ORIGINAL PAPER

doi: 10.5455/medarh.2023.77.451-454
MED ARCH. 2023; 77(6): 451-454
RECEIVED: OKT 16, 2023
ACCEPTED: NOV 21, 2023

Coleus Amboinicus Lour. Leaf Extract as an Antioxidant in Sepsis

Mutiara Indah Sari[1], R. Lia Kusumawati[2], Yunita Sari Pane[3], Sufitni Sufitni[4]

[1]Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

[2]Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

[3]Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

[4]Department of Anatomy, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Corresponding author: dr. Mutiara Indah Sari, M.Kes, Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara. Address: Jl. Dr. Mansyur No. 5, 20155, Medan, Indonesia. Phone: +62 82166260088. E-mail address: mutiara@usu.ac.id ORCID ID: https://orcid.org/0000-0001-6510-2196

ABSTRACT

Background: As broad-spectrum antibiotics can cause antimicrobial resistance in sepsis, there is the need for a complementary therapy to combat sepsis. Oxidative stress causes an increased severity and mortality in sepsis, whereas herbal medicines have been considered as an option due to its antioxidant potential. Coleus amboinicus Lour. has been documented for its therapeutic value due to the presence of flavonoid, an antioxidant compound. **Objective**: To study the effect of Coleus amboinicus Lour. leaf extract on total antioxidant capacity (TAC) and hepatic catalase (CAT) levels in septic rat model. **Methods:** Twenty-eight male Rattus norvegicus rats were divided into four groups: control (rats without sepsis induction and treatment), group 1 (septic rats treated with antibiotics), group 2 (septic rats treated with antibiotics and 250 mg/kg body weight of Coleus amboinicus Lour. leaf extract), and group 3 (septic rats treated with antibiotics and 500 mg/kg body weight of Coleus amboinicus Lour. leaf extract). The rats were sacrificed at the end of the eighth day of observation, and blood and liver tissues were gathered for examination. **Results**: Compared to the septic rat groups treated with only antibiotics, there was an increase in the TAC levels and CAT expression levels in septic rat groups given antibiotics and Coleus amboinicus Lour. leaf extract. However, the increase was not significant. **Conclusion:** Administering Coleus amboinicus Lour. leaf extract increases TAC levels and CAT expression levels in sepsis, decreasing oxidative stress. This will exert protective effects in the cells and therefore alleviate sepsis.

Keywords: antioxidant, Coleus amboinicus Lour., herbal, sepsis.

1. BACKGROUND

The standard therapy for sepsis according to the 2016 Surviving Sepsis Campaign (SSC) is an intravenous administration of broad-spectrum antibiotics (1). However, broad-spectrum antibiotics can cause antimicrobial resistance. Cases of multidrug-resistant (MDR) pathogens are increasing worldwide and estimated to cause approximately ten million deaths annually by 2050 (2). Therefore, there is the need for a complementary therapy to combat sepsis. A central mechanism that causes an increased severity and mortality in sepsis is the imbalance between the oxidant potential and the antioxidant defense system. In sepsis, the antioxidant defense system fails to counter the excess reactive oxygen species (ROS), leading to numerous physiological impairments. As a result, targeting oxidative stress has been considered as a therapeutic option in sepsis (3,4). While the usage of herbal medicines is notable in ancient cultures in Asia, in the last two decades, they have been considered as a complementary therapy. Herbal plants have been reported to exhibit antioxidant potential. Polyphenols, such as tannin, flavonoid, and phenolic acids, have been associated with the radical scavenging and antioxidant defense system enhancing potential of herbal medicines (5-7).

Coleus amboinicus Lour. or Plectranthus amboinicus Lour. Spreng is one of the species from the family Lamiaceae and known as bangunbangun or torbangun or cumin in Indonesia (8). The documentation of this plant has been attributed to its therapeutic value due to its common usage in folk medicines and the presence of important antioxidant compounds in the plant, such as flavonoid (9,10). A study shows that Coleus amboinicus Lour. leaf extract restores the antioxidant defense system in diabetic rat models through increasing blood glutathione (GSH) levels (11).

2. OBJECTIVE

We aim to study the effect of Coleus amboinicus Lour. leaf extract on total antioxidant capacity (TAC) and hepatic catalase (CAT) levels in septic rat model.

3. MATERIAL AND METHODS

Ethical approval and research location

This study is approved by Health Research Ethics Committee of Universitas Sumatera Utara (USU) with Letter No. 711/KEP/USU/2021. This research was conducted in the pharmacy laboratory in the Faculty of Pharmacy of Universitas Sumatera Utara as well as the pharmacology laboratory, integrated laboratory, and biochemistry laboratory in the Faculty of Medicine of Universitas Sumatera Utara.

Extract gathering and preparation

Coleus amboinicus Lour. is taken from Tanah Karo Berastagi, Medan, North Sumatra and identified in the pharmacy laboratory of USU faculty of pharmacy. The extract was made from dry powder by maceration using 70% ethanol. It was then soaked for 6 hours while stirred and kept in place for 18 hours. Then a centrifugation was performed to separate the powder from the solvent. This process was repeated at least once with the volume of solvent half the volume of the first dilution. The macerate was then evaporated with a low pressure vacuum or a rotary evaporator was used until a thick extract is obtained (12). Then we performed a phytochemical screening on the extract following standardized methods (12-15) to identify the presence of alkaloid, flavonoid, glycoside, saponin, tannin, and triterpenoid.

Research sample

The research population was male Rattus norvegicus rats. Samples were selected randomly with the inclusion criteria: male rats aged 10-12 weeks, body weight of 200-300 grams, and in good health. The exclusion criteria were rats that died during the study. The rats were taken from the pharmacology laboratory of USU faculty of medicine. Each rat was placed in a cage at 27 ± 5oC with a 12 h light/dark cycle of lighting. Animals were given the same standard laboratory treatment and feed for one week prior to the experiment.

Based on the Federer formula adjusted to the probability of loss to follow-up or drop out, the amount of sample is 28 rats in total, with 7 rats per group. The rats were divided into following groups: control (rats without sepsis induction and treatment), group 1 (rats with sepsis induction + 25 mg/kg body weight of imipenem cilastatin intraperitoneally), group 2 (rats with sepsis induction + 25 mg/kg body weight of imipenem cilastatin intraperitoneally on day 1-3 + 250 mg/kg body weight of Coleus amboinicus Lour. leaf extract orally on day 1-8), and group 3 (rats with sepsis induction + 25 mg/kg body weight of imipenem cilastatin intraperitoneally on day 1-3 + 500 mg/kg body weight of Coleus amboinicus Lour. leaf extract orally on day 1-8).

Sepsis induction and blood collection

The sepsis induction in this study was carried out using fecal slurry at a dose of 1 g/kg body weight of rats. The feces were suspended in saline to a concentration of 90 mg/ml and kept in place for 24 hours at 4°C (16). The injection was performed in the right lower quadrant of the abdomen using a 21 G cannula.

At the end of the eighth day of observation, the rats were anesthetized with 80-100 mg/kg ketamine and 10-12.5 mg/kg xylazine. They were then sacrificed via cardiac puncture using a 27G needle and 3 mL syringe. The blood gathered from the heart puncture was centrifuged to separate the serum and stored at -80 °C until the analysis. A post-mortem laparotomy was also performed to collect the liver tissues.

TAC levels examination

On the eighth day the rats were terminated to collect the serum. Serum was centrifuged at 2000-3000 RPM for 20 minutes at room temperature, and the supernatant was used for the ELISA procedure. TAC levels were examined using the kit (MAK187-1KT, Sigma-Aldrich) according to the manufacturer's instructions. The Optical Density value used was set at 450 nm.

Measurement of CAT expression level in the liver tissues

Total mRNA was extracted from liver tissue with RNAzol RT reagent (Sigma-Aldrich, USA) to determine expression levels. Total RNA was converted to cDNA with a reverse transcriptase kit (SensiFast cDNA Synthesis Kit, Bioline, USA) to create a template for RT-PCR. The master mix components consist of SsoFast EvaGreen SuperMix, USA, forward primers and reverse cDNA template samples and beta actin (SensiFast cDNA Synthesis Kit, Bioline, USA) and nuclease free water. The master mix volume and the primer sequences for CAT are based on a past study (17).

Statistical analysis

We used the Statistical Package for the Social Studies (SPSS) software to analyze the differences in TAC levels and CAT expression levels in all groups with ANOVA test. $p < 0.05$ indicates significance.

4. RESULTS

Phytochemical screening of Coleus amboinicus Lour.

The phytochemical screening of Coleus amboinicus Lour. leaf extract shows the presence of alkaloids, flavonoids, glycoside, saponin, and triterpenoid.

Analysis of TAC levels and CAT expression levels

On examination, group 2 and 3 showed an increase in TAC levels compared to group 1 (Figure 1). There was no significant difference in the increase of the TAC levels in the septic rat groups given Coleus amboinicus Lour. leaf extract, with $p > 0.05$ ($p = 0.511$).

Group 2 and 3 also showed an increase in CAT expression levels compared to group 1 (Figure 2). There

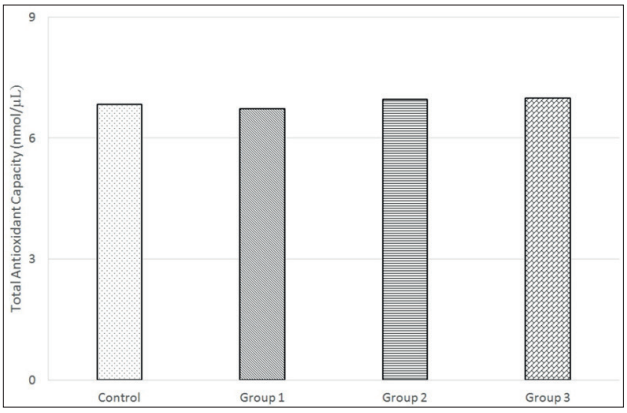

Figure 1. TAC levels of all rat groups

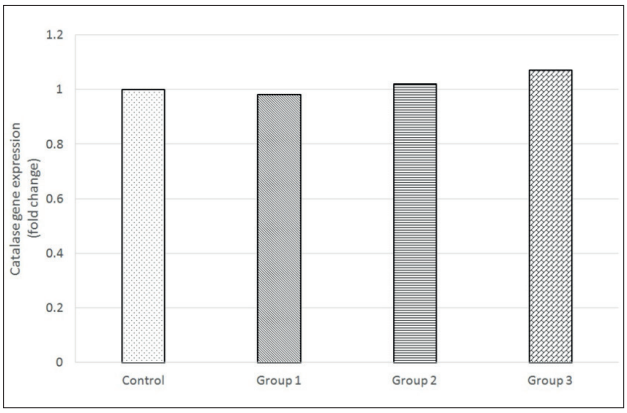

Figure 2. Hepatic CAT expression levels of all rat groups

was no significant difference in the increase of the CAT expression levels in the septic rat groups given Coleus amboinicus Lour. leaf extract, with $p > 0.05$ ($p = 0.175$).

5. DISCUSSION

In this study, we found that administering Coleus amboinicus Lour. leaf extract increased the TAC levels in sepsis-induced rats, although the increase is not significant. TAC, or non-enzymatic antioxidant capacity (NEAC), is defined as the moles of oxidants neutralized by one liter of body fluids. Radical scavenging or reducing capacity, including single electron transfer (SET) and hydrogen atom transfer (HAT), is measured in TAC assays (18).

One of the compounds found in the Coleus amboinicus Lour. leaf extract in our study is flavonoid, an antioxidant compound. Alkaloid and terpenoid, which are also found in the Coleus amboinicus Lour. leaf extract in our study, are classes of flavonoid (19). The antioxidant mechanism of flavonoid includes direct scavenging of ROS and inhibiting the enzymes or binding the elements involved in free radical formation. Altogether, these mechanisms improve TAC levels (18-20).

We also evaluated the CAT expression levels in the rat liver. Oxidative stress primarily targets the liver and damages the parenchymal cells, though Kupffer cells, hepatic stellate cells, and endothelial cells are vulnerable to it as well (2,21). In this study, the CAT expression levels in the liver were found decreased in the sepsis-induced rat group with only antibiotics treatment. Meanwhile, the sepsis-induced rat groups treated with antibiotics and Coleus amboinicus Lour. leaf extract showed an increase in the CAT expression levels in the liver compared to the control group, although not significant.

Flavonoid has been reported to enhance the antioxidant defense system through increasing the activity of antioxidant enzymes, including superoxide dismutase (SOD), CAT, glutathione (GSH), and glutathione peroxidase (GSH-Px) (7). A study showed that co-treatment with the flavonoid-rich Bridelia tomentosa leaf extract increased the levels of SOD, CAT, and GSH-Px in the liver in rat model of carbofuran-induced oxidative stress-mediated hepatotoxicity (22). Another study shows that flavonoid administration increases the CAT levels in rat model of arsenic-induced hepato-renal toxicity (23).

CAT is part of the antioxidant defense system in the body and can be found in most living tissues. This antioxidant enzyme protects cells by degrading or reducing H2O2 into water and oxygen with either iron or manganese as a cofactor. Although high levels of H2O2 are harmful to cells, at low amounts these molecules play a role in several physiological processes, such as cell proliferation and death, carbohydrate metabolism, mitochondrial function, and platelet activation (7,24). This suggests that extremely high levels of CAT are detrimental to the body. In our study, we found that the increase in CAT expression levels in the sepsis-induced rat groups treated with antibiotics and Coleus amboinicus Lour. leaf extract was not significant.

6. CONCLUSION

Administering Coleus amboinicus Lour. leaf extract increases TAC levels and CAT expression levels in sepsis, leading to a decrease in oxidative stress via scavenging and reduction of free radicals, as well as inhibition of free radical formation. This will exert protective effects in the cells and therefore alleviate sepsis.

- **Declaration of subject consent**: the authors certify that they have obtained all appropriate subject consent forms. In the form, the subjects have given their consent for their images and other clinical information to be reported in the journal.
- **Acknowledgments**: We would like to thank the Chancellor of Universitas Sumatera Utara, the head of the pharmacy laboratory in the Faculty of Pharmacy of Universitas Sumatera Utara as well as the head of pharmacology laboratory, integrated laboratory, and biochemistry laboratory in the Faculty of Medicine of Universitas Sumatera Utara
- **Authors's Contributions**: MIS conceptualized the study and contributed to the manuscript preparation. RLK and YSP gathered and analyzed the data. SS contributed to the manuscript revision. All authors have approved the final version of the manuscript
- **Conflict of interest**: There are no conflict of interest.
- **Financial support and sponsorship**: This study was supported by Universitas Sumatera Utara, Indonesia through the 2021 "Talenta" Research Grant No. 6789/UN5.1.R/PPM/2021, June 16, 2021.

REFERENCES

1. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International

Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017 Mar; 43(3): 304-377. doi: 10.1007/s00134-017-4683-6
2. Martínez ML, Plata-Menchaca EP, Ruiz-Rodríguez JC, Ferrer R. An approach to antibiotic treatment in patients with sepsis. J Thorac Dis. 2020 Mar; 12(3): 1007-1021. doi: 10.21037/jtd.2020.01.47
3. Abelli J, Méndez-Valdés G, Gómez-Hevia F, Bragato MC, Chichiarelli S, Saso L, Rodrigo R. Potential Antioxidant Multitherapy against Complications Occurring in Sepsis. Biomedicines. 2022 Dec 1; 10(12): 3088. doi: 10.3390/biomedicines10123088
4. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015 Jun 5; 97: 55-74. doi: 10.1016/j.ejmech.2015.04.040
5. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, RamaKrishna S, Berto F. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants (Basel). 2020 Dec 21; 9(12): 1309. doi: 10.3390/antiox9121309
6. Hassen G, Belete G, Carrera KG, Iriowen RO, Araya H, Alemu T, Solomon N, Bam DS, Nicola SM, Araya ME, Debele T, Zouetr M, Jain N. Clinical Implications of Herbal Supplements in Conventional Medical Practice: A US Perspective. Cureus. 2022 Jul 15; 14(7): e26893. doi: 10.7759/cureus.26893
7. Čobanová K, Váradyová Z, Grešáková Ľ, Kucková K, Mravčáková D, Várady M. Does Herbal and/or Zinc Dietary Supplementation Improve the Antioxidant and Mineral Status of Lambs with Parasite Infection? Antioxidants (Basel). 2020 Nov 24; 9(12): 1172. doi: 10.3390/antiox9121172
8. Lukhoba CW, Simmonds MSJ, Paton AJ. Plectranthus: Sebuah tinjauan penggunaan etnobotani. J Etnofarmaka. 2006; 103: 1–24. doi: 10.1016/j.jep.2005.09.011
9. Arumugam G, Swamy MK, Sinniah UR. Plectranthus amboinicus (Lour.) Spreng: Botanical, Phytochemical, Pharmacological and Nutritional Significance. Molecules. 2016 Mar 30; 21(4): 369. doi: 10.3390/molecules21040369
10. Bhatt P, Gilbert SJ, Pradeep SN, Mandyam CV. Chemical composition and nutraceutical potential of indian borage (Plectranthus amboinicus) stem extract. J Chemistry. 2013; 1: 1-7. doi: 10.1155/2013/320329
11. El-hawary SS, El-sofany RH, Abdel-Monem AR, Ashour RS, Sleem AA. Polyphenolics content and biological activity of Plectranthus amboinicus (lour.) spreng growing in Egypt (Lamiaceae). Pharmacogn J. 2012; 4(32): 45-54. doi: 10.5530/pj.2012.32.9
12. Ditjen POM. Farmakope Indonesia. 4th ed. Jakarta: Departemen Kesehatan RI; 1995.
13. Depkes RI. Materia Medika Indonesia. 6th Ed. Jakarta: Departemen Kesehatan RI; 1989.
14. Harborne JB. Metode Fitokimia. 2nd Ed. Bandung: Penerbit ITB; 1987.
15. Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH, Chen CY. Analgesic and Antiinflammatory Activities of the Aqueous Extract from Plectranthus amboinicus (Lour.) Spreng. Both In Vitro and In Vivo. Evid Based Complement Alternat Med. 2012; 2012: 508137. doi: 10.1155/2012/508137
16. Fang H, Gong C, Fu J, Liu X, Bi H, Cheng Y, Liu Y, Tang Y, Wang D. Evaluation of 2 Rat Models for Sepsis Developed by Improved Cecal Ligation/Puncture or Feces Intraperitoneal-Injection. Med Sci Monit. 2020 Jan 29; 26: e919054. doi: 10.12659/MSM.919054
17. Prasetyastuti, Rubi DS, Pramana AAC, Sadewa AH. Correlation between ratio of Nrf2/Keap1 and catalase gene expression in liver of hyperlipidemic rats after administration of 7-hydroxy-2-(4-hydroxy-3methoxyphenyl)-chromen-4-one. J Med Sci. 2018; 51(1): 24-301. doi: 10.19106/JMedSci005101201903
18. Marrocco I, Altieri F, Peluso I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. Oxid Med Cell Longev. 2017; 2017: 6501046. doi: 10.1155/2017/6501046
19. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016 Dec 29; 5: e47. doi: 10.1017/jns.2016.41
20. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. 2013 Dec 29; 2013: 162750. doi: 10.1155/2013/162750
21. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol. 2014 Jul 7; 20(25): 8082-8091. doi: 10.3748/wjg.v20.i25.8082
22. Mondal M, Hossen MS, Rahman MA, Saha S, Sarkar C, Bhoumik NC, Kundu SK. Antioxidant mediated protective effect of Bridelia tomentosa leaf extract against carbofuran induced oxidative hepatic toxicity. Toxicol Rep. 2021 Jul 8;8:1369-1380. doi: 10.1016/j.toxrep.2021.07.003
23. Ugbaja RN, Akinhanmi TF, James AS, Ugwor EI, Babalola AA, Ezenandu EO, Ugbaja VC, Emmanuel EA. Flavonoid-rich fractions from Clerodendrum volubile and Vernonia amygdalina extenuates arsenic-invoked hepato-renal toxicity via augmentation of the antioxidant system in rats. 2021 Feb; 35: 12-25. doi: 10.1016/j.nutos.2020.12.003
24. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018 Dec; 54(4): 287-293. doi: 10.1016/j.ajme.2017.09.001s