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Effect of *Moringa oleifera* Fruit Extract on Lactate Dehydrogenase Activity in Cardiac Biological Samples of Obese Rat Models

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Abstract

Obesity is a chronic metabolic disorder associated with oxidative stress, persistent inflammation, and cardiac tissue disturbances. Lactate dehydrogenase (LDH) is an intracellular enzyme commonly used as a biomarker of tissue damage. *Moringa oleifera* fruit contains bioactive compounds with potential antioxidant and anti-inflammatory effects. This study investigated the effects of *Moringa oleifera* fruit extract on cardiac LDH activity in stored cardiac tissue from obese rats. This post-test-only experimental study used tissue samples from thirty male Sprague–Dawley rats assigned to five groups: normal control, obesity control, once-daily extract (OEMO1), twice-daily extract (OEMO2), and vitamin C. Cardiac LDH activity was measured using a kinetic enzymatic assay, normalized to total protein concentration, and analyzed using one-way ANOVA followed by Bonferroni post hoc testing. LDH activity differed significantly among groups ($p < 0.001$), with the highest activity in the obesity control group. The OEMO1 group showed activity comparable to the normal control group and significantly lower activity than the vitamin C group ($p < 0.001$). These findings suggest that *Moringa oleifera* fruit extract may modulate obesity-related biochemical disturbances in cardiac tissue.

Keywords: cardiac injury; lactate dehydrogenase (LDH); *Moringa oleifera* fruit extract; obesity; oxidative stress.

Abbreviations: LDH, lactate dehydrogenase; OEMO1, obese rats receiving *Moringa oleifera* fruit extract once a day; OEMO2, obese rats receiving *Moringa oleifera* extract twice a day; PBS, phosphate-buffered saline; ROS, reactive oxygen species.

INTRODUCTION

Obesity has become one of the major health issues worldwide, affecting people in both developed and developing countries. Nowadays, obesity is no longer seen just as a result of excessive fat storage, but more as a complex metabolic disorder that can trigger various chronic diseases, such as cardiac disease, type 2 diabetes, high blood pressure, abnormal cholesterol, and even some types of cancer (WHO, 2025). A similar trend is happening in Indonesia. According to the 2023 Indonesian Health Survey (Survei Kesehatan Indonesia, SKI), the prevalence of obesity among adults rose from 21.8% in 2018 to 23.4% in 2023. This steady increase shows how important it is to understand obesity-related complications and to come up with strategies to reduce its impact on health (Badan Kebijakan Pembangunan Kesehatan, 2024).

The effects of obesity on the cardiovascular system aren't just due to an increase in fat mass, but also involve complex metabolic and inflammatory changes. Expansion of adipose tissue changes its normal function and promotes a state of mild chronic inflammation, oxidative imbalance, and mitochondrial dysfunction. As an endocrine organ, fat tissue releases several inflammatory mediators, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which contribute to excessive production of reactive oxygen species (ROS). When antioxidant defense mechanisms can't keep up with the increased ROS production, oxidative damage can occur through lipid peroxidation, protein modification, mitochondrial dysfunction, and DNA damage in cardiomyocytes. Over time, this process can affect the structure and function of the myocardium, making it more vulnerable to cardiac injury and remodeling. (Khafagy & Dash, 2021; Martínez-Martínez & Cachofeiro, 2022).

Besides oxidative stress, lipid accumulation in cardiac tissue is also an important mechanism involved in obesity-related cardiac damage. Increased circulating free fatty acids can enter cardiomyocytes and disrupt normal energy metabolism by impairing mitochondrial β-oxidation, triggering endoplasmic reticulum stress, reducing ATP availability, and activating cell death pathways. Continuous exposure to excess lipids can further weaken cardiomyocyte membrane stability and

impair contractile function, ultimately contributing to myocardial remodeling and cellular damage (Jiménez-González et al., 2020; Lipke et al., 2022; Rocca et al., 2023; Yarmohammadi et al., 2022). Since these cellular changes can occur before obvious structural alterations are detected, biochemical indicators are needed to identify early myocardial disturbances.

Lactate dehydrogenase (LDH) is an enzyme often associated with cell damage. This intracellular enzyme plays an important role in anaerobic glycolysis by catalyzing the reversible conversion of pyruvate to lactate. Under normal conditions, LDH stays inside the cell because the membrane integrity is intact. However, cellular stress caused by oxidative damage, inflammation, ischemia, or necrosis can damage the plasma membrane and allow LDH to leak into the extracellular environment. Increased LDH activity can reflect cell membrane disruption and has been widely used as a marker of tissue damage, including cardiac damage (Brigic et al., 2024; Farhana & Lappin, 2023; Zhou et al., 2022). While measuring serum LDH is often used in clinical assessments, looking at LDH activity directly in cardiac tissue can provide extra insights into the biochemical changes happening in the affected organ.

The search for natural compounds that can reduce oxidative damage and inflammation has been on the rise in recent years, especially to tackle metabolic complications linked to obesity. *Moringa oleifera* fruit has attracted attention as a potential source of natural bioactive compounds with beneficial effects on metabolic health. Previous experimental studies have investigated the potential of *Moringa oleifera* fruit extract in improving metabolic disturbances associated with obesity, including alterations in lipid profiles (Arif et al., 2026). In addition, *Moringa oleifera* fruit extract has demonstrated potential antioxidant effects against liver injury in experimental animal models, suggesting its possible role in reducing oxidative damage associated with metabolic stress (Thadeus et al., 2024). However, evidence regarding the effects of *Moringa oleifera* fruit extract on obesity-related myocardial injury is still limited, especially studies focusing on cardiac LDH activity.

Biological samples that are stored also provide an efficient approach to evaluating tissue biomarkers while optimizing the use of existing experimental samples and supporting the ethical principle of reducing animal use in biomedical research (Pelisek et al., 2019). Despite these advantages, studies investigating the effects of *Moringa oleifera* fruit extract on cardiac LDH activity in stored cardiac biological samples from obese rat models are still rare. Therefore, this study aims to evaluate the effects of *Moringa oleifera* fruit extract on cardiac LDH activity in stored biological samples obtained from an obese rat model. The findings from this study are expected to contribute to the growing evidence regarding the potential cardiac protective effects of *Moringa oleifera* fruit against obesity-related myocardial injury.

MATERIALS AND METHODS

Study area

This lab-based experimental study was carried out using stored biological specimens (SBS) obtained from a main research project titled *The Potential of Moringa oleifera Fruit Extract on Cardiac Aging Markers in an Obese Rat Model*. The study used a post-test-only design with a control group and analyzed preserved cardiac tissue collected from the obese rat model. Myocardial tissue samples from male Sprague–Dawley rats were processed and analyzed at the Faculty of Medicine Laboratory, Universitas Pembangunan Nasional "Veteran" Jakarta.



Figure 1. Fruits of *Moringa oleifera* used in this study.

Cardiac tissue was collected on October 15, 2025. After collection, each sample was rinsed with phosphate-buffered saline (PBS) and stored at −80°C until biochemical analysis was done in April 2026. Storing at such a low temperature was used to keep the tissue intact and minimize biochemical damage before laboratory examination (Wieser et al., 2022).

Procedures

Experimental Animals and Treatment Groups

The cardiac tissue samples came from male Sprague–Dawley rats that had previously participated in the main research project. These animals were previously randomly divided into five experimental groups (n = 6 per group): normal control (N), obese control (O), obese rats given Moringa oleifera fruit extract once daily (OEMO1), obese rats given the extract twice daily (OEMO2), and obese rats given vitamin C as a positive control (OC).

Obesity in the main study was induced with a high-fat and high-protein diet. Rats in the treatment groups received Moringa oleifera fruit extract orally at a dose of 500 mg/kg body weight, administered once daily in the OEMO1 group and twice daily in the OEMO2 group for 14 days. Meanwhile, rats in the positive control group received vitamin C at a dose of 15 mg/kg body weight for 14 days. All treatments were administered according to the experimental protocol established in the main study.

Cardiac Tissue Preparation and LDH Measurement

Cardiac tissue specimens that were stored were taken from −80°C storage and processed for biochemical analysis. Around 100 mg of myocardial tissue from each sample was collected and homogenized in 1 mL of phosphate-buffered saline (PBS; pH 7.4). The homogenization process continued until a uniform tissue suspension was obtained. The resulting homogenate was then used to measure LDH activity according to the lab protocol.

Cardiac LDH activity was measured using the LDH BR reagent kit from Glory Diagnostics (China) based on the ultraviolet (UV) kinetic enzyme method. The reaction absorbance was measured using an EZ Read 400 microplate reader according to the manufacturer's instructions. Total protein concentration was measured separately using the Bradford assay. Because variations in tissue protein content can affect enzyme activity measurements, LDH activity was normalized to total protein concentration and reported as the LDH-to-protein ratio (U/mg protein) for further statistical analysis.

Ethical Approval

This study used previously collected cardiac tissue specimens from an umbrella research project. The original animal study was approved by the Research Ethics Committee for Medicine and Health (KEPKK), Universitas Muhammadiyah Prof. Dr. HAMKA (UHAMKA) (Approval No. KEPKK/FK/091/09/2025). Animal procedures followed institutional ethical guidelines and the principles of Replacement, Reduction, and Refinement (3R). The specimens were collected in October 2025 and stored at −80°C. The present study received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta (Approval No. 26/II/2026/KEP).

Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics. Data are shown as mean ± standard deviation (SD) based on the LDH-to-protein ratio. The Shapiro–Wilk test was used to check data distribution, while Levene's test was used to assess variance homogeneity. Differences in LDH activity among experimental groups were analyzed using one-way ANOVA. If significant differences were found, a Bonferroni post hoc analysis was done for multiple comparisons. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

The effect of *Moringa oleifera* fruit extract on cardiac lactate dehydrogenase (LDH) activity was evaluated using stored cardiac tissue specimens from an obese Sprague–Dawley rat model. LDH activity was measured in myocardial homogenates and normalized to total protein concentration. The resulting LDH-to-protein ratios are summarized in Table 1, while statistical comparisons are shown in Figure 2.

Table 1. Cardiac Lactate Dehydrogenase (LDH) Levels Expressed as the LDH-to-Protein Ratio in Experimental Groups.

Group	Cardiac LDH Activity (Mean ± SD)
Normal	0.0355 ± 0.0019
Obesity	0.0571 ± 0.0017
OEMO1	0.0372 ± 0.0004
OEMO2	0.0460 ± 0.0021
OC	0.0476 ± 0.0049

The data are presented as the mean ± standard deviation (SD). N, normal control; O, obese control; OEMO1, obese rats given *Moringa oleifera* fruit extract (500 mg/kg body weight) once a day; OEMO2, obese rats given the same extract twice a day; and OC, obese rats given vitamin C (15 mg/kg body weight).

The numerical values differed among the five experimental groups. Cardiac LDH activity reached the highest level in the obese group, while the normal control showed the lowest value. Administration of *Moringa oleifera* fruit extract was associated with lower cardiac LDH activity in obese rats. The largest decrease was seen in the OEMO1 group, with LDH levels approaching the normal control. Lower LDH activity was also found in the OEMO2 and vitamin C groups, although both were still higher than OEMO1.

Overall, the distribution of LDH activity showed a consistent pattern across the treatment groups; compared to untreated obese rats, the animals that received *Moringa oleifera* fruit extract had lower LDH activity, especially those given it once daily.

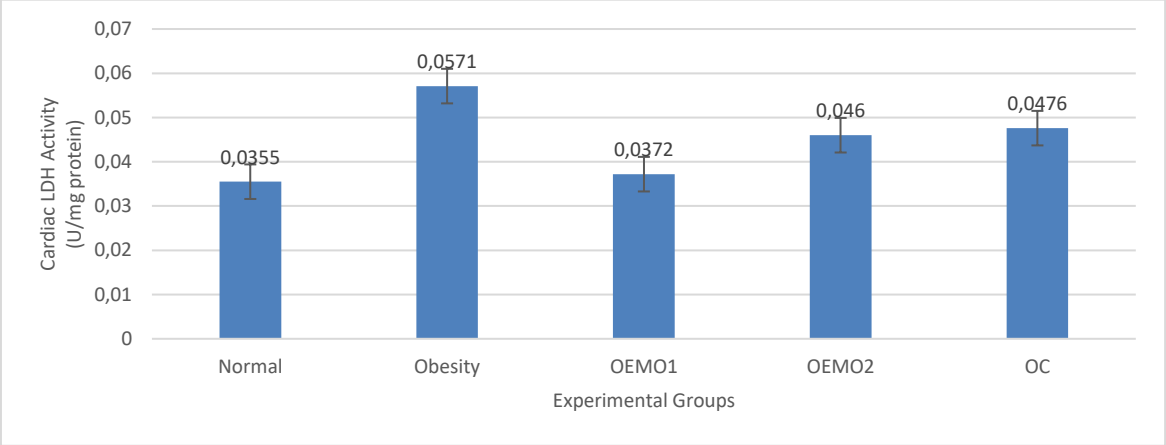


Figure 2. Cardiac lactate dehydrogenase (LDH) activity among the experimental groups. Cardiac LDH activity is shown as the ratio of LDH to total protein (U/mg protein). The values are presented as mean ± standard deviation (SD). N stands for normal control; O, obese control; OEMO1, obese rats given *Moringa oleifera* fruit extract (500 mg/kg body weight) once a day; OEMO2, obese rats given the same extract twice a day; and OC, obese rats given vitamin C (15 mg/kg body weight). Statistical analysis was done using one-way ANOVA followed by Bonferroni post hoc analysis.

A one-way ANOVA showed a significant difference in cardiac LDH activity among the experimental groups ($p < 0.001$). Pairwise comparisons using the Bonferroni test showed that the obesity group had significantly higher LDH activity than the normal control group ($p < 0.001$). No significant difference was detected between the OEMO1 and normal control groups ($p = 1.000$). In contrast, LDH activity in the OEMO2 and vitamin C groups remained significantly higher than that in the normal control group (both $p < 0.001$). No statistically significant difference was observed between the OEMO2 and vitamin C groups ($p = 1.000$). Additionally, the OEMO1 group showed significantly lower cardiac LDH activity than the vitamin C group ($p < 0.001$).

Among the treatment groups, the lowest mean LDH activity was observed in the OEMO1 group. Increasing the administration frequency to twice daily did not result in a further reduction in LDH activity under the conditions evaluated in this study.

Discussion

Cardiac LDH activity was much higher in the obesity group compared to the normal control group, indicating that obesity is associated with greater myocardial cellular disturbances in this experimental model. This finding is consistent with the role of LDH as an intracellular enzyme involved in anaerobic glycolysis. Changes in LDH activity within cardiac tissue may reflect alterations in cellular metabolism and biochemical conditions associated with tissue injury. For this reason, LDH has been widely used as a biochemical marker of cell damage, including damage that occurs in the myocardium (Martínez-Martínez & Cachofeiro, 2022; Zhou et al., 2022). The striking difference between the obesity and normal groups suggests that the obesity model is associated with detectable biochemical alterations in myocardial tissue.

The increase in LDH seen in obese rats can be explained by several biological changes that occur along with obesity. Excess fat tissue is not just an energy reserve, but also acts as an active endocrine organ that continuously releases inflammatory mediators. As obesity progresses, mild chronic inflammation arises along with excessive production of reactive oxygen species (ROS). When ROS production exceeds the body's antioxidant capacity, oxidative stress occurs and gradually damages cell structures. Mitochondria become less efficient, lipid peroxidation increases, and cardiomyocyte membranes become more prone to injury. Once membrane integrity is compromised, cellular injury may alter intracellular enzyme activity and contribute to biochemical disturbances in cardiac tissue. So, the high LDH activity found in the obese group may reflect obesity-related biochemical disturbances in myocardial tissue, rather than normal biological variation (Martínez-Martínez & Cachofeiro, 2022).

The current findings are also in line with previous reports. Othman et al. (2022) showed that long-term consumption of a high-fat diet increases oxidative stress and inflammatory responses in cardiac tissue, which eventually leads to structural and functional changes in the myocardium. Similar conclusions were reported by Martínez-Martínez & Cachofeiro (2022), who described oxidative stress as one of the main causes of myocardial remodeling in obesity. Although the specific outcome sizes differed between studies, the overall pattern remains consistent. In this study, obese animals also showed higher cardiac LDH activity compared to healthy controls, supporting the idea that obesity itself contributes to myocardial injury (Martínez-Martínez & Cachofeiro, 2022; Othman et al., 2022).

The use of stored cardiac biological specimens in this study provided an opportunity to investigate tissue biomarkers while supporting the principle of reducing animal use in biomedical research. The cardiac tissues were collected from the same umbrella research project and stored at −80°C until biochemical analysis. Although storage at ultra-low temperatures is generally effective in

preserving tissue quality and protein stability, prolonged storage may still affect protein conformation or enzyme activity to some extent (Park et al., 2021; Shabihkhani et al., 2014). In the present study, all samples underwent identical storage conditions and comparable storage periods before analysis. Therefore, storage-related effects alone are unlikely to explain the differences in cardiac LDH activity among the experimental groups, although their potential influence cannot be completely excluded.

Treatment with *Moringa oleifera* fruit extract was associated with lower cardiac LDH activity compared to the untreated obesity group, suggesting a potential beneficial effect on obesity-related biochemical disturbances in myocardial tissue. Both treatment groups showed lower LDH levels compared to the obese control, but the improvement levels were not identical. The once-a-day regimen (OEMO1) resulted in the largest decrease in LDH activity, with levels no longer significantly different from the normal control group. On the other hand, although the twice-a-day regimen (OEMO2) also lowered LDH activity, the decrease was smaller, and LDH levels remained much higher compared to healthy animals. These findings suggest that, under the conditions evaluated in this study, once-daily administration was associated with the lowest mean cardiac LDH activity among the treatment groups.

The observation that OEMO1 performs better than OEMO2 is interesting because increasing the dosage frequency actually doesn't improve the results. At first glance, one might expect that giving it more often would provide stronger protection. However, the biological response to plant-based compounds is often more complex than a simple dose-response relationship. Once absorbed, phytochemicals go through metabolism, distribution, and elimination, and these processes can affect how effectively the active compounds work in the target tissues (Azlan et al., 2022; Kashyap et al., 2022). So, giving the extract twice a day might not actually increase the amount of active compounds reaching the myocardium or extend their biological activity. Since pharmacokinetic parameters weren't evaluated in this study, this explanation is still speculative and needs confirmation in future research.

Interestingly, the response to different administration frequencies in the present study differed from the findings reported by Arif et al. (2026). In their study, twice-daily administration of *Moringa oleifera* fruit extract showed a greater numerical reduction in triglyceride levels than once-daily administration, although the difference between the two treatment groups was not statistically significant. In contrast, the present study showed a lower mean cardiac LDH activity in the once-daily treatment group than in the twice-daily treatment group. These differences may be related to the distinct biological parameters evaluated, as triglyceride levels reflect lipid metabolism, whereas cardiac LDH activity represents a different biochemical aspect of tissue metabolism and cellular disturbance. Therefore, the optimal administration frequency may depend on the biological outcome being investigated.

The decrease in cardiac LDH activity observed after treatment may be associated with the potential antioxidant properties of *Moringa oleifera* fruit extract. Previous research has demonstrated the antioxidant potential of *Moringa oleifera* fruit extract in experimental animal models (Thadeus et al., 2024). In obesity, excessive reactive oxygen species (ROS) production may contribute to mitochondrial dysfunction, lipid peroxidation, and disturbances in cellular integrity. These processes may be associated with alterations in intracellular enzyme activity, including LDH. Therefore, the lower cardiac LDH activity observed in the treated groups may reflect an improvement in obesity-related biochemical disturbances. However, since oxidative stress markers and specific bioactive compounds were not measured in the present study, this potential mechanism requires further investigation.

Rather than relying on a single compound, the antioxidant potential of *Moringa oleifera* may be associated with various bioactive phytochemicals. Previous studies have reported antioxidant properties of different *Moringa oleifera* plant parts, including flavonoids and phenolic compounds (Azlan et al., 2022; Kashyap et al., 2022). However, the phytochemical composition may vary depending on the plant part and extraction method. Research specifically examining *Moringa oleifera* fruit extract has also demonstrated its potential antioxidant activity in experimental animal models (Thadeus et al., 2024). Although these findings provide a possible basis for understanding the observed effects, the specific bioactive compounds and molecular pathways involved were not examined in the present study.

The current findings are also supported by previous studies that investigated the biological effects of *Moringa oleifera*. Louisa et al. (2022) summarized evidence showing that *Moringa oleifera* has antioxidant and anti-inflammatory activities that can improve metabolic disorders associated with obesity. In studies specifically examining *Moringa oleifera* fruit extract, Arif et al. (2026) reported lower triglyceride levels following fruit extract administration in obese rat models. Furthermore, Thadeus et al. (2024) reported the potential antioxidant effects of *Moringa oleifera* fruit extract against liver injury in an experimental animal model. Although these studies did not specifically investigate cardiac LDH activity, their findings provide supporting evidence for the potential metabolic and antioxidant benefits of *Moringa oleifera* fruit extract. Therefore, the lower cardiac LDH activity observed in the present study may be consistent with a potential reduction in obesity-related biochemical disturbances associated with oxidative stress.

The group given vitamin C also showed lower cardiac LDH activity than the untreated obesity group, suggesting a potential beneficial effect of antioxidant

supplementation. However, LDH activity in this group remained higher than in the normal control group. Interestingly, the vitamin C group showed a similar pattern to the OEMO2 group, with no statistically significant difference between them ($p = 1.000$). In contrast, the once-daily *Moringa oleifera* regimen (OEMO1) showed a significantly lower mean LDH activity than the vitamin C group ($p < 0.001$). These findings indicate that, among the treatment regimens evaluated, once-daily administration of *Moringa oleifera* fruit extract was associated with the lowest mean cardiac LDH activity.

Vitamin C is a well-known antioxidant that helps neutralize reactive oxygen species and reduce oxidative damage

(Luo et al., 2022). Unlike vitamin C, which represents a single antioxidant compound, *Moringa oleifera* fruit extract may contain multiple bioactive constituents that contribute to its potential antioxidant activity. Previous research has demonstrated the antioxidant potential of *Moringa oleifera* fruit extract in experimental animal models (Thadeus et al., 2024). These properties may partly explain the differences in cardiac LDH activity observed among the treatment groups. However, the specific mechanisms remain unclear because individual bioactive compounds and oxidative stress markers were not measured in the present study. Further investigations are therefore needed to clarify these potential effects.

Another aspect worth considering is the use of stored cardiac tissue in this study. Rather than collecting fresh specimens, this study utilized cardiac samples obtained from a previous parent research project. The use of existing biological materials supports the principle of reducing animal use in biomedical research. The tissue samples were stored at −80°C under controlled conditions until biochemical analysis was performed. Previous studies have shown that ultra-low temperature storage can help preserve tissue quality and protein stability for subsequent biochemical analysis. (Park et al., 2021).

Although frozen storage may cause slight changes to the structure or stability of some proteins, these effects are usually limited if the samples are handled and stored properly (Shabihkhani et al., 2014). In this study, all experimental groups went through the same storage procedures, were kept under identical conditions, and were analyzed using the same lab protocols. Since these conditions were applied consistently across all groups, it's unlikely that sample storage alone would explain the differences in cardiac LDH activity. The variations observed make more sense as being due to the biological effects of obesity and treatment. That said, the potential impact of long-term storage can't be completely ignored and should still be considered when interpreting the results (Park et al., 2021; Shabihkhani et al., 2014).

Some limitations also need to be considered. This study only focused on cardiac LDH activity as an indicator of myocardial injury. While LDH is a useful marker of cell damage, it doesn't fully represent the complex biological processes involved in obesity-related cardiac injury. Measuring additional biomarkers, such as cardiac troponin, CK-MB, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, or inflammatory cytokines, would give a more comprehensive understanding of myocardial injury and oxidative stress (Zhou et al., 2022). Similarly, oxidative stress pathways and inflammatory responses weren't examined directly, so it's not possible to pinpoint the exact mechanisms responsible for the protective effects observed.

Another limitation is related to the treatment protocol. Only a single dose of *Moringa oleifera* fruit extract (500 mg/kg body weight) was evaluated, while the treatment groups only differed in terms of dosing frequency. As a result, the optimal dose and duration of treatment are still unclear. Future studies should explore different dose levels, longer treatment periods, histopathological changes, pharmacokinetic profiles, and molecular biomarkers to explain the mechanisms involved and determine the most effective therapy regimen.

Overall, these findings suggest that obesity increases cardiac LDH activity, which reflects greater myocardial cell damage in obese rats. Giving *Moringa oleifera* fruit extract lowers LDH activity, indicating that this extract might help protect cardiac tissue from obesity-related damage. Among the treatment regimens evaluated, giving it once a day consistently produced the best results. Although the exact molecular mechanism still needs to be clarified, the observed reduction in cardiac LDH activity may be associated with the potential antioxidant properties of *Moringa oleifera* fruit extract, although the underlying mechanisms require further investigation.

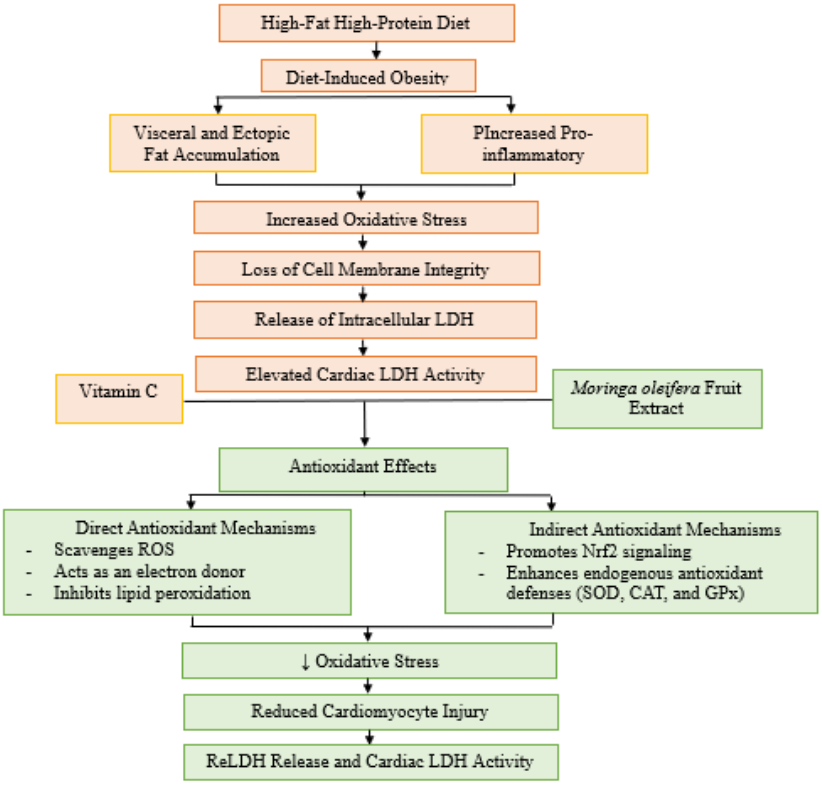


Figure 3. The proposed mechanism showing the potential cardiac-protective role of *Moringa oleifera* fruit extract in cardiac injury caused by obesity. Obesity development after a high-fat and high-protein diet is linked to excessive fat accumulation and increased oxidative stress in cardiac tissue. High production of free radicals (ROS) can disrupt cellular balance and trigger a series of pathological changes, including inflammation activation, mitochondrial dysfunction, and damage to cardiac cell membranes. These changes can harm cell integrity and contribute to the release of lactate dehydrogenase (LDH) from damaged cardiac cells.

Moringa oleifera fruit extract might be able to counter obesity-related changes through the activity of various phytochemicals it contains, including flavonoids, phenolic compounds, vitamin C, and β-sitosterol. The combined effects of these compounds are thought to support antioxidant defense, protect mitochondrial function, and maintain the stability of cardiac cell membranes. As a result, this extract could help preserve cardiac cell structure and reduce LDH leakage associated with obesity-induced myocardial stress.

CONCLUSIONS

This study shows that obesity is associated with increased cardiac LDH activity in Sprague–Dawley rats, suggesting obesity-related biochemical disturbances in cardiac tissue. Treatment with *Moringa oleifera* fruit extract at a dose of 500 mg/kg body weight was associated with lower cardiac LDH activity compared to the untreated obesity group.

Among the treatment groups, the rats that received the extract once daily (OEMO1) showed the lowest mean cardiac LDH activity, with no statistically significant difference from the normal control group ($p = 1.000$). The twice-daily extract group (OEMO2) and the vitamin C group also showed lower mean cardiac LDH activity than the obesity control group; however, their values remained higher than those of the OEMO1 group. These findings suggest that increasing the administration frequency from once daily to twice daily was not associated with a further reduction in cardiac LDH activity under the conditions of this study.

These findings suggest that *Moringa oleifera* fruit extract may have potential benefits in modulating obesity-related biochemical disturbances in cardiac tissue. However, further research is needed to confirm these findings and clarify the underlying mechanisms. Future studies should explore different doses and treatment periods, involve larger sample sizes, and include additional assessments such as cardiac biomarkers, histopathological evaluation, oxidative stress markers, and molecular analyses. These approaches may provide a better understanding of the potential effects of *Moringa oleifera* fruit extract on obesity-related cardiac alterations.

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Authors' Contributions: Wahyuni Patrianingsih, Dr. dr. Tiwuk Susantiningsih, M.Biomed., Sp.KKLP., and Seftiwan Pratami Djasfar, S.Si., M.Si. contributed to the planning and development of this study. Dr. dr. Tiwuk Susantiningsih, M.Biomed., Sp.KKLP., as the main research project initiator, provided the main research direction, supervised the study process, and guided the scientific aspects of this work.

Dr. Ratna Puspita, S.Si., M.Si. contributed through scientific evaluation and manuscript review, mainly by giving suggestions to improve the quality and clarity of the study. Seftiwan Pratami Djasfar, S.Si., M.Si. was involved in research supervision, helped interpret the findings, and contributed to manuscript improvements.

Wahyuni Patrianingsih carried out the lab procedures, analyzed the data, and prepared the first draft of the manuscript. Revisions and improvements were made based on discussions and input from co-authors. All authors reviewed the final manuscript, approved the version submitted, and took responsibility for the honesty and accuracy of this research.

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