BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 2, October 2026 | Pages: 2291-2299 | DOI: 10.14421/biomedich.2026.152.2291-2299 ISSN 2540-9328 (online)

Effect of *Moringa oleifera* Fruit Extract on Cardiac Myoglobin Levels in Obese Rat Models: A Study Using Archived Tissue Specimens

Nadhira Yulianajmi Chairunnisa[1], Tiwuk Susantiningsih[2]*, Yosha Putri Wahyuni[3], Kristina Simanjuntak[2]
[1]Medicine; [2]Departement of Biochemistry, [3]Departement of Biology, Faculty of Medicine,
Universitas Pembangunan Nasional "Veteran" Jakarta
Jl. R.S Fatmawati No. 1, Pondok Labu, Jakarta Selatan 12450. Tel. +62-765-6971, Indonesia.

Corresponding author*
tiwuksusantiningsih@upnvj.ac.id

Manuscript received: 16 July 2026. Revision accepted: 18 November 2026, Published: 21 November 2026.

Abstract

Obesity is one of the major cardiovascular disease risk factor that induces oxidative stress, damaging cardiac tissue, and reducing myoglobin, which is an oxygen-binding protein for cardiomyocyte function. To counter this condition, *Moringa oleifera* fruit extract that contains antioxidant compounds with potential cardioprotective effects was used to evaluate its impact on myoglobin levels in archived cardiac tissue specimens. This study used archived heart tissue specimens obtained from a previous animal experiment of an obese rat models, thereby supporting the 3R principles, particularly Reduction. Archived specimens from a previous experiment involving five groups of six rats each (total n=30) were analyzed: normal control (P1), obese control (P2), obese rats treated with *Moringa oleifera* fruit extract 500 mg/kg body weight once daily (P3), *Moringa oleifera* fruit extract 500 mg/kg body weight twice daily (P4), and obese rats treated with vitamin C 15 mg/kg body weight (P5). Myoglobin levels were measured by ELISA and then analyzed using one-way ANOVA followed by Bonferroni test. The results showed that myoglobin levels were significantly lower in obese than normal rats (0.0858 ± 0.0026 vs 0.0915 ± 0.0006 µg/mg; $p = 0.002$), whereas *Moringa oleifera* extract significantly increased myoglobin levels compared with obese controls ($p < 0.000$), with no significant differences observed between extract-treated groups and vitamin C, or between the two dosing regimens ($p > 0.05$). Taken together, these findings suggest that *Moringa oleifera* fruit extract preserves cardiac myoglobin levels and may protect against obesity-induced cardiac injury.

Keywords: Archived Tissue Specimens; Cardioprotective; *Moringa oleifera* Fruit; Myoglobin; Obesity.

INTRODUCTION

Obesity is a chronic metabolic disorder characterized by excessive adipose tissue accumulation that adversely affects health. According to the World Health Organization (WHO, 2024), more than 390 million children and adolescents aged 5–19 years were overweight in 2022, with the global prevalence increasing from 8% in 1990 to 20% in 2022. The World Obesity Federation (2025) reported that Southeast Asia Southeast faces the highest rates of obesity in the world, with Indonesia ranking fifth on the list. These findings explaining that obesity is a concerning public health problem in Indonesia.

Obesity promotes adipocyte hypertrophy and lipid accumulation and leading to excessive production of reactive oxygen species (ROS), chronic low-grade inflammation, insulin resistance, hyperglycemia, and dyslipidemia. Increasing levels of reactive oxygen species that are unbalanced relative to the body's oxidants can damage membrane phospholipids, mitochondria, and the sarcoplasmic reticulum of cardiomyocytes, thereby leading to endothelial dysfunction, atherosclerosis, and hypertension (Hildebrand & Pfeifer, 2025).

Chronic oxidative stress in cardiomyocytes can oxidize myoglobin which is a muscle protein in the cardiac that responsible for delivering oxygen to mitochondria, serving as an intracellular oxygen buffer, and scavenging reactive oxygen species. When myoglobin scavenging the reactive oxygen species, it can turned into metmyoglobin that is incapable of binding oxygen, thereby impairing cellular oxygen homeostasis in the cardiac. Furthermore, cardiomyocyte injury resulting from chronic inflammation and oxidative stress can damage the sarcolemma and leading to the release of myoglobin a reduction in its levels within cardiac tissue (Hayashida et al., 2024). With that reason, cardiac tissue myoglobin can serve as a biomarker of oxidative stress and obesity-induced myocardial injury.

The utility of myoglobin as a biomarker depends on the quality and integrity of the samples. Archived tissue specimens collected in prior studies and preserved under

specific conditions can be re-analyzed for their biomarkers. Long-term storage at −80°C in accordance with international biobanking standards can minimizes proteolytic activity, reduces protein denaturation, and preserves biomolecular integrity (Liang et al., 2025). The use of archived tissue specimens also enhances research efficiency by lowering costs, shortening study durations, and reducing animal usage while supporting the 3R principles (Replacement, Reduction, and Refinement) in animal research ethics (Azkona, 2023). By utilized previously collected and properly stored specimens, researchers can avoid using new animals for every study, thereby directly reducing the total number of animals used and minimizing repetitive invasive procedures.

Biomarkers such as myoglobin can be measured to evaluate the efficacy of therapeutic interventions in preventing obesity-induced cardiac injury. *Moringa oleifera* is known from various studies to contain flavonoids, phenolic compounds, and vitamin C, thereby exhibiting antioxidant and cardioprotective properties. Previous research has demonstrated that *Moringa oleifera* reduces oxidative stress, improves lipid metabolism, and attenuates obesity-associated cardiovascular damage (Pareek et al., 2023). Specifically regarding *Moringa oleifera* fruit, Thadeus et al. (2024) showed that administering *Moringa oleifera* fruit extract at a dose of 500 mg/kg body weight (BW) once and twice daily significantly increased the activity of catalase and manganese superoxide dismutase (MSOD) that indicating enhanced antioxidant defense. This dosage regimen was subsequently applied by Arif et al. (2026) in an experimental obese rat model, which their study is the one provided the archived cardiac tissue specimens used in this research. However, evidence regarding the effect of *Moringa oleifera* fruit extract on cardiac tissue myoglobin, particularly studies utilizing archived tissue specimens remains very limited.

Therefore, this study aims to evaluate the effect of *Moringa oleifera* fruit extract on cardiac myoglobin levels in an obese rat model using archived tissue specimens. This research is expected to expand knowledge regarding the potential of *Moringa oleifera* fruit as a complementary therapy for obesity-related heart disease. It is also expected to provide evidence supporting the feasibility of using archived tissue specimens for biomarker analysis.

MATERIALS AND METHODS

Study Area

This experimental study were using archived tissue specimens, specifically frozen cardiac tissue samples obtained from a previous obesity rat model experiment by Arif et al. (2026). The The study was conducted from September 2025 to April 2026 at the Stem Cell Laboratory, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta. The use of archived cardiac tissue specimens in this study was approved by the Research Ethics Committee of Universitas Pembangunan Nasional “Veteran” Jakarta with ethical approval No. 24/II/2026/KEP.

Procedures

Research Sample

Specimens were selected using purposive sampling based on predefined inclusion and exclusion criteria which is only samples stored at −80°C, available in sufficient quantity whic is 100 mg, preserved for less than 5 years, and free from visible degradation or contamination were included in the analysis. The previous experiment involved 30 male *Sprague Dawley* rats in 6-8 weeks old rats that were allocated into five groups (n = 6/group): (1) normal control receiving distilled water; (2) obese control receiving distilled water; (3) obese rats treated with *Moringa oleifera* fruit extract (500 mg/kgBW once daily); (4) obese rats treated with *Moringa oleifera* fruit extract (500 mg/kgBW twice daily); and (5) obese rats treated with vitamin C (15 mg/kgBW/day) as positive control. The sample size for the previous experiment was determined using the Federer formula, with an additional 10% allocated to compensate for potential sample loss. As this study is utilized the archived tissue rather than performing new animal treatments, there is no additional intervention, randomization, or in vivo procedures were conducted. This analysis was restricted to biomarker assessment of the archived specimens.

Preparation of Archived Tissue Specimens

Frozen cardiac tissue specimens were taken out from a −80°C freezer. Subsequently, the tissue was sectioned and weighed 100 mg for the analysis. The tissue was homogenized in 1000 µL of phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 6000 rpm for 10 minutes to obtain the tissue homogenate. The supernatant from the homogenized tissue will be collected.

Determination of Total Protein Concentration

Total protein concentration was determined using the Bradford method with *Coomassie Brilliant Blue G-250* reagent. A 10 µL of tissue supernatants was mixed with Bradford reagent that had been diluted in distilled water. The mixture was then placed in a microplate well and measured at a wavelength of 595 nm using a microplate reader to determine the total protein concentration for normalization purposes. This normalization step was performed to account for variability in tissue and cellular content across homogenates and to ensure that myoglobin levels were expressed relative to total protein.

Measurement of Myoglobin Levels by Enzyme-Linked Immunosorbent Assay (ELISA)

Myoglobin concentration was measured using a pre-coated, rat-specific ELISA kit. A series of standard solutions was prepared to generate a calibration curve.

The supernatants and standard solutions were loaded in duplicate into microplate wells pre-coated with anti-myoglobin antibodies. Subsequent steps involved sequential incubation with detection reagents A and B containing Horseradish Peroxidase (HRP) enzyme, washing stages, a reaction with tetramethylbenzidine (TMB) substrate, and the addition of a stop solution, causing the samples to change color from blue to yellow. Optical density (OD) was measured at a wavelength of 450 nm using a microplate reader.

Calculation of Normalized Myoglobin Concentration

Standard optical density (OD) were plotted against their respective myoglobin concentrations to generate a standard calibration curve. Each samples were interpolated using this calibration curve to determine the myoglobin concentration (µg/mL). The myoglobin concentration was then normalized to the corresponding total protein concentration that previously determined using the Bradford method and expressed in units of µg/mg protein.

Data Analysis

Data of this study were analyzed using IBM SPSS Statistics software. The descriptive statistics are presented as mean ± standard deviation. Data distribution normality was assessed using the Shapiro–Wilk test, while the homogeneity of variance was evaluated using Levene's test. If both assumptions were met ($p > 0.05$), inter-group comparisons were performed using One Way ANOVA, followed by Bonferroni *post hoc* analysis for pairwise comparisons.

RESULTS AND DISCUSSION

Statistic Descriptive

Table 1. Average Myoglobin Levels.

Treatment Group	Average Myoglobin Level (µg/mg) ± SD
Normal Contol (P1)	0.0915 ± 0.0006
Obese Control (P2)	0.0858 ± 0.0026
Moringa oleifera 1x Daily (P3)	0.0917 ± 0.0007
Moringa oleifera 2x Daily (P4)	0.0933 ± 0.0006
Vitamin C (P5)	0.0941 ± 0.0029

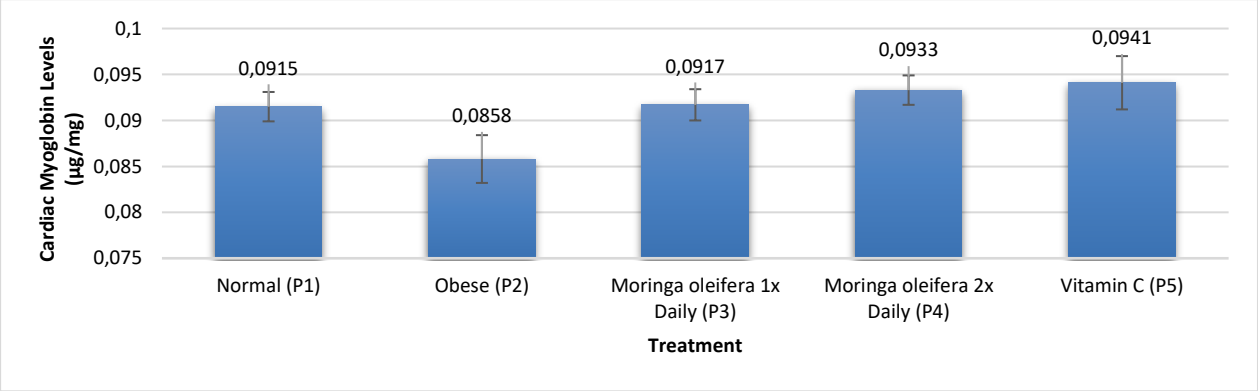


Figure 1. Cardiac tissue myoglobin levels in each experimental group. Data are presented as mean ± standard deviation (SD). P1 = normal control; P2 = obese control; P3 = obese treated with *Moringa oleifera* fruit extract (500 mg/kgBW, once daily); P4 = obese treated with *Moringa oleifera* fruit extract (500 mg/kg BW, twice daily); P5 = obese treated with vitamin C (15 mg/kgBW) as positive control.

As shown in Table 1, the highest mean myoglobin level was observed in the positive control group treated with vitamin C (0.0941 ± 0.0029), followed by the group receiving *Moringa oleifera* fruit extract twice daily (0.0933 ± 0.0006), the group receiving *Moringa oleifera* fruit extract once daily (0.0917 ± 0.0007), and the normal control group (0.0915 ± 0.0006). The lowest mean myoglobin level was observed in the obese group (0.0858 ± 0.0026). These findings indicate variation in mean cardiac myoglobin levels among the experimental groups, with the obese group showing the lowest value and the treatment groups showing values comparable to or higher than the normal control group.

One-Way ANOVA Test

The Shapiro–Wilk and Levene tests indicated that the data were normally distributed and had homogeneous variances ($p > 0.05$). Therefore, One Way ANOVA could

be performed to compare cardiac myoglobin levels among the experimental groups.

Table 2. One-Way ANOVA Test Results.

Myoglobin Levels	p-value
Between Groups	0.000

One Way ANOVA showed a significant difference in myoglobin levels among the groups ($p < 0.000$). Bonferroni *post hoc* analysis was performed to determine which pairwise group comparisons were significantly different.

Post Hoc Bonferroni Test

Table 3. Post Hoc Bonferroni Test Results

Group		p-value
Normal Control (P1)	Obese Control (P2)	0.000
	Moringa oleifera 1x Daily (P3)	1.000
	Moringa oleifera 2x Daily (P4)	1.000
	Vitamin C (P5)	0.463
Obese Control (P2)	Normal Control (P1)	0.002
	Moringa oleifera 1x Daily (P3)	0.000
	Moringa oleifera 2x Daily (P4)	0.000
	Vitamin C (P5)	0.000
Moringa oleifera 1x Daily (P3)	Normal Control (P1)	1.000
	Obese Control (P2)	0.000
	Moringa oleifera 2x Daily (P4)	1.000
	Vitamin C (P5)	1.000
Moringa oleifera 2x Daily (P4)	Normal Control (P1)	1.000
	Obese Control (P2)	0.000
	Moringa oleifera 1x Daily (P3)	1.000
	Vitamin C (P5)	1.000
Vitamin C as Positive Control (P5)	Normal Control (P1)	0.463
	Obese Control (P2)	0.000
	Moringa oleifera 1x Daily (P3)	1.000
	Moringa oleifera 2x Daily (P4)	1.000

Bonferroni analysis the obesity group exhibited significantly lower cardiac myoglobin levels compared to the normal control group ($p = 0.002$), indicating a reduction in cardiac myoglobin levels associated with obesity. The obesity group also differed significantly from the group receiving *Moringa oleifera* fruit extract once daily and twice daily ($p < 0.000$), and the vitamin C group as the positive control ($p < 0.000$). All three treatment groups showed higher cardiac myoglobin levels than the obesity group.

There is no significant differences were found between the normal control group and the groups receiving *Moringa oleifera* fruit extract once daily ($p = 1.000$) or twice daily ($p = 1.000$), nor the vitamin C group ($p = 0.463$). These findings indicate that all three interventions restored cardiac myoglobin levels in the obesity group to levels approaching those of the normal control group.

Comparisons between the *Moringa oleifera* fruit extract groups, once or twice daily and the vitamin C group showed no statistically significant differences ($p = 1.000$). These results indicate that administering *Moringa oleifera* fruit extract, whether once or twice daily, produces an effect on cardiac myoglobin levels comparable to vitamin C as positive control. Furthermore, no statistically significant difference was observed between the once-daily and twice-daily *Moringa oleifera* fruit extract groups ($p = 1.000$) explaining that increasing the frequency of *Moringa oleifera* fruit extract administration did not did not provide a statistically significant additional benefit.

Discussion

Effect of Obesity on Cardiac Myoglobin Levels in Rats

The obese group (0.0858 ± 0.0026) exhibited significantly lower cardiac myoglobin levels compared to the normal group (0.0915 ± 0.0006) ($p = 0.000$). This indicates impaired metabolic function and increased oxidative stress within cardiac tissue. This reduction aligns with the relationship between myoglobin expression and tissue metabolic status reported by Liskiewicz et al. (2021), who found that improving metabolic status through weight loss could enhance myoglobin expression. These findings suggest a potential adverse impact of obesity on myoglobin function and metabolism.

Obesity triggers insulin resistance, hyperlipidemia, and chronic inflammation, driving endothelial dysfunction, cardiomyocyte hypertrophy, and fibrosis (Ren et al., 2021). Reduced myoglobin levels may exacerbate these conditions by increasing myocardial susceptibility to hypoxia. At the molecular level, myoglobin interacts with lipid metabolism enzymes such as peroxisome proliferator-activated receptor alpha (PPARα) and carnitine palmitoyltransferase-1 (CPT-1) a decline in myoglobin levels impairs fatty acid oxidation, leading to the accumulation of toxic lipid intermediates—such as ceramides and diacylglycerols which subsequently trigger apoptosis and cardiac remodeling (Adepu et al., 2024).

This lipotoxic process is closely related to oxidative stress, where lipid accumulation occurs in the myocardium, disrupting mitochondrial structure and function (Adepu et al., 2024). Mitochondrial dysfunction caused by ectopic lipid accumulation in obese conditions may lead to decreased myoglobin levels because myoglobin expression is closely related to mitochondrial oxidative capacity (Jiménez-González et al., 2020). In accordance with this mechanism, Ballal et al. (2010) reported a significant decrease in the expression of myocyte enhancing factor 2C (MEF2C) and its downstream target, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) in a rat model of obesity induced by a high-fat/Western diet. MEF2C is known to directly activate myoglobin gene transcription through calcineurin-dependent dephosphorylation and subsequent binding to an A/T-rich regulatory element within the myoglobin promoter (Wu et al., 2000). A regulatory pathway also implicated in explaining variations in myoglobin mRNA expression in human skeletal muscle (Jacobs et al., 2023). Although this mechanism has been primarily characterized in skeletal muscle, MEF2C is also a key transcriptional regulator in the myocardium. MEF2 is part of a gene network that controls transcription factors driving cardiac gene expression (Desjardins & Naya, 2016). On top of that, MEF2C specifically needs to be working inside heart muscle cells (Materna et al., 2019). This study suggesting that a similar regulatory relationship may exist regarding cardiac myoglobin expression. However, this specific relationship has not yet been directly investigated in models of cardiac obesity and requires further study.

Adding to this, excessive production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) by adipose tissue in obesity induces cardiomyocyte dysfunction through activation of the nuclear factor kappa B (NF-κB) signaling pathway, which ultimately suppresses the expression of genes involved in oxidative metabolism, including myoglobin (Kirichenko et al., 2022). Collectively, lipotoxicity, mitochondrial oxidative stress, transcriptional suppression, and inflammatory signaling provide a plausible explanation for the significantly lower myoglobin levels observed in the obese group of the present study.

Comparison of the Effects of *Moringa oleifera* Fruit Extract Administration and the Obese Control on Cardiac Myoglobin Levels in Obese Rats

Both once-daily (0.0917 ± 0.0007) and twice-daily (0.0933 ± 0.0006) administration of *Moringa oleifera* fruit extract significantly increased myoglobin levels compared with the obese group ($p = 0.000$), restoring them to values comparable with the normal group ($p = 1.000$). This restorative effect can be explained by the rich antioxidant composition of *Moringa oleifera* fruit, which contain more than 111 bioactive compounds, predominantly polyphenols such as the flavonoids quercetin and kaempferol, along with phenolic compounds including gallic acid, chlorogenic acid, and ferulic acid (Xie et al., 2024).These compounds alleviate oxidative stress by targeting reactive oxygen species (ROS) and inhibiting NADPH oxidase and other ROS-generating pathways (Chiș et al., 2024). Mechanistically, the antioxidant properties of quercetin and kaempferol can be attributed to their aromatic rings with hydroxyl (–OH) groups. These groups are capable of donating electrons to neutralize reactive species of ROS such as $O_2\bullet^-$ and •OH. The electron donation of the unstable oxidants to the more stable chemically paired forms effectively reduces their potential to inflict damage on cellular structures (Zhang et al., 2023).

This antioxidant activity targets one of the main mechanisms underlying the oxidative stress-induced depletion of myoglobin. Excessive ROS can oxidize the heme's ferrous iron (Fe^{2+}) in myoglobin to the oxidized form of iron (Fe^{3+}), resulting in metmyoglobin and cellular damage of the globin protein, which promotes the leakage of myoglobin from cardiomyocytes to the bloodstream (Jiang et al., 2025).The ROS-scavenging activity of quercetin and kaempferol may explain why the treatment groups have higher myoglobin levels compared to the obese group. There is a potential of preserving the myoglobin by binding to the ROS before it attacks the myoglobin and helps it remain in the cardiomyocytes.

Excess reactive oxygen species (ROS) oxidize ferrous iron (Fe^{2+}) into ferric (Fe^{3+}) forming metmyoglobin. This compound continues to degrade into ferryl-myoglobin and globin proteins that cross-link and become damaged and disarrayed (Richards, 2013). This oxidation of iron occurs via a Fenton-type reaction, where heme-bound iron reacts with ROS such as hydrogen peroxide (Chaudhary & Janmeda, 2025). Myoglobin is a small protein and so is easily released into the blood from damaged myocardium. The release and appearance of myoglobin occurs within 2 hours while peak levels occur 4-12 hours post-injury (Duan et al., 2023). The ROS-scavenging antioxidants of *Moringa oleifera* fruit, specifically quercetin and kaempferol which display strong activity as free radical scavenging antioxidants

(Xie et al., 2024), could help sustain myoglobin's structural integrity and help retain it in cardiac muscle.

Besides direct scavenging of ROS, *Moringa oleifera* fruit can also boost the antioxidant system in the body. *Moringa oleifera* fruit extract can elevate the expression of Nrf2 and HO-1. This elevation can enhance glutathione redox balance and augment the activity of SOD, CAT, and GPx (Duranti et al., 2021). It is thought that the Nrf2 pathway is activated by *Moringa oleifera* fruit extract to protect cardiomyocytes from oxidative stress and damage and help to preserve cardiac myoglobin.

Moringa oleifera fruit likely protects myoglobin by reducing myocyte apoptosis. Quercetin, one of the bioactive compounds, crosses the blood brain barrier and inhibits NF-kB by suppressing IkB kinase beta (IKKβ), and possibly activates the PI3K/Akt pathway (Anjum et al., 2025). Both of these phenomena can upregulate the anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein, thus inactivating the pro-apoptotic Bad protein, improving cardiomyocyte apoptosis and preserving the integrity of the mitochondrial membranes (Alia et al., 2022). This also helps maintain the cardiac myoglobin (Mb) reserves and protects it from leaking into the circulation.

Comparison of *Moringa oleifera* Fruit Extract and Vitamin C on Cardiac Myoglobin Levels in Obese Rats

Moringa oleifera fruit extract exhibits a statistically similar effect on myoglobin recovery as vitamin C, the positive control (p = 1,000). This may be because Moringa oleifera fruit contains vitamin C in quantities of around 3.96-8.27 mg/100 g. However, the concentrations are dependent on the plant part and how it is cultivated (Klimek-Szczykutowicz et al., 2024). Being a strong water-soluble antioxidant, vitamin C can eliminate reactive oxygen species (ROS) and reduce lipid peroxidation to help protect cardiomyocytes from stress-induced damage (Zheng et al., 2024).

This likely indicates that the fruit of *Moringa oleifera* has more than one cardioprotective mechanism. *Moringa oleifera* has over one hundred identified bioactive compounds in different parts of the plant (Pareek et al., 2023). These potentially include alkaloids, flavonoids, anthraquinones, glycosides, terpenes and vitamins. Moringa oleifera fruit extract likely has a synergistic effect on cardiomyocyte protection and cell membrane damage from combined biocompounds, including vitamin C, which may also suppress ROS formation. This may explain why *Moringa oleifera* fruit has comparable effects to the positive control, vitamin C.

Comparison of Once-Daily and Twice-Daily Administration of *Moringa oleifera* Fruit Extract (500 mg/kg Body Weight) on Cardiac Myoglobin Levels in Obese Rats

This study showed that 500 mg/kgBW/d of *Moringa oleifera* fruit extract maximally increased myoglobin levels. Doubling the frequency to two times a day did not have a significant effect (p = 1.000). It showed a dose response, where the 200–800 mg/kgBW frequency plateau, where no increase in dosage or frequency of the extract had a therapeutic effect (Louisa et al., 2022).

This plateau effect can be explained by the concept of Nweke (2022). Nweke (2022). stated that it is biological plasticity, where cells have the capacity to adjust to a range of bioactive compound exposures relative to the dosage. Phytochemicals at a certain range encourage and stimulate the defense systems and strengthen the antioxidant levels and provide protection against oxidative stress to the cells. However, if it is greater than the cells' defense and protection, then the response is diminished because the biological response has already been outsaturated and the stimulus is decreasing over time (Nweke, 2022).

Calabrese & Kozumbo (2021) describe an example of Nrf2-mediated hormesis. In this example, Nrf2 serves as the main controller of the cellular antioxidant defense system. When mild stress is administered to cells, or bioactive compounds are given to cells in optimal dosage, Nrf2 is activated, and the expression of many stress-related genes is upregulated. The cells become more resistant to the stress. This response is called the hormetic response and is limited by biology. With the Nrf2 pathway, the response is mild and does not increase steadily with increasing dosage of the compounds (Calabrese & Kozumbo, 2021).

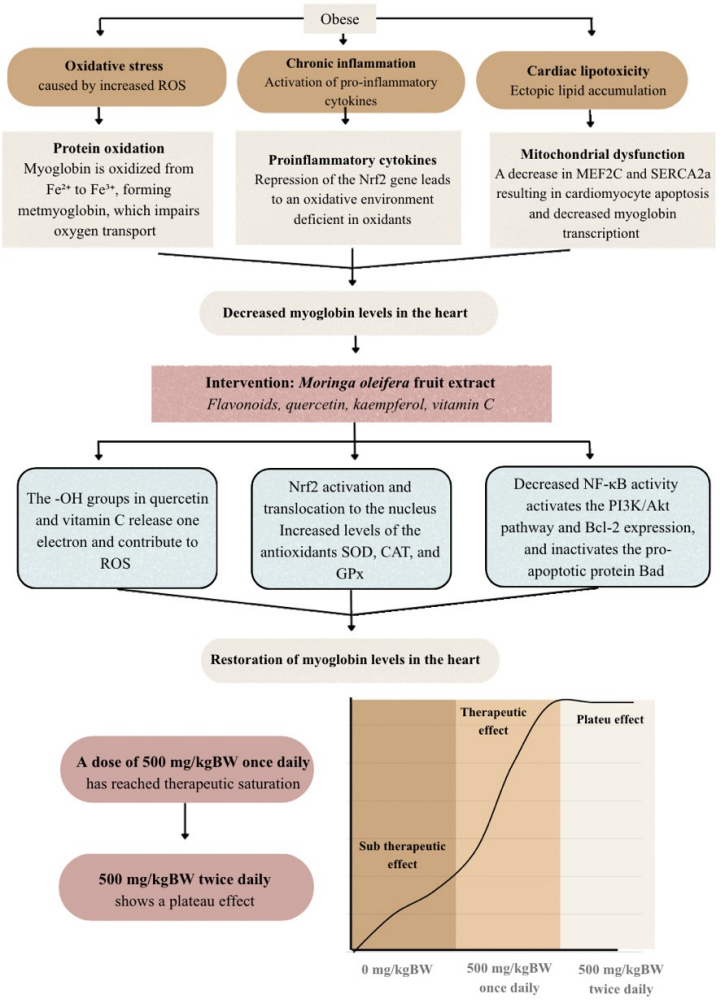


Figure 2. *Moringa oleifera* Fruit Extract and Its Predicted Mechanism of Action on Myoglobin Levels in the Hearts of Obese Rats.

CONCLUSIONS

The results of this research show that obesity can decrease levels of cardiac myoglobin. It was shown that *Moringa oleifera* fruit extract can increase the levels of cardiac myoglobin to values that are equivalent to the normal and vitamin C as the positive control groups. A 500 mg/kg body weight dose administered once a day had a restorative effect, and no additional effect was observed for a dose that was administered two times a day. The findings of this research suggest that *Moringa oleifera* fruit extract can serve as a natural cardioprotective agent when used as complementary therapy. Moreover, this research shows that the reuse of archived tissue samples can be used for biomarker research and shows a more efficient and sustainable research design in the future.

Acknowledgements: The authors extend their gratitude to the Faculty of Medicine of the Universitas Pembangunan Nasional "Veteran" Jakarta for providing the facilities, institutional support, and opportunity to conduct this study.

Authors' Contributions: The conceptualization and design of the study were carried out by Nadhira Yulianajmi Chairunnisa and Tiwuk Susantiningsih. Archived biological specimens for this study were made available Tiwuk Susantiningsih, M. Biomed., SP.KKLP, who also provided supervision for the study. Nadhira Yulianajmi Chairunnisa was responsible for the laboratory study, the data analysis, and preparation of the first draft of the study. Scientific inputs were provided by Yosha Putri Wahyuni and Kristina Simanjuntak, who also reviewed the manuscript and provided important input for subsequent revisions. All authors of the study provided their approval for the final draft.

Competing Interests: All of the authors declare that there is no competing interest.

Funding: This research forms part of a broader study conducted under the supervision of Dr. dr. Tiwuk Susantiningsih, M.Biomed., Sp.KKLP. Funding for this

activity was provided by the BIMA Kementerian Pendidikan Tinggi, Sains, dan Teknologi (Kemdiktisaintek).

REFERENCES

Adepu, K. K., Anishkin, A., Adams, S. H., & Chintapalli, S. V. (2024). A Versatile Delivery Vehicle For Cellular Oxygen And Fuels Or Metabolic Sensor? A Review And Perspective On The Functions Of Myoglobin. Physiological reviews, 104(4), 1611–1642. https://doi.org/10.1152/physrev.00031.2023

Alia, F., Putri, M., Anggraeni, N., & Syamsunarno, M. R. A. A. (2022). The potency of *Moringa oleifera* Lam. as protective agent in cardiac damage and vascular dysfunction. *Frontiers in Pharmacology*, 12, 724439. https://doi.org/10.3389/fphar.2021.724439

Anjum, F., Touqeer, S., Jamil, Q., Rida, A., Haji, E. M., Abdullah Alnasser, S. M., Almutairy, A. F., Alghamdi, H., Ahmad, A., & Iqbal, S. M. (2025). Cardioprotective effects of Bassia indica via NF-κB and BCL-2/BAX modulation in isoproterenol-induced myocardial injury. *Frontiers in Pharmacology*, 16, 1628715. https://doi.org/10.3389/fphar.2025.1628715

Arif, A., Susantiningsih, T., Wahyuningsih, S., Kristanti, M., & Zulfa, F. (2026). The Effect of *Moringa oleifera* Fruits Extract in Lowering Triglycerides Levels in Obesity. *Biology, Medicine, & Natural Product Chemistry, 15*(1), 623-629. doi:https://doi.org/10.14421/biomedich.2026.151.623-629

Azkona, G. (2023). Implementing the 3Rs in laboratory animal research—From theory to practice. *Animals*, 13(19), 3063. https://doi.org/10.3390/ani13193063

Ballal, K., Wilson, C. R., Harmancey, R., & Taegtmeyer, H. (2010). Obesogenic high fat western diet induces oxidative stress and apoptosis in rat heart. *Molecular and Cellular Biochemistry*, 344(1–2), 221–230. https://doi.org/10.1007/s11010-010-0546-y

Calabrese, E. J., & Kozumbo, W. J. (2021). The hormetic dose-response mechanism: Nrf2 activation. *Pharmacological Research*, 167, 105526. https://doi.org/10.1016/j.phrs.2021.105526

Chaudhary, P., & Janmeda, P. (2025). Major heme proteins hemoglobin and myoglobin with respect to their roles in oxidative stress – a brief review. *Frontiers in Chemistry*, *13*, 1543455. https://doi.org/10.3389/fchem.2025.1543455

Chiș, A., Noubissi, P. A., Pop, O.-L., Mureșan, C. I., Fokam Tagne, M. A., Kamgang, R., Fodor, A., Sitar-Tăut, A.-V., Cozma, A., Orășan, O. H., Hegheș, S. C., Vulturar, R., & Suharoschi, R. (2024). Bioactive compounds in *Moringa oleifera*: Mechanisms of action, focus on their anti-inflammatory properties. *Plants*, 13(1), 20. https://doi.org/10.3390/plants13010020

Desjardins, C. A., & Naya, F. J. (2016). The function of the MEF2 family of transcription factors in cardiac development, cardiogenomics, and direct reprogramming. *Journal of Cardiovascular Development and Disease*, *3*(3), 26. https://doi.org/10.3390/jcdd3030026

Duan, D., Fan, T., Zhang, L., Li, L., Wang, H., Guo, M., & Feng, Y. (2023). The correlation between cardiac oxidative stress and inflammatory cytokine response following myocardial infarction. *International Journal of Immunopathology and Pharmacology*. https://doi.org/10.1177/10760296231211907

Duranti, G., Maldini, M., Crognale, D., et al. (2021). *Moringa oleifera* leaf extract upregulates Nrf2/HO-1 expression and ameliorates redox status in C2C12 skeletal muscle cells. *Molecules*, 26(16), 5041. https://doi.org/10.3390/molecules26165041

Hayashida, T., Kuroko, Y., Shimizu, S., Akiyama, T., Suezawa, T., Kioka, Y., Kotani, Y., Shishido, T., & Kasahara, S. (2024). Effects of dapagliflozin on myoglobin efflux from cardiomyocyte during myocardial ischemia/reperfusion in anesthetized rats. *Scientific Reports*, 14(1), 16337. https://doi.org/10.1038/s41598-024-67195-3

Jacobs, N., Mos, D., Bloemers, F. W., van der Laarse, W. J., Jaspers, R. T., & van der Zwaard, S. (2023). Low myoglobin concentration in skeletal muscle of elite cyclists is associated with low mRNA expression levels. *European Journal of Applied Physiology, 123*(7), 1469–1478. https://doi.org/10.1007/s00421-023-05161-z

Jiménez-González, S., Marín-Royo, G., Jurado-López, R., Bartolomé, M. V., Romero-Miranda, A., Luaces, M., Islas, F., Nieto, M. L., Martínez-Martínez, E., & Cachofeiro, V. (2020). The crosstalk between cardiac lipotoxicity and mitochondrial oxidative stress in the cardiac alterations in diet-induced obesity in rats. *Cells*, 9(2), 451. https://doi.org/10.3390/cells9020451

Kirichenko, T. V., Markina, Y. V., Sukhorukov, V. N., Khotina, V. A., Karnaukhova, A. A., & Orekhov, A. N. (2022). The role of adipokines in inflammatory mechanisms of obesity. *International Journal of Molecular Sciences*, 23(23), 14982. https://doi.org/10.3390/ijms232314982

Klimek-Szczykutowicz, M., Gaweł-Bęben, K., Rutka, A., Blicharska, E., Tatarczak-Michalewska, M., Kulik-Siarek, K., Kukula-Koch, W., Malinowska, M. A., & Szopa, A. (2024). *Moringa oleifera* (drumstick tree)—nutraceutical, cosmetological and medicinal importance: A review. *Frontiers in Pharmacology*, 15, 1288382. https://doi.org/10.3389/fphar.2024.1288382

Liang, X., Xu, W., Chen, L., Liu, X., Hong, W., & Hu, X. (2025). Quality assessment from biobank plasma and serum specimens: A systematic review. *PeerJ*, 13, e20122. https://doi.org/10.7717/peerj.20122

Liśkiewicz, A. D., Marczak, Ł., Bogus, K., Liśkiewicz, D., Przybyła, M., & Lewin-Kowalik, J. (2021). Proteomic and structural manifestations of cardiomyopathy in rat models of obesity and weight loss. *Frontiers in Endocrinology*, 12, 568197. https://doi.org/10.3389/fendo.2021.568197

Louisa, M., Patintingan, C. G. H., & Wardhani, B. W. K. (2022). *Moringa oleifera* Lam. in cardiometabolic disorders: A systematic review of recent studies and possible mechanism of actions. *Frontiers in Pharmacology*, 13, 792794. https://doi.org/10.3389/fphar.2022.792794

Materna, S. C., Sinha, T., Barnes, R. M., Lammerts van Bueren, K., & Black, B. L. (2019). Cardiovascular development and survival require Mef2c function in the myocardial but not the endothelial lineage. *Developmental Biology*, *445*(2), 170–177. https://doi.org/10.1016/j.ydbio.2018.12.002

Nweke, C. O., Nwangwu, O. R., Okechi, R. N., Araka, N. N., & Ogbonna, C. J. (2022). Statistical modeling of hormesis quantities in inverted U-shaped dose-response relationships by reparameterization of a bilogistic model. *Journal of Environmental Science and Health, Part A*, 57(12), 1003–1023. https://doi.org/10.1080/10934529.2022.2138056

Pareek, A., Pant, M., Gupta, M. M., Kashania, P., Ratan, Y., Jain, V., Pareek, A., & Chuturgoon, A. A. (2023). *Moringa oleifera*: An updated comprehensive review of its pharmacological

activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *International Journal of Molecular Sciences*, 24(3), 2098. https://doi.org/10.3390/ijms24032098

Ren, J., Wu, N. N., Wang, S., Sowers, J. R., & Zhang, Y. (2021). Obesity cardiomyopathy: Evidence, mechanisms, and therapeutic implications. *Physiological Reviews*, 101(4), 1745–1807. https://doi.org/10.1152/physrev.00030.2020

Richards, M. P. (2013). Redox reactions of myoglobin. *Antioxidants & Redox Signaling*, *18*(17), 2342–2351.

Thadeus, M. S., Susantiningsih, T., Muktamiroh, H., Fauziah, C., Citrawati, M., Irmarahayu, A., Wahyuningsih, S., Harjono Hadiwiardjo, Y., Yusmaini, H., Bahar, M., Zulfa, F., Agustini, D., & Chairani, A. (2024). *Moringa oleifera* fruit extract as a potential antioxidant against liver injury by 2-nitropropane induction in obese male mice model: Pre-clinical study. *F1000Research*, 12, 300. https://doi.org/10.12688/f1000research.121695.2

World Health Organization. (2025). *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

World Obesity Federation. (2025). *World obesity atlas 2025: Overweight, obesity and non-communicable diseases – New global, regional and national estimates of the prevalence of overweight and obesity in adults from 2000 to 2030*. World Obesity Federation.

Wu, H., Naya, F. J., McKinsey, T. A., Mercer, B., Shelton, J. M., Chin, E. R., Simard, A. R., Michel, R. N., Bassel-Duby, R., Olson, E. N., & Williams, R. S. (2000). MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *The EMBO Journal, 19*(9), 1963–1973. https://doi.org/10.1093/emboj/19.9.1963

Xie, R., Ponnampalam, E. N., Ahmadi, F., Dunshea, F. R., & Suleria, H. A. R. (2024). Antioxidant potential and characterization of polyphenol compounds in *Moringa oleifera* pods. *Food Science & Nutrition*, 12(12), 10881–10902. https://doi.org/10.1002/fsn3.4628

Zhang, W., Zheng, Y., Yan, F., Dong, M., & Ren, Y. (2023). Research progress of quercetin in cardiovascular disease. *Frontiers in Cardiovascular Medicine*, 10, 1203713. https://doi.org/10.3389/fcvm.2023.1203713

Zheng, H., Xu, Y., Liehn, E. A., & Rusu, M. (2024). Vitamin C as scavenger of reactive oxygen species during healing after myocardial infarction. *International Journal of Molecular Sciences*, 25(6), 3114. https://doi.org/10.3390/ijms25063114

THIS PAGE INTENTIONALLY LEFT BLANK