EXPERIMENTAL STUDY

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The effect of dexketoprofen trometamol on the healing of diaphysis fractures of rat tibia

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Objective: The aim of this study was to analyze the effect of dexketoprofen trometamol, a non-steroidal anti-inflammatory drug, on fracture healing.

Methods: Closed tibia fracture was created in the right tibia of 60 male Wistar albino rats. Fixation was achieved by closed reduction and 0.5 mm intramedullary nails. Intramuscular dexketoprofen trometamol was administered at a dose of 5 mg/kg daily to the 30 rats in the study group. Rats were sacrificed in groups of 10 at the 2nd, 4th, and 6th weeks following the fracture. Fracture healing was compared mechanically, radiologically, and histopathologically between the groups.

Results: There was no statistically significant difference between the study and control groups in terms of mean values of radiological or histopathological scores at the 2nd, 4th and 6th weeks ($p>0.05$). Biomechanical evaluation could not be conducted in all rats in the study and control groups at the 2nd week due to early stage fracture healing. Mean biomechanical examination values were not statistically significant at the 4th and 6th weeks between the study and control groups ($p>0.05$).

Conclusion: No radiological, biomechanical, and histological effects were detected in the healing of closed fractures of the tibia fixed with intramedullary nail with the long-term use of dexketoprofen trometamol. Dexketoprofen trometamol may be used in patients undergoing surgical fixation for traumatic fractures, taking into account other drugs administered together.

Key words: Animal experiment; closed fracture; dexketoprofen trometamol; fracture healing; tibial fracture.

In addition to traffic and occupational accidents, the number of fractures has increased in the population in parallel with the increase in the elderly population and the problems accompanying fracture healing. Non-steroidal anti-inflammatory drugs are cheap and effective drugs widely used in the treatment of pain, edema, and heterotopic ossification accompanying trauma or degenerative changes. Non-steroidal anti-inflammatory drugs should be avoided due to their negative effects in situations where healing depending on biological process is desired, such as fractures and cementless arthroplasties. Although it is generally accepted that non-steroidal anti-inflammatory drugs have negative effects on the healing of the mesenchymal tissue, their effects on healing of fractures may be different.[1]

Studies on fracture healing investigate drugs that have either negative effects on fracture healing or lack of any effect. While indomethacin, aspirin, ibuprofen,

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piroxicam, tenoxicam, flunixin, ketorolac and diclofenac are molecules with negative effects on fracture healing, various studies have indicated that tramadol and paracetamol do not have any negative effects.[2,3] Naproxen in high doses can exert inhibitory effects on the healing of fractures.[4] Therefore, molecules with such inhibitory effect, such as indomethacin, may be frequently used to suppress increased osteoblastic activity such as in heterotrophic ossification.[5]

Dexketoprofen trometamol, a non-steroidal anti-inflammatory drug, has attracted attention for its use in pain management when required starting at the immediate postoperative period. Additionally, the drug's ability to be used for a longer duration is due to its strong analgesic effects and the fact that it does not prolong bleeding time. Dexketoprofen trometamol comes in parenteral, oral, and topical forms. To our knowledge, no previous studies on the effects of dexketoprofen trometamol on fracture healing exist in the literature.

This study aimed to investigate the effects of intermediate and long-term use of dexketoprofen trometamol starting at the first day of the healing of tibia fractures produced and fixated with intramedullary nails in rats.

Materials and Methods

Permission for the study was obtained from the Kahramanmaras Sütçü Imam University Ethics Committee and performed in an experimental investigation laboratory. The study included 60 Wistar albino male rats (mean age: 2.9 months, range: 2.5 to 3.2 months; mean weight: 190 g, range: 172 to 213 g). Animals were randomly and equally divided into control and study groups. These groups were divided into three sub-groups, for a total of six groups of ten animals in each cage. Rats were monitored for 48 hours preoperatively under laboratory conditions. Water and standard feed were given throughout the study. Animals were monitored at a temperature of 22°C and exposed to light for 12 hours and dark for 12 hours.

Intraperitoneal injection of 50 mg/kg of ketamine hydrochloride (Ketalar flacon, Parke Davis, Istanbul, Turkey) was administered for anesthesia. Anesthesia depth was monitored according to the response given to squeezing of the skin of the rat at 5-minute intervals. After local cleaning of the area with a betadine solution, rats were covered with sterile green dressings. An incision of 1 cm was made anteriorly to the upper end of the right tibia, the skin and the subcutaneous tissues were passed and the tibia plateau was exposed with the aid of hemostatic forceps. At the anterior surface of the tibia plateau, a dental needle tip of 0.3 mm (12-gauge) was advanced inside the medulla as a guide wire and placed. After the production of a tibia body fracture according to the three-point bending principle, the fracture was examined manually. Fractures were simple fractures composed of two main parts. The dental needle tip was cut and intramedullary fixation was achieved by advancing black injector needles of 0.5 mm (20-gauge) through the dental needle. The black injector needle tip was cut and embedded towards the bone by the help of a clamp without disturbing the skin. The incision was sutured using 4/0 silk. Formed fractures were confirmed radiologically immediately following clinical examination. The rats in which segmental and open fractures developed were excluded from the study (Fig. 1).

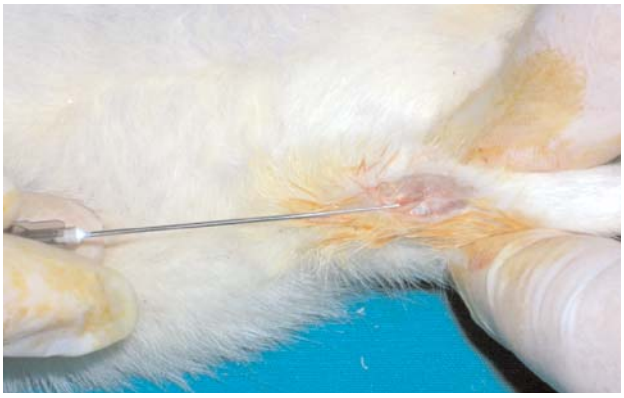

Fig. 1. A tibia body fracture was formed on the anterior surface of the tibia plateau according to the three-point bending principle by a dental needle tip of 0.3 mm (12-gauge) used as an intramedullary guide. After cutting the dental needle tip, the black injector needle of 0.5 mm (20-gauge) was advanced through the dental needle for intramedullary fixation. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

No antibiotic prophylaxis was administered during and after the surgical procedure. One rat died in the control group at the 4th postoperative week. During the follow-up, 2 rats in the control group were excluded from the study due to the development of osteomyelitis in the 2nd and 6th weeks. Sub-groups were labeled 1A, 1B, 1C, 2A, 2B and 2C (Table 1). Five mg/kg/day

Table 1. The distribution of rats in study and control groups.

	Group name	Number of rats	End date
Group 1 (study)	1A	9*	2nd week
Fracture+IM fixation+drug	1B	10	4th week
	1C	9*	6th week
Group 2 (Control)	2A	10	2nd week
Fracture + IM fixation	2B	9*	4th week
	2C	10	6th week

*These three test subjects were excluded from the study due to the development of osteomyelitis in one rat in both study groups at 2nd and 6th weeks and due to the death of one rat after the operation in the control group at the 4th week. IM: intramedullary.

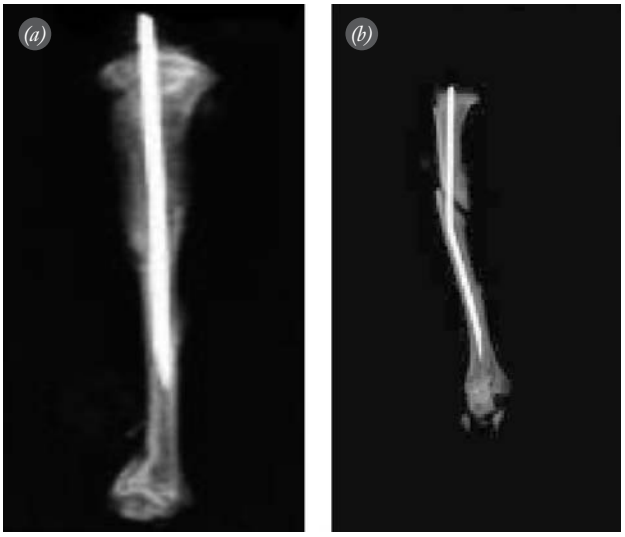

Fig. 2. Radiographies of **(a)** the control and **(b)** study groups at the 2nd week.

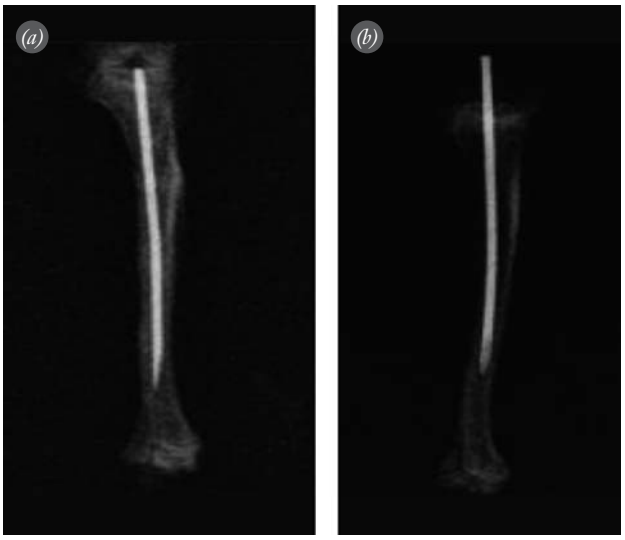

Fig. 3. Radiographies of the **(a)** control and **(b)** study groups at the 4th week.

dexketoprofen trometamol (Arveles® ampule 50 mg/2 ml; I.E. Ulagay, Istanbul, Turkey) was administered intramuscularly to groups 1A (10 rats), 1B (10 rats) and 1C (10 rats) starting on the day of surgery.[6] Dexketoprofen was administered for 2 weeks in Group 1A, for 4 weeks in Group 1B, and 6 weeks in Group 1C. All injections were administered in the left inguinal area with an insulin injector by the same person. No injections were made on rats in groups 2A (10 rats), 2B (10 rats) or 2C (10 rats). Sacrifice through cervical dislocation was performed on rats at the end of the 2nd week in groups 1A and 2A, at the end of the 4th week groups 1B and 2B, and at the end of the 6th week in groups 1C and 2C. The right tibias were disarticulated at the knee joint. Soft tissues over the tibia were properly scraped from the bone by a specialist pathologist using routine histopathological procedures without harming the callus tissue. All right tibias were radiologically, histopathologically and biomechanically examined.

For radiological evaluation, direct radiographs were taken with the feet placed anteroposteriorly at a distance of 105 cm with a conventional radiography device (Siemens) and magnified 100% (Figs. 2-4). A single cassette was used for each group. Radiographs were evaluated biweekly by the same orthopedist according to the Lane-Sandhu classification blinded to the groups (Table 2).[6]

Samples were taken from the area of fracture for histopathological evaluation. Bone tissue samples were held in 5% formic acid after being fixated in 10% neutral formaldehyde. After routine histopathological preparation, materials were placed into a paraffin complex and divided into 5 mm sections by a Leica rotary microtome. Sections were stained with hematoxylin-eosin and hematoxylin-van Gieson stains. The tissue micrographs were evaluated through a binocular study microscope connected to a digital camera by a specialist pathologist (Fig. 5).

All preparations were evaluated according to the ratios of fibrous tissue, cartilage, new bone and mature bone by the scale recommended by Huo et al. (Table 3).[7]

The radiographical and histopathological scores were compared for the control and study groups (Table 4).[6,7]

Rat tibias were preserved in 10% neutral formaldehyde until biomechanical evaluation. The thin wires

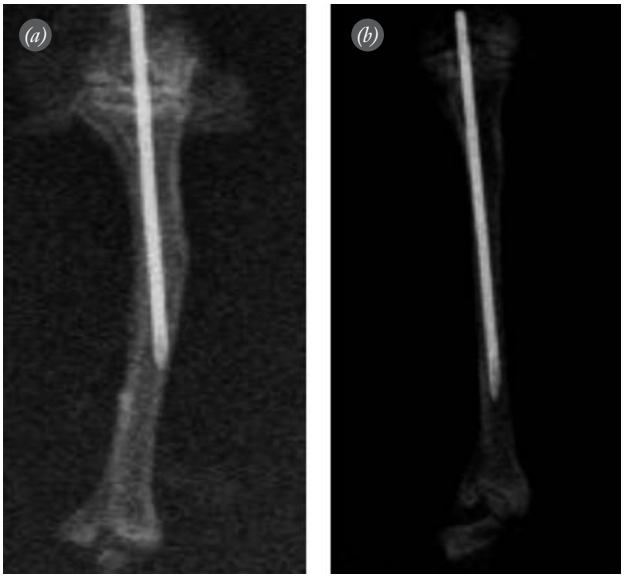

Fig. 4. Radiographies of the **(a)** control and **(b)** study groups at the 6th week.

used for intramedullary fixation were removed. Reduction was disturbed after removal of the intramedullary fixator in all rat tibias in groups 1A and 2A at the 2nd week and biomechanical evaluation could not be conducted. After removal of the intramedullary fixator, the three-point bending test was performed on tibias in groups 1B, 1C, 2B and 2C using the test device TA.XT2i Texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) which controls the lengthening, moves at a speed of 2 mm/sec and can translate the applied force to the computer screen as graphic and numeric data. By applying a force to the callus region, the resistance forces of the elements of each group were measured in Newton units and compared (Figs. 1 and 2). Results of the control and study groups were statistically compared using the Mann-Whitney U test. P values of greater than 0.05 were considered significant.

Results

There was no significant difference in the mean values of radiological or histopathological examination between the study and control groups at the 2nd, 4th or 6th weeks ($p>0.05$). Biomechanical evaluation could not be conducted on the tibias of all rats in the study and control groups at the 2nd week (Fig. 6). The difference in mean values of the biomechanical examination between the study and control groups at the 4th and 6th weeks was not statistically significant ($p>0.05$) (Fig. 7).

Table 2. Lane-Sandhu classification for the evaluation of radiological data.[6]

0	No callus
1	Callus formation present
2	Beginning of bone healing
3	No apparent fracture line
4	Complete bone healing

Table 3. Huo et al.[7] scoring system for the histological evaluation of healing of the fracture.

Score	Histological findings in the area of fracture
Grade 1	Fibrous tissue
Grade 2	Mostly fibrous tissue, small amount of cartilage
Grade 3	Equal amounts of fibrous and cartilage tissue
Grade 4	Mostly cartilage, small amount of fibrous tissue
Grade 5	Cartilage tissue
Grade 6	Mostly cartilage, small amount of immature bone
Grade 7	Equal amounts of cartilage and immature bone tissue
Grade 8	Mostly immature bone, small amounts of cartilage tissue
Grade 9	Healing of fracture with immature bone
Grade 10	Healing of fracture with mature bone

Discussion

Anatomical and functional integrity of the bone is disturbed during the formation of fracture due to trauma or other reasons. The surrounding soft tissues are also affected. Many factors with effects on the healing process of a bone have been defined, including fracture

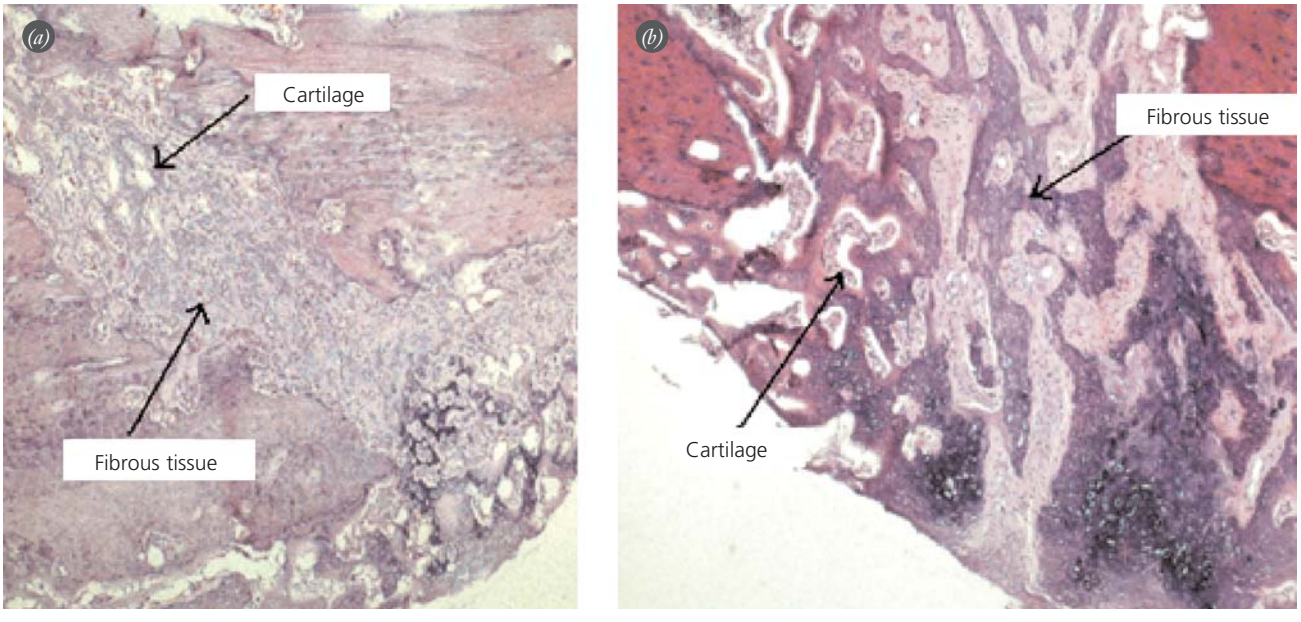

Fig. 5. Histopathological staining. Patchy areas of cartilage tissue formation in addition to the fibrous tissue in the control and study groups at the 2nd week (H&E ×100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 4. Radiological and histopathological scores.

	2nd week			4th week			6th week		
	DX group	Control group	p	DX group	Control group	p	DX group	Control group	p
Radiographical score	0.12±0.35	0.00	0.642	3.87±0.35	3.5±0.84	0.698	4	4	0.587
Histopathological score	6.37±0.51	6.7±0.48	0.655	8.8±0.64	9.33±0.50	0.458	9.75±0.46	10	0.387

DX: dexketoprofen trometamol

type, treatment options, fixation type, systemic problems and various drugs.[8,9]

Non-steroidal anti-inflammatory drugs are frequently used over the long-term in patients with chronic pain due to degenerative changes which frequently occur in the elderly. In cases where healing depending on biological process is desired, such as fractures and cementless arthroplasty, these drugs should be avoided due to their potential negative effects.[10] Non-steroidal anti-inflammatory drugs in which an anti-inflammatory effect has been found can be used in the treatment of heterotrophic ossification for long periods after hip surgeries.[11-13]

Non-steroidal anti-inflammatory drugs can be used for partial suppression of extensive inflammation and to benefit from its analgesic effect after fractures or surgical interventions, especially in cases in which greater edema and pain are expected.[13,14] However, the dosage and the duration of drug use should be carefully determined. Although its negative effects on bone healing have not been demonstrated, interaction with other drugs used concurrently should be considered.[15-17]

The duration of the use of non-steroidal anti-inflammatory drugs and their doses can differ in their effects on bone. Cyclooxygenase inhibitors are frequently used due to their anti-inflammatory effects. As their pain relief effect is satisfactory and gastrointestinal side effects are few, they are frequently preferred in orthopedic clinics. Dexketoprofen trometamol is often preferred due to its lack of gastrointestinal side effects and prolonging of bleeding time.[12]

Alien et al. detected a delay with aspirin and indomethacin depending on the drug and the dose but did not find a significant difference in the rate of pseudarthrosis.[2] Elves et al. demonstrated negative effects of indomethacin started one week prior to fracture formation in rats.[3] In a study conducted on rabbits, Törnkvist et al. detected that torsional endurance in the groups in which both indomethacin and ibuprofen were used did not return to normal in 5 to 8 weeks in contrast to the control group.[4]

More et al.[5] reported that bone healing in rabbits started on the first day and that piroxicam and flunixin administered for three weeks can delay but not disturb the healing process. They explained this delay by the anti-inflammatory effect of the drug. In a study conducted on rats with naproxen, it was reported that bone formation was delayed in only large doses, while naproxen in low doses slowed bone resorption, demonstrating the different possible effects of dose on the bone.[7]

In a study conducted with ibuprofen, Huo et al. failed to demonstrate that ibuprofen, when used at ani-

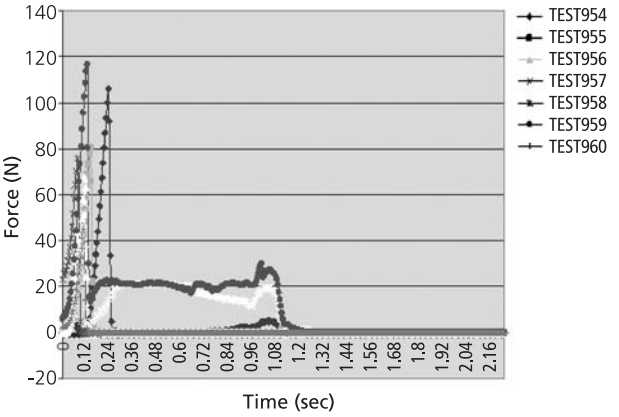

Fig. 6. The resistances of the callus tissues against bending that is formed in the 4th and 6th week groups (N/sec).

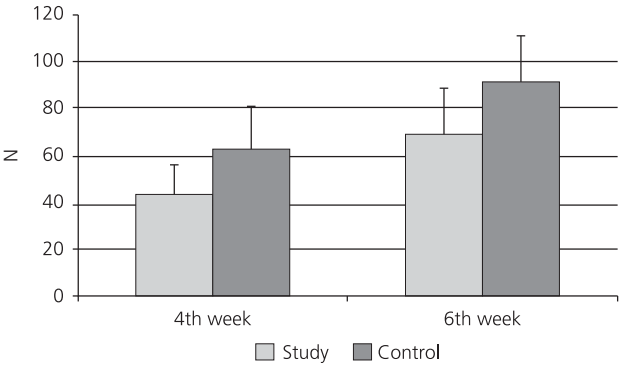

Fig. 7. Biomechanical results of the groups. No statistically significant difference was detected between the groups in the 4th and 6th weeks ($p<0.05$).

mal doses for 5 weeks, starting from the 1st day, produced a significant difference in both biomechanical and histomorphometric parameters of the fracture.[7] Ho et al. demonstrated a dose-dependent inhibitor effect in a study done with ketorolac.[18] In an experimental study on rat tibias, it was demonstrated that tenoxicam, a non-steroidal anti-inflammatory drug, when used intramuscularly immediately after the formation of the fracture, prevented bone healing.[11] It was shown that a non-steroidal anti-inflammatory drug, diclofenac, had negative effects in healing of bone defects that were formed in rats.[19] In another clinical study, it was mentioned that non-steroidal anti-inflammatory drugs that are used in the perioperative period delayed bone healing.[5] In an animal study, Hugo et al. compared the efficacy of dexketoprofen trometamol with morphine and paracetamol and reported a similar success with morphine.[20]

The effect of dexketoprofen trometamol on bone healing has yet to be studied in the literature. The effects of non-steroidal anti-inflammatory drugs on the musculoskeletal system, whose mechanism of action is not yet precisely known, require further study. Because these drugs are widely and frequently used, except for chronic inflammatory diseases which is the primary indication for their use.[15,21,22]

Dexketoprofen trometamol is a synthetic, non-steroidal, acidic drug that has anti-inflammatory, analgesic, and antipyretic effects.[23-25] Non-steroidal anti-inflammatory drugs can be used in the conservative treatment of fractures and after extremity surgeries to decrease pain. In addition to decreasing pain, it can be necessary to inhibit aseptic inflammatory reactions that occur in the tissues after trauma. The model of fracture healing was used in many studies in the literature.[25-28]

A limitation of the current study, which conducted mechanic and histopathological examinations on the same tibia, was that the exact fracture line could not be evaluated. However, we believe that histopathological investigations around the fracture line do not cause a major problem, as the healing was sufficient in all of the bones and it was radiologically determined previously that there was no significant difference between groups.

In conclusion, use of dexketoprofen trometamol from the day of operation until the 6th postoperative week has no effect on the healing of closed fractures of the tibia fixed with intramedullary nails in rat models. We believe that dexketoprofen trometamol can be used carefully considering the dependent effects of drugs used simultaneously.

Conflicts of Interest: No conflicts declared.

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