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Improving Lidocaine Delivery Efficiency in Canine Lumbosacral Injection: Formulation and Standardization of Liposomal Carrier

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ABSTRACT

Liposomes are thought to be promising and versatile drug vesicles. Liposomes outperform standard drug delivery systems in terms of site-targeting, sustained or controlled release, drug protection from degradation and clearance, superior therapeutic effects, and lesser toxic side effects. The Nano-Liposome lidocaine was prepared from lipid form lecithin-cholesterol 1:1 in elution of mixture of methanol-chloroform 1:2 and created liposome by lipid film empty liposome by the Bangham method, with the loaded buffed lidocaine hydrochloride 2% in the liposome within the lipid film by vortex dispersion. Accordingly, a total of 18 healthy adult male local breed dogs were meticulously selected, prepared, and then randomly allocated into three equal groups: the lidocaine group, which was injected with 4.5 mg/kg body weight of 2% lidocaine; and the liposomal lidocaine group, which injected an identical dosage of lidocaine encapsulated within liposomal carriers; and the control groups, which were injected with a placebo.

The purpose of this research is to produce liposomal lidocaine and standardize liposomes made from lecithin-cholesterol utilizing a lipid film process, with the lipid film hydrated by an alkaline phosphate-buffered saline (PBS) containing lidocaine hydrochloride to overcome the undesirable effects of lidocaine and enhance its delivery via lumbosacral injection of liposomal lidocaine in dogs.

Results showed the nanoliposomal lidocaine size was 100.4 nm and was spherical and multi-lamellar, as seen by a light microscope and scanning electron microscopy images. The osmo-tolarance and pH challenges of liposomes showed that nanoliposomal lidocaine has more coverage than empty liposomes in saline and acidic solutions (2–8) and (0.0–8.5), respectively.

After lumbosacral injection of liposome lidocaine in dogs, the results were safe without any complications (allergy, necrosis, and death) and more convenient (smooth induction and smooth recovery) in comparison with free lidocaine.

Conclusion: A positive pH and osmo-tolerance multilamellar liposome containing lidocaine hydrochloride 2% was successfully synthesized with highly effective encapsulation and positive loading, which is more stable in neutral saline and acidic solutions. Nano liposomal lidocaine was safe and had fewer unwanted reactions during lumbosacral injection in dogs.

Introduction

Liposomes offer promising drug delivery due to their capacity, biodegradability, and compatibility (1).

The classic liposome resembles dense living cells. Composed of a phospholipid bilayer with an aqueous inner chamber (2). This shape enables the liposome to saturate the insoluble drug molecules and shield the loaded medication from the abrasive physiological environment (3).

Additionally, the liposome's surface can be altered to speed up blood circulation and/or focus on particular tissues (4,5). With these above-mentioned advantages, various liposome systems have been clinically approved (6, 7).In the pharmaceutical industry, liposomes have a number of limitations, including quick degradation and the inability to achieve continuous drug administration over an extended period of time, although liposomes have received extensive research as an active ingredient delivery method. Additionally, it is well known that liposome-based systems have drawbacks such as instability brought on by changes in temperature and pH (8–10). Drugs can leak from vesicles due to stability issues with liposomes, which can harm storage facilities.

One of the most essential objectives in basic medical procedures is to achieve adequate preoperative, intraoperative, and postoperative anesthesia and analgesia (11, 12) via pain control by using local anesthetics, which are considered safer than general-type anesthetics (13, 14). The short time onsite is the most important problem for anesthetic action locally (15–17).

Lipid nanoparticles and hydrogels are used in scientific research to accelerate and prolong local anesthetic action, preventing adverse effects from other methods like epinephrine (18, 19). Or other agents or formulations like gels, ointments, and creams; injection methods (20). That does not fix the practical application and requirements, in addition to different anesthetic techniques like infusion and multiple injections (21).

Lidocaine is a widely used local anesthetic for dental, minor surgeries, and dermatological procedures, providing quick pain relief (22–24). Local anesthetics use stimulatory materials like clonidine, epinephrine, liposomes, and nanoparticles (25–27). Lidocaine as a local anesthetic drug Application is popularly used in different surgical procedures (28). Because of its poor bonding to proteins, it is a rapid-acting local anesthetic with a middle time between actions to promote and enhance its action by using a drug that releases lidocaine after topical application (29–31).

The present study aimed to prepare liposomal lidocaine (lidosome), standardize liposomes, and improve the lidocaine local anesthetic shortfall by achieving control of the duration of local anesthesia without synergistic additive agents and with a minimized dosed amount and concentration via encapsulation of lidocaine in smart nanoliposomes controlling release. The use of anesthetic nanoparticles in the quest to achieve greater effectiveness and safety of lidocaine agents while reducing the likelihood of toxicity and side effects is a breakthrough in medical practice and a great advantage for the safety and comfort of the patient.

MATERIALS AND METHODS

Ethical Statement

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine, University of Baghdad (number P.G. 1364 at 2022/7/3) before starting this study.

Liposome Preparation Technique

The Bangham technique (32) was utilized for the creation and formulation of liposomes, with the

following procedural steps: Initially, cholesterol and phosphatidylcholine were dissolved in a chloroform and methanol solvent mixture and agitated vigorously for 30 minutes to ensure thorough homogenization. Subsequently, the solvent was eliminated through the application of a vacuum pump, resulting in the formation of a lipid layer with a foam-like appearance. This lipid layer was then subjected to incubation at 40°C. Subsequently, it was integrated with a pH 7.2 phosphate buffer, lidocaine, and liposomes, as depicted in Figure 1. The subsequent phase of the process involved the sequential steps required for the entrapment of lidocaine within the liposomes.

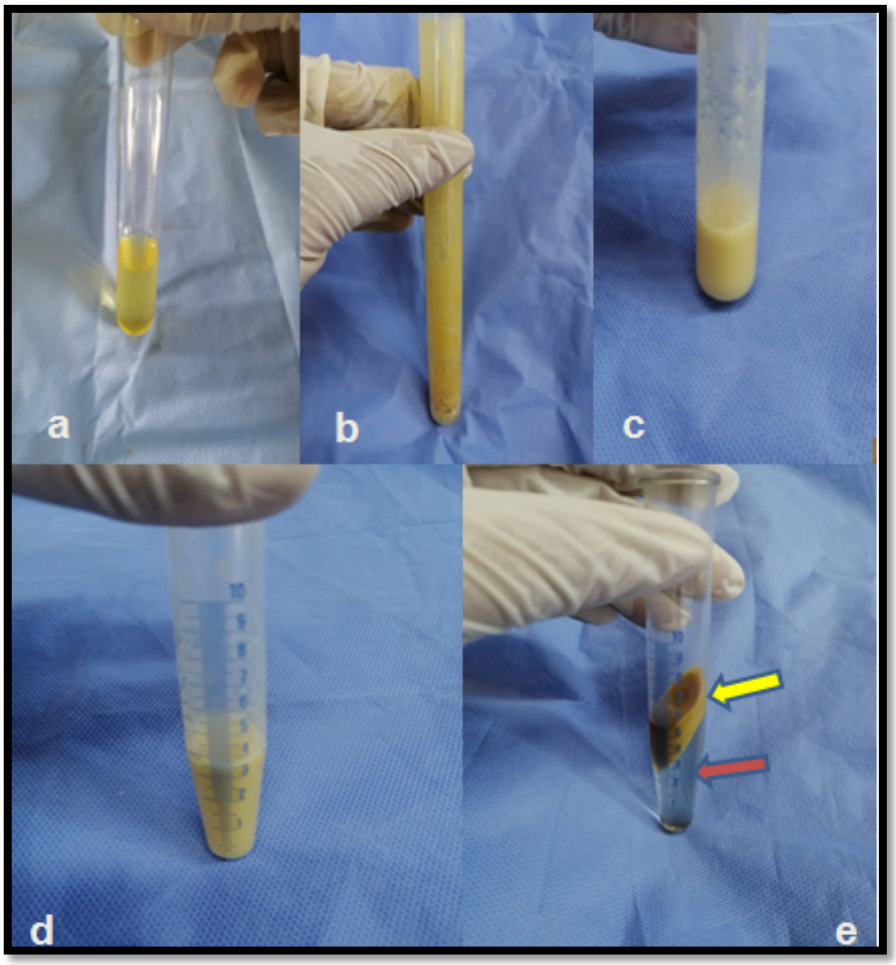

Figure1. Steps of liposome preparation: a. phosphatidylcholine and cholesterol dissolve in chloroform and methanol. b. The thin film deposited on the tube wall. c. Liposome loading lidocaine forms the appearance of pale color foamy shape creamy texture d. conical tube for centrifugation. e. fatty ring and the unloaded lidocaine, which appeared at the bottom of the tube: the upper part (yellow arrow) containing the loaded nanomaterial and the lower precipitate part (red arrow) containing the unloaded material.

Stock solution of Lidocaine

Lidocaine 2000 mg were dissolved in 100 ml of Phosphate buffer solution pH7.2, the final concentration of Lidocaine was 20 mg/ml.

Lidocaine entrapping

The solution was mixed with liposomes and vortexed at 40°C for 15 minutes. Following this, the Lidocaine Liposome solution was subjected to centrifugation at 4000 rpm for 15 minutes to separate the drug from the solution. The resulting residue was collected and then passed through filter membranes. The concentration of lidocaine was subsequently determined using high-

performance liquid chromatography (HPLC) in conjunction with the liposomes. The assessment of lidocaine concentration was conducted according to the method outlined by **Babaie *et al.***, (33).

Step 1: Opening and separation of liposome

The liposome vesicle breaks by adding 10ml methanol for 10 minutes with g gentile agitation to release the entrapped lidocaine.

Step 2: Certified HPLC equations were used to determine the calculated lidocaine (34, 35).

$$\mathbf{EE}\ (\%) = \frac{\mathbf{W(Intial\ drug) - W(Free\ drug)}}{\mathbf{W(Intial\ drug)}} \times \mathbf{100}$$

$$\mathbf{LC}\ (\%) = \frac{\mathbf{W(Entrapment\ drug)}}{\mathbf{W(Total\ lipid)}} \times \mathbf{100}$$

- **W (Initial drug) is the amount of initial drug used and W(Free drug) is the amount of free drug detected in the lower chamber of Amicon® tube after centrifugation of the Nano formulations.**
- **W (Entrapped drug) is the amount of loaded drug and W(Total lipid) is the amount of used phospholipids and cholesterol in the preparation process.**

Liposome standardization

By using a light and electron microscope (Inspect F-50 SEM FEI, Origin Holland), different liposome kinds, lamellar structures, and diameters were studied (36, 37).

Identification of liposomes under light microscopy

A light microscope analyzes

A liposome was carefully examined under a microscope with high magnification (1000X) to analyze its particle size and lamellar structure. A one percent solution was employed for this examination, and particular attention was given to assessing the overall dimensions and shape uniformity of liposomal lidocaine as outlined in reference 38.

Liposome pH stability

This method was modified in response to the need for research measurement. Investigated the pH-dependent stability of liposomes containing lidocaine. The liposome formula stock suspension was placed in acidified tubes labeled 2, 3, 4, 6, 7, and 8 pH and allowed to stand for one hour. Liposome concentrations were determined using the spectrophotometer (APEL PD-303UV and Origin India) at 418 nm for extrapolation of the standard curve and absorbance curve both at zero time and one hour, indicating the absorption characteristics of the substance at that wavelength.

Osmo-Tolerance

Tolerance osmolarity was determined by the dilution assay method (0.1 ml). From standard loading, liposome suspension was added to tubes containing different concentrations of NaCl (8.5, 7.5, 6.5, 5.5, 4.5, 3.5, 2.5, 1.5, 0.5, and 0.0 mg/ml). After 1 hour of incubation at 37 ºC, the tubes were measured, and the liposome count remaining in each tube was calculated by spectrophotometer (39).

The sterilization by filtration

The Lidocaine solution, empty liposome, and Liposomal Lidocaine were all sterilized by membrane filtering with a (0.2)µm zeta filter **(40).**

Animals

Eighteen healthy male dogs of the local breed, aged (3.167 ± 0.145) years, with an average weight of (25±0.714) kg, were included in this research and randomly divided into three groups. Group A (GA) included six (6) dogs injected with liposomal lidocaine epidurally; Group B (GB) included six (6) dogs injected with 4.5 mg/kg of lidocaine 2%; and Group C (GC) included six (6) dogs as controls; they were injected with a placebo. All the dogs were accommodated in individual cages at the College of Veterinary Medicine, University of Baghdad. They were maintained under standard environmental conditions, including climate control, management protocols, and feeding. The dogs were provided with commercial food and had unrestricted access to water throughout the study period.

Surgical interference

Prior to surgery, the dogs were fed for six hours before the epidural injection. The lumbosacral area was aseptically prepared by clipping and shaving the lumbosacral region, washing with tap water and soap, and disinfecting the area with 70% ethyl alcohol for an aseptic epidural puncture. 22G×90mm (90 millimeter = 9 cm) spinal needles were inserted into the lumbosacral epidural space at the intervertebral depression between the last lumbar and first sacral vertebrae (Figure 2) (41). The calculated dose for each animal in the group was equalized to 4.5 mg/kg, administered epidurally and slowly. (42).

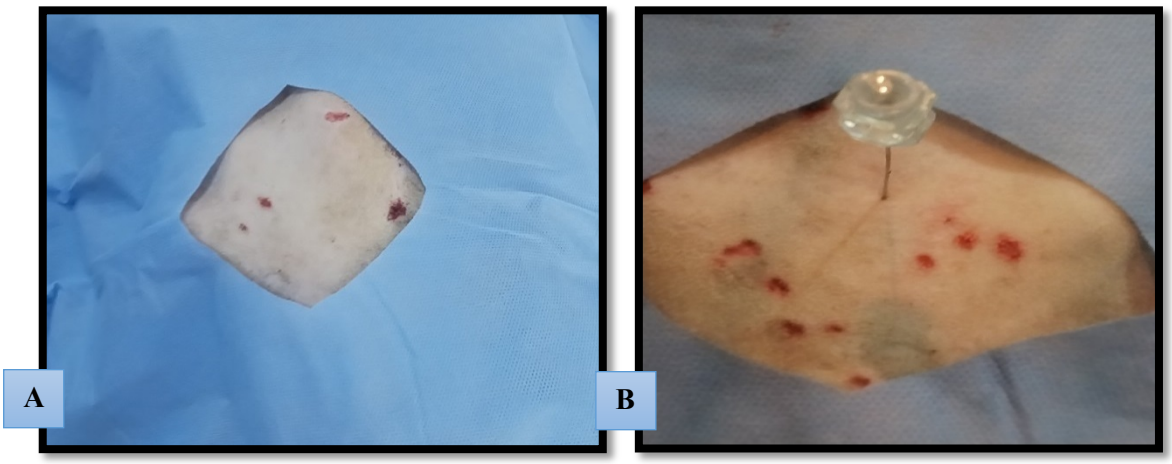

Figure 2. Surgical techniques: A. area of anesthetics injection. B. Insert the needle at the lumbosacral depression,

RESULTS

Light microscope examination

A Light microscope observation of the liposomes was performed to determind the morphology of the liposomes, as shown in (Figure 3), which showed clearly that the shape of the liposomes was spherical.

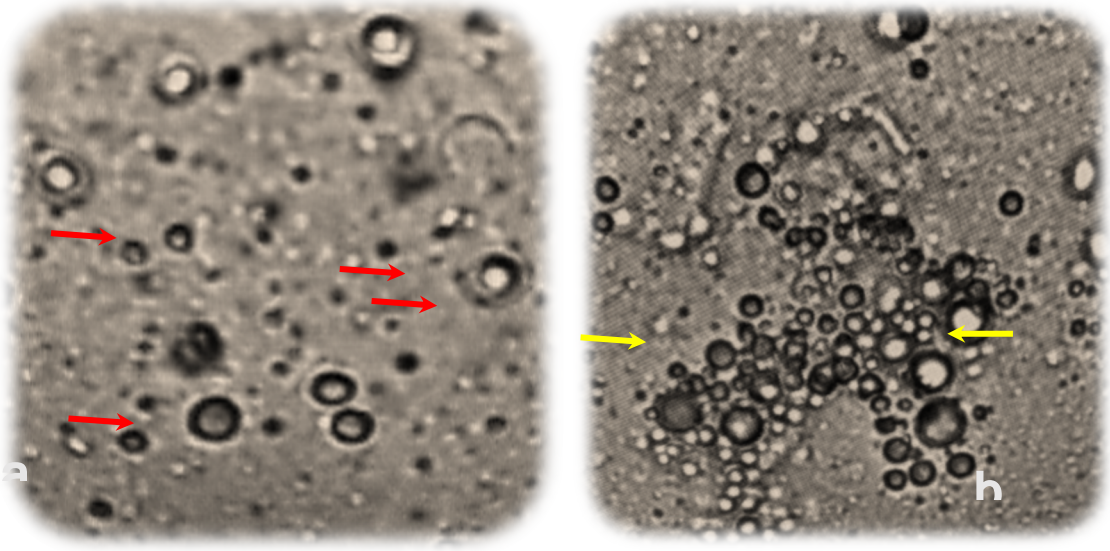

Figure 3. The figure depicts a light macroscopic field observed using a thin-film light microscope, revealing liposomes containing lidocaine. In Part A, the scanning mode image reveals liposomes of varying sizes (red arrow). Part B highlights the presence of multi-laminar layers within the multi-vesicular liposome (yellow arrow) magnification (1000X).

Electron Microscope Micrograph

The microscope was employed for the examination of topographical characteristics, encompassing liposome formation and dimensions, in electron micrographs. Figure 4 visually depicts the prevalent spherical morphology indicative of the global formation of unilamellar liposomes.

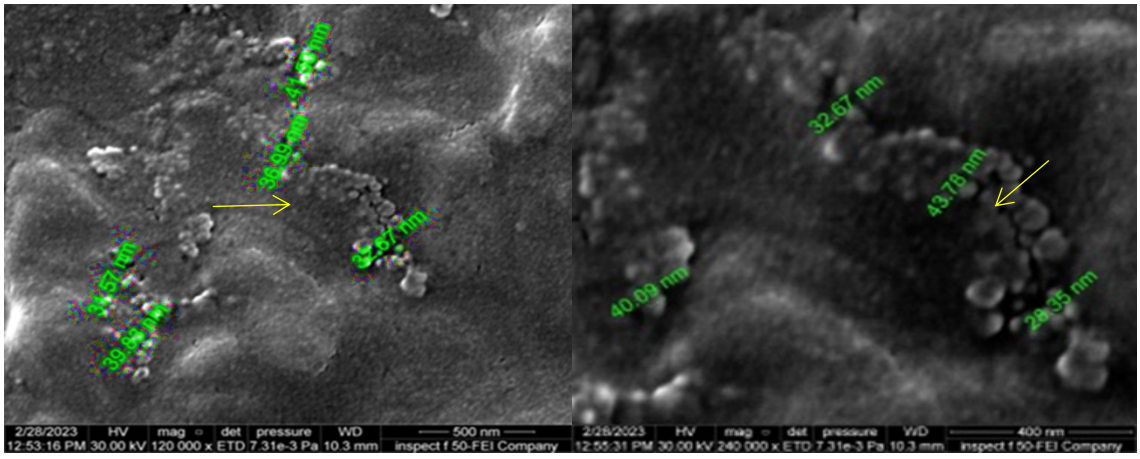

Figure 4. Electron macroscopic micrograph displaying liposomes loaded with lidocaine. The micrograph reveals the presence of multi-lamellar and multi-vesicular structures, showcasing liposomes of varying sizes (yellow arrow) measuring between 400 and 500) nanometers. The magnification scale used for this image ranges from 120,000 to 240,000 X.

Liposome pH stability

This method was modified according to the necessity of research measurement and the stability of liposomes containing lidocaine in pH changes. The liposome formula stock suspension had been added to the acidified tubes labeled as 2, 3, 4, 5, 6, 7, and 8 pH and left to stand for one hour. The liposome concentrations were measured at zero and one hour by spectrophotometer at 418 nm maximum. This concept plan determines the number of liposomes that survive during the serial increment in acidity values.

Table 1. The Absorbance at 418 nm of the Liposome carrying lidocaine in different pH values were measured on zero time and post one hour.

pH value	Absorbance measurements/nm	
	Zero time	after one hour
pH 2	2.139	2.185
pH 3	2.119	2.202
pH 4	2.401	2.482
pH 5	2.365	2.54
pH 6	2.735	2.503
pH 7	3.208	3.501
pH 8	3.210	3.501

Osmo-Tonicity

After introducing liposomes and lidocaine, the action of varied sodium chloride concentrations on them resulted in an increase in the absorbance curve, and after an hour, the tonicity of the solution gradually fell from isotonic to hypotonic, as shown in Figures 5 and 6.

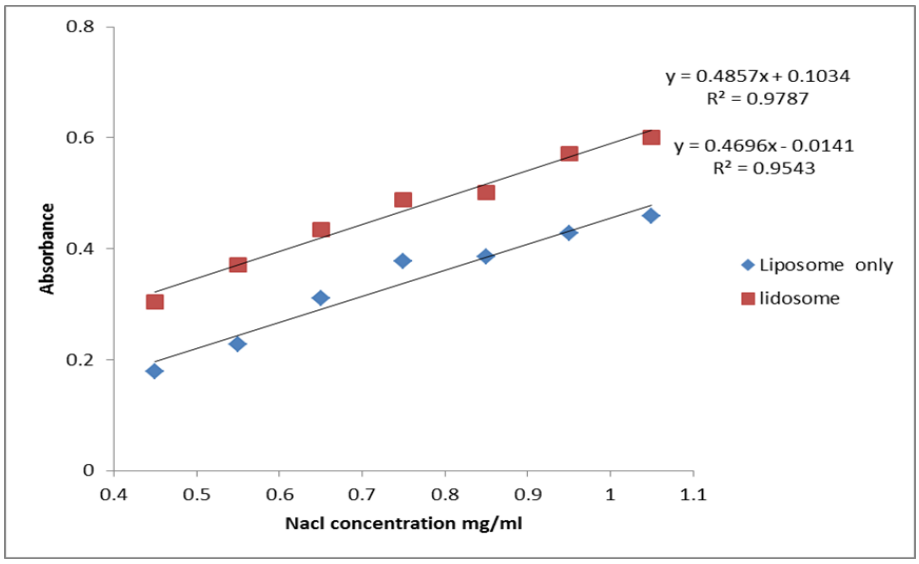

Figure 5. Effect of different osmolarity on Lidosome and empty liposome at zero time.

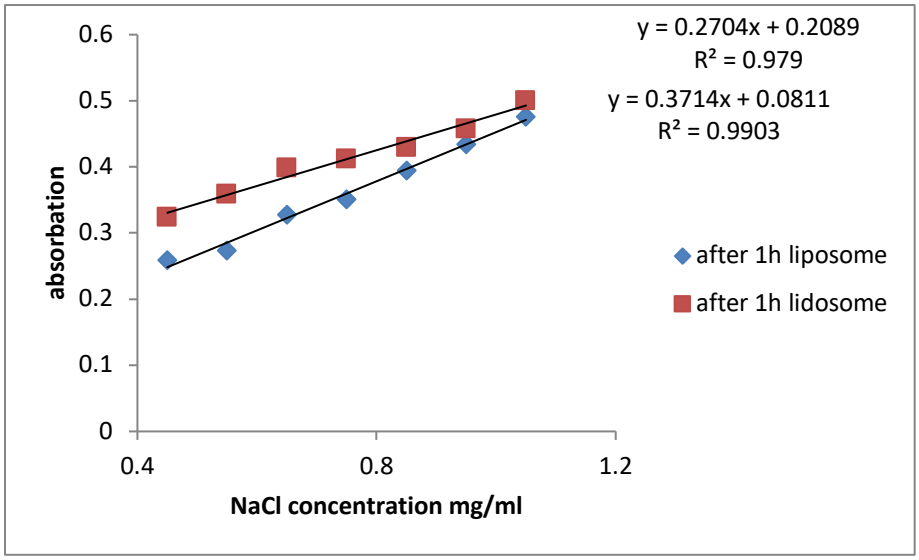

Figure 6. Effect of different osmolarity on Lidosome and empty liposome after one hour.

Assessment of Quality of Anesthesia

Clinically, all studied dogs were kept under inspection during the period of anesthesia to record their general health, behavior, and alertness.

The onset time for both the repeated-dose liposomal lidocaine groups (3.71 ± 0.40 minutes) was notably shorter compared to the Lidocaine group repeated dose (7.91 ± 0.56 minutes). The liposomal lidocaine group repeated dose demonstrated a significantly faster onset time when compared to the lidocaine group repeated dose (3.71 ± 0.44 minutes vs. 7.91 ± 0.56 minutes, respectively).

Furthermore, the repeated-dose Liposomal Lidocaine group exhibited a substantial ($P < 0.05$) increase in the duration of anesthesia, lasting 3.20 ± 0.06 hours compared to the single-dose group; in contrast, the repeated-dose Lidocaine group displayed a significantly ($P < 0.05$) prolonged anesthesia duration, spanning 1.23 ± 0.05 hours.

Significantly prolonged recovery times were observed in both the repeated-dose Liposomal Lidocaine group, with duration of 101.67 ± 4.59 minutes, and the Lidocaine group, with a duration of 53.33 ± 2.47 minutes ($P < 0.05$). Moreover, a noteworthy difference was evident when comparing the recovery times between the liposomal lidocaine and Lidocaine groups, with the liposomal lidocaine groups displaying a significantly extended recovery period.

Table 2. Comparison between Liposomal Lidocaine and lidocaine groups in Onset time, Duration time and Recovery time with repeated doses

Groups	Onset time/min.	Duration time/hr.	Recovery time/min.
Liposomal lidocaine	3.71 ±0.44	3.20 ±0.06	101.67 ±4.59
Lidocaine	7.91 ±0.56	1.23 ±0.05	53.33 ±2.47
Control	0.00±0.00	0.00±0.00	0.00±0.00

Discussion

The surface topography of liposomes was observed by using light and a scanning electron microscope, and the distribution of liposomal size was also determined by analyzing a scanning electron micrograph according to (43). Figure 2 clearly shows that the shape of the liposomes was spherical under scanning electron microscopic observation. As a result, the vesical spherical size shape of synthesis Liposome lidocaine agrees with the findings of (44, 45).

Liposomes consist of two structural components: the hydrophobic lipid bilayer and the hydrophilic inner aqueous chamber. The pH of the inner aqueous chamber significantly impacts drug stability, while lidocaine is a hydrophilic molecule found inside the aqueous. The pH of a lidosome's microenvironment influences its chemical stability. Liposomes, composed of lipids, can become unstable when external pH changes, affecting their chemical stability (46, 47).

Liposomes' micro-environmental pH, differing from their surroundings, affects their activity. Local anesthetics (Las) are stronger at alkaline pH levels and require a unionized form for axon membrane passage.

According to Ramana *et al.,* (48), liposome size significantly impacts media pH, with variations at acidic and basic levels. Initial size growth may be due to secondary particle development or Ostwald ripening. PH-sensitive medications' stability affects distribution, and liposomal architecture degradation may occur (49).

PH results are in agreement with (50) and (51) and pertain to the pH stability and behavior of the formulation over time, specifically addressing the modest pH drop and the potential implications of polymer degradation. Hyposmolality increased particle size and absorbance curve after an hour, causing swelling of lecithin and cholesterol. This swelling increased fluidity and elasticity, increasing compliance and size. Abrupt water inflow caused liposomes to expand in volume (52).

Quantitative description of the pulsatory liposome swelling phase in hypotonic baths, utilizing the osmotic transmembrane gradient for energy (53).Onset time was longer in the lidocaine group compared with the liposomal lidocaine group; this could be attributed to many factors, including the intrinsic properties of the anesthetic drug, its amount, the anesthetic technique, the onset time after injection, and the rate of administration (54); it is clear that all these factors affect the diffusion rate of the anesthetic agent, which in turn affects the time of its onset. The rapid onset of the liposomal lidocaine group could be due to the phospholipid structure of the liposome, which spontaneously forms a closed structure when dissolved in water in an internal aqueous environment bounded by phospholipid bilayer membranes; this makes the transport of drugs easy (55).

While the duration of anesthesia and recovery time were longer in the liposomal lidocaine group compared with the lidocaine group because a greater lipid solubility of a drug not only enhances its potency but also enables more rapid diffusion through cell membranes; high lipid solubility may impede dispersion throughout tissue fluids and also foster sequestration in neighboring adipose tissues or myelin sheaths. In either case, fewer molecules reach the neuronal membrane, and the onset is delayed (56). The spread of anesthetic solutions within the epidural space is known to be influenced by a number of factors, including age, obesity, pregnancy, and body position. In clinical practice, dogs would normally be sedated as indicated to facilitate epidural puncture (57).

The longer duration of action of the liposomal lidocaine group could be due to the phospholipid structures, which are the major liposome components, and although cholesterol is not required for their lamellar ensemble, it has a prominent role in determining bilayer fluidity, a crucial factor for prolonged release of drugs, mainly in ionic-gradient liposomes (58).

Liposomes act as drug reservoirs (59). Liposomal lidocaine acts to decrease local blood flow, slowing the systemic absorption of local anesthetic agents, which prolongs the anesthetic duration (60). Encapsulation of local anesthetics into liposomes results in slow drug release, prolonged duration of the anesthetic effect, and reduced side effects (61). The duration of epidural blockade in the liposomal lidocaine group was notably longer compared to that in the free lidocaine group. These findings imply that the extension of the epidural blockade by liposomal lidocaine results from the gradual release of the drug from the liposomal lidocaine. These results disagree with (62), who record a long duration of recovery in their research concerning both the liposomal lidocaine group and the free lidocaine group, and this disagreement could be due to the usage of pre-anesthetic agents in their research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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