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Anti-inflammatory activity of combination of ethanol extracts of ginger (*Zingiber officinale*) and bangle (*Zingiber cassumunar*) in carrageenan-induced rats

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ABSTRACT

Background: Inflammation is an essential innate immune response aimed at antigen elimination and preventing their spread. Ginger (*Zingiber officinale*) and bangle (*Zingiber cassumunar*) rhizomes have been empirically utilized as medicinal components due to their anti-inflammatory potential. However, the combined efficacy of these rhizomes has not been previously explored.

Objective: This study aimed to assess the anti-inflammatory activity of a combination of ginger and bangle rhizome extracts.

Method: Ginger and bangle rhizomes were individually subjected to extraction through maceration with 96% ethanol, followed by purification with n-hexane. The anti-inflammatory activity was evaluated via motility tests on subjects administered orally with 1% CMC-Na (control), diclofenac sodium (4.5 mg/kg body weight), ginger ethanol extract (200 mg/kg body weight), bangle ethanol extract (400 mg/kg body weight), and a combination of both extracts (100:200 mg/kg body weight). Observations were made over 6 hours, with motility scores subsequently analyzed using ANOVA and the LSD test for statistical significance.

Results: Treatments involving ginger extract, bangle extract, and their combination significantly improved motility scores compared to the negative control. Furthermore, these treatments displayed no significant difference in effectiveness compared to the diclofenac sodium group ($p > 0.05$).

Conclusion: The combined ethanol extracts of ginger and bangle rhizomes demonstrate anti-inflammatory activity comparable to diclofenac sodium, as evidenced by motility score evaluations. This suggests their potential as alternative anti-inflammatory agents.

Keywords: ginger, bangle, anti-inflammatory, motility score, *Zingiber officinale*, *Zingiber cassumunar*

Introduction

Inflammation is an innate immune response aimed at eliminating antigens to prevent damage to body tissues and their spread to healthy areas. Stimuli triggering inflammation include pathogenic bacteria, cell damage, toxic compounds, or radiation [1]. Prolonged inflammation can exacerbate symptoms and lead to diseases such as chronic inflammation, rheumatoid arthritis, asthma, and cancer [2,3]. The inflammatory mechanism involves enzymes scuh as cyclooxygenase (COX) and lipoxygenase (LOX), as well as components of the innate immune system, including neutrophils, mast cells, histamine, and macrophages, triggering pathways such as NF-κB, MAPK, and JAK-STAT [1].

Traditionally, non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, indomethacine, and piroxicam have been used to combat inflammation 4,5]. However, long-term use is associated with adverse effects, including gastrointestinal damage, bleeding,

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nephrotoxicity, and hypersensitivity [6,7]. Selective COX-2 inhibitors, such as celecoxib, were developed to mitigate gastrointestinal issues but present risks of thrombosis in patients with cardiovascular histories [5].

Ginger (*Zingiber officinale*) and bangle (*Zingiber cassumunar*) rhizomes, used in Indonesian traditional medicine, exhibit anti-inflammatory properties [3,8-14]. The active components in ginger, 6-shogaol and 6-gingerol, inhibit pro-inflammatory mediators, potentially reducing inflammation through the NF-κB pathway and decreasing specific mRNA transcriptions (iNOS, COX-2, IL-6, and IL-1β) [15]. Studies have also highlighted the anti-arthritic effects of ginger's essential oils and gingerols, along with significant reductions in TNF-α, ferritin, and MDA levels, marking them as effective anti-inflammatory and antioxidant agents [16,17].

Similarly, bangle has demonstrated antioxidant and anti-inflammatory activities, notably inhibiting COX-2 [18], and has been traditionally used for various inflammatory conditions, such as musculoskeletal and menstrual pain, arthritis, rheumatisms, and sprains [19]. Research indicates that formulations containing bangle extract can significantly inhibit inflammation [10]. This study explores the combined anti-inflammatory effects of ginger and bangle rhizome extracts in male Wistar rats induced with carrageenan, building on evidence that suggests potential synergistic benefits from their combination.

Methods

Extraction process

Ginger and bangle rhizomes were sourced from Lembang, Bandung, West Java, Indonesia. Five hundred grams each of dried, powdered ginger and bangle rhizomes underwent maceration with 2 liters of 96% ethanol for 72 hours, with solvent renewal and agitation occurring every 24 hours. The macerated mixture was then concentrated using a rotary evaporator at 70°C and 60 rpm for 12 hours. Post-evaporation, n-hexane was used for washing, followed by further evaporation to yield a thick extract. The yield was subsequently quantified.

Animals and ethical clearance

Male Wistar rats (*Rattus norvegicus*), aged 2-3 months and weighing 200-300 grams, were acclimatized for a week at the Laboratory of Pharmacology, Department of Pharmacy, Universitas Jenderal Soedirman. Ethical considerations, including humane treatment, proper nutrition, and regular cage cleaning, were strictly followed. The Health Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Soedirman, granted ethical clearance (130/EC/KEPK/VII/2020).

Anti-inflammatory activity assay

This study divided 35 male Wistar rats randomly into five groups, with seven rats per group, employing a posttest-only control group design. The dosage determination followed the Federer formula [20]. The treatments administered orally were as follows: 1% CMC-Na for the negative control, 4.5 mg/kg body weight (BW) of diclofenac sodium, 200 mg/kg BW of ginger extract, 400 mg/kg BW of bangle extract, and a combination of 100 mg/kg BW ginger and 200 mg/kg BW bangle extract.

The study assessed anti-inflammatory activity using a motility test, which observed the rats' behavior for 10 seconds at 30-minute intervals over six hours. The assessment criteria were as follows [21]:

- Score 0 : Difficulty in moving 10 cm without touching the inflamed paw to the floor.
- Score 1 : Walking 20 cm with difficulty, with feet touching the floor.
- Score 2 : Easily walking more than 20 cm.

Data analysis

The motility scores were analyzed using One-way ANOVA at a 95% confidence level, followed by the LSD test to identify significant differences between groups. A p-value of ⩽0.05 was considered statistically significant.

Results

The extraction yields for ginger and bangle were found to be 9.14% and 5%, respectively. These values are considered appropriate, as they exceed the minimum yield of 4.3% previously reported for ginger [22], and match the 5% yield observed in prior research for bangle [23].

The motility score is a metric to evaluate the rats' locomotor ability, with higher scores indicating improved walking capability [21]. This assessment is crucial for determining the efficacy of the administered treatments in mitigating inflammation-induced

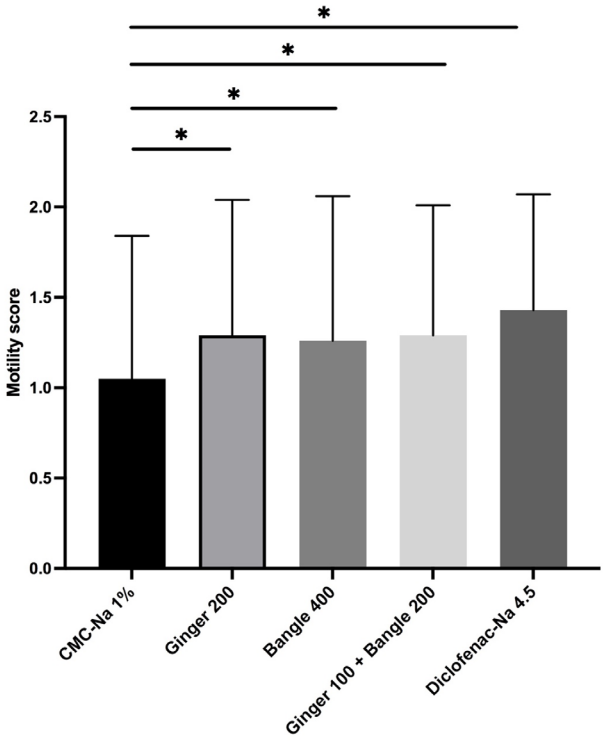

Figure 1. Rats ability to walk after being given treatment. The average walking ability of rats over a 6-hour observation period following carrageenan induction and subsequent treatment with ginger ethanol extract (200 mg/kg BW), bangle ethanol extract (400 mg/kg BW), a combination of ginger and bangle ethanol extracts (100:200 mg/kg BW), and diclofenac sodium (4.5 mg/kg BW).* $p \leq 0.05$

locomotor impairment. Notably, treatments resulting in significantly increased walking ability ($p \leqslant 0.05$) underscore their potential anti-inflammatory effects [Figure 1].

Discussion

The motility score, indicative of a rat's walking ability, is a critical measure in this study, where higher values denote better locomotor function [21]. Based on Figure 1, significant differences in motility scores between negative control and treatment groups suggest that the treatments effectively mitigate inflammation-induced locomotor impairment.

Ginger rhizomes, rich in gingerol and shogaol, play a pivotal role in suppressing cyclooxygenase activity and, consequently, the formation of pro-inflammatory mediators. Conversely, bangle extract, containing the cartilage-protective agent component D ((E)-4-(3', 4'-methoxyphenyl)but-3-en-1-ol), acts against degradation triggered by matrix metalloproteinases (MMP), which are regulated by pro-inflammatory cytokines [24,25]. This differential mechanism of action underscores the potential synergistic effect of combining ginger and bangle extracts, offering a promising alternative anti-inflammatory therapy.

However, this study's scope was confined to a single dose ratio of 100:200 mg/kg body weight, limiting insights into the optimum dosing for maximum anti-inflammatory efficacy. Future studies should explore various dose ratios to delineate the full therapeutic potential of these extracts in inflammation management.

Conclusion

A combination of ginger and bangle ethanol extracts, in a dose ratio of 100:200 mg/kg body weight, significantly reduces inflammation in carrageenan-induced rats. These findings suggest that the combined extracts may be an effective anti-inflammatory therapy alternative.

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Conflict of interest

The author declares no conflict of interest.

Author contributions

HE and EP designed this study; BH, IYN assisted with data collection. HE and BH wrote the initial script, supervising and statistical analysis. IYN contributed in reviewing and proofreading of the manuscript. HE, EP, and IYN contributed to the data interpretation and final approval of the manuscript.

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References

1. Chen L, Deng H, Cui H, Fang J, Zuo Z. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18. https://doi.org/10.18632/oncotarget.23208
2. Kandy AP. Uji Aktivitas Antiinflamasi Kombinasi Ekstrak Etanol Jahe Merah (Zingiber officinale var. rubrum) dan Daun

Sidaguri (Sida rhombifolia L.) Terhadap Jumlah Neutrofil tikus yang Diinduksi Karagenan. 2016.

3. Shallangwa GA, Musa H, Nyaga GT. In-Vitro Evaluation of Ethanolic Extracts of Zingiber Officinale , Sygzium Aromaticum and their 1 : 1 Extracts Blend on Protein Denaturation during Acute. 2015;1(1):1-8.
4. Walker C. Are All Oral COX-2 Selective Inhibitors the Same ? A Consideration of Celecoxib , Etoricoxib , and Diclofenac. International Journal of Rheumatology. 2018;2018(1):1-12. https://doi.org/10.1155/2018/1302835
5. Jin J. Nonsteroidal Anti-inflammatory Drugs. JAMA Patient Page. 2015;314(10):1084. https://doi.org/10.1001/jama.2015.9936
6. Royado GG, Navarro M, Lanas A. NSAID induced gastrointestinal damage and designing GI-sparing NSAIDs. Expert Review of Clinical Pharmacology. 2018;0(0):1-50.
7. Blumenthal KG, Lai KH, Huang M, Wallace ZS. Adverse and Hypersensitivity Reactions to Prescription Nonsteroidal Anti-Inflammatory Agents in a Large Health Care System. J Allergy Clin Immunol Pract. 2017;1(1):1-10. https://doi.org/10.1016/j.jaip.2016.12.006
8. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). Foods. 2019;8(6):185. https://doi.org/10.3390/foods8060185
9. Handrianto P. UJI Antibakteri Ekstrak Jahe Merah Zingiber officinale var . Rubrum Terhadap Staphylococcus aureus Dan Escherichia coli. Journal of Research and Technologies. 2016;2(1):1-4. https://doi.org/10.55732/jrt.v2i1.259
10. Wulansari ED, Wahyuono S, Widyarini S. Aktivitas Antiinflamasi Topikal Ekstrak Etanolik Rimpang Bangle (Zingiber cassumunar Roxb .) pada Mencit yang Diinduksi Karagenin. Trad Med J. 2018;23(2):122-6. https://doi.org/10.22146/mot.34640
11. Aryaeian N, Tavakkoli H. Ginger and its Effects on Inflammatory Diseases. Adv Food Technol Nutr Sci Open J. 2015;1(4):97-101. https://doi.org/10.17140/AFTNSOJ-1-117
12. Tang C, Dong X, He W, Cheng S, Chen Y, Huang Y, et al. Cerebral mechanism of celecoxib for treating knee pain : study protocol for a randomized controlled parallel trial. 2019;20(58):1-8. https://doi.org/10.1186/s13063-018-3111-8
13. Tangyuenyonhwatana P V Keeratinijakal, W Gritsanapan. Thin-Layer Chromatography-Densitometry Analysis of Dimethoxyphenylbutadiene Content in. Journal of AOAC International. 2012;95(6):1614-8. https://doi.org/10.5740/jaoacint.11-188
14. Bischoff-Kont I, Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. Pharmaceuticals. 2021 Jun 15;14(6):571. https://doi.org/10.3390/ph14060571
15. Liang N, Sang Y, Liu W, Yu W, Wang X. Anti-Inflammatory Effects of Gingerol on Lipopolysaccharide-Stimulated RAW 264.7 Cells by Inhibiting NF-κB Signaling Pathway. Inflammation. 2018 Jun;41(3):835-45. https://doi.org/10.1007/s10753-018-0737-3
16. Funk JL, Frye JB, Oyarzo JN, Chen J, Zhang H, Timmermann BN. Anti-inflammatory effects of the essential oils of ginger (Zingiber officinale Roscoe) in experimental rheumatoid arthritis. PharmaNutrition. 2016 Jul;4(3):123-31. https://doi.org/10.1016/j.phanu.2016.02.004
17. Kulkarni RA, Deshpande AR. Anti-inflammatory and antioxidant effect of ginger in tuberculosis. Journal of Complementary and Integrative Medicine. 2016 Jun 1;13(2):201-6. https://doi.org/10.1515/jcim-2015-0032
18. Leelarungrayub J, Manorsoi J, Manorsoi A. Anti-inflammatory activity of niosomes entrapped with Plai oil (Zingiber cassumunar Roxb.) by therapeutic ultrasound in a rat model. IJN. 2017 Mar;Volume 12:2469-76. https://doi.org/10.2147/IJN.S129131
19. Chongmelaxme B, Sruamsiri R, Dilokthornsakul P, Dhippayom T, Kongkaew C, Saokaew S, et al. Clinical effects of Zingiber cassumunar (Plai): A systematic review. Complementary Therapies in Medicine. 2017 Dec; 35:70-7. https://doi.org/10.1016/j.ctim.2017.09.009
20. Randomization And Sample Size in Experimentation. In Washington D.C: Cornell University; 1966.
21. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-Inflammatory Activity of Lactobacillus on Carrageenan-Induced Paw Edema in Male Wistar Rats. International Journal of Inflammatio. 2012;2012(1):1-7. https://doi.org/10.1155/2012/752015
22. Indonesia DKR. Sumber ebook. In: Farmakope Herbal Indonesia. 2011.
23. Soleha M, Purba AV. Uji Daya Antelmetika Ekstrak n-Heksan Rimpang Bangle (Zingiber cassumunar) pada Cacing Ascaris suum secara In Vitro. Jurnal Kefarmasian Indonesia. 2013;3(1):19-25.
24. Id MS rad, Maria E, Id V, Salehi B, Sharifi-rad J. Plants of the Genus Zingiber as a Source of Bioactive Phytochemicals : From Tradition to Pharmacy. Molecules. 2017;22(2145):1-20. https://doi.org/10.3390/molecules22122145
25. Ikawati Z. Farmakologi Molekuler. In: Gadjah Mada University Press. 2019. p. 141.