Amerta Nutrition
e-ISSN: 2580-1163 (Online)
p-ISSN: 2580-9776 (Print)
Tsuraya dkk. | Amerta Nutrition Vol. 9 Issue 2 (June 2025). 350-354

RESEARCH STUDY
English Version
OPEN ACCESS

Effect of Combination of Probiotics, *Ipomoea Batatas*, and Zinc on Thickness of Intestinal Epithelium in Mice Model of Acetic Acid-Induced Colitis

Pengaruh Kombinasi Probiotik, Ubi Jalar Ungu, dan Zinc terhadap Tebal Epitel Usus Mencit Jantan BALB/c Kolitis Ulseratif terinduksi Asam Asetat

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ARTICLE INFO

Received: *08-05-2024*
Accepted: *05-05-2024*
Published online: *20-06-2025*

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DOI:
10.20473/amnt.v9i2.2025.350-354

Available online at:
https://e-journal.unair.ac.id/AMNT

Keywords:
Probiotics, Purple sweet potatoes, Zinc, Epithelial thickness, Colitis

ABSTRACT

Background: The use of probiotics to treat colitis is unsatisfactory, leading to a need for the combination with purple sweet potatoes (Ipomoea Batatas) and zinc.
Objectives: This study aimed to determine the effect of the combination of probiotics, purple sweet potatoes, and zinc on epithelial thickness.
Methods: This was a post-test-only randomized controlled group experimental study using 30 mice divided into six groups, including KN, K-, K+, P1, P2, and P3. All groups except KN were induced with 4% acetic acid on day 1, then each received treatment for five days. KN, K+, P1, and P2 groups were given distilled water, sulfasalazine 1.3 mg/20 g BW, probiotics 0.078 mg/20 gBW + purple sweet potatoes 0.00169 g/20 gBW, and probiotics 0.078 mg/20 gBW + zinc 0.052 mg/20 gBW, respectively. Meanwhile, P3 received a combination of probiotics 0.078 mg/20 gBW + purple sweet potatoes 0.00169 g/20 gBW + zinc 0.0052 mg/20 gBW. Histological preparations were stained with Hematoxylin-Eosin and examined under 400× magnification across 10 fields of view.
Results: The average epithelial thicknesses were KN (75.48±5.09), K− (28.02±5.60), K+ (69.1±13.82), P1 (47.05±9.41), P2 (48.6±9.73), and P3 (71.57±14.31). One-way analysis of variance (ANOVA) showed a significant difference among the groups with a p-value <0.001. Post Hoc LSD test identified significant differences (p-value<0.05) among all group pairs except between K+ and P3 (p-value=0.134) as well as P1 and P2 (p-value=0.349), but P3 had the highest average thickness value (71.57±14.31).
Conclusions: The combination of probiotics, purple sweet potatoes, and zinc had an effect on intestinal epithelial thickness in male BALB/c mice with ulcerative colitis.

INTRODUCTION

Ulcerative colitis is a chronic inflammatory disorder that affects the digestive tract, specifically the colon[1]. This can initiate death, as evidenced by the increasing mortality rate from the disease in the 20th century[2]. Ulcerative colitis is a disorder that causes damage to the digestive tract, changes in intestinal bacteria, and an increased permeability originating from the epithelial barrier disruption[3]. Clinical manifestations that can arise in this disease include bloody diarrhea, severe abdominal pain, mucus discharge from the anus, and tenesmus[4]. The effect of ulcerative colitis damages the intestinal epithelial barrier, leading to increased permeability which is associated with tight junction dysregulation and the capability to promote the absorption of luminal antigens[5].

Alternative treatments that can be provided for ulcerative colitis include probiotics combination. A 2020 study stated that various benefits originated from giving a combination of probiotics at a dose of 6.12 mg/day and zinc at 20 mg/day. The benefits include accelerating the regeneration process of the epithelial layer, improving water and electrolyte absorption, increasing enzyme activity in brush-border enterocytes, as well as enhancing the immune response to eliminate pathogens from the intestine[6].

Probiotics treatment can be combined with purple sweet potatoes (*Ipomoea Batatas*) because of the ability to increase Short Chain Fatty Acid (SCFA) which is useful for acidifying intestinal pH and inhibiting pathogenic bacteria proliferation. Moreover, SCFA in the form of butyrate acts as fuel to provide energy for epithelial cells, increasing the intestinal barrier to avoid the translocation of pathogens and antigens. Purple

How to cite: *Tsuraya, G., Yuniarifa, C., Hussaana, A., Riza, M., & Masfiyah, M. (2025). Effect of Combination of Probiotics, Ipomoea Batatas, and Zinc on Thickness of Intestinal Epithelium in Mice Model of Acetic Acid-Induced Colitis: Pengaruh Kombinasi Probiotik, Ubi Jalar Ungu, dan Zinc terhadap Tebal Epitel Usus Mencit Jantan BALB/c Kolitis Ulseratif terinduksi Asam Asetat. Amerta Nutrition, 9(2), 350–354.*

sweet potatoes contain anthocyanins indirectly associated with the modulation of intestinal microbiota and metabolite production due to bacterial fermentation activity, which improves several parameters related to intestinal health[7]. Therefore, this study aimed to determine the effect of giving a combination of probiotics, purple sweet potatoes, and zinc on intestinal epithelial thickness in BALB/c male mice with Ulcerative Colitis induced by acetic acid.

METHODS

This experimental study used animals in the form of BALB/c male mice, and measurement was conducted with a post-test-only control group design. Furthermore, the population of BALB/c male mice investigated were kept in the Animal House Laboratory IBL FK UNISSULA.

The experimental subjects were 30 healthy active BALB/c male mice, aged two to three months, weighing 20-30 g, and showing no anatomical abnormalities. The exclusion criteria applied were BALB/c male mice with anatomical abnormalities, congenital abnormalities, or physical disabilities, which died during the study. The subjects were divided into six groups consisting of one control and five experimental groups used for effect determination.

Ethical Clearance with Number. 61/II/2024/ was obtained from the Bioethics Commission for Medical/Health Studies, Faculty of Medicine, Sultan Agung Islamic University, and the ethical study prerequisites were met on February 15, 2024. Therefore, the Bioethics Commission recommended the execution of this study by considering the principles stated in the Declaration of Helsinki and the guidelines contained in the National Guidelines for Health Research Ethics (PNEPK) of the Indonesian Ministry of Health in 2004.

Mice in the treatment group were anesthetized with Ketamine 100 mg/kgBW intraperitoneally, and 0.5 ml of 4% acetic acid was given intrarectally on day 1 to produce BALB/c male mice model of colitis. These animals were considered to have colitis when the stool showed a liquid consistency and blood was present in the stool or around the anus. A mice sample was fasted for 4 hours before being selected to examine the development through surgery, but in a case of no colitis detection, the treatment of 4% acetic acid was continued for two days.

All study samples except KN were induced with 0.5 ml of 4% acetic acid perianally on day 1, and oral treatment was administered on days 1 to 5. KN, K-, and K+ groups were given standard feed, treatment, and standard oral treatment of sulfasalazine 1.3 mg/20 gBW, respectively. P1, P2, and P3 were given oral treatments of probiotics 0.078 mg/kgBW + purple sweet potatoes 0.00169 g/kgBW, probiotics 0.078 mg/20 kgBW + zinc 0.052 mg/kgBW, and probiotics 0.078 mg/kgBW + purple sweet potatoes 0.00169 g/kgBW + zinc 0.052 mg/kgBW, respectively.

Mice samples were euthanized on day 6 using chloroform before the colon organs were collected and sequentially processed into histological preparations in the same segment in all experimental animals. The preparations were stained with Hematoxylin Eosin (HE), then observed histologically using an Olympus Cx23 microscope with a 400× magnification in 10 pandan fields, and the colonic epithelial thickness was measured following the Histomorphometry method.

The epithelial thickness data collected were processed using the Shapiro-Wilk method to examine normality and Levene's test to determine homogeneity. The results showed normally distributed and homogeneous data, then a One-way analysis of variance (ANOVA) and Post Hoc least significant difference (LSD) tests were performed.

RESULTS AND DISCUSSIONS

This study was conducted at the Integrated Biomedic Laboratory, FK UNISSULA, on December 27, 2023, using 30 BALB/c male mice aged two to three months and weighing 20-30 g, which were randomly divided into six groups, each consisting of five individuals. KN was a group without induction, and K- was only induced with 4% acetic acid on day 1 and given distilled water treatment. Meanwhile, K+, P1, P2, and P3 were administered with 4% acetic acid on day 1 as well as the treatments of sulfasalazine 1.3 mg/20 gBB, probiotics 0.078 mg/20 gBW + purple sweet potatoes 0.00169 g/20 gBW, probiotics 0.078 mg/20 gBW + zinc 0.052 mg/20 gBW, and probiotics 0.078 mg/20 gBW + purple sweet potatoes 0.00169 g/20 gBW + zinc 0.052 mg/20 gBW for five days, respectively.

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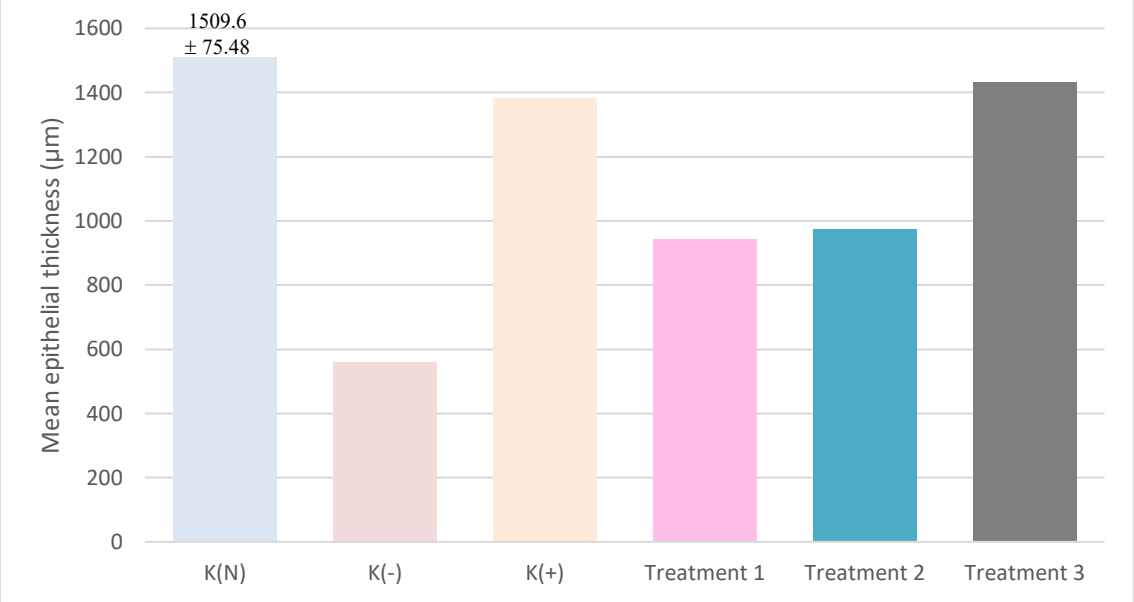
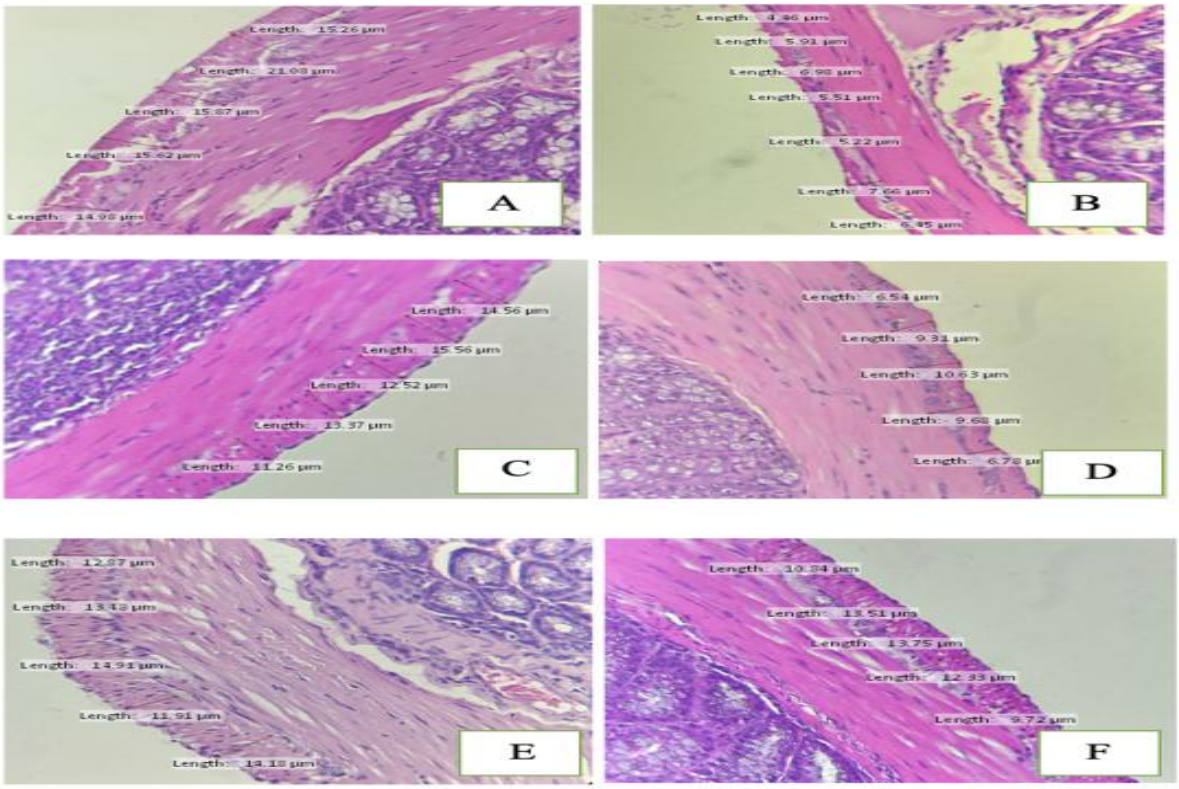


The average epithelial thickness results decreased significantly post induction with acetic acid in the K-group and increased significantly after treatment was given to each group.

Figure 1. Average Epithelial Thickness of the Control and Treatment Groups

The results of the Shapiro-Wilk normality and Levene's homogeneity tests showed homogeneous and normally distributed average epithelial thickness data because the p-value obtained in both tests was 0.05. Therefore, analysis was performed using parametric statistics in the form of One-way ANOVA, which produced significant results for all variables because the p-value = 0.000 <0.05 (Table 2).



(A) KN no epithelial damage, (B) K- epithelial damage occurs, and inflammatory cells appear,
(C) K+, (D) P1, (E) P2, and (F) P3 show epithelial cell repair after being treated.

Figure 2. Histological Image of Mice Colon Thickness with HE staining using (magnification 400×)

Administering P3 with a combination of 0.078 mg probiotics, 0.00169 mg purple sweet potatoes, and 0.052 mg zinc for five days succeeded in increasing the epithelial thickness, although not to the same extent as

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the normal or control group (Figure 1). The combination of probiotics and zinc can clinically work synergistically because zinc supports cell growth and acts as an antioxidant to protect membranes against damage caused by oxidation. Meanwhile, the purpose of administering probiotics is to inhibit pathogen adhesion and immunomodulation[8]. This corresponds with a theory stating that giving a combination of probiotics at a dose of 6.12 mg/day and zinc at a dose of 0.36 mg/day to experimental animals causes intestinal mucosal atrophy and reduces villi damage[9]. The combination of probiotics and purple sweet potatoes reduces blood lipid peroxidation by increasing antioxidant activity, leading to a decrease in cell damage due to free radicals[10]. This is consistent with the theory, which states giving purple sweet potatoes at a dose of 0.84 g/kgBW/day or approximately 0.00169 g/20 gBW of BALB mice/c/day induced by ovalbumin, can reduce the degree of inflammation in the duodenum lining[11].

The K+ group in Figure 1 and Table 2 shows that giving Sulfasalazine 1.3 mg/20 gBW for five days can increase the epithelial thickness after acetic acid induction. Sulfasalazine has components including mesalazine/5-ASA and sulfapyridine, which are both bound by azo bonds and broken down when exposed to colon bacteria[12]. Only 10% of consumed sulfasalazine appears to be absorbed in the small intestine, while the remaining 90% is absorbed in the large intestine. The 5-ASA portion is considered the main active component in the treatment, which produces an anti-inflammatory effect after being released in the colon lumen with the destruction of azo bonds by azoreductase from the luminal microflora[13]. This corresponded with previous studies stating that administration of sulfasalazine at a dose of 1-2 g/day was effective in treating patients with Ulcerative Colitis[14].

Administering probiotics 0.078 mg + zinc 0.052 mg was significant in increasing the epithelial thickness after being induced by acetic acid. This can prevent the attachment of pathogenic bacteria to the gastrointestinal mucosa when the epithelial cells are saturated with probiotics, hindering further colonization. Providing sufficient zinc can improve the mucosa through the function of regenerating epithelial cells and stabilizing disturbed intestinal membranes[15]. This is consistent with studies showing that probiotics and zinc can stimulate the growth of epithelial cells, thereby increasing the height of intestinal villi[9].

P1 given 0.078 mg probiotics + 0.00169 mg purple sweet potatoes had significantly increased epithelial thickness after acetic acid induction. This treatment combination has the benefit of suppressing inflammation because purple sweet potatoes contain anthocyanin compounds, which provide natural purple coloring agents. Anthocyanins are a group of pigments that can cause a reddish appearance[16] and comprise part of the flavonoid compounds acting as an antioxidant to slow down damage triggered by the oxidation process[17]. This description is supported by studies reporting that the synbiotics of purple sweet potatoes function to increase bacteria producing SCFA to suppress inflammation[7].

Post Hoc LSD test results showed that nearly all pairs of experimental groups had a significant difference in the average epithelial thickness (p-value <0.05). This suggested that the administration of sulfasalazine, probiotics + purple sweet potatoes, probiotics + zinc, and a combination of probiotics + purple sweet potatoes + zinc in mice for five days substantially increased the thickness. There were no differences found between K+ and P3 (p-value=0.134), as well as P1 and P2 (p-value=0.349), signifying that the administration of sulfasalazine, a combination of probiotics + purple sweet potatoes + zinc, probiotics + purple sweet potatoes, and probiotics + zinc was equally good for increasing epithelial thickness after acetic acid induction.

The strength of this study is the opportunity to manufacture traditional probiotics products using natural ingredients. Probiotics of purple sweet potatoes are very beneficial, healthy, composed of high vitamin content, and easy to prepare. However, the limitation is that the traditional probiotics manufactured by investigators cannot last long, leading to compulsory preservation in the refrigerator and immediate consumption when opened.

CONCLUSIONS

In conclusion, this study found that the combination of probiotics, purple sweet potatoes, and zinc affected the intestinal epithelial thickness in BALB/c Male Mice with ulcerative colitis. The most effective combination affecting the intestinal epithelial thickness was observed in group P3 where the damage induced by acetic acid was repaired, as shown by increased colonic epithelial thickness.

More studies should be conducted to prove the effect of the combination of probiotics + purple sweet potatoes + zinc before and after treatment. These could use different doses as well as examine various proinflammatory cytokine markers including IL-6, TNF-a, iFN-y, and IL-1B triggered by acetic acid induction in BALB/c male mice with ulcerative colitis.

ACKNOWLEDGEMENT

The authors are grateful to Sultan Agung Islamic University for supporting this study.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare that there is no conflict of interest in this study, and the authors provided necessary funding for the execution of this study.

AUTHOR CONTRIBUTIONS

GT: Original draft writing and editing, conceptualization, investigation, and methodology. CY: correspondence, conceptualization, supervision, and review writing. AH: conceptualization, supervision, and review writing. MR & M: formal analysis, supervision, and validation.

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How to cite: *Tsuraya, G., Yuniarifa, C., Hussaana, A., Riza, M., & Masfiyah, M. (2025). Effect of Combination of Probiotics, Ipomoea Batatas, and Zinc on Thickness of Instestinal Epithelium in Mice Model of Acetic Acid-Induced Colitis: Pengaruh Kombinasi Probiotik, Ubi Jalar Ungu, dan Zinc terhadap Tebal Epitel Usus Mencit Jantan BALB/c Kolitis Ulseratif terinduksi Asam Asetat. Amerta Nutrition, 9(2), 350–354.*