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Bayyinatul Muchtaromah, Siti Imroatul Maslikah, Lailil Mufarrichah, and Prilya Dewi Fitriasari

Histological Description of Small Intestine and Kidney of White Rats (*Rattus norvegicus*) Infected with *Salmonella typhi* by Giving Earthworm Flour

Bayyinatul Muchtaromah[1, a)], Siti Imroatul Maslikah[2, b)], Lailil Mufarrichah[1, c)] and Prilya Dewi Fitriasari[1, d)]

[1]*Department of Biology, Faculty of Science and Technology, The State Islamic University of Maulana Malik Ibrahim Malang, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Science, State University of Malang, Indonesia*

[a)]Corresponding author: bayyinatul@bio.uin-malang.ac.id
[b)] siti.imroatul.fmipa@um.ac.id
[c)] laililmufar@gmail.com
[d)] prilyadewi@bio.uin-malang.ac.id

Abstract. Earthworms (*Lumbricus rubellus*) can be used as medicine for typhus caused by *Salmonella typhi*. The active compounds in earthworms include lysozyme, agglutinin, lytic factor, and lumbricine. The treatment of flour worms with concentration and duration of administration improperly influence the *S. typhi* inhibition in vivo. This study aimed to determine the effect of concentration and duration of worm flour administration on the histological description of the small intestine and kidney of white rats (*R. norvegicus*) infected with the *S. typhi*. The first factor was the concentration of worm flour (32%, 48% and 60%), and the second factor was the duration of administration (7 days and 14 days). Data were analyzed by two-way ANOVA. The results showed that the concentration and duration of worm flour administration affected improving the histological damage of the small intestine and kidneys of rats, but the interaction of concentration and duration of worm flour administration did not show a significant effect. The effective treatment was found at a concentration of 60%, with a duration of 14 days.

INTRODUCTION

Typhoid is a disease caused by the bacterium *S. typhi* which is an enteropathogenic bacterium from the genus Salmonella which causes infection which then becomes typhoid fever [1]. Typhus in the body infects several soft tissues, bones, digestive tract and urinary tract [2]. As a result of the complications of typhoid, a small intestinal disorder appears, which is experiencing intestinal bleeding and perforation. Besides that, it can also cause acute kidney injury (AKI) [3].

Earthworms were contained arachidonic acid, a peroxidase enzyme, catalase, cellulase enzyme, and ligase. The content of earthworms were efficacious, including arachidonic acid to reduce body temperature fever due to infection, lumbrokinase enzymes to help cope with blood pressure, cellulase and ligase enzymes efficacious in supporting the digestive process of food, while the peroxidase enzyme and catalase help overcome degenerative diseases such as diabetes mellitus, high cholesterol and rheumatism [4]. Earthworms are processed by society in a simple way, such as boiling and drinking water. The community believes that earthworms can be used as an alternative medicine for typhus, a lack of knowledge about related sciences, making the process of making worm flour less standardized such as processing fried or heated stoves with no temperature gauge. Processing using high temperatures without contro can lead to denaturation of proteins. It can damage the active substance so that it cannot work as expected [5].

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This study aimed to determine the effect of concentration and duration of worm flour administration on the histological description of the small intestine and kidneys of white rats (*R. norvegicus*) infected with the *S. typhi*, as well as the interaction of both.

EXPERIMENTAL DETAILS

The research design used in this study is a Completely Random Design (CRD) factorial pattern. In this study, two factors and four replications were used. The first factor is the concentration of worm flour from the type of *L. rubellus* which consists of three levels of treatment, A = concentration of 32%, B = concentration of 48%, C = concentration of 60%, The second factor is the duration of earthworm flour administration consisting of two levels of treatment, namely treatment 1 = for 7 days and treatment 2 = for 14 days. The procedure in the study was a combination of factors from all levels of treatment, which consisted of 6 treatments and two controls (positive and negative controls) each consisting of four replications. The *S. typhi* used as pathogens with a density of 8.57×10^5 in 0.5 ml of suspension. The test animals used were 32 white rats (*R. norvegicus*) Sprague-Dawley strain, 2.5 months old with a weight of 300 g.

The worm flour was carried out using the modified method [6]. The *L. rubellus* earthworms washed using running water, ground in a temperature of 50 ^{o}C, 8 h. The earthworm is mashed by pounding it into worm flour and then sifted. Sterilization of the appliance is done by dry sterilization with a heating oven. Wet sterilization is done using an autoclave. Media for *S. typhi* cultivation used were NB (Nutrient Broth), SS Agar (Salmonella-Shigella Agar) Media Plate, McFarland Standard 0.5 solution. White rats were acclimatized in the laboratory for two weeks, fed and drank ad libitum. The blood was taken for the first serological test (widal test). The test was to determine the health status of rats (not infected with *S. typhi*).

Furthermore, 24 rats in the treatment group and four positive control rats were infected by *S. typhi* with a density of 8.57×10^5 cells/ml as much as 0.5 ml. After 34 h later the rats were taken for blood to do the second serological test (widal test) to determine the antibody status due to *S. typhi* infection. After the treatment, surgery was carried out for organ harvesting (duodenum and kidney small intestine) to make histological preparations.

Score data on the level of the small intestine and kidney damage were analyzed by a two-way ANOVA test, then proceed with the BNJ test with a significance level of 1% if there are significant differences. Histology profile data was determined by observation under the microscope and observed cell damage in the organ. This observation was done using a 400× magnification.

RESULT AND DISCUSSION

Effect of Earthworm (*L. rubellus*) Flour on Small Intestine Histology

The histological picture of the small intestine was assessed based on the degree of histological damage in the form of the nucleus pyknosis, karyorrhexis, karyolysis, and the presence of a gap in the edge of the villi, Fig.1. Based on the results of observations of the level of damages, the scores obtained were then analyzed statistically by the Two Way ANOVA test with a significance level of 1% known that there is a concentration effect of earthworm flour on the histology of the small intestine of *R. norvegicus* infected with *S. typhi*. The duration of earthworm flour administration was influenced by histology of *R. norvegicus* kidney infected with *S. typhi*. In the interaction between concentration and length of time, there is an effect of interaction between concentration and duration of administration earthworm flour to histology of *R. norvegicus* small intestine infected with *S. typhi* Fig. 2.

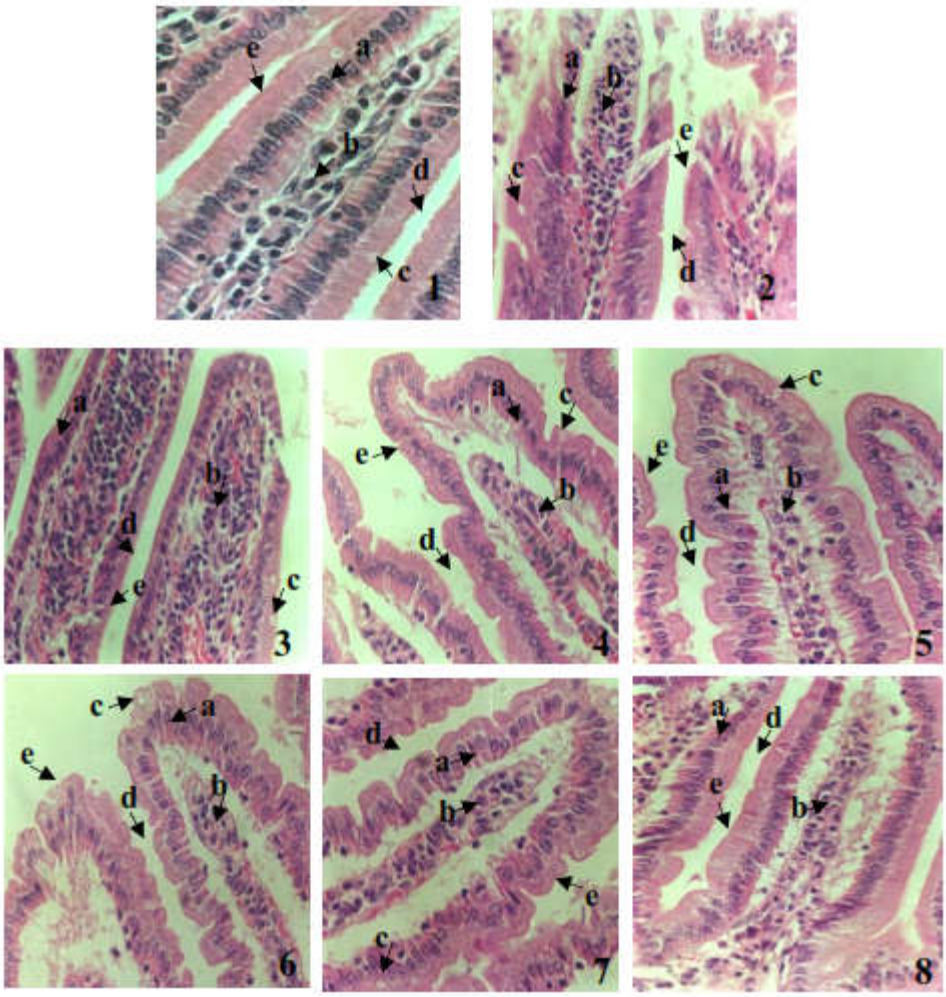

FIGURE 1. Epithelial Histology of White Rat (400× magnification). Information: (1) Negative Control (without treatment); (2) Positive Control (infected with *S. typhi*); (3) Treatment 1 (infected rats with 32% earthworm flour, 7 days); (4) Treatment 2 (infected rats with 48% earthworm flour, 7 days); (5) Treatment 3 (infected rats with 60% earthworm flour, 7 days), (6) Treatment 4 (infected rats with 32% earthworm flour, 14 days), (7) Treatment 5 (infected rats with 48% earthworm flour, 14 days, (8) treatment 6 (infected rats with 60% earthworm flour, 14 days), (a) Columnum epithelium cells, (b) Lamina propria, (c) Goblet cells (d) Lumen (e) Villi

Based on the Fig. 2, it was found that negative controls (normal *R. novergicus*) had the lowest histological damage of the small intestine compared to the treatments. The concentration of 60% of earthworm flour has lower damage level than the concentration of 48% and 32%. The concentrations of 60%, 48%, and 32% had more moderate histological damage to the small intestine than positive controls (*R. novergicus* with kidney damage). So, the best dose in affecting the decrease in damage is a 60% earthworm flour concentration.

The negative control had the lowest level of small intestinal villi epithelial damage compared to the treatment duration of 14 days and seven days. But at the time of administration of 14 days, the level of renal glomerulus damage was lower than the length of administration of seven days. The positive control was higher in the level of intestinal villi epithelial damage than in all treatment periods. So it can be concluded that the most effective treatment duration is at the time of giving 14 days. From the results of the previous studies [1], it is known that *L. rubellus* produced antimicrobial substances from his body that were used to protect himself from attacks of pathogenic microorganisms, with high-quality antimicrobial substances in earthworms.

From the results of previous studies, it can be understood that the optimal temperature in making worm flour is a temperature of 50 °C (or maybe lower than 50 °C). The bacterial compounds or substances contained in the body of earthworms are mostly proteins, so it is assumed that temperatures above 50 °C damage the chemical structure of

functional and structural proteins in worms [5]. From the observations of the histological features of the small intestine of white rats (*R. norvegicus*) known that *S. typhi* can cause mucosal damage to the small intestine of white rats (*R. norvegicus*) such as swelling of the cell nucleus with pyknosis, the nucleus that experiences injury due to cell pressure and cell swelling due to increased plasma membrane permeability [7]. *S. typhi* infects the intestine so that an acute inflammatory process occurs on the intestinal mucosa and submucosa caused by the release of inflammatory cytokines which act as chemoattractants for lymphocyte, neutrophil, and macrophage cells, causing inflammatory cells to produce reactive oxides which cause small intestinal villi experiencing atrophy and not being able to absorb fluids and food properly [8]. Other studies regarding the use of earthworms on broiler carcass and ileum characteristics showed that broiler carcass and ileum characteristics had an increase in the total number of lactic acid bacteria and reduced intestinal microflora pathogen population (the doses used were 10-30 g/kg yield) [9].

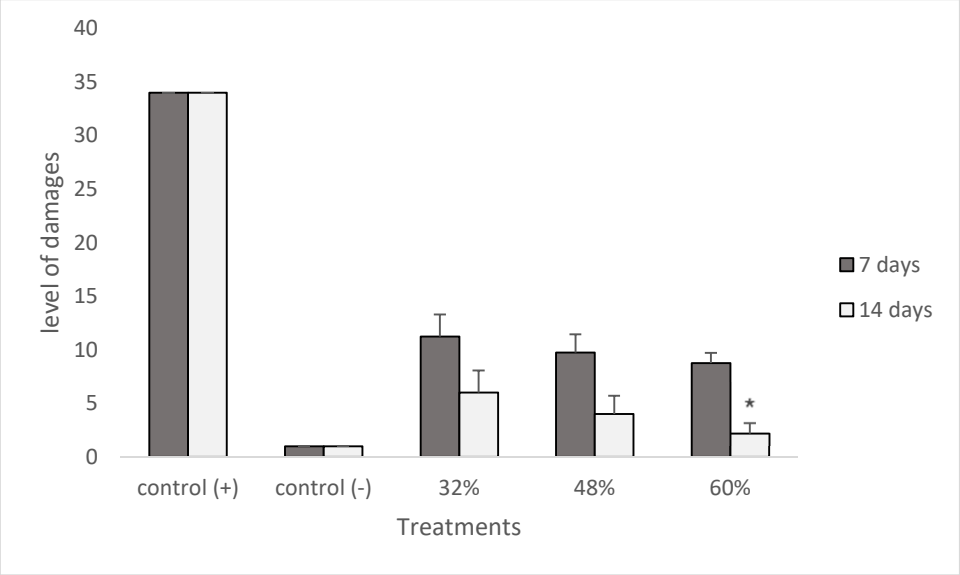

FIGURE 2. Level of damages on the small intestine histology of white rats after treatments

The pathogenicity of *S.typhi* is likely to be inhibited by antibacterial compounds or substances found in the body of earthworms. The antibacterial compound is mostly a protein consisting of lumbrifebrine, terestrolimbrocyline, hypoxanthine, amino acids, xanthine, guanine, choline, and guanidine [5]. In earthworms also contain arachidonic acid which is effective in reducing fever due to infection, lumbrokinase enzymes can help overcome blood pressure disease, cellulase and ligninase enzymes can help digest the food, while the peroxidase enzyme and catalase to overcome degenerative diseases such as diabetes mellitus, high cholesterol and rheumatism [4].

The inhibition mechanism of *S. typhi* infection by extract of *L. rubellus* earthworm is seen in prevention with a treatment of 14 days, while for prevention with the treatment of seven days administration there is almost no mechanism for inhibition of pathogenicity. The interference mechanism of the rat body where nonspecific antimicrobial material is produced due to the response of a bacterial infection called interferon. Interferon is a low molecular weight protein that can inhibit the antibacterial activity of *L. rubellus* extract. The interferons produced were interferon α and γ [10]. The interference mechanism will be optimal when the infection rate is still low, while at high levels of infection interferon will be produced in tiny amounts [11].

In treatment three to treatment eight, where the infection was given simultaneously, will provide an opportunity for *S. typhi* bacteria to stimulate rat body cells. The rat's cells were a response by producing interferon which works to inhibit the antibacterial effect of earthworms extracts before reaching the optimal. If the antibacterial effect of worms has worked optimally, there will be a mechanism for inhibiting *S. typhi* bacterial infection against the body of the rat. Treatment using earthworm extract when rats were sick will be an effective sting, and it is known that *S. typhi* have an incubation period of 7-14 days after infection [12]. In this case, the interfacing mechanism to produce interferon, especially to inhibit antibacterial originating from earthworm extract, will be less effective, but treatment for bacterial infections will run optimally.

Effect of Earthworm (*L. rubellus*) Flour on Kidney Histology

The histological picture of the kidney was assessed based on the level of histological damage in the form of the nucleus pyknosis, karyorrhexis, karyolysis, widening the distance between the two ends of the Bowman capsule, and narrowing of the glomerulus cells, Fig. 3.

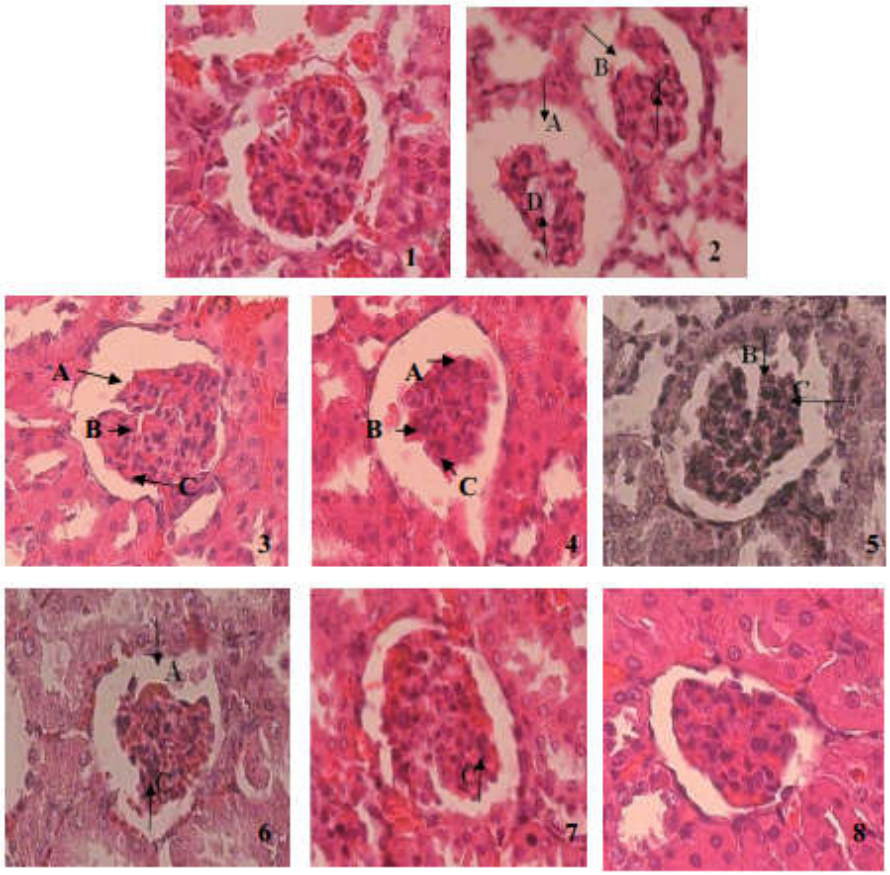

FIGURE 3. Glomerulus Cell Histology of White Rat with 400x magnification. Information: (1) Negative Control (without treatment); (2) Positive Control (infected with *S. typhi*); (3) Treatment 1 (infected rats with 32% earthworm flour,7 days); (4) Treatment 2 1 (infected rats with 48% earthworm flour,7 days); (5) Treatment 3 1 (infected rats with 60% earthworm flour,7 days), (6) Treatment 4 1 (infected rats with 32% earthworm flour,14 days), (7) Treatment 5 1 (infected rats with 48% earthworm flour, 14 days, (8) treatment 6 1 (infected rats with 60% earthworm flour,14 days), (A) Distance of the Second Bowman Capsule Wall, (B) Glomerular Depreciation, (C) Core pyknosis, (D) Core karyolysis.

Based on the results of the level of the damage of glomerulus cell, [Fig. 4] there is an effect of earthworm flour concentration on the histology of the *R. norvegicus* kidney infected with *S. typhi*. The duration of earthworm flour administration was influenced by histology of *R. norvegicus* kidney infected with *S. typhi*. More effective treatment in the administration of earthworm flour from different concentrations can be done using a further test with 1% Post Hoc Test which showed that there was a significant difference in decreasing renal histological damage in *R. norvegicus* kidney. It can be seen that the best dosage in affecting damage to kidney histology is a concentration of 60%. Based on the results of observations of the level of damage to kidney glomerulus in *R. norvegicus* infected with *S. typhi*.

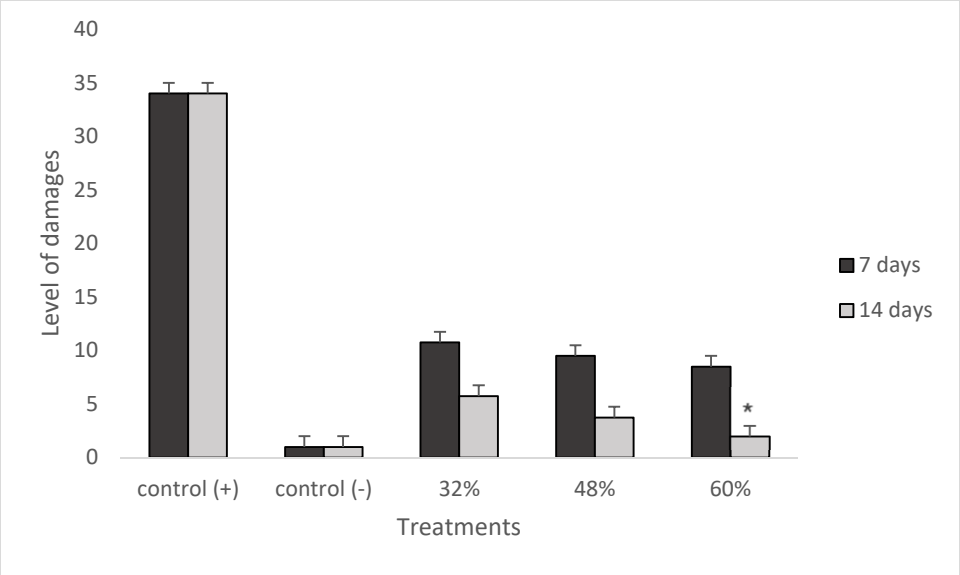

FIGURE 4. Level of damages on the kidney histology of white rats after treatments

The duration of earthworm flour administration is a very significant difference from each treatment. Negative control has the lowest level of renal glomerular damage compared to the treatment duration of 14 days and seven days, but at the time of administration of 14 days, the level of renal glomerulus damage was lower than the period of administration of 7 days. The positive control was higher in the level of renal glomerular impairment than all the length of treatment. So it can be concluded that the most effective treatment duration is at the time of giving 14 days.

The desired result from the process of making worm flour is the production of worm flour with optimal antibacterial function. The parameter of the ability of a substance as an antibacterial ingredient is its ability to inhibit damage to body cells caused by pathogenic bacteria. While the desired worm flour is worm flour which is effective in inhibiting and restoring cell damage, this bacterium secretes exotoxin to the mucosal surface. Then exotoxins attack the host cell locally which is carried around the bloodstream and attacks vulnerable organs such as the kidneys [13]. The level of urine and creatinine in the blood is a parameter of kidney function. If kidney function is impaired, blood and creatinine concentrations in the blood exceed the threshold [14]. Another study on the use of earthworms on renal histology of rats has been carried out by 13 which distinguishes the earthworm species used is *Pheretima javanica* K and with lower doses of 0.4-3.2 g, after treatment for 14 days, the results show that administration this worm does not affect the physiology and histopathology of the kidneys.

Kidney damage due to toxic substances can be identified based on changes in histological structure, such as Acute Tubular Necrosis (ATN) which is morphologically characterized by the decay of the proximal tubular epithelium. Another factor that can cause kidney damage is the accumulation of xenobiotic substances in cells. If a chemical is released actively from the direction of urine, the first chemicals accumulate in the proximal tubule, as a result of the concentration process, these toxic substances will gather in the kidneys. The excretion process that takes place in the kidney can affect the histological picture of the kidney [13]. The organs affected by nephrotoxic material will make significant repairs at one to two weeks of the healing phase and repair can continue for up to 12 months or until kidney function is restored [15].

SUMMARY

The worm flour affects improving the histological picture of the small intestine and kidneys of white rats (*R. norvegicus*). The effective concentration at a concentration of 60% and duration of effective administration for 14 days. The interaction of concentration and duration of worm flour administration has not shown a significant improvement in improving the histological picture of the small intestine and kidney of white rats (*R. norvegicus*) infected with the *S. typhi*.

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