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SCIENTIFIC ARTICLE

# The effect of intra-articular levobupivacaine on shoulder cartilage at different doses—experimental study

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**KEYWORDS**
Glenohumeral joint;
Chondrolysis;
Levobupivacaine

**Abstract**
*Background and objectives:* In this study it was aimed to examine the histological and morphometric effects on cartilage structure of intra-articular application of levobupivacaine to the shoulder joint.
*Methods:* In twenty New Zealand adult male rabbits, 35 shoulders were used for the study and prepared in 5 groups of 7. These groups were defined as Groups L1, L2, L3 and L4 which were right shoulders administered with 0.25% and 0.5% levobupivacaine, Group C which were left shoulders as the control group and Groups S1 and S2 which were left shoulders administered with 0.9% saline. On the 2nd and 15th days the animals were killed, the glenohumeral joints were evaluated macroscopically then cartilage samples were taken. These samples were evaluated with Mankin score, and histomorphometrically by measuring the thickness of the cartilage between the superficial cartilage layer and the tidemark and the thickness of calcified cartilage between the tidemark and the subchondral bone.
*Results:* Macroscopically, on the 15th day the joint fluid was seen to have reduced in all the groups. After microscopic evaluation, the highest Mankin score (mean: $3.14 \pm 2.1/14$) was in the L4 group (15th day 0.5% levobupivacaine) and was found to be statistically significant ($p < 0.05$). No statistically significant difference was determined between the other groups.

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*Conclusions:* Histologically, as the highest Mankin score was in the L4 group, this indicates that in a single intra-articular injection of levobupivacaine a low concentration should be selected.
*Level of evidence:* Level 5, animal study.


**PALAVRAS-CHAVE**
Articulação glenoumeral;
Condrólise;
Levobupivacaína

**O efeito de levobupivacaína intra-articular na cartilagem do ombro em doses diferentes—estudo experimental**

**Resumo**
*Justificativa e objetivo:* Neste estudo o objetivo foi examinar os efeitos histológicos e morfométricos sobre a estrutura da cartilagem da aplicação intra-articular de levobupivacaína em articulação do ombro.
*Métodos:* Trinta e cinco ombros de 20 coelhos New Zealand, machos e adultos, foram usados para o estudo e divididos em cinco grupos de sete. Os grupos foram definidos como L1, L2, L3 e L4, consistindo em ombros direitos nos quais levobupivacaína a 0,25% e 0,5% foi administrada; o Grupo C, consistindo em ombros esquerdos foi o grupo controle; grupos S1 e S2, consistindo em ombros esquerdos receberam solução salina a 0,9%. Os animais foram sacrificados no segundo e no décimo quinto dia; as articulações glenoumerais foram avaliadas macroscopicamente e, em seguida, amostras de cartilagem foram coletadas. As amostras foram avaliadas com o escore de Mankin e, histomorfometricamente, medindo-se a espessura da cartilagem entre a camada superficial e a ''linha de maré'' (*tidemark*) e a espessura da cartilagem calcificada entre a *tidemark* e o osso subcondral.
*Resultados:* Macroscopicamente, observou-se no décimo quinto dia que o líquido articular havia reduzido em todos os grupos. Após a avaliação microscópica, o maior escore de Mankin (média: $3{,}14 \pm 2{,}1/14$) foi observado no grupo L4 (15° dia levobupivacaina a 0,5%), considerado estatisticamente significativo ($p < 0{,}05$). Nenhuma diferença estatisticamente significativa foi determinada entre os outros grupos.
*Conclusões:* Histologicamente, como o maior escore de Mankin foi observado no Grupo L4, isso indica que em uma única injeção intra-articular de levobupivacaína, uma concentração baixa deve ser selecionada.
*Nível de evidência:* Nível 5, estudo em animais.


## Introduction

Despite better current understanding of pain mechanisms and recorded developments in treatment, clinicians remain inadequate in the management of postoperative pain control and the majority of patients have complaints of pain following surgery.[1] Insufficient pain treatment is one of the elements affecting the recovery process of the patient and by extending the hospital stay, has a negative effect on morbidity and mortality rates. The most important aim in the treatment of postoperative pain is to provide effective analgesia without causing any serious side effects. Therefore, to avoid side effects of high doses of morphine, which is the gold standard, pre-emptive analgesia, peripheral nerve blocks, wound site infiltration and multimodal analgesia programmes have been developed. In this context, intra-articular injections are one of the most popular techniques.

The use of local anaesthetic by intra-articular single injection or continuous infusion are widely used pain management methods in shoulder and knee joint surgery.[2,3] They are used to obtain both perioperative analgesia and local and regional anaesthesia.[4–7] The local anaesthetic which is most frequently used intra-articularly is bupivacaine.[8] The injection of different doses of intra-articular bupivacaine has been shown to be an effective local anaesthetic agent in intraoperative anaesthesia and post-operative analgesia.[4,9–12] However, in in vitro studies, there has been reported to be a chondrotoxic effect of bupivacaine associated with dose and time and this has been the reason for its limited use.[13–17]

In previous voluntary human trials, levobupivacaine has been shown to be as effective as bupivacaine providing a longer and strong analgesic effect and with lower cardiac and central nervous system toxicity.[18–21] However, there are a limited number of studies showing the intra-articular use of levobupivacaine in shoulder and knee arthroscopy.[22–24]

It was aimed in this study to examine histologically and morphometrically, the effects on the cartilage structure of

the intra-articular application to the shoulder joint of levobupivacaine.

## Materials and methods

Approval for the study was granted by Local University Animal Experiments Ethics Committee. The study was conducted at Local University Morphology Campus Animal Laboratory using 20 New Zealand adult male rabbits, aged 12–15 months and with a mean weight of 3 kg. Using both shoulders of the rabbits, 35 shoulders were prepared in 5 groups of 7. The grouping was made blind and after the procedure, the ears were labelled with the group letter and number, then the animals were housed 2 to a cage. The right shoulders of the rabbits were defined as Group L receiving 0.25% and 0.5% levobupivacaine, the left shoulders as the control group, Group C and those receiving 0.9% saline, Group S.

The levobupivacaine and saline groups were subdivided on Day 2 (0.25% and 0.5% levobupivacaine as Groups L1 and L2 and Group S1) and on Day 15 (0.25% and 0.5% levobupivacaine as Groups L3 and L4 and Group S2) for the taking of necropsy material. As there was no previous data to be taken as a reference defining the capsular volume of the rabbit glenohumeral joint or showing how and at what dosage levobupivacaine should be administered to rabbits, the clinical and experimental studies of bupivacaine on rabbits were taken into consideration and the amount to be administered was calculated as 0.5 mL for 3 kg.[15,25]

### Surgical procedure

After 6 h fasting, sedoanalgesia was obtained in the rabbits through intramuscular administration of xylazine hydrochloride (5 $mg \cdot kg^{-1}$) and ketamine hydrochloride (5 $mg \cdot kg^{-1}$). After sterile preparation, the glenuhumeral joint was located by palpation. With a 38 G insulin injector, firstly the joint space was confirmed by aspiration of the intra-articular fluid, then the experimental agent was injected into the joint space. The previously defined doses of 0.25% levobupivacaine were administered to the right shoulders of 10 rabbits and 0.5% levobupivacaine to the right shoulders of the other 10 rabbits. To the left shoulders of 10 rabbits, 0.9% NaCl was administered at the defined dose. The rabbit shoulders were then separated into 7 groups of 5 for the taking of necropsy material at 2 and 15 days after the procedure.

### Macroscopic analysis and cartilage sampling

The rabbits were sacrificed on the 2nd or 15th day by intraperitoneal high dose anaesthetic agent (sodium thiopental 150 $mg \cdot kg^{-1}$) and the glenohumeral joints were opened. After macroscopic evaluation of the surface and colour of the glenohumeral joint cartilage and amount of intra-articular synovial fluid, cartilage samples were taken from the humerus head with a 4 mm diameter, sharp-ended trocar, which is used for cartilage transfer in orthopaedic surgery (mosaicplasty). The purpose of using this was not to cause any iatrogenic damage to the cartilage field.

**Table 1** Histology histopathology grading system.

| | |
|---|---|
| *1. Structure* | |
| (A) Normal | 0 |
| (B) Surface irregularities | 1 |
| (C) Pannus and surface irregularities | 2 |
| (D) Clefts to transitional zone | 3 |
| (E) Clefts to radial zone | 4 |
| (F) Clefts to calcified zone | 5 |
| (G) Complete disorganisation | 6 |
| *2. Cells* | |
| (A) Normal | 0 |
| (B) Diffuse hypercellularity | 1 |
| (C) Cloning | 2 |
| (D) Hypocellularity | 3 |
| *3. Safranin-O staining* | |
| (A) Normal | 0 |
| (B) Slight reduction | 1 |
| (C) Moderate reduction | 2 |
| (D) Severe reduction | 3 |
| (E) No dye noted | 4 |
| *4. Tidemark integrity* | |
| (A) Intact | 0 |
| (B) Crossed by blood vessels | 1 |

Mankin HHGS score is the sum of structure, cells, Safranin-O staining and tidemark integrity.

### Histological analysis

The samples of cartilage tissue taken were placed in 10% buffered formalin solution for examination by light microscope. After 48 h fixation in the solution, the tissue samples were decalcified in a controlled decalcification solution prepared at the ratio of 1:1 of 8% formic acid and 8% hydrochloric acid solutions. Following the decalcification process, tissue samples were dehydrated through a graded series of ethanol and embedded in parafin. The 7 $\mu$m sections taken from the paraffin blocks were stained with haematoxylin–eosin (H&E) for routine examination and stained with Giemsa and Gomori's single stage trichome for evaluation of the cartilage extracellular matrix. The sections were analysed and photographed with light microscopy (Leica DM 3000 photomicroscope) for histomorphological structure.

For histomorphometric measurements H–E stained sections from each animal were selected. Three fields were chosen randomly on each section and by using an ocular micrometre at 10× magnification, the cartilage thickness between the superficial cartilage layer and the tidemark and the calcified cartilage thickness between the tidemark and the subchondral bone were measured. To achieve standardisation, the measurements were taken parallel to the cartilage cell columns. In each section, the number of tidemarks was stated. All sections were evaluated by 2 experienced histologists blinded to the groups according to the histological/histochemical grading system (HHGS), the Mankin's Scoring System, and the points of the Mankin score were determined (Table 1).

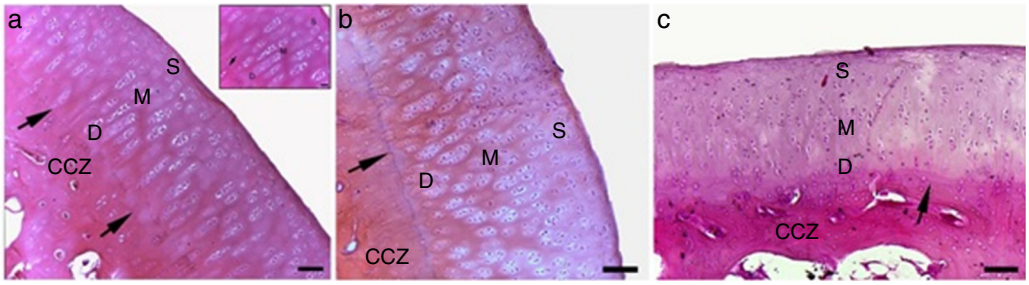


**Figure 1** Photomicrograph of articular cartilage from the control (a), S1 (b) and S2 (c) groups. S, superficial zone; M, middle zone; D, deep zone; CCZ, calcified cartilage zone; arrow, tide mark, H&E, 10× obj., bars: 100 μm (inlet 40×, bar: 30 μm).

### Statistical analysis

All the data were compared between the groups separately using the Kruskal–Wallis test. The cartilage thickness between the superficial layer and the tidemark and the calcified cartilage thickness between the tidemark and the subchondral bone were statistically analysed with the Anova test. A value of $p < 0.05$ was accepted as statistically significant.

## Results

### Macroscopic evaluation

The appearance of the glenohumeral joint exposed during the experiment was examined. Macroscopically, in all the groups, the joint surface was regular and no abnormal colour changes were observed. The intra-articular synovial fluid was clear in consistency but the amount was small. When the groups were compared, the intra-articular fluid in the shoulders of the rabbits on which 15th day necroscopy was applied, was seen to have reduced to what could be described as none.

### Microscopic evaluation

In the control group (Group C) and the groups to which 0.5 mL saline was administered intra-articularly (Groups S1–2), the appearance was normal in the cartilage cells and the structure of the hyalin cartilage superficial, middle (transitional) and deep (radial) layers and calcified cartilage zone in the examined joint cartilage sections. The tidemark (the transit area line) was observed to be uninterrupted and dark basophilic stained (Fig. 1A–C).

In the group to which 0.25% levobupivacaine was administered and 2nd day necropsy was applied (Group L1), mild irregularity on the cartilage surface, an increased number of tidemarks and chondrocyte hypertrophy in the middle layer were observed and in the group to which 0.5% levobupivacaine was administered (Group L2), there were scattered breaks in the tidemark and irregularity in the column of chondrocytes in the deep layer (Fig. 2A and B). An increase was seen in the Mankin score of both groups compared to the control group. When the characteristics stained with Giemsa were examined, there was observed to be a reduction in cartilage extracellular matrix staining at both doses compared to the control group. In the trichrome stained preparates, in the group to which 0.5% levobupivacaine was administered (Group L2) staining was shown in the extracellular matrix structure in the calcified cartilage area (Fig. 3A–D).

In the group to which 0.25% levobupivacaine was administered and 15th day necropsy was applied (Group L3), there was irregularity on the cartilage surface and hypocellularity and in the group to which 0.5% levobupivacaine was applied (Group L4), there was observed to be an increase in the number of tidemarks and a reduction in the number of cells in the middle and deep layers (Fig. 4). When the characteristics were examined with Giemsa and trichrome staining, there was a reduction in cartilage extracellular matrix staining at both doses compared to the control group and fissures were observed extending from the surface as far as the transit

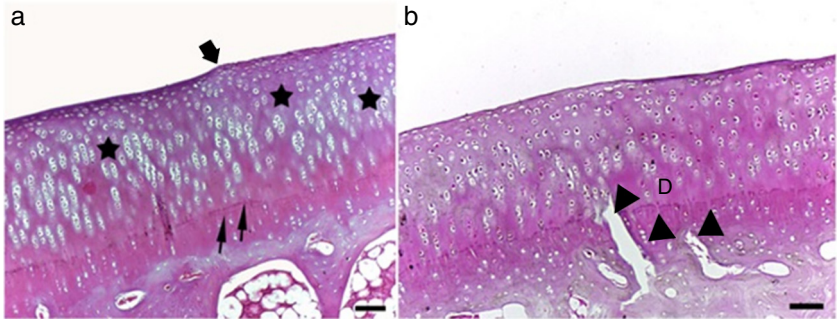


**Figure 2** Photomicrograph of articular cartilage from the L1 (a), L2 (b) groups. (a) Thick arrow, irregularity on the cartilage surface; stars, chondrocyte hypertrophy in the middle layer; arrows, tide mark. (b) D, irregular organisation of chondrocytes in the deep layer; arrowheads, interruptions in the tide mark, H&E, 10× obj., bars: 100 μm.

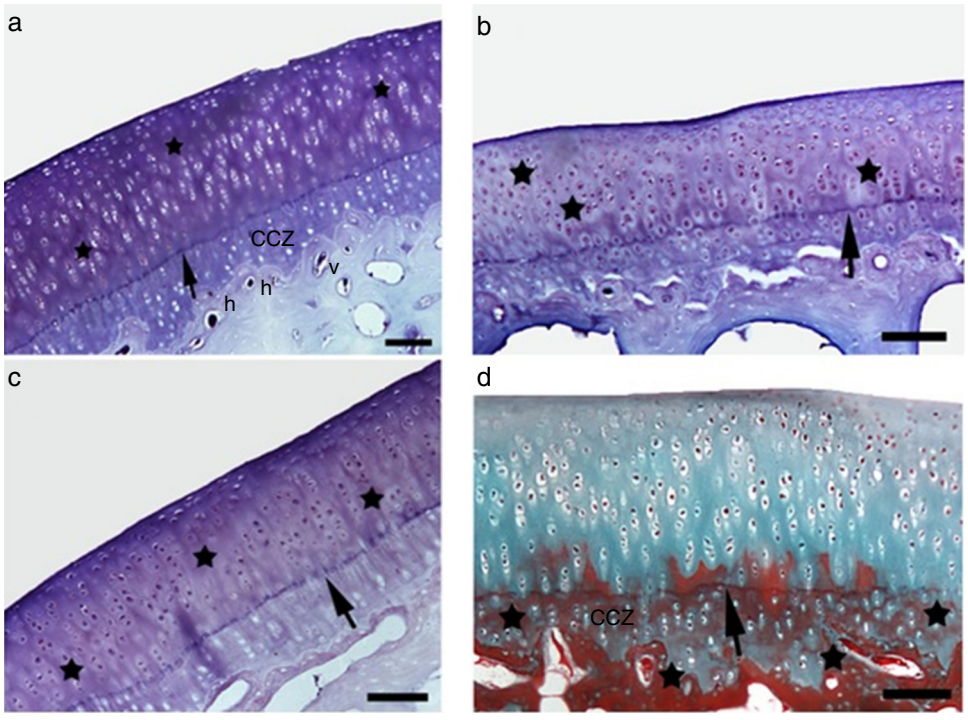


**Figure 3** Extracellular matrix staining of articular cartilage from the C (a), L1 (b), L2 (c and d) groups. (a) Arrow, tide mark; stars, normal staining of extracellular matrix; CCZ, calcified cartilage zone; h, havers canal; v, valkmann canal. (b and c) Stars, reduction in cartilage extracellular matrix staining, Giemsa. (d) Stars, extracellular matrix structure in the calcified cartilage area, Gomori's trichrome stain, 10× obj., bars: 100 μm.

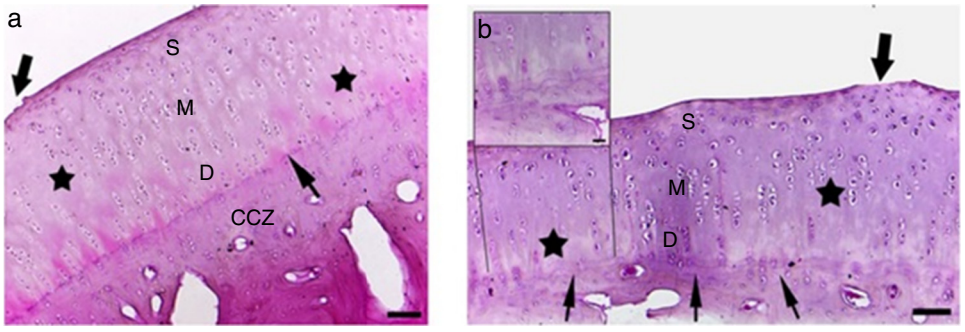


**Figure 4** Photomicrograph of articular cartilage from the L3 (a), L4 (b) groups. S, superficial zone; M, middle zone; D, deep zone; CCZ, calcified cartilage zone; arrows, tide mark; stars, hypocellularity; thick arrow, surface irregularity, H&E, 10× obj., bars: 100 μm (inlet: multiple tide marks, 40× obj., bar: 30 μm).

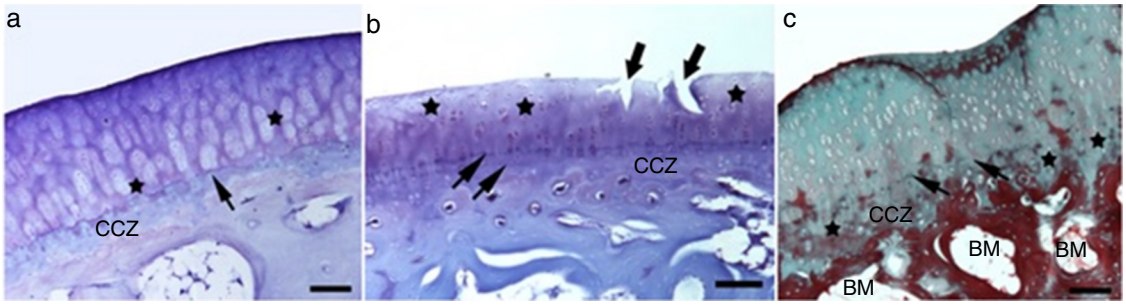


**Figure 5** Extracellular matrix staining of articular cartilage from the L3 (a), L4 (b and c) groups. (a and b) CCZ, calcified cartilage zone; arrows, multiple tide marks; stars, reduction in cartilage extracellular matrix staining; thick arrow, superficial fissures, Giemsa, 10× obj., bars: 100 μm. (c) Stars, calcified cartilage area showing extracellular matrix staining; BM, bone marrow, Gomori's trichrome stain, 10× obj., bars: 100 μm.

**Table 2** Histopathological data and statistical analysis of the groups.

| | Control | 0.25% levobupivacain | | 0.5% levobupivacain | | Saline 0.5 mL | | *p*-value |
|---|---|---|---|---|---|---|---|---|
| | | 2 day (L1) | 15 day (L3) | 2 day (L2) | 15 day (L4) | 2 day (S1) | 15 day (S2) | |
| | | Mean ± SD | | | | | | |
| Mankin score (0–14) | 0.86 ± 0.378 | 3.57 ± 1.134 | 4.43 ± 1.718 | 3.86 ± 1.215 | 5 ± 1.155[a] | 0.86 ± 1.069 | 3.14 ± 2.193 | |
| SL-TM[b] (μm) | 280 ± 68.07 | 260 ± 75.41 | 272 ± 45.11 | 221 ± 56.02 | 282 ± 97.30 | 276 ± 55.60 | 222 ± 49.29 | n.s. |
| TM-SCB[c] (μm) | 90 ± 18.70 | 73 ± 24.67 | 79 ± 30.20 | 98 ± 13.19 | 88 ± 13.61 | 96 ± 10.42 | 71 ± 18.40 | n.s. |
| No of TM | Median (min–max) 1 (1– 4) | Median (min–max) 2 (1– 4) | Median (min–max) 3 (2– 5) | Median (min–max) 3 (1– 4) | Median (min–max) 2 (1– 5) | Median (min–max) 1 (1– 4) | Median (min–max) 3 (2– 5) | n.s. |

[a] The highest Mankin score was seen in Group L4 ($p < 0.05$).
[b] Cartilage thickness between the superficial layer (SL) and the tidemark (TM).
[c] Calcified cartilage thickness between the tidemark (TM) and the subchondral bone (SCB).

region. In the trichrome stained preparates, in the group to which 0.25% levobupivacaine was administered (Group L3) there was reduced staining in the extracellular matrix in the surface cartilage area and hypocellularity. In the group to which 0.5% levobupivacaine was administered (Group L4) staining was shown in the extracellular matrix structure in the calcified cartilage area (Fig. 5). An increase was seen in the Mankin score in both groups compared to the control group and the highest score was seen to be in Group L4 ($p < 0.05$) (Figs. 4 and 5) (Tables 1 and 2).

The Mankin score was higher in Groups L1, L2 and L3 than in the control group and Group S1 and this was statistically significant ($p < 0.05$). The Mankin score in Group S1 was a similar value to that of the control group. In the histological examination in Group S2, the Mankin score was found to be at a statistically high value compared to that of the control group ($p < 0.05$).

No statistically significant difference was determined between the groups in the comparison of the mean values of cartilage thickness between the superficial layer and the tidemark and the calcified cartilage thickness between the tidemark and the subchondral bone ($p > 0.05$).

No statistically significant difference was determined in the comparison between the control and study groups in terms of tidemark numbers ($p > 0.05$).

## Discussion

Although perioperative intra-articular local anaesthesia administration is thought to be safe, in recent years, there have been many studies examining the effects of local anaesthetics on joint cartilage.[13–17,25–29] Previous studies have been oriented to draw the attention of orthopedists and anesthesiologists to the potential damage which could occur in the joint cartilage from the intra-articular use of local anaesthesia either alone or with other medications and as a single injection or as a continuous infusion. As a result of a review by Piper et al. of chondrotoxicity of local anaesthetics, it was reported that high doses should be avoided and the results of a single intra-articular injection were not clear and there was a need for further studies.[25]

In a study by Baker et al., the 24 h effect of in vitro different doses of bupivacaine, ropivacaine and levobupivacaine was examined in human chondrocyte culture and it was reported that a significantly increased level of chondrocyte damage was seen at high doses of the local anaesthetic groups. It was reported that the chondrotoxic effect was dose-dependent.[27] Similarly, in a study by Güngör et al., different concentrations of levobupivacaine and bupivacaine were added to rat cartilage cell cultures and after 48 h the chondrotoxic effect was reported to be dose-dependent.[28] In the current study, parallel to both of those previous studies, it was observed that in groups administered with different concentrations of levobupivacaine as a single intra-articular injection, at both the 2nd and the 15th day, the cartilage was affected.

The Mankin score was significantly high in Group L4. Different from the previous studies, in Group L4, histologically there was seen to be the formation of fissures extending to the mid transit layer, a hypocellular appearance, tidemark irregularity, a mild and moderate degree of loss of staining in the cartilage extracellular matrix. As there was seen to be greater degeneration of the joint cartilage, it was thought that the chondrotoxic effect could continue over time. Taking into consideration that cartilage damage may develop over time with the use of intra-articular levobupivacaine, this finding shows also the necessity of using a low dose. In support of the findings of our study, Molinos et al. have considered that the chondrotoxic effect of levobupivacaine was time-dependent, so following total knee replacement surgery, samples taken from human cartilage were exposed for 15, 30 and 60 min to 0.5% levobupivacaine, 0.5% bupivacaine and 0.5% physiological saline in cell cultures. The greatest chondrotoxic effect as observed to occur after 1 h associated with exposure to levobupivacaine.[29]

In our study, macroscopic observation of reduced synovial fluid in all the study groups, including saline, it was

considered that diminished cartilage nutrition due to the fluid dilution may be the toxic effects of levobupivacaine and this effect would become more evident in the late stage. Hyalin cartilage, which covers the surface of synovial joints, is an avascular structure dependent on synovial fluid for metabolic needs. Any fluid administered into the joint space can change the composition of synovial fluid or dilute it and thus in this situation the joint cartilage nutrition is disrupted. As the knee joint cartilage is thick and the intra-articular cavity is wide, there is a lower possibility of impaired nutrition of the joint cartilage which would occur due to pressure and fluid dilution. Because of the anatomical and histological structure of the shoulder joint and cartilage, it is thought that it could be more greatly affected by a single intra-articular injection of high-dose local anaesthetic.

As no statistically significant difference was found in the measurements of the cartilage layers compared to those of the control group, it can be thought that there was no change related to the cartilage layer thickness. The values were similar to those reported in literature in studies related to the calcified cartilage layer.[30]

Although main limitation of our study is that the choosen animal model does not represent the clinical situation, as we deal with injured or diseased shoulders.

In our animal study it has been shown that, a single intra-articular injection of levobupivacaine a low concentration and low amount of total intra-articular fluid should be preferred in consideration of long-term chondrotoxic effects.

## Summary

Although further studies must be done for final recommendations, according to our study, clinical use of single dose intra-articular levobupivacaine should be choosen at low doses for artroscopic shoulder surgery.

## Conflicts of interest

The authors declare no conflicts of interest.

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