rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Abstract Submission: ICPHS 2022

1 message

scientific icphs2022 <scientific.icphs2022@gmail.com> Wed, Aug 31, 2022 at 2:27 PM
To: riza.ambar.sari-2020@ff.unair.ac.id, rr-retno-w@ff.unair.ac.id

Penang, 31 August 2022

Ms. Riza Ambar Sari
Department of Pharmaceutical Sciences
Faculty of Pharmacy
University of Airlangga
60115 Indonesia

Dear Ms. Sari,

Thank you for your abstract submission to the International Conference of Pharmacy & Health Science (ICPHS) 2020. The Scientific Committee has the pleasure of informing you that your abstract, entitled:

Antiosteoarthritis Activities of *Eleutherine bulbosa* (Mill.) Urb. Extract on Monosodium Iodoacetate-induced Osteoarthritis in a Rat Model

was submitted and has been accepted as **POSTER PRESENTATION**. The abstract code is **NPDD-P-04**. Please use this code for further communication, including naming your ePoster and/or narrative ePoster video as follows:

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Kindly find the abstract in the attachment and acknowledge the corrections, if any. If you would like to address the changes, please reply to this email.

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Kindly ensure to follow the attached guidelines in preparing the ePoster and narrative ePoster video. Both ePoster and narrative ePoster video should be submitted to scientific.icphs2022@gmail.com by 15 September 2022 at the latest.

The details concerning the presentation sessions will be given on the website (https://www.icphs2022.com) a few weeks before the Conference. We also would like to gently

remind you to register on the website if you have yet to do so.

Should you have any queries, please do not hesitate to contact us.

Sincerely yours,

Dr. Roza Dianita
Scientific Committee
Organizing Committee of the ICPHS 2022

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2 attachments

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2 messages

ICPHS 2022 <scicommicphs2022@gmail.com> Fri, Dec 16, 2022 at 6:08 AM
To: rr-retno-w@ff.unair.ac.id

Ref: Submission ID ICPHS-12

Dear Dr. Retno Widyowati,

Your manuscript, "Antiosteoarthritis activities of 70% ethanol extract of Eleutherine bulbosa (Mill.) Urb. on rats monosodium iodoacetate-induced osteoarthritis", has now been reviewed, and the reviewer comments are appended below. While the reviewers find your work of interest, you will see that they have raised points that need to be addressed.

Therefore, we invite you to revise your paper, considering the points raised. At the same time, we ask you to make sure your manuscript complies with our format by reviewing our guidelines for preparing your manuscript, as attached to this email. After revision, the manuscript should have a similarity index of less than 20%.

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Kind regards,

Tutik Sri Wahyuni
Andang Miatmoko

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Reviewer Comments:

Reviewer 1

The author has described the studies well. However, detail points need to be added. Please check the manuscript.
Detailed revisions are provided in the comments of the manuscript.

Reviewer 2

The article is well written. There are some suggestions to improve the clarity and quality of the manuscript (details are attached):
1. Title: please include the part of the plant used
2. Introduction: State clearly what is the difference between the current study and the previous study
3. Methods: The method used for drying the sample should be included. Please check on the term used and use the standard terms
4. Results: Based on the data in Fig 3b, is it possible to discuss the results from each week? In the treated group, doses 12 and 24, the response is very similar for each week compared to the dose 6 group. This trend is also different from the meloxicam group, in which the response is increasing each week
5. Discussion: Second paragraph: This paragraph should focus on the compounds reported from E. bulbosa. However, if the author wants to discuss other compounds in the extract, sufficient information in the introduction should be provided. If this is the case, the author should explain other metabolites that are possibly present in the introduction. Perhaps compounds from the same plant genus.
6. Figure: Can you explain why the SD for meloxicam is large? what is the value for SD that is

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Antiosteoarthritis activities of 70% ethanol extract of *Eleutherine bulbosa* (Mill.) Urb. on rats monosodium iodoacetate-induced osteoarthritis

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Keywords : osteoarthritis, antiinflammatory, *Eleutherine bulbosa*, MIA, IL-1β

Authors' Contributions:

Riza Ambar Sari	: literature search, experimental studies, data analysis
Fina Luthfiana	: literature search, experimental studies, data acquisition
Irawati Sholihah	: Statistical analysis, research materials
Katsuyoshi Matsunami	: Manuscript editing and review
Sukardiman	: Manuscript editing and review
Retno Widyowati	: conception and design research, Manuscript editing and review

Conflict of Interests Statement: none of the authors declared any conflicts of interest

Abstract:

Background**:** osteoarthritis (OA) is the most typical degenerative joint condition that induces pain and disability in the elderly. Traditionally, *Eleutherine bulbosa* bulb from Pasuruan, East Java, is used to treat many diseases, including as an anti-inflammatory.

Objective: in this study, we used an in vivo model to examine the effects of 70% ethanol extracts of *E. bulbosa* (EBE) on the progression and development of OA.

Methods**:** a single injection of intraarticular monosodium iodoacetate (MIA) was used to create the OA model in rats. The progression of OA was observed for three weeks. Furthermore, treatment of EBE at a dose of 6, 12, and 24 mg/200g BW orally for four weeks was conducted to assess the effects on decreasing IL-1ß level, joint swelling, and hyperalgesia.

Results**:** induction was successful, indicated by a significant difference ($p<0.05$) in decreasing latency time, increasing joint swelling, and IL-1ß level. EBE 24 mg/200 g BW treatment has significantly ($p<0.05$) reduced IL-1ß levels, joint swelling, and response to hyperalgesia.

Conclusion**:** 70% ethanol extract of *E. bulbosa* bulb has therapeutic effects on inflammation through reducing IL-1ß in experimental MIA-induced osteoarthritis in a rat model. According to this study, EBE may have an effective potential new agent for OA therapy.

1. Introduction

Osteoarthritis (OA) is the most prevalent type of degenerative joint disease in the elderly.[1] Clinically, the disease is associated with joint pain caused by the entire joint becoming dysfunctional, particularly the articular cartilage, synovium, subchondral bone, and other tissues with close mechanical and molecular biological interactions.[2,3] Some characteristics of OA are tenderness, stiffness, joint swelling, immobility, and joint deformity. The risk factors of OA are gender, genetic, and joint injury, which then increase along with obesity and age.[1,4] This disease is the leading cause of disability, with nearly 10-15% of adults over 60 years suffering from OA.[5] The prevalence of knee OA is 240 per 100,000 individuals per year. In Indonesia, knee OA is 65% of people over 61 years old, 30% of people aged 40-60, and 5% less than 40 years. This incidence is estimated at 15.5% in men and 12.7% in women.[6]

IL-1 is implicated in the pathophysiology of knee OA due to increased production of this cytokine associated with the severity of symptoms.[7] Furthermore, IL-1 has been shown to inhibit chondrocytes.[8,9] IL-1Ra specifically inhibits the action of IL-1β by binding to IL-1R1.[10] Strong evidence for the critical role of interleukin-1β (IL-1β) and OA-associated regulation genes such as matrix metalloproteinase (MMP), tumor necrosis factor-a (TNF-α), cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6) in the development of OA have been reported. IL-1β is a proinflammatory substance that is important for responding to the immune system's defenses against injury.[11,12] The activation of signaling events by IL-1β was associated with the upregulation of MMP-13.[9] Reduced chondrocyte proteoglycan synthesis, increases in the production of MMP, and the release of nitric oxide are all caused by IL-1β in the joint.[13] Another mechanism is the binding of IL-1β to the IL-1 receptor (IL-1R1), triggering a proinflammatory response that leads to cartilage destruction followed by subchondral destruction. The pro-inflammatory response also causes synovial membrane inflammation, which manifests as pain.[7]

The osteoarthritis model using monosodium iodoacetate (MIA) in rats has been used for a long time and is well established. An intraarticular injection of MIA causes histopathological abnormalities in articular cartilage, similar to degenerative OA in

humans. MIA is a metabolic inhibitor that disrupts the aerobic glycolytic pathway of cells and causes cell death by inhibiting the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes.[14]

Eleutherine bulbosa (Mill.) Urb is the Iridaceae family's oriental medicinal plant. This plant is used in traditional medicine for treating several conditions, such as cough, anemia, heart failure, cancer, infertility, skin disease, and inflammation.[15,16] Previous phytochemical compounds have been isolated from *E. bulbosa,* such as naphthalene, anthraquinone, naphthoquinone and their derivatives eleutherinone, eleutherine, isoeleutherine, eleutherol, eleuthone, eleutherinol 8-*O*- β -D-glucoside, eleuthoside.[16–18] According to Tessele *et al.* (2011), eleutherine and isoeleutherine have antihypernociceptive and anti-inflammatory effects due to their decrease in paw edema mice that induced carrageenan. *E. bulbosa* also contains luteolin, which inhibits cyclooxygenase and reduces pain.[20]

Therefore, inhibition of IL-1β is considered a potential target to lessen the pain and inflammation in the development of OA. This research investigated the anti-osteoarthritis activity of 70% ethanol extract of *E. bulbosa* (EBE) in an osteoarthritis rat model induced by MIA.

2. Materials and Methods

2.1 Ethical Considerations

Thirty male Wistar rats (*Rattus norvegicus*) were purchased from the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia, in healthy condition. Rats aged 3-4 months (200-300 g) were acclimatized for seven days in the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. The animals were kept in ideal lighting (12-hour light-dark cycle), a temperature of 22±1°C, and a humidity of 60-80%. They also had full access to drink and food. The entire process has been authorized by the Animal Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, with Ethical Clearance No. 2.KEH.120.09.2022.

2.2 Plant materials

Eleutherine bulbosa (Mill.) Urb. were found and collected from Pasuruan, East Java, Indonesia. Determined by UPT Laboratorium Herbal Materia Medica, Batu, East Java, Indonesia (Certificate of Determination No. 074/722/102-7-A/2021). A dried powder of *E. bulbosa* bulb was extracted by maceration using 70% ethanol and then concentrated using a rotary evaporator. The extractive value of 70% ethanol extract of *E. bulbosa* bulb was calculated as % w/w yield and found at 15,98%.

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2.3 Animal Model

Animals were divided into six groups: the naïve group was given food and water *ad libitum*, the negative group was assigned 0,5% Carboxy Methyl Cellulose (CMC), the positive group received meloxicam (Indofarma, Indonesia) 0.135 mg/200 g BW, and 70% ethanol extract of *E. bulbosa* bulb in 3 kinds of dose that given Dose 1: 6 mg/200 g BW; Dose 2: 12 mg/200 g BW; Dose 3: 24 mg/200 g BW, respectively. Intraarticular injection of MIA (4 mg MIA dissolved in 50 µl of saline) (Sigma-Aldrich, Darmstadt, Germany) was performed on all groups except the naïve group under anesthesia using a combination of 10% ketamine (80 mg/kg) (Agrovet, Nicaragua) and 1% xylazine (5 mg/kg) (Holland, Netherlands) to obtain the condition of OA. Three weeks after MIA induction, all rats were checked for OA success on day 21 with the measured IL-1β levels (pretest), followed by an oral administration of 70% ethanol extract daily for 28 days based on their group. Furthermore, the response of hyperalgesia and knee joint swelling was measured with a hot plate and calibrated screw micrometer for seven weeks (on days 0, 7, 14, 21, 28, 35, 42, and 49), respectively. Latency response was measured with a stopwatch. After the treatment, the determination of IL-1β levels in blood serum was measured on the seventh week (day 49 as a posttest).

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2.4 Evaluation of Parameters

Hyperalgesia testing

This study used a hot plate to observe the response hyperalgesia of all groups on days 0, 7, 14, 21, 28, 35, 42, and 49. A rat was left unrestrained on a metal surface kept at a

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constant temperature of 55±0.5°C for the hot plate test. The investigator documented the response latency, and the time it takes to generate a nocifensive reaction. Nocifensive behavior includes withdrawal or licking of the hind paw, leaning posture, stamping, and jumping. After observing the response, the rats were immediately removed from the hot plate.

Joint swelling measurement

Joint swelling was measured on the ipsilateral knee of the rat. All groups were measured on days 0, 7, 14, 21, 28, 35, 42, and 49 using a calibrated screw micrometer.

IL-1β Cytokine Assay

Blood samples from the tail vein were collected into a blood collection tube on days 21 and 49. The centrifugation at 3000 rpm was performed to take the blood serum. The levels of IL-1β were determined using commercial rat IL-1β ELISA kits (Bioenzy, Germany), following the manufacturer's protocols.

Statistical Analysis

Data from animal experiments were collected, and the results were presented as mean ± SD of 5 animals in each group using Graph Pad Prism 7 while the analytical statistic using SPSS 25. Two-way analysis of variance (ANOVA) was used to compare the data statistically, followed by the LSD post hoc test. Statistical significance was defined as a p value < 0.05.

3. Results

After the treatment, data were collected and analyzed. According to Figure 1. the knee joint was swelling as the increase of the diameter around 20-30%. Figure 1. also shows that from the second week up to the third week the progression of OA seems at a steady state. It indicates a specific set of beginning condition, because a steady state is an example of an attractor that can be attained.[21,22] Based on Figure 3, in the third week IL-1ß level of the naïve group and negative control have a significant difference (p<0.01). Thereby, in the fourth week, the rats were separated into groups and given the

treatment for 28 days. The negative control rats were inflamed and marked by swollen joints. The knee diameter, time latency, and IL-1ß level have been measured.

In comparison, there was a significant difference between the rats who were given EBE 24 mg/200 g BW and the other dose but no significant difference with the positive control (meloxicam) group. In addition, to evaluate osteoarthritis based on the clinical data, time latency and level of IL-1ß of the joints were assessed. The time latency of the negative control group showed a significantly differed from the other groups ($p<0.05$). Treatment with EBE 6, 12, and 24 mg/g BW increased the time latency in comparison with the negative control group, but there was no significant difference among these groups (Figure 3).

Therefore, the proinflammatory cytokine IL-1ß was measured in rats receiving EBE. According to Figure 2, EBE 12 and 24 mg/200 g BW significantly ($p<0.01$) suppressed the amount of IL-1ß in the serum of rats compared with negative control after 28 days. EBE dose 24 mg/200 g BW is also significantly different from EBE 6 mg/200 g BW.

4. Discussion

Inflammation and pain are recognized as the primary cause of OA symptoms and development. MIA disrupts the metabolism of chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase, which affects the production of reactive oxygen species (ROS) and the breakdown of the cartilage matrix. MIA also increases proinflammatory cytokines such as IL-1ß and TNF-α that may cause the production of COX-2 and matrix metalloproteinases (MMPs).[8] Additionally, IL-1ß has been shown to accelerate cartilage breakdown in articular chondrocytes and inhibit the synthesis of cartilage matrix.[5] MMPs and ADAMTS are zinc-dependent endopeptidases that play an important role in cartilage matrix destruction.[23] The production of MMPs and ADAMTS is significantly increased during OA in response to proinflammatory mediators, including IL-1ß and TNF-α.[24]

Administration of EBE at various doses could increase latency time as well as decrease joint swelling and IL-1ß in the OA rats model. It was due to the compounds in EBE, such as flavonoids and naphthoquinone. Quercetin is a basic structure that forms other flavonoids. Before having the first pass effect, the small intestine absorbs quercetin, then delivered it to the liver by portal circulation. The quercetin will diffuse throughout the body's tissues at that point. It is well known that quercetin significantly binds to plasma albumin. Quercetin inhibits the Transient Receptor Potential Cation Channel Subfamily V member 1 (TRPV1) receptor and has an opioid action.[20] In naphthoquinone derivatives, Hong et al. (2008) showed clear evidence that T helper cell proliferation is partially inhibited by both eleutherine and isoeleutherine. The substances also elevated levels of the cytokine IL-2 and apoptosis. Through the secretion of several cytokines, the biological response to inflammation is managed by T cells. In addition, another study showed that eleutherine and isoeleutherine might affect the mechanical hypernociception induced by lipopolysaccharide (LPS). Eleutherine has been reported to be able to interfere with paw edema caused by PGE_2 or histamine significantly. It is well known that the release of many inflammatory mediators occurs as a result of inflammatory hypernociception.[19] Otherwise, *in silico* prediction of β-sitosterol in the *Eleutherine americana* shows that it has anti-inflammatory activity and has a strong affinity for COX-2 greater than celecoxib. The *in silico* toxicity prediction indicated that the compound was not toxic.[26] It is interesting that β-sitosterol is a steroid molecule with high closeness to corticosteroids, which are known as anti-inflammatory drugs. This may explain the high affinity of β-sitosterol to COX-2 as well as the intermolecular connections created.[27]

EBE at various doses has been able to increase latency time as well as decrease joint swelling and IL-1ß in OA rats model, but only EBE 24 mg/200 g BW reduced joint swelling and IL-1ß level as well as meloxicam. Enhancing EBE dosage reduces the sign of antiosteoarthritis which helps decrease pain and inflammation.

Limitations

The study's limitations were recognized. First, this research did not histologically examine the rat knee's articular cartilage The quantitative results revealed some distinct tendencies, and current study findings are important and useful for creating an OA model. The present study's findings significantly contributed to the knowledge of mechanisms in the treatment of osteoarthritis. Second, induced OA using the chemical compound MIA. Some previous studies claimed that chemical has a different pathophysiology unrelated to post-traumatic OA.[28,29] However, several studies have revealed morphological and histological alterations in the articular tissues that are identical to the characteristics of human OA.[14,30]

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5. Conclusion

The present study demonstrated that EBE decreased the IL-1β induced inflammatory response, reduced joint swelling, and increased time latency. Additionally, MIA caused OA in the rat model, and EBE had a protective effect against OA degeneration. These findings suggested that EBE 12 and 24 mg/200 g BW might be a potential treatment for OA as well as meloxicam.

References

1. Yunus MHM, Nordin A, Kamal H. Pathophysiological perspective of osteoarthritis. *Medicina* 2020;56:614–27.
2. Grässel S, Zaucke F, Madry H. Osteoarthritis: Novel molecular mechanisms increase our understanding of the disease pathology. *J Clin Med* 2021;10:1–22.
3. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage–bone crosstalk. *Nat Rev Rheumatol* 2016;12:632–44.
4. Cui A, Li H, Wang D, et al. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EClinicalMedicine* 2020;29–30:1–13.
5. Huang X, Pei W, Ni B, et al. Chondroprotective and antiarthritic effects of galangin in osteoarthritis: An in vitro and in vivo study. *Eur J Pharmacol* 2021;906:1–7.
6. Siddik M, Haryadi RD. The risk factors effect of knee osteoarthritis towards postural lateral sway. *Indian J Forensic Med Toxicol* 2020;14:1787–92.
7. Budhiparama NC, Lumban-Gaol I, Sudoyo H, et al. Interleukin-1 genetic polymorphisms in knee osteoarthritis: What do we know? A meta-analysis and systematic review. *J Orthop Surg* 2022;30:1–14.
8. Khotib J, Pratiwi AP, Ardianto C, et al. Attenuation of IL-1ß on the use of glucosamine as an adjuvant in meloxicam treatment in rat models with osteoarthritis. *J Basic Clin Physiol Pharmacol* 2020;30:1–9.
9. Rasheed Z, Al-Shobaili HA, Rasheed N, et al. MicroRNA-26a-5p regulates the expression of inducible nitric oxide synthase via activation of NF-κB pathway in human osteoarthritis chondrocytes. *Arch Biochem Biophys* 2016;594:61–7.
10. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011;117:3720–32.
11. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev* 2011;22:189–95.
12. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* 2012;11:633–52.
13. Joosten LAB, Smeets RL, Koenders MI, et al. Interleukin-18 promotes joint inflammation and induces interleukin-1-driven cartilage destruction. *Am J Clin Pathol* 2004;165:959–67.

14. Takahashi I, Matsuzaki T, Kuroki H, et al. Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. *PLoS ONE* 2018;13:1–15.

15. Hanh PTB, Thao DT, Nga NT, et al. Toxicity and anti-inflammatory activities of an extract of the *Eleutherine bulbosa* rhizome on collagen antibody-induced arthritis in a mouse model. *Nat Prod Commun* 2018;13:883–6.

16. Ha LM, Huyen DTT, Kiem PV, et al. Chemical constituents of the rhizome of *Eleutherine bulbosa* and their inhibitory effect on the pro-inflammatory cytokines production in Lipopolysaccharide-stimulated bone marrow-derived dendritic cells. *Bull Korean Chem Soc* 2013;34:633–36.

17. Insanu M, Kusmardiyani S, Hartati R. Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr. *Procedia Chem* 2014;13:221–28.

18. Kamarudin AA, Sayuti NH, Saad N, et al. *Eleutherine bulbosa* (Mill.) Urb. Bulb: Review of the pharmacological activities and its prospects for application. *Int J Mol Sci* 2021;22:6747–67.

19. Tessele P, Delle Monache F, Quintão N, et al. A New naphthoquinone isolated from the bulbs of *Cipura paludosa* and pharmacological activity of two main constituents. *Planta Med* 2011;77:1035–43.

20. Hafizh M, Indiastuti DN, Mukono IS. Analgesic effect of Dayak Onion (*Eleutherine americana* (Aubl.) Merr.) on mice (*Mus musculus*) by hot plate test method. *BHSJ* 2021;4:22–5.

21. Chen H, Qin J, Shi H, et al. Rhoifolin ameliorates osteoarthritis via the Nrf2/NF-κB axis: in vitro and in vivo experiments. *Osteoarthr Cartil* 2022;30:735–45.

22. Sobie EA. An introduction to dynamical systems. *Sci Signal* 2014;4:1–5.

23. Malemud CJ. Inhibition of MMPs and ADAM/ADAMTS. *Biochem Pharmacol* 2019;165:33–40.

24. Vincent TL. IL-1 in osteoarthritis: time for a critical review of the literature. *F1000Res* 2019;8:934–43.

25. Hong J-H, Yu ES, Han A-R, et al. Isoeleutherin and eleutherinol, naturally occurring selective modulators of Th cell-mediated immune responses. *Biochem Biophys Res Commun* 2008;371:278–82.

26. Damayanti S, Puspaningrum D, N. Muhammad H, et al. Interaction of the chemical constituents of *Eleutherine americana* (AUBL.) MERR. EX K. HEYNE with cyclooxygenase and H5N1 RNA polymerase: an in-silico study. *Rasayan J Chem* 2021;14:844–54.

27. Raghavamma STV, Rama Rao N, Devala Rao G. Inhibitory potential of important phytochemicals from *Pergularia daemia* (Forsk.) chiov. on snake venom (Naja naja). *J Genet Eng Biotechnol* 2016;14:211–17.

28. Kuyinu EL, Narayanan G, Nair LS, et al. Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res* 2016;11:19–46.

29. Teeple E, Jay GD, Elsaid KA, et al. Animal models of osteoarthritis: Challenges of model selection and analysis. *AAPS J* 2013;15:438–46.

30. Jiang L, Li L, Geng C, et al. Monosodium iodoacetate induces apoptosis via the mitochondrial pathway involving ROS production and caspase activation in rat chondrocytes in vitro: apoptosis induced by monosodium iodoacetate. *J Orthop Res* 2013;31:364–9.

Acknowledgements

This research was supported by Faculty of Excellence Research (Penelitian Unggulan Fakultas) of Airlangga University with the contract No. 545/UN3.15/PT/2022

Tables and figures

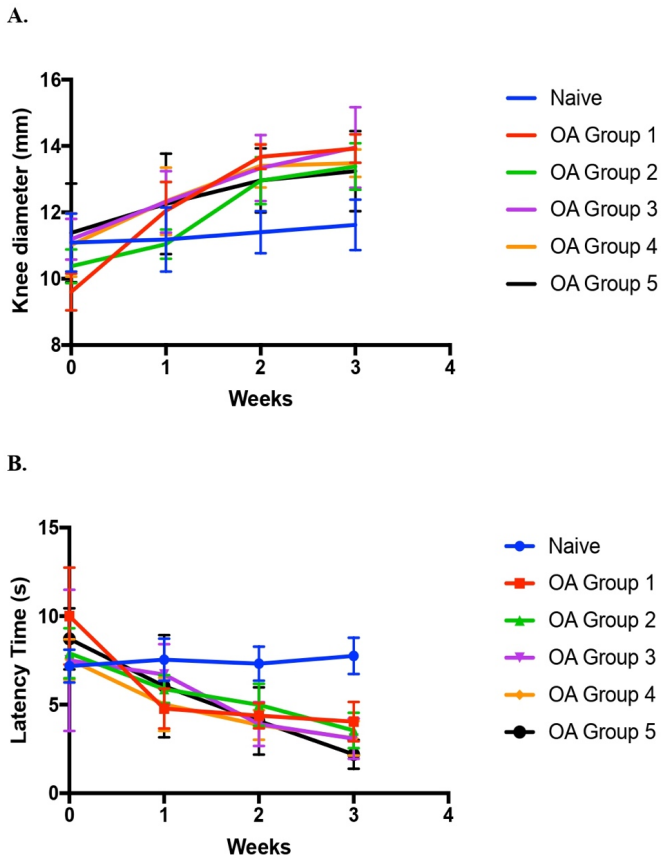

FIGURE 1: The development of OA after injecting MIA in three weeks (A) rat knee diameter; (B) time latency. Data are present as Mean±SD base on N=5 for all groups.

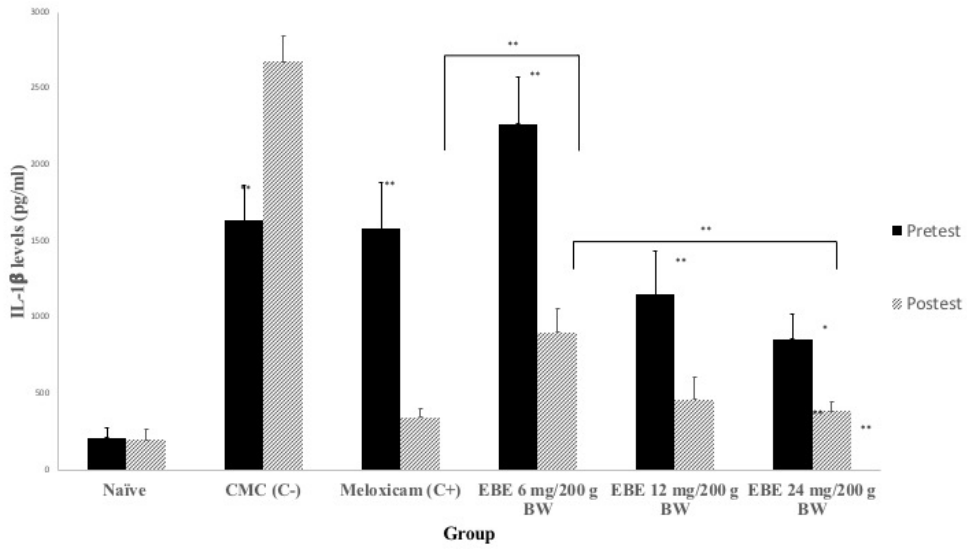

FIGURE 2: The IL-1ß levels on the pretest increased in rat serum compared with the naïve group. The cytokine was measured in the serum of EBE treated rats at three doses (6, 12, and 24 mg/200 g BW) together with negative (CMC) and positive (meloxicam) groups. The levels of IL-1ß were decreased after treatment as compared with the negative control group ($P<0.01$). Data are present as Mean±SD base on n=5 for all groups. *$P<0.05$; **$P<0.01$.

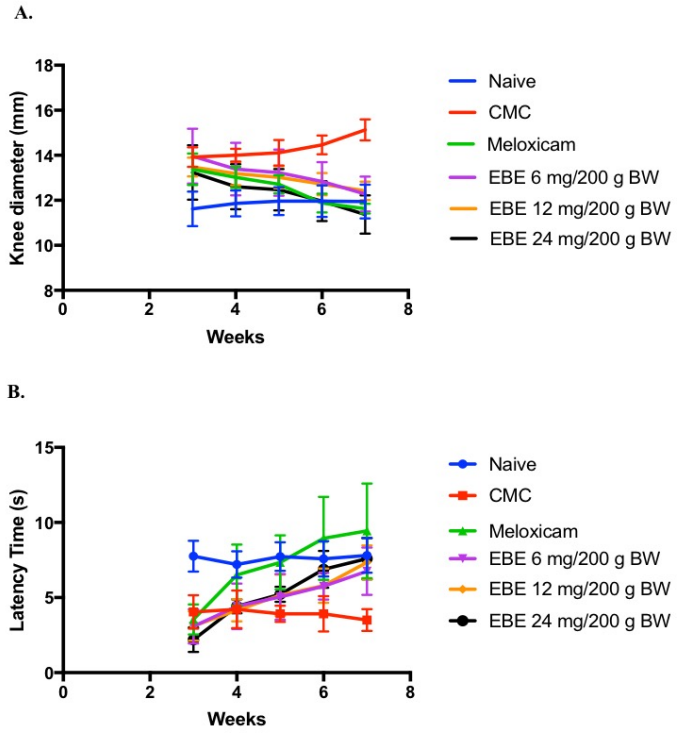

FIGURE 3: The effects of EBE in vivo MIA induced OA model (A) Rat's knee diameter was significantly decreased after the treatment of EBE as compared to the negative control group ($P<0.01$); (B) Time latency was significantly increased after the treatment of EBE as compared to the negative control group ($P<0.05$). Data are present as Mean±SD base on n=5 for all groups.

Antiosteoarthritis activities of 70% ethanol extract of *Eleutherine bulbosa* (Mill.) Urb. on rats monosodium iodoacetate-induced osteoarthritis

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[1]*Master Program in Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia*

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Keywords : osteoarthritis, antiinflammatory, *Eleutherine bulbosa*, MIA, IL-1β

Authors' Contributions:

Riza Ambar Sari	: literature search, experimental studies, data analysis
Fina Luthfiana	: literature search, experimental studies, data acquisition
Irawati Sholihah	: Statistical analysis, research materials
Katsuyoshi Matsunami	: Manuscript editing and review
Sukardiman	: Manuscript editing and review
Retno Widyowati	: conception and design research, Manuscript editing and review

Conflict of Interests Statement: none of the authors declared any conflicts of interest

Abstract:

Background**:** osteoarthritis (OA) is the most typical degenerative joint condition that induces pain and disability in the elderly. Traditionally, *Eleutherine bulbosa* bulb from Pasuruan, East Java, is used to treat many diseases, including as an anti-inflammatory.

Objective: in this study, we used an in vivo model to examine the effects of 70% ethanol extracts of *E. bulbosa* (EBE) on the progression and development of OA.

Methods**:** a single injection of intraarticular monosodium iodoacetate (MIA) was used to create the OA model in rats. The progression of OA was observed for three weeks. Furthermore, treatment of EBE at a dose of 6, 12, and 24 mg/200g BW orally for four weeks was conducted to assess the effects on decreasing IL-1ß level, joint swelling, and hyperalgesia.

Results**:** induction was successful, indicated by a significant difference ($p<0.05$) in decreasing latency time, increasing joint swelling, and IL-1ß level. EBE 24 mg/200 g BW treatment has significantly ($p<0.05$) reduced IL-1ß levels, joint swelling, and response to hyperalgesia.

Conclusion**:** 70% ethanol extract of *E. bulbosa* bulb has therapeutic effects on inflammation through reducing IL-1ß in experimental MIA-induced osteoarthritis in a rat model. According to this study, EBE may have an effective potential new agent for OA therapy.

1. Introduction

Osteoarthritis (OA) is the most prevalent type of degenerative joint disease in the elderly.[1] Clinically, the disease is associated with joint pain caused by the entire joint becoming dysfunctional, particularly the articular cartilage, synovium, subchondral bone, and other tissues with close mechanical and molecular biological interactions.[2,3] Some characteristics of OA are tenderness, stiffness, joint swelling, immobility, and joint deformity. The risk factors of OA are gender, genetic, and joint injury, which then increase along with obesity and age.[1,4] This disease is the leading cause of disability, with nearly 10-15% of adults over 60 years suffering from OA.[5] The prevalence of knee OA is 240 per 100,000 individuals per year. In Indonesia, knee OA is 65% of people over 61 years old, 30% of people aged 40-60, and 5% less than 40 years. This incidence is estimated at 15.5% in men and 12.7% in women.[6]

IL-1 is implicated in the pathophysiology of knee OA due to increased production of this cytokine associated with the severity of symptoms.[7] Furthermore, IL-1 has been shown to inhibit chondrocytes.[8,9] IL-1Ra specifically inhibits the action of IL-1β by binding to IL-1R1.[10] Strong evidence for the critical role of interleukin-1β (IL-1β) and OA-associated regulation genes such as matrix metalloproteinase (MMP), tumor necrosis factor-a (TNF-α), cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6) in the development of OA have been reported. IL-1β is a proinflammatory substance that is important for responding to the immune system's defenses against injury.[11,12] The activation of signaling events by IL-1β was associated with the upregulation of MMP-13.[9] Reduced chondrocyte proteoglycan synthesis, increases in the production of MMP, and the release of nitric oxide are all caused by IL-1β in the joint.[13] Another mechanism is the binding of IL-1β to the IL-1 receptor (IL-1R1), triggering a proinflammatory response that leads to cartilage destruction followed by subchondral destruction. The pro-inflammatory response also causes synovial membrane inflammation, which manifests as pain.[7]

The osteoarthritis model using monosodium iodoacetate (MIA) in rats has been used for a long time and is well established. An intraarticular injection of MIA causes histopathological abnormalities in articular cartilage, similar to degenerative OA in

humans. MIA is a metabolic inhibitor that disrupts the aerobic glycolytic pathway of cells and causes cell death by inhibiting the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes.[14]

Eleutherine bulbosa (Mill.) Urb is the Iridaceae family's oriental medicinal plant. This plant is used in traditional medicine for treating several conditions, such as cough, anemia, heart failure, cancer, infertility, skin disease, and inflammation.[15,16] Previous phytochemical compounds have been isolated from *E. bulbosa,* such as naphthalene, anthraquinone, naphthoquinone and their derivatives eleutherinone, eleutherine, isoeleutherine, eleutherol, eleuthone, eleutherinol 8-*O*- β -D-glucoside, eleuthoside.[16–18] According to Tessele *et al.* (2011), eleutherine and isoeleutherine have antihypernociceptive and anti-inflammatory effects due to their decrease in paw edema mice that induced carrageenan. *E. bulbosa* also contains luteolin, which inhibits cyclooxygenase and reduces pain.[20]

Therefore, inhibition of IL-1β is considered a potential target to lessen the pain and inflammation in the development of OA. This research investigated the anti-osteoarthritis activity of 70% ethanol extract of *E. bulbosa* (EBE) in an osteoarthritis rat model induced by MIA.

2. Materials and Methods

2.1 Ethical Considerations

Thirty male Wistar rats (*Rattus norvegicus*) were purchased from the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia, in healthy condition. Rats aged 3-4 months (200-300 g) were acclimatized for seven days in the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. The animals were kept in ideal lighting (12-hour light-dark cycle), a temperature of 22±1°C, and a humidity of 60-80%. They also had full access to drink and food. The entire process has been authorized by the Animal Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, with Ethical Clearance No. 2.KEH.120.09.2022.

2.2 Plant materials

Eleutherine bulbosa (Mill.) Urb. were found and collected from Pasuruan, East Java, Indonesia. Determined by UPT Laboratorium Herbal Materia Medica, Batu, East Java, Indonesia (Certificate of Determination No. 074/722/102-7-A/2021). A dried powder of *E. bulbosa* bulb was extracted by maceration using 70% ethanol and then concentrated using a rotary evaporator. The extractive value of 70% ethanol extract of *E. bulbosa* bulb was calculated as % w/w yield and found at 15,98%.

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Comment [1]: Mention here how many g of simplicia and the total of solvent

2.3 Animal Model

Animals were divided into six groups: the naïve group was given food and water *ad libitum*, the negative group was assigned 0,5% Carboxy Methyl Cellulose (CMC), the positive group received meloxicam (Indofarma, Indonesia) 0.135 mg/200 g BW, and 70% ethanol extract of *E. bulbosa* bulb in 3 kinds of dose that given Dose 1: 6 mg/200 g BW; Dose 2: 12 mg/200 g BW; Dose 3: 24 mg/200 g BW, respectively. Intraarticular injection of MIA (4 mg MIA dissolved in 50 µl of saline) (Sigma-Aldrich, Darmstadt, Germany) was performed on all groups except the naïve group under anesthesia using a combination of 10% ketamine (80 mg/kg) (Agrovet, Nicaragua) and 1% xylazine (5 mg/kg) (Holland, Netherlands) to obtain the condition of OA. Three weeks after MIA induction, all rats were checked for OA success on day 21 with the measured IL-1β levels (pretest), followed by an oral administration of 70% ethanol extract daily for 28 days based on their group. Furthermore, the response of hyperalgesia and knee joint swelling was measured with a hot plate and calibrated screw micrometer for seven weeks (on days 0, 7, 14, 21, 28, 35, 42, and 49), respectively. Latency response was measured with a stopwatch. After the treatment, the determination of IL-1β levels in blood serum was measured on the seventh week (day 49 as a posttest).

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Does the animal followed the sacrifice process, please explaine.

Microsoft Office User 11/12/22 18.57
Comment [3]: Mention Rat rather than animal

2.4 Evaluation of Parameters

Hyperalgesia testing

This study used a hot plate to observe the response hyperalgesia of all groups on days 0, 7, 14, 21, 28, 35, 42, and 49. A rat was left unrestrained on a metal surface kept at a

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Comment [4]: type of instrument, specification condition?

constant temperature of 55±0.5°C for the hot plate test. The investigator documented the response latency, and the time it takes to generate a nocifensive reaction. Nocifensive behavior includes withdrawal or licking of the hind paw, leaning posture, stamping, and jumping. After observing the response, the rats were immediately removed from the hot plate.

Joint swelling measurement

Joint swelling was measured on the ipsilateral knee of the rat. All groups were measured on days 0, 7, 14, 21, 28, 35, 42, and 49 using a calibrated screw micrometer.

IL-1β Cytokine Assay

Blood samples from the tail vein were collected into a blood collection tube on days 21 and 49. The centrifugation at 3000 rpm was performed to take the blood serum. The levels of IL-1β were determined using commercial rat IL-1β ELISA kits (Bioenzy, Germany), following the manufacturer's protocols.

Statistical Analysis

Data from animal experiments were collected, and the results were presented as mean ± SD of 5 animals in each group using Graph Pad Prism 7 while the analytical statistic using SPSS 25. Two-way analysis of variance (ANOVA) was used to compare the data statistically, followed by the LSD post hoc test. Statistical significance was defined as a p value < 0.05.

3. Results

After the treatment, data were collected and analyzed. According to Figure 1. the knee joint was swelling as the increase of the diameter around 20-30%. Figure 1. also shows that from the second week up to the third week the progression of OA seems at a steady state. It indicates a specific set of beginning condition, because a steady state is an example of an attractor that can be attained.[21,22] Based on Figure 3, in the third week IL-1ß level of the naïve group and negative control have a significant difference (p<0.01). Thereby, in the fourth week, the rats were separated into groups and given the

treatment for 28 days. The negative control rats were inflamed and marked by swollen joints. The knee diameter, time latency, and IL-1ß level have been measured.

In comparison, there was a significant difference between the rats who were given EBE 24 mg/200 g BW and the other dose but no significant difference with the positive control (meloxicam) group. In addition, to evaluate osteoarthritis based on the clinical data, time latency and level of IL-1ß of the joints were assessed. The time latency of the negative control group showed a significantly differed from the other groups ($p<0.05$). Treatment with EBE 6, 12, and 24 mg/g BW increased the time latency in comparison with the negative control group, but there was no significant difference among these groups (Figure 3).

Therefore, the proinflammatory cytokine IL-1ß was measured in rats receiving EBE. According to Figure 2, EBE 12 and 24 mg/200 g BW significantly ($p<0.01$) suppressed the amount of IL-1ß in the serum of rats compared with negative control after 28 days. EBE dose 24 mg/200 g BW is also significantly different from EBE 6 mg/200 g BW.

4. Discussion

Inflammation and pain are recognized as the primary cause of OA symptoms and development. MIA disrupts the metabolism of chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase, which affects the production of reactive oxygen species (ROS) and the breakdown of the cartilage matrix. MIA also increases proinflammatory cytokines such as IL-1ß and TNF-α that may cause the production of COX-2 and matrix metalloproteinases (MMPs).[8] Additionally, IL-1ß has been shown to accelerate cartilage breakdown in articular chondrocytes and inhibit the synthesis of cartilage matrix.[5] MMPs and ADAMTS are zinc-dependent endopeptidases that play an important role in cartilage matrix destruction.[23] The production of MMPs and ADAMTS is significantly increased during OA in response to proinflammatory mediators, including IL-1ß and TNF-α.[24]

Administration of EBE at various doses could increase latency time as well as decrease joint swelling and IL-1ß in the OA rats model. It was due to the compounds in EBE, such as flavonoids and naphthoquinone. Quercetin is a basic structure that forms other flavonoids. Before having the first pass effect, the small intestine absorbs quercetin, then delivered it to the liver by portal circulation. The quercetin will diffuse throughout the body's tissues at that point. It is well known that quercetin significantly binds to plasma albumin. Quercetin inhibits the Transient Receptor Potential Cation Channel Subfamily V member 1 (TRPV1) receptor and has an opioid action.[20] In naphthoquinone derivatives, Hong et al. (2008) showed clear evidence that T helper cell proliferation is partially inhibited by both eleutherine and isoeleutherine. The substances also elevated levels of the cytokine IL-2 and apoptosis. Through the secretion of several cytokines, the biological response to inflammation is managed by T cells. In addition, another study showed that eleutherine and isoeleutherine might affect the mechanical hypernociception induced by lipopolysaccharide (LPS). Eleutherine has been reported to be able to interfere with paw edema caused by PGE_2 or histamine significantly. It is well known that the release of many inflammatory mediators occurs as a result of inflammatory hypernociception.[19] Otherwise, *in silico* prediction of β-sitosterol in the *Eleutherine americana* shows that it has anti-inflammatory activity and has a strong affinity for COX-2 greater than celecoxib. The *in silico* toxicity prediction indicated that the compound was not toxic.[26] It is interesting that β-sitosterol is a steroid molecule with high closeness to corticosteroids, which are known as anti-inflammatory drugs. This may explain the high affinity of β-sitosterol to COX-2 as well as the intermolecular connections created.[27]

EBE at various doses has been able to increase latency time as well as decrease joint swelling and IL-1ß in OA rats model, but only EBE 24 mg/200 g BW reduced joint swelling and IL-1ß level as well as meloxicam. Enhancing EBE dosage reduces the sign of antiosteoarthritis which helps decrease pain and inflammation.

Limitations

The study's limitations were recognized. First, this research did not histologically examine the rat knee's articular cartilage The quantitative results revealed some distinct tendencies, and current study findings are important and useful for creating an OA model. The present study's findings significantly contributed to the knowledge of mechanisms in the treatment of osteoarthritis. Second, induced OA using the chemical compound MIA. Some previous studies claimed that chemical has a different pathophysiology unrelated to post-traumatic OA.[28,29] However, several studies have revealed morphological and histological alterations in the articular tissues that are identical to the characteristics of human OA.[14,30]

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5. Conclusion

The present study demonstrated that EBE decreased the IL-1β induced inflammatory response, reduced joint swelling, and increased time latency. Additionally, MIA caused OA in the rat model, and EBE had a protective effect against OA degeneration. These findings suggested that EBE 12 and 24 mg/200 g BW might be a potential treatment for OA as well as meloxicam.

References

1. Yunus MHM, Nordin A, Kamal H. Pathophysiological perspective of osteoarthritis. *Medicina* 2020;56:614–27.
2. Grässel S, Zaucke F, Madry H. Osteoarthritis: Novel molecular mechanisms increase our understanding of the disease pathology. *J Clin Med* 2021;10:1–22.
3. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage–bone crosstalk. *Nat Rev Rheumatol* 2016;12:632–44.
4. Cui A, Li H, Wang D, et al. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EClinicalMedicine* 2020;29–30:1–13.
5. Huang X, Pei W, Ni B, et al. Chondroprotective and antiarthritic effects of galangin in osteoarthritis: An in vitro and in vivo study. *Eur J Pharmacol* 2021;906:1–7.
6. Siddik M, Haryadi RD. The risk factors effect of knee osteoarthritis towards postural lateral sway. *Indian J Forensic Med Toxicol* 2020;14:1787–92.
7. Budhiparama NC, Lumban-Gaol I, Sudoyo H, et al. Interleukin-1 genetic polymorphisms in knee osteoarthritis: What do we know? A meta-analysis and systematic review. *J Orthop Surg* 2022;30:1–14.
8. Khotib J, Pratiwi AP, Ardianto C, et al. Attenuation of IL-1ß on the use of glucosamine as an adjuvant in meloxicam treatment in rat models with osteoarthritis. *J Basic Clin Physiol Pharmacol* 2020;30:1–9.
9. Rasheed Z, Al-Shobaili HA, Rasheed N, et al. MicroRNA-26a-5p regulates the expression of inducible nitric oxide synthase via activation of NF-κB pathway in human osteoarthritis chondrocytes. *Arch Biochem Biophys* 2016;594:61–7.
10. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011;117:3720–32.
11. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev* 2011;22:189–95.
12. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* 2012;11:633–52.
13. Joosten LAB, Smeets RL, Koenders MI, et al. Interleukin-18 promotes joint inflammation and induces interleukin-1-driven cartilage destruction. *Am J Clin Pathol* 2004;165:959–67.

14. Takahashi I, Matsuzaki T, Kuroki H, et al. Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. *PLoS ONE* 2018;13:1–15.

15. Hanh PTB, Thao DT, Nga NT, et al. Toxicity and anti-inflammatory activities of an extract of the *Eleutherine bulbosa* rhizome on collagen antibody-induced arthritis in a mouse model. *Nat Prod Commun* 2018;13:883–6.

16. Ha LM, Huyen DTT, Kiem PV, et al. Chemical constituents of the rhizome of *Eleutherine bulbosa* and their inhibitory effect on the pro-inflammatory cytokines production in Lipopolysaccharide-stimulated bone marrow-derived dendritic cells. *Bull Korean Chem Soc* 2013;34:633–36.

17. Insanu M, Kusmardiyani S, Hartati R. Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr. *Procedia Chem* 2014;13:221–28.

18. Kamarudin AA, Sayuti NH, Saad N, et al. *Eleutherine bulbosa* (Mill.) Urb. Bulb: Review of the pharmacological activities and its prospects for application. *Int J Mol Sci* 2021;22:6747–67.

19. Tessele P, Delle Monache F, Quintão N, et al. A New naphthoquinone isolated from the bulbs of *Cipura paludosa* and pharmacological activity of two main constituents. *Planta Med* 2011;77:1035–43.

20. Hafizh M, Indiastuti DN, Mukono IS. Analgesic effect of Dayak Onion (*Eleutherine americana* (Aubl.) Merr.) on mice (*Mus musculus*) by hot plate test method. *BHSJ* 2021;4:22–5.

21. Chen H, Qin J, Shi H, et al. Rhoifolin ameliorates osteoarthritis via the Nrf2/NF-κB axis: in vitro and in vivo experiments. *Osteoarthr Cartil* 2022;30:735–45.

22. Sobie EA. An introduction to dynamical systems. *Sci Signal* 2014;4:1–5.

23. Malemud CJ. Inhibition of MMPs and ADAM/ADAMTS. *Biochem Pharmacol* 2019;165:33–40.

24. Vincent TL. IL-1 in osteoarthritis: time for a critical review of the literature. *F1000Res* 2019;8:934–43.

25. Hong J-H, Yu ES, Han A-R, et al. Isoeleutherin and eleutherinol, naturally occurring selective modulators of Th cell-mediated immune responses. *Biochem Biophys Res Commun* 2008;371:278–82.

26. Damayanti S, Puspaningrum D, N. Muhammad H, et al. Interaction of the chemical constituents of *Eleutherine americana* (AUBL.) MERR. EX K. HEYNE with cyclooxygenase and H5N1 RNA polymerase: an in-silico study. *Rasayan J Chem* 2021;14:844–54.

27. Raghavamma STV, Rama Rao N, Devala Rao G. Inhibitory potential of important phytochemicals from *Pergularia daemia* (Forsk.) chiov. on snake venom (Naja naja). *J Genet Eng Biotechnol* 2016;14:211–17.

28. Kuyinu EL, Narayanan G, Nair LS, et al. Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res* 2016;11:19–46.

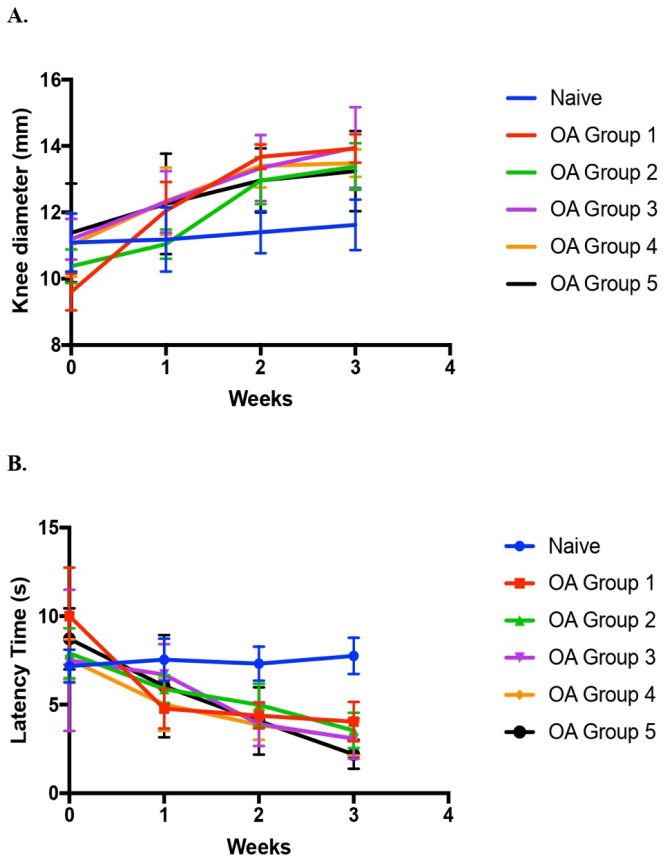
29. Teeple E, Jay GD, Elsaid KA, et al. Animal models of osteoarthritis: Challenges of model selection and analysis. *AAPS J* 2013;15:438–46.

30. Jiang L, Li L, Geng C, et al. Monosodium iodoacetate induces apoptosis via the mitochondrial pathway involving ROS production and caspase activation in rat chondrocytes in vitro: apoptosis induced by monosodium iodoacetate. *J Orthop Res* 2013;31:364–9.

Acknowledgements

This research was supported by Faculty of Excellence Research (Penelitian Unggulan Fakultas) of Airlangga University with the contract No. 545/UN3.15/PT/2022

Tables and figures



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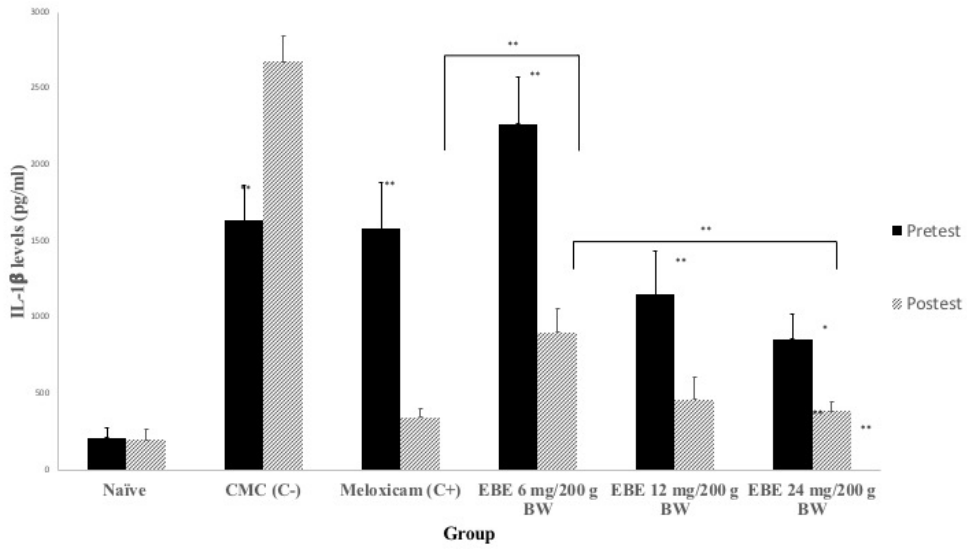


FIGURE 2: The IL-1ß levels on the pretest increased in rat serum compared with the naïve group. The cytokine was measured in the serum of EBE treated rats at three doses (6, 12, and 24 mg/200 g BW) together with negative (CMC) and positive (meloxicam) groups. The levels of IL-1ß were decreased after treatment as compared with the negative control group ($P<0.01$). Data are present as Mean±SD base on n=5 for all groups. *$P<0.05$; **$P<0.01$.

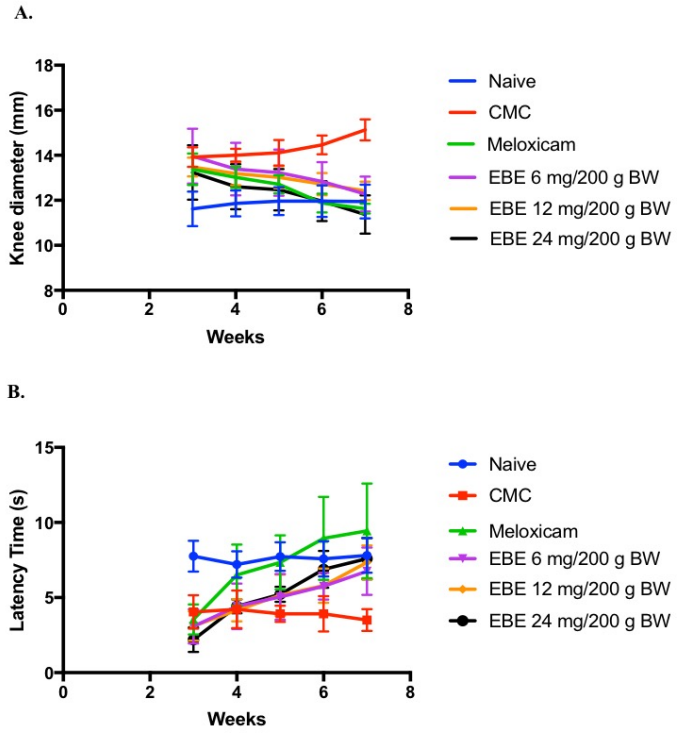


FIGURE 3: The effects of EBE in vivo MIA induced OA model (A) Rat's knee diameter was significantly decreased after the treatment of EBE as compared to the negative control group ($P<0.01$); (B) Time latency was significantly increased after the treatment of EBE as compared to the negative control group ($P<0.05$). Data are present as Mean±SD base on n=5 for all groups.

Response to Reviewers

Thank you for the opportunity to submit a revised draft of our manuscript titled "Antiosteoarthritis Activities of 70% Ethanol Extract of *Eleutherine Bulbosa* (Mill.) Urb. Bulb on Rats Monosodium Iodoacetate-Induced Osteoarthritis" to *Journal of Public Health of Africa*. We are grateful for the time and work that you and the reviewers have dedicated to delivering your insightful comments on our manuscipt. We appreciate of the reviewers' valuable feedback on our manuscript. We have been able to edit the document to reflect the majority of the reviewers' recommendations. We have highlight the manuscript's alterations and track the change we have made. Here is a point-by-point answer to the criticisms and queries raised by the reviewers.

Title	: Antiosteoarthritis Activities of 70% Ethanol Extract of *Eleutherine Bulbosa* (Mill.) Urb. Bulb on Rats Monosodium Iodoacetate-Induced Osteoarthritis
Manuscript number	: Submission ID ICPHS-12
Revision Version	: 1
Editor's Decision Received Date	: Friday, December 16th 2022
Revision Submission Date	:

Author Response 1st revision

Reviewer 1

Reviewer Comments:

The author has described the studies well. However, detail points need to be added. Please check the manuscript.

Detailed revisions are provided in the comments of the manuscript.

Comment 1: *Mention here how many g of simplicia and the total of solvent*

Author Response: Thank you for pointing this out, we have revised this point.

"Fresh bulb of *E. bulbosa* were cut into small parts, dried , and mashed with blender to obtained a dried powder of *E. bulbosa* bulb (1000 gram). The simplicia was extracted by maceration method using 5000 mL 70% ethanol (1:5) at room temperature for 24 h."

Comment 2: *Explaine to manage the rat after treatment. Does the animal followed the sacrifice process, please explaine*

Author Response: Thank you for the constructive comment on how to manage the rat after treatment. Yes, the animal has followed the sacrifice process and we have added as your suggestion.

"The euthanasia process was using anesthetic overdose with the combination of 300 mg/kg ketamine and 30 mg/kg xylazine intraperitoneal."

Comment 3: *Mention Rat rather than animal*
Author Response: We agree with your suggestion and made the change according to this.

"2.3 Rat Model
Rats were divided into six groups: the naïve group was given food and water *ad libitum*…"

Comment 4: T*ype of instrument, specification condition?*

Author Response: You made a valid point that the paper should add the type of instrument, and the spesification. So, we have incorporated your suggestion to the manuscript.

"This study used a hot plate (Ugo Basile Hot/Cold Plate 35100, Gemonio, Italy) to observe the response hyperalgesia of all groups on days 0, 7, 14, 21, 28, 35, 42, and 49. The hot plate method was a traditional method, using unrestrained animals, and depends on a rat own visual to express thermal pain"

Comment 5: *Include this explanation* (limitation) *in the discussion section*

Author Response: thank you for the reminder, we have done to include the limitation paragraph on the discussion section.

Reviewer 2
Reviewer Comments:
Comment 1: *Title: please include the part of the plant used*

Author Response: Thank you for your kind reminder. We revised the title as follows.

"Antiosteoarthritis activities of 70% ethanol extract of *Eleutherine bulbosa* (Mill.) Urb. bulb on rats monosodium iodoacetate-induced osteoarthritis"

Comment 2: *Introduction: State clearly what is the difference between the current study and the previous study*

Author Response:
Thank you for the excellent suggestion, we have revised and state about the previous and current study clearly.

"Previous study declared that *E. bulbosa* from Vietnam in mice with colagen antibody induced arthritis can used as antinflammatory agent at dose 1000 mg/kg b.w. suppressed IL-16 and TNF-α, while the IL-10 was increased. This current study focused on IL-1β, which play an crucial role in pain response. IL-1β is able to increase the regulation of pronociceptive mediators, such as nerve growth factor (NGF) which plays an important role in pain processes. IL-1β signaling via cascades leads to the release and/or activation of nociceptive molecules such as prostaglandins, IL-6, substance P, and matrix metalloproteinase-9 (MMP-9)."

Comment 3: *Methods: The method used for drying the sample should be included. Please check on the term used and use the standard terms*

Author Response: thank you for the valuable comment, we have rewrite the method that used for drying the sample as follow.

"The filtrate was obtained after filtered using Whatman's paper no.41 and remacerated for twice. The filtrate was evaporated using a rotary evaporator at 40°C with 40 rpm until the thick extract was obtained with the constant weight. The extractive value of 70% ethanol extract of *E. bulbosa* bulb was calculated as % w/w yield and found at 15,98%."

Comment 4: *Results: Based on the data in Fig 3b, is it possible to discuss the results from each week? In the treated group, doses 12 and 24, the response is very similar for each week compared to the dose 6 group. This trend is also different from the meloxicam group, in which the response is increasing each week*

Author Response: Thank you for this suggestion. It would have been interesting to explore this aspect. We have add about the increasing time latency each week.

"Treatment with EBE 6, 12, and 24 mg/200g BW were increased in each week. On the first week after treatment, the time latency of positive control and EBE 24 mg/200 g BW group were increased up to 80%, but EBE 6 and 12 mg/200 g BW group were increased up to 40%. On the second and third week, time latency of all the treatment group were increased up to 20%, but on the last week, the increasing of time latency decrease around 10% of all the treatment groups (Figure 3b)."

Comment 5: *Discussion: Second paragraph: This paragraph should focus on the compounds reported from E. bulbosa. However, if the author wants to discuss other compounds in the extract, sufficient information in the introduction should be provided. If this is the case, the author should explain other metabolites that are possibly present in the introduction. Perhaps compounds from the same plant genus.*

Author Response: Thank you for your assessment. We have add them into the manuscript before and have added the compound from the same family (Iridaceae) in the introduction. The compound from the extract has been highlighted in the manuscript as follow.

Introduction:

"According to Tessele *et al.* (2011), eleutherine and isoeleutherine from *Cipura paludosa* (Iridaceae) have antihypernociceptive and anti-inflammatory effects due to their decrease in paw edema mice that induced carrageenan."

Discussion:

"In naphthoquinone derivatives, Hong et al. (2008) showed clear evidence that T helper cell proliferation is partially inhibited by both eleutherine and isoeleutherine. The substances also elevated levels of the cytokine IL-2 and apoptosis. Through the secretion of several cytokines, the biological response to inflammation is managed by T cells. In addition, another study showed that eleutherine and isoeleutherine might affect the mechanical hypernociception induced by lipopolysaccharide (LPS). Eleutherine has been reported to be able to interfere with paw edema caused by PGE_2 or histamine significantly."

Comment 6: *Figure: Can you explain why the SD for meloxicam is large? what is the value for SD that is acceptable in this study (Fig 3b)*

Author Response: Thank you for the questions. The SD of the meloxicam is large because each rat show the different reaction to express the thermal pain. You are right, the SD is large. We have 7 data and select best of 5 data to include in this journal. This is our seven data and the five one.

Control (+)	4,60	6,10	6,10	6,40	5,80
	5,50	3,80	7,90	4,10	5,33
	7,60	6,20	13,10	16,60	10,88
	6,70	6,50	7,20	7,40	6,95
	9,20	10,40	10,20	11,80	10,40
	6,50	7,20	6,80	7,80	7,08
	4,40	7,50	8,10	8,60	7,15
MEAN	6,36	6,81	8,49	8,96	7,65
SD	1,70	1,98	2,41	4,09	

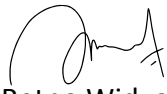
Positive Control (Meloxicam Group)	4,60	6,10	6,10	6,40	5,80
	6,50	7,20	6,80	7,80	7,08
	6,70	6,50	7,20	7,40	6,95
	9,20	10,40	10,20	11,80	10,40
	4,40	7,50	8,10	8,60	7,15
MEAN	6,28	7,54	7,68	8,40	7,48
STD	1,94	1,69	1,58	2,06	

Comment 7: *English: Some sentences should be revised for clarity*

Author Response: Thank you for the reminder. In addition to the above comments, all spelling and grammatical errors corrected.

We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.

Sincerely,



Retno Widyowati

Antiosteoarthritis activities of 70% ethanol extract of *Eleutherine bulbosa* (Mill.) Urb. bulb on rats monosodium iodoacetate-induced osteoarthritis

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Keywords : osteoarthritis, antiinflammatory, *Eleutherine bulbosa*, MIA, IL-1β

Authors' Contributions:

Riza Ambar Sari : drafting the work, analysis, interpretation the data

Fina Luthfiana : analysis, data acquisition

Irawati Sholihah : data analysis, interpretation the data

Sukardiman : revising critically, final approval

Retno Widyowati : conception and design the work, revising critically, final approval

Conflict of Interests Statement: none of the authors declared any conflicts of interest

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Abstract:

Background**:** osteoarthritis (OA) is the most typical degenerative joint condition that induces pain and disability in the elderly. Traditionally, *Eleutherine bulbosa* bulb from Pasuruan, East Java, is used to treat many diseases, including as an anti-inflammatory.

Objective: in this study, we used an in vivo model to examine the effects of 70% ethanol extracts of *E. bulbosa* (EBE) on the progression and development of OA.

Methods**:** a single injection of intraarticular monosodium iodoacetate (MIA) was used to create the OA model in rats. The progression of OA was observed for three weeks. Furthermore, treatment of EBE at a dose of 6, 12, and 24 mg/200g BW orally for four weeks was conducted to assess the effects on decreasing IL-1ß level, joint swelling, and hyperalgesia.

Results**:** induction was successful, indicated by a significant difference ($p<0.05$) in decreasing latency time, increasing joint swelling, and IL-1ß level. EBE 24 mg/200 g BW treatment has significantly ($p<0.05$) reduced IL-1ß levels, joint swelling, and response to hyperalgesia.

Conclusion**:** 70% ethanol extract of *E. bulbosa* bulb has therapeutic effects on inflammation through reducing IL-1ß in experimental MIA-induced osteoarthritis in a rat model. According to this study, EBE may have an effective potential new agent for OA therapy.

1. Introduction

Osteoarthritis (OA) is the most prevalent type of degenerative joint disease in the elderly.[1] Clinically, the disease is associated with joint pain caused by the entire joint becoming dysfunctional, particularly the articular cartilage, synovium, subchondral bone, and other tissues with close mechanical and molecular biological interactions.[2,3] Some characteristics of OA are tenderness, stiffness, joint swelling, immobility, and joint deformity. The risk factors of OA are gender, genetic, and joint injury, which then increase along with obesity and age.[1,4] This disease is the leading cause of disability, with nearly 10-15% of adults over 60 years suffering from OA.[5] The prevalence of knee OA is 240 per 100,000 individuals per year. In Indonesia, knee OA is 65% of people over 61 years old, 30% of people aged 40-60, and 5% less than 40 years. This incidence is estimated at 15.5% in men and 12.7% in women.[6]

IL-1 is implicated in the pathophysiology of knee OA due to increased production of this cytokine associated with the severity of symptoms.[7] Furthermore, IL-1 has been shown to inhibit chondrocytes.[8,9] IL-1Ra specifically inhibits the action of IL-1β by binding to IL-1R1.[10] Strong evidence for the critical role of interleukin-1β (IL-1β) and OA-associated regulation genes such as matrix metalloproteinase (MMP), tumor necrosis factor-a (TNF-α), cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6) in the development of OA have been reported. IL-1β is a proinflammatory substance that is important for responding to the immune system's defenses against injury.[11,12] The activation of signaling events by IL-1β was associated with the upregulation of MMP-13.[9] Reduced chondrocyte proteoglycan synthesis, increases in the production of MMP, and the release of nitric oxide are all caused by IL-1β in the joint.[13] Another mechanism is the binding of IL-1β to the IL-1 receptor (IL-1R1), triggering a proinflammatory response that leads to cartilage destruction followed by subchondral destruction. The proinflammatory response also causes synovial membrane inflammation, which manifests as pain.[7]

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The osteoarthritis model using monosodium iodoacetate (MIA) in rats has been used for a long time and is well established. An intraarticular injection of MIA causes histopathological abnormalities in articular cartilage, similar to degenerative OA in

humans. MIA is a metabolic inhibitor that disrupts the aerobic glycolytic pathway of cells and causes cell death by inhibiting the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes.[14]

Eleutherine bulbosa (Mill.) Urb is the Iridaceae family's oriental medicinal plant. This plant is used in traditional medicine for treating several conditions, such as cough, anemia, heart failure, cancer, infertility, skin disease, and inflammation.[15,16] Previous phytochemical compounds have been isolated from *E. bulbosa,* such as naphthalene, anthraquinone, naphthoquinone and their derivatives eleutherinone, eleutherine, isoeleutherine, eleutherol, eleuthone, eleutherinol 8-*O*- β -D-glucoside, eleuthoside.[16–18] According to Tessele *et al.* (2011), eleutherine and isoeleutherine from *Cipura paludosa* (Iridaceae) have antihypernociceptive and anti-inflammatory effects due to their decrease in paw edema mice that induced carrageenan. *E. bulbosa* also contains luteolin, which inhibits cyclooxygenase and reduces pain.[20] Previous study declared that *E. bulbosa* from Vietnam in mice with colagen antibody induced arthritis can used as antinflammatory agent at dose 1000 mg/kg b.w. suppressed IL-16 and TNF-α, while the IL-10 was increased.[15] This current study focused on IL-1β, which play an crucial role in pain response. IL-1β is able to increase the regulation of pronociceptive mediators, such as nerve growth factor (NGF) which plays an important role in pain processes. IL-1β signaling via cascades leads to the release and/or activation of nociceptive molecules such as prostaglandins, IL-6, substance P, and matrix metalloproteinase-9 (MMP-9).[21]

Therefore, inhibition of IL-1β is considered a potential target to lessen the pain and inflammation in the development of OA. This research investigated the antiosteoarthritis activity of 70% ethanol extract of *E. bulbosa* (EBE) in an osteoarthritis rat model induced by MIA.

2. Materials and Methods

2.1 Ethical Considerations

Thirty male Wistar rats (*Rattus norvegicus*) were purchased from the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia, in healthy condition. Rats aged

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3-4 months (200-300 g) were acclimatized for seven days in the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. The animals were kept in ideal lighting (12-hour light-dark cycle), a temperature of 22±1°C, and a humidity of 60-80%. They also had full access to drink and food. The entire process has been authorized by the Animal Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, with Ethical Clearance No. 2.KEH.120.09.2022.

2.2 Plant materials

Eleutherine bulbosa (Mill.) Urb. were found and collected from Pasuruan Regency, East Java, Indonesia. The collected plants was determinated by UPT Laboratorium Herbal Materia Medica, Batu, East Java, Indonesia (Certificate of Determination No. 074/722/102-7-A/2021). Fresh bulb of *E. bulbosa* were cut into small parts, dried , and mashed with blender to obtained a dried powder of *E. bulbosa* bulb (1000 gram). The simplicia was extracted by maceration method using 5000 mL 70% ethanol (1:5) at room temperature for 24 h. The filtrate was obtained after filtered using Whatman's paper no.41, and remacerated for twice. The filtrate was evaporated using a rotary evaporator at 40°C with 40 rpm until the thick extract was obtained with the constant weight. The extractive value of 70% ethanol extract of *E. bulbosa* bulb was calculated as % w/w yield and found at 15,98%.

2.3 Rat Model

Rats were divided into six groups: the naïve group was given food and water *ad libitum*, the negative group was assigned 0,5% Carboxy Methyl Cellulose (CMC), the positive group received meloxicam (Indofarma, Indonesia) 0.135 mg/200 g BW, and 70% ethanol extract of *E. bulbosa* bulb in 3 kinds of dose that given Dose 1: 6 mg/200 g BW; Dose 2: 12 mg/200 g BW; Dose 3: 24 mg/200 g BW, respectively. Intraarticular injection of MIA (4 mg MIA dissolved in 50 μl of saline) (Sigma-Aldrich, Darmstadt, Germany) was performed on all groups except the naïve group under anesthesia using a combination of 10% ketamine (80 mg/kg) (Agrovet, Nicaragua) and 1% xylazine (5 mg/kg) (Holland, Netherlands) to obtain the condition of OA. Three weeks after MIA

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induction, all rats were checked for OA success on day 21 with the measured IL-1β levels (pretest), followed by an oral administration of 70% ethanol extract daily for 28 days based on their group. Furthermore, the response of hyperalgesia and knee joint swelling was measured with a hot plate and calibrated screw micrometer for seven weeks (on days 0, 7, 14, 21, 28, 35, 42, and 49), respectively. Latency response was measured with a stopwatch. After the treatment, the determination of IL-1β levels in blood serum was measured on the seventh week (day 49 as a posttest). The euthanasia process was using anesthetic overdose with the combination of 300 mg/kg ketamine and 30 mg/kg xylazine intraperitoneal.

2.4 Evaluation of Parameters

Hyperalgesia testing

This study used a hot plate (Ugo Basile Hot/Cold Plate 35100, Gemonio, Italy) to observe the response hyperalgesia of all groups on days 0, 7, 14, 21, 28, 35, 42, and 49. The hot plate method was a traditional method, using unrestrained animals, and depends on a rat own visual to express thermal pain. A rat was left unrestrained on a metal surface kept at a constant temperature of 55±0.5°C for the hot plate test. The investigator documented the response latency, and the time it takes to generate a nocifensive reaction. Nocifensive behavior includes withdrawal or licking of the hind paw, leaning posture, stamping, and jumping. After observing the response, the rats were immediately removed from the hot plate.

Joint swelling measurement

Joint swelling was measured on the ipsilateral knee of the rat. All groups were measured on days 0, 7, 14, 21, 28, 35, 42, and 49 using a calibrated screw micrometer.

IL-1β Cytokine Assay

Blood samples from the tail vein were collected into a blood collection tube on days 21 and 49. The centrifugation at 3000 rpm was performed to take the blood serum. The levels of IL-1β were determined using commercial rat IL-1β ELISA kits (Bioenzy, Germany), following the manufacturer's protocols.

Statistical Analysis

Data from rat experiments were collected, and the results were presented as mean ± SD of 5 animals in each group using Graph Pad Prism 7 while the analytical statistic using SPSS 25. Two-way analysis of variance (ANOVA) was used to compare the data statistically, followed by the LSD post hoc test. Statistical significance was defined as a p value < 0.05.

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3. Results

After the treatment, data were collected and analyzed. According to Figure 1. the knee joint was swelling as the increase of the diameter around 20-30%. Figure 1. also shows that from the second week up to the third week the progression of OA seems at a steady state. It indicates a specific set of beginning condition, because a steady state is an example of an attractor that can be attained.[22,23] Based on Figure 3, in the third week IL-1ß level of the naïve group and negative control have a significant difference (p<0.01). Thereby, in the fourth week, the rats were separated into groups and given the treatment for 28 days. The negative control rats were inflamed and marked by swollen joints. The knee diameter, time latency, and IL-1ß level have been measured.

In comparison, there was a significant difference between the rats who were given EBE 24 mg/200 g BW and the other dose but no significant difference with the positive control (meloxicam) group. In addition, to evaluate osteoarthritis based on the clinical data, time latency and level of IL-1ß of the joints were assessed. The time latency of the negative control group showed a significantly differed from the other groups (p<0.05). Treatment with EBE 6, 12, and 24 mg/200g BW were increased in each week. On the first week after treatment, the time latency of positive control and EBE 24 mg/200 g BW group were increased up to 80%, but EBE 6 and 12 mg/200 g BW group were increased up to 40%. On the second and third week, time latency of all the treatment group were increased up to 20%, but on the last week, the increasing of time latency decrease around 10% of all the treatment groups (Figure 3b).

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Therefore, the proinflammatory cytokine IL-1ß was measured in rats receiving EBE. According to Figure 2, EBE 12 and 24 mg/200 g BW significantly (p<0.01) suppressed the amount of IL-1ß in the serum of rats compared with negative control after 28 days. EBE dose 24 mg/200 g BW is also significantly different from EBE 6 mg/200 g BW.

4. Discussion

Inflammation and pain are recognized as the primary cause of OA symptoms and development. MIA disrupts the metabolism of chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase, which affects the production of reactive oxygen species (ROS) and the breakdown of the cartilage matrix. MIA also increases proinflammatory cytokines such as IL-1ß and TNF-α that may cause the production of COX-2 and matrix metalloproteinases (MMPs).[8] Additionally, IL-1ß has been shown to accelerate cartilage breakdown in articular chondrocytes and inhibit the synthesis of cartilage matrix.[5] MMPs and ADAMTS are zinc-dependent endopeptidases that play an important role in cartilage matrix destruction.[24] The production of MMPs and ADAMTS is significantly increased during OA in response to proinflammatory mediators, including IL-1ß and TNF-α.[25]

Administration of EBE at various doses could increase latency time as well as decrease joint swelling and IL-1ß in the OA rats model. It was due to the compounds in EBE, such as flavonoids and naphthoquinone. Quercetin is a basic structure that forms other flavonoids. Before having the first pass effect, the small intestine absorbs quercetin, then delivered it to the liver by portal circulation. The quercetin will diffuse throughout the body's tissues at that point. It is well known that quercetin significantly binds to plasma albumin. Quercetin inhibits the Transient Receptor Potential Cation Channel Subfamily V member 1 (TRPV1) receptor and has an opioid action.[20] In naphthoquinone derivatives, Hong et al. (2008) showed clear evidence that T helper cell proliferation is partially inhibited by both eleutherine and isoeleutherine. The substances also elevated levels of the cytokine IL-2 and apoptosis. Through the secretion of several cytokines, the biological response to inflammation is managed by T cells. In addition, another study showed that eleutherine and isoeleutherine might affect the mechanical

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hypernociception induced by lipopolysaccharide (LPS). Eleutherine has been reported to be able to interfere with paw edema caused by PGE_2 or histamine significantly. It is well known that the release of many inflammatory mediators occurs as a result of inflammatory hypernociception.[19] Otherwise, *in silico* prediction of β-sitosterol in the *Eleutherine americana* shows that it has anti-inflammatory activity and has a strong affinity for COX-2 greater than celecoxib. The *in silico* toxicity prediction indicated that the compound was not toxic.[27] It is interesting that β-sitosterol is a steroid molecule with high closeness to corticosteroids, which are known as anti-inflammatory drugs. This may explain the high affinity of β-sitosterol to COX-2 as well as the intermolecular connections created.[28]

The study's limitations were recognized. First, this research did not histologically examine the rat knee's articular cartilage The quantitative results revealed some distinct tendencies, and current study findings are important and useful for creating an OA model. The present study's findings significantly contributed to the knowledge of mechanisms in the treatment of osteoarthritis. Second, induced OA using the chemical compound MIA. Some previous studies claimed that chemical has a different pathophysiology unrelated to post-traumatic OA.[29,30] However, several studies have revealed morphological and histological alterations in the articular tissues that are identical to the characteristics of human OA.[14,31]

EBE at various doses has been able to increase latency time as well as decrease joint swelling and IL-1ß in OA rats model, but only EBE 24 mg/200 g BW reduced joint swelling and IL-1ß level as well as meloxicam. Enhancing EBE dosage reduces the sign of antiosteoarthritis which helps decrease pain and inflammation.

5. Conclusion

The present study demonstrated that EBE decreased the IL-1β induced inflammatory response, reduced joint swelling, and increased time latency. Additionally, MIA caused

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Moved up [1]: The study's limitations were recognized. First, this research did not histologically examine the rat knee's articular cartilage The quantitative results revealed some distinct tendencies, and current study findings are important and useful for creating an OA model. The present study's findings significantly contributed to the knowledge of mechanisms in the treatment of osteoarthritis. Second, induced OA using the chemical compound MIA. Some previous studies claimed that chemical has a different pathophysiology unrelated to post-traumatic OA.[28,29] However, several studies have revealed morphological and histological alterations in the articular tissues that are identical to the characteristics of human OA.[14,30]

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OA in the rat model, and EBE had a protective effect against OA degeneration. These findings suggested that EBE 12 and 24 mg/200 g BW might be a potential treatment for OA as well as meloxicam.

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References

1. Yunus MHM, Nordin A, Kamal H. Pathophysiological perspective of osteoarthritis. *Medicina* 2020; 56: 614–27.
2. Grässel S, Zaucke F, Madry H. Osteoarthritis: Novel Molecular Mechanisms Increase Our Understanding of the Disease Pathology. *J Clin Med* 2021; 10: 1938–50.
3. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage–bone crosstalk. *Nat Rev Rheumatol* 2016; 12: 632–44.
4. Cui A, Li H, Wang D, et al. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EClinicalMedicine* 2020; 29–30: 100587–100.
5. Huang X, Pei W, Ni B, et al. Chondroprotective and antiarthritic effects of galangin in osteoarthritis: An in vitro and in vivo study. *Eur J Pharmacol* 2021; 906: 174232–9.
6. Siddik M, Haryadi RD. The risk factors effect of knee osteoarthritis towards postural lateral sway. *Indian J Forensic Med Toxicol* 2020; 14: 1787–92.
7. Budhiparama NC, Lumban-Gaol I, Sudoyo H, et al. Interleukin-1 genetic polymorphisms in knee osteoarthritis: What do we know? A meta-analysis and systematic review. *J Orthop Surg (Hong Kong)* 2022; 30: 1–14.
8. Khotib J, Pratiwi AP, Ardianto C, et al. Attenuation of IL-1ß on the use of glucosamine as an adjuvant in meloxicam treatment in rat models with osteoarthritis. *J Basic Clin Physiol Pharmacol* 2020; 30: 1–9.
9. Rasheed Z, Al-Shobaili HA, Rasheed N, et al. MicroRNA-26a-5p regulates the expression of inducible nitric oxide synthase via activation of NF-κB pathway in human osteoarthritis chondrocytes. *Arch Biochem Biophys* 2016; 594: 61–7.
10. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011; 117: 3720–3732.
11. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev* 2011; 22: 189–195.

12. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* 2012; 11: 633–652.

13. Joosten LAB, Smeets RL, Koenders MI, et al. Interleukin-18 promotes joint inflammation and induces interleukin-1-driven cartilage destruction. *Am J Clin Pathol* 2004; 165: 959–67.

14. Takahashi I, Matsuzaki T, Kuroki H, et al. Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. *PLoS ONE* 2018; 13: e0196625.

15. Hanh PTB, Thao DT, Nga NT, et al. Toxicity and anti-inflammatory activities of an extract of the *Eleutherine bulbosa* rhizome on collagen antibody-induced arthritis in a mouse model. *Nat Prod Commun* 2018; 13: 883–86.

16. Kim, Seung Hyun, Hong, Hye-Jin, Kim, Sohyun, et al. Chemical constituents of the rhizome of *Eleutherine bulbosa* and their inhibitory effect on the pro-inflammatory cytokines production in lipopolysaccharide-stimulated bone marrow-derived dendritic cells. *Bull Korean Chem Soc* 2013; 34: 633–636.

17. Insanu M, Kusmardiyani S, Hartati R. Recent studies on phytochemicals and pharmacological effects of *Eleutherine Americana* Merr. *Procedia Chem* 2014; 13: 221–228.

18. Kamarudin AA, Sayuti NH, Saad N, et al. *Eleutherine bulbosa* (Mill.) Urb. Bulb: review of the pharmacological activities and its prospects for application. *Int J Mol Sci* 2021; 22: 6747–67.

19. Tessele P, Delle Monache F, Quintão N, et al. A new naphthoquinone isolated from the bulbs of *Cipura paludosa* and pharmacological activity of two main constituents. *Planta Med* 2011; 77: 1035–43.

20. Hafizh M, Indiastuti DN, Mukono IS. Analgesic effect of dayak onion (*Eleutherine americana* (Aubl.) Merr.) on mice (*Mus musculus*) by hot plate test method. *BHSJ* 2021; 4: 22.

21. Ren K, Torres R. Role of interleukin-1β during pain and inflammation. *Brain Research Reviews* 2009; 60: 57–64.

22. Chen H, Qin J, Shi H, et al. Rhoifolin ameliorates osteoarthritis via the Nrf2/NF-κB axis: in vitro and in vivo experiments. *Osteoarthr Cartil* 2022; S1063458422000152.

23. Sobie EA. An introduction to dynamical systems. *Sci Signal*; 4. Epub ahead of print 20 September 2011. DOI: 10.1126/scisignal.2001982.

24. Malemud CJ. Inhibition of MMPs and ADAM/ADAMTS. *Biochem Pharmacol* 2019; 165: 33–40.

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25. Vincent TL. IL-1 in osteoarthritis: time for a critical review of the literature. *F1000Res* 2019; 8: 934–43.

26. Hong J-H, Yu ES, Han A-R, et al. Isoeleutherin and eleutherinol, naturally occurring selective modulators of Th cell-mediated immune responses. *Biochem Biophys Res Commun* 2008; 371: 278–82.

27. Damayanti S, Puspaningrum D, N. Muhammad H, et al. Interaction of the chemical constituents of *Eleutherine americana* (AUBL.) MERR. EX K. HEYNE with cyclooxygenase and H5N1 RNA polymerase: an *in-silico* study. *Rasayan J Chem* 2021; 14: 844–54.

28. Raghavamma STV, Rama Rao N, Devala Rao G. Inhibitory potential of important phytochemicals from *Pergularia daemia* (Forsk.) chiov., on snake venom (Naja naja). *J Genet Eng Biotechnol* 2016; 14: 211–17.

29. Kuyinu EL, Narayanan G, Nair LS, et al. Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res* 2016; 11: 19–46.

30. Teeple E, Jay GD, Elsaid KA, et al. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J* 2013; 15: 438–46.

31. Jiang L, Li L, Geng C, et al. Monosodium iodoacetate induces apoptosis via the mitochondrial pathway involving ROS production and caspase activation in rat chondrocytes in vitro: apoptosis induced by monosodium iodoacetate. *J Orthop Res* 2013; 31: 364–69.

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Acknowledgements

This research was supported by Faculty of Excellence Research (Penelitian Unggulan Fakultas) of Airlangga University with the contract No. 545/UN3.15/PT/2022

Tables and figures

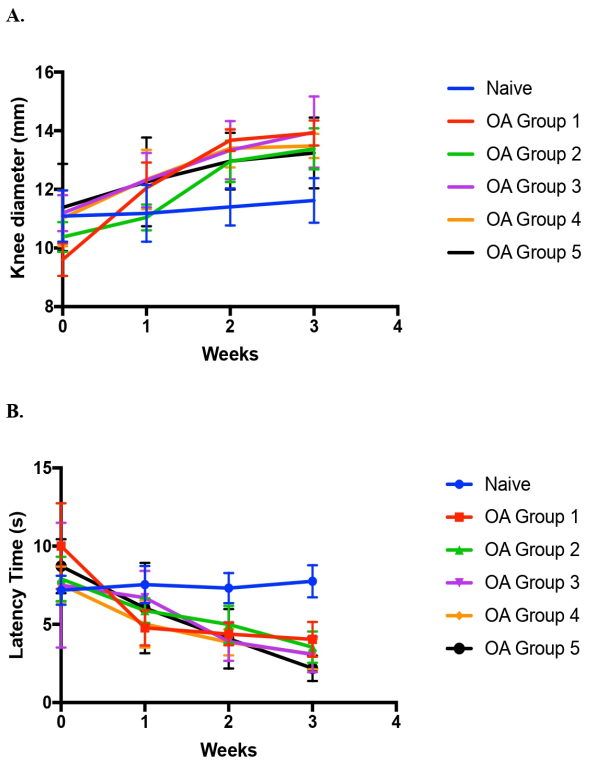

FIGURE 1: The development of OA after injecting MIA in three weeks (A) rat knee diameter; (B) time latency. Data are present as Mean±SD base on N=5 for all groups.

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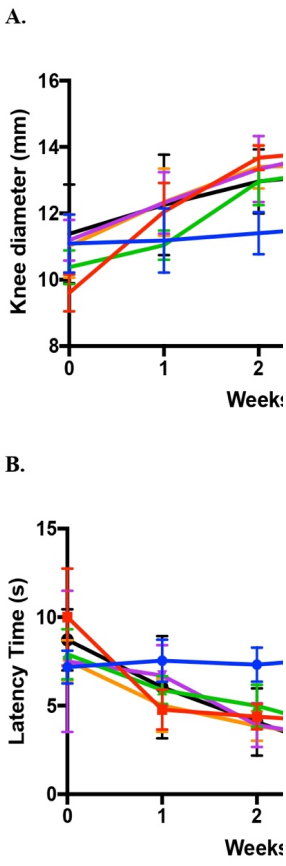

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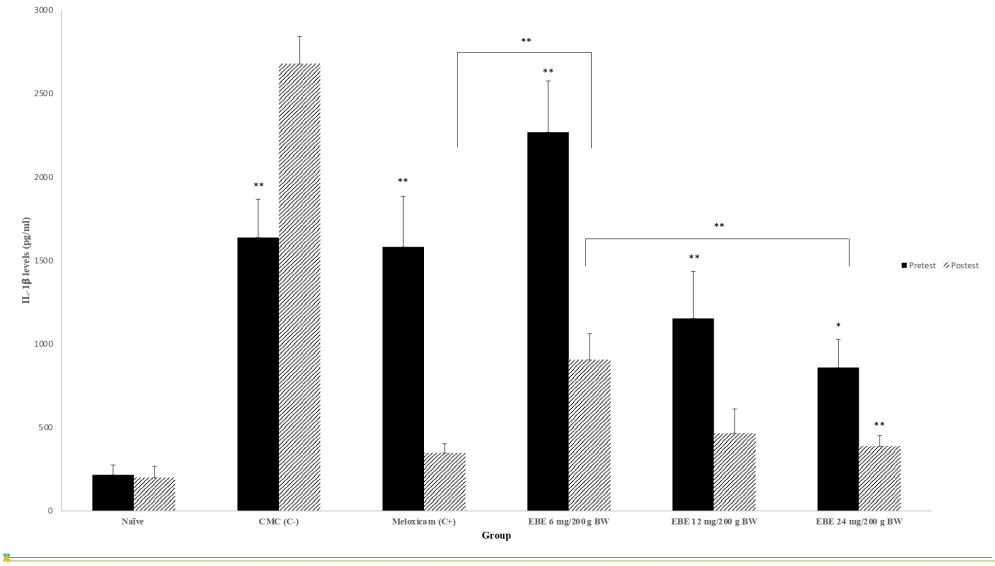

FIGURE 2: The IL-1ß levels on the pretest increased in rat serum compared with the naïve group. The cytokine was measured in the serum of EBE treated rats at three doses (6, 12, and 24 mg/200 g BW) together with negative (CMC) and positive (meloxicam) groups. The levels of IL-1ß were decreased after treatment as compared with the negative control group (p<0.01). Data are present as Mean±SD base on n=5 for all groups. *p<0.05; **p<0.01.

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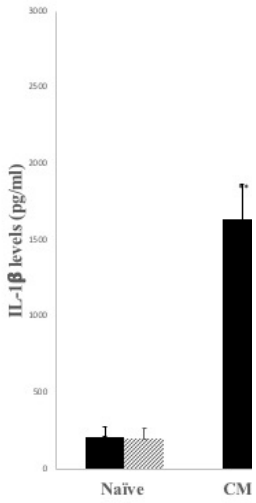

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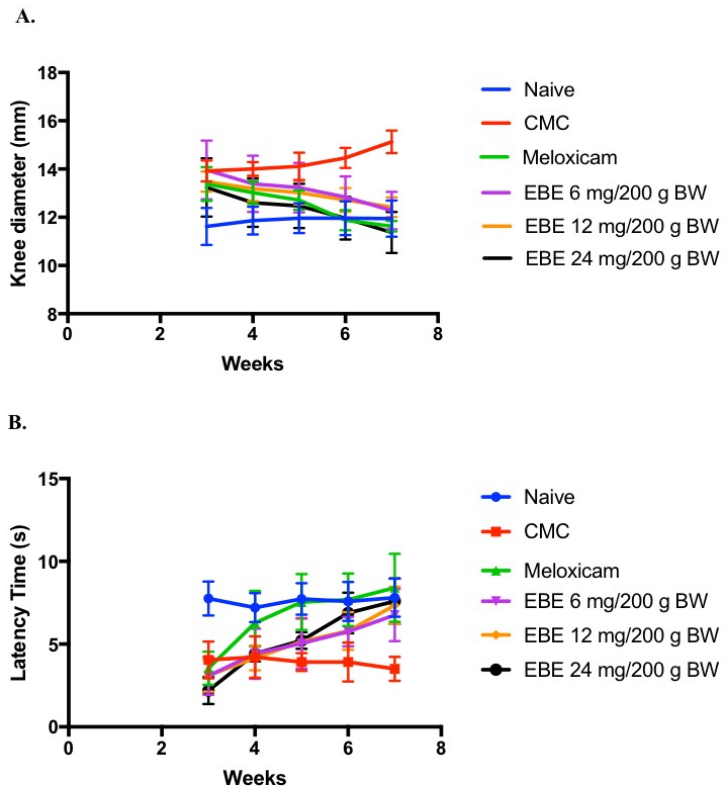

FIGURE 3: The effects of EBE in vivo MIA induced OA model (A) Rat's knee diameter was significantly decreased after the treatment of EBE as compared to the negative control group (p<0.01); (B) Time latency was significantly increased after the treatment of EBE as compared to the negative control group (p<0.05). Data are present as Mean±SD base on n=5 for all groups.

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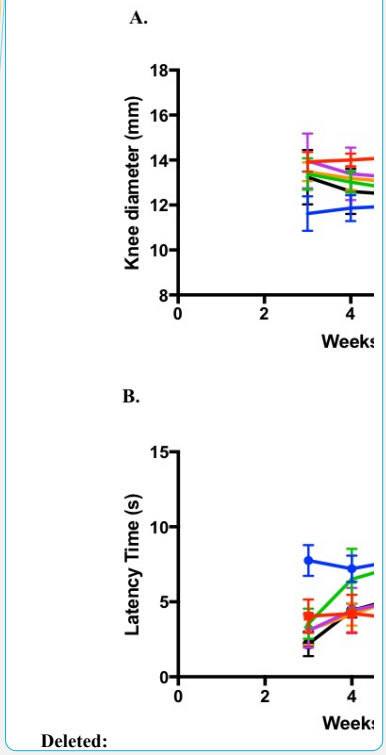

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