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Antipyretic Effects Of Dried Earthworm (Pheretima Javanica K.) In Male White Rat (Rattus Norvegicus) With Typhoid Fever

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Abstract: Typhoid fever is a disease caused by Salmonella typhi bacterial infection, The symptoms commonly caused by this disease are usually fever, dizziness, nausea and even diarrhea. Using Chloramphenicol as a medicine has been experienced resistance for the last few years and has side effects. The aim of this research is to produce an alternative medicine of typhoid fever without side effects from dried earthworm (Pheretima javanica K.) in male white rats (Rattus norvegicus L.). This research is an experimental study, with 2 control group and 4 treatments group of each 10 white rats. The 4 tests are consist of Widal Test, body weight measurement, body temperature measurement and feces test. 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 $p = 0,000$ ($p < 0,05$) means that dried earthworm has a significant effect on the reduction of typhoid fever on male white rats. The most optimal dose was gained from treatment 2 (dried earthworm induction with dose 0,8 gs/0,2 Kg of weight) which had similar value with the positive control with chloramphenicol induction.

Index Terms : Typhoid Fever, Salmonella typhi, Pheretima javanica powder, Widal Test

1 INTRODUCTION

Typhoid fever is systemic infectious disease caused by Salmonella typhi bacteria which is still considered as a health problem for the world people, Enteric fever continues to be a global health problem, with an estimated 21.6 million people incidence of 3.6 per 1,000 population and kills an estimated 200,000 people every year [1] especially for developing countries with tropical climate including Indonesia. [2] This acute disease was indicated by the emergence of some symptoms like prolonged fever, nausea, headache, decreased appetite, and diarrhea. [2] Typhoid fever is a disease that can affect many people from different types of age from toddlers, children, adolescents, adults to old age. [3] Typhoid fever is a contagious disease that causes acute infection on small intestines. The large number of the cases makes this people health problem needs more attention. In Indonesia, typhoid fever is also apprehensive and needs more attention because this disease is endemic and threatens the people's health from different types of age. The data results from some huge hospitals in Indonesia showed that there is an increase in typhoid fever cases per year with total average 500/100.000 people and death cases about 0,6%-5%. [5]

The handling usually used to treat typhoid fever cases was by using synthetic medicines like chloramphenicol, tiamphenicol, amoxicillin, and ampicillin. Some hospitals use chloramphenicol as the medicine which is often used to treat typhoid fever cases.

Chloramphenicol still becomes the first choice to treat typhoid fever since it is effective, inexpensive, and easily obtained. However, it has been reported that in last five years there has occurred multiple drug resistance on Salmonella typhi which is the main cause of typhoid fever cases. Besides, the use of chloramphenicol as the typhoid fever medicine can cause some side effects like bone marrow suppression and the occurrence of aplastic anemia. [6] Based on those large numbers of typhoid fever cases and the occurrence of drug resistance on the bacteria that cause typhoid fever, the researcher conducted a research to produce a natural medicine that can be used as alternative medicine to cure typhoid fever. This research utilized earthworm (Pheretima javanica) as the alternative medicine to cure typhoid fever. Some people stated that earthworms have many efficacies. Earthworms are one type of animals that live under the ground. Every type of earthworms has different tolerance limitation on their habitat as the place for them to make living. [7] The existence of earthworms has given many benefits which one of them is giving soil fertility especially on agricultural fields. Beside fertilizing soil, the other benefits of earthworms are repairing and maintaining soil structure, increasing water absorption capacity of soil, feeding fish and making bait for fishing. In health, earthworms are often used as fever reducing medicine and also used as a cosmetic mixer. [8] The body of earthworms type Pheretima javanica contains antibacterial compounds that is capable to inhibit the growth of bacteria. [8] 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. [13] So, in this experiment the widal test will be used to detect the bacteria salmonella typhi cause typhoid fever.

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2 MATERIALS AND METHODS

This research consisted of some stages. They are animal model preparation stage, dried 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 dried earthworm making stage was started by collecting samples of earthworm type *Pheretima javanica* which were washed out and dried in about 6-7 days. After being dried, the earthworms were baked in the oven for 4 hours with temperature 400C and then they were blended until smooth. The treatment stage was started with *Salmonella typhi* infection up to earthworm induction. The doses used in this research were 0,4 gs; 0,8 gs; 1,6 gs and 3,2 gs/0,2 kgs body weight, one negative control and one positive control with 10 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, they are widal first test was performed after acclimation, widal second test was performed after mouse showed symptoms of typhoid fever, widal third test was done after 2 weeks of treatment using dried earthworm, and the widal fourth 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 rats body weight measurement would be analyzed descriptively. While the result of rat body temperature measurement would be analyzed by using ANOVA for parametric data.

3. RESULTS AND DISCUSSION

This research was done Pharmacology Laboratory at the Faculty of Dentistry of Jember University. The research was started by doing animal model preparation in the form of male white rats of Wistar species. The animal model was firstly acclimated in a week. Then, the animal model was tested using Widal Test for the first time to know that the rats were really in a good health and ready to be treated. After the Widal test was finished, it was continued by infecting *Salmonella typhi* bacteria which was already standardized with McFarland 0,5 as much as 1 mls orally. This research used dried earthworms type *Pheretima javanica* as the alternative medicine to cure typhoid fever. The dried *Pheretima javanica* was considered to be capable to cure typhoid fever because those earthworms consisted of antibacterial compounds aimed to inhibit the growth of bacteria especially the bacteria which caused typhoid fever. This statement was supported by Waluyo (2006) who stated that antibacterial protein compounds in the earthworm body can inhibit the growth of *Salmonella typhi*. [9] This research resulted in some data gained. The first data was the result of body temperature measurement, which was done to know the difference of the rat body temperature when acclimation, when it was infected with *Salmonella typhi* per oral, and when the dried earthworm (*Pheretima javanica*) induction. The body temperature was the indication to know whether or not the animal model experienced fever. Fever was the main characteristic of typhoid fever.

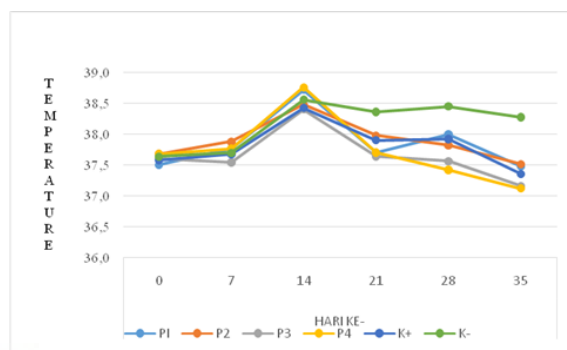


Figure 1: Temperature of Body Variables Between Different Groups of Treatment and Time of Acclimation, Infection and Induction

NOTES

- ↓ : Acclimation (day 0 - 7)
- ↓ : Infection (day 8-14)
- ↓ : Induction (day 15 - 35)

- K(+) : Positive control with chloramphenicol induction (which is converted by dose in humans: 0,0018 gs)
- K(-) : Negative control with CMC Na 1% induction 2 mls
- P1 : Treatment 1 (dried earthworm induction with dose 0,4 gs/0,2 KgBB)
- P2 : Treatment 2 (dried earthworm induction with dose 0,8 gs/0,2 KgBB)
- P3 : Treatment 3 (dried earthworm induction with dose 1,6 gs/0,2 KgBB)
- P4 : Treatment 4 (dried earthworm induction with dose 3,2 gs/0,2 KgBB)
- U : Replication

From the graph above, it could be known that on day 0 until day 7 the average of the rat body temperature was in normal condition because during those days the animal model was still in acclimation phase. On day 8, the animal model was orally infected with *Salmonella typhi* as much as 1 ml. On day 14, the average of body temperature of the animal model increased since the bacteria has managed to infect the animal model and the animal model could be said to have fever. After that, on day 15, the animal model was induced by dried earthworm with a certain dose. Based on the result of ANOVA test, it could be gained that the substitution value (p) for the effect of dried earthworm type *Pheretima javanica* on the reduction of typhoid fever was 0,000, in which this case meant that the substitution ($p < 0,05$) so it could be concluded that the dried earthworm has a significant effect on the reduction of typhoid fever on the animal model. Decrease in body temperature in animal testing due to the presence of antipyretic substances in earthworms such as arachidonic acid, antipurin, anti-toxins and vitamins. this substance that can lower body temperature and inhibit bacterial growth. *Pheretima javanica* earthworms also has lumbricin which is a group of antimicrobial proteins in earthworms that have a natural defense against the presence of pathogenic microbes in its environment. This research result showed that dried earthworm type *Pheretima javanica* has an effect on the reduction of typhoid fever. In addition to body temperature measurement, this research also performed serological test. The most commonly used diagnostic test is the Widal test, for

decades. This test is used to measure agglutinating antibodies against H and O antigens of *Salmonella typhi*.^[11] Widal test was done to strengthen typhoid fever diagnosis by knowing antibody titers in blood. Commonly, diagnosis of typhoid fever is done in laboratory to strengthen the diagnosis itself.^[2] The Widal test in this research was done in Jember "Piramida" Clinic Laboratory. This test was done four times. Widal Test I was done to know the animal model condition, Widal Test II was done to know whether or not the animal model positively got typhoid fever after being infected by the bacteria, Widal Test III was done after two weeks post dried earthworm induction, and Widal Test IV was done to know whether or not the animal could be said as negatively got typhoid fever in post induction except the real negative control that was not given any 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	1/160	1/80	1/40
P2	1/40	1/160	1/80	1/40
P3	1/40	1/320	1/80	-
P4	1/40	1/320	1/40	-
K(+)	-	1/160	1/80	1/40
K(-)	1/40	1/160	1/320	1/320

The result of Widal Test I could be said as negative and in a good health condition. It could be seen from the result of the Widal test that the antibody titers indicated negative value. The result of Widal Test II showed that there was an increment of the animal model antibody titers because it was infected by *Salmonella typhi*. The animal model was said to be positively got typhoid fever if the antibody titers experienced increment up to 1/160 and 1/320.^[12] Widal Test III and IV were done after the induction of dried earthworm which aimed to reduce the antibody titers so that the animal model could be said as negatively got typhoid fever. From result of Widal test, it can be gained that earthworm has an effect on antibody titers reduction so that the animal model can be said to be negative from typhoid fever. The next data of this research was feces test. Feces test was done to strengthen the diagnosis. The feces test was conducted in Microbiological Laboratory at Medical Faculty of Jember University. This test was done by culturing the feces in a medium and then it was incubated for several hours to get the bacterial colony in the medium. In this research, the experiment of feces test was done twice. The first test was done after *Salmonella typhi* infection which aimed to know that the animal model was positively got typhoid fever that could be seen from the result of bacterial culturing on SSA (*Salmonella Shigella Agar*) medium. It could be said as positive if there was an existence of black colony on the SSA. That colony indicated that *Salmonella typhi* was growing in the medium. The first feces test resulted in almost all of the body of the sample was overgrown by black colony which indicated that the animal model positively got typhoid fever. The second feces test was done on day 35 or the last day of dried earthworm type *Pheretima javanica* induction. This test aimed to know that the animal model which had been given a treatment of dried earthworm (*Pheretima javanica*) induction was recovered from typhoid fever since the dried earthworm could inhibit the growth of bacteria. The result of the second feces test showed that almost all the treatments signified negative since there was no indication of the growth of black

colony on SSA medium, except the negative control itself which indicated positively got typhoid fever because it was not given any treatment of dried earthworm induction. After the previous stage, the next data was gained from body weight measurement. The animal model body weight measurement aimed to know the differences between the body weight of the animal model in acclimation phase post infection and the body.

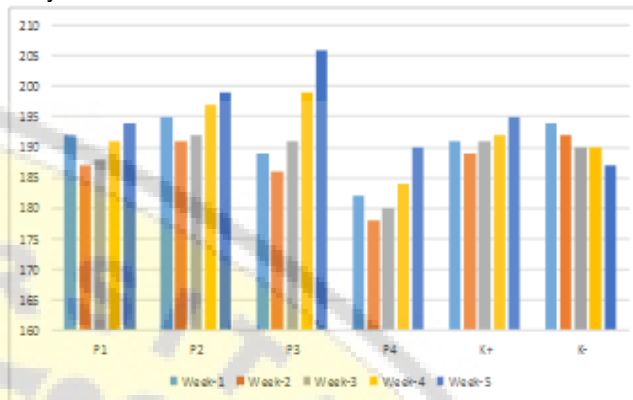


Figure 2: Body Weight Variations According to Weeks of Treatment and Between Groups

Based on the graph above, it can be known that there was body weight loss on week-2 in every treatment. The body weight loss of that week was accrued after the infection of *Salmonella typhi* which caused the animal model got typhoid fever and lost its appetite and resulted in its body weight loss. On week-3 until week-5 each of treatments, except the negative control, started to experience weight gain as a result of recovery process and dried earthworm type *Pheretima javanica* induction process. Beside it is capable to cure typhoid fever, according to Waluyo (2004), earthworm is such kind of food that has high nutritional value. The nutrient contents of earthworms are proteins, fats, minerals and water.^[8] So, it can help the animal model to improve the nutrition post infection of *Salmonella typhi* bacteria. Based on the capabilities possessed by the *Pheretima javanica* earthworm, It should be done a further research on the utilization of earthworms by determine the antibacterial compounds related to its effects on typhoid fever, so that are not only used for the alternative treatment, but can also be a weight in dried earthworm type *Pheretima javanica* induction phase. The body weight measurement was done five times every week. The graph of the rat body weight measurement could be seen as follows commercial drug that can be traded and consumed by the people with minimize the side effects.

4. CONCLUSION

The result of this research showed that dried earthworm type *Pheretima javanica* has an effect on the reduction of typhoid fever. The most optimal dose was gained from treatment 2 (dried earthworm induction with dose 0,8 gs/0,2 Kg of weight) which had similar value with the positive control with chloramphenicol induction. Further studies are necessary to determine the antibacterial compounds related to its effects on typhoid fever for perfect this research. The result of this research showed that dried earthworm type *Pheretima javanica* has an effect on the reduction of typhoid fever. The most optimal dose was gained from treatment 2 (dried

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