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The effect of preemptive use of nonsteroidal anti-inflammatory drug on inflammation, oxidative stress, and wound healing

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This study evaluated the effects of preemptively administered nonsteroidal anti-inflammatory drugs (NSAIDs) on wound healing, inflammation, and oxidative stress in rats. 24 male Sprague-Dawley rats were assigned to four groups: no NSAID (Control), preemptive NSAID (Group 1), postoperative NSAID (Group 2), and pre- and postoperative NSAID (Group 3). As a NSAID, Celecoxib (20 mg/kg/day) was administered via oral gavage. A standardized mandibular burr-hole bone defect was created. Animals were euthanized at 1, 2, or 4 weeks postoperatively. Bone healing was evaluated histologically using the Emery scoring system. M1 and M2 macrophages were identified via immunohistochemistry. Interleukin-1β, interleukin-6, interleukin-10, transforming growth factor-β, prostaglandin E_2, and oxidative stress markers including total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were measured using enzyme-linked immunosorbent assay. No statistically significant differences were observed among groups in bone healing scores, macrophage counts, or cytokine levels; however, TAS was significantly higher in Group 1 and lower in Group 2 at week 1 ($p = 0.023$). OSI showed a consistent temporal pattern, with Group 1 exhibiting the lowest OSI at week 1, although this difference was not statistically significant. Given the small sample size, these findings should be interpreted as preliminary and hypothesis-generating rather than conclusive. Preemptive celecoxib administration appeared to modulate early inflammatory and oxidative responses without impairing bone regeneration. Further adequately powered studies are required to confirm these early trends.

Keywords Bone healing, Celecoxib, Inflammation, Oxidative stress, Preemptive analgesia

Preemptive analgesia refers to the administration of analgesic interventions prior to surgical trauma to prevent or attenuate postoperative pain[1]. This concept is based on inhibiting central sensitization and reducing the likelihood of persistent or chronic pain[2–4]. The effectiveness of this strategy is highly dependent on timing, as the intervention must be delivered before the initiation of nociceptive signaling triggered by incisional or inflammatory injury[5,6].

Although preemptive analgesia has shown promising results, particularly with nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, clinical evidence, including studies in third-molar surgery, remains inconsistent[7–9]. Pain and edema continue to be among the most common postoperative complications, both reflecting the local inflammatory response. Despite several proposed mechanisms, the precise initiation and regulation of postoperative inflammation are still not fully understood[10].

Previous research has predominantly evaluated postoperative NSAID administration or focused solely on clinical outcomes, leaving limited understanding of how preoperative NSAID use may influence early inflammatory responses, oxidative stress, and bone healing[10].

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Macrophages have emerged as key regulators of postoperative inflammation and tissue repair due to their dynamic polarization capability. M1 macrophages contribute to the early proinflammatory phase by releasing cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), whereas M2 macrophages promote resolution and tissue regeneration through mediators such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β)[11,12]. Understanding how the timing of NSAID exposure influences macrophage behavior may therefore be essential for optimizing postoperative healing.

Accordingly, this study aimed to evaluate the effects of preemptive NSAID administration on bone healing, inflammatory cytokine patterns, oxidative stress status, and macrophage polarization in a rat model with surgically induced burr-hole–type mandibular defects. We hypothesized that administering NSAIDs before surgical trauma would attenuate early inflammatory activation and reduce oxidative stress, thereby supporting a more favorable early healing environment.

Methods

The study protocol was reviewed and approved by the Bezmialem Vakıf University Local Ethics Committee for Animal Experiments (Decision No: 2024-15-1), and all procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. The study adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Twenty-four male Sprague–Dawley rats (approximately 500 g, 11 weeks old) were obtained from the Bezmialem Vakıf University Experimental Animals Application and Research Centre (Istanbul, Turkey). All animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and provided ad libitum access to chow and water. Three rats were housed per cage, and all animals were acclimatized for one week prior to surgery. Only male rats were used to avoid hormonal fluctuations associated with the estrous cycle in females, which can influence inflammatory responses, macrophage activity, oxidative stress parameters, pain sensitivity, and bone turnover. Reducing sex-related physiological variability was necessary to maintain experimental consistency. Evaluation of sex-dependent differences was not within the scope of this study.

Randomization and blinding procedure

All animals were randomly allocated to the experimental groups using a computer-generated random sequence (http://www.randomizer.org). Randomization was performed by an investigator not involved in surgical procedures or outcome assessments to minimize allocation bias. All histological evaluations, macrophage counts, and the Enzyme-Linked Immunosorbent Assay (ELISA) analyses were conducted in a blinded manner. Tissue samples and serum tubes were coded by an independent researcher, and investigators performing scoring and biochemical assays remained unaware of group assignments throughout data collection and analysis.

Operative procedure

Rats underwent mild sedation with inhaled isoflurane and oxygen prior to surgical intervention. General anesthesia was then induced via intraperitoneal injection of 10% ketamine (35 mg/kg) and 2% xylazine (15 mg/kg). Each rat was positioned on a custom-designed operating table. To minimize intraoperative bleeding and provide local anesthesia, 500 µL of lidocaine diluted at 1:80000 was administered subcutaneously at the surgical site.

The surgical field was shaved and disinfected with povidone-iodine solution. Under sterile conditions, a 1 cm midline incision was made in the submental region using a #15 scalpel to access the subcutaneous. The platysma was dissected, and the anterior belly of the digastric and masseter muscles were gently retracted to expose the mandible.

A standardized defect was created in the anterior tooth region of the mandible, as described by Liu et al.[13]. A 0.5 mm-deep, 2×4 mm^2 rectangular defect was prepared under continuous saline irrigation using a surgical micromotor and a 1.2 mm diameter flat-tipped cylindrical diamond bur (Fig. 1). The defect size was confirmed using a 0.5 mm-tipped modified periodontal probe. Muscle and skin layers were then repositioned and sutured primarily using 5 – 0 resorbable sutures.

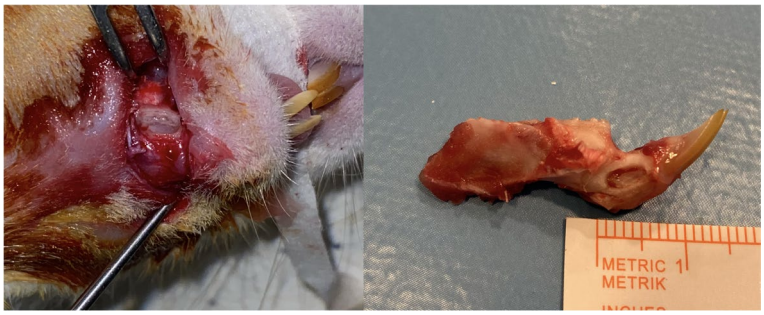

Fig. 1. Standardized burr-hole bone defect ($2 \times 4 \times 0.5$ mm^2) at anterior mandible.

Postoperatively, animals were fed a soft commercial diet for the first seven days, followed by a standard laboratory diet until euthanasia. As antibiotic prophylaxis, Clamoxyl® L.A. (10 mg/kg, intramuscularly) was administered.

Drug administration protocol

Celebrex® 200 mg (Pfizer Pharmaceuticals LLC, Caguas, Puerto Rico) was prepared by dissolving the capsule contents in 40 mL of dimethyl sulfoxide (DMSO) (Emplura®, Merck, Japan). The selected dose (20 mg/kg/day) was based on body surface area (BSA) conversion, which is recommended for interspecies dose extrapolation. Although the therapeutic adult human dose is 200–400 mg/day (approximately 3.3–6.6 mg/kg), previous animal studies have frequently used much lower doses (3–8 mg/kg/day), which underestimate human-equivalent exposure. According to Food and Drug Administration (FDA)-approved BSA conversion guidelines and prior reports, the dose used in the present study (20–21 mg/kg/day) approximates the human therapeutic dose range and provides a more clinically relevant exposure level[14].

The rats in Groups 1 and 3 received celecoxib via oral gavage twice daily for three consecutive days before surgery (Day −3 to Day −1). Although DMSO has been reported to exert anti-inflammatory and antioxidant properties at higher concentrations, the vehicle used in this study was a highly diluted DMSO solution (2 mL/day) administered uniformly across all groups to minimize potential carrier-related influence. Control animals received either physiological saline ($n=3$) or the DMSO vehicle alone ($n=3$); as no differences were detected between these subgroups in any histological, immunohistochemical, or biochemical parameter, they were analyzed together as a single control group.

Surgery was performed on Day 0. Beginning on the day of surgery, celecoxib was administered in Group 2 for three days (Day 0 to Day + 2), while Group 3 and the control group continued receiving the same dosing protocol. In Group 1, treatment was discontinued after surgery (Fig. 2).

Animals allocated to each time point were euthanized on postoperative days 7, 14, and 28 ($n=8$ per time point) under ketamine–xylazine anesthesia.

Blood sampling timeline

Blood samples were collected at four defined time points: immediately before surgery (Day 0) and on postoperative days 7, 14, and 28. Importantly, blood was obtained from all animals that were alive at each time point, not only from those scheduled for euthanasia. Thus, serum sample numbers at each time point were: Day 0: $n=24$, Day 7: $n=24$, Day 14: $n=16$, Day 28: $n=8$.

At each postoperative time point, animals designated for euthanasia ($n=8$ per time point) were sampled terminally under anesthesia, whereas animals continuing in the study ($n=16$ on Day 7; $n=8$ on Day 14) were sampled non-terminally using identical handling and blood draw procedures (Fig. 2).

All samples were collected into gel-containing biochemistry tubes (Vacusera, DISERA A.Ş., İzmir, Türkiye), centrifuged at 4000 rpm for 10 min (NF1200, Nüve, Ankara, Türkiye), and the resulting sera were aliquoted into Eppendorf tubes and stored at − 80 °C until analysis.

This design allowed each animal to contribute a single serum sample per time point, while maximizing sample availability for biochemical analyses without increasing the total number of animals.

Preparation of tissue specimens

At each postoperative time point (days 7, 14, and 28), two animals per group were euthanized, and mandibular bone specimens were harvested from the defect region. Samples were fixed in 10% neutral-buffered formalin, decalcified using formic acid for 14 days, dehydrated through graded ethanol, cleared in xylene for 3 h, and embedded in paraffin. Serial sections of 3–4 µm thickness were prepared using a rotary microtome (Leica RM2245).

Histological evaluation

Sections designated for routine staining were processed with Hematoxylin–Eosin (H&E) and Masson's Trichrome (MT) and examined under a light microscope. Bone healing was evaluated using Emery's histological scoring

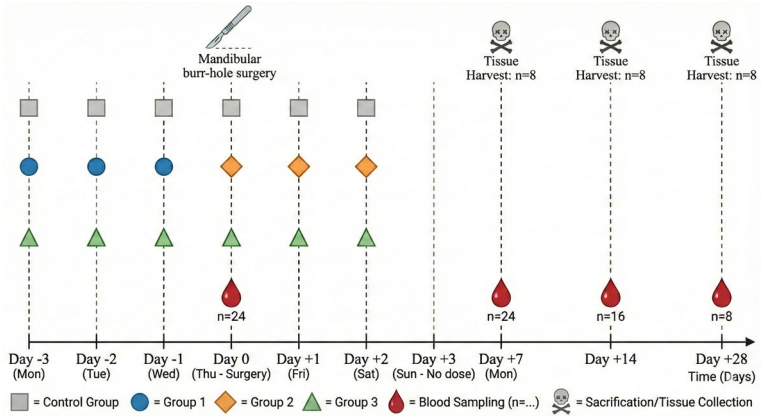

Fig. 2. Experimental timeline showing preoperative celecoxib dosing, Day-0 baseline blood sampling, mandibular defect surgery, postoperative drug protocols, and terminal sampling at postoperative days 7, 14, and 28.

system by two independent blinded investigators (CA and ERH). In case of disagreement, slides were jointly reviewed to establish a consensus score; therefore, inter-observer reliability statistics were not calculated. The scoring criteria are detailed in Supplementary Figure.

Immunohistochemical evaluation

Sections for immunohistochemistry (IHC) were mounted on positively charged slides (Epiglass EG-SP9172, Epifarma, Turkiye), deparaffinized, and subjected to heat-induced antigen retrieval using sodium citrate buffer (pH 6.0). Primary antibodies used were NOS2 (M1 macrophage marker; FNab04325, FineTest®, China) and CD206 (M2 macrophage marker; FNab01442, FineTest®, China). A polymer-based detection system was applied (SensiTek HRP Anti-Polyvalent, ScyTek Laboratories, USA).

Images were captured using a Nikon Eclipse i5 microscope with a DS-Fi1c camera at 400X magnification and analyzed in NIS-Elements software (v4.2). Immunopositive M1 and M2 macrophages within the entire defect area were manually counted by two blinded investigators (CA and ERH). Inter-observer agreement was quantified using the intraclass correlation coefficient (ICC), and mean values were used for statistical analyses, as illustrated in Fig. 3.

Biochemical analysis

The collected serum samples were used to evaluate inflammatory responses by measuring levels of IL-1β, IL-6, IL-10, TGF-β, and Prostaglandin E_2 (PGE_2). Oxidative stress was assessed by determining the total oxidant status (TOS) and total antioxidant status (TAS). The oxidative stress index (OSI), reflecting the balance between pro-oxidants and antioxidants, was calculated as: 'OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L) / TAS (mmol Trolox equivalent/L) × 100'. All parameters were quantified using ELISA method, and the kit specifications are provided in Supplementary Table.

Statistical analyses

The sample size calculation, conducted using G*Power (version 3.0.10), was based on a one-way ANOVA test design. Assuming a large effect size (f = 0.83), the a priori power analysis indicated that a total of 24 animals would be required to achieve 80% power and a 5% type I error rate.

All statistical analyses were performed using IBM SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed prior to analysis, and numerical variables were summarized as mean ± standard deviation or median (25th–75th percentile), as appropriate.

Because different animals contributed to each time point and the number of animals per group at each point was very limited ($n = 2$), repeated-measures analyses were not applied. Instead, temporal changes within each group and independent comparisons between groups at each time point were evaluated using non-parametric tests (primarily Kruskal–Wallis and Mann–Whitney U tests). Parametric tests (one-way ANOVA) were used only when distributional assumptions were satisfied.

Given the exploratory nature of the design and the limited sample size per condition, all statistical findings should be interpreted cautiously and regarded as descriptive and hypothesis-generating rather than confirmatory. A p-value < 0.05 was considered statistically significant.

Results

Histological findings

At week 1, corresponding to the early healing phase, minimal bone formation was noted, with scores ranging from 0 in the control group to 1 ± 0 in Group 2. At week 2, reflecting ongoing repair, scores increased across all groups, indicating moderate new bone formation (Control: 2.5 ± 0.71; Group 1: 3.5 ± 0.71; Group 2: 3 ± 0; Group

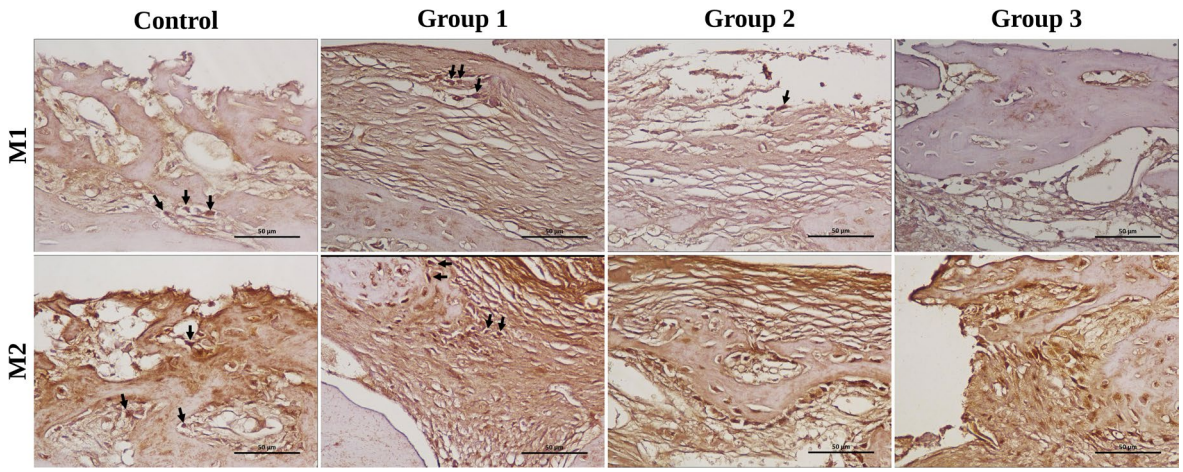

Fig. 3. Quantifying of M1 and M2 macrophages in bone defect healing (black arrows). Images captured at 400X were analyzed in Image-analysis software.

3: 4 ± 0). At week 4, scores further increased, indicating more advanced bone regeneration (Control: 5 ± 0; Group 1: 5 ± 2.83; Group 2: 6 ± 0; Group 3: 5 ± 0).

All groups showed a time-dependent increase in Emery scores, demonstrating progressive bone healing at the defect site. However, no statistically significant differences were observed in scores among groups at any time point ($p > 0.05$ for all comparisons) (Table 1; Fig. 4).

Immunohistochemical analysis

The number of M1 and M2 macrophages tended to be higher in Group 2 than in the other groups, except at week 2, where values were comparable across Groups 1, 2, and 3. The greatest numbers of M1 macrophages were observed at week 2 in the control group and in Groups 1 and 3, whereas Group 2 demonstrated its peak M1 count at week 1.

M2 macrophages peaked at week 2 in all groups. The M1/M2 ratio was relatively higher at week 1 in Groups 1, 2, and 3 but converged to similar levels by weeks 2 and 4 (Fig. 5). No significant differences in M1 or M2 counts or in the M1/M2 ratio were detected among the groups (Table 1).

Inter-observer reliability for macrophage quantification was also high (ICC = 0.89), confirming excellent agreement between blinded investigators.

Biochemical analysis

All biochemical outcomes, including inflammatory cytokine levels (IL-1β, IL-6, IL-10, TGF-β), PGE_2, and oxidative stress markers (TAS, TOS, OSI), are summarized in Tables 2 and 3, with cytokine trends illustrated in Fig. 6 and PGE_2/oxidative stress trends presented in Fig. 7.

Inflammatory cytokine levels

Intragroup comparisons also revealed no statistically significant changes over time (Control: $p = 0.092$; Group 1: $p = 0.191$; Group 2: $p = 0.406$; Group 3: $p = 0.406$). In Group 1, IL-1β levels peaked at week 1 and reached their lowest point at week 2. At week 4, the highest IL-1β level was recorded in the control group, with lower levels in Group 1, Group 2, and Group 3. However, there were no statistically significant differences in IL-1β levels among the groups at any time point ($p > 0.05$ for all).

At baseline (T0), a statistically significant difference was observed in IL-6 levels among the groups ($p = 0.03$), with Group 3 showing the highest level. No significant differences were detected at subsequent time points. Intragroup comparisons showed no statistically significant changes over time (Control: $p = 0.199$; Group 1: $p = 0.147$; Group 2: $p = 0.406$; Group 3: $p = 0.406$). In Group 3, IL-6 levels decreased from baseline to the day of operation, followed by an increase at week 1, peaking at week 2, and subsequently declining at week 4. At week 4, the control group had the highest IL-6 value, followed by Group 2, Group 3, and Group 1.

No statistically significant differences in IL-10 or TGF-β levels were observed among the groups at any time point ($p > 0.05$ for all). Intragroup comparisons also showed no significant changes over time for IL-10 (Control:

		Week 1	Week 2	Week 4	*p*
Emery score	Control	0	2.5 ± 0.71	5 ± 0	0.135
	Group 1	0.5 ± 0.71	3.5 ± 0.71	5 ± 2.83	0.223
	Group 2	1 ± 0	3 ± 0	6 ± 0	0.135
	Group 3	0.5 ± 0.71	4 ± 0	5 ± 0	0.135
	p	0.321	0.161	0.512	
M1 macrophage	Control	1 ± 0	5 ± 1.41	1.5 ± 2.12	0.223
	Group 1	2.5 ± 2.12	13.5 ± 7.78	4 ± 0	0.156
	Group 2	23 ± 2.83	14 ± 4.24	14.5 ± 2.12	0.223
	Group 3	3 ± 2.83	14.5 ± 2.12	3 ± 0	0.223
	p	0.161	0.261	0.087	
M2 macrophage	Control	1 ± 0	5 ± 1.41	1.5 ± 2.12	0.156
	Group 1	1 ± 0	23 ± 4.24	7 ± 7.07	0.135
	Group 2	4.5 ± 0.71	31 ± 14.14	16 ± 1.41	0.135
	Group 3	1 ± 0	36.5 ± 4.95	13 ± 7.07	0.135
	p	0.077	0.160	0.183	
M1/M2	Control	1 ± 0	0.47 ± 0.04	1.5 ± 2.12	0.607
	Group 1	2.5 ± 2.12	0.57 ± 0.23	1.17 ± 1.18	0.607
	Group 2	5.23 ± 1.45	0.54 ± 0.38	0.91 ± 0.05	0.135
	Group 3	3 ± 2.83	0.41 ± 0.11	0.28 ± 0.15	0.223
	p	0.249	0.919	0.761	

Table 1. Histological and immunohistochemical findings with intragroup and intergroup comparisons. p values in rows represent intragroup comparisons across weeks (Week 1, Week 2, and Week 4), indicating time-dependent changes within each group. p values in columns represent intergroup comparisons at each time point. A p value less than 0.05 was considered statistically significant.

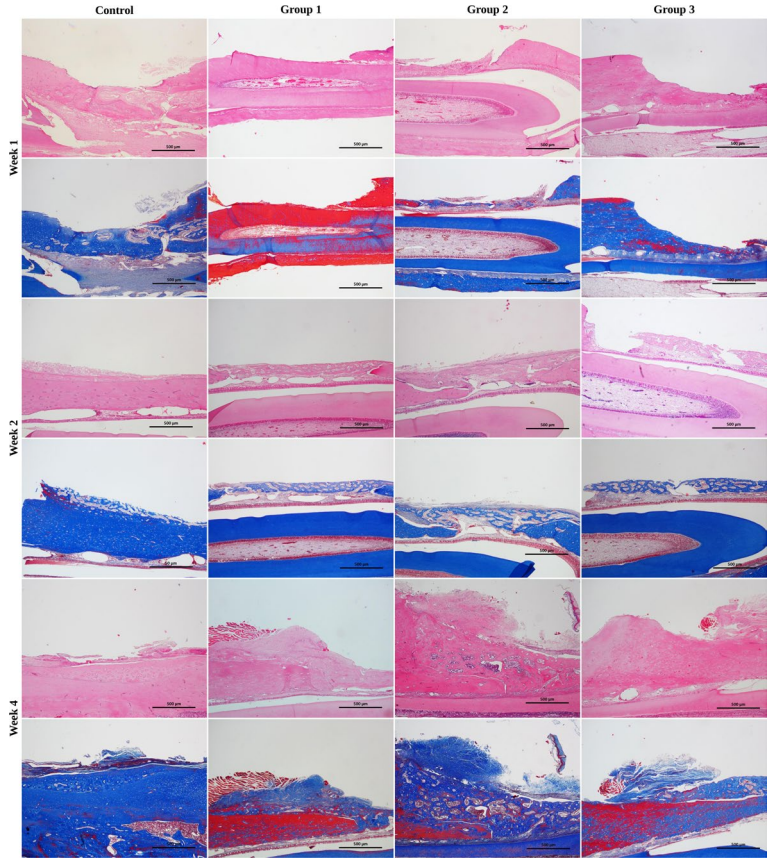

Fig. 4. Bone healing progression evaluated by Emery histopathological scoring at weeks 1, 2, and 4 post-surgery (H&E and MT 40X, Masson-Trichrome 40X). Scores indicate minimal bone formation at week 1, moderate new bone formation at week 2, and more advanced regeneration by week 4 across all groups (Control, Group 1, Group 2, and Group 3). No statistically significant differences were found between groups at any time point ($p > 0.05$).

$p = 0.525$; Group 1: $p = 0.809$; Group 2: $p = 0.406$; Group 3: $p = 0.406$) or TGF-β (Control: $p = 0.147$; Group 1: $p = 0.126$; Group 2: $p = 0.406$; Group 3: $p = 0.406$).

PGE_2 levels

In the control group and Group 2, the highest PGE_2 levels were observed on the day of operation, followed by a decrease at week (1) In Groups 1 and 3, a mild decline in PGE_2 was recorded from the operation day to week 1, followed by a modest increase at week (2) At week 4, PGE_2 levels varied among the groups without a consistent trend.

However, no statistically significant differences in PGE_2 levels were found among the groups at any time point ($p > 0.05$). Intragroup comparisons also revealed no significant changes over time (Control: $p = 0.171$; Group 1: $p = 0.231$; Group 2: $p = 0.406$; Group 3: $p = 0.406$).

Oxidative stress parameters

No statistically significant differences in TAS or TOS levels were found among the groups at most time points ($p > 0.05$), except for TAS at week 1 ($p = 0.023$). Intragroup comparisons indicated no statistically significant changes over time (TAS: Control: $p = 0.107$; Group 1: $p = 0.147$; Group 2: $p = 0.406$; Group 3: $p = 0.406$; TOS: Control: $p = 0.525$; Group 1: $p = 0.147$; Group 2: $p = 0.406$; Group 3: $p = 0.406$). At week 1, Group 1 exhibited the highest and TOS levels, whereas Group 2 had the lowest TAS. At week 2, both TAS and TOS remained lowest in Group 2.

OSI levels did not differ significantly among the groups at any time point ($p > 0.05$). Intragroup comparisons also showed no statistically significant changes (Control: $p = 0.107$; Group 1: $p = 0.092$; Group 2: $p = 0.406$; Group 3: $p = 0.406$). At week 1, Group 1 had the lowest OSI level, while the highest value was observed in Group 2. At week 2, OSI levels increased across all groups, with the highest value again recorded in Group 2, followed by Group 1, Group 3, and the control group.

Discussion

NSAIDs are widely used for preemptive analgesia due to their well-established anti-inflammatory and analgesic effects. These agents reduce nociceptive sensitization primarily through inhibition of cyclooxygenase (COX)

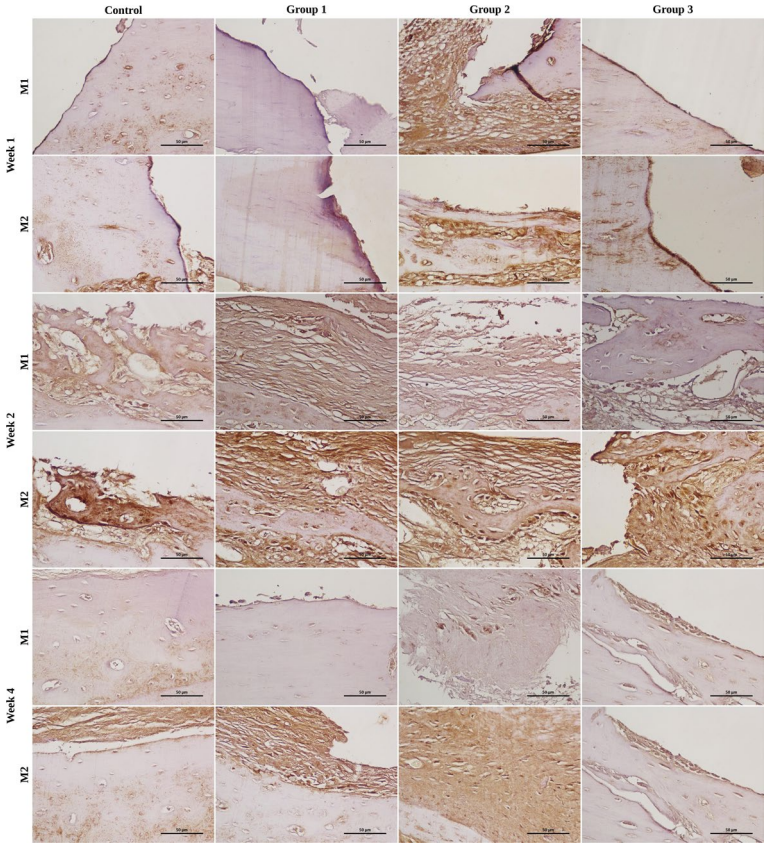

Fig. 5. Immunohistochemical analysis for M1 and M2 macrophages in bone defect healing at weeks 1, 2, and 4 post-surgery in rats. Group 2 showed generally higher numbers of both macrophage types except at week 2, where Groups 1, 2, and 3 had comparable counts. Peak M1 macrophage levels occurred at week 2 in Control, Group 1, and Group 3, while Group 2 peaked at week 1. M2 macrophages peaked at week 2 across all groups. The M1/M2 ratio was higher at week 1 in Groups 1, 2, and 3, becoming similar across groups by weeks 2 and 4. No significant differences were found among groups at any time point.

enzymes, thereby decreasing prostaglandin production and early postoperative inflammatory signaling[15]. Although selective COX-2 inhibitors such as celecoxib have improved safety profiles regarding gastrointestinal and bleeding complications[16,17], their potential effects on bone healing remain controversial[18–21].

To investigate this relationship in a clinically relevant context, the present study utilized the standardized mandibular burr-hole defect model introduced by Liu et al.[13]. This model mimics small cortical perforations frequently created during common maxillofacial surgeries—including miniplate fixation, guided bone regeneration, and implant site preparation—while avoiding major biomechanical instability. The approach allows controlled assessment of the early inflammatory and reparative phases, making it particularly suitable for evaluating the biological influence of preoperative NSAID administration.

Histological evaluation using Emery criteria showed progressive bone healing at weeks 1, 2, and 4 in all groups, and celecoxib administration—whether preoperative, postoperative, or perioperative—did not result in statistically significant differences. Previous studies have similarly reported minimal adverse effects of short-term NSAID use on bone healing[22,23]. In contrast, other investigations have demonstrated impaired bone regeneration with COX-2 inhibition. Li et al. showed that celecoxib suppressed fracture healing in a rat model at both early and late phases[14]. Nagano et al. further reported that celecoxib can impair osteoblast differentiation and mineralization in vitro, suggesting potential inhibitory effects on bone-forming activity[24]. A meta-analysis by Al-Waeli et al. noted delayed bone healing within the first 21 days in NSAID-treated rats,[15] and a review by Huss et al. advised caution regarding non-selective COX inhibitors in orthopedic settings, while suggesting that short-term selective COX-2 inhibition may still be appropriate as part of multimodal analgesia[25]. Clinical studies have also reported higher rates of nonunion in patients receiving NSAIDs to prevent heterotopic ossification[26–28], and Jeffcoach et al. identified increased postoperative complications associated with NSAID use during the healing period[19]. In the current work, descriptive inspection indicated slightly lower early histological scores in groups receiving preoperative NSAIDs, but with only two rats contributing to each time point, these temporal tendencies should not be interpreted beyond their exploratory nature. Previous evidence indicates that COX-2 inhibition may exert timing-dependent postoperative effects[15,24,29], which requires further validation in adequately powered studies.

		T0	Week 1	Week 2	Week 4	p
IL-1β (pg/mL)	Control	58.1 ± 4.9	67.8 ± 9.84	162.07 ± 89.56	91.47 ± 17.17	0.092
	Group 1	64.6 ± 12.95	95.66 ± 29.66	86.73 ± 15.54	71.52 ± 4.27	0.191
	Group 2	67.66 ± 13.98	67.2 ± 14.17	156.38 ± 81.92	82.16 ± 23.96	0.406
	Group 3	73.37 ± 23.14	80.5 ± 33.97	109.77 ± 36.57	81.61 ± 6.67	0.406
	p	0.684	0.124	0.311	0.418	
IL-6 (pg/mL)	Control	144.91 ± 106.42	163.77 ± 121.84	116.57 ± 65.9	182.7 ± 180.96	0.261
	Group 1	76.81 ± 22.28	128.38 ± 111.89	94.84 ± 40.82	67.68 ± 17.6	0.147
	Group 2	46.42 ± 7.14*	44.19 ± 6.88	53.87 ± 4.51	125.6 ± 92.29	0.406
	Group 3	157.12 ± 83.5*	139.32 ± 0.91	214.04 ± 208.84	100.99 ± 20.11	0.406
	p	0.03*	0.186	0.081	0.761	
IL-10 (pg/mL)	Control	12.98 ± 0.46	13.7 ± 1.14	13.94 ± 0.54	14.56 ± 0.14	0.525
	Group 1	12.72 ± 0.8	13.5 ± 1.06	13.66 ± 0.74	14.74 ± 0.38	0.809
	Group 2	12.5 ± 0.21	12.74 ± 0.41	13.81 ± 2.6	13.61 ± 0.74	0.406
	Group 3	13.69 ± 2.21	13.24 ± 0.91	13.63 ± 0.26	14.13 ± 1.42	0.406
	p	0.475	0.464	0.583	0.406	
TGF- β (pg/mL)	Control	5.15 ± 0.4	5.19 ± 0.72	5.81 ± 0.77	5.85 ± 0.97	0.147
	Group 1	5.08 ± 0.43	5.32 ± 0.57	5.84 ± 0.58	4.82 ± 0.17	0.126
	Group 2	4.65 ± 0.11	5.02 ± 0.54	6.39 ± 2.82	5.55 ± 0.69	0.406
	Group 3	6.34 ± 2.08	5.37 ± 0.54	5.44 ± 0.57	4.69 ± 0.02	0.406
	p	0.126	0.437	0.696	0.114	

Table 2. Inflammatory cytokine levels with intragroup and intergroup comparisons. p values in rows represent intragroup comparisons across weeks (T0, Week 1, Week 2, and Week 4), indicating time-dependent changes within each group. p values in columns represent intergroup comparisons at each time point. A p value less than 0.05 was considered statistically significant.

		T0	Week 1	Week 2	Week 4	*p*
PGE_2 (pg/mL)	Control	14.13 ± 8.51	11.07 ± 4.58	23.31 ± 5.2	17.23 ± 0.61	0.171
	Group 1	9.27 ± 2.33	14.08 ± 7.62	19.36 ± 6.11	13.68 ± 4.75	0.231
	Group 2	11.09 ± 1.12	13.61 ± 4.49	14.36 ± 3.22	23.07 ± 13.18	0.406
	Group 3	11.51 ± 3.21	14.32 ± 0.89	15.56 ± 7.87	18.9 ± 10.57	0.406
	p	0.354	0.588	0.154	0.761	
TAS (mmol Trolox equivalent/L)	Control	2.59 ± 1.27	2.46 ± 0.84	1.29 ± 0.48	1.45 ± 0.58	0.107
	Group 1	1.67 ± 0.49	4.95 ± 4.31	1.25 ± 0.54	1.28 ± 0.33	0.147
	Group 2	1.61 ± 0.44	1.97 ± 0.27	0.7 ± 0.07	2.37 ± 1.74	0.406
	Group 3	3.42 ± 1.82	2.13 ± 0.35	1.49 ± 0.47	1.91 ± 0.38	0.406
	p	0.345	0.023*	0.09	0.539	
TOS (μmol H_2O_2 equivalent/L)	Control	14.61 ± 3.55	10.96 ± 2.81	17.31 ± 6.55	13.29 ± 2.52	0.525
	Group 1	15.45 ± 4.12	12.27 ± 3.12	19.94 ± 8.59	14.23 ± 3.7	0.147
	Group 2	9.76 ± 1.5	10.76 ± 1.45	11.33 ± 0.95	15.9 ± 4.54	0.406
	Group 3	13.69 ± 3.31	10.37 ± 2.77	18.63 ± 3.41	14.5 ± 5.29	0.406
	p	0.066	0.563	0.084	0.761	
OSI	Control	6.85 ± 3.1	4.84 ± 1.51	14.08 ± 3.78	9.6 ± 2.17	0.107
	Group 1	9.34 ± 0.8	3.83 ± 2.06	15.97 ± 0.004	11.13 ± 0	0.092
	Group 2	6.64 ± 2.75	5.46 ± 0.001	16.18 ± 0.43	8.21 ± 4.12	0.406
	Group 3	5.59 ± 3.78	4.9 ± 1.11	13.41 ± 4.43	8.04 ± 4.37	0.406
	p	0.253	0.349	0.820	0.208	

Table 3. PGE_2 levels and oxidative stress levels with intragroup and intergroup comparisons. p values in rows represent intragroup comparisons across weeks (T0, Week 1, Week 2, and Week 4), indicating time-dependent changes within each group. p values in columns represent intergroup comparisons at each time point. A p value less than 0.05 was considered statistically significant.

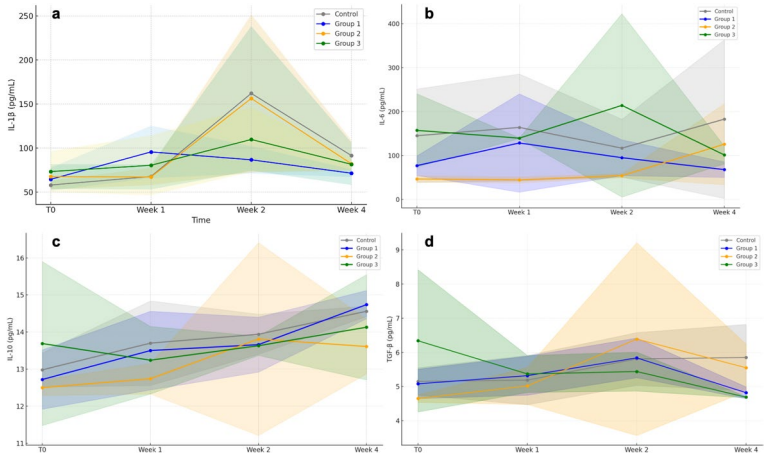

Fig. 6. Temporal cytokine profiles across experimental groups. (a) IL-1β (pg/mL), (b) IL-6 (pg/mL), (c) IL-10 (pg/mL), and (d) TGF-β (pg/mL) measured at baseline (T0) and postoperative weeks 1, 2, and 4 in the Control (grey), Group 1 (blue), Group 2 (orange), and Group 3 (green) animals. Data are presented as mean ± SD.

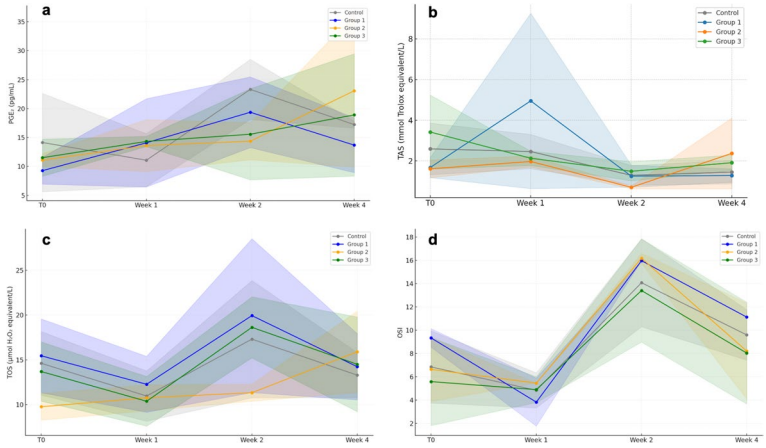

Fig. 7. Temporal trends in PGE_2 and oxidative stress markers. (a) PGE_2 (pg/mL), (b) TAS (mmol Trolox equivalent/L), (c) TOS (μmol H_2O_2 equivalent/L), and (d) OSI (arbitrary units) measured at baseline (T0) and postoperative weeks 1, 2, and 4 in the Control (grey), Group 1 (blue), Group 2 (orange), and Group 3 (green) animals. Data are presented as mean ± SD.

The relatively wide standard deviations observed at week 4 are likely attributable to inherent biological variability in late-stage bone remodeling, influenced by factors such as vascularity, immune dynamics, and mastication-related functional loading[30,31]. Such variation becomes more apparent when sample size is limited and should not be attributed to treatment.

Surgical trauma triggers pro-inflammatory cytokine activation through pathways involving COX-2 and prostaglandin synthesis[10,32]. Previous studies have reported that preemptive COX-2 inhibition reduces postoperative cytokine release and analgesic requirements[33–37]. In the present study, IL-1β increased at week 1 in all groups except Group 2, which received celecoxib only after surgery, whereas Group 1 showed no further rise between weeks 1 and 2. IL-6 demonstrated a different pattern, with relatively higher values in the control and Group 3 at week 1 and peaks at week 2 across groups, followed by reduction at week 4. Anti-inflammatory cytokines (IL-10, TGF-β) and PGE_2 did not show notable early postoperative fluctuations.

Because none of the cytokine differences reached statistical significance, these temporal variations should be interpreted cautiously as exploratory trends rather than evidence of treatment-related modulation. Confirmation in adequately powered studies is required to determine whether NSAID timing meaningfully influences postoperative inflammatory responses.

Baseline IL-6 levels were unexpectedly higher in Group 3 despite the absence of any intervention at that stage. Given the small sample size ($n = 6$ per group at baseline) and known variability related to circadian rhythm, handling sensitivity, and individual stress responses, this imbalance likely reflects random distribution rather than a biological effect. This further supports cautious interpretation of early cytokine differences in underpowered experimental designs.

Macrophage polarization plays a key role in coordinating inflammatory response and tissue repair through dynamic shifts between pro-inflammatory M1 and pro-resolving M2 phenotypes. In this study, different NSAID administration protocols were evaluated in relation to macrophage behavior using a standardized rat mandibular burr-hole defect model. Although no statistically significant differences were found in M1 and M2 macrophage counts or in M1/M2 ratios among the groups, these results are limited by the small number of animals per time point and should therefore be interpreted cautiously.

The highest M1/M2 ratio at week 1 was observed in Group 2, which received NSAID only after surgery. Given that a higher ratio reflects a stronger pro-inflammatory phenotype, this pattern may suggest a more pronounced early inflammatory response in animals that did not receive preoperative COX-2 inhibition. Because celecoxib was not present at the moment of surgical trauma in this group, the initial prostaglandin-driven inflammatory surge likely proceeded unmodulated, allowing M1-dominant activation to predominate during the early postoperative phase[25,34]. Thus, preemptive celecoxib dosing may have modulated the initial macrophage-mediated inflammatory cascade. However, these interpretations remain speculative due to the limited number of animals per time point and the absence of statistically significant differences. By weeks 2 and 4, the ratios converged across groups, indicating the expected progression toward immune resolution, although these temporal trends should also be considered exploratory.

Previous reports indicate that NSAIDs can influence macrophage phenotypes, including reductions in M1-associated markers and support for macrophage maturation under inflammatory conditions[38,39]. Evidence specifically focusing on macrophage polarization in the context of preemptive NSAID administration in bone healing models, however, remains scarce. The present findings therefore offer a novel, biologically relevant perspective on macrophage-mediated healing responses, while emphasizing the need for larger, adequately powered studies to clarify whether the timing of COX-2 inhibition meaningfully modulates macrophage behavior during bone repair.

Oxidative stress, a key contributor to delayed tissue repair and inflammatory dysregulation. Although no statistically significant differences were detected among the groups, OSI values showed a similar temporal pattern across the study, declining during the first postoperative week, peaking at week 2, and decreasing again by week 4. Group 1, which received celecoxib preoperatively, demonstrated the greatest early reduction in OSI. While a lower OSI indicates reduced oxidative burden, these observations must be interpreted cautiously as exploratory findings in an underpowered model. Nonetheless, the temporal trend may imply a potential influence of preemptive NSAID administration on early redox balance, although this interpretation remains speculative due to the limited statistical power.

Previous studies have demonstrated that NSAIDs can variably affect oxidative status depending on dose, timing, and formulation. Some reports associate COX inhibition with increased oxidative stress[40,41], while others describe elevations in antioxidant defenses or reduced oxidative byproducts under certain conditions[38,42]. In this context, the current findings may suggest a transient influence of preemptive NSAID administration on redox homeostasis; however, the absence of statistically significant results precludes any definitive biological conclusions. Larger, adequately powered studies incorporating biochemical and mechanistic endpoints are required to clarify whether NSAID timing meaningfully modulates postoperative oxidative dynamics.

This study has several limitations. Behavioral pain assessment was not included, which restricts interpretation of functional analgesic benefit. Orofacial bone defect models do not consistently generate quantifiable nocifensive behaviors (e.g., withdrawal, guarding, or alterations in feeding) in rodents[43]; therefore, objective biochemical markers were prioritized. Future studies should integrate validated nociceptive metrics to strengthen translational relevance. Celecoxib was administered via oral gavage due to solubility constraints; although procedures were standardized across all groups to minimize variability, minor handling-related stress cannot be fully excluded. DMSO, used as a vehicle at a highly diluted dose, exhibits dose-dependent anti-inflammatory and antioxidant activity. Despite the absence of differences between saline- and DMSO-treated controls in any parameter, intrinsic carrier bioactivity remains a methodological limitation. Micro-CT or biomechanical assessments were not feasible due to the small and shallow nature of the burr-hole defect, making histology and immunohistochemistry the most reliable and biologically informative primary outcomes for early healing. Finally, although sample size was determined by power analysis, ethical constraints on animal use reduced statistical sensitivity and limit generalizability, requiring cautious interpretation of group comparisons[44].

Despite these limitations, this work provides novel insight by concurrently evaluating bone healing, inflammatory cytokines, oxidative stress markers, and macrophage polarization within a clinically relevant mandibular defect. To our knowledge, this is the first study to explore how the timing of COX-2 inhibition may influence macrophage-mediated wound biology in a maxillofacial bone healing model, addressing a gap not previously examined in preemptive analgesia research. Across all outcomes, celecoxib timing did not produce statistically significant differences; however, preoperative dosing showed distinct trend-level reductions in early oxidative stress and M1/M2 imbalance, suggesting a potential modulatory effect on immediate postoperative inflammatory dynamics.

These biological patterns should be regarded as preliminary and hypothesis-generating rather than indicative of a treatment effect. Larger, adequately powered studies integrating molecular, imaging-based, biomechanical, and behavioral endpoints are warranted to determine whether preemptive COX-2 inhibition can meaningfully modulate postoperative inflammation and bone repair in clinically relevant oral and maxillofacial surgery settings.

Data availability

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

Received: 19 September 2025; Accepted: 7 January 2026
Published online: 13 January 2026

References

1. Kissin, I. Preemptive analgesia. *Anesthesiology* **93**, 1138–1144 (2000).
2. Woolf, C. J. & Chong, M. S. Preemptive analgesia—treating postoperative pain by preventing the establishment of central sensitization. *Anesth. Analg.* **77**, 362–379 (1993).
3. Zhou, Y. et al. Positive preemptive analgesia effectiveness of pregabalin combined with celecoxib in total knee arthroplasty: a prospective controlled randomized study. *Pain Res. Manag.* **2023**, 7088004 (2023).
4. Penprase, B., Brunetto, E., Dahmani, E., Forthoffer, J. J. & Kapoor, S. The efficacy of preemptive analgesia for postoperative pain control: a systematic review of the literature. *AORN J.* **101**, 94–105e8 (2015).
5. Vadivelu, N. et al. Preventive analgesia for postoperative pain control: a broader concept. *Local. Reg. Anesth.* **7**, 17–23 (2014).
6. Ramires, G. A. D. et al. Combination of Etodolac and dexamethasone improves preemptive analgesia in third molar surgery: a randomized study. *Clin. Oral Investig.* **25**, 2297–2305 (2021).
7. Falci, S. G. M., Lima, T. C., Martins, C. C., Santos, C. R. R. & Pinheiro, M. L. P. Preemptive effect of dexamethasone in third-molar surgery: a meta-analysis. *Anesth. Prog.* **64**, 136–143 (2017).
8. Costa, F. W. G. et al. Does the preemptive use of oral nonsteroidal anti-inflammatory drugs reduce postoperative pain in surgical removal of third molars? A meta-analysis of randomized clinical trials. *Anesth. Prog.* **62**, 57–63 (2015).
9. Cetira Filho, E. L. et al. Preemptive use of oral nonsteroidal anti-inflammatory drugs for the relief of inflammatory events after surgical removal of lower third molars: a systematic review with meta-analysis of placebo-controlled randomized clinical trials. *J. Cranio-Maxillofac. Surg.* **48**, 293–307 (2020).
10. Albuquerque, A. F. M. et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind randomized placebo-controlled study. *Int. J. Oral Maxillofac. Surg.* **46**, 1615–1625 (2017).
11. Zhang, B., Yang, Y., Yi, J., Zhao, Z. & Ye, R. Hyperglycemia modulates M1/M2 macrophage polarization via reactive oxygen species overproduction in ligature-induced periodontitis. *J. Periodontal Res.* **56**, 991–1005 (2021).
12. Yunna, C., Mengru, H., Lei, W. & Weidong, C. Macrophage M1/M2 polarization. *Eur. J. Pharmacol.* **877**, 173090 (2020).
13. Liu, G. et al. A standardized rat burr-hole defect model to study maxillofacial bone regeneration. *Acta Biomater.* **86**, 450–464 (2019).
14. Li, K. H., Cheng, L., Zhu, Y., Deng, G. B. & Long, H. T. Effects of a selective cyclooxygenase-2 inhibitor (celecoxib) on fracture healing in rats. *Indian J. Orthop.* **47**, 395–401 (2013).
15. Al-Waeli, H. et al. Non-steroidal anti-inflammatory drugs and bone healing in animal models: a systematic review and meta-analysis. *Syst. Rev.* **10**, 1–20 (2021).
16. Panchal, N. K. & Sabina, E. P. Non-steroidal anti-inflammatory drugs (NSAIDs): a current insight into its molecular mechanism eliciting organ toxicities. *Food Chem. Toxicol.* **172**, 113598 (2023).
17. Teerawattananon, C., Tantayakom, P., Suwanawiboon, B. & Katchamart, W. Risk of perioperative bleeding related to highly selective cyclooxygenase-2 inhibitors: a systematic review and meta-analysis. *Semin Arthritis Rheum.* **52**, 520–528 (2022).
18. Zura, R. et al. Epidemiology of fracture nonunion in 18 human bones. *JAMA Surg.* **151**, e162775 (2016).
19. Jeffcoach, D. R. et al. Nonsteroidal anti-inflammatory drugs' impact on nonunion and infection rates in long-bone fractures. *J. Trauma. Acute Care Surg.* **76**, 779–783 (2014).
20. Pountos, I., Panteli, M., Walters, G. & Giannoudis, P. V. NSAIDs inhibit bone healing through downregulation of TGF-β3 expression during endochondral ossification. *Injury* **52**, 1294–1299 (2021).
21. Menger, M. M. et al. Diclofenac, a NSAID, delays fracture healing in aged mice. *Exp. Gerontol.* **178**, 112201 (2023).
22. Sevimli, R., Uzel, M., Sayar, H., Kalender, A. M. & Dokmeci, O. The effect of dexketoprofen Trometamol on the healing of diaphysis fractures of rat tibia. *Acta Orthop. Traumatol. Turc.* **47**, 423–429 (2013).
23. Inal, S. et al. Comparison of the effects of dexketoprofen trometamol, meloxicam and diclofenac sodium on fibular fracture healing, kidney and liver: an experimental rat model. *Injury* **45**, 494–500 (2014).
24. Nagano, A., Arioka, M., Takahashi-Yanaga, F., Matsuzaki, E. & Sasaguri, T. Celecoxib inhibits osteoblast maturation by suppressing the expression of Wnt target genes. *J. Pharmacol. Sci.* **133**, 18–24 (2017).
25. Huss, M. K., Felt, S. A. & Pacharinsak, C. Influence of pain and analgesia on orthopedic and wound-healing models in rats and mice. *Comp. Med.* **69**, 535–545 (2019).
26. Burd, T., Hughes, M. & Anglen, J. Heterotopic ossification prophylaxis with indomethacin increases the risk of long-bone nonunion. *J. Bone Joint Surg. Br.* **85**, 700–705 (2003).
27. Lavernia, C. J., Contreras, J. S., Villa, J. M. & Rossi, M. D. Celecoxib and heterotopic bone formation after total hip arthroplasty. *J. Arthroplasty*. **29**, 390–392 (2014).
28. Migliorini, F. et al. Selective versus non-selective NSAIDs as prophylaxis for heterotopic ossification following hip arthroplasty: a meta-analysis. *J. Orthop. Traumatol.* **23**, 30 (2022).
29. Mills, L., Tsang, J., Hopper, G., Keenan, G. & Simpson, A. The multifactorial aetiology of fracture nonunion and the importance of searching for latent infection. *Bone Joint Res.* **5**, 512–519 (2016).
30. Ghiasi, M. S., Chen, J., Vaziri, A., Rodriguez, E. K. & Nazarian, A. Bone fracture healing in Mechanobiological modeling: a review of principles and methods. *Bone Rep.* **6**, 87–100 (2017).
31. Ma, Q., Miri, Z., Haugen, H. J., Moghanian, A. & Loca, D. Significance of mechanical loading in bone fracture healing, bone regeneration, and vascularization. *J. Tissue Eng.* **14**, 20417314231172573 (2023).
32. Megha, K., Joseph, X., Akhil, V. & Mohanan, P. Cascade of immune mechanism and consequences of inflammatory disorders. *Phytomedicine* **91**, 153712 (2021).
33. Shavit, Y., Fridel, K. & Beilin, B. Postoperative pain management and Proinflammatory cytokines: animal and human studies. *J. Neuroimmune Pharmacol.* **1**, 443–451 (2006).
34. Albuquerque, A. F. M. et al. Does non-steroidal anti-inflammatory drugs-related preemptive analgesia modulate SOCS3/IL-6 pathway in oral surgery? *Inflammopharmacology* **32**, 1017–1024 (2024).
35. Ye, G. et al. Quercetin alleviates neuropathic pain in the rat CCI model by mediating AMPK/MAPK pathway. *J. Pain Res.* **14**, 1289–1301 (2021).
36. Gallelli, L. et al. The effects of nonsteroidal anti-inflammatory drugs on clinical outcomes, synovial fluid cytokine concentration and signal transduction pathways in knee osteoarthritis. A randomized open label trial. *Osteoarthr. Cartil.* **21**, 1400–1408 (2013).
37. Beilin, B. et al. Effects of preemptive analgesia on pain and cytokine production in the postoperative period. *Anesthesiology* **98**, 151–155 (2003).
38. Álvarez, M. S. et al. New formulation of ibuprofen-arginate reduces oxidative stress and prevents macrophage polarization toward M1 phenotype. *Biomed. Pharmacother.* **183**, 117819 (2025).
39. Pennock, N. D. et al. I ibuprofen supports macrophage differentiation, T cell recruitment, and tumor suppression in a model of postpartum breast cancer. *J. Immunother. Cancer* **6**, 1–23 (2018).
40. Kahraman, E., Cevik, B. & Saracoglu, K. T. The effects of pre-emptive intravenous ibuprofen on the thiol/disulfide homeostasis and C-reactive protein level as the markers of oxidative stress and inflammation during gynecologic laparoscopy: a randomised clinical trial. *Int. J. Clin. Pract.* **75**, e14872 (2021).

41. Bindu, S., Mazumder, S. & Bandyopadhyay, U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. *Biochem. Pharmacol.* **180**, 114147 (2020).
42. Cetira Filho, E. L. et al. Effect of preemptive photobiomodulation associated with nimesulide on the postsurgical outcomes, oxidative stress, and quality of life after third molar surgery: a randomized, split-mouth, controlled clinical trial. *Clin. Oral Investig.* **26**, 6941–6960 (2022).
43. Liu, S., Crawford, J. & Tao, F. A assessing orofacial pain behaviors in animal models: a review. *Brain Sci.* **13**, 390 (2023).
44. Gracely, E. J. Justification of the number of animals to be used. In *The IACUC Handbook* 237–245 (CRC Press, 2014).

Author contributions

C.A: Methodology, Resources, Animal study, Data Curation, and writing of the manuscript, P.A: Methodology, supervision and planning of the research, Review of the manuscript, E.R.H: Methodology, Animal study, Data Curation, and Review of the manuscript, H.D: Methodology, Data Curation, S.K: Formal analysis.

Funding

No funding was received for conducting this study.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-35629-9.

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