GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

The capability of *Piper betle* leaves ethanolic extract to inhibit neutrophil infiltration in sepsis-induced BALB/c Mice

Shod Abdurrachman Dzulkarnain [1,*], Irene Ratridewi [2], Yessy Chairani Puspita Sari [3], Vanianisa Hanareta Hantoko [3] and Dewi Santosaningsih [4]

[1] *Department of Medical Science, Faculty of Medicine, Universitas Negeri Surabaya, Surabaya, East Java, 60213, Indonesia.*
[2] *Department of Pediatrics of dr. Saiful Anwar General Hospital, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, 65145, Indonesia.*
[3] *Medical Degree, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, 65145, Indonesia.*
[4] *Department of Clinical Microbiology of dr. Saiful Anwar General Hospital, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, 65145, Indonesia.*

GSC Biological and Pharmaceutical Sciences, 2024, 26(03), 212–216

Publication history: Received on 13 February 2024; revised on 25 March 2024; accepted on 28 March 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.26.3.0103

Abstract

Background: Sepsis due to ESBL-producing *Escherichia coli* infection requires innovative management to prevent a higher prevalence of meropenem overuse. The antimicrobial effect of *Piper betle* leaves ethanolic extract has been previously proven and should be tested on sepsis-induced animal models.

Objectives: This study assesses the antimicrobial effect of *Piper betle* leaves ethanolic extract towards the bacterial loads and its anti-inflammatory effect on neutrophil infiltration in the lung, kidney, and liver of sepsis-induced mice.

Methods: After sepsis inducement, 3 mg/mL of *Piper betle* leaves ethanolic extract was administered orally. After the mice died, the bacterial load of the lung, kidney, and liver was measured, while neutrophil infiltration was observed using hematoxylin-eosin staining.

Results: There is no significant decreasing the number of bacterial loads in the treatment group but there is a significant decrease in the number of neutrophils infiltration in the treatment group.

Conclusion: Single dose administration of *Piper betle* leaves ethanolic extract is sufficient to inhibit neutrophil infiltration significantly.

Keywords: *Piper betle* ethanolic extract; ESBL-producing *Escherichia coli*; sepsis-induced mice; Bacterial load; Neutrophil infiltration

1. Introduction

Sepsis is life life-threatening organ dysfunction due to the deregulation of the immune response related to infectious disease [1]. Sepsis contributes to 36% of death cases in high-income countries [2], while in low-middle-income countries, sepsis contributes to 70% of death cases every year [3]. The most frequent bacteria causing sepsis is *Escherichia coli* (*E. coli*). The emergence of extended-spectrum beta-lactamase (ESBL) producing *E. coli* leads to therapeutic failure and a higher mortality rate [4][5].

* Corresponding author: Shod Abdurrachman Dzulkarnain

Focus infection should be confirmed in sepsis cases, usually in certain specific organs or widely distributed as bacteremia. The common sources of infection include urinary organs such as the kidney or bladder, respiratory organs such as the lung, or gastrointestinal organs such as the intestine or liver. When bacteria infect the organ, the inflammatory response towards bacteria can disrupt the organ function causing organ damage [6].

Neutrophil is the leading cellular immune response towards bacterial infection. However excessive inflammatory response of neutrophils will damage the organ. Phagocytosis and neutrophil degranulation release high reactive oxygen species and degrading enzymes such as collagenase leading to tissue damage and organ dysfunction [7].

ESBL-producing *E. coli* is resistant to several antibiotics hence meropenem is widely used as the antibiotic therapy for sepsis due to ESBL-producing *E. coli*. However, meropenem overused will increase the prevalence of meropenem resistance. To solve this problem, several studies recommend the use of plant extract to eliminate multidrug-resistant microorganisms. A previous study mentioned the antibacterial; activity of *Piper betle* leaves ethanolic extract toward ESBL-producing *E. coli* [8]. *Piper betle* leaves ethanolic extract also inhibits neutrophil infiltration due to the antioxidant effect of the hydroxychavicol component which is capable of inhibiting reactive oxygen species' role as chemoattractants [9].

This study will assess the antibacterial effect of *Piper betle* leaves ethanolic extract towards ESBL-producing *E. coli* bacterial load in the liver, lung, and kidney of sepsis-induced mice. This study will also measure the number of neutrophils in the aforementioned organs.

2. Material and Methods

2.1. ESBL-producing *E. coli* isolates

The ESBL-producing *E. coli* isolate was provided by the Department of Clinical Microbiology, Faculty of Medicine, Brawijaya University from clinical isolates. It was identified and confirmed using the Vitek-2 system and double disk synergy test, respectively [10].

2.2. Plant material and extract preparation

Plant material and extract preparation was performed based on a previous study [8]. Shortly, *Piper betle* leaf powder was macerated in 20 ml 96% ethanol for 6 hours at room temperature before filtration and evaporation. The extract was administered orally to sepsis-induced mice, 30 minutes after sepsis inducement.

2.3. Animal infection, bacterial load, and neutrophil count.

The selection of animal model (BALB/c mice) and infecting procedures was performed based on the previous study [8]. Shortly, there were 3 tested groups, negative control, positive control, and treatment group (receiving 3mg/mL of *Piper betle* leaves ethanolic extract, single dose). After the mice died, the lung, kidney, and liver were removed aseptically. 100 mg of each organ were homogenized and inoculated on eosin-methylene blue agar for bacterial count [11]. To determine neutrophil infiltration in the organs, a histopathological examination was performed. The organ was fixed in a 10% formalin solution and embedded in paraffin blocks. 5 mm thick sections were obtained, then staining with hematoxylin-eosin was performed to identify the neutrophil and evaluate the tissue morphology in 400 times magnification [12].

2.4. Statistical analysis

The number of bacterial counts and observed neutrophils was analyzed using an independent T-test followed by simple linear regression analysis.

3. Results

3.1. Antimicrobial effect of *Piper betle* leaves ethanolic extract toward Bacterial Load of ESBL producing *E. coli* in Lung, Kidney, and Liver of Sepsis Induced BALB/c mice

We identified a non-significant decrease in the number of bacterial loads between the positive control group and the treated group, either lung, kidney, or liver. Non-significant results were proven by the p-value provided by the independent T-test, >0.050.

Table 1 Bacterial Load of Lung, Kidney, and Liver (Table 1)

	Negative Control	**Positive Control**	**Treatment**	***p-value***
Bacterial Load of Lung (10^7 CFU/g)	0±0	16.8±5.5	9.2±2.5	0.236
Bacterial Load of Kidney (10^7 CFU/g)	0±0	17.3±5.2	24.2±2.8	0.246
Bacterial Load of Liver (10^7 CFU/g)	0±0	55.9±9.2	52.3±7.6	0.760

3.2. Anti-Inflammatory effect of *Piper betle* leaves ethanolic extract toward Neutrophil Infiltration \ in Lung, Kidney, and Liver of Sepsis Induced BALB/c mice

We identified a significant decrease in the number of neutrophil infiltrations between the positive control group and the treated group, either lung, kidney, or liver. Significant results were proven by the p-value provided by the independent T-test, <0.001.

Table 2 Neutrophil Infiltration of Lung, Kidney, and Liver (Table 2)

	Negative Control	**Positive Control**	**Treatment**	***p-value***
Neutrophil Infiltration of Lung (Cells/20 OF)	0±0	348.1±19.5	174.0±16.0	<0.001
Neutrophil Infiltration of Kidney (Cells/20 OF)	0±0	86.9±6.1	40.1±2.2	<0.001
Neutrophil Infiltration of Liver (Cells/20 OF)	0±0	393.3±16.0	180.2±14.6	<0.001

Hematoxylin-eosin staining shows increasing infiltration of inflammatory cells including neutrophils. The highest number of neutrophil infiltrations can be observed in the positive control group (Figure 1).

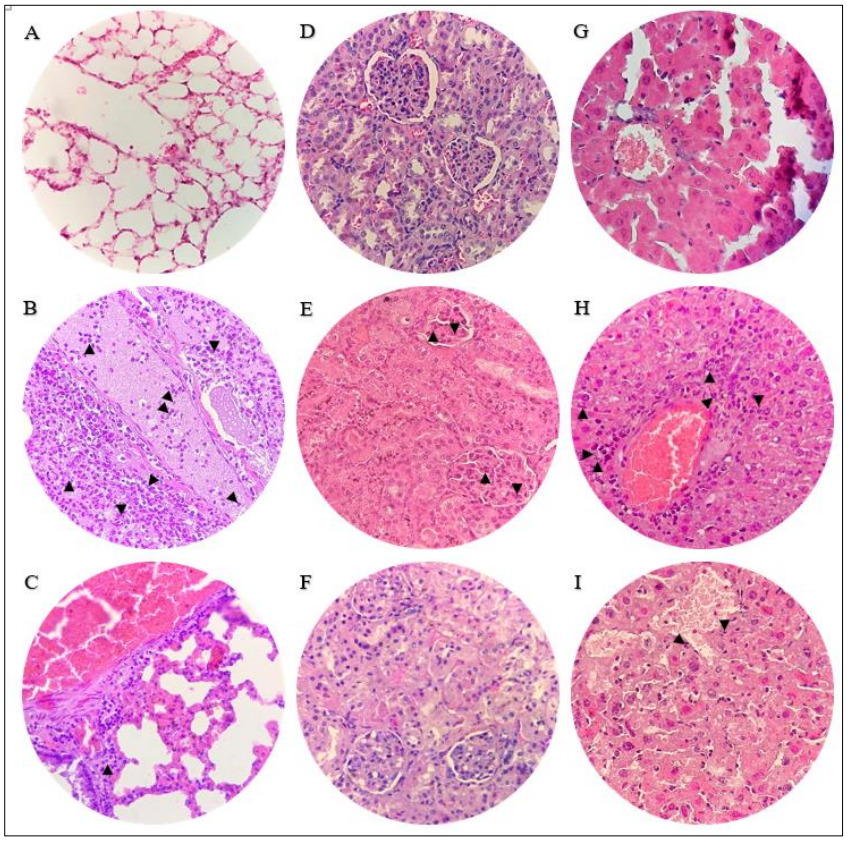

Figure 1 Hematoxylin-eosin staining of Lung Tissue, Kidney Tissue, and Liver Tissue. A: Lung from Negative Control Group; B: Lung from Positive Control Group; C: Lung from Treatment Group; D: Kidney from Negative Control Group; E: Kidney from Positive Control Group; F: Kidney from Treatment Group; G: Liver from Negative Control Group; B: Liver from Positive Control Group; C: Liver from Treatment Group; the arrow shows neutrophil cell

4. Discussion

The insignificant difference in bacterial load between the positive control group and treatment group in each organ was caused by decreasing the concentration of *Piper betle* leaves ethanolic extract in the mice plasma. The decreasing concentration happened due to the pharmacological effect after *Piper betle* leaves ethanolic extract ingestion and absorption through the intestine. In this study, we did not consider the half-life of *Piper betle* leaves ethanolic extract, and simply focused on the antimicrobial effect of *Piper betle* leaves ethanolic extract which has been proven in previous studies [8]. Due to the scarce number of publications discussing the effect of *Piper betle* leaves ethanolic extract on sepsis-induced mice, a proper comparison is difficult to be performed. A similar study performed in Malang, assessing the antimicrobial effect of lime leaves (*Cytrus hytrix* L.) extract towards *Salmonella typhimurium-infected* mice showed a significant decrease of bacterial loads in the mice's intestine, spleen, and liver. However, the lime leaf extract was administered three times after the mice were infected instead of a single dose as in our study. Using this method, the optimal dose could be achieved and maintained hence antimicrobial effect of the extract could affect *Salmonella typhimurium* for a longer duration [13].

However, this study showed a significantly decreasing number of neutrophil infiltration in the tissue of the lung, kidney, and liver. A previous study performed in Malaysia showed decreasing bactericidal activities of neutrophil culture after 5 mg/mL of *Piper betle* leaves ethanolic extract was administered into the system [14]. Decreased neutrophil infiltration happened due to eugenol and polyphenols contained in *Piper betle* leaves ethanolic extract. A previous study detected the eugenol and polyphenols in *Piper betle* leaves ethanolic extract [8]. Both compounds have potent anti-inflammatory effect and antioxidant effects which are capable of inhibiting neutrophil chemotaxis. Another study in India listed the active compound of *Piper betle* leaves ethanolic extract and showed several compounds with anti-inflammatory activity such as α-pinene, eugenol, and caryophyllene [15].

5. Conclusion

Although single dose administration of 3 mg/mL of *Piper betle* leaves ethanolic extract cannot affect the bacterial load, it could inhibit neutrophil infiltration in the lung, kidney, and liver of sepsis-induced mice significantly. Further investigation is required to determine the therapeutical dose and half-life of *Piper betle* leaves ethanolic extract.

Compliance with ethical standards

Acknowledgments

The author thanks support staff from the Clinical Microbiology Department of the Faculty of Medicine and Animal Laboratory of the Faculty of Medicine Brawijaya University, the Technical Chemistry Laboratory of the State Polytechnic of Malang, and UPTD Materia Medica. This research was funded by the PNBP FKUB (214.48/SK/UN.10F08.06/PN/2020).

Disclosure of conflict of interest

All author declares no conflict of interest.

Statement of ethical approval

The study was approved by the Health Research Ethics Committee Faculty of Medicine, Brawijaya University (172/EC/KEPK/10/2020), and the methods were carried out by the approved guidelines.

References

[1] Rello, J., Valenzuela-Sanchez, F., Ruiz-Rodriguez, A., Moyano, S. Sepsis: A Review of Advances in Management. Adv Ther. 2017. 34: 2393-2411

[2] Keeley, A., Hina, P., Nsutebu, E. The Recognition and Management of Sepsis and Septic Shock: A Guide for Non-Intensivist. Postgrad Med J. 2017. 93: 626-634

[3] Ding, Y., Lin, Y., Zhu, T., Huang, M., Xu, Q. Interleukin 6 Increases Dysfunction of Organs in Sepsis Rats Through Sirtuin 1. Int J Clin Exp Med. 2014. 7(9): 2593-2598

[4] Nasir, N., Ahmed, S., Razi, S., Awan, S., Mahmood, S.F. Risk Factor for Mortality of Patients with Ceftriaxone Resistant *E. coli* Bacteremia Receiving Carbapenem versus Beta-Lactam/Beta-Lactamase Inhibitor Therapy. BMC Research Notes. 2019. 12(611):1-5

[5] Brooks, G.E., Carrol, K.C., Butel, J.S., Morse, S.A., Mietzner, T.A. Jawetz, Melnick, & Adelberg's Microbiology Twenty-Sixth Edition. USA: McGraw Hill; 2013. p: 229-236; 372

[6] Hotchkiss, R.S., *et al*. Sepsis and Septic Shock. Nat Rev Dis Primers. 2016. 2 (16045): 1-47

[7] Abbas, A.K., Lichtman, A.H., Pillai, S. Cellular and Molecular Immunology, Ninth Edition. Elsevier; 2018. p: 14-15 Harris, 46-47. 351-355

[8] Dzulkarnain, S.A, Ratridewi, I., Hantoko, V.H., Sari, Y.C.P., Santosaningsih, D. Antibacterial activity of *Piper betle* ethanolic extract against ESBL producing *Escherichia coli* in sepsis-induced BALB/c mice. GSC Biological and Pharmaceutical Sciences. 2021. 15(2):1-7

[9] Singh, D., Majumdar, A.G., Gamre, S., Subramanian, M. Membrane damage precedes DNA damage in hydroxychavicol-treated *E. coli* cells and facilitates cooperativity with hydrophobic antibiotics. Biochimine. 2021. 180: 158-168

[10] Paterson, D.L., Bonomo, R.A. Extended-Spectrum β-Lactamases: a Clinical Update. American Society for Microbiology. 2005. 18 (4): 657-686

[11] Tille, P.M. Bailey & Scott's Diagnostic Microbiology Fourteenth Edition. Elsevier. 2017. p:177-188

[12] Chauhan, A.K., Kim, J., Lee, Y., Balasubramanian, P.K., Kim, Y. Isorhamnetin Has Potential for the Treatment of *Escherichia coli*-Induced Sepsis. Molecules. 2019. 24 (3984): 1-13

[13] Ulhaq, Z.S., Hendyatama, T.H., Hameed, F., Santosaningsih, D. Antibacterial Activity of *Citrus hystrix* toward *Salmonella spp.* Infection. Enfermedades Infecciosas Microbiologia Clinica. 2020. 5 (16): 1-4

[14] Hussain, R.M., Din, N.A.M., Nasir, N.A.N.M. *Piper betle* Ehtanolic Extract Reduces Neutrophil Scavenging Ability and Possibly Catalase Activityin in *S. aureus.* Int. J. Pharm. Sci. Rev. Res. 2013. 22(1): 35-40

[15] Das, S., Parida, R., Sandeep, I.S., Nayak, S., Mohanty, S. Biotechnological Intervention in Betelvine (*Piper bettle* L.): A Review on Recent Most Advances and Future Prospects. Asia Pacific Journal of Tropical Medicine. 2016. 9 (10): 938-946