

Healing Effects of Fresh Earthworms (*Pheretima Javanica* K.) for Typhoid Fever Induced in Male White Rat (*Rattus Norvegicus* L.)



Joko Waluyo, Dwi Wahyuni, Wiwien S. Utami

Abstract: Typhoid fever is systemic infectious disease caused by *Salmonella typhi* bacteria which is still considered as a health problem for the world people. This research aims to know the healing effects of fresh earthworm species *Pheretima javanica* to reduction typhoid fever replace the treatment using chloramphenicol. This research using male white rats (*Rattus norvegicus* L.) with negative control and positive control by using chloramphenicol. and 4 treatments group of each 5 rats. The test consists of Widal test, feces test and body weight measurement were analyzed descriptively, while body temperature measurement was analyzed by using ANOVA test and continued with Duncan test with confidence rate at 95%. The analyzed result got probably result $p = 0,0001$ ($p < 0,05$) means that fresh earthworm has a significant effect on the reduction of typhoid fever on male white rats with optimal dosage is 3 gram / 0.2 kg BB.

Index Terms: Fresh *Pheretima javanica* K., typhoid fever, *Salmonella typhi*, Widal Test..

I. INTRODUCTION

Typhoid fever is a global health problem caused by *Salmonella typhi* [6]. In developing countries, an estimated incidence is 540 / 100,000 in a year. It is estimated that there are about 17 million cases worldwide each year. *Salmonella typhi* is transmitted from impurities which enter the body through the digestive tract [10]. Fever or warm are the main symptoms of typhoid fever [7]. The common treatment for typhoid fever patients is antibiotics [3]. Antibiotics which are often used in the treatment of typhoid fever are chloramphenicol, ampicillin, cotrimoxazole, norfloxacin, neomycin, ciproflaksasin and ofloxacin. Using antibiotics is caused new problems, that cause emergence of resistance, especially on the unprocedural and uncontrolled antibiotics [9].

To overcome those problems, it takes a natural medicine which comes from animals and plants. Earthworms are often used to treat typhoid fever and reduce heat for common people.^[4] In rural areas, earthworms are often used as typhus or typhoid fever in various ways, fried without oil, boiled, and crushed mixed with water of *siyem* pumpkin then drunk [8] or typhoid fever in various ways, fried without oil, boiled, and crushed mixed with water of *siyem* pumpkin then drunk [8]. The types of earthworms found in Java are *Pontoscolex coretrurus*, *Lumbricus rubellus*, *Pheretima capensis* and *Pheretima javanica*. Among those earthworms, *Pheretima javanica* K. are the highest population and have relatively larger bodies than others [8].

In the body of earthworm (*Pheretima javanica* K.), there are antipyretics like arakhidonat acid, antipurin, anti-poison, and vitamin. The content of that substances makes earthworms can decrease body temperature and inhibit the growth of bacteria [9].

A study was done by Purwangingtyas (2009), who said that fresh earthworms (*Pheretima javanica* K.) can inhibit *Salmonella typhi* growth in vivo. So that, this research conducted further research with the addition of dosage. It is expected to heal typhoid fever faster by using fresh earthworms (*Pheretima javanica* K.) rather than chloramphenicol.

Typhoid fever is usually diagnosed by blood culture, stool culture, bone marrow culture, bile culture and serological techniques, among these blood culture is considered as gold standard and becomes positive in first week of fever, Serological techniques including Widal is still commonly used in endemic areas of developing countries.^[11] So, in this experiment the widal test will be used to detect the bacteria *Salmonella typhi* cause typhoid fever.

II. MATERIALS AND METHODS

This research consisted of some stages. They are animal model preparation stage, fresh earthworm making stage, and treatment stage. The animal model preparation stage was done by preparing animal model namely male white rats of Wistar species which was first acclimated for a week. The fresh earthworm making stage was started by collecting samples of earthworm type *Pheretima javanica* K. The first step is fresh earthworm was washed and blended, then weighed with a dosage of 1.8 grams / 0.2 kg BB; 2.4 grams / 0.2 kg BB; 3 grams / 0.2 kg BB and 3.6 grams / 0.2 kg BB.

Revised Manuscript Received on 30 July 2019.

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Healing Effects of Fresh Earthworms (*Pheretima Javanica* K.) for Typhoid Fever Induced in Male White Rat (*Rattus Norvegicus* L.)

The treatment stage was started with *Salmonella typhi* infection up to earthworm induction. one negative control and one positive control use cloramphenicol with 5 times replication for each treatment group.

This research consisted of some tests, like Widal test, feces test, rat body temperature measurement, and rat body weight measurement. This study has several tests such as widal Test, fecal test, rat body temperature measurement and rat body weight measurement. The widal test was performed in 4 stages, widal I test was performed after acclimation, widal II test was performed after mouse showed symptoms of typhoid fever, widal III test was done after 2 weeks of treatment using fresh earthworm, widal IV test was done to ensure animal experiments negative salmonella typhi. Temperature measurements and faeces tests are performed every day while weight is performed every week

The results of Widal test, feces test, and rat body weight measurement would be analyzed descriptively. While the result of rat body temperature measurement would be analyzed by using ANOVA for parametric data

III. RESULT AND DISCUSSION

The parameters observed in this study are the decrease of white rat (*Rattus norvegicus* B.) body temperature post-induction of earthworm (*Pheretima javanica* K.), the decrease of antibody titer to *Salmonella typhi* in white rats (*Rattus norvegicus* B.) blood, feces test, and rat body weight as an additional data.

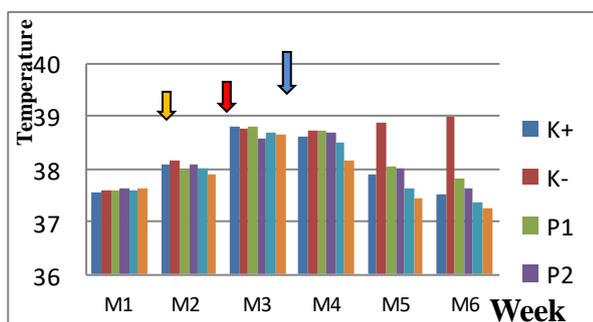


Fig 1. Temperature Body Along Acclimation, Infection of Salmonella Typhi and Fresh Earthworms Administration Periods

NOTES :

- K+ : Cloramfenicol induction 0,018 g/0,2 kg BB
- K- : Aquades induction 2 ml
- P1 : Fresh Earthworms induction (*Pheretima javanica* K.) dosage 1,8 gram/0,2 kg BB
- P2 : Fresh Earthworms induction (*Pheretima javanica* K.) dosage 2,4 gram/0,2 kg BB
- P3 : Fresh Earthworms induction (*Pheretima javanica* K.) dosage 3 gram/0,2 kg BB
- P4 : Fresh Earthworms induction (*Pheretima javanica* K.) dosage 3,6 gram/0,2 kg BB
- ↓ : Acclimation Period/M1
- ↓ : Infection Period of *Salmonella typhi*/M2-M3
- ↓ : Induction Period of Fresh Earthworms (*Pheretima javanica*)/M3-M6

Figure 1 shows that rats' body temperature is about 37.6 ° C in acclimation period of all treatments. After bacterial infection, the average of the rats' temperature was increased to 38.7 ° C. At the third week post treatment fresh earthworm (*Pheretima javanica* K.) for 23 days, it was resulted in significant temperature decreasing in K +, P1, P2, P3 and P4 rats' group. In contrast to K-rats which are treated in negative control and aquades induction.

Based on white mouse body temperature measurement, one way ANOVA analysis was conducted. Fresh earthworms have significantly effect on decreasing rat body temperature presenting typhoid fever ($p=0.0001$) The results of ANOVA test also has a significant difference of the treatment for each group, so that it was needed Duncan test. Duncan test resulted significant value $p < 0,05$. This indicates that any treatment given in the study provides a significant difference in decreasing rats' body temperature.

The second parameter observed is widal test. Widal test aims to detect the presence of antibodies of *Salmonella typhi* bacteria by measuring the levels of antibody agglutination against O and H antigens in blood samples. The widal titer is usually a multiple of 1/40, 1/80, 1/160, 1/320, 1/640.[6]

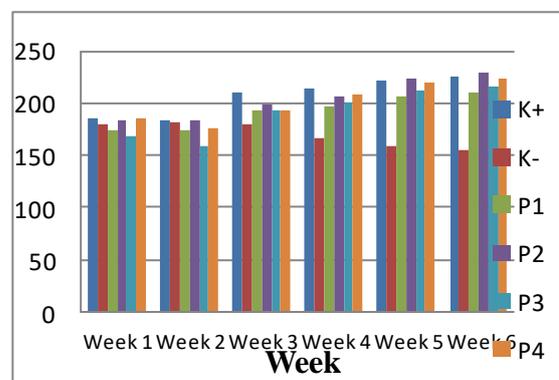


Figure 2. Comparison of Weight Variations Along The Treatment Weeks and Groups or Treatment

Table 1. The Result of Widal Test In Male White Rats

Treatments Group	Widal I	Widal II	Widal III	Widal IV
P1	1/40	1/320	1/80	1/40
P2	-	1/160	1/80	1/40
P3	-	1/320	1/40	1/40
P4	1/40	1/160	1/40	-
K(+)	1/40	1/160	1/80	1/40
K(-)	-	1/160	1/160	1/320

The table showed that there was an increase of antibody titer in white blood samples after *Salmonella typhi* infection, and decreased after fresh earthworm induced (*Pheretima javanica* K.), except in K(-) treatment which increased antibody titer continuously. This indicates that more bacteria *Salmonella typhi* are in the blood of the rats, this happens because the negative control is not given antibacterial substances but only given aquades.

To support the statements, feces test was also done. Positive results were indicated by the thick and black growth in the SSA medium. Positive results indicated that white rats actually had typhoid fever. After the induction of fresh earthworms (*Pheretima javanica* K.), negative results were obtained on the treatment of K (+), P3 and P4, while P1 and P2 were still seen the growth of *Salmonella typhi* but not as much as before. In the K(-) treatment, *Salmonella typhi* growth was increasing, indicating that white rats are still positively typhoid fever.

In this study, researchers also performed weight measurements to determine the effect of *Salmonella typhi* bacterial infection against white mice. In Figure 2, it is seen that the weight of white rats decreased after infection with *Salmonella typhi* bacteria. However, after fresh earthworms, the weight of rats increases again, this is because fresh earthworms can inhibit *Salmonella typhi* bacteria and it also contains high protein that improves cells damaged by salmonella typhi infection so that the rats are getting healthy.

Use of the whole fresh earthworms (*Pheretima javanica* K.) with the contents of their stomach can reduce symptoms of typhoid fever that were influenced by the content of the antipyretic substance in earthworms, such as arachidonic acid, antipurin, antitoxic, and vitamins. This substance can decrease earthworms' body temperature and inhibit the growth of bacteria [9]. Earthworm also has Lumbricin compounds. Lumbricin is an antibacterial compound in earthworms which belongs to the class of antimicrobial peptides. It is a natural defense against the presence of pathogenic microbes in its environment [4].

The compound used as a drug is not only pure from the earthworm itself, but also there are microorganisms which have antibiotic compounds. In the body of earthworms, it was found various *Streptomyces* that produce antibacterial compounds [7]. Antibacterials produced by *Streptomyces* can inhibit the growth of gram-negative bacteria such as *Salmonella typhi* [2].

IV. CONCLUSION

Based on the result of the research, it can be concluded that the induction of fresh earthworms (*Pheretima javanica* K.) influenced the healing typhoid fever in white rats (*Rattus norvegicus* B.). the best dosage with faster healing of symptoms of typhoid fever experimentally induced in rats was 3.6g of fresh earthworm/0.2kg of weight.

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