats may serve as an effective and rapid intervention against hypotension and bradycardia induced by high or total spinal anesthesia.

While our findings are promising, they are derived from a single experimental design and the data collected are limited. Nevertheless, we are optimistic that the results of this study will stimulate further research in this field.

To substantiate the potential of this method and consider its clinical application, more rigorous experimental and clinical trials are essential. Future research should aim to confirm our findings, investigate long-term safety and efficacy, and determine the optimal protocol for intrathecal lipid emulsion administration.

Peer-review: Externally peer-reviewed.

Ethics Committee Approval: The Koç University Animal Experiments Ethics Committee granted approval for this study (date: 21.03.2017, number: 2017.HADYEK.001).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Author Contributions: Concept – KD; Design – KD, MAK; Supervision – ÖE; Resource – KD; Materials – KD, BY; Data Collection and/or Processing – AÜ, SÇ; Analysis and/or Interpretation – KD, İK; Literature Search – MAK, YY; Writing – KD, MAK; Critical Reviews – KD, YY.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Jenkins JG. Some immediate serious complications of obstetric epidural analgesia and anaesthesia: a prospective study of 145,550 epidurals. Int J Obstet Anesth 2005; 14(1): 37–42. [CrossRef]
2. Ting HY, Tsui BC. Reversal of high spinal anesthesia with cerebrospinal lavage after inadvertent intrathecal injection of local anesthetic in an obstetric patient. Can J Anaesth 2014; 61(11): 1004–7. [CrossRef]
3. Kar GS, Jenkins JG. High spinal anaesthesia: Two cases encountered in a survey of 81 322 obstetric epidurals. Int J Obstetric Anesthesia 2001; 10(3): 189–91. [CrossRef]
4. Skowronski GA, Rigg JR. Total spinal block complicating epidural analgesia in labour. Anaesth Intensive Care 1981; 9(3): 274–6. [CrossRef]
5. Morgan GE, Mikhail MS, Murray MJ. Clinical Anesthesiology, 4th edition. New York: McGraw-Hill; 2006.p. 263–75.
6. Zhang Y, Lin H, Yi WB. Evaluation of the effects of ketamine on spinal anesthesia with levobupivacaine or ropivacaine. Exp Ther Med 2016; 12(4): 2290–6. [CrossRef]
7. Tan H, Wan T, Guo W, Fan G, Xie Y. Mepivacaine versus bupivacaine for spinal anesthesia: A systematic review and meta-analysis of random controlled trials. Adv Ther 2022; 39(5): 2151–64. [CrossRef]
8. Eldor J, Nguyen TA. Lipid emulsion for local anesthesia reversal (LAR) after prolonged spinal/epidural anesthesia. Jor Health Sci Development 2018; 1(1): 43–7.
9. Tsui BCH, Malherbe S, Koller J, Aronyk K. Reversal of an unintentional spinal anesthetic by cerebrospinal lavage. Anesth Analg 2004; 98(2): 434–6. [CrossRef]
10. Wan L, Shen PY, Zhang SX, Wang LZ. Leg compression versus control for prevention of spinal anesthesia induced hypotension in elective cesarean delivery: A meta-analysis of randomized controlled trials. J Perianesth Nurs 2022; 37(4): 501–8. [CrossRef]
11. Yahalom B, Athiraman U, Soriano SG, Zurakowski D, Carpino EA, Corfas G, et al. Spinal anesthesia in infant rats: Development of a model and assessment of neurologic outcomes. Anesthesiology 2011; 114(6): 1325–35. [CrossRef]
12. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ. Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anesthesiology 1998; 88(4): 1071–5. [CrossRef]
13. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, Strichartz G, et al. Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. Reg Anesth Pain Med 2006; 31(4): 296–303. [CrossRef]
14. Neal JM, Barrington MJ, Fettiplace MR, Gitman M, Memtsoudis SG, Mörwald EE, et al. The Third American Society of Regional Anesthesia and Pain Medicine Practice Advisory on Local Anesthetic Systemic Toxicity: Executive Summary 2017. Reg Anesth Pain Med 2018; 43(2): 113–23. [CrossRef]
15. Rahman MM, Lee JY, Kim YH, Park CK. Epidural and ıntrathecal drug delivery in rats and mice for experimental research: Fundamental concepts, techniques, precaution, and application. Biomedicines 2023; 11(5): 1413. [CrossRef]
16. Liu Y, Zhang J, Yu P, Niu J, Yu S. Mechanisms and efficacy of ıntravenous lipid emulsion treatment for systemic toxicity from local anesthetics. Front Med (Lausanne) 2021; 8: 756866. [CrossRef]
17. Kuo I, Akpa BS. Validity of the lipid sink as a mechanism for the reversal of local anesthetic systemic toxicity: A physiologically based pharmacokinetic model study. Anesthesiology 2013; 118(6): 1350–61. [CrossRef]
18. Mazoit JX, Le Guen R, Beloeil H, Benhamou D. Binding of long-lasting local anesthetics to lipid emulsions. Anesthesiology 2009; 110(2): 380–6. [CrossRef]
19. Wolfe JW, Butterworth JF. Local anesthetic systemic toxicity: update on mechanisms and treatment. Curr Opin Anaesthesiol 2011; 24(5): 561–6. [CrossRef]
20. Sakaeda T, Hirano K. Effect of composition on biological fate of oil particles after intravenous injection of O/W lipid emulsions. J Drug Target 1998; 6(4): 273–84. [CrossRef]