RESEARCH PAPER

Defining Safe Intracameral Lidocaine Concentrations through Dose–Response Histopathological Assessment of Corneal Toxicity in Rabbits

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Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 30th June, 2026; Available Online: 10th July, 2026

ABSTRACT

Background: Intracameral lidocaine is widely used as an adjunctive anesthetic during cataract surgery because of its rapid onset and favorable analgesic profile. However, concerns remain regarding its potential toxic effects on corneal tissues, particularly the corneal endothelium. Existing evidence suggests that lidocaine-induced toxicity is concentration- and exposure-dependent, yet a definitive safe concentration threshold has not been established.

Objective: To determine the safety profile of intracameral lidocaine by evaluating dose-dependent histopathological changes in the corneal epithelium, stroma, and endothelium of rabbits and to identify a safe concentration threshold for clinical application.

Methods: An experimental post-test-only controlled study was conducted using rabbit eyes exposed to intracameral lidocaine at concentrations of 0.2%, 0.5%, 1%, and 2%. Histopathological examinations were performed to assess structural alterations in the corneal epithelium, stroma, and endothelium at predetermined observation periods. Corneal toxicity was graded based on cellular degeneration, vacuolization, inflammatory changes, and endothelial damage. Comparative analyses were conducted among concentration groups and observation times. Receiver operating characteristic (ROC) analysis was performed to determine the optimal concentration threshold associated with corneal toxicity.

Results: Histopathological alterations increased progressively with higher lidocaine concentrations and longer exposure durations. The epithelium demonstrated the earliest and most pronounced structural changes, characterized by cellular degeneration and vacuolization. Stromal alterations were generally mild, whereas endothelial injury became increasingly evident at higher concentrations. Significant dose-dependent differences were observed between treatment groups, indicating a direct relationship between lidocaine concentration and corneal tissue damage. ROC analysis identified a concentration threshold below which histopathological toxicity remained minimal, supporting the existence of a clinically safer concentration range for intracameral administration.

Conclusions: Intracameral lidocaine induces concentration-dependent histopathological changes in rabbit corneal tissue. Corneal toxicity increases with both concentration and duration of exposure, with epithelial tissue exhibiting greater susceptibility than stromal and endothelial layers. Lower concentrations demonstrated a more favorable safety profile, suggesting that careful dose selection is essential to minimize corneal toxicity during intraocular procedures.

Keywords: *Intracameral lidocaine; Corneal toxicity; Histopathology; Corneal endothelium; Cataract surgery; Rabbit model; Dose–response relationship.*

How to cite this article: Ismail MA, Ohorella VYCI, Muhiddin HS, Bahar B, Eka H, Umar BT., Defining Safe Intracameral Lidocaine Concentrations through Dose–Response Histopathological Assessment of Corneal Toxicity in Rabbits. Int J Drug Deliv Technol. 2026;16(76s): 1862-1872; DOI: 10.25258/ijddt.16.76s.163
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Cataract remains the leading cause of preventable blindness worldwide and continues to impose a substantial burden on healthcare systems, particularly in low- and middle-income countries (WHO, 2023). Advances in cataract surgery, especially phacoemulsification, have significantly improved visual outcomes while reducing surgical trauma and postoperative recovery time (Moshirfar et al., 2024). Alongside these developments, anesthetic techniques have evolved to maximize patient comfort and procedural safety. Topical anesthesia

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supplemented with intracameral lidocaine has become a widely accepted anesthetic approach in modern cataract surgery. Compared with retrobulbar and peribulbar anesthesia, intracameral administration avoids injection-related complications such as globe perforation, retrobulbar hemorrhage, optic nerve injury, and extraocular muscle damage (Shah, 2010; Henderson, 2014). Furthermore, intracameral lidocaine provides additional analgesia by anesthetizing intraocular structures, particularly the iris and ciliary body, thereby improving patient comfort during surgery (Hamid & Hamid, 2022).

Despite its clinical benefits, concerns regarding the ocular safety of intracameral lidocaine persist. The corneal endothelium is particularly susceptible to injury because of its limited regenerative capacity. Corneal endothelial cells play a critical role in maintaining corneal transparency through regulation of stromal hydration via the pump–leak mechanism (Srinivas, 2010; O'Connor & Gomez, 2014). Endothelial cell loss beyond a critical threshold may result in corneal edema, decompensation, and irreversible visual impairment (Proulx & Brunette, 2012). Previous experimental studies have suggested that lidocaine-induced corneal toxicity is both concentration- and exposure-dependent. Kadonosono et al. (1998) demonstrated that intracameral administration of 2% lidocaine in rabbit eyes resulted in significant ultrastructural abnormalities, including irregular endothelial cell morphology and loss of surface microvilli, whereas lower concentrations (0.02% and 0.2%) produced no detectable morphological damage. Similarly, Eggeling et al. (2000) reported progressive endothelial injury with increasing lidocaine concentrations, ranging from minimal cellular damage at 1% to widespread endothelial necrosis at 10%.

The mechanisms underlying lidocaine-induced toxicity are multifactorial. Beyond its primary action on voltage-gated sodium channels, lidocaine has been shown to induce mitochondrial dysfunction, reduce intracellular adenosine triphosphate (ATP) production, increase reactive oxygen species (ROS) generation, and activate apoptotic pathways (Bielory et al., 2017; Yang et al., 2020). Excessive oxidative stress may disrupt endothelial integrity and contribute to cellular degeneration and death (Karnina et al., 2021). Experimental evidence further suggests that prolonged exposure to lidocaine exacerbates these toxic effects, indicating that both concentration and duration of exposure are important determinants of corneal safety (Yang et al., 2020).

Although several studies have investigated the effects of intracameral lidocaine on corneal endothelial cells, a definitive safety threshold has not yet been established. Existing studies differ substantially in terms of experimental design, concentration range, exposure duration, and outcome assessment, resulting in inconsistent conclusions regarding safe clinical use. Moreover, most investigations have focused primarily on endothelial damage, while histopathological changes involving the corneal epithelium and stroma remain less extensively characterized. Determining a safe intracameral lidocaine concentration is of considerable clinical importance because the drug continues to be routinely used in cataract surgery worldwide. A comprehensive assessment of concentration-dependent histopathological changes across all corneal layers may provide valuable evidence for optimizing intraocular anesthetic protocols and minimizing treatment-related toxicity.

Therefore, the present study aimed to evaluate dose-dependent histopathological changes in the corneal epithelium, stroma, and endothelium following intracameral administration of lidocaine in a rabbit model. In addition, this study sought to identify a concentration threshold associated with minimal corneal toxicity, thereby providing experimental evidence to support safer clinical application of intracameral lidocaine during intraocular surgery.

METHODS

Study Design

This experimental post-test controlled study was conducted to investigate dose-dependent histopathological changes in rabbit corneas following intracameral lidocaine administration. The study evaluated four concentrations of preservative-free lidocaine (0.2%, 0.5%, 1.0%, and 2.0%) and assessed corneal histopathological changes at predetermined observation periods. Histopathological scores of the corneal epithelium, stroma, and endothelium were defined as the primary outcome measures.

Animals and Housing

Healthy adult New Zealand White rabbits (Oryctolagus cuniculus) were obtained from an accredited laboratory animal facility. Animals were acclimatized for at least seven days before experimentation and housed individually under controlled environmental conditions, including a temperature of 22–25°C, relative humidity of 50–70%, and a 12-hour light–dark cycle. Standard rabbit chow and water were provided ad libitum throughout the study period.

Sample Size

The sample size was determined based on the Federer formula for experimental animal studies, resulting in a minimum number of animals required for each treatment group. Additional animals were included to compensate for potential losses during the experimental period. Only clinically healthy rabbits with normal anterior segment findings on ophthalmic examination were included. Animals with pre-existing corneal abnormalities, ocular inflammation, infection, trauma, or systemic illness were excluded. Animals experiencing procedural complications that could interfere with histopathological assessment were excluded from the final analysis.

Randomization and Blinding

Animals were randomly allocated to treatment groups using a computer-generated randomization sequence. Histopathological evaluation was performed by a

pathologist who was masked to treatment allocation and study groups to minimize observer bias.

Experimental Procedures

All procedures were performed under general anesthesia using a standardized anesthetic protocol. Following aseptic preparation, a corneal paracentesis was created under operating microscopy. A predetermined volume of preservative-free lidocaine solution was injected into the anterior chamber through the paracentesis site. Care was taken to avoid direct mechanical injury to the corneal endothelium, iris, and crystalline lens during the procedure. At the designated observation periods, animals were humanely euthanized and corneal tissues were harvested immediately. Specimens were fixed in 10% neutral buffered formalin and processed for histopathological examination.

Histopathological Evaluation

Paraffin-embedded corneal tissues were sectioned at 4–5 μm thickness and stained with hematoxylin and eosin. Histopathological assessment was performed using light microscopy. Structural alterations involving the corneal epithelium, stroma, and endothelium were evaluated, including epithelial degeneration, stromal disorganization, vacuolization, endothelial cell loss, and overall tissue integrity. Histopathological changes were graded according to a predefined scoring system.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version XX (IBM Corp., Armonk, NY, USA). Data distribution was assessed prior to analysis. Because histopathological scores were ordinal variables, non-parametric methods were used. Differences among treatment groups were evaluated using the Kruskal–Wallis test followed by post hoc pairwise comparisons where appropriate. Receiver operating characteristic (ROC) curve analysis was performed to identify the concentration threshold associated with corneal toxicity. A two-sided p value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Experimental Animals

A total of 50 eyes from healthy adult male New Zealand White rabbits were included in this study. The baseline characteristics of the experimental animals are presented in Table 1.

Table 1. Baseline Characteristics of Experimental Animals

Variable	n	Value
Body weight (kg)	50	Mean 1.53 (1.03–2.05)
Age (weeks)	50	Mean 14.6 (16–20)
Sex	50	Male 50 (100%)
Observation period		
• Day 1	25	50%
• Day 7	25	50%
Treatment group		
• BSS	10	20%
• Lidocaine 0.2%	10	20%
• Lidocaine 0.5%	10	20%
• Lidocaine 1.0%	10	20%
• Lidocaine 2.0%	10	20%

The study population consisted entirely of male rabbits, with a mean body weight of 1.53 kg (range 1.03–2.05 kg) and a mean age of 14.6 weeks. The distribution of samples across treatment groups was balanced, with each group contributing 10 eyes (20% of the total sample). Likewise, the observation periods were equally distributed, with 25 eyes evaluated on Day 1 and 25 eyes evaluated on Day 7. The relatively homogeneous body weight and age ranges among experimental animals minimized the likelihood that differences in histopathological outcomes were influenced by baseline biological variation rather than the administered intracameral lidocaine concentrations.

Descriptive Analysis of Corneal Histopathological Scores

Descriptive statistics for all histopathological parameters evaluated in the study are presented in Table 2.

Table 2. Descriptive Statistics of Corneal Histopathological Scores in All Samples

Variable	N	Minimum	Maximum	Mean	SD
Epithelial nuclear vacuolization	50	0	3	1.42	0.97
Stromal keratocyte necrosis	50	0	3	0.56	0.84
Stromal cytoplasmic vacuolization	50	0	3	1.46	1.09
Eosinophilic stromal keratocytes	50	0	3	0.90	0.97
Endothelial cell loss	50	0	3	0.70	0.86
Endothelial cell vacuolization	50	0	3	0.70	0.86

Descriptive analysis demonstrated that the highest histopathological scores were observed in stromal cytoplasmic vacuolization (mean 1.46 ± 1.09) and epithelial nuclear vacuolization (mean 1.42 ± 0.97). These findings indicate that the corneal epithelium and stroma were the earliest and most susceptible structures affected by intracameral lidocaine exposure. In contrast, endothelial alterations were less prominent, with mean scores of 0.70 ± 0.86 for both endothelial cell loss and endothelial vacuolization. Stromal keratocyte necrosis exhibited the lowest overall mean score (0.56 ± 0.84), suggesting that severe stromal cellular destruction occurred less frequently than epithelial vacuolar degeneration and stromal cytoplasmic injury. Overall, the data demonstrate that histopathological injury was not uniformly distributed across corneal layers, with epithelial and stromal changes predominating over endothelial abnormalities.

Comparison of Histopathological Changes Among Lidocaine Concentrations

The effect of intracameral lidocaine concentration on corneal histopathological alterations was assessed by comparing scores among the five treatment groups. The results are summarized in Table 3.

Table 3. Comparison of Corneal Histopathological Scores Among Lidocaine Concentrations

Treatment	Epithelial Nuclear Vacuolization Mean ± SD	Rank	Stromal Keratocyte Necrosis Mean ± SD	Rank	Stromal Cytoplasmic Vacuolization Mean ± SD	Rank	Eosinophilic Stromal Keratocytes Mean ± SD	Rank	Endothelial Cell Loss Mean ± SD	Rank	Endothelial Cell Vacuolization Mean ± SD	Rank	p-value
BSS	0.20 ± 0.42	7.80	0.00 ± 0.00	12.50	0.10 ± 0.31	7.65	0.10 ± 0.31	13.60	0.00 ± 0.00	12.50	0.10 ± 0.31	12.50	0.049
Lidocaine 0.2%	1.40 ± 0.96	21.80	0.80 ± 0.92	22.50	1.60 ± 1.17	23.00	0.80 ± 0.92	22.40	0.80 ± 0.92	22.50	1.60 ± 1.17	22.50	0.004
Lidocaine 0.5%	1.70 ± 0.67	25.35	1.00 ± 1.05	24.50	1.90 ± 0.87	26.25	1.00 ± 1.05	24.40	1.00 ± 1.05	24.50	1.90 ± 0.87	24.50	<0.001
Lidocaine 1.0%	1.90 ± 0.87	27.05	0.80 ± 0.92	22.50	1.80 ± 1.03	25.10	0.70 ± 0.82	21.60	0.80 ± 0.92	22.50	1.80 ± 1.03	22.50	0.039
Lidocaine 2.0%	1.90 ± 0.73	5.00	0.20 ± 0.42	1.30	1.90 ± 0.73	5.00	1.90 ± 0.73	5.00	0.90 ± 0.73	2.35	1.90 ± 0.73	5.00	0.001

Significant differences in histopathological injury were observed among treatment groups. The BSS control group consistently demonstrated the lowest scores across all evaluated parameters, indicating minimal spontaneous tissue damage. In contrast, all lidocaine-treated groups exhibited higher histopathological scores, supporting the presence of lidocaine-associated corneal toxicity. Among epithelial parameters, nuclear vacuolization increased substantially after lidocaine exposure. Mean scores increased from 0.20 ± 0.42 in the BSS group to 1.40 ± 0.96, 1.70 ± 0.67, 1.90 ± 0.87, and 1.90 ± 0.73 in the 0.2%, 0.5%, 1.0%, and 2.0% lidocaine groups, respectively. This pattern indicates progressive epithelial injury with increasing lidocaine concentration. Within the stromal layer, cytoplasmic vacuolization represented the most prominent pathological alteration. Mean scores increased from 0.10 ± 0.31 in the control group to approximately 1.60–1.90 in the lidocaine-treated groups. Similar trends were observed for eosinophilic stromal keratocytes and keratocyte necrosis, suggesting progressive stromal cellular injury following exposure to intracameral lidocaine. Endothelial abnormalities were likewise observed, although the magnitude of injury was generally lower than that seen in the epithelium and stroma. Endothelial cell loss increased from 0.00 ± 0.00 in the control group to values ranging between 0.80 ± 0.92 and 1.00 ± 1.05 in the lidocaine groups. Similarly, endothelial vacuolization increased markedly following lidocaine administration. The Kruskal–Wallis analysis demonstrated statistically significant differences among groups, indicating that intracameral lidocaine concentration significantly influenced corneal histopathological injury.

Comparison of Histopathological Changes According to Observation Period

To evaluate the effect of exposure duration, histopathological scores were compared between Day 1 and Day 7. The findings are shown in Table 4.

Table 4. Comparison of Corneal Histopathological Scores According to Observation Period

Observation Period	Epithelial Nuclear Vacuolization Mean ± SD	Rank	Stromal Keratocyte Necrosis Mean ± SD	Rank	Stromal Cytoplasmic Vacuolization Mean ± SD	Rank	Eosinophilic Stromal Keratocytes Mean ± SD	Rank	Endothelial Cell Loss Mean ± SD	Rank	Endothelial Cell Vacuolization Mean ± SD	Rank	p-value
Day 1	1.00 ± 0.82	4.58	0.28 ± 0.79	2.70	1.04 ± 0.98	4.70	0.56 ± 0.92	3.38	0.36 ± 0.81	2.82	0.36 ± 0.81	2.82	<0.001
Day 7	1.84 ± 0.94	4.96	0.84 ± 0.80	2.38	1.88 ± 1.05	5.08	1.24 ± 0.93	3.22	1.04 ± 0.79	2.68	1.04 ± 0.79	2.68	<0.001

Histopathological injury progressed substantially between Day 1 and Day 7. Epithelial nuclear vacuolization increased from 1.00 ± 0.82 to 1.84 ± 0.94, while stromal cytoplasmic vacuolization increased from 1.04 ± 0.98 to 1.88 ± 1.05. These findings indicate ongoing cellular degeneration within the epithelial and stromal layers following exposure. Endothelial injury also demonstrated temporal progression. Mean endothelial cell loss increased from 0.36 ± 0.81 on Day 1 to 1.04 ± 0.79 on Day 7. A nearly identical increase was observed for endothelial cell

vacuolization. These findings suggest that endothelial damage became more apparent during later observation periods and may represent a delayed manifestation of lidocaine toxicity. The Friedman test demonstrated a statistically significant effect of observation period on all histopathological parameters ($p < 0.001$).

Comparison of Histopathological Changes Among Lidocaine Concentrations According to Observation Period

Table 5. Comparison of Corneal Histopathological Scores Among Lidocaine Concentrations According to Observation Period

Treatment	Day 1 Mean ± SD	Rank	Day 7 Mean ± SD	Rank	p-value
BSS	0.00 ± 0.00	3.50	0.40 ± 0.55	4.30	0.283
Lidocaine 0.2%	0.60 ± 0.55	21.80	2.20 ± 0.45	23.00	0.001
Lidocaine 0.5%	1.80 ± 0.84	25.35	1.60 ± 0.55	26.25	<0.001
Lidocaine 1.0%	1.20 ± 0.45	27.05	2.60 ± 0.55	25.10	<0.001
Lidocaine 2.0%	1.40 ± 0.55	5.00	2.40 ± 0.55	5.00	<0.001

Further stratified analysis revealed that histopathological damage generally increased between Day 1 and Day 7 within lidocaine-treated groups. The control group showed minimal changes over time and no statistically significant difference ($p = 0.283$). Conversely, all lidocaine groups demonstrated significant increases in histopathological injury. The most marked increase was observed in the 1.0% lidocaine group, where the mean score increased from 1.20 ± 0.45 on Day 1 to 2.60 ± 0.55 on Day 7. Similar increases were observed in the 0.2% and 2.0% groups.

Distribution of Histopathological Severity According to Observation Period

To further characterize the progression of corneal injury following intracameral lidocaine administration, the severity distribution of histopathological changes was analyzed according to observation period. Histopathological alterations were categorized into four severity grades: no damage, involvement of 0–25% of the tissue layer, involvement of 25–50% of the tissue layer, and involvement of more than 50% of the tissue layer. The results are presented in Table 6.

Table 6. Distribution of Histopathological Severity According to Observation Period

Histopathological Change	Severity Grade	Day 1 n (%)	Day 7 n (%)
Epithelium			
Epithelial nuclear vacuolization	None	7 (28%)	3 (12%)
	0–25% of layer	12 (48%)	4 (16%)
	25–50% of layer	5 (20%)	12 (48%)
	>50% of layer	1 (4%)	6 (24%)
Stroma			
Stromal keratocyte necrosis	None	22 (88%)	10 (40%)
	0–25% of layer	0 (0%)	9 (36%)
	25–50% of layer	2 (8%)	6 (24%)
	>50% of layer	1 (4%)	0 (0%)
Stromal cytoplasmic vacuolization	None	8 (32%)	4 (16%)
	0–25% of layer	11 (44%)	3 (12%)
	25–50% of layer	3 (12%)	10 (40%)
	>50% of layer	3 (12%)	8 (32%)
Eosinophilic stromal keratocytes	None	17 (68%)	6 (24%)
	0–25% of layer	3 (12%)	9 (36%)
	25–50% of layer	4 (16%)	8 (32%)
	>50% of layer	1 (4%)	2 (8%)
Endothelium			
Endothelial cell loss	None	20 (80%)	7 (28%)
	0–25% of layer	2 (8%)	10 (40%)
	25–50% of layer	2 (8%)	8 (32%)
	>50% of layer	1 (4%)	0 (0%)
Endothelial cell vacuolization	None	20 (80%)	7 (28%)
	0–25% of layer	2 (8%)	10 (40%)
	25–50% of layer	2 (8%)	8 (32%)
	>50% of layer	1 (4%)	0 (0%)

The distribution analysis demonstrated a clear progression of histopathological injury between Day 1 and Day 7. Within the epithelial layer, mild injury predominated on Day 1, with 48% of samples showing involvement of only 0–25% of the epithelial layer. By Day 7, however, the proportion of moderate and severe injury increased substantially, with 48% of samples demonstrating involvement of 25–50% of the epithelial layer and 24% showing injury involving more than 50% of the epithelial thickness. Simultaneously, the proportion of samples without epithelial damage decreased from 28% to 12%. These findings indicate progressive epithelial degeneration following intracameral lidocaine exposure.

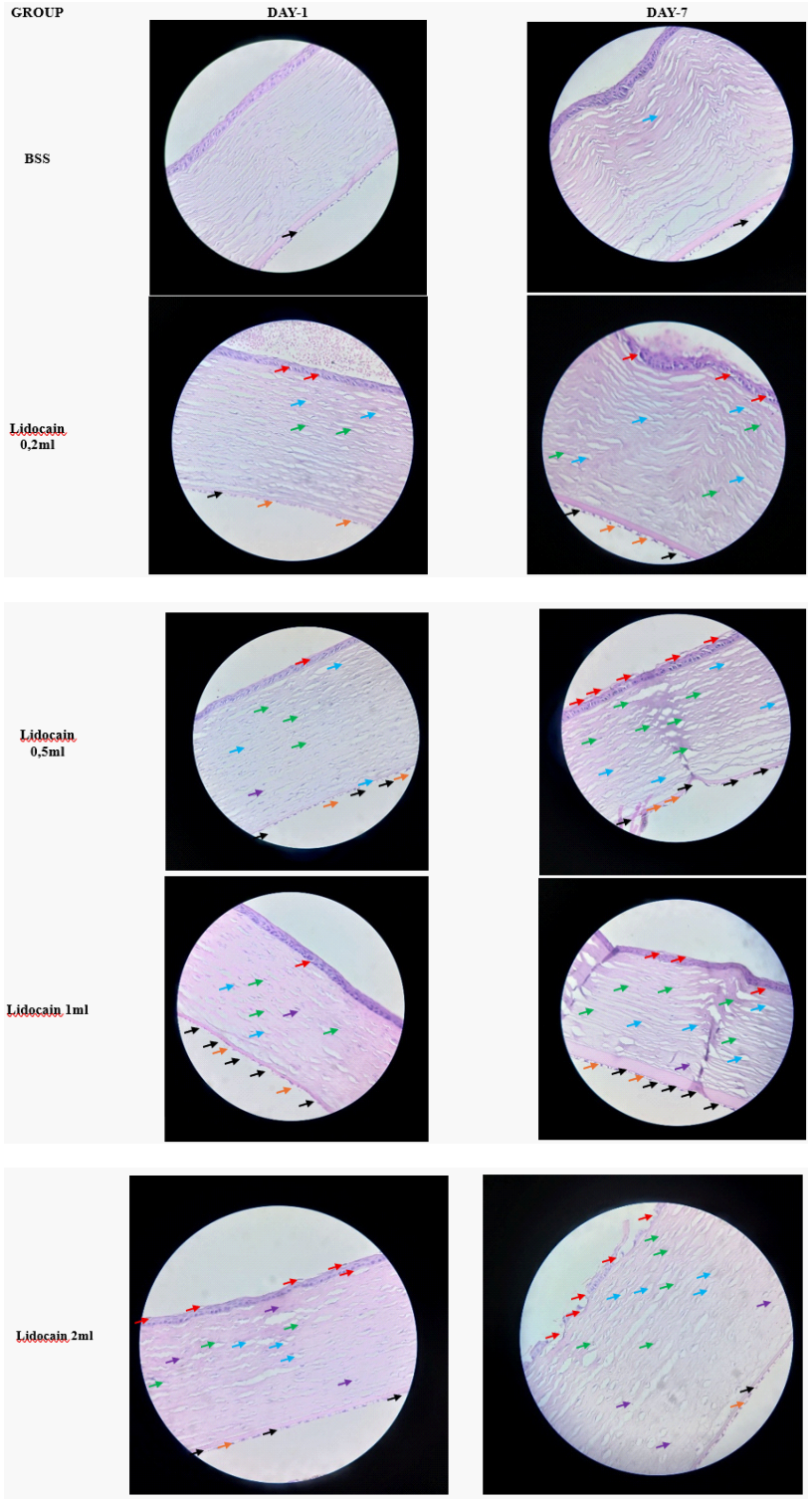

Figure 1. Histopathological examination

Within the stromal layer, stromal cytoplasmic vacuolization represented the predominant pathological alteration. On Day 1, most samples exhibited either no stromal vacuolization (32%) or mild involvement limited to 0–25% of the stromal thickness (44%). By Day 7, the proportion of moderate stromal injury increased markedly, with 40% of samples exhibiting involvement of 25–50% of the stromal layer and an additional 32% demonstrating severe injury affecting more than 50% of the stroma. Similar progression was observed for eosinophilic stromal keratocytes and stromal keratocyte necrosis, suggesting ongoing stromal cellular degeneration over time.

Endothelial alterations were less prominent during the early observation period but became increasingly apparent by Day 7. The proportion of samples without endothelial cell loss decreased dramatically from 80% on Day 1 to only 28% on Day 7. Concurrently, mild endothelial injury involving 0–25% of the endothelial layer increased from 8% to 40%, while moderate injury involving 25–50% of the layer increased from 8% to 32%. An identical pattern was observed for endothelial vacuolization. These findings indicate that endothelial injury developed progressively during the later stages of exposure and support the concept of delayed endothelial toxicity following intracameral lidocaine administration. Overall, the severity distribution analysis demonstrated a progressive shift from absent or mild injury on Day 1 toward moderate and severe injury on Day 7 across all corneal layers. These findings further support the time-dependent nature of lidocaine-induced corneal toxicity.

Histopathological Safety Profile According to Lidocaine Concentration

To provide a clinically relevant interpretation of the observed histopathological findings, the severity of corneal injury was summarized according to lidocaine concentration. This analysis integrated mean histopathological scores, severity distribution patterns, mean rank values, and statistical comparisons with the control group. The results are presented in Table 7.

Table 7. Histopathological Safety Profile According to Intracameral Lidocaine Concentration

Treatment Group	Epithelial Changes	Stromal Changes	Endothelial Changes	Difference from Control	Interpretation
BSS (Control)	Minimal	Minimal	Minimal	None	Safe
Lidocaine 0.2%	Mild	Mild	Mild	Observed primarily on Day 7	Relatively Safe
Lidocaine 0.5%	Moderate	Moderate	Moderate	Inconsistent	Safety Threshold
Lidocaine 1.0%	Moderate-to-Severe	Moderate-to-Severe	Moderate-to-Severe	Present	Unsafe
Lidocaine 2.0%	Severe	Severe	Severe	Present	Unsafe

A clear concentration-dependent increase in histopathological injury was observed across all corneal layers. The BSS control group demonstrated minimal alterations throughout the study period and served as the reference standard for normal corneal morphology. No meaningful histopathological damage was identified in the control group, confirming that the observed tissue changes were attributable to lidocaine exposure rather than the injection procedure itself. The 0.2% lidocaine group demonstrated only mild epithelial, stromal, and endothelial alterations. Histopathological abnormalities were generally limited and became more apparent during the later observation period, suggesting that this concentration was associated with a relatively favorable safety profile. Consequently, 0.2% lidocaine was categorized as relatively safe for intracameral administration. The 0.5% lidocaine group demonstrated moderate histopathological alterations involving all corneal layers. Although tissue injury was evident, the degree of damage was less severe than that observed with higher concentrations. Because the observed changes occupied an intermediate position between mild and severe toxicity, 0.5% lidocaine was considered the practical safety threshold for intracameral use. In contrast, the 1.0% and 2.0% lidocaine groups consistently demonstrated moderate-to-severe and severe injury, respectively. Histopathological alterations involved all corneal layers and were associated with statistically significant differences compared with the control group. These findings indicate substantial tissue toxicity at higher concentrations and support their classification as unsafe concentrations for intracameral administration.

Receiver Operating Characteristic Analysis

The predictive value of intracameral lidocaine concentration for clinically significant corneal toxicity was further evaluated using receiver operating characteristic (ROC) analysis.

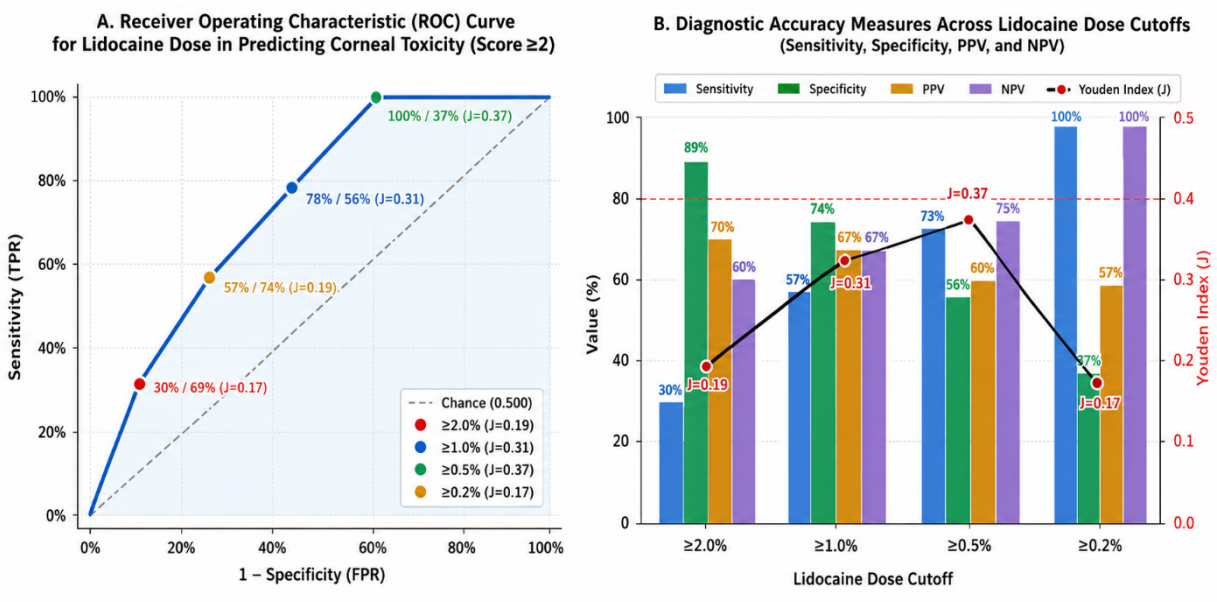

Figure 2. ROC analysis

The ROC analysis demonstrated that lidocaine concentration was a statistically meaningful predictor of histopathological corneal toxicity, yielding an area under the curve (AUC) of 0.741. According to conventional diagnostic performance criteria, this value corresponds to a fair level of discrimination. An AUC of 0.741 indicates a 74.1% probability that a randomly selected sample with clinically significant corneal toxicity would have been exposed to a higher lidocaine concentration than a randomly selected sample without significant toxicity.

The ROC findings confirmed the dose-dependent relationship observed throughout the histopathological analyses. Increasing lidocaine concentration was consistently associated with increasing severity of epithelial, stromal, and endothelial injury. Collectively, these findings suggest that concentrations exceeding the safety threshold are associated with a substantially greater risk of clinically relevant corneal toxicity. Moreover, the descriptive, comparative, severity distribution, and ROC analyses consistently demonstrated that intracameral lidocaine induces concentration-dependent and time-dependent corneal toxicity. The epithelium and stroma exhibited the earliest and most pronounced alterations, whereas endothelial injury became increasingly apparent with prolonged exposure. Based on the overall histopathological findings, 0.2% lidocaine demonstrated the most favorable safety profile, whereas 0.5% represented the upper limit of the safety threshold. Concentrations of 1.0% and 2.0% were associated with substantial corneal toxicity and were therefore classified as unsafe.

DISCUSSION

The present study demonstrated that intracameral lidocaine induced concentration-dependent and time-dependent histopathological alterations in rabbit corneal tissue. Histopathological changes were observed in all corneal layers, including the epithelium, stroma, and endothelium, with increasing severity at higher lidocaine concentrations and longer observation periods. Among the evaluated parameters, epithelial nuclear vacuolization and stromal cytoplasmic vacuolization exhibited the highest mean scores, indicating that the corneal epithelium and stroma were the earliest and most susceptible tissues affected by intracameral lidocaine exposure. In contrast, endothelial alterations were less pronounced during the early observation period but became increasingly evident by Day 7. These findings support the hypothesis that intracameral lidocaine exerts toxic effects on corneal tissue in a dose-dependent manner and that tissue injury progresses with prolonged exposure.

The most important finding of this study is that increasing intracameral lidocaine concentration was associated with progressively greater histopathological injury across all corneal layers. Compared with the BSS control group, all lidocaine-treated groups demonstrated higher histopathological scores, and statistically significant differences were observed among treatment groups. Furthermore, the severity distribution analysis revealed a progressive shift from absent or mild injury on Day 1 toward moderate and severe injury on Day 7. These observations indicate that both concentration and duration of exposure contribute substantially to corneal toxicity.

Another important finding was the differential susceptibility of corneal layers. Histopathological alterations were most prominent in the epithelium and stroma, particularly epithelial nuclear vacuolization and stromal cytoplasmic vacuolization. Endothelial injury was present but generally less severe than epithelial and stromal changes. This pattern suggests that lidocaine-induced toxicity may initially affect metabolically active superficial tissues before progressing to deeper corneal structures.

The ROC analysis further supported the concentration-dependent relationship between lidocaine exposure and corneal toxicity. An AUC value of 0.741 indicated fair discriminatory performance, confirming that increasing lidocaine concentration was associated with a progressively higher probability of clinically relevant histopathological injury. Collectively, these findings suggest that lidocaine concentration is an important determinant of corneal safety following intracameral administration.

Effects of Lidocaine on Corneal Epithelial Injury

The corneal epithelium demonstrated the highest degree of histopathological alteration among all evaluated structures. Epithelial nuclear vacuolization increased consistently with increasing lidocaine concentration and longer observation periods. The severity distribution analysis demonstrated that moderate and severe epithelial injury became substantially more common by Day 7, indicating progressive epithelial degeneration.

These findings are biologically plausible because epithelial cells are directly exposed to intracameral agents through diffusion within the aqueous humor and are highly dependent on intact cellular membrane function for maintenance of tissue homeostasis. Local anesthetics such as lidocaine exert their primary pharmacological effect through blockade of voltage-gated sodium channels; however, at higher concentrations they may also disrupt membrane integrity, impair mitochondrial function, and induce intracellular calcium dysregulation. These processes may result in cellular swelling, vacuole formation, and eventual cell death.

Previous experimental studies have similarly reported epithelial toxicity following exposure to local anesthetics. Laboratory investigations have demonstrated that lidocaine can reduce cellular viability and induce apoptosis through mitochondrial dysfunction and oxidative stress pathways. The prominent epithelial vacuolization observed in the present study may therefore represent an early morphological manifestation of these cellular injury mechanisms.

Stromal Changes Following Intracameral Lidocaine Exposure

The stromal layer exhibited substantial histopathological alterations, particularly stromal cytoplasmic vacuolization. Stromal keratocyte necrosis and eosinophilic keratocyte changes also increased with increasing lidocaine concentration and longer exposure duration. These findings suggest progressive stromal degeneration following intracameral lidocaine administration. Keratocytes play a critical role in maintaining stromal architecture and extracellular matrix integrity. Toxic injury to keratocytes may disrupt collagen organization, alter stromal hydration, and impair corneal transparency. The predominance of stromal cytoplasmic vacuolization observed in this study suggests that intracellular metabolic disturbances may occur before irreversible cellular necrosis develops.

Experimental studies have proposed several mechanisms for local anesthetic-induced stromal toxicity. These include mitochondrial membrane depolarization, reactive oxygen species generation, ATP depletion, and activation of apoptotic signaling pathways. Such mechanisms may explain the progressive increase in stromal injury observed between Day 1 and Day 7. The delayed progression of stromal damage further suggests that cellular injury continues after the initial exposure period, likely reflecting ongoing metabolic dysfunction rather than direct chemical toxicity alone.

Endothelial Toxicity and Corneal Safety

Although endothelial injury was less pronounced than epithelial and stromal alterations, progressive endothelial cell loss and endothelial vacuolization were clearly observed. The proportion of specimens without endothelial abnormalities decreased substantially from Day 1 to Day 7, indicating that endothelial toxicity became increasingly apparent during later observation periods. The corneal endothelium is particularly important because of its limited regenerative capacity. Endothelial cells maintain corneal deturgescence through active ionic transport mechanisms commonly described by the pump–leak hypothesis. Loss of endothelial cells beyond a critical threshold may compromise corneal transparency and result in persistent corneal edema.

The findings of the present study are consistent with those reported by Kadonosono and colleagues, who demonstrated ultrastructural endothelial abnormalities following intracameral lidocaine administration in rabbit eyes. Their study reported endothelial surface irregularities and loss of microvilli at higher lidocaine concentrations, whereas lower concentrations produced minimal morphological changes. Similarly, Eggeling et al. observed concentration-dependent endothelial injury, ranging from mild cellular alterations at lower concentrations to extensive endothelial necrosis at higher concentrations.

Although endothelial injury in the present study was generally less severe than epithelial and stromal alterations, its clinical significance should not be underestimated. Even modest endothelial cell loss may have important implications in patients with pre-existing endothelial dysfunction, such as those with Fuchs endothelial corneal dystrophy, pseudophakic bullous keratopathy, or reduced endothelial cell density associated with aging.

Influence of Exposure Duration

A notable finding of the present study was the significant effect of observation period on histopathological outcomes. All evaluated parameters demonstrated higher scores on Day 7 compared with Day 1. This temporal progression indicates that corneal injury continues to evolve after initial lidocaine exposure. Several mechanisms may explain this observation. Initial cellular injury may trigger secondary inflammatory and oxidative pathways that continue to damage tissue beyond the immediate exposure period. Furthermore, mitochondrial

dysfunction induced by local anesthetics may result in persistent impairment of cellular energy metabolism, leading to progressive structural degeneration. The delayed increase in endothelial abnormalities observed in this study supports the hypothesis that some toxic effects become apparent only after a period of continued cellular stress. These findings emphasize the importance of considering not only drug concentration but also exposure duration when evaluating the safety of intracameral agents.

The present findings have direct clinical relevance because intracameral lidocaine remains widely used during cataract surgery and other anterior segment procedures. The safety interpretation developed from the histopathological findings suggests that lower concentrations are associated with substantially less tissue injury. Among the evaluated concentrations, 0.2% lidocaine demonstrated only mild histopathological changes and was categorized as relatively safe. In contrast, 1.0% and 2.0% lidocaine consistently produced moderate-to-severe and severe injury, respectively, indicating a substantially higher risk of corneal toxicity. The 0.5% concentration appeared to represent the practical upper safety threshold, producing moderate but not uniformly severe tissue damage. These findings suggest that minimizing lidocaine concentration whenever clinically feasible may reduce the risk of corneal injury while preserving anesthetic efficacy. Nevertheless, extrapolation of these experimental findings to human clinical practice should be undertaken cautiously because species differences, surgical conditions, aqueous humor dynamics, and postoperative healing responses may influence toxicity profiles.

Strengths and Limitations

The present study possesses several strengths. First, multiple lidocaine concentrations were evaluated, allowing characterization of dose-dependent toxicity. Second, histopathological assessment included all major corneal layers, providing a comprehensive evaluation of tissue injury. Third, the inclusion of two observation periods enabled assessment of temporal progression and demonstrated the importance of exposure duration. Several limitations should also be acknowledged. The study was conducted in a rabbit model, and therefore direct extrapolation to human eyes may be limited. Histopathological evaluation was performed using light microscopy without ultrastructural assessment by transmission electron microscopy. Additionally, endothelial cell density measurements and molecular biomarkers of apoptosis, oxidative stress, and inflammation were not evaluated. Future studies incorporating specular microscopy, immunohistochemistry, and molecular analyses may provide further insight into the mechanisms underlying lidocaine-induced corneal toxicity.

Future investigations should evaluate endothelial cell density, apoptotic markers, oxidative stress pathways, and inflammatory mediators following intracameral lidocaine exposure. Comparative studies involving alternative intracameral anesthetics and larger animal models may further clarify the optimal balance between anesthetic efficacy and corneal safety. Clinical studies evaluating endothelial outcomes after exposure to low-concentration intracameral lidocaine would also be valuable for translating these experimental findings into clinical practice.

CONCLUSION

This study demonstrated that intracameral lidocaine induces concentration-dependent and time-dependent histopathological injury in rabbit corneal tissue. The epithelium and stroma exhibited the earliest and most pronounced alterations, while endothelial injury became increasingly evident with prolonged exposure. Increasing lidocaine concentration was consistently associated with greater tissue damage, and ROC analysis confirmed a significant relationship between concentration and corneal toxicity. Based on the overall histopathological findings, 0.2% lidocaine exhibited the most favorable safety profile, whereas 0.5% represented the practical safety threshold. Concentrations of 1.0% and 2.0% produced substantial corneal toxicity and should be used with caution when considering intracameral administration.

Ethics Approval and Consent to Participate

The study protocol was approved by the Health Research Ethics Committee of Hasanuddin University, Makassar, Indonesia (Approval No. [insert approval number]). All experimental procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and complied with institutional guidelines for animal welfare.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' Contributions

VYCIO conceptualized the study, conducted the experiments, collected the data, performed the statistical analysis, and drafted the manuscript. MAI and HSM supervised the research process, contributed to study design, and critically revised the manuscript. BB contributed to statistical analysis, interpretation of data, and manuscript revision. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the Laboratory of Experimental Animals, Faculty of Medicine, Hasanuddin University, for technical assistance during the

experimental procedures. The authors also acknowledge the Department of Anatomical Pathology for assistance with histopathological processing and evaluation.

ABBREVIATIONS

ATP	Adenosine Triphosphate
AUC	Area Under the Curve
ARVO	Association for Research in Vision and Ophthalmology
BSS	Balanced Salt Solution
H&E	Hematoxylin and Eosin
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SD	Standard Deviation

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