DOI: https://doi.org/10.30742/jv.v14i1.285 -ISSN 2685-8894; p-ISSN 2460-9773
Jurnal Vitek Bidang Kedokteran Hewan Vol.14 No.1, Mei 2024

The effect of *Averrhoa bilimbi* extract on the histopathology of duodenum in rats infected with *Escherichia coli*

Raynard Putra Umbu Pidi[1], Aldin Akbar Rahmatullah[2*], Nurul Hidayah[3], Indra Rachmawati[4], Yos Adi Prakoso[5]

[1345] Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya
[2*] Faculty of Veterinary Medicine, Universitas Airlangga
*email: aldin.akbar01@yahoo.com

Received : 28 April 2024
Accepted : 10 Mei 2024
Published : 07 Juni 2024

Abstract

This study aimed to prove the efficacy of Averrhoa bilimbi extract on histopathological changes in the duodenum of rats infected with Escherichia coli. It was a laboratory experimental study involving 30 male white rats aged 3 months, weight ±250 g divided into six treatment groups: P0 (no E. coli induction + no Averrhoa bilimbi extract), P1 (E. coli induction + no Averrhoa bilimbi extract), and P2, P3, P4, and P5 (E. coli induction + various concentrations of Averrhoa blimbi extract 20%, 30%, 50%, and 60% respectively). All treatments lasted for 14 days. On the 15th day, necropsy was performed to collect duodenal tissues, which were then histologically examined using Hematoxylin-Eosin staining. Microscopic observations focused on detecting necrosis, inflammatory cell infiltration, and hemorrhage in the duodenal villi. Data were analyzed using the Kruskall-Wallis test continued with the Mann-Whitney test, revealing significant differences between treatments ($P < 0.05$). The result show that the Averrhoa bilimbi extract did not demonstrate preventive effects against duodenal villi damage, including necrosis, inflammatory cell infiltration, and hemorrhage. In conclusion, the administration of Averrhoa bilimbi extract to rats infected with Escherichia coli did not show effectiveness in preserving the histopathological integrity of the duodenal small intestine in white rats.

Keywords: *Averrhoa bilimbi, Duodenum, Escherichia coli, Rattus norvegicus.*

INTRODUCTION

Escherichia coli bacteria are bacteria from the genus Escherichia that produce toxins and disrupt the digestive system (Peng et al., 2024). Several types of *Escherichia coli* bacteria that cause disorders in the digestive system are Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), and Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) (Sania et al., 2020). *Escherichia coli* bacterial toxins will cause an inflammatory reaction in the digestive system. This reaction is influenced by genetic factors, imbalanced immune responses, and the presence of gastroenteritis disturbances (Sobrinho et al, 2020). During inflammation caused by foreign bodies or microorganisms such as bacteria, viruses, and parasites, the body will respond by phagocytosis as a defense against infectious agents (Purnamasari et al., 2014).

The first immune response that occurs during bacterial invasion is neutrophils (Malech et al., 2020). Neutrophils will respond to acute inflammation with an increase in the number of neutrophils in the blood (Bongers et al., 2021). Then the remnants of dead and damaged cells will be digested by monocytes and respond to disease-causing organisms with immunological resistance (Tethool, 2015). In the process of phagocytosis of foreign bodies or microorganisms called antigens, monocytes will produce free radicals that react with bacterial cell membranes and damage bacterial cells (Supriyana et al., 2019). In enhancing the immune response or the body's defense against foreign bodies and microorganisms through the administration of immunostimulants as an additional therapy for preventive measures in disease prevention and increasing immunity (Aldi, 2016).

There are two groups of immunostimulants known as biological and synthetic immunostimulants. Among

biological immunostimulants are phytochemicals and vitamin C contained in herbal plants (Kumar et al., 2022). One of the utilized plants is *Averrhoa bilimbi*. This tropical fruit that can bear fruit all year round originates from the genus Averrhoa, which can grow in backyards or forests (Fahrunida et al., 2015). From the fruit extract, *Averrhoa blimbi* is beneficial as an immunomodulator, anti-inflammatory, antioxidant, antibacterial, antimycotic, analgesic, sedative, antidiabetic, antifertility (Alhassan et al., 2016).

MATERIALS AND METHODS

This research was conducted at the Bacteriology and Mycology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga to obtain *E. coli* bacterial isolates. Animal Research Facility of the Faculty of Veterinary Medicine, Universitas Airlangga to conduct the treatment. Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya for termination and data collection. Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga for the preparation of histopathological slides and interpretation of results. The research was carried out from February to March 2021.

This study was an experimental research using the Completely Randomized Design (CRD) method with random sampling technique. Thirty male rats aged 3 months with a weight of ±250 grams were used, divided into 6 groups with 5 rats per group. Group P0 was without *E. coli* induction and *Averrhoa Bilimbi* extract administration,

Group P1 was only induced with *E. coli* 10^6 cfu/ml at 1 ml/day, Groups P2, P3, P4, and P5 were induced with E. coli 10^6 cfu/ml at 1 ml/day and given *Averrhoa bilimbi* extract in escalating doses (20% in P2, 30% in P3, 50% in P4, and 60% in P5). Bacteria were administered via intraperitoneal injection and *Averrhoa Bilimbi* extract was given orally. This treatment was conducted for 14 days, and on the 15th day, the rats were terminated by

cervical dislocation and laparotomy was performed to collect the duodenum organ, which was then immersed in 10% formalin phosphate buffer solution. The duodenum tissue was processed and histological slides were made using Hematoxylin Eosin (HE) staining.

Histopathology of the duodenum was examined using a microscope with magnifications of 40x, and 100x to detect the necrosis, inflammatory cell infiltration and hemorrhage in the duodenal villi. The data obtained in the form of values on changes in the duodenum histopathological images of white rats (*Rattus norvegicus*) were arranged in tabular form and then analyzed. To find out different changes in the duodenum histopathological images of white rats, statistical tests were carried out using the Kruskal-Wallis test. The degree of change was processed using ranking research and if there was a significant difference, it continued with the Mann-Whitney test. All analyses were performed using computer software (Imalaya *et al*., 2023).

RESULTS AND DISCUSSION

The results of data analysis in the study on the effectiveness of *Averrhoa bilimbi* extract on the histopathological appearance of the duodenum, including necrosis, inflammatory cell infiltration, and hemorrhage in the duodenal villi of rats (*Rattus norvegicus*) infected with *Escherichia coli*, showed significant differences among treatments (Table 1).

Tabel 1. Mean scores of histopathological findings in the duodenum of rats (*Rattus norvegicus*) infected with *Escherichia coli* after administration of extract *Averrhoa bilimbi*.

Group	Necrosis	Inflammation cells infiltration	Haemorrhage
P0	2.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.50 ± 1.73^{a}
P1	2.50 ± 1.00^{ab}	1.75 ± 0.50^{b}	5.00 ± 0.00^{b}
P2	3.50 ± 1.00^{bc}	2.00 ± 0.00^{b}	5.00 ± 0.00^{b}
P3	4.00 ± 0.00^{c}	2.00 ± 0.00^{b}	5.00 ± 0.00^{b}
P4	4.00 ± 0.00^{c}	2.00 ± 0.00^{b}	5.00 ± 0.00^{b}
P5	4.00 ± 0.00^{c}	2.00 ± 0.00^{b}	5.00 ± 0.00^{b}

Note: Different superscripts a, b, c indicate significant differences among treatments ($p < 0.05$).

The analysis of data in Table 1 shows that the scores of cells experiencing necrosis in the

duodenal villi exhibit significant differences among treatments. Specifically, treatment P0 significantly differs from treatments P2, P3, P4 and P5 but not from treatments P1. Treatment P1 significantly differs from P3, P4 and P5 but not from P0 and P2. Histopathological images of cells experiencing necrosis in each treatment can be seen in Figure 1.

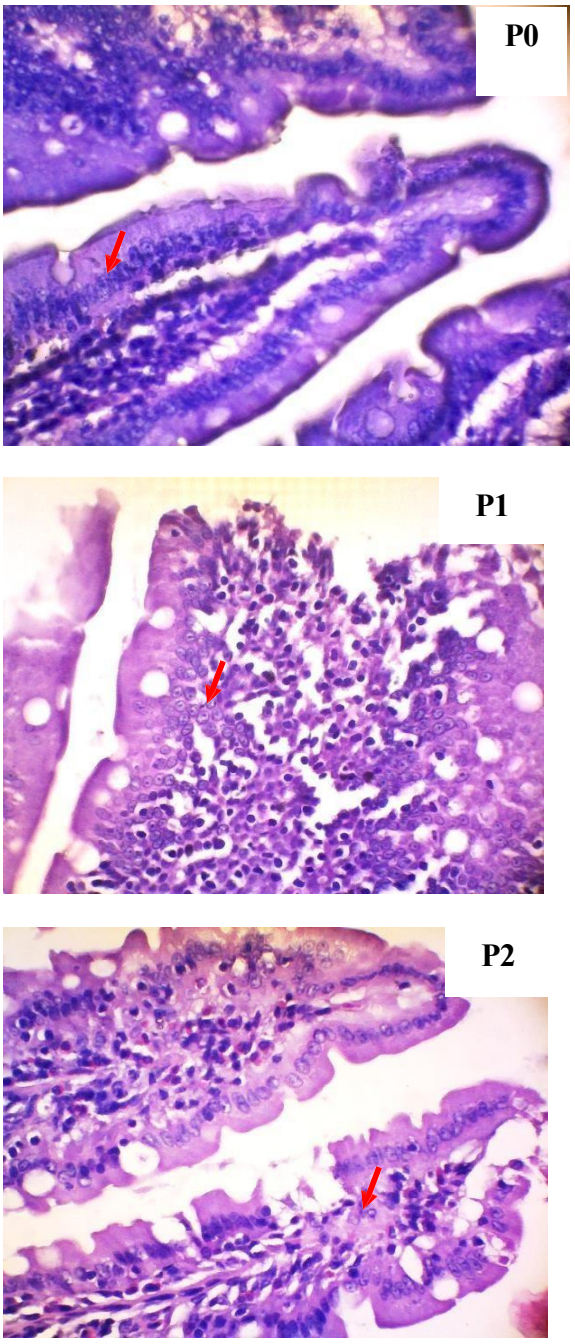

Figure 1. Histopathology of the duodenal villi of rats (*Rattus norvegicus*) infected with *Escherichia coli* after administration of *Averrhoa bilimbi* extract. Magnification 100x. Hematoxylin-Eosin staining. Cells undergoing necrosis karyorrhexis (red arrows).

Scores of cells undergoing inflammatory cell infiltration in the duodenal villi show significant differences among treatments (Table 1). Specifically, treatment P0 significantly differs from all other treatments, but there are no significant differences among

treatments P1, P2, P3, P4, and P5. Histopathological images of cells undergoing inflammatory cell infiltration in each treatment can be seen in Figure 2.

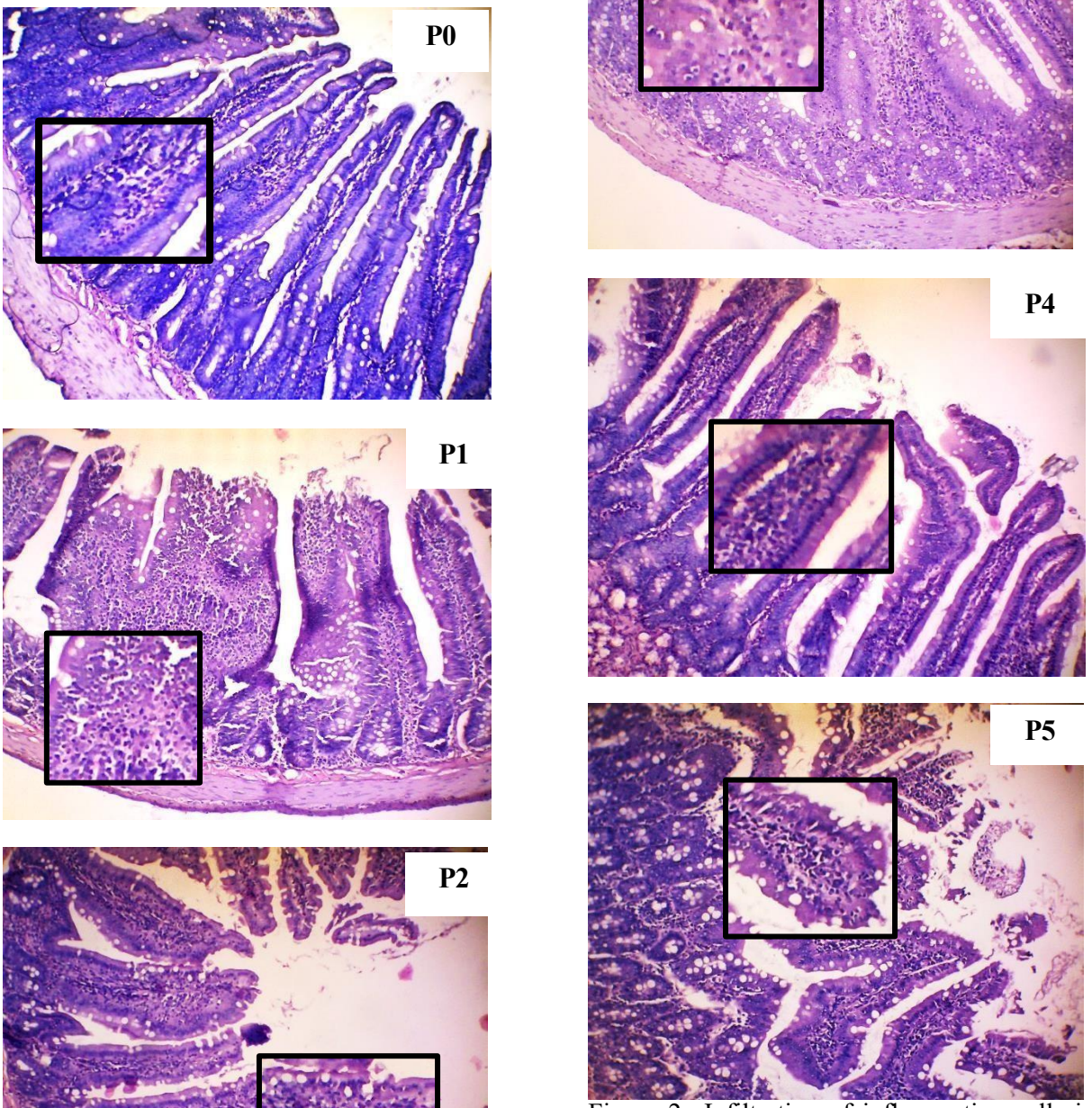

Figure 2. Infiltration of inflammation cells in the duodenal villi of rats (*Rattus norvegicus*) infected with *Escherichia coli* after administration of *Averrhoa bilimbi* extract. Magnification 400x. Hematoxylin-Eosin staining

Scores of hemorrhage in the duodenal villi indicate significant differences among treatments ($P < 0.05$) (Table 1). Treatment P0 significantly differs from all other treatments, while treatment P1 does not significantly differ from treatments P2, P3, P4, and P5

given *Averrhoa bilimbi* extract. Histopathological images of hemorrhage in each treatment can be seen in Figure 3.

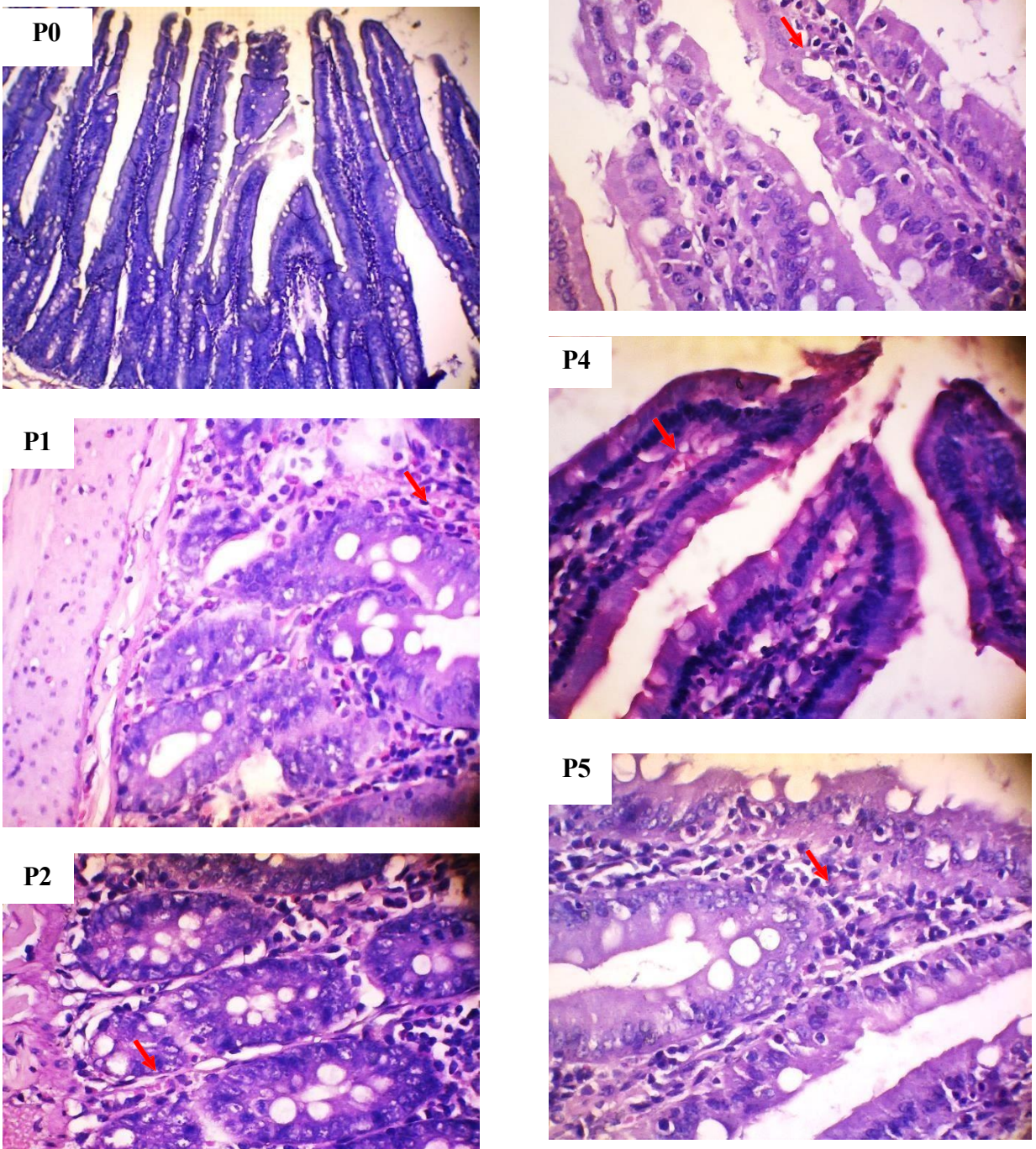

Figure 3. Histopathology of the duodenal villi of rats (*Rattus norvegicus*) infected with *Escherichia coli* after administration of *Averrhoa bilimbi* extract. Magnification 40x and 100x. Hematoxylin-Eosin staining. Haemorrhage (red arrow).

Wiadnyana et al. (2015) state that histological changes in the intestines can provide insights into their ability to digest food

and the effects thereof. Damage to the intestines can be caused by microbes, pathogens, and toxic substances entering the intestines. Histopathological observations of the duodenum of rats (*Rattus norvegicus*) infected with *Escherichia coli* after the administration of *Averrhoa bilimbi* extract have a significant impact. In treatment P1, where rats were infected with *Escherichia coli* without the administration of *Averrhoa bilimbi* extract, shortened and damaged villus surface observed. In contrast, in treatment P0, where rats were not infected with *Escherichia coli* and received no *Averrhoa bilimbi* extract, and in treatments P2, P3, P4, and P5, where rats were infected with *Escherichia coli* after the administration of *Averrhoa bilimbi* extract, the mucosa in the duodenal villi appears normal.

However, in the bacterial infection treatment, damage to the cells in the duodenal small intestine of rats is evident. Despite being infected with *Escherichia coli* after the administration of *Averrhoa bilimbi* extract, the intestinal mucosa appears normal due to the presence of flavonoid compounds found in *Averrhoa bilimbi*, which are antibacterial compounds capable of disrupting bacterial synthesis processes, leading to bacterial plasma damage and eventual lysis (Biharee et al., 2020). Additionally, phenolic content in *Averrhoa bilimbi* can interfere with bacterial growth by denaturing proteins and damaging cell membranes (Nassarawa et al., 2023).

Histological changes occurring in the duodenal of rats include necrosis, inflammatory cell infiltration, and hemorrhage, which are observed in all treatments. However, treatment P0 exhibits the lowest damage scoring, followed by treatment P1. Treatments accompanied by the administration of *Averrhoa bilimbi* extract (P2, P3, P4, and P5) show higher histopathological changes scores compared to those without the administration of *Averrhoa bilimbi* extract.

The most severe necrosis in the duodenal villi was found with the highest scores in treatments P3, P4 and P5. Necrosis is characterized by damaged intestinal tissue, which may be caused by the presence of bioactive compounds found in *Averrhoa bilimbi*. Prolonged use of high doses *Averrhoa bilimbi* which contains antioxidant can lead to cell wall damage and disruption duodenal tissue, resulting in tissue necrosis (Dutta et al, 2022). This is evidenced by the administration of *Averrhoa bilimbi* extract at concentrations of 30-60%, resulting in high necrosis scores. Although *Averrhoa bilimbi* extract has antimicrobial potential such flavonoid, oxalic acid, tannin, ascorbic acid, saponin, and alkaloid (Prastiyanto et al., 2020). Its bioactive components, such as oxalic acid and ascorbic acid, can become toxic and irritating when administered in excessively high doses. These bioactive components can also disrupt the integrity of epithelial cells in the duodenum, especially if the tissue is already infected and experiencing oxidative stress (Thakur et al., 2019).

Additionally, the immunomodulatory effects of Averrhoa bilimbi extract component such flavonoid, ascorbic acid, tannin, saponin, and alkaloid can exacerbate the inflammatory response in the duodenal mucosa (de Barros Cardoso et al., 2019). Induction of E. coli in rats, which causes an inflammatory reaction, combined with the administration of *Averrhoa bilimbi* extract, will enhance the inflammatory response, further aggravating the damage to the duodenal tissue. However, treatment P0 had low scoring because it consisted of healthy white rats without treatment or *E. coli* infection. Nevertheless, necrotic cells were still found in treatment P0, as D'arcy (2019) suggest that cells in normal conditions can undergo necrosis, although this is not pathological. Necrosis may occur due to simultaneous body disturbances leading to cell damage.

Inflammatory cell infiltration is another histopathological change observed in this study. However, the lowest scoring for inflammatory cell infiltration was observed in treatment P0 compared to *E. coli* infection treatments in white rats (P1, P2, P3, P4, and P5). Inflammatory cell infiltration in the duodenal small intestine of white rats occurs due to *E. coli* infection. The bacterial cell wall of *E. coli* contains α-hemolysin (HlyA) protein and lipopolysaccharide (LPS), which can cause tissue damage (Jiang et al., 2021). HlyA protein and LPS entering host cells are perceived as foreign proteins, triggering a host immune response. This immune response increases cytokine production, leading to

interleukin (IL-6 and IL-8) production, triggering an inflammatory response by infiltrating inflammatory cells such as leukocytes and macrophages (Ledwaba et al, 2020).

Histopathological changes such as hemorrhage in the duodenal of rats occurred in all treatments, with the lowest scoring in P0 due to the absence of treatment and *E. coli* infection compared to treatments with treatment and *E. coli* infection (P1, P2, P3, P4, and P5). Bader et al. (2023) reported that hemorrhage or bleeding observed in small intestine tissues indicates vascular or organ damage caused by viruses or other microorganisms. This condition can disrupt the biochemical processes of the organ, ultimately leading to disturbances in carbohydrate, protein, and lipid metabolism in cells. Intracellular metabolic disturbances eventually lead to structural changes in cells.

Based on Agnesa et al. (2017), when *E. coli* enters the small intestine, goblet cells produce mucus, a fluid that functions to expel foreign bodies such as pathogenic bacteria. If the mucus fails to expel pathogenic bacteria *E. coli*, it will persist and penetrate the intestinal epithelial cells, breaking through the top layer of villi. Its growth is inhibited by lymphocytes, but if lymphocytes fail to inhibit *E. coli* growth, the bacteria will enter the bloodstream, causing bleeding within the blood vessels.

CONCLUSION

Administration of *Averrhoa bilimbi* extract to rats (*Rattus norvegicus*) infected with Escherichia coli is deemed ineffective based on the histopathological observations of the duodenal in rats (*Rattus norvegicus*), encompassing necrosis, inflammatory cell infiltration, and hemorrhage.

REFERENCES

Agnesa, O. S., Susilo, H., & Lestari, S. R. 2017. Aktivitas imunostimulan ekstrak bawang putih tunggal pada mencit yang diinduksi Escherichia coli. Pharmaciana, 7(1), 105.

Aldi,Y. 2016. *Uji Efek Imunomodulator Dari Ekstrak Daun Manggis (Garcinia Mangostana L.) Dengan Metode Carbon Clearence Dan Menghitung Jumlah Leukosit Pada Mencit Putih Jantan*. Jurnal farmasi. 8(1)

Alhassan, A. M, Ahmed Q. U. 2016. *Averrhoa bilimbi Linn.: A Review of its Ethnomedicinal Uses, Phytochemistry, and Pharmacology.* J Pharm Bioallied Sci. 8(4):265–71.

Bader, S. M., Preston, S. P., Saliba, K., Lipszyc, A., Grant, Z. L., Mackiewicz, L., Baldi, A., Hempel, A., Clark, M. P., Peiris, T., Clow, W., Bjelic, J., Stutsz, M. D., Arandjelovic, P., Teale, J., Du, F., Coultas, L., Murphy, J. M., Allison, C. C., Pellegrini, M., & Samson, A. L. 2023. Endothelial Caspase-8 prevents fatal necroptotic hemorrhage caused by commensal bacteria. Cell Death & Differentiation, 30(1), 27-36.

Biharee, A., Sharma, A., Kumar, A., & Jaitak, V. 2020. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia, 146, 104720.

Bongers, S. H., Chen, N., van Grinsven, E., van Staveren, S., Hassani, M., Spijkerman, R., Roi, A. T. L. T., Leijte, G. P., Hietbrink, F., Koenderman, L., & Vrisekoop, N. 2021. Kinetics of neutrophil subsets in acute, subacute, and chronic inflammation. Frontiers in immunology, 12, 674079.

D'arcy, M. S. 2019. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell biology international, 43(6), 582-592.

de Barros Cardoso, C. R., de Castro Habka, A., Pinzan, C. F., Oliveira, C. N. S., da Silva, J. L., & Duarte-Silva, M. 2019. Traditional drugs: mechanisms of immunosuppressor and corticosteroid therapies for inflammatory bowel diseases. In Biological Therapy for Inflammatory Bowel Disease. IntechOpen.

Dutta, S., Sengupta, P., Roychoudhury, S., Chakravarthi, S., Wang, C. W., & Slama, P. 2022. Antioxidant paradox in male infertility:'A blind eye'on inflammation. Antioxidants, 11(1), 167.

Fahrunida dan Rarastoeti P. 2015. *Kandungan Saponin Buah, Daun, dan Tangkai Daun Belimbing Wuluh (Averrhoa blimbi L.).* Seminar Nasional

Konservasi dan Pemanfaatan Sumber Daya Alam: 220 – 224.

Imalaya, K. A. W., Widjiati, W., Rahmahani, J., Widiyatno, T. V., Santoso, K. P., Rachmawati, K., & Plumeriastuti, H. 2023. The Effect of Administering Apis dorsata Forest Honey on Liver Histopathological Changes of Rats (Rattus norvegicus) Exposed to Physical Stress. IJSCIA, 4(3): 433-436.

Jiang, L., Zhu, Y., Luan, P., Xu, J., Ru, G., Fu, J. G., Sang, N., Xiong, Y., He, Y., Lin, G., Wang, J., Zhang, J., & Li, R. 2021. Bacteria-anchoring hybrid liposome capable of absorbing multiple toxins for antivirulence therapy of Escherichia coli infection. ACS nano, 15(3), 4173-4185.

Kumar, S., Choubey, A. K., & Srivastava, P. K. 2022. The effects of dietary immunostimulants on the innate immune response of Indian major carp: A review. Fish & Shellfish Immunology, 123, 36-49.

Ledwaba, S. E., Costa, D. V., Bolick, D. T., Giallourou, N., Medeiros, P. H., Swann, J. R., Traore, A. N., Potgieter, N., Nataro, J. P., & Guerrant, R. L. 2020. Enteropathogenic Escherichia coli infection induces diarrhea, intestinal damage, metabolic alterations, and increased intestinal permeability in a murine model. Frontiers in cellular and infection microbiology, 10, 595266.

Malech, H. L., DeLeo, F. R., & Quinn, M. T. 2020. The role of neutrophils in the immune system: an overview. Neutrophil: Methods and Protocols, 3-10.

Nassarawa, S. S., Nayik, G. A., Gupta, S. D., Areche, F. O., Jagdale, Y. D., Ansari, M. J., Hemeg, H. A., Al-Farga, A., & Alotaibi, S. S. 2023. Chemical aspects of polyphenol-protein interactions and their antibacterial activity. Critical Reviews in Food Science and Nutrition, 63(28), 9482-9505.

Peng, Z., Wang, X., Huang, J., & Li, B. 2024. Pathogenic Escherichia coli. In Molecular Medical Microbiology (pp. 1065-1096). Academic Press.

Prastiyanto, M. E., Wardoyo, F. A., Wilson, W., & Darmawati, S. 2020. Antibacterial activity of various extracts of Averrhoa bilimbi against multidrug resistant bacteria. Biosaintifika: Journal of Biology & Biology Education, 12(2), 163-168.

Purnamasari, I. W., Astuti P., Ermawati T. 2014. *Viabilitas neutrofil yang diinkubasi dalam ekstrak rimpang temulawak (CurcumaXanthorrhiza) dan dipapar dengan Streptococcus mutans.* Jurnal Dentofasial. 2014: 13(3): 135- 406.

Raharjaningtyas, E. R. P. 2013. Pengaruh Pemberian Infusa Daun Sirsak (Annona muricata L) Secara Subkronis Terhadap Gambaran Histopatologis Lambung dan Usus Tikus Putih. [Skripsi]. Fakultas Farmasi Universitas Sanata Dharma Yogyakarta.

Sania, E., Sandy VK., Yohanna A. 2020. *Perbandingan Efektivitas Antibakteri Moringa oleifera dan Ziziphus mauritiana dengan Ekstrak Etanol 96% terhadap Escherichia Coli.* Sriwijaya Journal of Medicine 3(1):39-46.

Sobrinho, C. Pimentel., J. Lima Godoi, F. Neves Souza., C. Graco Zeppelini, V. Espirito Santo, D. Carvalho Santiago, R. Sady Alves, H. Khalil, T. Carvalho Pereira, M. Hanzen Pinna., M. Begon ., S. Machado Cordeiro ., J. Neves Reis ., F. Costa . 2020. *Prevalence of Diarrheagenic Escherichia coli (DEC) and Salmonella spp. with zoonotic potential in urban rats in Salvador,* Brazil.Epidemiology & Infection is published by Cambridge University Press.

Supriyana., Endah A., Sadimin., Wahyu J. D. U. 2019. *Kemampuan Obat Kumur Jinten Hitam Sediaan Kantong Celup Terhadap Monosit dan Neutrofil pada Adhesi Streptococcus mutan.* Jurnal Link, 15(2):36-41.

Tethool, S. P. 2015. *Efek Fraksi Etanol Air Rumput Kebar (Byophitum Petersianum Klotzch) Terhadap Diferensiasi Leukosit Kelinci Hiperlipidemia*. Jurnal Ilmu Ternak danTanaman 5 (1).

Thakur, A., Sharma, V., & Thakur, A. 2019. An overview of anti-nutritional factors in food. Int. J. Chem. Stud, 7(1), 2472-2479.

Wiadnyana, I. M. P., Budiasa, K., & Berata, I. K. 2015. Histopatologi Usus Halus Mencit Pasca Pemberian Ekstrak Etanol Daun Ashitaba Buletin Veteriner Udayana 7(1): 73-79.