



Effects of *Kaempferia galanga* L. extract on monosodium iodoacetate-induced osteoarthritis in rats

[Efectos del extracto de *Kaempferia galanga* L. sobre la osteoartritis inducida por yodoacetato monosódico en ratas]

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Abstract

Context: Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the progressive degradation of cartilage, synovial inflammation, and functional impairment. *Kaempferia galanga* L. extract (KGE) demonstrates analgesic, anti-inflammatory, and antioxidant properties, yet its effects on OA remain unclear.

Aims: To evaluate the chondroprotective and anti-inflammatory effects of KGE on a monosodium iodoacetate (MIA)-induced OA rat model.

Methods: Male Wistar rats were allocated into six groups: normal control, MIA control, MIA + KGE (90, 180, and 360 mg/kg), and MIA + ibuprofen (20 mg/kg). OA was induced via intra-articular MIA injection. Treatments were administered orally for 21 days. Knee joint diameter, plasma IL-1 β , histopathology (OARSI scoring), and immunohistochemical markers (type II collagen, SOX-9, aggrecan, NF- κ B, TNF- α , and MMP-13) were assessed.

Results: KGE reduced plasma IL-1 β levels, suppressed NF- κ B, TNF- α , and MMP-13, while concurrently enhancing type II collagen, SOX-9, and aggrecan in a dose-dependent manner. Administration of KGE at 360 mg/kg demonstrated the most pronounced chondroprotective effects.

Conclusions: KGE attenuated MIA-induced OA by modulating inflammatory pathways and preserving cartilage integrity, thereby supporting its potential as an alternative therapeutic option for osteoarthritis. Further research is warranted to elucidate its molecular mechanisms and assess its long-term safety profile.

Keywords: cartilage protection; inflammation; *Kaempferia galanga*; MIA model; OARSI; osteoarthritis.

Resumen

Contexto: La osteoartritis (OA) es una enfermedad articular degenerativa crónica que se caracteriza por la degradación progresiva del cartílago, la inflamación sinovial y el deterioro funcional. El extracto de *Kaempferia galanga* L. (KGE) presenta propiedades analgésicas, antiinflamatorias y antioxidantes; sin embargo, sus efectos sobre la OA aún no están claros.

Objetivos: Evaluar los efectos condroprotectores y antiinflamatorios del KGE en un modelo de rata con OA inducida por yodoacetato monosódico (MIA).

Métodos: Se asignaron ratas Wistar machos a seis grupos: control normal, control con MIA, MIA + KGE (90, 180 y 360 mg/kg) y MIA + ibuprofeno (20 mg/kg). La OA se indujo mediante inyección intraarticular de MIA. Los tratamientos se administraron por vía oral durante 21 días. Se evaluaron el diámetro de la articulación de la rodilla, la IL-1 β plasmática, la histopatología (puntuación OARSI) y los marcadores inmunohistoquímicos (colágeno tipo II, SOX-9, agregano, NF- κ B, TNF- α y MMP-13).

Resultados: KGE redujo los niveles plasmáticos de IL-1 β , suprimió NF- κ B, TNF- α y MMP-13, y mejoró el colágeno tipo II, SOX-9 y el agregano de forma dosis-dependiente. La administración de KGE a 360 mg/kg mostró los efectos condroprotectores más pronunciados.

Conclusiones: KGE atenuó la OA inducida por MIA mediante la modulación de las vías inflamatorias y la preservación de la integridad del cartílago, lo que respalda su potencial como opción terapéutica alternativa para la osteoartritis. Se requieren más investigaciones para dilucidar sus mecanismos moleculares y evaluar su perfil de seguridad a largo plazo.

Palabras Clave: inflamación; *Kaempferia galanga*; modelo de MIA; OARSI; osteoartritis; protección del cartílago.

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Abbreviations: ADAMTS-4: a disintegrin and metalloproteinase with thrombospondin motifs-4; BMP-2: bone morphogenetic protein-2; ECM: extracellular matrix; EPMC: Ethyl p-methoxycinnamate; IA: intra-articular; Ibu: ibuprofen; IL-1 β : interleukin-1 β ; IL-6: interleukin-6; KGE: *Kaempferia galanga* L. extract; MIA: monosodium iodoacetate; MMP-13: matrix metalloproteinase-13; NF- κ B: nuclear factor kappa B; OA: osteoarthritis; OARSI: Osteoarthritis Research Society International; PO: oral administration; ROS: reactive oxygen species; SOX-9: transcription factor SRY-related HMG box 9; TNF- α : tumor necrosis factor- α .

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive degeneration of articular cartilage, weakening of the joint capsule, formation of osteophytes, subchondral bone sclerosis, and narrowing of the joint space (Indonesian Rheumatology Association, 2014). The global incidence of OA has escalated markedly, increasing from 247.51 million cases in 1990 to 527.81 million in 2019, with knee OA affecting approximately 16% of the elderly population (Cui et al., 2020; Long et al., 2022). The pathophysiology of the disease is attributed to an imbalance between anabolic and catabolic processes in cartilage, which is influenced by pro-inflammatory cytokines such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6). These cytokines activate nuclear factor kappa B (NF- κ B), leading to the upregulation of matrix-degrading enzymes, including matrix metalloproteinase-13 (MMP-13) and a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4) (Molnar et al., 2021). This inflammatory response downregulates critical anabolic markers, such as the transcription factor SRY-related HMG box 9 (SOX-9), type II collagen, and aggrecan, thereby facilitating OA progression (Ouyang et al., 2019). Current pharmacological interventions for OA primarily focus on alleviating symptoms, including pain and stiffness. However, these medications, particularly non-steroidal anti-inflammatory drugs, are often associated with adverse effects, especially with prolonged use (Leopoldino et al., 2019). Consequently, there is an increasing interest in investigating natural compounds with anti-inflammatory and chondroprotective properties as potential alternative treatments for osteoarthritis (Abo-Zalam et al., 2021; Choi et al., 2020; Chun et al., 2021; Hamdalla et al., 2022; Kim et al., 2020; Song et al., 2025; Tseng et al., 2023).

Indonesia is home to a diverse array of medicinal plants used in traditional herbal remedies. One significant species is *Kaempferia galanga* L., which achieved a production volume exceeding 44 million kilograms in 2020, with substantial cultivation occurring in East Java, where it yielded approximately 8,759 tons annually in 2018 (Central Bureau of Statistics, 2018). *K. galanga* is recognized for its broad pharmacological properties, particularly its anti-inflammatory and analgesic effects (Umar et al., 2012; Wahyuni et al., 2022). Ethyl p-methoxycinnamate (EPMC), the principal bioactive compound in *K. galanga*, can constitute up to 80.05% of the total extract (Nurani et al., 2020). EPMC exerts anti-

inflammatory effects by inhibiting the production of pro-inflammatory cytokines, such as IL-1 β and TNF- α (Umar et al., 2014). Additionally, another constituent, kaempferol, has been shown to stimulate chondrogenesis by enhancing matrix proteoglycan synthesis and upregulating chondrogenic markers, including SOX-9, as well as signaling molecules such as bone morphogenetic protein-2 (BMP-2) (Nepal et al., 2013). Recent clinical evidence has illuminated the potential of *K. galanga* L. in the management of osteoarthritis. In a double-blind randomized clinical trial, the administration of a *K. galanga* extract (KGE) capsule at a dosage of 160 mg/day over ten days resulted in a reduction in pain score, stiffness, physical function, and total score on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), demonstrating an efficacy comparable to that of meloxicam at 15 mg/day (Syahrudin et al., 2017). Although previous studies have demonstrated the anti-inflammatory effects of KGE, the precise molecular mechanisms underlying these effects and their impact on an OA model have not been thoroughly investigated. Consequently, this study aimed to evaluate the therapeutic potential of KGE in a rat model of monosodium iodoacetate (MIA)-induced OA. KGE was administered orally for 21 days at doses of 90, 180, and 360 mg/kg. The severity of OA was assessed using the Osteoarthritis Research Society International (OARSI) score, plasma IL-1 β levels, and the expression of critical biomarkers in articular cartilage, including type II collagen, SOX-9, TNF- α , NF- κ B, MMP-13, and aggrecan.

MATERIAL AND METHODS

Preparation of *Kaempferia galanga* extract

The rhizome of *K. galanga* was harvested in Blitar, East Java, Indonesia (GPS: 8°08'44.3"S 112°20'13.2"E), cultivated within an agroforestry system managed by the Faculty of Agriculture at Universitas Brawijaya. The botanical identity of the plant material was taxonomically verified by the UPT Laboratory of Herbal Materia Medica in Batu, Indonesia (certificate of determination no. 074/400/102.20-A/2022). The extract was prepared by macerating 200 g of *K. galanga* powder in 2 L of 96% ethanol for 72 hours. Following maceration, the mixture was filtered, and the filtrate was concentrated using a continuous rotary evaporator (IKA RV 10 basic, IKA Werke, Staufen im Breisgau, Germany) at approximately 40°C. Subsequently, the concentrated extract was dried in a laboratory oven (ED 53, Binder,

Germany) at 50°C for three days (Adianingsih et al., 2023). The extraction yield of the *K. galanga* extract (KGE) was approximately 10.405%.

Experimental animals

Thirty adult male Wistar rats (weight range: 160–220 g; age: 8–10 weeks) were sourced from the Murine Farm located in East Java, Indonesia. All experimental protocols involving animal subjects were conducted in accordance with the principles of laboratory animal care, and the study protocol received approval from the Ethics Committee of Universitas Brawijaya (Approval No. 076-KEP-UB-2022, dated May 12, 2022). Measures were implemented to minimize animal suffering throughout the study. The rats were housed in the Animal Laboratory, Bioscience Institute, Universitas Brawijaya, Indonesia, under controlled conditions of 24–26°C, 40–60% humidity, and a 12-hour light/12-hour dark cycle. Body weight was measured at the onset of the experiment and weekly thereafter. All animals underwent a seven-day acclimatization period and were provided with standard feed and ad libitum access to water. The experimental subjects were randomly assigned to 6 groups (n = 5), with 2–3 rats per cage. The study design is illustrated in Fig. 1. The experimental groups included: (1) normal control; (2) MIA-induced osteoarthritis control; (3) MIA + KGE 90 mg/kg; (4) MIA + KGE 180 mg/kg; (5) MIA + KGE 360 mg/kg; and (6) MIA + ibuprofen 20 mg/kg.

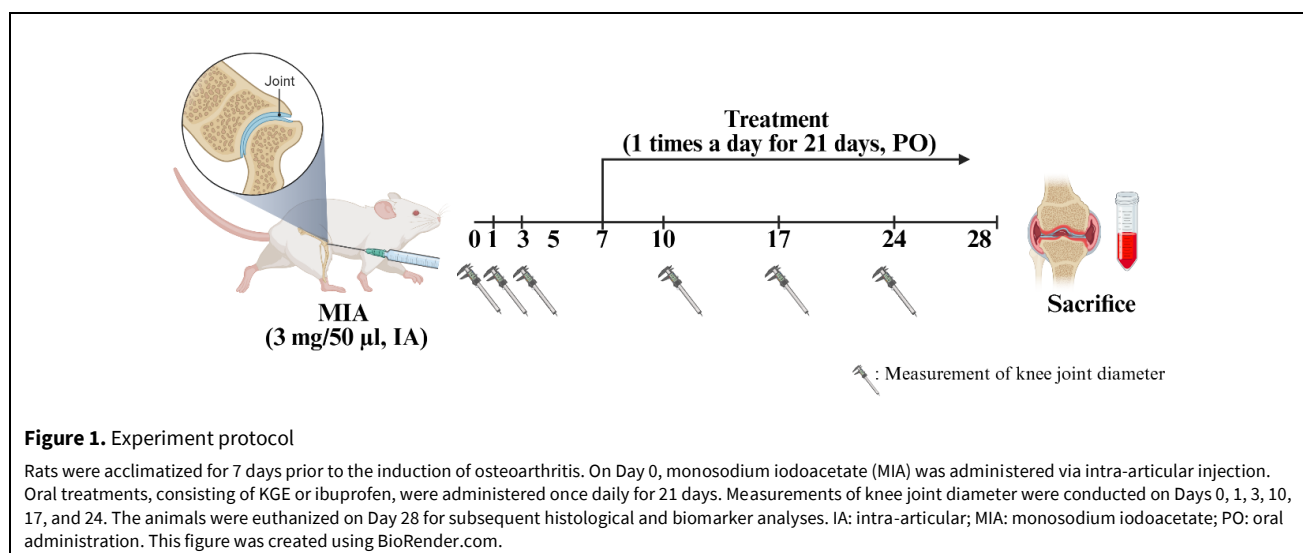
MIA-induced osteoarthritis

Osteoarthritis was induced via a single intra-articular injection of 3 mg/50 µL monosodium iodoacetate (MIA) (cat. #I2512; Sigma, St. Louis, MO, USA), as previously described (Abo-Zalam et al., 2021). The animals were briefly anesthetized with ketamine (40–87 mg/kg; concentration 50 mg/mL), administered intramuscularly at a volume of 0.3 mL (Hall et al., 2001). The

right knee region was shaved and disinfected with a 96% ethanol swab. Three milligrams of MIA were dissolved in 0.9% sterile saline (total volume of 50 µL) and injected into the right knee joint cavity using a sterile hypodermic syringe. All procedures were conducted under aseptic conditions. Following the injection, the animals were returned to their cages and monitored until they fully recovered from anesthesia. Joint diameter was measured using a digital caliper (Monotaro RoHS) on days 0 (prior to and 1 h post-injection), 1, 3, 10, 17, and 24 post-injections (Abo-Zalam et al., 2021).

Drug administration

KGE was administered orally at doses of 90, 180, and 360 mg/kg, along with ibuprofen (IOL Chemical and Pharmaceutical Limited, India), on day 7 following the MIA injection. The selected doses of KGE (90, 180, and 360 mg/kg) were informed by a prior study utilizing carrageenan-induced rat models, which demonstrated that oral administration of KGE at 45, 90, and 180 mg/kg led to a significant reduction in paw edema in a dose-dependent manner (Samodra & Febrina, 2019). The inclusion of the 360 mg/kg dose in this study aimed to investigate the potential upper limit of therapeutic efficacy. Both KGE extracts and ibuprofen were suspended in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) and administered orally once daily for 21 consecutive days (Abo-Zalam et al., 2021). Ibuprofen was utilized at a reference dose of 20 mg/kg to represent conventional drug therapy (Kim et al., 2020). All animals were sacrificed on day 28. Anesthesia was induced using a 1:1 mixture of 2% xylazine and ketamine (100 mg/mL), administered intramuscularly. Blood samples were collected for the measurement of plasma IL-1β levels, and the right knee joint was harvested for subsequent histopathological and immunohistochemical analyses.



Measurement of plasma IL-1 β levels

Blood samples were collected, and plasma was separated from whole blood utilizing a high-speed centrifuge (TOMY LC-200) at 3,000 rpm for 10 min. Plasma levels of IL-1 β were quantified using a rat IL-1 β enzyme-linked immunosorbent assay (ELISA) kit (catalog number E-EL-R0012; Elabscience Technology, Wuhan, China), and all analyses were conducted according to the manufacturer's instructions. The optical density (OD) of each sample was measured at 450 nm using an ELISA reader (LABTRON).

Histopathological examination using the OARSI scoring system

The right knee joint was excised and subsequently fixed in 10% neutral-buffered formalin for 24 h (Kim et al., 2020). Following fixation, the knee joint underwent a series of processing steps, including decalcification with 10% EDTA for 33 days, dehydration, paraffin embedding, and sectioning at 4 μ m. The sections were then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). Histopathological alterations were assessed utilizing the Osteoarthritis Research Society International (OARSI) scoring system. This grading system, which ranges from 0 to 6, reflects the vertical depth of cartilage lesions and indicates their biological severity (Ma et al., 2018; Pritzker et al., 2006). The tissue sections were analyzed under an optical microscope (AmScope Trinocular Microscope, USA) at magnifications of 100 $\times$ and 400 $\times$.

Immunohistochemical analysis

Immunohistochemical staining was employed to evaluate the expression levels of type II collagen, SOX-9, aggrecan, NF- κ B, TNF- α , and MMP-13. The immunostaining procedure utilized a Super Plus High-Sensitive and Rapid Immunohistochemical kit (cat. #E-IR-R221, Elabscience Technology, Wuhan, China). In brief, sections of knee joint tissue (4 μ m) were subjected to Dewaxing/Antigen Retrieval Buffer treatment for 30 minutes under heating conditions, followed by incubation with SP Reagent B Peroxidase Blocking Buffer at room temperature for 15 minutes. Following a wash with PBS, the sections were incubated at 37°C for two hours with the following primary antibodies: a mouse monoclonal anti-collagen type II antibody (1:50, cat. #sc-52658, Santa Cruz, USA), a mouse monoclonal anti-aggrecan antibody (1:50, cat. #ab-3773, Abcam, Cambridge, UK), a mouse monoclonal anti-SOX-9 antibody (1:200, cat. #sc-166505, Santa Cruz, CA, USA), a mouse monoclonal anti-p65 NF- κ B antibody (1:50, cat. #sc-8008, Santa Cruz, USA), a mouse monoclonal anti-TNF- α antibody (1:50, cat. #sc-52746, Santa Cruz, CA,

USA), and a mouse monoclonal anti-MMP-13 antibody (1:200, cat. #sc-515-284, Santa Cruz, CA, USA). After the incubation period, sections were treated with a secondary antibody, followed by color development using a 3,3'-diaminobenzidine solution, and subsequently counterstained with hematoxylin. The stained tissues were examined under an AmScope trinocular microscope (USA) at 400 $\times$ magnification, and images were captured from five random locations per slide. Quantitative analysis of type II collagen and aggrecan expression was conducted using ImageJ 1.53k software (National Institutes of Health, Maryland, USA) by calculating the percentage of the positively stained area. The expressions of SOX-9, NF- κ B, TNF- α , and MMP-13 were quantified by determining the percentage of positively stained chondrocytes (brown in color) relative to the total number of chondrocytes within the visual field.

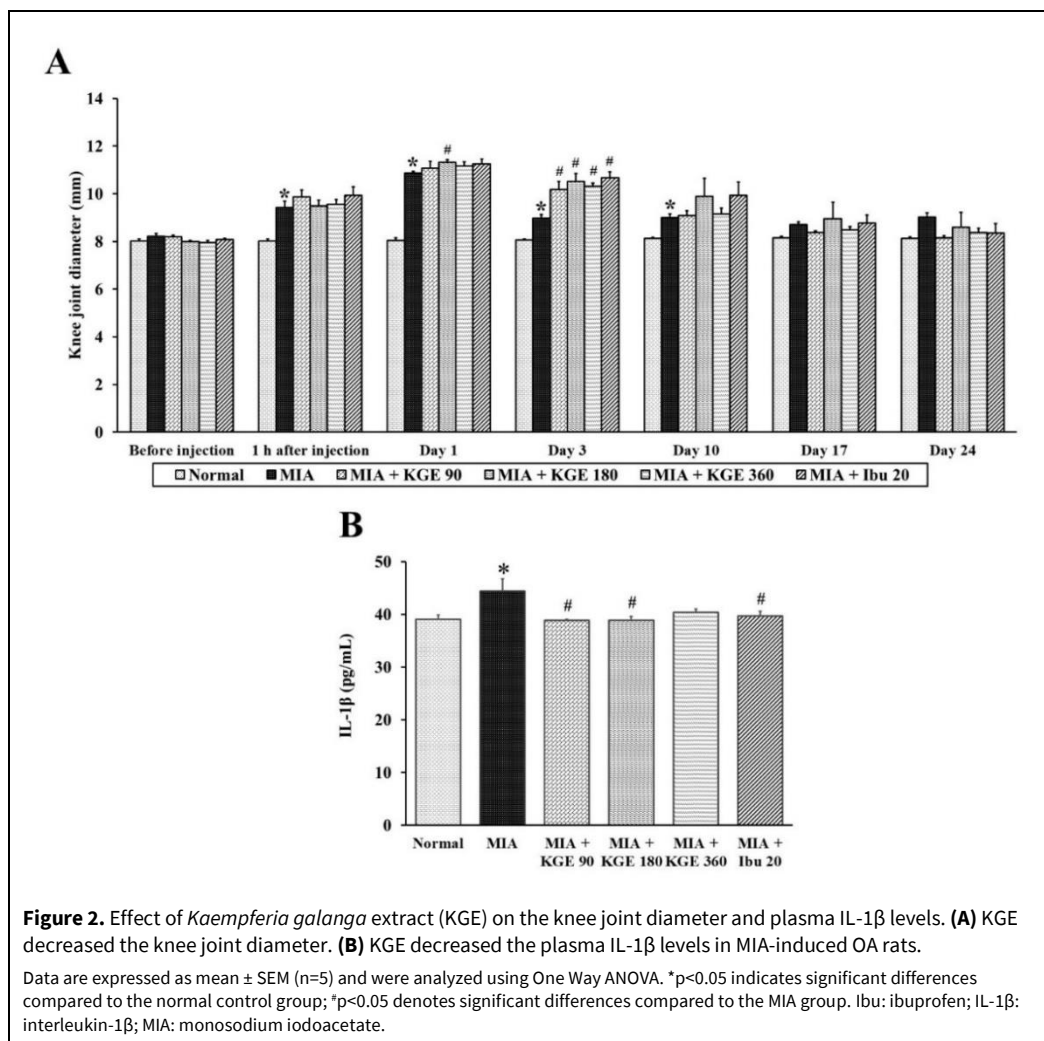
Statistical analysis

Results are presented as means $\pm$ standard error of the mean (SEM). Data analysis was conducted using SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was employed to compare differences among groups, followed by Tukey's Honestly Significant Difference post hoc test. For the non-parametric analysis of the OARSI score, the Kruskal-Wallis test and the Mann-Whitney test were utilized. Statistical significance was established at $p < 0.05$.

RESULTS

Effect of KGE on knee joint diameter and plasma IL-1 β levels

Knee joint diameter was monitored at multiple time points to evaluate joint inflammation following MIA injection (Fig. 2A). A significant increase in joint diameter was observed in the MIA-induced OA group, peaking on day 1 post-injection ($p = 0.013$), followed by a gradual decline across all groups over time. Notably, animals treated with KGE, particularly at doses of 90 and 360 mg/kg, demonstrated a reduction in swelling on days 17 and 24, comparable to the ibuprofen treatment group; however, this difference was not statistically significant ($p > 0.05$). To evaluate systemic inflammatory responses, plasma IL-1 β levels were measured (Fig. 2B). The MIA group showed significantly elevated IL-1 β levels compared with the normal control group ($p = 0.018$). Oral administration of KGE resulted in a significant reduction in IL-1 β levels at 90 mg/kg ($p = 0.020$) and 180 mg/kg ($p = 0.013$), with effects like those observed in the ibuprofen-treated group ($p = 0.043$).



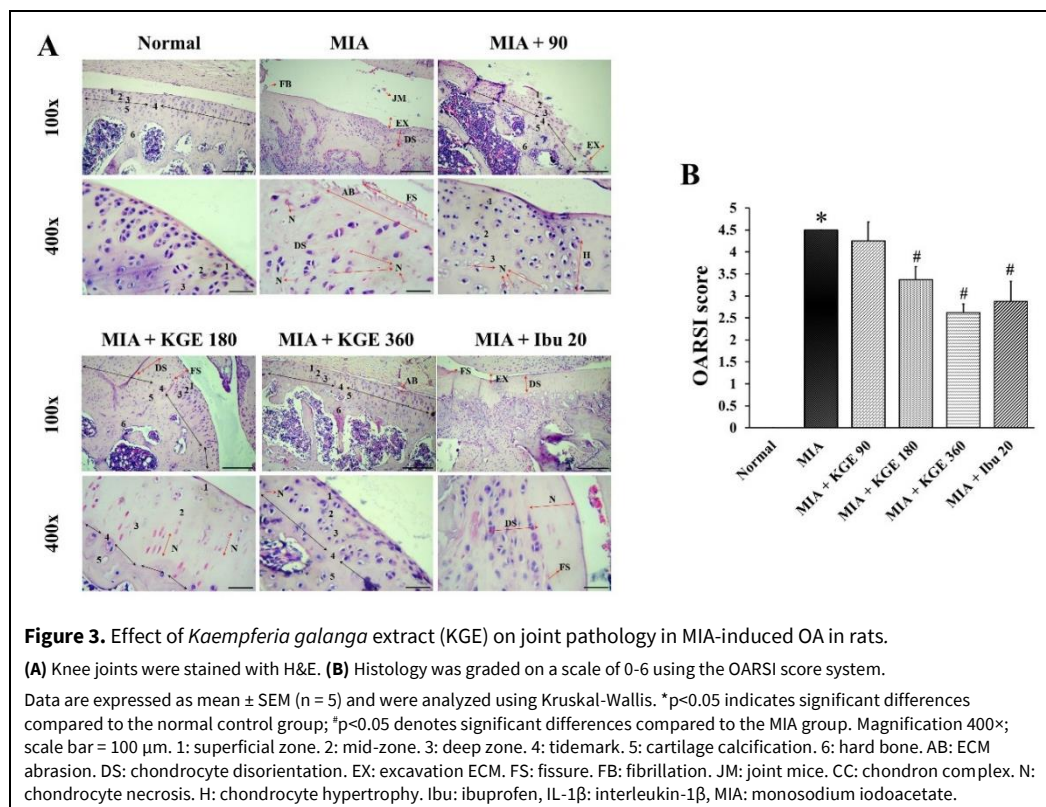
Effect of KGE on histopathology and OARSI score

Histopathological alterations in articular cartilage were assessed utilizing the OARSI scoring system (Fig. 3). In the control group, cartilage exhibited a normal structure, corresponding to an OARSI score of 0. Conversely, cartilage from the MIA-induced OA group demonstrated significant degeneration, as evidenced by a markedly elevated OARSI score compared with the normal group ($p = 0.048$). Administration of KGE at a dosage of 90 mg/kg yielded minimal enhancement in cartilage integrity, resulting in a marginal reduction in the OARSI score ($p > 0.05$). However, treatment with KGE at 180 mg/kg facilitated moderate structural restoration, resulting in a lower OARSI score than in the MIA group ($p = 0.040$). Notably, at a dosage of 360 mg/kg, further improvements were observed, with cartilage structure being significantly preserved and a substantial reduction in the OARSI score ($p = 0.013$). Similarly, rats treated with ibuprofen exhibited improved cartilage histology ($p = 0.046$), although the reduction in the OARSI score was slightly less

pronounced than that observed in the KGE 360 mg/kg group.

Effect of KGE on type II collagen, SOX-9, aggrecan, NF-κB, TNF-α, and MMP-13 expression in articular cartilage

Immunohistochemical analysis was performed to assess the expression of cartilage matrix and inflammatory markers within the articular cartilage (Fig. 4A). In the MIA-induced OA group, there was a significant reduction in the expression of type II collagen ($p = 0.022$), SOX-9 ($p = 0.041$), and aggrecan ($p = 0.014$) when compared to the normal control group. Conversely, treatment with KGE at all administered doses, in combination with ibuprofen, increased the expression of these cartilage-related proteins. Notably, the expression levels of type II collagen, SOX-9, and aggrecan were significantly elevated in the KGE-treated groups, with the 360 mg/kg dosage demonstrating the most pronounced effect ($p > 0.05$) (Fig. 4B–D). In contrast, the expression levels of NF-κB ($p = 0.018$), TNF-α ($p = 0.014$), and MMP-13 ($p = 0.003$) were significantly increased in the MIA group compared with the normal control



group (Fig. 4E–G). Nonetheless, the administration of KGE resulted in a dose-dependent reduction in the expression of these inflammatory and catabolic markers, with the most substantial reduction observed in the KGE 360 mg/kg group ($p > 0.05$).

DISCUSSION

MIA acts as a chondrotoxic agent, disrupting chondrocyte metabolism and leading to chondrocyte necrosis and subsequent articular cartilage degradation (Chun et al., 2021). Furthermore, MIA triggers systemic inflammatory responses by promoting the production of reactive oxygen species (ROS), which activate the NF- κ B signaling pathway and upregulate pro-inflammatory mediators and apoptosis-related proteins (Lepetsos et al., 2019; Mabey et al., 2016). Additionally, MIA is implicated in the induction of acute joint inflammation and edema, leading to increased knee joint diameter (Moilanen et al., 2015; Tseng et al., 2023). This observation is consistent with prior research indicating that edema reaches its peak on day 1 and remains evident for approximately 10 days (Abo-Zalam et al., 2021). Another investigation reported analogous findings, with a significant increase in knee joint diameter observed on days 1, 3, and 10, returning to baseline levels by day 17 (McDougall et al., 2017). MIA is also characterized by biphasic pain behavior, encompassing an early inflammatory phase (2–6 days post-injection)

followed by a later chronic pain phase (greater than 14 days post-injection) (Lockwood et al., 2019). This biphasic pattern may elucidate the transient nature of joint swelling observed in the current study, in which edema peaked early and gradually resolved, despite the persistence of cartilage damage and inflammation.

Given the extensive molecular and structural damage induced by MIA, effective therapeutic intervention is essential for restoring cartilage integrity and exerting chondroprotective effects. In this context, the present study demonstrates that KGE exhibits chondroprotective and anti-inflammatory properties in MIA-induced OA rat models. These effects were observed to be dose-dependent, with a dosage of 360 mg/kg yielding outcomes comparable to those of ibuprofen. The underlying mechanism is likely mediated through the suppression of NF- κ B and its downstream cytokines, resulting in decreased MMP-13 activity and increased expression of sex-determining region Y-box 9 (SOX-9) and type II collagen. KGE also improved the histological architecture of rat articular cartilage by reducing IL-1 β and TNF- α expression, thereby inhibiting the NF- κ B signaling pathway, which ultimately led to a reduction in cartilage damage. A key bioactive constituent responsible for these effects is EPMC, which was present in this extract at a concentration of 54.13%, as determined using the validated HPLC method from a previously published study (Adianingsih et al., 2023).

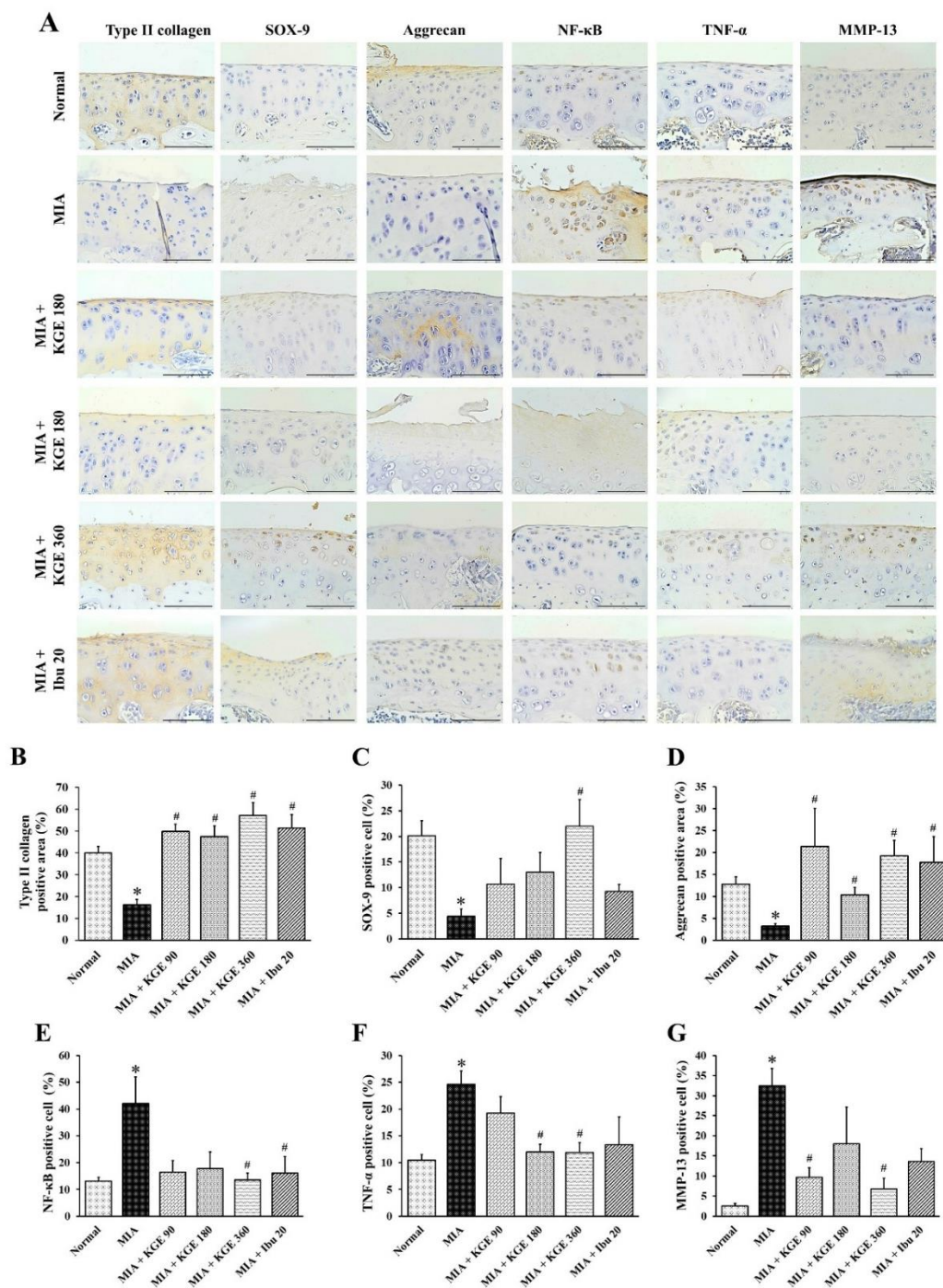


Figure 4. Effect of *Kaempferia galanga* extract (KGE) on the cartilage of OA rats.

(A) Immunohistochemistry. The rat knee joints were examined via immunohistochemistry of joint sections from different treatment groups for type II collagen, SOX-9, aggrecan, NF-κB, TNF-α, and MMP-13. (B-G) *Kaempferia galanga* extract increased the expression of type II collagen, SOX-9, and aggrecan compared to the MIA group and decreased the expression of NF-κB, TNF-α, and MMP-13.

Data are expressed as mean ± SEM (n = 5). *p<0.05 indicates significant differences () compared to the normal control group, while #p<0.05 denotes significant differences compared to the MIA group. Magnification 400×; scale bar = 100 μm. Ibu: Ibuprofen, IL-1β: interleukin 1β, MIA: monosodium iodoacetate.

EPMC exerts its anti-inflammatory effects through multiple mechanisms, including the inhibition of ROS and the suppression of NF-κB signaling. Research indicates that EPMC can reduce intracellular ROS levels by over 50%, thereby mitigating oxidative stress, a known activator of NF-κB (Srivastava et al., 2019). In

cancer cell models, EPMC also inhibits NF-κB transcriptional activity by downregulating p38 and Akt (Ser473) phosphorylation (Lallo et al., 2022), further substantiating its extensive anti-inflammatory potential. Additionally, both *in vitro* and *in vivo* studies have demonstrated the antioxidant properties of *K. galanga*,

reinforcing its significance as a natural therapeutic agent in inflammation-driven diseases (Wang et al., 2023; 2025). IL-1 β contributes to chondrocyte apoptosis by activating caspase-8 and caspase-3 (Yang et al., 2022). In these findings, KGE significantly decreased plasma IL-1 β levels compared to the MIA-induced osteoarthritis group. This anti-inflammatory effect is consistent with prior studies demonstrating that KGE at doses of 45, 90, and 180 mg/kg significantly reduced paw edema in carrageenan-induced rat models (Samodra & Febrina, 2019).

KGE exerts chondroprotective effects by attenuating ROS production, thereby downregulating NF- κ B signaling. This modulation results in decreased levels of MMP-13, preservation of SOX-9 and type II collagen, and protection against chondrocyte hypertrophy and apoptosis. EPMC and kaempferol, the principal bioactive compounds in KGE, function synergistically to diminish pro-inflammatory cytokine levels and inhibit NF- κ B signaling, thereby supporting the observed structural and molecular enhancements. Treatment with KGE led to downregulation of markers of cartilage degradation and a reduction in OARSI scores, indicating structural recovery. Notably, the group receiving 360 mg/kg of KGE exhibited the lowest OARSI score among all treatment cohorts. Bone morphogenetic proteins play a critical role in maintaining cartilage homeostasis and promoting SOX-9 expression. However, pro-inflammatory cytokines can suppress SOX-9 expression in chondrogenic cells (Ushita et al., 2009). SOX-9 subsequently facilitates the synthesis of type II collagen, thereby creating a positive feedback loop that sustains extracellular matrix (ECM) integrity (Chen et al., 2021; Lepetsos et al., 2019; Quintana et al., 2009). The observed increases in SOX-9 and type II collagen expression in the KGE-treated groups support this proposed mechanism. This effect may be mediated by the inhibition of ROS and the suppression of NF- κ B, which decrease MMP-13, an enzyme responsible for the degradation of type II collagen (Lian et al., 2019; Srivastava et al., 2019). Furthermore, kaempferol, a flavonoid derived from *K. galanga*, has been shown to significantly enhance SOX-9 mRNA expression in OA mice via the NF- κ B signaling pathway in an anterior cruciate ligament method-induced OA mouse model (Estakhri et al., 2020). Additionally, kaempferol promotes chondrogenesis by inducing SOX-9 through the activation of the BMP signaling pathway (Heinegård, 2009). Collectively, these findings underscore the potential role of KGE in modulating cartilage-specific and inflammatory markers.

KGE has demonstrated efficacy comparable to ibuprofen in reducing pro-inflammatory cytokines (IL-1 β and TNF- α) and MMP-13 expression, while maintaining cartilage integrity (Kim et al., 2020). Ibuprofen at a

dose of 20 mg/kg effectively reduced IL-1 β levels, consistent with its established role in lowering inflammatory markers and alleviating pain in OA rat models (Kim et al., 2020). Moreover, ibuprofen has been shown to decrease IL-6 and interleukin-8 expression and to inhibit NF- κ B signaling, thereby reducing MMP-1 and MMP-13 expression and ultimately mitigating cartilage degradation in mice with MIA-induced OA (Sun et al., 2017). Therefore, KGE represents a promising natural alternative to non-steroidal anti-inflammatory drugs, providing both anti-inflammatory and chondroregenerative benefits through its phytochemical constituents. In a similar vein, multi-herbal formulations containing curcuminoids, gingerols, and boswellic acid have demonstrated significant efficacy in reducing OA severity in MIA-induced models, primarily by reducing oxidative stress and inflammatory responses (Erten et al., 2024). These plant-derived agents not only reduced the expression of IL-1 β , TNF- α , and MMPs but also improved joint structural integrity, a trend consistent with the current findings.

In summary, this study demonstrated that KGE attenuates OA progression in a dose-dependent manner, with 360 mg/kg exhibiting the most significant effects across structural, molecular, and inflammatory parameters. Despite some biomarkers displaying relatively high SEM values, likely attributable to individual variability and the limited sample size ($n = 5$), the dose-dependent effects of KGE remained consistent and statistically significant. Nevertheless, the specific molecular mechanisms and optimal therapeutic dosage remain to be explored. Previous acute toxicity studies have shown that oral administration of KGE at 5 g/kg does not cause mortality in experimental animals (Kanjapothi et al., 2004), suggesting a favorable safety profile. Several complementary experimental methodologies are recommended to enhance future research. These include behavioral pain assessments, such as the hind-paw weight-bearing incapacitance test (Kim et al., 2020), locomotor function evaluations utilizing the rotarod apparatus (Abo-Zalam et al., 2021), and nociceptive behavioral assessments employing the von Frey filament method (Choi et al., 2020). Furthermore, direct measurement of oxidative stress biomarkers, such as malondialdehyde, superoxide dismutase, and catalase, would provide additional mechanistic insights into the antioxidant role of KGE. Gene expression analyses via quantitative polymerase chain reaction targeting NF- κ B, SOX-9, MMP-13, and type II collagen would further elucidate transcriptional regulation. Phytochemical profiling of the extract utilized in this study is also recommended to ensure reproducibility and standardization of active constituents, such as kaempferol and EPMC. Additionally, future investigations should encompass pharmacokinetic evaluations to assess the systemic exposure and

bioavailability of KGE, which are essential for understanding dose-response relationships and therapeutic potential. Longer treatment durations should be considered to evaluate sustained cartilage protection beyond 21 days, alongside comprehensive toxicity assessments, including liver and kidney histopathology, to confirm systemic safety. These complementary approaches will contribute to a deeper understanding of the broader therapeutic effects of *K. galanga* extract in osteoarthritis, extending beyond histological and molecular outcomes.

CONCLUSION

KGE demonstrated dose-dependent anti-inflammatory and chondroprotective effects in an MIA-induced OA rat model. The extract attenuated joint swelling, suppressed proinflammatory mediators, and preserved cartilage structural integrity. These findings suggest KGE as a promising phytotherapeutic candidate for OA management. However, further studies are required to confirm its molecular mechanisms, determine optimal dosing, and assess functional and long-term safety prior to clinical application.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used ChatGPT (OpenAI) to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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Concepts or ideas	x	x	x					x	x
Design	x	x	x					x	x
Definition of intellectual content	x	x	x						x
Literature search	x	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x	x	x
Data acquisition									
Data analysis	x	x	x	x	x	x	x		
Statistical analysis	x			x	x	x	x		
Manuscript preparation	x	x	x	x	x	x	x	x	x
Manuscript editing	x	x	x					x	x
Manuscript review	x	x	x	x	x	x	x	x	x

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