**Pharmaciana**
Vol.15, No.1, March 2025, Page. 154-164
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v12i1.21830 

# Antioxidant and antiinflammatory of clove extract (*Syzigium aromaticum)* in skeletal muscle damage

**Tyas Sari Ratna Ningrum[1]*, Riska Risty Wardhani[1], Nining Sugihartini[2]**
*[1]Physiotherapy Program, Aisyiyah University of Yogyakarta*
*Jl. Siliwangi (Ring Road Barat) No. 63 Mlangi, Nogotirto, Gamping, Sleman, Yogyakarta, Indonesia*
*[2]Pharmacy Program, Universitas Ahmad Dahlan,*
*Jl. Prof. Dr. Soepomo, S.H, Warungboto, Umbulharjo, Yogyakarta, Indonesia*

***Submitted: 20-07-2024*** ***Reviewed: 10-12-2024*** ***Accepted: 23-01-2025***

## ABSTRACT

Clove extract is a natural ingredient abundant in Indonesia that has various benefits, including being an antioxidant and anti-inflammatory. Although Research has been conducted, it has been limited to discussing the effects of clove extract on muscle damage. This study aims to determine the effect of clove extract on skeletal muscle damage due to excessive eccentric exercise in mice. We used experimental research methods by a post-test only with control group design. Fifteen male mice were randomized into 5 groups. Group I: Normal control, Group II: Negative control, treatment group with a dose of 100 mg/kg BW, treatment group with a dose of 150 mg/kg BW, and treatment group with a dose of 250 mg/kg BW. Measurement of Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NOX) and Toll Like Receptor4 (TLR 4) levels were carried out using the ELISA method via Quadriceps muscle samples, and skeletal muscle damage was viewed through an x1000 light microscope. The results of this study carried out using ANOVA showed results of p 0.004 and 0.006, which means that there was a decrease in oxidative stress as measured using NOX and a decrease in the inflammatory stimulator TLR 4 in skeletal muscle damage due to eccentric exercise.ANOVA test results show that there are differences in influence between treatment groups and show that clove extract has benefits as an antioxidant and anti-inflammatory in muscle damage. According to this study, clove extract has the potential to be an antioxidant and anti-inflammatory which can reduce NOX levels and reduce the inflammatory, mediator TLR4 in muscle damage caused by excessive eccentric exercise.

.

**Keywords**: clove extract, NOX, TLR4, muscle damage, antioxidant, antiinflammatory

***Corresponding author:***
Tyas Sari Ratna Ningrum
Aisyiyah University of Yogyakarta
Jl. Siliwangi (Ring Road Barat) No. 63 Mlangi, Nogotirto, Gamping, Sleman, Yogyakarta, Indonesia
Email: tyassariratnaningrum@unisayogya.ac.id

## INTRODUCTION

Strenuous exercise is very likely to cause injury to the musculoskeletal system, especially to the skeletal muscles. Muscle conditions with heavy activity can become severe because they not only involve the musculoskeletal system but also other systems. This risk of excessive exercise is experienced by various individual sports, sports that require low physical positions (such as golf), sports played by women, and professional athletes (Kreher & Schwartz, 2012). Research conducted by Liao et al. (2020) shows that excessive eccentric exercise will begin to affect systems and organs in the body within 7 days after excessive eccentric exercise (Liao et al., 2020), while according to Dewangga, et al (2021) show that within 1 day and 2 weeks after heavy exercise it will increase ROS (Dewangga et al., 2021; Liao et al., 2020) through the NADPH Oxidase (NOX) mechanism which will suppress the endogenous antioxidant enzyme (Os et al., 2019). Thus, skeletal muscle damage suggests a link between inflammation, oxidative stress, and muscle damage caused by mechanical insults applied to the muscle (Järvinen et al., 2000).

Long-term high-intensity exercise lasting more than a few hours promotes increases in muscle inflammatory concentrations during exercise. Eccentric exercise for a long time induces Tool Like Receptor 4 (TLR4) (Ali et al., 2021). During stress or cellular injury, patterns of muscle cell destruction can be released into the extracellular environment and blood circulation from damaged cells (Dewangga et al., 2021). Tool Like Receptor 4 activates mitogen-activated protein kinases (MAPKs) and nuclear factor kappa Beta (IκB) kinase (IKK) inhibitors to increase the production of inflammatory cytokines from leukocytes which are then activated through the activation of transcption factor activator protein 1 (AP-1) and other factors. nuclear κB (NFκB) (Tu & Li, 2023).

Treatment of muscles that are damaged due to excessive eccentric exercise is often ignored because it is not immediately visible (Zuo et al., 2018). However, over time muscle strength will decrease due to the increasing number of muscle cells being damaged, which will reduce daily activities due to increasingly severe damage to muscle tissue (Hody et al., 2019). Muscle damage due to excessive eccentric exercise will cause oxidative stress and inflammation in the muscles, so medical treatment is given in various ways, such as providing rest or medication. Nonsteroidal Anti-inflammatory Drugs (NSAIDs) are medications used to treat muscle damage (Louw, 2023). NSAIDs are chemical drugs that have side effects if consumed long term, Therefore, the author is trying to find herbal alternatives that are local wisdom and have properties that are no less good than chemical drugs (Jahromi et al., 2021). Mateen et al. (2019)'s Research used cinnamaldehyde and clove flowers as anti-inflammatories, and Sugihartini et al. (2017) showed that the eugenol contained in cloves has anti-inflammatory and antioxidant properties (Sugihartini et al., 2019). This is the basis for the author to use clove flowers *(Syzygium aromaticum*) because they have anti-inflammatory and natural antioxidant benefits obtained through clove flower extract, which is a natural ingredient that has low side effects (Han & Parker, 2017).

Cloves have good antioxidant and anti-inflammatory properties, but the best solvent for consumption is still a consideration. One of the factors that influences the maceration method is the type of solvent. Selecting the type of solvent must consider several factors, including selectivity, ability to extract, toxicity, ease of evaporation, and price of the solvent (Harborne et al., 1987). Various sources have found that methanol is one of the extraction solvents that has certain properties. In this study, we tested the effectiveness of administering clove extract with methanol solvent on levels of oxidative stress and inflammation represented by NOX, and TLR4, reviewed in 3 doses, namely 100, 150, and 250 mg/kg bw after induction of muscle damage with excessive eccentric training.

The author considers it important to evaluate the effect of a mixture of clove extracts that is safe to use for conditions of oxidative stress and skeletal muscle inflammation due to muscle damage induced through excessive eccentric exercise. Therefore, the researchers took the title regarding the effect of a single dose of clove flower extract *(S. aromaticum*) on skeletal muscle damage in terms of NOX and TLR 4 expression after excessive eccentric exercise.

## MATERIALS AND METHOD

The materials used in this study were mice as experimental animals, a treadmill used as a tool to induce muscle damage, clove extract used for treatment, and an ELISA kit for NOX and TLR 4 proteins. The Research was carried out in several stages where the condition of healthy mice was induced by eccentric running exercise on a treadmill with the downhill $15^0$ at a speed of 25 m/minute for 20-75 minutes, followed by clove extract treatment with 3 doses, namely 100, 150 and 250 mg/kg bw. The treatment group was divided into 5 groups: 3 groups consisted of clove extract treatment in 3 doses, and 2 groups were control groups consisting of a healthy control group and a negative control group. Clove flowers from Magelang, Central Java, Indonesia, were air-dried, ground, and sieved using a 40-mesh filter. Extraction was performed with a 1:10 maceration and diluted in carboxymethylcellulose sodium (CMC Na) to achieve a final concentration of 0.1 mg per gram of mouse body weight. The extract was administered at doses 100, 150, and 250 mg/kg bw mg/kg, dissolved in 0.2 mL of CMC Na.

The extraction results were stored in a freezer (-10° C) until the sample was used or tested on test animals. The procedure of clove extract is done orally, where the clove extract is given a CMC Na as a diluent. After extraction, a thickening process is carried out with a ratio of 1:10. After being in the form of a thick extract, clove extract was taken at 0.1 mg per gram of mouse body weight.

### Animals

This Research was carried out in the pharmacy and biochemistry laboratory at Airlangga University, Surabaya, East Java, with a total sample size of 15 samples divided into 5 treatment groups. This treatment has received ethical approval for the Research. Universitas Respati Yogyakarta. Yogyakarta. Indonesia (Approval number: 0231.3/FIKES/PL/X/2023).

The sample criteria used were mice aged 8-12 weeks weighing between 20-25 grams with healthy conditions in terms of extremities that move normally, clean fur, and healthy extremities, while the exclusion criteria indicate that the mice died during the experiment.

The experimental animals used in this Research were male mice (Mus musculus) aged 8-12 weeks, weighing 20-25 grams. Before being given treatment, male mice were given acclimatization treatment for 1 week before being treated in the animal house at the experimental animal laboratory at Airlangga University, Surabaya. Mice were kept in cages covered with wire. The condition of animal cages is ventilated with a lighting system that changes every 12 hours and is equipped with places to eat and drink ad libitum. For cleanliness, the husks are changed every 2 days.

The treatment group was compared with 2 control groups (Table 1), a negative and a health group. Clove extract was given using the oral method, which was given immediately after training, and then the mice were sacrificed to have their skeletal muscles taken.

**Table 1. Treatment group**

| Treatment Group | Code | Description |
|---|---|---|
| Health Control | KS | No exercise and no treatment |
| Negative Control | KN | Exercise without treatment |
| Treatment 1 | T1 | Exercise and treatment 100mg/kg BW |
| Treatment 2 | T2 | Exercise and treatment 150mg/kg BW |
| Treatment 3 | T3 | Exercise and treatment 250mg/kg BW |

Calculations are performed using the formula
Equation 1:

$$\frac{\text{Dosage} = \mu \text{ mg}}{1000 \text{ gr} \qquad \alpha \text{ gr}}$$

Description :
Dosage: treatment dosage
μ mg: weight of clove extract
α gr: weight of mice

**Eccentric exercise**

Induce muscle damage with eccentric exercise using a treadmill as long as the mice can run at a speed of 25 m/minute with a 15-degree downhill position.

**Figure 1. Treadmill schematic** (Commat Ltd., 2025)

The implementation procedure used the treadmill as seen in Figure 1. It was positioned according to the requirements, and then the mice were placed on the treadmill track. Eccentric training was used for the treatment group in 3 doses, namely 100 mg, 150 mg, and 250 mg, and negative control.

Muscle damage is seen through an examination of muscle samples obtained for diagnostic purposes via open biopsy with the euthanasia method. The biopsy underwent histopathological examination to determine muscle tissue damage using the Hematoxyllin-Eosin staining method with longitudinal sections.

.

**Clove extract**

Clove flowers from Magelang, Central Java, Indonesia, were air-dried, ground, and sieved using a 40-mesh filter. Extraction was performed with a 1:10 maceration ratio in 90% ethanol, followed by 6 hours of reflux, filtration through Whatman No. 41 paper, and washing with 50 mL of ethanol. The filtrate was concentrated to 6 mL using a rotary evaporator at 45°C and stored at -10°C until further use [23]. For administration, the extract was diluted in carboxymethylcellulose sodium (CMC Na) to achieve a final concentration of 0.1 mg per gram of mouse body weight. The extract was administered at doses 100, 150, and 250 mg/kgBW, dissolved in 0.2 mL of CMC Na with an amount per dose of 3.5 mg.

**Protein checking procedure**

The protein measurement used is the Enzyme-linked Immunosorbent Antibody (ELISA) method, which is a technique that combines antibody specificity with enzyme sensitivity using antibodies or antigens combined with an enzyme for NOX and TLR 4, and HE examination for muscle damage. The mice's skeletal muscle tissue in each bunch was homogenized by including 0.3 mL of 9% ordinary saline in each 10 g. Tissue supernatant was delivered by centrifugation, and the concentration of the muscle-derived homogenate was balanced (Jin et al., 2023). Estimations were carried out according to the information given by the distributor (Gamma Scientific BioLab, Malang, Indonesia). The analysis was carried out at the Laboratory of Biomedicine, Universitas Airlangga, Surabaya, Indonesia, consisting of two stages: protein examination using ELISA and histological evaluation via hematoxylin-eosin staining. A team of blinded laboratory technicians conducted the assessments, ensuring the integrity of the data. The reaction was terminated by the addition of an acidic stop solution, and absorbance was measured at 450 nm. Skeletal muscle tissue was homogenized with 0.3 mL of 9% saline per 10 g, followed by centrifugation to obtain the supernatant. Concentrations were

standardized according to the manufacturer's instructions. Histopathological evaluation of muscle damage was performed using hematoxylin and eosin staining.

**Statistical method**

To analyze and interpret the data collected by the research objectives, the following procedure was carried out: the data was analyzed using the SSPS version 26 statistical program. The normality test used the Shapiro-Wilk test, followed by the homogeneity test with Lavene's test, and finally, the hypothesis test to determine the effect of cloves extracts on NOX and TLR4 expression using the one-way ANOVA test with a 95% confidence level.

## RESULT AND DISCUSSION

**Muscle damage**

Skeletal muscle not only functions as a means of movement, but also acts as an endocrine organ with the ability to secrete various bioactive molecules known as myokines (Nielsen & Pedersen, 2007). Myokines are cytokines and other proteins released by muscle fibers during contraction, which have paracrine and endocrine effects on various body tissues, each of which has a specific function in regulating metabolism, muscle growth, and cognitive function, thus contributing to the improvement of organ function and the body's systems (de Sousa et al., 2021). Hence, it has been contended that cytokines and other peptides that are created, communicated, and discharged by skeletal muscle strands and apply paracrine, autocrine, or endocrine impacts ought to be classified as "myokines ."Skeletal muscles can deliver and express cytokines, which have a place in distinctive families. Be that as it may, the primary recognized and most studied myokine may be a proinflammatory cytokine receptor that increments up to 100-fold in circulation amid physical exercise (Pedersen & Febbraio, 2008).

Excessive exercise showed that mice caused tissue structure damage and fire cell penetration in skeletal muscle and caused quantitative changes indicating an increase in the number of inflammatory cells and changes in skeletal muscle structure. Skeletal muscle damage induced by excessive exercise can result to sarcolemma integrity and inflammatory cell infiltration. Disruption of the sarcolemma can lead to inflammatory cell infiltration and enzymatic release, whereas elevated inflammatory cell counts can contribute to inflammation, tissue damage, and impaired wound healing processes.

This Research is specifically discusses inflammation that occurs in skeletal muscle. There were 5 clove extract treatment groups with 2 dependent variables, namely NOX and TLR4 measurements as markers of ROS and inflammation.

**Table 2. Concentration of NOX and TLR4 of mice with clove extract treatment and control groups**

| Group | N | NOX (U/gprot) | TLR4 (ng/ml) | p(sig) | p(sig) |
|---|---|---|---|---|---|
| | | Mean±SD | Mean±SD | NOX | TLR4 |
| NEGATIVE CONTROL | 3 | 2738.98±209.87 | 5.75±0.73 | | |
| HEALTHY CONTROL | 3 | 1680.29±106.68 | 3.37±0.10 | | |
| DOSAGE 1 100 mg/kg BW | 3 | 1868.61±355.37 | 4.43±0.71 | 0.004 | 0.006 |
| DOSAGE 2 150 mg/kg BW | 3 | 2119.58±238.39 | 5.24±0.57 | | |
| DOSAGE 3 250 mg/kg BW | 3 | 2024.09±247.57 | 4.94±0.64 | | |

Based on Table 2, giving clove extract can reduce NOX and TLR4. The SPSS analysis test results using the ANOVA test also show a significance value of <0.05, where the NOX test results show a value of 0.004 and the TLR4 test shows a value of 0.006. ANOVA test results show that there are differences in influence between treatment groups.

Based on the results of the one way ANOVA test, it was found that the results were significant (>0.05) so that the next test was the Bonferroni and Holm post hoc test, the results obtained were that:

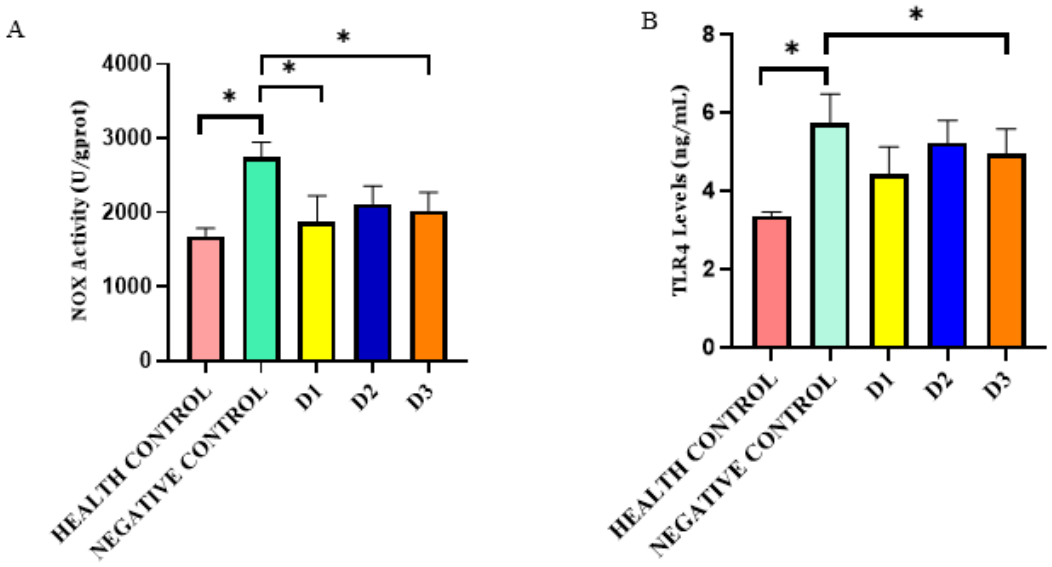


**Figure 2. (A) Post hoc Bonferroni and Holm test of NOX activity among groups; (B) Post hoc Bonferroni and Holm test of TLR4 levels among groups. D1 is dosage 1 (100 mg/kg bw), D2 is dosage 2 (150 mg/kg bw), D3 is dosage 3 (250 mg/kg bw)**

Post hoc Bonferroni and Holm test revealed significant differences in NOX activity between the Negative Control Group and the Health Control Group, D1 (p=0.012), D3 (*p*=0.001). These findings support the hypothesis that clove extract (100mg/kg bw and 250 mg/kg bw) can decreased NOX activity on skeletal muscle damage due to excessive eccentric exercise in mice. Post hoc Bonferroni and Holm test revealed significant differences in TLR4 levels between the Negative Group and the Health Group (p=0.006), then the Heath Control and D3 (250mg/kg bw) (p=0.029). These findings support the hypothesis that clove extract (250mg/kg bw) can decreased TLR4 levels on skeletal muscle damage due to excessive eccentric exercise in mice.

Clove extract has the potential to be an antioxidant and anti-inflammatory in muscle damage caused by excessive eccentric exercise. Clove flowers have a high eugenol content, and eugenol was produced from clove extract in this study. Free radicals in the body, which can increase due to excessive exercise, are associated with elevated levels of NADPH oxidase (NOX). The repair effect of eugenol is shown by a remarkable decrease in DNA damage levels and ROS mediators along with a significant increase in antioxidant enzyme activity, and may be explained by the high content of anti-inflammatory and antioxidant eugenol (Mohamed et al., 2024).

Eugenol contained in clove extract affects neutrophils as an initial defense in the healing process of muscle injuries (Park et al., 2024). The neutrophil defense pathway will stimulate a large amount of superoxide anions (O2) and then NOX will be formed through the enzyme responsible for O2, which causes tissue injury and prolonged inflammation. All of these mechanisms are related to the anti-radical properties of some constituents of clove extract and their ability to capture radicals, which reduces oxidative stress and, in turn, prevents damage to lipids, proteins, and DNA (Park et al., 2024). The mechanism of ROS emergence begins through NOS used for NO synthesis, which inhibits the activation of NOX subunits in mice after short eccentric exercise (Ghozali et al., 2023).

Eugenol is known to be promptly retained after oral consumption and quickly comes to plasma and blood with a half-life of 14 to 18 hours, and its collective impacts have been watched after its daily administration to treat neuropathic pain. After that, eugenol is metabolized into glucuronic acid or sulfate conjugates within the liver (Batiha et al., 2020). This is in line with a few past considerations

that expressed that the eugenol contained in cloves is a dynamic substance that's valuable as an antioxidant and anti-inflammatory (Mateen et al., 2019).

According to the statistical test applied, it shows that clove extract, which contains a percentage of the active substance eugenol, has a strong function as an antioxidant and anti-inflammatory. Clove extract containing eugenol derived from S. aromaticum has been documented to have an effective anti-inflammatory effect on murine macrophages by suppressing the production of pro-inflammatory cytokines (Batiha et al., 2020).

Eugenol has been used for its pain help and sedative impacts, among other applications, and is broadly used for its pain relieving and anti-inflammatory qualities. On the other hand, eugenol has antioxidant and antiseptic properties (Davoudi et al., 2017). A number of Research have described the effects of eugenol as an antiinflammatory agent and modulator of its role in tissue remodeling processes. Research by Barboza et al (2018) appeared that twelve weeks of eugenol organization constricted alveolar bone loss and remodeling related to estrogen insufficiency using the ovariectomized (OVX) Wistar rat model, which is comparable to what happens after menopause in people, appearing an impact Eugenol's anti-inflammatory properties are especially critical, because it goes with by a lessening in IL-8, IL-6, and TNF-α levels coming about in a diminishment in provocative cells (Barboza et al.,2018).

The highest component of clove extract compounds, such as eugenol and acetyl eugenol, also contributes to antioxidant activity (Sheweita et al., 2016). Essential antioxidants will respond with peroxide radicals which are at that point changed over into more steady radicals subsequently suppressing ROS (Davoudi et al., 2017). Eugenol is the dynamic component found in cloves, can diminish levels of incendiary go betweens at the quality and protein expression level, pro-inflammatory proteins such as cytokines, prostaglandin amalgamation and neutrophil chemotaxis, as well as hindering the NF-kB calculate in lessening pro-inflammatory cytokines and Cyklooxygenase (COX-2) expression (Marmouzi et al, 2019).

The methanol extract of clove leaves had a higher reduction power than other extracts. The most elevated total antioxidant movement and lessening control were gotten from the extract and both were closely related to the entire phenol substance of cloves (Mu'nisa et al., 2012). The number of inflammatory cells can be measured. Based on test comes about by Sugihartini, et al (2019), it appears that the number of incendiary cells, and the thickness of the epidermis so that the expansion of the upgrading composition gives great anti-inflammatory action (Sugihartini et al., 2019).

Excessive eccentric exercise damages skeletal muscle cells. The greater the muscle damage, the more ROS or oxidative stress reprecentage with NOX level will increase and stimulate the amount of inflammation. This condition of muscle damage is given natural ingredients in the form of clove extract. In this study, clove extract was compared with a healthy control group and a negative control group. Free radicals will stimulate the TLR4 protein as an inflammatory response, which will then inhibit IK-β, thereby stimulating Nfk-β, which will increase levels of pro-inflammatory cytokines. So, the fewer the number of free radicals, the lower the TLR4 levels. This study shows that the clove extract treatment group had better results compared to the negative control group, which was only given rest without any treatment. This can be seen in the histopathological examination, which shows that the muscle that was given treatment experienced muscle repair while the negative control still experienced damage. Therefore, more attention must be paid to the condition of muscle damage so the skeletal muscle cells can be repaired immediately.

The benefits of clove content are supported by several studies, there are study by Mateen et al (2019). Eugenol provides protection against biomolecules from rheumatoid arthritis patients.The results of molecular examination show the effectivity of cinnamaldehyde and eugenol with the pro-inflammatory mediators. Cinnamaldehyde and eugenol were proven to be anti-inflammatory and antioxidant in PBMC cultures from RA patients. Thus, this compound may be used as an adjunct in RA management (Mateen et al., 2019). The effectiveness of clove and ginger oil managed orally and antiinflammatory drugs (Indomethacin) at a dosage of 3 mg/kg/day orally appeared that there was

antirheumatic activity by diminishing joint pain scores, repressing the expression levels of proinflammatory cytokines (Fathy et al., 2022).

Skeletal muscle harm infers that ROS delivered by the respiratory burst coming about from overexercise may be included within the acceptance of DNA harm. The discharge of ROS, intervened by NOX amid the respiratory burst, not only causes lipid peroxidation within the blood but also causes a series of distinctive peroxidation responses in other cells. Others considerations moreover report that free radicals incorporate a harmful impact on DNA and then cause lasting harm. As a result, harm to cellular DNA can alter the organic action of cells and cause quality changes and even cell death (Dong et al., 2011). An overview of skeletal muscle is shown in Figure 3.

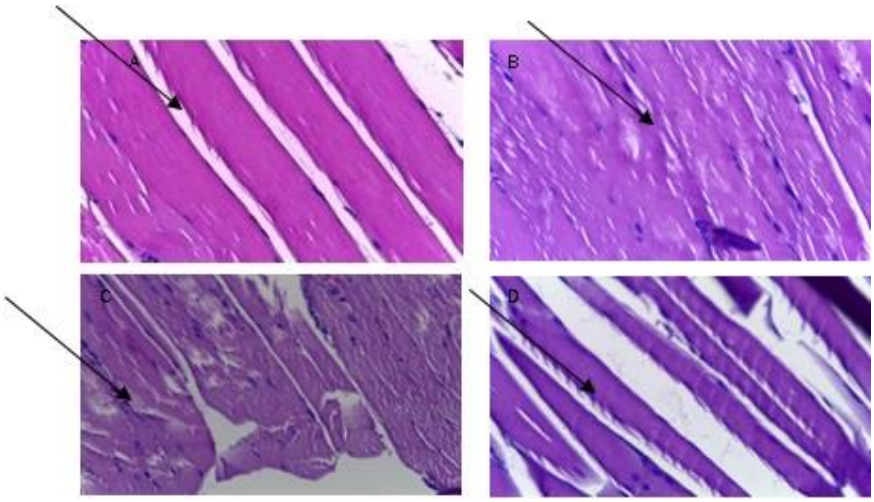


**Figure 3. Histopathological examination using HE staining on skeletal muscle damage**

Note:
A is the muscles in the healthy group. The muscle tissue shows muscle cells with intact sarcolemma and no inflammatory cells. Pictures B and C are the muscle groups that were damaged, the tissue shows muscle cells with sarcolemma that are starting to experience damage and have inflammatory cells and picture D is the muscles that were repaired after clove extract treatment. the tissue shows repair of the sarcolemma wall and fewer inflammatory cells

The clove extract was administered orally according to the schedule, and skeletal muscles were collected post-sacrifice for analysis. Muscle damage was assessed via biopsy, conducted either as an open biopsy under general anesthesia or as a needle biopsy under mild sedation. The quadriceps muscle was collected post-sacrifice by incising at the muscle origin (iliac spine) and the tendon insertion. The excised muscle was placed into a storage container, with the left portion immersed in formalin buffer for histological preparation to assess muscle cell condition, and the right portion stored in a sealed dry container at -80°C for protein analysis. Histopathological examination using hematoxylin-eosin staining on longitudinal sections, conducting using a light microscope at 1000x magnification.

These provocative changes are followed by increments in serum chemical levels/activities that serve as utilitarian markers of organ harm. Information propose that, as a result of multi-organ intuitive, it can cause a maintained fiery reaction for at slightest 24 hours, coming about in tissue injuries and eventually multiple-organ damage (Liao et al., 2020). The muscle damage that occurs due to excessive eccentric exercise increases the number of inflammatory cells that occur in the muscles. Apart from that, it will damage the Z disc component and reduce the integrity of the sarcolemma in skeletal muscles compared to healthy muscles, such as in Figure 3A, which is healthy skeletal muscle, and in Figures 3B and 3C, which are damaged skeletal muscles. Clove extract contains the active

substance eugenol, which has antioxidant and anti-inflammatory benefits. After administering clove extract treatment to muscle damage, there appeared to be improvements in the z disc and sarcolemma integrity show in the Figure 3D.

## CONCLUSION

Based on the results of this study, it shows clear results that a single dose of clove extract has significant results in reducing NOX levels as a ROS parameter and TLR4 as an inflammation parameter. The conclusion of this study shows that clove extract has benefits as an antioxidant and anti-inflammatory against skeletal muscle damage.

## ACKNOWLEDGEMENT

We would like to thank Aisyiyah University of Yogyakarta and the PP Muhammadiyah Diktilitbang Council for providing the opportunity and funding for this Research.

## REFERENCES

Ali, M. M., McMillan, R. P., Fausnacht, D. W., Kavanaugh, J. W., Harvey, M. M., Stevens, J. R., Wu, Y., Mynatt, R. L., & Hulver, M. W. (2021). Muscle-specific deletion of toll-like receptor 4 impairs metabolic adaptation to wheel running in mice. *Medicine and Science in Sports and Exercise*, *53*(6), 1161–1169. https://doi.org/10.1249/MSS.0000000000002579

Barboza, J. N., da Silva Maia Bezerra Filho, C., Silva, R. O., Medeiros, J. V. R., & de Sousa, D. P. (2018). An overview on the anti-inflammatory potential and antioxidant profile of eugenol. *Oxidative Medicine and Cellular Longevity*, *2018*. https://doi.org/10.1155/2018/3957262

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum l. (myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2). https://doi.org/10.3390/biom10020202

Commat Ltd. (2025). *Treadmill (TME 0804)*.

Davoudi, K. A., Mohammadi, R., Pourfathollah, A. A., Siery, Z., & Davoudi-Kiakalayeh, S. (2017). Effects of Eugenol on pain response to the formalin test and Plasma antioxidant activity in high fructose drinking water in male Rats. *International Journal of Preventive Medicine*, *8*. https://doi.org/10.4103/ijpvm.IJPVM

de Sousa, C. A. Z., Sierra, A. P. R., Martínez Galán, B. S., Maciel, J. F. de S., Manoel, R., Barbeiro, H. V., Souza, H. P. de, & Cury-Boaventura, M. F. (2021). Time course and role of exercise-induced Cytokines in Muscle damage and repair after a marathon race. *Frontiers in Physiology*, *12*(October), 1–13. https://doi.org/10.3389/fphys.2021.752144

Dewangga, M. W., Irianto, D. P., Dimyati, Sumaryanto, Nasihun, T., Febrianta, Y., Wahyuni, Wijianto, & Agustiyawan. (2021). Different effects of acute and Chronic Strenuous physical exercise on superoxide dismutase (SOD), Malondialdehyde (MDA) Levels, and Sperm Quality of the Wistar Rats. *Journal of Kerman University of Medical Sciences*, *28*(6), 539–547. https://doi.org/10.22062/jkmu.2021.91825

Dong, J., Chen, P., Wang, R., Yu, D., Zhang, Y., & Xiao, W. (2011). NADPH oxidase: a target for the modulation of the excessive oxidase damage induced by overtraining in rat neutrophils. *International Journal of Biological Sciences*, *7*(6), 881–891. https://doi.org/10.7150/ijbs.7.881

Fathy, A. T., Kholief, T. E., Moram, G. S. E., & Elmasry, S. (2022). Anti-inflammatory and antioxidant activities of clove and Ginger oils on induced Rheumatoid arthritis in rats. *Teikyo Medical Journal*, *45*(02), 5453–5468.

Ghozali, D. A., Doewes, M., Soetrisno, S., Indarto, D., & Ilyas, M. F. (2023). Dose-response effect of L-citrulline on skeletal muscle damage after acute eccentric exercise: an in vivo study in mice. *PeerJ*, *11*, e16684. https://doi.org/10.7717/peerj.16684

Han, X., & Parker, T. L. (2017). Anti-inflammatory activity of clove (Eugenia caryophyllata) essential

oil in human dermal fibroblasts. *Pharmaceutical Biology*, *55*(1), 1619–1622. https://doi.org/10.1080/13880209.2017.1314513

Harborne, J. B., Padmawinata, K. S., & Iwang. (1987). Menganalisis, metode fitokimia penuntun cara modern tumbuhan, edisi kedua,. In *Metode Fitokimia* , 69-76.

Hody, S., Croisier, J., Bury, T., & Rogister, B. (2019). *Eccentric Muscle contractions : risks and benefits*. *10*(May), 1–18. https://doi.org/10.3389/fphys.2019.00536

Jahromi, B., Pirvulescu, I., Candido, K. D., & Knezevic, N. N. (2021). *Herbal Medicine for Pain Management : Efficacy and Drug Interactions*. 1–30.

Järvinen, T. A. H., Kääriäinen, M., Järvinen, M., & Kalimo, H. (2000). Muscle strain injuries. *Current Opinion in Rheumatology*, *12*(2), 155–161. https://doi.org/10.1097/00002281-200003000-00010

Jin, J., Yang, Z., Liu, H., Guo, M., Chen, B., Zhu, H., Wang, Y., Lin, J., Wang, S., & Chen, S. (2023). *Effects of acupuncture on the miR-146a-mediated IRAK1 / TRAF6 / NF- κ B signaling pathway in rats with sarcopenia induced by*. *11*(2), 1–15. https://doi.org/10.21037/atm-22-6082

Kreher, J. B., & Schwartz, J. B. (2012). *Sindrom overtraining: panduan praktis*. *02114*, 128–138.

Liao, P., He, Q., Zhou, X., Ma, K., Wen, J., Chen, H., Li, Q., & Pecchiari, M. M. (2020). *Repetitive bouts of exhaustive exercise induces a systemic inflammatory response and multi-organ damage in rats*. *11*(June), 1–14. https://doi.org/10.3389/fphys.2020.00685

Louw, M. (2023). *Anti-inflammatory drugs for muscle pain and injuries – Why you should think twice*.

Marmouzi, I., Karym, E. M., Alami, R., El Jemli, M., Kharbach, M., Mamouch, F., Attar, A., Faridi, B., Cherrah, Y., & Faouzi, M. E. A. (2019). Modulatory effect of Syzygium aromaticum and Pelargonium graveolens on oxidative and sodium nitroprusside stress and inflammation. *Oriental Pharmacy and Experimental Medicine*, *19*(2), 201–210. https://doi.org/10.1007/s13596-018-0335-9

Mateen, S., Rehman, M. T., Shahzad, S., Naeem, S. S., Faizy, A. F., Khan, A. Q., Khan, M. S., Husain, F. M., & Moin, S. (2019). Anti-oxidant and anti-inflammatory effects of cinnamaldehyde and eugenol on mononuclear cells of rheumatoid arthritis patients. *European Journal of Pharmacology*, *852*, 14–24. https://doi.org/10.1016/j.ejphar.2019.02.031

Mohamed, H. R. H., El-Shamy, S., Abdelgayed, S. S., Albash, R., & El-Shorbagy, H. (2024). Modulation efficiency of clove oil nano-emulsion against genotoxic, oxidative stress, and histological injuries induced via titanium dioxide nanoparticles in mice. *Scientific Reports*, *14*(1), 1–10. https://doi.org/10.1038/s41598-024-57728-1

Mu'nisa, A., Wresdiyati, T., Kusumorini, N., & Manalu, W. (2012). Aktivitas antioksidan ekstrak daun Cengkeh. *Jurnal Veteriner*, *13*(3), 272–277.

Nielsen, A. R., & Pedersen, B. K. (2007). The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Applied Physiology, Nutrition and Metabolism*, *32*(5), 833–839. https://doi.org/10.1139/H07-054

Os, J., Pereira, L. M., & Cabral, I. (2019). Strenuous acute exercise induces slow and fast twitch-dependent NADPH oxidase expression in rat skeletal muscle. *Antioxidants 2020*, *9*(57). https://doi.org/10.3390/antiox9010057

Park, J. Y., Kim, T. Y., Woo, S. W., & Moon, H. Y. (2024). Effect of exercise-induced Neutrophil maturation on skeletal muscle repair in vitro. *Biochemistry and Biophysics Reports*, *38*(December 2023), 101699. https://doi.org/10.1016/j.bbrep.2024.101699

Pedersen, B. K., & Febbraio, M. A. (2008). Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiological Reviews*, *88*(4), 1379–1406. https://doi.org/10.1152/physrev.90100.2007

Sheweita, S. A., El-hosseiny, L. S., & Nashashibi, M. A. (2016). *Protective effects of essential oils as natural antioxidants against Hepatotoxicity induced by Cyclophosphamide in Mice*. 1–17. https://doi.org/10.1371/journal.pone.0165667

Sugihartini, N., Prabandari, R., Yuwono, T., & Rahmawati, D. R. (2019). The anti-inflammatory activity of essential oil of clove (Syzygium aromaticum) in absorption base ointment with addition of oleic acid and propylene glycol as enhancer. *International Journal of Applied Pharmaceutics*, *11*(Special Issue 5), 106–109. https://doi.org/10.22159/ijap.2019.v11s5.T0081

Tu, H., & Li, Y. (2023). In fl ammation balance in skeletal muscle damage and repair. *Immunologi*, *January*, 1–14. https://doi.org/10.3389/fimmu.2023.1133355

Zuo, Q., Wang, S., Yu, X., & Chao, W. (2018). Response of macrophages in rat skeletal muscle after eccentric exercise. *Chinese Journal of Traumatology*, *21*(2), 88–95. https://doi.org/10.1016/j.cjtee.2017.12.001