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RESEARCH ARTICLE

Activities of sondhep herbs to cause changes in IL-1 β in an osteoarthritis rat model

Retno Widyowati^{1,2} , Iffatur Rosyidah³, Via Qurrota A'yun³, Rice Disi Oktarina^{1,2}, Irawati Sholikhah⁴, Ram Kumar Sahu⁵

¹ Department of Pharmaceutical Sciences, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

² Natural Products Drug Discovery and Development Research Group, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

³ Undergraduate Program, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

⁴ Department of Chemistry, Faculty of Sains and Technology, Universitas Airlangga, Surabaya, Indonesia

⁵ Department of Pharmaceutical Sciences, Assam University, India

Keywords

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Correspondence

Retno Widyowati
Gedung Nanizar Zaman Joenoes
Kampus C UNAIR
Mulyorejo
Indonesia
rr-retno-w@ff.unair.ac.id

Abstract

Background: Osteoarthritis (OA) is a slowly progressive and degenerative joint disease. Symptoms of pain and inflammation are influenced by IL-1 β , which increases COX-2 enzyme activity and PGE2 production. Sumenep Sondhep herbs are used by the community for joint pain. **Objective:** To evaluate the activities of the Sumenep Sondhep herb in reducing the cytokine IL-1 β in the OA rats model. **Methods:** Monosodium iodoacetate (MIA) induction was carried out on animal OA models. Rats were treated with Sondhep extract for 28 days. Knee diameter and hyperalgesia responses were recorded every week until the 7th week. The IL-1 β levels were observed on the 21st and 49th days using the ELISA method. **Results:** There was a significant difference in decreasing the diameter of the rat's knee ($p < 0.05$), increasing the hyperalgesia test in the rats ($p < 0.05$), and decreasing IL-1 β levels between before and after treatment ($p < 0.05$). **Conclusion:** The Sondhep Sumenep herb has activity against osteoarthritis by reducing pro-inflammatory cytokine IL-1 β in OA rat models.

Introduction

Osteoarthritis (OA) is a degenerative joint disorder associated with cartilage deficiency, loss of meniscus tissue, and joint misalignment. It also increases the pro-inflammatory cytokines (Heijink *et al.*, 2012). Osteoarthritis is one of the most common diseases in the world and affects more than 50% of the elderly population (Oliveira *et al.*, 2019). In Indonesia, the prevalence is 5% at ages less than 40 years, 30% at the age of 40-60 years, and 65% at the age of more than 60 years (Siddik & Haryadi, 2020). The pain experienced by people with osteoarthritis is "strong" to "severe".

The IL-1 β plays a role in the development of pain and inflammation (Oliveira *et al.*, 2019). The Ministry of Health recommends the use of traditional medicine by

the community to maintain and care for their health, as well as to prevent diseases.

Jamu is a traditional medicine made from a natural cultural heritage passed down from generation to generation for health. Jamu is a combination of several ingredients from plants, animal materials, mineral materials, serial (generic) preparations, or mixtures of these materials which have been used for generations for treatments based on experience. In Gapura Sub-district, Sumenep, there are 96 types of herbs and 100 plants that are efficacious for 67 types of diseases (Mangestuti *et al.*, 2007). The most famous herb is the Sondhep herb (Hadiwinata, 2016). It is empirically used for joint pain. It consists of red ginger rhizome, aromatic ginger, garlic bulb, single garlic, and leek bulb. Scientifically, each ingredient of the Sondhep herb can reduce IL-1 β as a mediator of inflammation and pain. It

is necessary to research the activity of *Sondhep* herb against osteoarthritis rats induced by monosodium iodoacetate (MIA).

Methods

Design

This research was administered and divided into six groups. The groups were divided into 1) The normal group (rats not induced by MIA); 2) The negative group (rats injected with four milligrams MIA and treated with CMC-Na); 3) The positive group (rats injected with four milligrams MIA and treated with 0.7 mg/kg BW meloxicam); 4) *Sondhep* herbs dose I group (rats injected with four milligrams MIA and treated with 28 mg/kg BW of *Sondhep* herbs extract); 5) *Sondhep* herbs dose II group (rats injected with four milligrams MIA and treated with 56 mg/kg BW of *Sondhep* herbs extract); and 6) *Sondhep* herbs dose III group (rats injected with four milligrams MIA and treated with 112 mg/kg BW of *Sondhep* herbs extract). Osteoarthritis induction in rats was done using MIA 4 mg/kg BW and observed after 21 days. Then, rats were further given treatment for 28 days. Knee diameter and hyperalgesia response were measured every week until the 7th week. The IL-1 β levels were observed on the 21st and 49th days using the ELISA method (Sari *et al.*, 2023; Widyowati *et al.*, 2023).

Assessment

Data were analysed using a two-way ANOVA test with a 95% confidence level to determine significant differences. The LSD post hoc test was also carried out to determine the significant difference between the treatment groups. Paired sample *t*-test analysis also was performed to determine the significant difference in IL-1 β levels before and after treatment in the group. A significant difference in the groups was observed from the value ($p < 0.05$).

Results

During the treatment process, the diameter of the rat's right knee was measured using a calliper. Figure 1 shows the results of measuring the knee diameter of rats.

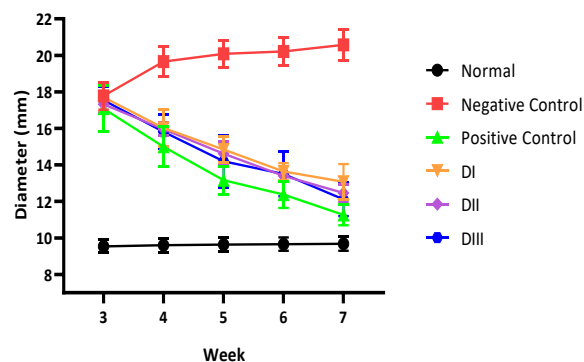


Figure 1: Rat knee diameter during treatment

The graph shows a decrease in knee diameter in rats in the positive group given meloxicam and *Sondhep* herbs group. In the negative controls, given CMC Na, there was an increase in diameter, and for the normal group, the knee diameter was constant until the 7th week.

There were significant differences ($p < 0.05$) in the knee diameter of rats in the pre-test and post-test groups with appropriate time intervals.

Furthermore, the hot plate method was adopted for the measurement of resistance time (hyperalgesia). Figure 2 shows the results of resistance time (hyperalgesia) during treatment.

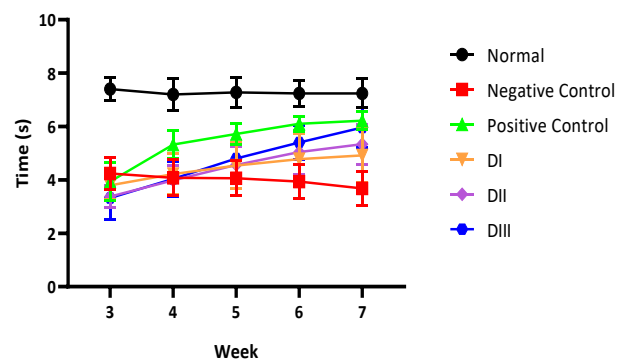


Figure 2: Heat resistance time of rats during treatment

After giving therapy, there was an increase in the resistance time in the positive and dose groups while the negative group had a decrease in resistance time from the 4th week to the 7th week.

The statistical test results showed a p -value < 0.05 , which means there was a significant difference in the heat resistance of the rats in the pretest and post-test groups at the appropriate time intervals.

Based on Table I, the results in the meloxicam group, *Sondhep* dose I, *Sondhep* dose II, and *Sondhep* dose III decreased significantly in IL-1 β levels. The reduction in

IL-1 β levels occurred in meloxicam > *Sondhep* dose III > *Sondhep* dose II > *Sondhep* dose I. Based on statistical

analysis, there was a significant difference before and after treatment ($p < 0.05$).

Table I: Rat IL-1 β level before and after treatment

Groups (n=5)	Rat IL-1 β level (pg/ml) before treatment	Rat IL-1 β level (pg/ml) after treatment	Rat IL-1 β level (pg/ml) reduction after treatment
Normal	416.87 \pm 57.22	378.65 \pm 73.01	38.22
OA	1385.67 \pm 324.58	1319.00 \pm 99.48	66.19
Meloxicam (0.675 mg/kg BW)	1603.67 \pm 239.01	302.67 \pm 46.10	1301.00
<i>Sondhep</i> dose I (28 mg/kg BW)	1234.27 \pm 276.74	536.43 \pm 160.01	697.84
<i>Sondhep</i> dose II (56 mg/kg BW)	1578.93 \pm 150.26	469.06 \pm 220.49	1109.87
<i>Sondhep</i> dose III (112 mg/kg BW)	1613.50 \pm 201.32	356.73 \pm 92.00	1256.77

Discussion

This study aimed to determine the activities of the *Sonchep* herb against osteoarthritis using a rat model induced by MIA. The parameters used in this study were measurements of rat knee diameter, latency time to heat stimulus (Hyperalgesia), and measurement of IL-1 β cytokine levels. The knee diameter measurement was carried out to determine the presence of MIA-induced swelling in rats intra-articularly. The swelling occurred due to the MIA induction which triggered changes in peripheral involvement including acute localised inflammation followed by cartilage erosion, joint disruption, and increased expression of pro-inflammatory cytokines in the joints (Bao *et al.*, 2022).

There was a measurement of the latency time of the rats to the heat stimulus and the occurrence of hyperalgesia which is a sign of the development of a painful state in the MIA-induced OA rats. The model of osteoarthritis with injection intra-articular MIA shows a pain response that occurs due to mediators inflammation and neuropathic pain caused by nerve injury. Besides, the source of pain due to MIA induction occurs due to local inflammation in the knee joint, namely in the synovial membrane, which can produce pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6. Such mediators can promote cartilage degradation and induce hyperalgesia (Orita *et al.*, 2011).

The use of IL-1 β level parameters is because IL-1 β is a pro-inflammatory cytokine that plays a role in osteoarthritis. The role of IL-1 β in osteoarthritis, among others, can induce the expression of tumour necrosis factor (TNF) and matrix metalloproteinase (MMP). Increased IL-1 β and TNF can increase the activity of COX-2 enzymes, microsomal PGE synthetase-1 (mPGES-1), and phospholipase A2, which can increase

the production of prostaglandin E2 (PGE2) (Goldring & Otero, 2014). Prostaglandins are one of the pain neurotransmitters (Bahrudin, 2017) and inflammatory mediators (Anggraini & Prasetyo, 2018). Meanwhile, the increase in MMP plays a role in collagen degradation which aggravates the condition of osteoarthritis (Jabłońska-trypuć *et al.*, 2016). IL-1 β plays a role in the development of pain and inflammation so inhibition of IL-1 β can overcome pain and inflammation in various conditions (Ren & Torres, 2011).

The OA induction was carried out by intra-articular injection of MIA in the rats' right knee. Monosodium iodoacetate (MIA) is a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitor which can result in reduced glycolysis and can cause changes in articular cartilage as seen in osteoarthritis by inhibiting the integration of chondral structures and causing chondrocyte cell death (Kim *et al.*, 2016). MIA can cause acute inflammation associated with increased expression of proinflammatory cytokines such as IL-1 β , IL-6, IL-5, Inducible Nitric Oxide Synthase (iNOS), COX 2, and matrix metalloproteinase-13 (MMP-13) (Moilanen *et al.*, 2015).

In addition to causing articular cartilage damage, MIA can increase inflammatory cells and induce chondral deformation, so the use of MIA in induction in mice is considered a suitable model for creating conditions that resemble the phenomena observed in human osteoarthritis (Kim *et al.*, 2016). Osteoarthritis can occur several weeks after intra-articular MIA injection (Moilanen *et al.*, 2015).

Table I showed the group that received MIA induction had higher IL-1 β levels compared to the healthy group. This indicates the success of MIA induction in causing an increase in IL-1 β levels resulting in a condition resembling osteoarthritis in humans. After the

osteoarthritis condition, on the 21st day, each rat in the “*Sondhep herbs group*” was given 28 mg/kg BW (dose I), 56 mg/kg BW (dose II), and 112 mg/kg BW (dose III), then for the group receiving positive given meloxicam (0.7 mg/kg BW) orally for 28 days. Meloxicam was chosen as a positive control because meloxicam affects inflammation by decreasing IL-1 β in rats (Khotib *et al.*, 2020), and meloxicam can reduce IL-6 and TNF (Li *et al.*, 2021).

On the 49th day, rat blood samples were taken to evaluate changes in levels of the pro-inflammatory cytokine IL-1 β . The results of the statistical test pair sample *t*-test showed a significant difference between IL-1 β levels before and after treatment (sig <0.05). The decrease in IL-1 β levels in the meloxicam group was because meloxicam, a class of NSAIDs, also reduced the IL-1 β levels in rats (Khotib *et al.*, 2020). Meanwhile, the *Sondhep* herb can reduce IL-1 β levels because all components in this herb contain compounds that have an activity to reduce IL-1 β levels. These compounds are six-gingerol in red ginger (*Zingiber officinale* var. *Rubrum*) rhizomes (Srikandi *et al.*, 2020), ethyl-*p*-methoxycinnamate in aromatic ginger (*Kaempferia galanga* Linn) rhizomes (Umar *et al.*, 2014), diallyl-disulfide (DADS) in garlic (*Allium sativum*) bulbs (Kiess *et al.*, 2003) and solo garlic (*Allium sativum*) (Harmita *et al.*, 2020).

Meanwhile, D-limonene in leeks (*Allium fistulosum*) can prevent the production of NO, TNF, IL-1 β , IL-6, and PGE₂, which are mediators in the inflammatory process (Yoon *et al.*, 2010). In the healthy group, there was a slight decrease in IL-1 β levels. This was thought to be due to differences in stress conditions in the rats. IL-1 β levels are not only affected by inflammation and pain but also by stress conditions in rats. The presence of a stressor in rats causes stress or anxiety so it can increase IL-1 β levels (Badowska-Szalewska *et al.*, 2009).

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References

- Anggraini, O.D., Komariah, C., & Prasetyo, A. (2018). Effects of arumanis mango peel extract on reducing carrageenin-induced leg edema in male white mice. *Journal of Health Library*, **6**(2), 267–271
<https://doi.org/10.19184/pk.v6i2.7666>
- Badowska-Szalewska, E., Klejbor, I., Sidor-Kaczmarek, J., Cecot, T., Lietzau, G., Spodnik, J.H., & Moryś J. (2009). Stress-induced changes of interleukin-1 β within the limbic system in the rat. *Folia Morphologica*, **68**(3), 119–128.
- Bahrudin, M. (2018). Patofisiologi Nyeri (Pain). *Saintika Medika*, **13**(1), 7 <https://doi.org/10.22219/sm.v13i1.5449>
- Bao, Z., Chen, M., Li, C., Shan, Q., Wang, Y., & Yang, W. (2022). Monosodium iodoacetate-induced subchondral bone microstructure and inflammatory changes in an animal model of osteoarthritis. *Open Life Science*. **17**(1), 781-793
<https://doi.org/10.1515/bio-2022-0079>
- Goldring, M.B., & Otero, M. (2014). Inflammation in osteoarthritis. *Curr Opin Rheumatol* **23**(5), 471–478
<https://doi.org/10.1097/BOR.0b013e328349c2b1>
- Hadiwinata, A.P. (2016). Pemanfaatan jamu *Sondhep* dan salekarang pada wanita pasca menopause keluarga Keraton Sumenep.
- Harmita, H., Suryadi, H., & Likasa, D.L. (2020). Validation and analysis of diallyl disulfide and diallyl trisulfide in garlic (*Allium sativum* L.) using gas chromatograph. *International Journal of Applied Pharmaceutics*, **12**(1), 1–4
<https://doi.org/10.22159/ijap.2020.v12s1.FF035>
- Heijink, A., Gomoll, H.A., Madry, H., Drobnic, M., Filardo G, Espregueira-Mendes J., & Van Dijk C.N. (2012). Biomechanical considerations in the pathogenesis of osteoarthritis of the knee. *Knee Surgery, Sports Traumatology, Arthroscopy*, **20**(3), 423–435
<https://doi.org/10.1007/s00167-011-1818-0>
- Jabłońska-trypuć, A., Matejczyk, M. & Rosochacki, S. (2016). Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs enzymes in collagen degradation, as a target for anticancer drugs. *Journal Enzyme Inhibitor Medicinal Chemistry*, **31**(1), 177-183
<https://doi.org/10.3109/14756366.2016.1161620>
- Khotib, J., Pratiwi, A.P., Ardianto, C. & Rahmadi, M. (2020). Attenuation of IL-124 β on the use of glucosamine as an adjuvant in meloxicam treatment in rat models with osteoarthritis. *Journal of Basic and Clinical Physiology and Pharmacology*, **30**(6) <https://doi.org/10.1515/jbcpp-2019-0332>
- Kiess, P.H., Dirsch, M.V., Hartung, T., Haffner, T., Trueman, L., Auger, J., Kahane, R. & Vollmar, A.M. (2003). Garlic (*Allium sativum* L.) Modulates Cytokine Expression in Lipopolysaccharide Activated Human Blood Thereby

Inhibiting NF-kappaB activity. *Journal of Nutritional*.

133(7):2171-2175 <https://doi.org/10.1093/jn/133.7.2171>

Kim, W.K., Chung, J.H., Pyee, Y., Choi, J.T., Park H.J., Hong J.Y., Shin J.S., Lee, J.H., Ha, I.H. & Lee, S.K. (2016). Effects of intra-articular SHINBARO treatment on monosodium iodoacetate-induced osteoarthritis in rats. *Chinese Medicine*, **11**(1), 1–10 <https://doi.org/10.1186/s13020-016-0089-6>

Li, J.Y., Wang, N.S., & Dong, M.Y. (2021). The effect of topical application of meloxicam on inflamed dental pulp. *Journal of Dental Sciences*, **16**(3), 915–921 <https://doi.org/10.1016/j.jds.2020.11.010>

Mangestuti, Subehan, Widyawaruyanti, A., Zaidi, S.F.H., Awale, S., & Kadota, S. (2007). Traditional medicine of Madura island in Indonesia. *Journal of Traditional Medicines*, **24**(3). <https://doi.org/10.11339/jtm.24.90>

Moilanen, L.J., Hamalainen, M., Nummenmaa, E., Ilmarinen, P., Vuolteenaho K., Nieminen R.M., Lehtimäki L., & Moilanen E. (2015). Monosodium iodoacetate induced inflammation and joint pain are reduced in TRPA1 deficient rat – Potential role of TRPA1 in osteoarthritis. *Osteoarthritis and Cartilage*, **23**(11), 2017–2026 <https://doi.org/10.1016/j.joca.2015.09.008>

Oliveira, C.N., Alfieri, M.F. Lima, A.R.S., & Portes, A. (2019). Lifestyle and pain in women with knee osteoarthritis. *American Journal of Lifestyle Medicine*, **13**(6), 606–610 <https://doi.org/10.1177/1559827617722112>

Orita, S., Ishikawa, T., Miyagi, M., Ochiai, N., Inoue, G., Eguchi, Y., Kamoda, H., Arai, G., Toyone, T., Aoki, Y., Kubo, T., Takahashi, K., & Ohtori, S. (2011). Pain-related sensory innervation in monoiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. *BMC Musculoskeletal Disorders*, **12**(1), 1-12 <https://doi.org/10.1186/1471-2474-12-134>

Ren, K. & Torres, R. (2011). Role of interleukin-1 β during pain and inflammation. *Brain Res Rev*, **60**(1), 57–64 <https://doi.org/10.1016/j.brainresrev.2008.12.020>

Sari, R.A., Luthfiana, F., Sholihah, I., Matsunami, K., Sukardiman, & Widyowati R. (2023). Antiosteoarthritis activities of 70% ethanol extract of *Eleutherine bulbosa* (mill.) urb. bulb on rats monosodium iodoacetate-induced osteoarthritis. *Journal of Public Health in Africa*, **14**(S1), 2506 <https://doi.org/10.4081/jphia.2023.2506>

Siddik, M., & Haryadi, R. D. (2020). The risk factors effect of knee osteoarthritis towards postural lateral sway. *Indian Journal of Forensic Medicine and Toxicology*, **14**(2), 1787–1792 <https://doi.org/10.37506/ijfvt.v14i2.3196>

Srikandi, S., Humaeroh, M., & Sutamirhadja, R.T.M. (2020). Kandungan gingerol dan shogaol dari ekstrak jahe merah (*Zingiber officinale* Roscoe) dengan metode maserasi bertingkat. *Jurnal ilmu kimia & terapan*, **7**(2), 75–81 <https://doi.org/10.15575/ak.v7i2.6545>

Umar, I.M., Asmawi, Z.A., Sadikun, A., & Majid, S.M.A. (2014). Ethyl-*p*-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin-1, tumor necrosis factor- α , and angiogenesis by blocking endothelial functions. *CLINICS (Sao Paulo)*, **69**(2), 134–144 [https://doi.org/10.6061/clinics/2014\(02\)10](https://doi.org/10.6061/clinics/2014(02)10)

Widyowati, R., Suciati, S., Hariyadi, D.M., Chang, H., Ipg Suryawan N., Tarigan N., Sholikhah I., Ardianto C., Nurhan A.D., & Sagitaras I.B. (2023). The pro-inflammatory cytokine IL-1 β alteration by deer (*Rusa unicolor*) antler extract on an osteoarthritis rat model. *Saudi Pharmaceutical Journal*. **31**(6), 1109–1114 <https://doi.org/10.1016/j.jsps.2023.03.022>

Yoon, W.J., Lee, H.N., & Hyun, G.C. (2010). Limonene suppresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2, and pro-inflammatory cytokines in RAW 264.7 macrophages. *Journal of Oleo Science*. **59**(8), 415–421 <https://doi.org/10.5650/jos.59.415>

Appendix

Data available at this link

https://drive.google.com/drive/folders/14QGf1SuMSWqhilqdxTFaD03CCnR031_f?usp=sharing