

Digital Repository Universitas Jember

Brawijaya

Jurnal Kedokteran

M e d i c a l J o u r n a l o f B r a w i j a y a

JKB Vol. 32 : Number 3, February 2023



Editorial Team

Editor in Chief

Viera Wardhani, <https://scholar.google.co.id/citations?user=TkieHskAAAAJ&hl=id&oi=ao>, Faculty of Medicine Universitas Brawijaya, Indonesia

Editorial Board

Asti Melani Astari, <https://scholar.google.com/citations?user=ajXwW8EAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Dewi Kartikawatiningsih, <https://scholar.google.co.id/citations?user=dK8uLgUAAAAJ&hl=en>, SintaID: 5994340, Faculty of Medicine Universitas Brawijaya. School of Social Welfare School of Public Health Graduate Certificate Program in Health Disparities, State University of New York, Albany-New York, United States

Desak Ketut Indrasari Utami, <https://scholar.google.co.id/citations?user=VfoGfpEAAAAJ&hl=id>, Neurology Departemen Medical Faculty of Udayana University, Indonesia

Dian Handayani, (ScopusID: 56035896000), Jurusan Gizi - Faculty of Medicine Universitas Brawijaya, Indonesia

Dian Nugrahenny, (ScopusID: 55907116600), Faculty of Medicine Universitas Brawijaya, Indonesia

Diana Lyrawati, (ScopusID: 6503907038), Faculty of Medicine Universitas Brawijaya, Indonesia

Holipah Holipah, <http://orcid.org/0000-0002-6143-8239>, Faculty of Medicine Universitas Brawijaya. Departemen of Public Health, University of Miyazaki

Husnul Khotimah, <https://scholar.google.co.id/citations?user=2yxMSREAAAAJ&hl=id&oi=ao>, Faculty of Medicine Universitas Brawijaya, Indonesia

Ira Kusumawaty, <https://scholar.google.co.id/citations?user=HEPuAsUAAAAJ&hl=id>, Poltekkes Kemenkes Palembang, Indonesia

Loeki Enggar Fitri, (ScopusID: 36080235500), Faculty of Medicine Universitas Brawijaya, Indonesia

Syahrul Chilmi, https://scholar.google.com/citations?user=y_jfIk0AAAAJ&hl=en, Faculty of Medicine Universitas Brawijaya, Indonesia

Tri Siswati, <https://scholar.google.co.id/citations?user=1zc3ED0AAAAJ&hl=id>, Poltekkes Kemenkes Yogyakarta, Indonesia

Language Editor

Reza Nugraha, Founder of inFocus English, Malang, East Java, Indonesia

Peer Reviewers

Ahmad Dian Wahyudiono, <https://scholar.google.co.id/citations?user=D9Jw9BQAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Ahsan Ahsan, <https://scholar.google.co.id/citations?user=DDQYBaQAAAAJ&hl=id&oi=ao>, Faculty of Medicine Universitas Brawijaya, Indonesia

Agustina Tri Endharti, (ScopusID: 6505560171), Faculty of Medicine Universitas Brawijaya, Indonesia

Aryo Dewanto, Post Graduate Program in Hospital Management, Faculty of Medicine Universitas Brawijaya, Indonesia

Basuki Bambang Purnomo, https://scholar.google.com/scholar?hl=id&as_sdt=0%2C5&q=basuki+purnomo&btnG=, Saiful Anwar Hospital, Indonesia

Besut Daryanto, <https://orcid.org/0000-0002-0776-1633>, Saiful Anwar Hospital, Indonesia

Cholid Tri Tjahjono, (ScopusID: 57192893732), Faculty of Medicine Universitas Brawijaya, Indonesia

Djoko Wahono S, <https://scholar.google.co.id/citations?user=VnIz2A4AAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Dearisa Surya Yudhantara, https://scholar.google.com/citations?user=N_IVxOAAAAJ&hl=id&authuser=1&oi=ao, Faculty of Medicine Universitas Brawijaya, Indonesia

Dewi Santosaningsih, <https://scholar.google.com/citations?user=3umANTUAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Devita Rahmani Ratri, <https://scholar.google.com/citations?user=PpWVjcoAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Edi Widjajanto, (ScopusID: 56006172100), Faculty of Medicine Universitas Brawijaya, Indonesia

Efta Triastuti, <https://scholar.google.co.id/citations?user=nv0bjBgAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Ema Pristi Yunita, <https://scholar.google.co.id/citations?user=ClfT1GMAAAAAJ&hl=id&oi=ao>, Faculty of Medicine Universitas Brawijaya, Indonesia

Eviana Norahmawati, <https://scholar.google.co.id/citations?user=MEcuBTYAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Fajar Ari Nugroho, <https://scholar.google.co.id/citations?user=L7oACfIAAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Friyia Rachma Putri, <https://scholar.google.co.id/citations?user=J7DoBtIAAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Harun Al Rasyid, <https://scholar.google.co.id/citations?user=EnP9WYAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Nikma Fitriasaki, <https://scholar.google.com/citations?user=HQBWIoAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Hidayat Suyuti, (ScopusID: 56231098000), Faculty of Medicine Universitas Brawijaya, Indonesia

Hikmawan Wahyu Sulistomo, <https://scholar.google.com/citations?user=uGoNLIIAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Inggita Kusumastuty, <https://scholar.google.co.id/citations?user=wEerbaUAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

I Wayan Arsana Wiyasa, https://scholar.google.co.id/citations?user=r_t92NgAAAAJ&hl=en, Faculty of Medicine Universitas Brawijaya

Krisni Subandijah, <https://scholar.google.co.id/citations?user=yQ-DdtwAAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Kurnia Widyaningrum, <https://scholar.google.co.id/citations?user=wHDeIAgAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Lilik Zuhriyah, <https://scholar.google.com/citations?user=ZJNU2DUAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Lukman Hakim, Faculty of Medicine Universitas Brawijaya, Indonesia

Muhammad Saifurrohman, (ScopusID: 8325126900), Faculty of Medicine Universitas Brawijaya, Indonesia

Muhammad Sasmito Djati, (ScopusID: 56403414400), Fakultas MIPA Universitas Brawijaya, Indonesia

Nadia Artha Dewi, <https://scholar.google.com/citations?user=CcARsGgAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Novi Arfarita, https://scholar.google.co.id/citations?user=_ZCTEacAAAAJ&hl=id, Universitas Islam Malang & IRC-MEDMIND, Universitas Brawijaya, Indonesia

Novi Khila, <https://www.scopus.com/authid/detail.uri?authorId=57188973746>, <https://scholar.google.co.id/citations?user=7FwnZqkAAAAJ&hl=id>, Faculty of Dentistry Universitas Brawijaya, Indonesia

Nur Samsu, <https://scholar.google.co.id/citations?user=mIbOS3MAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Putu Eka Sudaryatma, https://www.researchgate.net/profile/Putu_Sudaryatma, Balai Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan Denpasar Bali, Veterinary Microbiology, Miyazaki University Japan, Indonesia

Retty Ratnawati, <https://scholar.google.com/citations?user=ZoVj1nEAAAAJ&hl=en>, Department of Physiology Faculty of Medicine Universitas Brawijaya, Indonesia

Respati Suryanto Dradjat, <https://scholar.google.co.id/citations?user=P-Cvbh0AAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Sanarto Santoso, (ScopusID: 56118471300), Faculty of Medicine Universitas Brawijaya, Indonesia

Susanthy Djajalaksana, <https://scholar.google.co.id/citations?user=jm3t94AAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Saptadi Yulianto, <https://scholar.google.co.id/citations?user=X6t23HIAAAJ&hl=id>, Faculty of Medicine Universitas Brawijaya, Indonesia

Septi Dewi Rachmawati, <https://scholar.google.co.id/citations?user=PrKyPTAAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya

Seskoati Prayitnaningsih, <https://scholar.google.co.id/citations?user=mMASaz8AAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Setyawati Soeharto Karyono, (ScopusID: 56367333100), Faculty of Medicine Universitas Brawijaya, Indonesia

Shahdevi Nandar Kurniawan, <https://scholar.google.ca/citations?user=CwTE1i4AAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Sri Sunarti, (ScopusID: 56483311200), Faculty of Medicine Universitas Brawijaya, Indonesia

Sutiman Bambang Sumitro, (ScopusID: 55218596400), Fakultas MIPA Universitas Brawijaya, Indonesia

Taufiq Nur Budaya, <https://scholar.google.co.id/citations?user=iVUt3ZAAAAAJ&hl=en>, Faculty of Medicine Universitas Brawijaya, Indonesia

Teguh Wahyu Sardjono, (ScopusID: 57192300853), Faculty of Medicine Universitas Brawijaya, Indonesia

Tita Hariyanti, <https://scholar.google.co.id/citations?user=oDMAJTsAAAAJ&hl=id&oi=ao>, Faculty of Medicine Universitas Brawijaya, Indonesia

Valentina Yurina, (ScopusID: 56176863500), Faculty of Medicine Universitas Brawijaya, Indonesia

Widodo Widodo, (ScopusID: 57190247745), Fakultas MIPA Universitas Brawijaya, Indonesia

Wisnu Barlianto, (ScopusID: 56639963100), Faculty of Medicine Universitas Brawijaya, Indonesia

Yuyun Yueniwati, <https://scholar.google.co.id/citations?user=Cox9BfMAAAAJ&hl=id>, Radiology Department, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Assistant Editor

Dini Suryaning Mentari, Fakultas Kedokteran Universitas Brawijaya, Indonesia

Vol. 32 No. 4 (2023)

Published: 2023-08-31

Research Article

The Potency of Polyphenols Extract of Robusta Coffee Bean (*Coffea robusta*) on COX-2 Inhibition in Neutrophil Cells

Tantni Ermawati, Qurratul Aini Wahyu Pratiwi, Zahara Meilawaty, Happy Harmono

p.205-210



Aloe vera Extract Sheet and Egg Albumin (*Gallus domesticus*) Reduce the Number of Lymphocytes on Gingival Incision Wound

Wizni A'dila A'ziza, Christiana Cahyani Prihastuti, Mutia Rochmawati, Alya Ghina Rosyada, Myrna Evana Amanda Putri

p.211-216



The Effect of *Psychotria malayana* Extract on Wound Healing in Male White Rats (*Rattus Novergicus*) Sprague Dawley Strain

Fairuz Fairuz, Khory Aurora Berty, Humaryanto Humaryanto

p.217-222



The Relationship between APGAR Score and Delivery Mode with Neonatal Sepsis in Ulin Regional Hospital Banjarmasin

Monica Anggriana Salim, Pricilia Gunawan Halim, Ari Yunanto, Pudji Andayani

p.223-227



The Effect of Antiretroviral Therapy (ART) Duration on CD4 Counts in Human Immunodeficiency Virus (HIV) Patients in dr. Doris Sylvanus Regional Hospital

Oberson Nainggolan, Lia Sasmithae, Ratna Widayati

p.228-232



The Profile of Osteogenesis Imperfecta in Dr. Moewardi General Hospital Year 2020-2021: A Retrospective Study

Udi Heru Nefihancoro, Ricat Hinaywan Malik

p.233-239



The Correlation between Sleep Quality and Level of Stress among Students in Islamic Boarding School Bogor

Bety Semara Lakshmi, Irena Ujjanti, Fahrozi Fahrozi, Ibnu Albantani, Ananda Putri, Nofrizal Nofrizal, Agus Rahmadi, Endin Nokik Stujanna, Zahra Nurushhafa, Wawang S Sukarya p.240-245



The Effect of Personality Types on Blood Pressure in Female Patients Over 17 Years of Age

Bunga Fardah Nahdiyah Achmada, Dian Mutiasari, Abi Bakring Balyas, Lia Sasmithae, Helena Jelita p.246-250



Study of Dysgeusia and Anosmia Frequency and Risk Factors in Pre-Elderly Post-Covid-19

Ari Tri Wanodyo Handayani, Zahreni Hamzah, Dwi Prijatmoko, Dyah Indartin Setyowati, Hosea Ferdinan p.251-257



Case Report

Emphysematous Cystitis with Bladder Mass caused by Colovesical Fistula: A Rare Case Report

Yosi Rizal, Eka Yudha Rahman p.258-261



Octreotide Treatment for Hypoglycemic Refractory Case Caused by Sulfonylureas with Impaired Renal Function

Taufiq Abdullah, Ali Haedar, Budi Soenarto p.262-264



Iatrogenic Penile Amputation Due to Circumcision Clamp in an Eight-Year-Old Boy

Muhammad Baihaqy Ibnu Hakim, Taufiq Nur Budaya, Besut Daryanto p.265-268



Management of Iatrogenic Ureteral Trauma Post Caesarean Section

Prillian Akbaril Satriyo Pamungkas, Besut Daryanto, Andri Kustono p.269-273



Research Article

The Potency of Polyphenols Extract of Robusta Coffee Bean (*Coffea robusta*) on COX-2 Inhibition in Neutrophil Cells

Potensi Ekstrak Polifenol Biji Kopi Robusta (*Coffea robusta*) terhadap Penghambatan COX-2 pada Sel Neutrofil

Tantin Ermawati¹, Qurratul Aini Wahyu Pratiwi², Zahara Meilawaty³, Happy Harmono⁴

^{1,3,4}Department of Dentistry-Biomedical Science, Oral Pathology and Maxillofacial Faculty of Dentistry Universitas Jember Jember

²Faculty of Dentistry Universitas Jember Jember

ABSTRACT

Inflammation is a physiological response to various stimuli including infection and tissue injury. One cause of inflammation is gram-negative bacteria that release various toxins, e.g. lipopolysaccharide endotoxin (LPS). The first body defense response is neutrophils. Body defense response is expressed by the cyclooxygenase (COX) enzyme that functions to transform arachidonic acid into prostaglandin. Polyphenols extract from Robusta coffee bean is known to play an anti-inflammatory role, but the mechanism for inhibiting COX-2 remains unknown. This study aimed to determine the potential of polyphenols extracted from Robusta coffee beans on COX-2 inhibition in neutrophils exposed to *E. coli* LPS. The research design was experimental *in vitro* with a post-test-only control group design. The sample consisted of 6 treatment groups, neutrophil isolates was incubated in concentrations of Robusta coffee polyphenols extract of 3.13%, 6.25%, 12.5%, and 25% and exposed to *E. coli* LPS. Measurement of COX-2 expression was conducted using immunohistochemical methods. ANOVA and LSD test results showed COX-2 expression in the treatment groups, namely neutrophils which were incubated with Robusta coffee polyphenols extract and exposed to *E. coli* LPS, was lower than the neutrophil group exposed only to *E. coli* LPS. The conclusion of this study is that the polyphenols extract of the Robusta coffee bean can inhibit COX-2 expression on neutrophils exposed to *E. coli* LPS.

Keywords: COX-2, LPS, neutrophils, polyphenols, robusta coffee

ABSTRAK

Inflamasi merupakan respon fisiologis terhadap berbagai rangsangan seperti infeksi dan cedera jaringan. Penyebab inflamasi salah satunya adalah bakteri gram negatif dengan mengeluarkan berbagai toksin antara lain endotoksin lipopolisakarida (LPS). Respon pertahanan tubuh pertama adalah neutrofil. Respon pertahanan tubuh diekspresikan oleh enzim siklooksigenase (COX) yang berfungsi mengubah asam arakidonat menjadi prostaglandin. Ekstrak Polifenol biji kopi robusta diketahui berperan sebagai antiinflamasi, tetapi mekanisme dalam menghambat COX-2 belum diketahui. Penelitian ini bertujuan untuk mengetahui potensi ekstrak polifenol biji kopi robusta dalam menghambat COX-2 pada neutrofil yang dipapar LPS *E. coli*. Rancangan penelitian adalah eksperimental *in vitro* dengan *the posttest only control group design*. Sampel terdiri dari 6 kelompok perlakuan, isolat neutrofil diinkubasi ekstrak polifenol biji kopi robusta konsentrasi 3,13%, 6,25%, 12,5%, dan 25% dan dipapar LPS *E. coli*. Pengukuran ekspresi COX-2 menggunakan metode imunohistokimia. Hasil uji ANOVA dan LSD menunjukkan ekspresi COX-2 pada kelompok perlakuan yakni neutrofil yang diinkubasi ekstrak polifenol biji kopi robusta dan dipapar LPS *E. coli* lebih rendah dibandingkan kelompok neutrofil yang hanya dipapar LPS *E. coli*. Kesimpulan dari penelitian ini adalah ekstrak polifenol biji kopi robusta mampu menghambat ekspresi COX-2 pada netrofil yang dipapar LPS *E. coli*.

Kata Kunci: COX-2, kopi robusta, LPS, neutrofil, polifenol

Correspondence: Tantin Ermawati. Department of Dentistry-Biomedical Science Oral Pathology and Maxillofacial Faculty of Dentistry Universitas Jember, Jl. Kalimantan 37 Jember Tel. +6281336785431 Email: tantin.ermawati@unej.ac.id

DOI: <http://dx.doi.org/10.21776/ub.jkb.2023.032.04.1>

INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, such as pathogens or irritants (1). In the cellular phase of the inflammatory process, the first cells chemically attracted to the area of inflammation are neutrophils. Neutrophil cells are phagocytic cells that become a factor in the natural immune system and function in pathogenic microbes' phagocytosis process. Neutrophils have a short life span, are active in the inflammatory process, and are responsible for tissue damage (2-4). Increased vascular permeability, vasodilation, edema formation, and pain in the inflammatory process are caused by the influence of prostaglandins as inflammatory mediators (5).

Inflammatory reactions can be caused by bacterial invasion and its products. Bacteria will produce endotoxin in the form of lipopolysaccharide (LPS), a large molecule containing lipids and carbohydrates (6). LPS induces the production of local factors, which are proinflammatory cytokines, such as interleukin 1 α (IL-1 α), interleukin-1 β (IL-1 β), IL-6, TNF- α , and prostaglandins (7).

Cyclooxygenase (COX) is an enzyme that catalyzes the formation of prostaglandins, an inflammatory mediator, and a product of arachidonic acid metabolism. The two COX isoenzymes in the body are COX-1 and COX-2. COX-1 is a constitutive enzyme that catalyzes the formation of regulatory prostanoids in various tissues, especially in the mucous membranes of the gastrointestinal tract, kidney, platelets, and vascular epithelium. COX-1 is almost entirely expressed in most cells and tissues. Cyclooxygenase 2 (COX-2) is a family of myeloperoxidases located on the luminal side of the endoplasmic reticulum and nuclear membrane. In general, COX-2 is undetectable but is rapidly induced when cells receive inflammatory stimuli. COX-2 also acts at inflammation sites (8-10).

In inflammatory conditions, the standard therapy is the administration of non-steroidal anti-inflammatory drugs (NSAIDs). Pharmacological studies have shown that non-steroidal anti-inflammatory drugs on the market can inhibit both forms of COX enzymes. Some medicines used to reduce pain are aspirin, indomethacin, ibuprofen, and piroxicam (11). Administration of indomethacin in rats could significantly decrease COX-2 expression (12,12). On the contrary, long-term use of NSAIDs has a toxic effect, especially on the liver, kidneys, and gastrointestinal tract (11,14-17).

Coffee, which is currently favored by the public and has become a lifestyle, is a beverage beneficial for health (18,19). Study results show that coffee intake modifies various immune functions (20-22). One kind of coffee that is widely consumed is Robusta. Robusta coffee beans naturally contain caffeine, phenolic compounds, trigonelline, and chlorogenic acid with anti-bacterial and anti-inflammatory activities (23). Polyphenols have health benefits (24-26). In recent decades, several benefits of polyphenols from plants as antioxidants and anti-inflammatory have been recognized. Research conducted by Willenberg *et al.*, stated that polyphenols could reduce COX-2 expression in monocyte cells exposed to LPS (27). Polyphenols in coffee plants can be used to inhibit the development of diseases, such as cancer, cardiovascular disease, diabetes, osteoporosis, and neurodegenerative diseases (22,28). However, little research has been done on the potency of polyphenol extract of Robusta coffee beans on COX-2 expression in neutrophils. Therefore, this

study aimed to determine the potency of the polyphenol extract of Robusta coffee beans on COX-2 inhibition in neutrophils exposed to LPS *E. coli*.

METHOD

Experimental Design

The *In vitro* laboratory experimental with research design The Post Test Only Control Group Design. The independent variable in this study is the polyphenol extract of robusta coffee beans. The dependent variable is the amount of COX-2 expression of neutrophil cells. The controlled variables in this study were the concentration of robusta coffee bean polyphenol extract, neutrophil isolate, and LPS *E. coli* 0111: B4 (List Biology Lab).

Extraction of Robusta Coffee Bean Polyphenols

The extraction used the sonication method. The procedure for making the extract is as follows. 721.1 grams of coffee beans were baked at 60°C for 2 to 3 days. The coffee beans were put in a hammer mill to become coffee powder. The sonication process was carried out by adding 96% ethanol solvent and rotating with 80KHz vibration for one hour. Mixing with ethanol was done 3 times to obtain pure flavonoid content, then centrifuged at 4000 rpm for 5 minutes to precipitate particles from coffee powder so that an oil-like liquid was obtained. The liquid is dried in an oven at 60°C so that the ethanol evaporates and coffee bean polyphenol extract is obtained.

Neutrophil Preparation

Neutrophil isolation was performed using a modified gradient density technique using Double Ficoll Hypaque Centrifugation material (29). Six ml of cubital venous blood was added to the heparin tube and mixed thoroughly. Pipette 3ml histopage M119 into a sterile falcon tube and add 3ml Lymphoprep slowly through the tube wall, and 2 layers were formed. Pipette 6ml of blood into the 2-layer tube, slowly through the wall of the falcon tube, and 3 layers are formed (Histopage 119, Lymphoprep, and blood). Centrifuge at 700 g for 30 minutes at 20°C. Carefully pipette the 4th layer of Polymorphonuclear (fog ring) into a sterile tube. Dilute the Mononuclear sample using HBSS (1:1) homogenize. Centrifuge at 700g (gravity) for 10 minutes at 20°C do 3 repetitions. Add 1ml of HBSS pH 7.4 to the supernatant obtained. Add 5 μ l fungizone and 20 μ l Penicilin- Streptomycin. Prepare Well Plate Culture, and insert sterile poly L-lysin coverslip in each well as needed. Drip 100 μ l of cell isolation supernatant on the prepared coverslip. Incubate for 20-30 minutes at 37°C. Take and add 1ml of RPMI culture medium, and incubate again for 20-30 minutes at 37°C. Observe under an inverted microscope, by gently shaking to see the cell attachment. Wash using RPMI media 3 times, carefully to release cell contamination. Once the sterile cells are free from contamination. Replace the culture medium using M.199 culture medium, the cells are ready for cell treatment.

Treatment and Immunocytochemical Assay

Neutrophils were resuspended with 1000 μ l RPMI, then discarded. Add 100 μ l of polyphenol extract with a certain concentration (3.13%, 6.25%, 12.5%, 25%) to the cell culture well according to the treatment and homogenize. Each treatment group was slowly added with 100 μ l of *E. coli* LPS and homogenized. Incubate for 2 hours at 37°C and 5% CO₂. Observed the changes and development of neutrophils every hour during the incubation time with

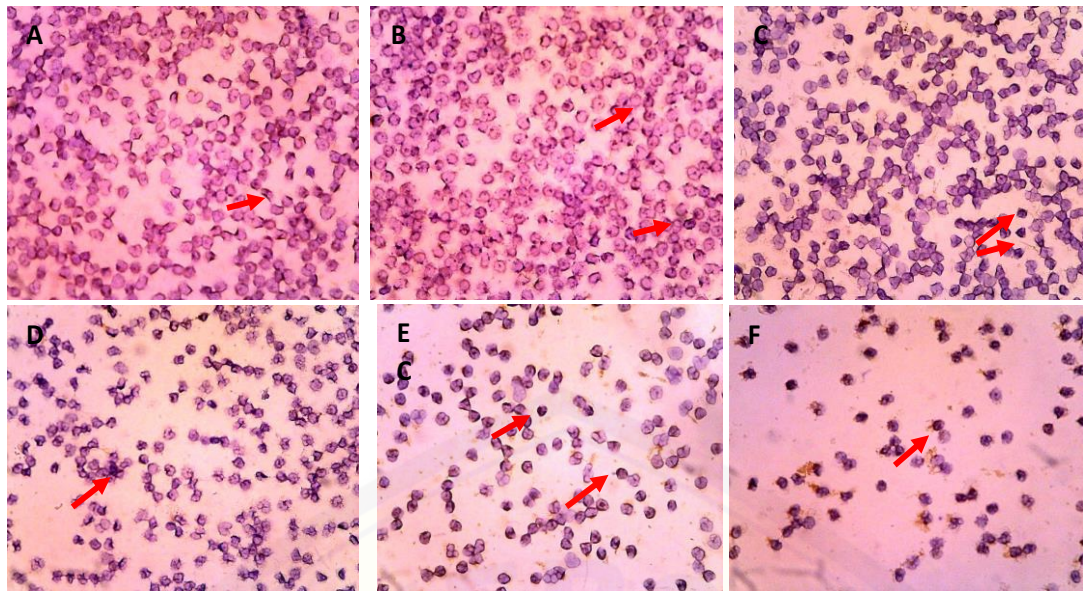


Figure 2. COX-2 expression in neutrophil cells (microscope magnification at 400 times)

Note: Arrows indicate COX-2 expression in neutrophil cells

LPS for 4 hours. After incubation of LPS exposure for 4 hours, the immunostaining procedure was continued using COX-2 monoclonal antibody. COX-2 expression was analyzed by immunocytochemical method. COX-2 expression was shown by neutrophils whose cell membranes were brown in color, observations were made under a microscope with 400 magnification. The study data was the average number of neutrophils expressing COX-2 counted per 100 cells.

Statistical Analysis

The data obtained were analyzed using the Kolmogorov-Smirnov test for the normality test and the Levene test for the homogeneity test. The normality test was performed with the parametric statistical test, namely One Way ANOVA, and continued with the LSD test. All tests used a significance level of 95% ($\alpha=0.05$)

RESULTS

The results showed that there were differences in COX-2 expression between the control and treatment groups. The average number of neutrophil cells expressing COX-2 in the control and treatment groups can be seen in Figure 1.

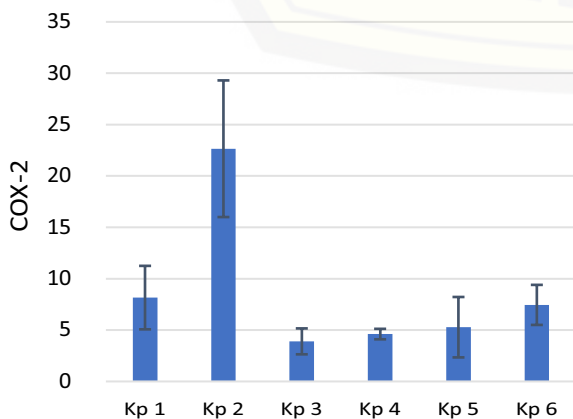


Figure 1. Graph of the mean COX-2 expression

Note:

- KP1 = Control
- KP2 = Positive control (neutrophils exposed to LPS)
- KP3 = Neutrophils incubated with 3.13% polyphenols and exposed to LPS E. coli
- KP4 = Neutrophils incubated with 6.25% polyphenols and exposed to LPS E. coli
- KP5 = Neutrophils incubated with 12.5% polyphenols and exposed to LPS E. coli
- KP6 = Neutrophils incubated with 25% polyphenol and exposed to LPS E. coli
- =SD (Standard Deviasion)

The lowest mean COX-2 expression (3.9 ± 1.26) was found in the treatment group with exposure to polyphenol extract with a concentration of 3.13%, and the highest COX-2 expression (22.65 ± 6.65) was found in the positive control group (LPS). These results indicate that polyphenol incubation treatment show lower COX2 expression in neutrophils than those in positive controls, nearly the same as normal conditions (control).

The neutrophils expressing COX-2 can be seen in Figure 2. COX-2 expressions yield a dark or brown color. The results of the Kolmogrov-Smirnov test showed that the data were normally distributed ($p > 0.05$). Based on the Levene test, it had a homogeneous variance ($p = 0.118$), meaning that the data obtained was homogeneous. One-way ANOVA test showed a significant difference in COX-2 between the control and treatment groups ($p = 0.00$). LSD analysis showed a significant difference between the negative control group (neutrophil cells exposed to LPS) and the treatment groups (neutrophil cells incubated with polyphenol extracts and exposed to LPS). There were no significant differences between treatment groups given different doses of coffee extract. These results indicate that polyphenol extract of coffee beans can reduce COX-2 levels in neutrophil cells exposed to LPS, and increasing the dose did not provide a significant difference.

DISCUSSION

This research was an in vitro experimental study using neutrophil cells and aimed to know the potency of polyphenol extract of Robusta coffee beans as an anti-inflammatory agent by determining COX-2 expression. Neutrophils are immune cells in the body that act as the

first line of defense against injuries, including bacteria and their products, such as LPS (2,7). Neutrophils migrate to the inflamed area by releasing elastase to kill bacteria, but it results in tissue damage if elastase is produced in large quantities (30). Neutrophils use three ways to fight and kill pathogenic microbes and their products, namely phagocytosis, degranulation, and Neutrophil Extracellular Trap (NET) formation (31-35). Based on research conducted by Pieterse *et al.*, LPS *E. coli* exposed to neutrophil cells was shown to release NET production as a defense response against damaging agents (36). In the phagocytosis process, neutrophils engulf microbes into phagosomes, while in the degranulation process, neutrophils release protease granules, which can cause damage to host cells (36-38).

The results showed that the mean COX-2 expression in the LPS-induced and polyphenol extracts groups tended to decrease compared to the group only exposed to LPS. LPS exposure to neutrophils showed the highest COX-2 expression compared to the treatment and control groups. It is because LPS is a potent endotoxin that is responsible for inflammation. LPS *E. coli* is a bacterial product that can bind to the CD14/Toll-like receptor 4 (TLR4) on the cell surface of macrophages and monocytes. The binding between TLR4 and LPS activates inflammatory signaling pathways. The inflammatory response results in the secretion of proinflammatory cytokines from several cell types. Macrophages that bind to bacteria due to TLR4 will secrete cytokines (IL-1 α , IL-1 β , TNF- α , and prostaglandins (PGE₂) (39). These results are in line with studies conducted by Nieves *et al.*, (39) that LPS exposure in astrocyte cell culture was proven to increase COX-2 expression and increase prostaglandin E2 (PGE₂).

The research data obtained prove that the polyphenol extract of Robusta coffee beans can inhibit COX-2

expression in neutrophil cells exposed to LPS. Polyphenols with various concentrations tend to reduce COX-2 expression. The group given polyphenols extract of Robusta coffee beans with 3.13% concentration showed the lowest COX-2 expression compared to higher treatment doses. The potential of polyphenols in suppressing inflammation is by blocking the cyclooxygenase (COX) and lipoxygenase (LOX) cycles. Polyphenols inhibit cell membranes that trigger the release of phospholipase enzymes and will inhibit the formation of arachidonic acid so that COX-2 expression and the formation of prostaglandins can be suppressed. Protein kinase C (PKC) activates phospholipase A₂ (PLA₂) in an inflammatory state and releases arachidonic acid from membrane phospholipids. Arachidonic acid is metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) to produce eicosanoids, including prostaglandins and leukotrienes (40). Flavonoids, such as quercetin and myricetin, are COX and LOX inhibitors (40-44). The results obtained are in accordance with findings from Yue *et al.*, (45) that polyphenol extracts from pomace apple can reduce COX-2 expression in rat macrophage cell culture. Polyphenol extracts are shown to decrease the expression of pro-inflammatory cytokines IL-6, TNF- α , PGE₂, and COX-2 in LPS-induced macrophage culture cells (46). The results of the LSD test showed that the variation of the polyphenol extract with higher doses (3.13%, 6.25%, 12.5%, and 25%) did not show significant differences in COX-2 expression. Polyphenols have different chemical structure characteristics. In addition, polyphenols have different mechanisms for controlling neutrophils depending on the receptors that act on the cells. This study proves that the polyphenol extract of Robusta coffee beans has anti-inflammatory properties, marked by a decrease in COX-2 in neutrophil cells. Therefore, polyphenols of Robusta coffee beans can be developed as alternative medicine in the health sector.

REFERENCES

1. Takei N, Newman MG, Klokkevold PR, and Carranza FA. *Carranza'S Clinical Periodontology*. Volume 53. Canada: Elsevier; 2019; p. 32–52.
2. Mócsai A. *Diverse Novel Functions of Neutrophils in Immunity, Inflammation, and Beyond*. The Journal of Experimental Medicine. 2013; 210(7): 1289–1299.
3. Yunanto A, Chandra M, Widjajanto E, and Widodo MA. *Kuantitas, Kualitas, dan Daya Fagositosis Neutrofil pada Saliva dan Darah Bayi Baru Lahir dengan Faktor Risiko Sepsis*. Jurnal Kedokteran Brawijaya. 2012; 27(2): 89–95.
4. Houghton AM. *Matrix Metalloproteinases in Destructive Lung Disease*. Matrix Biology. 2015; 44-46: 167-174.
5. Ma'at S. *Inflamasi*. Surabaya: Airlangga University Press; 2012.
6. Pussinen PJ, Kopra E, Pietiäinen M, *et al.* *Periodontitis and Cardiometabolic Disorders: The Role of Lipopolysaccharide and Endotoxemia*. Periodontology 2000. 2022; 89(1): 19–40.
7. Naegelen I, Beaume N, Plançon S, Schenten V, Tschirhart EJ, and Bréchar S. *Regulation of Neutrophil Degranulation and Cytokine Secretion: A Novel Model Approach Based on Linear Fitting*. Journal of Immunology Research. 2015; 2015: 1-15.
8. Ruslin R, Yamin Y, Kasmawati H, *et al.* *The Search for Cyclooxygenase-2 (COX-2) Inhibitors for the Treatment of Inflammation Disease: An in-silico Study*. Journal Multidisciplinary Healthcare. 2022; 15: 783–791.
9. Bell CR, Pelly VS, Moeini A, *et al.* *Chemotherapy-Induced COX-2 Upregulation by Cancer Cells Defines Their Inflammatory Properties and Limits the Efficacy of Chemoimmunotherapy Combinations*. Nature Communication. 2022; 13(1): 1-18.
10. Fajrin FA. *Keterkaitan Cyclooxygenase (Cox)-2 terhadap Perkembangan Terapi Kanker*. Stomatognatic. 2013; 10(1): 6–11.
11. Modi CM, Mody SK, Patel HB, Dudhatra GB, Kumar A, and Avale M. *Toxicopathological Overview of Analgesic and Anti-Inflammatory Drugs in Animals*. Journal Applied Pharmaceutical Science. 2012; 2(1): 149–157.
12. Hermawan D, Soejono SK, Sunarti S, Astuti I, and Agus ZAN. *Pengaruh Vitamin D terhadap Ekspresi COX-2, Kadar cAMP, Kadar Renin Darah dan Tekanan Darah Sistolik dalam Sistem Renin Angiotensin Aldosteron*. Jurnal Kedokteran Brawijaya. 2016; 29(2): 125–131.

13. Takeuchi K. *Pathogenesis of NSAID-Induced Gastric Damage: Importance of Cyclooxygenase Inhibition and Gastric Hypermotility*. World Journal of Gastroenterology. 2012; 18(18): 2147–2160.
14. Bindu S, Mazumder S, and Bandyopadhyay U. *Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Organ Damage: A Current Perspective*. Biochemical Pharmacology. 2020; 180: 1-21.
15. Orkaby AR, Ward R, Chen J, et al. *Influence of Long-term Nonaspirin NSAID Use on Risk of Frailty in Men ≥60 Years: The Physicians' Health Study*. The Journal of Gerontology. Series A, Biological Sciences and Medical Sciences. 2022; 77(5): 1048–1054.
16. Lucas GNC, Leitão ACC, Alencar RL, Xavier RMF, Daher EDF, and da Silva Junior GB. *Pathophysiological Aspects of Nephropathy Caused by Non-Steroidal Anti-Inflammatory Drugs*. Jornal Brasileiro de Nefrologia. 2019; 41(1): 124–130.
17. Fokunang C, Fokunang ET, Frederick K, Ngameni B, and Ngadjui B. *Overview of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in Resource-Limited Countries*. MOJ Toxicology. 2018; 4(1): 5–13.
18. Coso JD, Salinero JJ, and Lara B. *Effects of Caffeine and Coffee on Human Functioning*. Nutrients. 2020; 12(1): 1–5.
19. Bae JH, Park JH, Im SS, and Song DK. *Coffee and Health*. Integrative Medicine Research. 2014; 3(4): 189–191.
20. Açıkalin B and Sanlier N. *Coffee and Its Effects on the Immune System*. Trends in Food Science & Technology. 2021; 114: 625–632.
21. dePaula J and Farah A. *Caffeine Consumption Through Coffee: Content in the Beverage, Metabolism, Health Benefits and Risks*. Beverages. 2019; 5(2): 1-50.
22. Loftfield E, Shiels MS, Graubard BI, et al. *Associations of Coffee Drinking with Systemic Immune and Inflammatory Markers*. Cancer Epidemiology, Biomarkers & Prevention. 2015; 24(7): 1052–1060.
23. Dias RCE and Benassi MDT. *Discrimination between Arabica and Robusta Coffees Using Hydrosoluble Compounds: Is the Efficiency of the Parameters Dependent on the Roast Degree?* Beverages. 2015; 1(3): 127–139.
24. Cory H, Passarelli S, Szeto J, Tamez M, and Mattei J. *The Role of Polyphenols in Human Health and Food Systems: A Mini-Review*. Frontiers in Nutrition. 2018; 5: 1-9.
25. Khurana S, Venkataraman K, Hollingsworth A, Piche M, and Tai TC. *Polyphenols: Benefits to the Cardiovascular System in Health and in Aging*. Nutrients. 2013; 5(10): 3779–3827.
26. Rasouli H, Farzaei MH, and Khodarahmi R. *Polyphenols and Their Benefits: A Review*. International Journal of Food Properties. 2017; 20(2): 1700–1741.
27. Willenberg I, Meschede AK, Gueler F, Jang M, Shushakova N, and Schebb NH. *Food Polyphenols Fail to Cause a Biologically Relevant Reduction of COX-2 Activity*. PLoS One. 2015; 10(10): 1–16.
28. Rudrapal M, Shubham KJ, Khan J, et al. *Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action*. Frontiers in Pharmacology. 2022; 13: 1–15.
29. Kuhns DB, Priel DAL, Chu J, and Zarembek KA. *Isolation and Functional Analysis of Human Neutrophils*. Current Protocols in Immunology. 2015; 111(7): 1–18.
30. Gramegna A, Amati F, Terranova L, et al. *Neutrophil Elastase in Bronchiectasis*. Respiratory Research. 2017; 18(1): 1–13.
31. Rosales C. *Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types?* Frontiers in Physiology. 2018; 9: 1-17.
32. Mohanty T, Fisher J, Bakochi A, et al. *Neutrophil Extracellular Traps in the Central Nervous System Hinder Bacterial Clearance during Pneumococcal Meningitis*. Nature Communications. 2019; 10(1): 1-13.
33. Kobayashi SD, Malachowa N, and DeLeo FR. *Neutrophils and Bacterial Immune Evasion*. Journal of Innate Immunity. 2018; 10(5–6): 432–441.
34. Teng TS, Ji AL, Ji XY, and Li YZ. *Neutrophils and Immunity: From Bactericidal Action to Being Conquered*. Journal of Immunology Research. 2017; 2017: 1-14.
35. Kobayashi SD, Malachowa N, and DeLeo FR. *Influence of Microbes on Neutrophil Life and Death*. Frontiers in Cellular and Infection Microbiology. 2017; 7: 1-9.
36. Pieterse E, Rother N, Yanginlar C, Hilbrands LB, and van der Vlag J. *Neutrophils Discriminate between Lipopolysaccharides of Different Bacterial Sources and Selectively Release Neutrophil Extracellular Traps*. Frontiers in Immunology. 2016; 7: 1-13.
37. van Kessel KPM, Bestebroer J, van Strijp JAG. *Neutrophil-Mediated Phagocytosis of Staphylococcus aureus*. Frontiers in Immunology. 2014; 5: 1–12.
38. Tecchio C, Micheletti A, and Cassatella MA. *Neutrophil-Derived Cytokines: Facts Beyond Expression*. 2014; 5: 1–7.
39. Cirmi S, Maugeri A, Russo C, Musumeci L, Navarra M, and Lombardo GE. *Oleacein Attenuates Lipopolysaccharide-Induced Inflammation in THP-1-Derived Macrophages by the Inhibition of TLR4/MyD88/NF-κB Pathway*. International Journal of Molecular Sciences. 2022; 23(3): 1-17.
40. Font-Nieves M, Sans-Fons MG, Gorina R, et al. *Induction of COX-2 Enzyme and Down-Regulation of COX-1 Expression by Lipopolysaccharide (LPS) Control Prostaglandin E 2 Production in Astrocytes*. Journal of Biological Chemistry. 2012; 287(9): 6454–6468.
41. Kim H, Zamel R, Bai XH, and Liu M. *PKC Activation Induces Inflammatory Response and Cell Death in Human Bronchial Epithelial Cells*. PLoS One. 2013; 8(5): 1-12.
42. Panche AN, Diwan AD, and Chandra SR. *Flavonoids: An Overview*. Journal of Nutritional Science. 2016; 5:

- 1-15.
43. David AVA, Arulmoli R, and Parasuraman S. *Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid*. *Pharmacognosy Review*. 2016; 10(20): 84–89.
44. Yahfoufi N, Alsadi N, Jambi M, and Matar C. *The Immunomodulatory and Anti-Inflammatory Role of Polyphenols*. *Nutrients*. 2018; 10(11): 1-23.
45. Yue T, Bai X, Zhang H, and Yuan Y. *Fractionation and Anti-Inflammatory Effects of Polyphenol Enriched Extracts from Apple Pomace*. *Bangladesh Journal of Pharmacology*. 2012; 7(1): 28–32.
46. Rupasinghe HPV, Boehm MMA, Sekhon-Loodu S, Parmar I, Bors B, and Jamieson AR. *Anti-Inflammatory Activity of Haskap Cultivars is Polyphenols-Dependent*. *Biomolecules*. 2015; 5(2): 1079–1098.

