Original Article

The Potential of Cogon Grass (*Imperata cylindrica*) Ethanol Extract in Inhibiting Nitric Oxide Secretion in Fibroblast

Sherlyn Mangkulion, Moehamad Orliando Roeslan, Paopanga Monthanpisut[1]

Department of Oral Biology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia, [1]Oral Biology Laboratory, Faculty of Dentistry, Thammasat University, Pathumthani, Thailand

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ABSTRACT

Background: *Imperata cylindrica* or cogon grass is a kind of sharp-leaved grass that often becomes a weed on agricultural land. In traditional medicine, *I. cylindrica* is often used as a fever-lowering and anti-inflammatory drug. Research has shown that *I. cylindrica* can provide anti-inflammatory effects. Nitric oxide (NO) is a mediator produced in various mammalian cells and can be used as an inflammatory marker. **Objective:** To evaluate whether the ethanol extract of *I. cylindrica* can affect the ability of fibroblasts to secrete NO. **Methods:** Fibroblasts were stimulated using *Escherichia coli* lipopolysaccharide and treated with the ethanol extract of *I. cylindrica* at concentrations of 50, 80, 160, 320, and 640 part per million, and then the Griess test was performed. **Results:** There was a significant difference between the ethanol extract of *I. cylindrica* (640 part per million) and the negative control group. **Conclusion:** The ethanol extract of *I. cylindrica* (L.) Raeusch at a concentration of 640 part per million has the effect of lowering NO secretion in fibroblasts.

Keywords: *Cogon grass (Imperata cylindrica), fibroblast, nitric oxide*

Background

Inflammation is a natural reaction of the body to fight against diseases and various attacks from microorganisms.[1] Inflammation can be caused by immune disorders, cancer, infections, exposure to chemicals, and viral, fungal, or bacterial infections.[2] Once the inflammation has occurred, the wound-healing process can begin. Healing is a complex process, and separate parts of a wound may be at different stages of healing at any one time. There are four main phases in wound healing: the coagulation and hemostasis phase, the inflammatory phase, the proliferative phase, and remodeling.[3]

Nitric oxide (NO) is a free radical molecule produced naturally by the body.[4] Under normal physiological conditions, NO provides an anti-inflammatory effect. However, when NO production occurs excessively, it becomes a pro-inflammatory mediator.[5]

Research has shown that the amount of NO can have an effect on the wound-healing process. When tissue is injured and undergoes an inflammatory process, fibroblasts secrete NO spontaneously. The amount of NO produced is influenced by *interferon gamma* and lipopolysaccharide (LPS).[6]

LPSs are large molecules in the form of a complex between lipid compounds and polysaccharides with covalent bonds. LPSs are an integral structural component of the outer membrane of gram-negative bacteria.[7,8] In general, the structure of LPS consists of lipid A, the core oligosaccharide, and the O antigen.[9]

Lipid A, the hydrophobic portion of LPS, is an acylated β-1′-6-linked glucosamine disaccharide that forms the outer leaflet of the outer membrane.[10] The core

Address for correspondence: Dr. Moehamad Orliando Roeslan, Department of Oral Biology, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia.
E-mail: orliando.roeslan@trisakti.ac.id

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oligosaccharide is nonrepeating and is linked to the glucosamines of lipid A.[11] The O antigen is an extended polysaccharide attached to the core oligosaccharide, and it is composed of a repeating oligosaccharide composed of two to eight sugars.[12,13]

Fibroblasts are cells that synthesize and integrate structural proteins, such as collagen and elastin, into the extracellular matrix of most mesenchymal tissue.[14] When fibroblasts are injured, they undergo inflammatory processes, decreased proliferation activity, and increased contractile matrix and collagen synthesis.[6]

Imperata cylindrica is a plant that has often been used as a traditional medicine in several countries as an anti-inflammatory drug. It has been proven in previous studies that some compounds in *I. cylindrica* (such as flavonoids, alkaloids, and impecyloside) have anti-inflammatory effects.[15,16] These potential of these compounds as anti-inflammatory agents emphasizes the importance of this study, which could be the first step in further research on anti-inflammatory effects of *I. cylindrica*. Moreover, this plant also has never been studied regarding NO secretion in fibroblasts. Taking into account the above-mentioned information, ethanol extracts of *I. cylindrica* may have the potential to inhibit NO secretion in fibroblasts.

Materials and Methods

The study was an *in vitro* laboratory experimental study to investigate the effects of the ethanol extract of *I. cylindrica* leaves on NO inhibition in fibroblast cells. The research had a posttest-only control group design. Lemeshow's formula was used to calculate the sample size.[17] The study was conducted at the Biocore Laboratory, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia.

The plant, *I. cylindrica*, was extracted using the Soxhlet method, with ethanol as the solvent. The extracted solution was then filtered and tested. A phytochemical test was conducted at Biocore Laboratory to identify the active compounds contained in the ethanol extract of *I. cylindrica* leaves. In this research, we tested for the flavonoid, alkaloid, tannin, steroid, and terpenoid compounds.

The fibroblasts were isolated from skin and obtained from Faculty of Medicine, YARSI University Laboratory, Jakarta, Indonesia. The fibroblasts were cultured in Dulbecco's Modified Eagle Medium (Gibco, New York, USA), supplemented with 10% fetal bovine serum (Gibco), 1% amphotericin, and 1% penicillin/streptomycin (Invitrogen, Massachusetts, USA) in 5% CO_2 incubators. The substrate was replaced regularly, and fibroblasts are subcultured every three to four days or when the cells reached 80%–90% confluency in the flask.

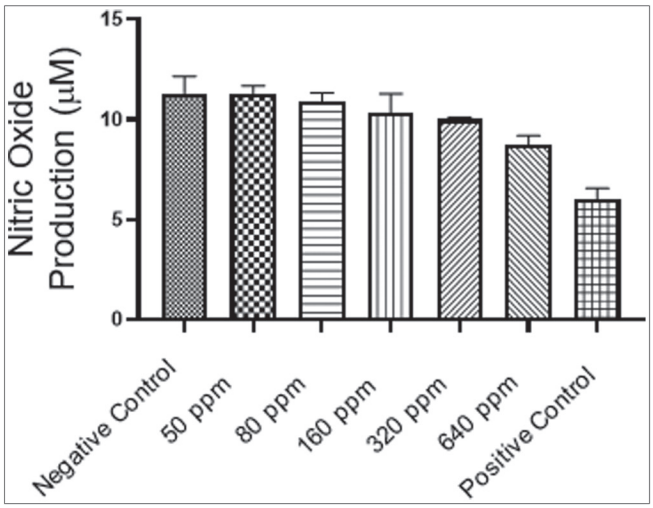

Figure 1: Effects of ethanol extracts of *Imperata cylindrica* at various concentrations on nitric oxide inhibitions by liposaccharide-stimulated fibroblasts. The data are expressed as mean ± SD (n = 3). * Indicates a significant difference

Fibroblasts (5×10^5 in 24-well plates) were incubated at 37°C and 5% CO_2. Then the medium was aspirated and replaced with various concentrations of the ethanol extract of *I. cylindrica*, and Genistein 1000 μM (Sigma-Aldrich) was used as positive controls. After four hours, the cell was stimulated using 2 μg/mL of *Escherichia coli* LPS (Sigma-Aldrich) for 24 h. The negative control was LPS-stimulated fibroblasts without extract treatment. NO concentration was measured using the NO detection kit (Griess Reagent System, Promega). A 96-well plate containing 50 μL supernatant and Griess reagent was incubated at room temperature for 10 min.[18] The absorbance was measured at a wavelength of 540 nm in a microplate reader. The nitrite concentration was determined by the sodium nitrite standard curve.

Data were reported as mean values ± standard deviation. The Shapiro–Wilk test was used to assess normality. Data distribution was considered normal at $P < 0.05$. Significant differences were determined using a one-way analysis of variance test and the post-hoc Tukey test. Results were considered significant at $P < 0.005$.

Results

In accordance with the nomenclature determination test conducted by the National Research and Innovation Agency in Indonesia, the plant used in this study was *I. cylindrica*. From the results of the phytochemical tests conducted in the Biocore Laboratory, it is known that the ethanol extract of *I. cylindrica* contains active

Table 1: Results of the phytochemical tests

No.	Substance	Methods	Results
1	Flavonoid	5 mL NH_3 + 1 mL H_2SO_4	+
2	Alkaloid	Dragendorff's reagent	+
3	Tannin	1 mL FeCl 0.1%	+
4	Steroid	3 drops of CH_3COOH + 1 mL H_2SO_4	+
5	Terpenoid	2 mL $CHCl_3$ + 3 mL H_2SO_4	+

substances in the form of flavonoids, alkaloids, tannins/phenols, steroids, and terpenoids. The results of the phytochemical tests are shown in Table 1.

The result shows that the ethanol extract of *I. cylindrica* at a concentration of 640 ppm and the positive control group had significant differences when compared to the negative control group. For other concentrations (50, 80, 160, and 320 ppm), there were no significant differences when compared to the negative control group [Figure 1].

Discussion

A previous study proved that flavonoid, alkaloid, tannin, phenol, steroid, triterpenoid, and terpenoid compounds exhibit anti-inflammatory effects.[19] The mechanism of how steroids act as anti-inflammatory agents is by inhibiting the release of prostaglandins from the source cells.[20] Some of the mechanisms of action of flavonoid compounds acting as anti-inflammatory agents are inhibiting the release of serotonin and histamine, the action of cyclooxygenase and lipoxygenase, leukocyte accumulation, and arachidonic acid, as well as secreting lysosome enzymes.[21] Tannins' mechanism of action as anti-inflammatory agents is by inhibiting the production of oxidants by neutrophils, monocytes, and macrophages.[22]

Previous research has shown that NO can mediate muscle relaxation and neurotransmission, as well as modulate inflammation in several organ systems and pathophysiological conditions.[4] When inflammation occurs, pro-inflammatory cytokines increase the induction of nitric oxide synthase in macrophages and granulocyte neutrophils.

E. coli LPS has been proven to be able to produce a powerful inflammatory response.[23] *E. coli* LPS can generate an inflammatory response by activating toll-like receptor 4, which results in the activation of nuclear factor κB and NO production.[18]

Several factors may account for the differences in the effect of the concentrations of extracts used in previous studies, such as the difference in the solvent used[24] and biological variations of *I. cylindrica*. A few examples of the cause of such variations are environmental factors such as temperature, humidity, wind, sunlight, and water.[25]

Genistein is an isoflavone compound from the flavonoid family. Genistein has been widely used in the health sector as a medicine to prevent osteoporosis and heart disease, to reduce postmenopausal symptoms, and as an anticancer agent. There has been extensive *in vitro* and *in vivo* research to determine the potential of genistein as an anti-inflammatory drug, and the results indicate that genistein can produce anti-inflammatory effects by inhibiting various signaling pathways.[26]

The discrepancy between the concentrations used in this research and previous research was caused by the cells used. In this research, the cells used as the reference are macrophages. On the basis of previous studies, it has been proven that macrophages are more sensitive to inflammation.[27] A limitation of this study is the possibility that bias could have occurred in the data collection. To avoid this, future studies can utilize "blind" protocols when performing experiments and recording data.[28]

Conclusion

This study was conducted to investigate the effects of ethanol extract of *I. cylindrica* leaves towards fibroblast cells. Previous study has shown that *I. cylindrica* contains some compounds with anti-inflammatory effects. However, extensive research regarding the effects of ethanol extract of *I. cylindrica* leaves toward NO secretion in fibroblasts has never been conducted. Considering the results of this study, it can be concluded that ethanol extract *I. cylindrica* (L.) Raeusch at a concentration of 640 ppm has the effect of lowering NO secretion in fibroblasts. Further research on the effectiveness of the ethanol extract of *I. cylindrica* on the anti-inflammatory effects of fibroblasts, using a variety of test methods, concentrations, extraction methods, and solvents, need to be conducted for utilization and development by the community and other dentists.

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Conflicts of interest

There are no conflicts of interest.

References

1. Wang Z, Qi F, Luo H, Xu G, Wang D. Inflammatory microenvironment of skin wounds. Front Immunol 2022;13:789274.
2. Prasad S, Tyagi AK, Aggarwal BB. Detection of inflammatory biomarkers in saliva and urine: Potential in diagnosis, prevention, and treatment for chronic diseases. Exp Biol Med 2016;241:783-99.

3. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing. J Chin Med Assoc 2018;81:94-101.
4. Santolini J, André F, Jeandroz S, Wendehenne D. Nitric oxide synthase in plants: Where do we stand? Nitric Oxide 2017;63:30-8.
5. Forstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. Eur Heart J 2012;33:829-37, 837a.
6. Kwesiga M P, Cook E, Hannon J, Wayward S, Gwaltney C, Rao S, *et al*. Investigative study on nitric oxide production in human dermal fibroblast cells under normal and high glucose conditions. Med Sci 2018;6:99.
7. Li H, Liao T, Debowski AW, Tang H, Nilsson H, Stubbs KA, *et al*. Lipopolysaccharide structure and biosynthesis in *Helicobacter pylori*. Helicobacter 2016;21:445-61.
8. Whitfield C, Trent MS. Biosynthesis and export of bacterial lipopolysaccharides. Annu Rev Biochem 2014;83: 99-128.
9. Liu B, Furevi A, Perepelov AV, Guo X, Cao H, Wang Q, *et al*. Structure and genetics of *Escherichia coli* O antigens. FEMS Microbiol Rev 2020;44:655-83.
10. Bertani B, Ruiz N. Function and biogenesis of lipopolysaccharides. EcoSal Plus. 2018;8:10.1128/ecosalplus.
11. Merino S, Gonzalez V, Tomás JM. The first sugar of the repeat units is essential for the Wzy polymerase activity and elongation of the O-antigen lipopolysaccharide. Future Microbiol 2016;11:903-18.
12. Wang L, Wang Q, Reeves PR. The variation of O antigens in gram-negative bacteria. In: Wang X, Quinn PJ, editors. Endotoxins: Structure, Function and Recognition. Dordrecht: Springer Netherlands; 2010. p. 123–52.
13. Kalynych S, Morona R, Cygler M. Progress in understanding the assembly process of bacterial O-antigen. FEMS Microbiol Rev 2014;38:1048-65.
14. Lynch MD, Watt FM. Fibroblast heterogeneity: Implications for human disease. J Clin Invest 2018;128:26-35.
15. Jung YK, Shin D. Imperata cylindrica: A review of phytochemistry, pharmacology, and industrial applications. Molecules 2021;26:1454.
16. Kwok AHY, Wang Y, Ho WS. Cytotoxic and pro-oxidative effects of *Imperata cylindrica* aerial part ethyl acetate extract in colorectal cancer in vitro. Phytomedicine 2016;23: 558-65.
17. Lwanga SK, Lemeshow S. Sample Size Determination in Health Studies: A Practical Manual. Geneva: World Health Organization; 1991. p. 80.
18. Roeslan MO, Ayudhya TDN, Yingyongnarongkul B, Koontongkaew S. Anti-biofilm, nitric oxide inhibition and wound healing potential of purpurin-18 phytyl ester isolated from *Clinacanthus nutans* leaves. Biomed Pharmacother 2019;113:108724.
19. An HJ, Nugroho A, Song BM, Park HJ. Isoeugenin, a novel nitric oxide synthase inhibitor isolated from the rhizomes of *Imperata cylindrica*. Molecules 2015;20:21336-45.
20. Tia Santika Dewi AA, Suarya P, Puspawati NM. Aktivitas Antiinflamasi Ekstrak Eter Kulit Batang Tenggulun (*Protium javanicum* Burm) Terhadap Edema pada Tikus Wistar yang Diinduksi dengan Karagenan. Jurnal Kimia 2015;9:13-19.
21. Audina M, Khaerati K. Efektivitas Antiinflamasi Ekstrak Ethanol Daun Sumambu (*Hyptis capitata* Jacq.) Pada Tikus Putih Jantan (*Rattus norvegicus* L.) yang Diinduksi dengann Karagenan. Audina 2018;12:17-23.
22. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9:7204-18.
23. Zhang W, Xie X, Wang J, Song N, Lv T, Wu D, *et al*. Increased inflammation with crude *E. coli* LPS protects against acute leptospirosis in hamsters. Emerg Microbes Infect 2020;9:140-7.
24. Sanaei-Zadeh H. The case files: Methanol or ethanol poisoning? Correct diagnosis influences treatment. Emerg Med News 2012;34:1.
25. Driesen E, Van den Ende W, De Proft M, Saeys W. Influence of environmental factors light, CO_2, temperature, and relative humidity on stomatal opening and development: A review. Agronomy 2020;10:19751975.
26. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: A review on its anti-inflammatory properties. Front Pharmacol 2022;13:820969.
27. Jang D, Lee MJ, Kim KS, Kim CE, Jung JH, Cho M, *et al*. Network pharmacological analysis on the herbal combinations for mitigating inflammation in respiratory tracts and experimental evaluation. Healthcare 2023;11:143.
28. Holman L, Head ML, Lanfear R, Jennions MD. Evidence of experimental bias in the life sciences: Why we need blind data recording. PLoS Biol 2015;13:e1002190.