

Comparison of Activity of Eudrilus Eugeniae and Hydrogel on Inflammatory Cells in the Wound Healing Process

Suandy¹, Setia Budi Tarigan², Catherine³, Janstine Firstiandy⁴

Bachelor of Medical Sciences, Faculty of Medicine, Universitas Prima Indonesia, Indonesia^{1,2,3,4}



Keywords:

Earthworms, Coelomic Fluid, anti-inflammatory, wound healing

ABSTRACT

Earthworms are widely used as ingredients for medicinal herbs to cure various diseases in the world of traditional medicine. Earthworm extract has antimicrobial, antipyretic, anti-inflammatory, fibrinolytic, and antioxidant abilities. Based on previous research, Eudrilus eugeniae extract has anti-inflammatory activity similar to aspirin. Earthworm paste can cause a reduction in inflammation, normalization of the number of erythrocytes and leukocytes, as well as differences in levels of neutrophils, lymphocytes, eosinophils, hemoglobin, and serum biochemical content, as well as acid electrolytes in cells. Coelomic Fluid Eudrilus eugeniae has similar effectiveness to Indomethacin, so it can be ascertained that coelomic fluid has potential as an anti-inflammatory. This study aims to determine the effect of Eudrilus eugeniae extract and hydrogel on the wound healing process. This study used the independent variables, namely Duoderm, NaCl, Ethanol Extract and Coelomic fluid. The dependent variable is inflammatory cells in the wound healing process. The sample of this research was Rattus norvegicus. Collecting data from the size of the wound which was measured using a caliper every day 0, 7, 14, 21, and 28, the results of this study showed that the positive control had 98.5% effectiveness of wound healing, treatment group 2 had 93.9% effectiveness, treatment group 1 had 85.3% effectiveness, and in the negative control only 69.1%. For inflammatory cells, on microscopic observation, it was found that on day 0 there were no inflammatory cells, on day 14 inflammatory cells such as PMN and Leukocytes were seen, and inflammatory cells had been seen reduced in day 28.



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.

1. INTRODUCTION

The development and mass production of chemically synthesized pharmaceuticals in the medical field has evolved in most countries of the world over the last 100 years. However, traditional medicine and the use of herbal medicines are still used by people in developing countries, such as 70% of the Indian population and 90% of the African population still relying on traditional medicine as their main treatment. Traditional medicine is not only used in developing countries, but the interest of people living in industrialized countries has also grown rapidly in the last 2 decades. In China, the use of traditional medicine is 40% and almost all

public hospitals in China also have traditional medicine units [2]. Since 1340, there have been records of the long association of earthworms with medicine, for example doctors in Burma and India used earthworms to treat certain ailments. One of the oldest disciplines in the world underlies most traditional medicine, namely Traditional Chinese Medicine in China and Ayurveda in India. Ayurvedic medicine using earthworms has revealed biological mechanisms and early guided approaches to understanding integrative medicine [3].

Earthworm extract has the ability as antimicrobial, antipyretic, anti-inflammatory, fibrinolytic, and antioxidant [1]. Based on experiments conducted by [3] conducted on albino wister rats (*Rattus Novergicus* Wistar) with earthworm paste compared with anti-inflammatory drugs, which focused on anti-inflammatory therapeutics, antioxidants, hematology, and serum biochemistry of worm pastes. land. As a result, there was a reduction in inflammation, normalization of the number of erythrocytes and leukocytes, as well as differences in the levels of neutrophils, lymphocytes, eosinophils, hemoglobin, and serum biochemical content, as well as acid electrolytes in the cells. Another study conducted by [6] used the earthworm *Eudrilus eugeniae* to see the anti-inflammatory activity of extracts of *Eudrilus eugeniae*. The study was conducted to compare the anti-inflammatory activity between extracts of *eudrilus eugeniae* at 95% ethanol and extracts of 0.2 M phosphate buffer with a pH of 7, and both will be compared with aspirin.

Between the body wall and intestine of earthworms, there is body fluid or Coelomic Fluid (CF), which contains various molecules and has antimicrobial, hemagglutinating, and anticoagulant properties [8]. Based on a study conducted by [7] to observe anti-inflammatory activity in wistar rats by the method of Carrageenan-induced rat paw edema and Croton oil induced inner ear edema, which were then compared with Indomethacin. The results showed that the administration of coelomic fluid has similar effectiveness to Indomethacin, so it can be ascertained that coelomic fluid has potential as an anti-inflammatory.

Because only a few people are aware of the use of earthworm-based herbal medicines [3], so researchers are interested in comparing inflammatory cells in the inflammatory process in *Eudrilus eugeniae* extract with coelomic fluid *Eudrilus eugeniae*.

2. Findings and Discussion

2.1 Macroscopic

Wound size of mice on day 28



Figure 1. Wound size of mice on day 28, “a” is a positive control group using duoderm ointment, “b” is a negative control group using NaCl, “c” is a treatment group 1 using a gel made from 70% ethanol extract of *Eudrilus eugeniae*, and “d” is treatment group 2 which uses a gel based on coelomic fluid

The parameter to see the shrinkage of the wound size is to measure the size of the wound with a caliper. The data obtained in the form of an average of the size of the wound in rats. The average size of the mice's wounds

taken every day 0, day 7, day 14, day 21, and day 28 can be seen in the table below.

Table 1. Comparison of Mean Wound Size: Positive Control: Duoderm Ointment, Negative Control: NaCl, Treatment Group 1: Gel based on Eudrilus Eugeniae Ethanol Extract 70%, and Treatment Group 2: Gel based on Coelomic Fluid

		Area of the wound				
		Day				
Group		Day 0 Mean	Day 7 Mean	Day 14 Mean	Day 21 Mean	Day 28 Mean
	Positive Control	176.8	112.4	51.6	16.1	2.6
	Negative Control	176.8	175.4	91.3	67.9	54.6
	Test Group 1	176.8	133.3	75.0	46.3	26.0
	Test Group 2	176.8	114.3	44.2	33.2	10.7

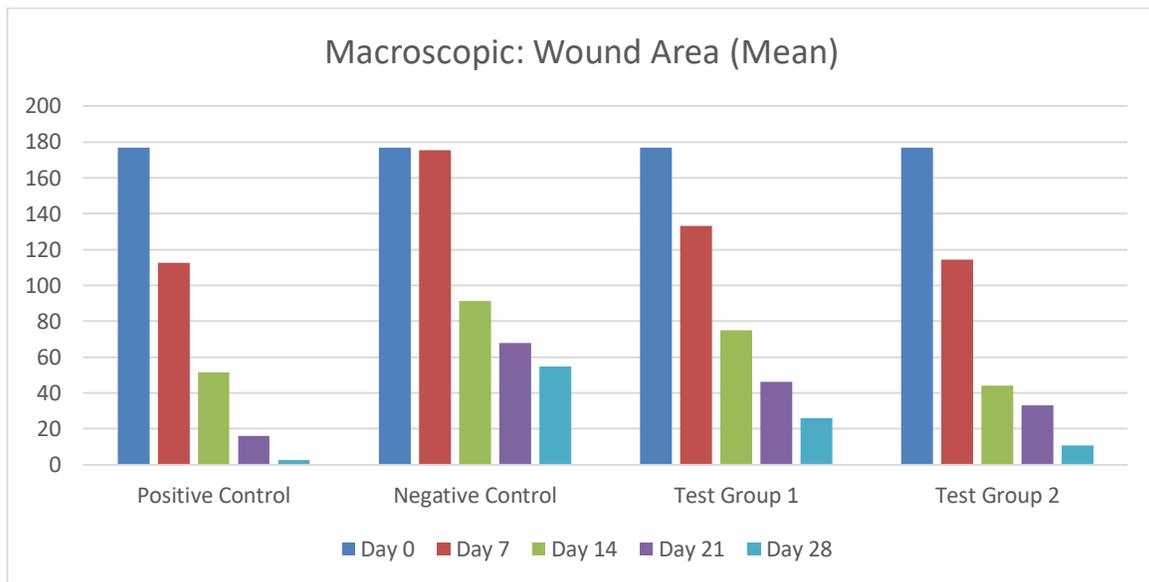


Figure 2. Comparison of Mean Wound Size: Positive Control: Duoderm Ointment, Negative Control: NaCl, Treatment Group 1: Gel based on Eudrilus Eugeniae Ethanol Extract 70%, and Treatment Group 2: Gel based on Coelomic Fluid

From the results of the treatment carried out for 28 days, it was found that the positive control had the best effectiveness among all, where the wound healing rate reached 98.5%. Furthermore, in treatment group 2, the wound healing rate reached 93.9%. The healing rate in treatment group 1 reached 85.3%, and in the negative treatment group, the wound healing rate only reached 69.1%.

2.2 Microscopic

Inflammatory cells were seen under the microscope on day 0, day 14, and day 28.

Inflammatory cells on day 0

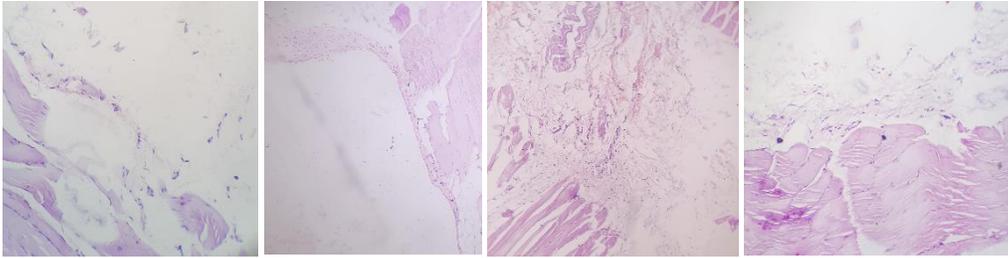


Figure 3. Microscopic appearance of inflammatory cells on Day 0

“a” is a positive control group using duoderm ointment, “b” is a negative control group using NaCl, “c” is a treatment group 1 using a gel based on 70% ethanol extract of *Eudrilus eugeniae*, and “d” is a treatment group 2 using a gel based on coelomic fluid.

On day 0, no inflammatory cells were seen in the four experimental groups.

Inflammatory cells on day 14

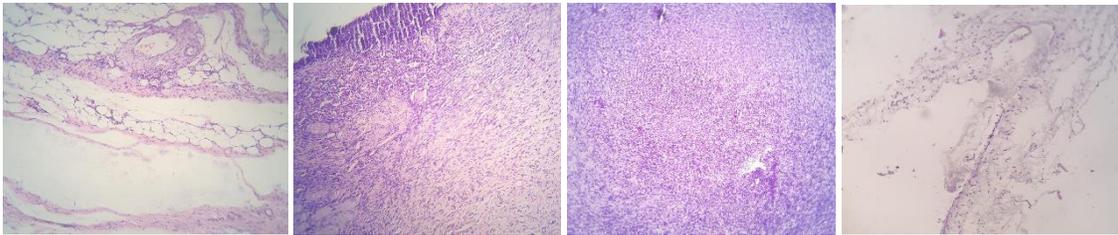


Figure 4. Microscopic Overview of Inflammatory Cells on Day 14

“a” is a positive control group using duoderm ointment, “b” is a negative control group using NaCl, “c” is a treatment group 1 using a gel based on 70% ethanol extract of *Eudrilus eugeniae*, and “d” is a treatment group 2 using a gel based on coelomic fluid.

On day 14, the number of inflammatory cells increased in the four experimental groups. In the positive control group (a): many inflammatory cells were found, namely PMN and leukocytes began to appear. In the negative control group (b): Many inflammatory cells were found, namely PMN. Likewise with treatment group 1 (c): many inflammatory cells were found, namely PMN. In treatment group 2 (d): many inflammatory cells were found, namely lymphocytes, while the number of PMN cells began to decrease.

Inflammatory cells on day 28

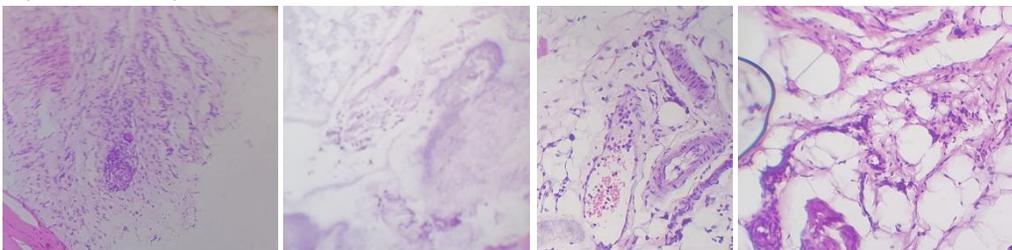


Figure 5. Microscopic Overview of Inflammatory Cells on Day 28

“a” is a positive control group using duoderm ointment, “b” is a negative control group using NaCl, “c” is a treatment group 1 using a gel based on 70% ethanol extract of *Eudrilus eugeniae*, and “d” is a treatment group 2 using a gel based on coelomic fluid.

On day 28, Inflammatory Cells such as PMN and Leukocytes in all four groups were reduced.

2.3 Organoleptic Test

Organoleptic test done on duoderm ointment as positive control, 70% etanol extract of eudrilus eugeniae as group test 1, and coelomic fluid as group test 2.

Table 2. Comparison of Organoleptic Test: Aroma, Colour, Form, Homogenous, and pH of Coelomic Fluid (Treatment 2), 70% Ethanol Extract of Eudrilus Eugeniae (Treatment 1), and Duoderm (Positive Control)

Test	Coelomic Fluid	70% Ethanol Extract of Eudrilus Eugeniae	Duoderm
Aroma	Distinctive Aroma	Distinctive Aroma	No Odor
Colour	Cloudy White	Dark Brown (Typical)	Clear
Form	Liquid Gel	Gel	Gel
Homogenous	Homogenous	Homogenous	Homogenous
pH	6,70	5,73	6,00

3. Conclusion

Wounds are damage to the normal anatomical structure and function of the skin due to pathological processes originating from internal and external and affecting certain organs. Another definition of wound is continuous damage to the skin, mucous membranes and bones or other body organs. Some of the effects that will occur if there is an injury such as loss of all or part of organ function, sympathetic stress response, bleeding and blood clotting, bacterial contamination, and cell death [4].

Wound healing is a form of business process to repair the damage that has occurred. Collagen and epithelial cells are the main components in the wound healing process. Cells that play a role in the synthesis of collagen are fibroblasts. Wound healing physiology naturally undergoes a hemostatic phase, an inflammatory phase, a proliferative phase, and a remodeling phase [5].

After an injury occurs, the broken blood vessels will constrict and retract accompanied by a hemostatic reaction because platelet aggregation and fibrin mesh coagulate the blood. The inflammatory phase is when the hemostatic component releases and activates cytokines, namely Epidermal Growth Factor (EGF), Insulin-like Growth Factor (IGF), Platelet Derived Growth Factor (PDGF), and Transforming Growth Factor Beta (TGF- β), which causes chemotaxis. neutrophils, macrophages, mast cells, endothelial cells and fibroblasts. Furthermore, there is vasodilation and accumulation of polymorphonuclear leukocytes (PMN). Platelet aggregates and macrophages will release inflammatory mediators, namely Transforming Growth Factor Beta 1 (TGF 1), which will activate fibroblasts to synthesize collagen [5].

This study aims to determine the effect of Eudrilus eugeniae extract and hydrogel on the wound healing process. This study used the independent variables, namely Duoderm as positive control, Nacl as negative control, gel based on 70% Ethanol Extract of Eudrilus eugeniae as treatment group 1 and Gel based on coelomic fluid treatment group 2. Collecting data from the size of the wound which was measured using a caliper every day 0, 7, 14, 21, and 28. The results of this study showed that the positive control had an effectiveness of wound healing reaching 98.5%, treatment group 2 had an effectiveness of 93.9%, treatment group 1 had an effectiveness of 85.3%, and in the negative control, the wound healing rate was only 69.1%. For inflammatory cells, mice were biopsied on days 0, 14, and 28 to see microscopic inflammatory cells. On microscopic observation, it was found that on day 0 there were no inflammatory cells, on day 14 inflammatory cells such as PMN and Leukocytes were seen, and on day 28, inflammatory cells had been seen reduced.

4. Acknowledgement

I acknowledge the generous financial support from the “Penelitian Dosen Pemula”(PDP) who gave me the golden opportunity to do this wonderful project on the topic Comparison of Activity of Eudrilus Eugeniae and Hydrogel on Inflammatory Cells in the Wound Healing Process which also helped me in doing a lot of research and I came to know about so many new things I am really thankful to them. Secondly, I would also like to thank my parents and friends who helped me a lot in finalizing this project within the limited time frame.

5. References

- [1] Augustine, D. et al. (2019) ‘In vitro cytotoxic and apoptotic induction effect of earthworm coelomic fluid of Eudrilus eugeniae, Eisenia foetida, and Perionyx excavatus on human oral squamous cell carcinoma-9 cell line’, *Toxicology reports*, 6, pp. 347–357.
- [2] Benzie, I. F. F. and Wachtel-Galor, S. (2011) ‘Herbal medicine: biomolecular and clinical aspects’.
- [3] Cooper, E. L. et al. (2012) ‘Earthworms dilong: ancient, inexpensive, noncontroversial models may help clarify approaches to integrated medicine emphasizing neuroimmune systems’, *Evidence-Based Complementary and Alternative Medicine*, 2012.
- [4] ISWARA, A. D. (2015) ‘POTENSI ANTIOKSIDAN GETAH BATANG PISANG AMBON (MUSA PARADISIACA VAR. SAPIENTUM) SEBAGAI KANDIDAT BIOMATERIAL PENYEMBUHAN LUKA IN VITRO’. UNIVERSITAS AIRLANGGA.
- [5] M. Alsen, M. and Sihombing, R. (2014) ‘Infeksi Luka Operasi’, *Majalah Kedokteran Sriwijaya*, 46(3), pp. 229–235.
- [6] Mathur, A. et al. (2011) ‘Anti-inflammatory activity of earthworm extracts’, *International Journal of Pharmaceutical Sciences and Research*, 2(2), p. 278.
- [7] Sruthy, P. B. et al. (2017) ‘of Biomedical AND Pharmaceutical sciences PRE CLINICAL STUDIES FOR THE ANTI-INFLAMMATORY AND TOXICITY’, 4(2).
- [8] Umamaheswari, S. and Murugan, S. (2021) ‘Identification of Lysenin Protein Function in Coelomic Fluid Of Eudrilus Eugeniae’, *European Journal of Molecular & Clinical Medicine*, 8(03), p. 2021.