

Test of the Effectiveness of Earthworm Flour (*Lumbricus rubellus*) Gastroretentive Mucoadhesive Granule Formulation on Male White Rats (*Rattus norvegicus*) Infected with *Salmonella typhi*

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Abstract: *Lumbricus rubellus* contains lumbricin which is efficacious for treating typhoid fever. The aim of the research was to determine the effectiveness of gastroretentive mucoadhesive granule preparations from earthworm flour. This study used 30 male Wistar rats which were divided into 6 groups which were given orally, the normal and negative control groups were given Na-CMC, the positive control group was given chloramphenicol, and the treatment group was given doses of 200 mg/kgBW, 400 mg/kgBW and 800 mg/kgBW. mg/kgBB gastroretentive mucoadhesive granules earthworm flour. The results of body temperature measurements, on day 7 the control group obtained results of ± 37.08 , ± 39.00 and ± 38.09 respectively, and the treatment group obtained results of ± 38.09 , ± 38.09 and ± 38.07 respectively. On day 21, the control group obtained results of ± 37.08 , ± 39.00 and ± 38.09 , respectively, and the treatment group obtained results of ± 38.09 , ± 38.09 and ± 38.07 , respectively. The Widal test results on the 7th day were positive and after 14 days of administering the preparation on the 21st day the results were negative. It was concluded that the effective dose for typhoid fever therapy was a dose of 400 mg/kgBW.

Keywords: Body temperatur; Earthworm; Granule mucoadhesive gastroretentive; *Salmonella typhi*

Introduction

Typhoid fever is a public health problem (Nathania et al., 2022) that is spread throughout the world (Nabani et al., 2023). The disease is predominant in low-income communities, where public health infrastructure is poorly resourced (Argimón et al., 2021). The majority of the disease burden falls on children in low and middle income countries (LMICs) (Ingle et al., 2019). Typhoid fever is a disease that attacks the digestive tract resulting in an infection of the small intestine caused by *Salmonella typhi* bacteria (Juniah & Arianti, 2023). The

incubation period for this disease is on average 7 to 14 days (Masyrofah et al., 2023).

Typhoid fever is still a serious medical condition that causes a great deal of suffering for people all over the world (Ahmed et al., 2024). Infection that affects an estimated 20.6 million people globally each year, causing an estimated 223,000 (Wong et al., 2016). The highest incidence rates occur in south Asia, which contains 70% of the global disease burden (da Silva et al., 2022). Typhoid fever is an acute infectious disease of the small intestine caused by *Salmonella typhi* (Dewi et al., 2019).

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Salmonella typhi infections in humans are very variable, so they can heal spontaneously (gastroenteritis), but can also be serious if enteric fever spreads systemically (Wulandari et al., 2024). Treatment of typhoid fever or salmonellosis can be done medically and traditionally, the appeal of traditional medicine mainly comes from its natural nature so that it is considered safer and better tolerated than modern medicine (Bahri et al., 2023).

Salmonella typhi is a rod, Gram-negative (Sartini et al., 2023) rod-shaped bacterium that usually enters the human body through contaminated water or food and causes a systemic infectious disease called typhoid fever (Zhao et al., 2021). *Salmonella typhi* infections may result in lipid peroxidase and subsequently causes tissue damage caused by ROS (*Reactive Oxygen Species*) (Samatra et al., 2017).

Earthworm (*Lumbricus rubellus*) is a traditional medicine from animal materials that is used to treat fever, especially typhoid fever caused by *Salmonella typhi* bacteria earthworms have properties in the form of phenols and antioxidants. In addition, that earthworms are rich in peptides, such as lumbricin. Lumbricin is composed of complete amino acids, especially proline as a secondary metabolite and in vitro has been shown to have a broad-spectrum antimicrobial activity that can inhibit gram-positive bacteria, gram-negative bacteria, and several fungi. Some researchers have suggested the potential of *Lumbricus rubellus* extract in inhibiting the proliferation of *Salmonella typhi*, thereby reducing the symptoms of fever associated with infection (Mercya et al., 2024).

Earthworms (*Lumbricus rubellus*) (Heděnc et al., 2020), species of earthworm, displays more potent antibacterial activity (Ulhaq et al., 2021). Several studies have reported that the bioactive compounds and antimicrobial properties of earthworms can inhibit pathogenic bacteria (Lestari et al., 2019). Earthworms (*Lumbricus rubellus*) have quite high active protein content, which is around 58–71% in dried worms (Fauzi et al., 2022). Enzymatic substances in earthworms include peroxidase, catalase, cellulose, and lumbrokinase. Lumbrokinase is easily absorbed in the digestive system without disrupting normal metabolic processes (Faradist et al., 2024).

Indiscriminate use of antimicrobials in humans and animals has led to the emergence of antimicrobial resistance (AMR) to conventional drugs (ampicillin, chloramphenicol, tetracycline, and cotrimoxazole) and new drugs (third generation cephalosporins, fluoroquinolones, and azithromycin) in NTS, which is a global concern. Some researchers found antimicrobials in earthworms (*Lumbricus rubellus*). *Lumbricus rubellus* has an antimicrobial peptide (AMP) called Lumbricin-I, which plays an important role in natural defense against

pathogenic microbes (Sara et al., 2023). Earthworms also produce lysosomal enzymes (lysozyme) which are important in protection from pathogenic microbes (Mughtaridi et al., 2022). Apart from that, *L. rubellus* also improves the humoral immune system based on its antimicrobial properties (Nurmansyah, 2017).

The mechanism used by the proteins possessed by earthworms is to create pores in the bacterial cell walls. In this way, it becomes more difficult for bacteria to fight because what is damaged is the cell structure of the bacteria itself (Bestari et al., 2022). Lumbricin can cause bacterial cell walls to become hollow so that the bacterial cytoplasm will be exposed to the external environment and cause disruption of bacterial activity and even death of the bacteria (Besmaya & Laksono, 2022). *Lumbricus rubellus* worms contain the enzyme lumbrokinase which works as a fibrinolytic by dissolving fibrin and increasing t-PA activity (Nasirotozahroh & Susanti, 2023).

Research and development of drugs into controlled release formulations will release the drug slowly into the gastrointestinal tract so that it will maintain effective drug concentrations in the systemic circulation for a longer period of time. Mucoadhesive is the attachment of a substance with mucus to the surface of biological tissue. Mucoadhesive agents can be natural or synthetic polymers. Commonly used mucoadhesive polymers include carbopol, chitosan, sodium alginate, HPMC, polyethylene glycol, and polyacrylic acid. Mucoadhesive polymers help bind drug substances to the mucosal surface and prolong the residence time of the drug at the site of application (Rosmawati et al., 2023). The mucoadhesive dosage form allows the material to be retained and localized in the stomach thereby increasing its effect in treating gastric ulcers (Nofianti, 2024).

The Widal test is a laboratory examination to detect the presence or absence of antibodies in typhoid fever sufferers against *Salmonella typhi* antigens (Fitriyani et al., 2021). The basic principle of the Widal Test is the agglutination reaction between antigen and antibody (Hidayati, 2020). The Widal test can detect *Salmonella* by determining the agglutinin titer contained in the patient's serum against the O and H antigens of the *Salmonella typhoid* antigen (Norsiah & Oktiyani, 2020).

This research was conducted to determine the effectiveness of earthworm flour mucoadhesive granule preparations against white rats infected with *Salmonella typhi*.

Method

This research is a laboratory experimental study with a modified pre-test-post test randomized control group design consisting of two treatment groups,

namely: control group (group I: normal control, group II: negative control and group III: positive control) and experimental group (group IV: gastroretentive mucoadhesive granule preparation for earthworms 200 mg/kgBB, group V: gastroretentive mucoadhesive granule preparation for earthworms 400 mg/kgBB, group VI: gastroretentive mucoadhesive granule preparation for earthworms 800 mg/kgBB).

This research has received ethical approval from the ethics committee of Tadulako University with an ethical approval letter number: 22/UN 28.1.30/KL/2024. This research was carried out in July-August 2024, carried out in the pharmacology laboratory, microbiology laboratory, and pharmaceutical technology laboratory of STIFA Pelita Mas Palu.

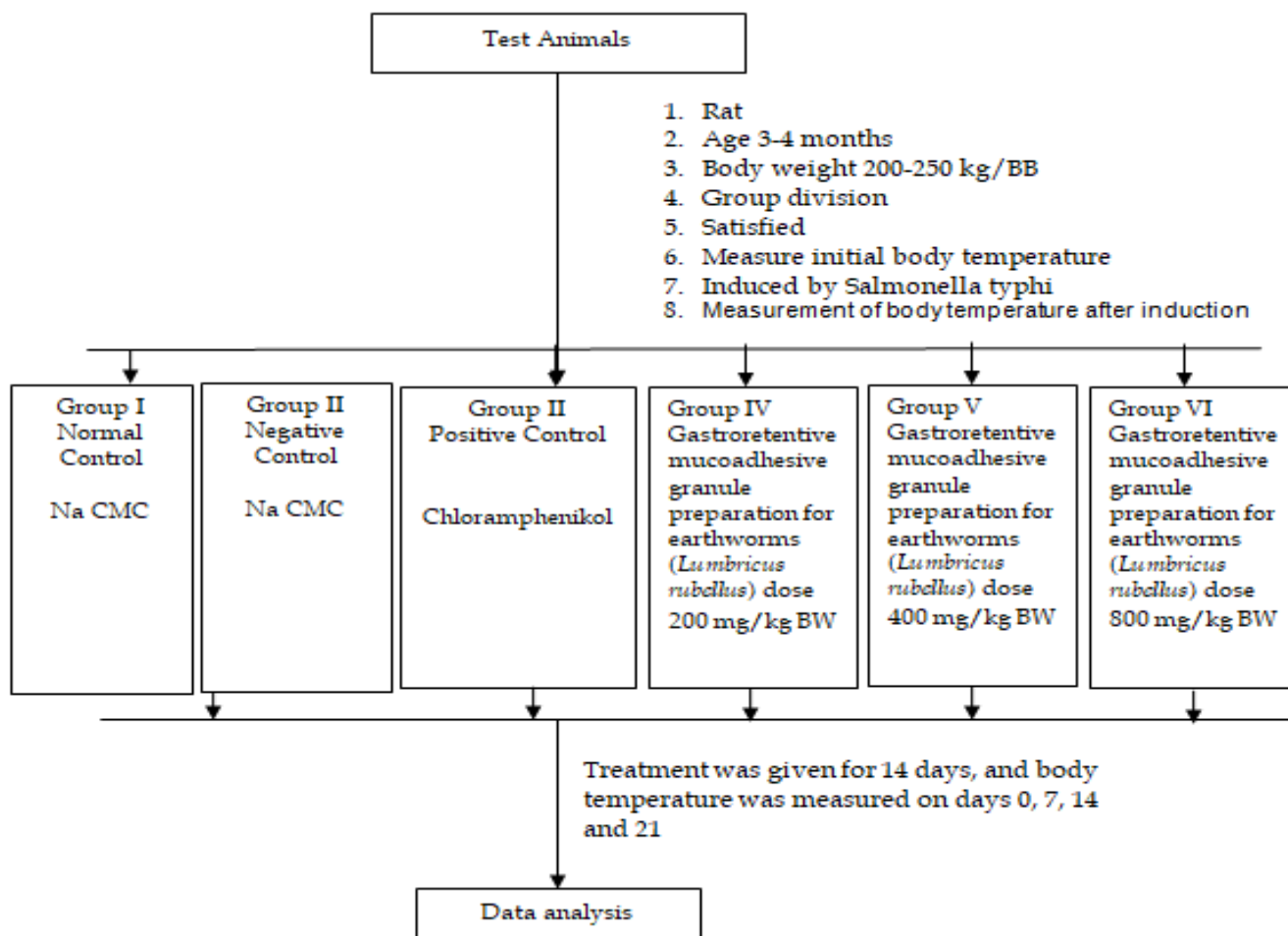


Figure 1. Research work schema

Research Procedure

Test Animal Setup

White rats were used as test subjects in this study (Wardani et al., 2024), with the criteria are 3-4 months old (Wardani et al., 2024) with a body weight of 150-200 grams (Hartika et al., 2024), have white fur, be in good condition, are male, and have been acclimatized for 14 days (Dipayana et al., 2024). This test animal is a type of rat commonly used for research. In this research, male white rats were used because they are known as good experimental animal models, are easy to handle, and can be obtained in large quantities and the research results are more stable because they are not influenced by the menstrual cycle and pregnancy as in female white rats.

The wistar strain was chosen because Wistar rats have a relatively fast metabolic capacity so they are more sensitive when used in research (Wardani et al., 2024). Dalam penelitian ini, Six groups of experimental animals were created at random (Safrida et al., 2023). The group consisted of 5 Wistar rats.

Preparation of Chloramphenicol Solution

Positive control is made from chloramphenicol capsules 250 mg (Ronauli, 2022). The maximum dose for adult humans is 50 mg, so the dose that is converted first from the results of the calculation of the human dose (70 kg) to the rat dose (200 grams) is 0.018 so that the dose of chloramphenicol given to white rats is 4.5 mg/kg BB.

7.2 mg equivalent tablet powder in 20 ml of 0.5% Na CMC weighed according to the optimization results that have been carried out.

Manufacture of Mucoadhesive Granular Suspension Gastroretentive, Earthworm Flour, Earthworm

Making Gastroretentive Earthworm Flour Mucoadhesive granule suspension is made with 3 dosage variations, namely 200 mg/kg BB, 400 mg/kg BB and 800 mg/kg BB. Mucoadhesive granule preparations Gastroretentive granules of earthworm flour are weighed according to the calculations. Furthermore, at each dose, a mucoadhesive preparation of gastroretentive granules of earthworm flour is added to taste with aquadest with a volume of up to 20 ml, then stirred until homogeneous.

Rejuvenation and Manufacture of Salmonella typhi Suspension Inocular Suspension

The rejuvenation of Salmonella typhi bacteria was carried out in 5 test tubes with SSA oblique media of 5 ml per test tube. After that, S. typhi bacteria were rejuvenated by the streak plate method by taking 1 dose of S. typhi bacteria into a test tube containing SSA (Salmonella Shigella Agar) inclined medium and then incubated at 37°C for 24 hours (Falakh & Astri, 2022). The preparation of bacterial suspension was carried out by mixing 1-2 doses of Salmonella typhi bacteria culture that had been rejuvenated for 24 hours and suspended into 5 ml of homogenized 0.9% NaCl solution (Rachmatiah et al., 2020). Homogenized using vortex and its turbidity was standardized to a concentration of 0.5 Mc Farland (Guplin et al., 2017).

Treatment of White Rats (Rattus norvegicus)

Some of the steps that must be taken in this research stage are as follows:

Measurement of Body Temperature of White Rats

The body temperature measurement of white rats (*Rattus norvegicus*) in this study was carried out to determine the initial condition of the experimental animals before being treated and used as preliminary data as well as a comparison in this study. The temperature was measured through the rectal rats using a digital thermometer. Measurements are taken on the rectum because the rectal temperature is 0.50-10C higher than the oral temperature, which is the core temperature of the body and changes the least to changes in ambient temperature.

Induction of Salmonella typhi Bacteria

Induction of Salmonella typhi bacteria in rats as much as 2.5 ml orally using a sonde device.

Mucoadhesion Suspension of Gastroretentive Granules of Earthworm Flour

The suspension of mucoadhesive granules of gastroretentive granules of earthworm flour was given to rats as much as 2.5 ml orally using a sonde device.

Measurement of Body Temperature of Rats

The body temperature measurement of white rats (*Rattus norvegicus*) was carried out to determine the condition of the experimental animals after being treated and used as final data as well as a comparison in this study. The temperature was measured through the rectal rats using a digital thermometer. Measurements were taken on the rectum because the rectal temperature was 0.5⁰-1⁰C higher when compared to the oral temperature, was the core body temperature, and changed the least to changes in environmental temperature.

Determination of Widal Test

The determination of the widal test uses a typhoid test kit (typhoid kit). The device works quickly, drips blood, and supports the typhoid test in the sample well, then the results will be obtained after 20 minutes or less.

Results and Discussion

This study used 30 male white rats (*Rattus norvegicus*) as test animals White rats (*Rattus norvegicus*) are one of the experimental animals in the laboratory that gives more stable results because they are not affected by the phase of menstruation and pregnancy like in female rats. The use of white rats as test animals is because white rats have been known as good experimental animal models, easy to handle, can be obtained in large quantities, and provide reliable test scores because white rats have relatively fast metabolic abilities so they are more sensitive when used in research related to metabolism (Lahamendu et al., 2019). The test animals used have obtained permission from the Research Ethics Committee of Tadulako University.

The test animals were first adapted for 14 days so that the rats could adapt to their new environment such as food, drink, temperature and surrounding conditions. A sample of 30 male mice were grouped randomly into six groups, namely Group 1 normal control group; group 2 Negative control group with CMC Na (Lubis et al., 2023), Group 3 controlled chloramphenicol suspension, and 3 treatment groups with different doses, namely earthworm flour gatsroretent mucoadhesive granules (*Lumbricus rubellus*) at doses of 200 mg/kgBB, 400 mg/kgBB, and 800 mg/kg BB.

This study used gastroretentive mucoadhesive granules of earthworm flour with wet granulation method. Wet granulation The wet granulation method is

the only method to be used in the granulation of large doses of active substances. The wet granulation method can improve the flow properties and compatibility of the material and can increase compressibility, this method can also improve the uniform distribution of the content (Fadhilah & Saryanti, 2019). The binding material used in wet granulation is Na CMC. The reason for using Na CMC as a binder is because the use of CMC-Na has strong adhesion, is non-toxic, and is non-irritant. Na CMC has good flow properties and percent compressibility (Anindhita et al., 2022).

The study began with the initial body temperature measurement (day 0) before the study

using a rectal digital thermometer, the results obtained were 37.04-37.090 C, indicating that the results of body temperature measurements differed insignificantly between all treatment groups. This is seen from the value of $p = 0.37$ ($P > 0.05$). Data from preliminary test results of changes in body temperature after the administration of bacterial suspension can be seen in Table 1. Meanwhile, the results of the widal test in each treatment group can be seen in Table 2. Ambient temperature is the most crucial factor that affects productivity Earthworm (Marnelis et al., 2024).

Table 1. Average \pm SD Rat Body Temperature

Day	Normal group	Negative group	Positive group	Granules dosage 200 mg/kgBB	Granules dosage 400 mg/kgBB	Granules dosage 800 mg/kgBB	P value
0	37.4 \pm 0.60	37.9 \pm 0.48	37.4 \pm 0.57	37.6 \pm 0.56	37.9 \pm 0.51	37.9 \pm 0.52	0.37
7	37.8 \pm 0.47	39.0 \pm 0.11	38.9 \pm 0.11	38.9 \pm 0.19	38.9 \pm 0.27	38.7 \pm 0.35	0.01
14	38.0 \pm 0.04	39.1 \pm 0.27	38.2 \pm 0.14	38.9 \pm 0.08	38.4 \pm 0.29	38.3 \pm 0.16	0.00
21	38.0 \pm 0.04	39.1 \pm 0.27	38.2 \pm 0.14	38.9 \pm 0.08	38.4 \pm 0.29	38.3 \pm 0.16	0.00

Table 2. Widal Test

Day	Normal group	Negative group	Positive group	Granules dosage 200 mg/kgBB	Granules dosage 400 mg/kgBB	Granules dosage 800 mg/kgBB
0	Negative	Negative	Negative	Negative	Negative	Negative
7	Negative	Positive	Negative	Negative	Negative	Negative
14	Negative	Positive	Negative	Negative	Negative	Negative
21	Negative	Positive	Negative	Negative	Negative	Negative

On the 7th day, the results of the Kruskal Wallis statistical test showed that the results of different body temperatures were not significant in each treatment group with a value of ($P > 0.05$), so it was continued with a further test by Mann Whitney. The results of the further test by Mann Whitney showed that the dose group of 200 mg/kg BB, dose 400 mg/kg BB, dose 800 mg/kg BB, positive control group and negative control were significantly different from normal control, this showed that all test animals in all groups except the normal control group were sick due to the effects of Salmonella typhi. Administration of Salmonella typhi can trigger an increase in body temperature directly related to the level of pyrogen cytokines produced to overcome various stimuli, especially infections. The larger the dose of Salmonella typhi ingested, the more people who show clinical symptoms, the shorter the incubation period does not change the clinical syndrome that arises (Martha Ardiaria, 2019).

On the 14th day, the results of the Kruskal wallis statistical test showed that the results of body temperature were significantly different from the value ($P < 0.05$) so it was continued with the Mann Whitney test to see the difference between all treatment groups. The results of the Mann Whitney test showed that the dose group of 200 mg/kg BB, 400 mg/kg BB, 800 mg/kg BB

and the positive group was significantly different from the normal group, this shows that the body temperature of the three dose groups has not decreased in proportion to the normal control. The dose group of 200 mg/kg BB, 400 mg/kg BB and 800 mg/kg BB was significantly different from the negative control group, this showed that the body temperature did not decrease because only 0.5% Na cmc was given so that it did not affect the body temperature in the rats. The dose group of 400 mg/kg BB was not significantly different from the positive control, which suggests that the administration of chloramphenicol has a mechanism that inhibits the synthesis of microbial cell proteins and is still the main choice for the treatment of typhoid fever because it is effective (Rahmasari & Lestari, 2018).

On the 21st day, the results of the Kruskal wallis statistical test showed that the body temperature results were significantly different from the value ($P < 0.05$) so it was continued with the Mann Whitney test to see the difference between all treatment groups. The results of the Mann Whitney test showed that the doses of 200 mg/kg BB, 400 mg/kg BB and 800 mg/kg BB were significantly different from the negative control group, this showed that the body temperature did not decrease because only 0.5% Na cmc was given so that it did not affect the body temperature in the rats. The dose group

of 200 mg/kg BB, 400 mg/kg BB, 800 mg/kg BB and the positive group differed significantly from the normal group, this shows that the body temperature of the three dose groups has not decreased which is comparable to the normal control. The dose group of 400 mg/kg BB was not significantly different from the positive control, which suggests that the administration of chloramphenicol has a mechanism that inhibits the synthesis of microbial cell proteins and is still the main choice for the treatment of typhoid fever because it is effective (Rahmasari & Lestari, 2018).

The results of the Widal I test showed negative results because the mice had not been induced with *S. typhi*, while the results of the Widal II test, which was the 7th day, showed negative results for normal control, positive control and treatment groups, while negative controls showed positive results. The results of the Widal III test, which is the 14th day after the administration of the test material, showed negative results in the positive control group given chloramphenicol at a dose of 7.2 mg/250 g BB, and in the mucoadhesive gastroretentive granules of earthworm flour at a dose of 200 mg/Kg BB, 400 mg/Kg BB and 800 mg/Kg BB, while the negative control showed positive results.

Based on the results of the research carried out, the mucoadhesive gastroretentive granules of earthworm flour (*Lumbricus rubellus*) at doses of 200 mg/kg BB, 400 mg/kg BB and 800 mg/kg BB can provide a decrease in body temperature comparable to normal control. The dose of 400 mg/kg BB granules of groundworm flour gastroretentive mucoadhesion (*Lumbricus rubellus*) is effective because it provides a decreasing effect that is close to normal control and positive control where the protein possessed by earthworm has an antimicrobial mechanism, namely by creating pores in the bacterial cell wall (Bestari et al., 2022), instability in the cell wall and cytoplasmic membrane of bacteria causes the function of selective permeability, active transport function, control of protein arrangement of bacterial cells to be disturbed, which will result in the escape of macromolecules, and ions from the cell. So that the bacterial cell loses its shape, and lysis occurs (Hariyati et al., 2015).

Conclusion

This study was conducted on wistar rats for 14 days. Gastroretentive preparations of mucoadhesive granules from earthworm flour (*Lumbricus rubellus*) at doses of 200 mg/Kg BB, 400 mg/Kg BB and 800 mg/Kg BB had an effect on the decrease in body temperature of male white rats induced by *Salmonella typhi*. A gastroretentive preparation of mucoadhesive granules

from earthworm flour (*Lumbricus rubellus*) at a dose of 400 mg/Kg BB is an effective dose in lowering the body temperature of male white rats with an average of 37.70 C with a negative widal test.

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Author's Contribution

Conceptualization, SGB, and Magfirah; methodology, SGB, Magfirah, and VA; validation, Magfirah; data analysis, Magfirah, and VA; investigation, SGB; resources, JT; data curation, Magfirah, and VA; writing—preparation of the original draft, SGB; writing—review and editing, SGB, Magfirah, and VA; supervision, Magfirah All authors have read and approved the published version of the manuscript.

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Conflict of Interest

The author stated that there was no conflict of interest.

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