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The in-vivo anti-inflammatory effect of red earthworm (*Lumbricus rubellus*) ethanolic extract from organic farmland in Bali, Indonesia



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ABSTRACT

Background: One of the typical responses to the protection of the body is inflammation. Extensive and severe inflammation can result in tissue damage. Earthworms have been used empirically for years to treat diseases that are based on inflammatory events. This study evaluates the anti-inflammatory properties of red earthworm (*Lumbricus rubellus*) ethanolic extract from organic farmland in Bali, Indonesia.

Methods: An experimental study by post-test only control group design was conducted among 30 adult male Wistar rats, divided into 6 groups. Each group was given 0.5% NaCMC (negative control), Diclofenac sodium 9 mg/kgBW (positive control), Red earthworm ethanolic extracts of 50 mg/kgBW (P1) and 100 mg/kgBW (P2) at 1 hour before carrageenan injection. In addition, the ethanolic extracts of Red earthworms, respectively 50 mg/kgBW (P3) and 100 mg/kgBW (P4), which had been given once a day, during 1 week

before and at the eighth day 1 hour before carrageenan injection. The measurements of edema were performed 4 hours after the administration of the test material. Data were analyzed using SPSS version 20 for Windows.

Results: The median score of Rats Hind Paw Edema was 0.3 (0.2-0.4) ml in negative control, 0.1 (0.0-0.2) ml in positive control, 0.2 (0.1-0.2) ml in P1, 0.2 (0.1-0.3) ml in P2, 0.2 (0.1-0.2) ml in P3, and 0.1 (0.1-0.2) ml in P4 groups. There was a significant differences between the NaCMC group (negative control) with the Diclofenac sodium group (positive control), P1, P3, and P4 groups ($p < 0.05$), but not with P2 group ($p > 0.05$).

Conclusion: We conclude that red earthworm (*Lumbricus rubellus*) ethanolic extract from Bali's organic farmland exhibits an anti-inflammatory effect.

Keywords: *Lumbricus rubellus* Extract, Anti-Inflammation, Edema

Cite this Article: Dewi, N.W.S., Mahendra, A.N. 2020. The in-vivo anti-inflammatory effect of red earthworm (*Lumbricus rubellus*) ethanolic extract from organic farmland in Bali, Indonesia. *Bali Medical Journal* 9(3): 545-548. DOI: [10.15562/bmj.v9i3.1788](https://doi.org/10.15562/bmj.v9i3.1788)

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INTRODUCTION

Inflammation is a protective response essential for survival due to critical to eliminate the disease's causative agent.¹ Inflammation is a protective response, whereas ongoing and excessive inflammation can result in tissue damage.¹ Inflammation originated from an exaggerated immune response, leading to a pathological condition and requiring treatment.^{1,2}

Medication is needed for some instances related to inflammation. Most treatments for inflammation use Non-Steroidal Anti-Inflammatory Drugs (NSAIDs).³ Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is useful for reducing inflammation, but on the other hand, causes many side effects, including disorders of the stomach, intestine, liver, and kidney.^{3,4} Long-term use of NSAIDs can also cause severe disorders in the digestive system and excretion system.⁵ Based on the severe side effects of drugs, it is necessary

to develop natural ingredients easily obtained, without or with minimal side effects, that have anti-inflammatory properties as traditional medicine.

Earthworms or other alternative medicines have been used empirically for years in traditional Chinese, Arabic, and Indian medicine to treat inflammation and hematological disorders.⁶⁻⁸ Dry powder of Red earthworms have been used empirically in Bali to treat diseases based on inflammatory events. Still, there is a few of research or scientific evidence about them. According to a previous study, ethanolic extract of Red earthworms from organic farmland in Bali is known to have high polyphenol content and antioxidant activity, so the possibility earthworms from organic farming land in Bali also has anti-inflammatory abilities.⁹ Based on those mentioned above, this study aims to evaluate the in-vivo anti-inflammatory effect of red earthworm (*Lumbricus rubellus*) ethanolic extract from organic farmland in Bali, Indonesia.

Received: 2020-04-01
Accepted: 2020-09-25
Published: 2020-10-13

METHODS

L. rubellus Extraction

Bali Organic Association (BOA) provides raw materials in Red earthworms bred on several organic agricultural lands in Bali. Red earthworms are then processed at the Faculty of Agriculture of Udayana University into Red earthworm powder. Red earthworm powder was macerated in 80% ethanol solution for at least 2 days, after which it is evaporated using a rotary vacuum evaporator becomes a crude extract. The extraction process approach was carried out at the Laboratory of Pharmacology and Therapy-Division of Drug Development and Therapy, Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University based on the previous study.⁹

Sample Preparation

Samples consisted of 30 male Wistar rats, healthy, weighing 200-250 mg, aged 2-2.5 months obtained from the Laboratory of Pharmacology and Therapy-Division of Drug Development and Therapy, Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University. Samples were adapted 1 week before treatment. The sample was divided into 6 groups; the first group was the negative control, given a 0.5% NaCMC. The second group was a positive control who received

Non-Steroid Anti-inflammation Drug (NSAID) Diclofenac sodium 9 mg/kgBW. The third and fourth groups were given Red earthworm ethanolic extracts 50 and 100 mg/kgBW, respectively, with a gavage 1 hour before carrageenan induction. The fifth and sixth groups were received 50 and 100 mg/kgBW ethanolic extract of Red earthworm, respectively, with a gavage once a day during a week, and on the eighth day, they were given 1 hour before carrageenan induction.

Rats paw edema induced by carrageenan and Data Analysis

All samples were given test material by gavage at 1 hour before 0.1 ml (1%) carrageenan injection into sub plantar tissue. The effect of test material between groups on the hind paw edema was evaluated 4 hours after using a plethysmometer. This study used a post-test only control group design method, which compares the groups at 4 hours after carrageenan induction. Data were analyzed using SPSS version 20 for Windows.

RESULTS

The median Rats Hind Paw Edema score in negative control was 0.3 (0.2-0.4) ml, followed by 0.1 (0.0-0.2) ml in positive control, 0.2 (0.1-0.2) ml in P1, 0.2 (0.1-0.3) ml in P2, 0.2 (0.1-0.2) ml in P3, and 0.1 (0.1-0.2) ml in P4 groups (Table 1 and Figure 1).

Based on Table 1 and Figure 1, the largest mean edema volume was in the NaCMC group (negative control). The smallest edema volume was found in the Diclofenac sodium (positive control) and the P4 groups (Figure 1). There were significant differences between the NaCMC group (negative control) with the Diclofenac sodium group (positive control), P1, P3, and P4 groups ($p < 0.05$), but not with P2 ($p > 0.05$). There was no significant difference between the Diclofenac sodium groups (positive control) with all treatment groups ($p > 0.05$).

DISCUSSION

Carrageenan is a natural carbohydrate obtained from Red seaweed.¹⁰ It is widely used to determine anti-inflammatory activity. Inflammatory signs, such as edema, were created by the carrageenan injection into Rat hind paw.¹¹ Previous studies were conducted by Hafeez A et al. and Cong HH et al. found that the maximum volume of edema was observed in 3-5 hours after the injection of carrageenan.^{12,13} In contrast, another study observed edema every 30 minutes in 6 hours or 6 hours to prove the substance's anti-inflammatory activity.¹⁴ However, in our study, a post-test-only control group design has been observed the anti-

Table 1. The Rats Hind Paw Edema score among groups

Group	Median	Min-Max
NaCMC (Negative control)	0.3	0.2-0.4 ^a
Diclofenac sodium (Positive control)	0.1	0.0-0.2 ^b
P1: REEE 50 mg/kgBW	0.2	0.1-0.2 ^b
P2: REEE 100 mg/kgBW	0.2	0.1-0.3 ^{ab}
P3: REEE 50 mg/kgBW, 1 week	0.2	0.1-0.2 ^b
P4: REEE 100 mg/kgBW, 1 week	0.1	0.1-0.2 ^b

REEE: Red Earthworm Ethanolic Extract; Different letter superscripts (a,b) in the same column show significant differences ($p < 0.05$).

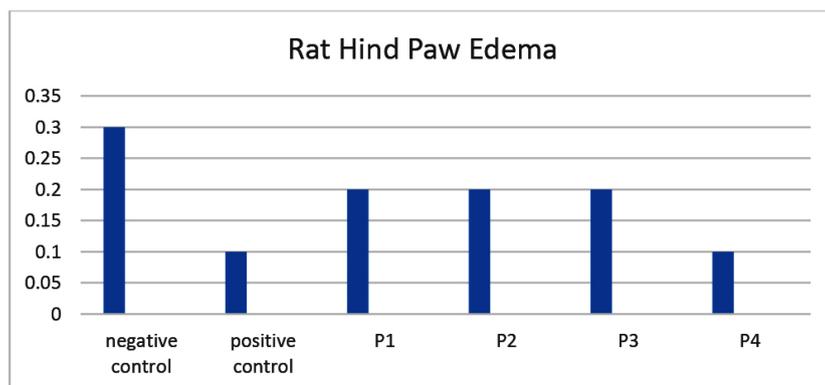


Figure 1. Rat Hind Paw Edema by Bar Chart.

inflammatory effect of Red earthworm ethanolic extract at 4 hours after carrageenan injected.

In Bali, red earthworm from organic farmland is based on previous research known to have high polyphenol content and antioxidant activity.⁹ Polyphenols have been extensively studied and proven that these compounds have anti-inflammatory activity. Terrestrial and marine plant species and organisms are also a source of polyphenol.¹⁵ A study conducted by Chang YM et al. demonstrated that Earthworm Extract exhibits an anti-inflammatory activity on Schwann cell.⁷ Another study was also demonstrated that earthworm powder from other areas has the same anti-inflammatory activity as Diclofenac sodium.¹⁶ Diclofenac Sodium is a widely used analgesic (pain relief) drug and also exerts anti-inflammatory activity.¹⁷ This drug was used as a positive control in this study. Earthworm extract, in this case, has the ability to approach anti-inflammatory analgesic drugs.

Our study also observed no significant differences in anti-inflammatory activity in experimental animals given material 1 hour before induction and those given 1 hour and 1 week before. We also like to observe the long-time use of Red earthworm's ethanolic extract in the future.

CONCLUSION

Based on the results, there was a significant difference in the edema volume between negative control compare with positive control and other treatment groups. However, no significant differences between positive control and treatment groups indicate that the treatment group has almost similar edema volume with positive control. These results show that Red earthworm (*Lumbricus rubellus*) ethanolic extract from Bali's organic farmland exhibits anti-inflammatory properties.

ACKNOWLEDGMENT

The authors would also like to thank I Gede Wiranata, S.Si. (technician, Division of Drug Development and Laboratory Animal, Integrated Biomedical Laboratory Unit, Faculty of Medicine), Amie Yelli Kusmawardani (technician, Division of Biochemistry, Integrated Biomedical Laboratory Unit, Faculty of Medicine) for their assistance.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

Ethics approvals have been obtained from the Ethics Committee, Faculty of Medicine, Universitas Udayana, Bali, Indonesia prior to the study being conducted.

FUNDING

This research was financially supported by LPPM Udayana University under DIPA PNPB Universitas Udayana TA-2019.

AUTHOR CONTRIBUTIONS

All of the authors equally contribute to the study from the conceptual framework, data gathering, data analysis, until reporting the results of study through publication.

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