

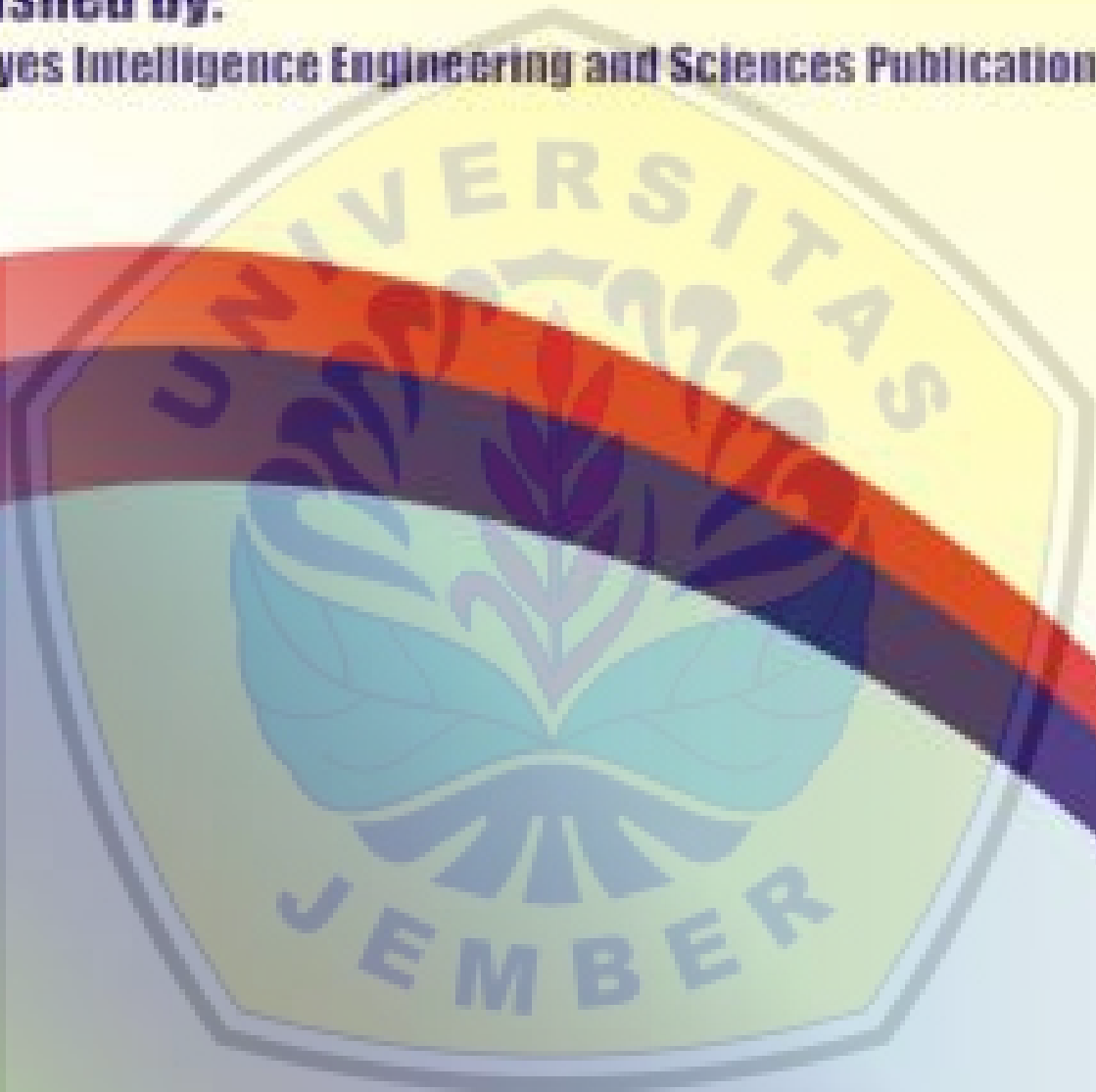
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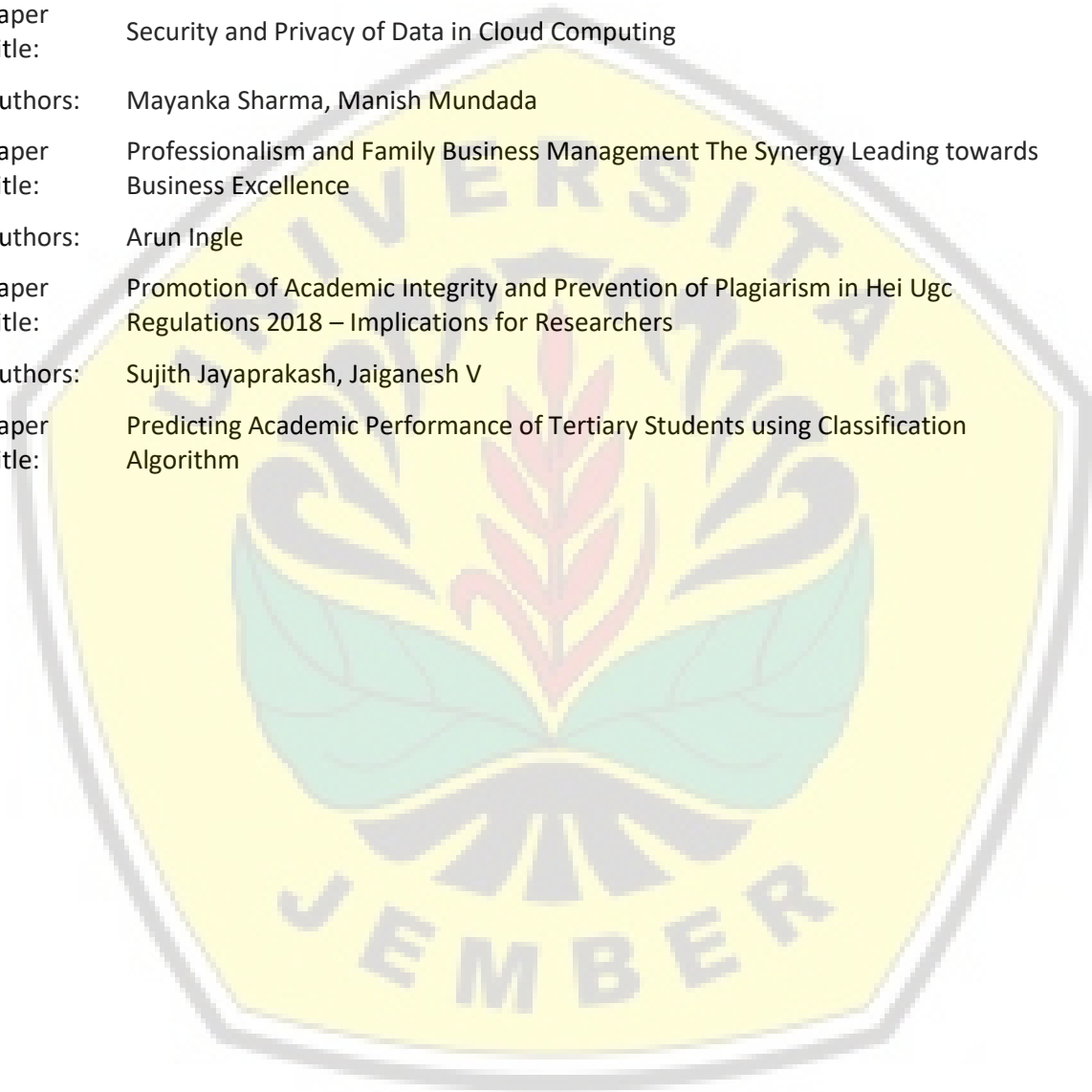


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1086	Authors:	Mayanka Sharma, Manish Mundada	6549-6553
	Paper Title:	Professionalism and Family Business Management The Synergy Leading towards Business Excellence	
1087	Authors:	Arun Ingle	6554-6557
	Paper Title:	Promotion of Academic Integrity and Prevention of Plagiarism in Hei Ugc Regulations 2018 – Implications for Researchers	
1088	Authors:	Sujith Jayaprakash, Jaiganesh V	6558-6561
	Paper Title:	Predicting Academic Performance of Tertiary Students using Classification Algorithm	



# Safety of Bioinsecticide Ekstrakt Sugar Apple Seed's Granule (*Annona squamosa* L.) on Histology of White Rat (*Rattus norvegicus* B.)

Dwi Wahyuni, Rifqi Fuadatul Lathifa, Vendi Eko Susilo

**Abstract:** The granules of sugar apple seeds extract (*Annona squamosa* L.) have been taken as the natural larvacide an economical in eradicating mosquito flicks of *Aedes aegypti* L. because of its the content of toxic compounds such as acetogenin, alkaloids, and flavonoids. In its use it is feared to cause toxic in the environment, especially the class of mammals as non-target animals, especially kidney organ. The kidney is an organ that is susceptible to toxic effects, since it receives 25-30% of the blood circulation to be cleaned, so that as a filtration organ there is a possibility of pathological changes. This study aims to determine the effect granules of sugar apple seeds extract (*Annona squamosa* L.) on the histology of white rat (*Rattus norvegicus* B.). Kidney histology observed as fat degeneration, hydrophic degeneration, and necrosis of tubular cells occurred in one field of view. The concentration given was 1 mg, 2 mg, 4 mg, and 8 mg while the negative control induced 2 ml aquades and positive control using abate 100 mg for 14 days with sonde method. The analysis used in this research is descriptive analysis with scoring. The results showed that granules of sugar apple seeds extract did not give effect to histology of white rat (*Rattus norvegicus* B.).

**Index Terms:** Granules Extract, Safety Bioinsecticide, Histology, Sugar Apple Seed's Granule

## I. INTRODUCTION

The sugar apple plant (*Annona squamosa* L.) is now scattered throughout Indonesia, although it is still limited to planting. People only use fruits for consumption [1]. The seed of sugar apple is one part of the sugar apple plant (*Annona squamosa* L.) that secretes into a natural insecticide. The seeds of contain chemical compounds of acetogenin consisting of annonain, squamosin, and asimisin [2].

Sugar apple seeds are oval, shiny and smooth, black or dark brown, and measuring 1.3-1.6 cm with a weight of 5-18 grams [3]. The seeds of srikaya also contain alkaloid compounds that can cause insect death through the mechanism of contact toxins and stomach toxins. In addition, alkaloids are thought to have the effect of inhibiting lipase enzyme activity because it has a chemical structure similar to orlistat (the presence of N elements). While flavonoids are respiratory inhibitors by working to damage the siphon and weaken the nervous system by inhibiting the action of acetylcholinesterase enzyme [4]. Efforts to control insects have long been done and almost all people use synthetic insecticides. Abate is one of the synthetic insecticides that are often used in the community to eradicate mosquito larvae. Increased use of synthetic insecticides results in resistance to

targeted insects, as well as residues that can pollute the environment [5]. Based on these facts need further action with security testing on mammals to be made as bioinsektisida that is safe for the surrounding environment, especially in mammals.

In addition to using extracts, the seed granules of srikaya seed (*Annona squamosa* L.) has now become an economical natural insecticide in the control of *Aedes aegypti* L larvae. The use of natural insecticides in granule form is more stable if stored in a long period of time and more applicable in its use by the community [6].

Srikaya plants (*Annona squamosa* L.) can grow on all soil types with pH 6-6.5. Thus, cultivated srikaya planted in a rather acidic environment. The most preferred type of soil is the soil containing lots of sand and lime. The area with an altitude of 100-1000 m above sea level is the height favored by srikaya plants, but the best height is at an altitude of 100-300 m above sea level. While the preferred temperature of srikaya plants ranges from 20-25°C [1].

The seed of srikaya (*Annona squamosa* L.) is one part of the srikaya plant (*Annona squamosa* L.) that has the potential to be developed as bioinsecticide. As in the research which states that the seeds of srikaya can be used to kill lice and insects. The seeds of srikaya consist of various toxic compounds such as acetogenin, alkaloids, and flavonoids [7]. The toxicity possessed by this plant has a level of toxic residue that is easily lost which is about 48 hours after use [8]. The compound of acetogenin in srikaya seed consists of annonain which has chemical formula C<sub>35</sub>H<sub>64</sub>O<sub>7</sub> and squamosin with chemical formula C<sub>37</sub>H<sub>66</sub>O<sub>7</sub>. These compounds have properties as insecticides, acaricides, antiparasites and bactericides with inhibitor mechanism [9].

Alkaloids in somatic seeds can act as insecticides, larvacides, insect repellents and inhibitors [10]. Alkaloids are secondary metabolites that are plant antioxidants and are capable of causing insect death through toxic mechanisms of contact and abdominal toxins and are easily broken down if stored for long periods [11]. Alkaloid compounds are suspected to have the effect of inhibiting lipase enzyme activity because it has a chemical structure similar to orlistat (the presence of N elements). While flavonoids are respiratory inhibitors by destructive siphon work and weaken the nervous system by inhibiting the action of enzyme acetylcholinesterase [12]. Granules are clumps of small particles that can be stored for a long time, and are more stable in their use. Granules have low concentrations of active ingredients and varying sizes. The size of the granules

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**Dwi Wahyuni**, Biologi Education Departemen, University of Jember, Jember, Indonesia

**Rifqi Fuadatul Lathifa**, Biologi Education Departemen, University of Jember, Jember, Indonesia

**Vendi Eko Susilo**, Medical Departemen, University of Jember, Jember, Indonesia.



usually ranges from 4-12 mesh sieve, but the granular size can be adjusted according to the research needs [13]. Granules are products produced by the granulation process which will then be used as solid dosage. Method of making granules there are 2 types, namely wet granulation and dry granulation [14].

Research conducted found that the granule of extracts of seeds srikaya (*Annona squamosa* L.) can kill 50% larvae of *Aedes aegypti* L. mosquitoes of 1.08 ppm within 24 hours [15]. Biopesticides are usually biodegradable, so they do not pollute the environment and are relatively safe for humans and livestock because the residue is quickly lost. However, it is possible that organic compounds present in biopesticides can affect organs in non-target animals [16]. The use of herbs as traditional medicine for long periods of time can cause symptoms of toxicity. Any drug taken orally will undergo a process of absorption, metabolism and excretion. The kidneys have an important function in the process of excretion of ingredients that enter the body orally.

The kidney is an organ that is susceptible to toxic effects, since it receives 25-30% of the blood circulation to be cleaned, so that as a filtration organ there is a possibility of pathological changes. Therefore, there is a need for further research on the effects that will be generated by the extract of the seed granules srikaya (*Annona squamosa* L.) against the kidneys of white rats (*Rattus norvegicus* B.).

As a result of the use of natural larvacide is seen from the histology of the kidneys that occur in non target animals. This study uses test animals that can be synonymous with events that may arise when the granules are used by humans. Humans are a class of mammals so that researchers use a test animal that is white mouse (*Rattus norvegicus* B.). these wistar white rat strains are included in the mammalian class to allow for the same effects as humans. The observed histologies are hydrophic degeneration, fat degeneration, and necrosis.

## II. MATERIALS AND METHODS

This research is a type of laboratory experimental research. This series of research was conducted in Biomedical Laboratory of Faculty of Dentistry of Jember University for acclimation, treatment of white rat (*Rattus norvegicus* B.), treatment to histology preparation, while histology observation was done at Biology Education Laboratory of FKIP University of Jember. The implementation of this study began in January 2018.

The procedures include sterilization of tools and materials, maintenance and treatment of white rats (*Rattus norvegicus* B.), administration of extracts of somatic seed extract (*Annona squamosa* L.), surgery, renal histology preparation, histologic observation. The concentration given was 1 mg, 2 mg, 4 mg, and 8 mg while the negative control induced 2 ml aquades and positive control using abate 100 mg for 14 days. The analysis used in this research is descriptive analysis.

## III. RESULT AND DISCUSSION

The results of histologic observation of rats of white rat (*Rattus norvegicus* B.) after granula extract of seed srikaya (*Annona squamosa* L.) orally. Figure 1, 2, 3 and 4 represents the renal histology of white rats (*Rattus norvegicus* B.)

treatment group, figure 5 is positive control group, and figure 6 is negative control can be seen in the following.

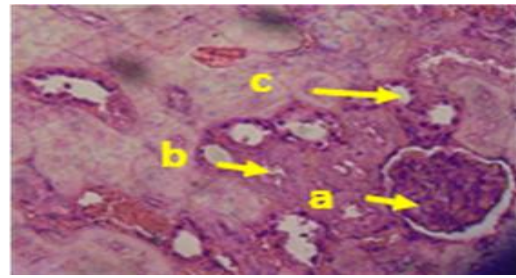


Figure 1. Renal histology of treatment group 1 (P1) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

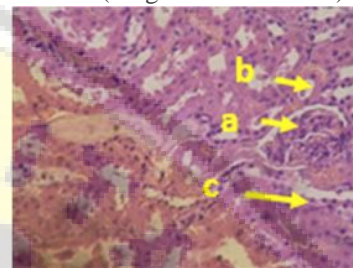


Figure 2. Renal histology of treatment group 2 (P2) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

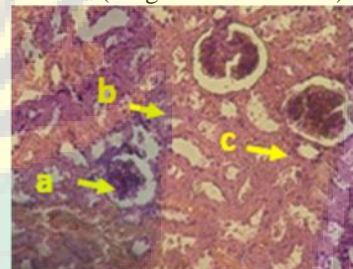


Figure 3. Renal histology of treatment group 3 (P3) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

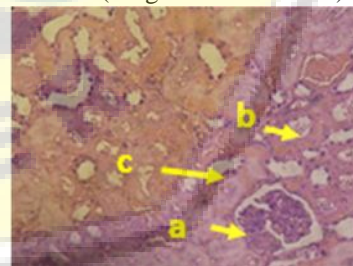


Figure 4. Renal histology of treatment group 4 (P4) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

Figure 5. Renal histology of treatment group (K+) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

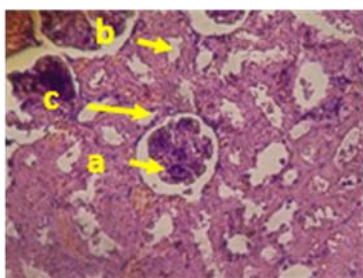


Figure 6. Renal histology of treatment group (K-) with HE staining. a = glomerulus; b = proximal tubules; c = distal tubule (Magnification 400 X)

From this research it can be seen that the histology of kidney rats in the treatment group (figure 1, 2, 3 and 4) and control group figure 5 (K+) and figure 6 (K-) did not show any difference. In this microscopic observation there is no sign of damage such as degeneration of fat which is usually characterized by a yellow vacuole surrounding the tubule epithelial nucleus, a hydrophic degeneration characterized by tubular epithelial tubular epithelium, and necrosis characterized by loss of the tubule epithelial nucleus. Thus, from the observation it can be seen that there is no effect of extract of somatic seed extract (*Annona squamosa* L.) on the histology of rats white rat (*Rattus norvegicus* B.) [15].

The mosquito's digestive system is different from mammals. Mammalian digestive system is much more complex than the digestive system in mosquitoes. Mosquitoes have digestion outside the intestinal tract (Elstrainstestinal Digestion) in which mosquitoes will release a special fluid to suck blood without blood clotting. The mosquito has blood on its body that resembles a mammal called hemolymph. Hemolymph is a liquid consisting of water, inorganic salts, proteins and fats. These materials serve to regulate the body temperature of mosquitoes, transporting nutrients, and immune. Blood circulation in mosquitoes is different from mammals. Circulatory system in the mosquito that is an open circulation system, whereas in mammals, especially white mice have a closed circulatory system.

One of the additional ingredients used in the manufacture of granules is lactose. Lactose ( $C_{12}H_{22}O_{11}$ ) can condense granule masses and readily mix homogeneously. Lactose is a form of disaccharide of carbohydrates that can be broken down in the simpler form of galactose and glucose. This glucose will be through the digestive mechanism to the small intestine and absorbed into the bloodstream. This blood will later be filtered in the kidney which is one of the organs of excretion in mammals.

Kidney is the main organ of drug excretion. Through urine, the kidneys excrete ingredients that are no longer needed by the body. Urine is the main path of excretion of toxicants, consequently the kidney has a high volume of blood flow. Kidney is prone to toxic substances, if too many toxic substances in the kidney will result in organ damage. The proximal part of the proximal tubule is the part that has an important role in the reabsorption of glucose and other important substances that are still needed by the body [16]. Proximal tubular epithelial cells are particularly susceptible to toxic substances. This is because in the proximal tubules occurs the process of absorption and active secretion, and levels of cytochrome P450 in the proximal tubulus is higher

to activate toxic substances, so often become the main target of toxic substances. Exposure to proximal tubules by toxic substances will continuously lead to cell death or necrosis. Renal damage the response of kidney damage produced in the direction of the higher concentration of toxic compounds that concern the organs of the kidney. Kidney damage is also associated with the ability of the kidneys to concentrate xenobiotic substances in the cells. Damage to proximal tubules caused by toxic substances is influenced by several factors including the type of chemical compound, dose, and duration of exposure of the compound. Higher concentrations will result in damage to the proximal tubules becoming wider [17].

#### IV. CONCLUSION

Granules extract of sugar apple seeds (*Annona squamosa* L.) are safe against the histology of the white rat (*Rattus norvegicus* B.) because the results are not found in tissues such as hydrophic degeneration, lipid degeneration, and necrosis of tubular epithelial cells.

#### V. ACKNOWLEDGMENT

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### AUTHORS PROFILE



**First Author** Graduate from Universitas Sebelas Maret, Master from Airlangga University, Doctoral from Airlangga University. The focus of research in Microbiology and medical. Lecture in Biologi Education Departemen University of Jember



**Second Author** Graduate Student of Biology Education Departemen from University of Jember



**Third Author** Graduate from Universitas of Jember. Master from Bogor Agricultural Institute. The focus of research in Biosystematic. Lecture in Biologi Education Departemen University of Jember.





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

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**Joko Waluyo**, Biologi Education Departemen, University of Jember, Jember, Indonesia

**Dwi Wahyuni**, Biologi Education Departemen, University of Jember, Jember, Indonesia

**Wiwien S. Utami**, Medical Departemen, University of Jember, Jember, Indonesia

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.

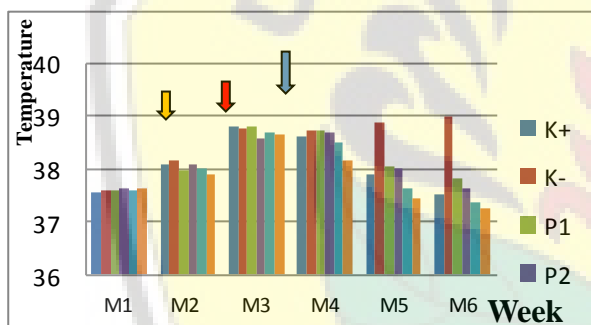


Fig 1. Temperature Body Along Acclimation, Infection of Salmonella Typhi and Fresh Earthworms Administration Periods

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]

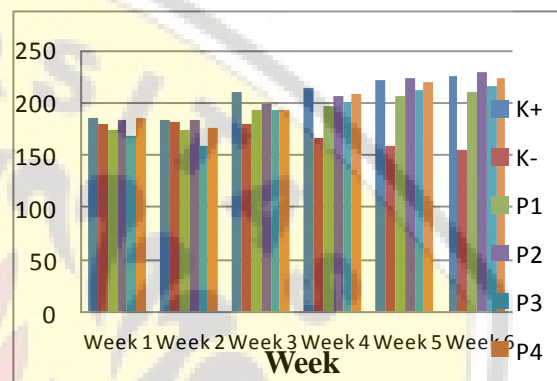


Figure 2. Comparison of Weight Variations Along The Treatment Weeks and Groups or Treatment

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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#### AUTHORS PROFILE



**First Author** Graduate from Universitas Sebelas Maret, Master from Airlangga University, Doctoral from Airlangga University. The focus of research in Microbiology and medical. Lecture in Biologi Education Departemen University of Jember



**Second Author** Graduate from Universitas Sebelas Maret, Master from Airlangga University, Doctoral from Airlangga University. The focus of research in Microbiology and medical. Lecture in Biologi Education Departemen University of Jember



**Third Author** Graduate from Gajah Mada University, Master from Gajah Mada University, Doctoral from Gajah Mada University. The focus of research in Microbiology and medical. Lecture in Medicaln Departemen University of Jember