

Research article

Potential Of *Salvia Hispanica* On Myocardial Histopathology In Overtraining

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Abstract: Overtraining can cause oxidative stress and damage heart muscle cells. Chia seeds have potential as an antioxidant to overcome overtraining. The objectives of this study were to determine the potential of chia seed extract (*Salvia Hispanica* L.) to prevent myocardial muscle damage, as indicated by histopathological features in an overtraining rat model. This study used a true experimental design with a post-test only control group design. The sample consisted of 15 rats, randomly divided into three groups. The control group was fed standard feed, while group I was overtrained with swimming exercises. Group II was overtrained and given chia seed extract at a dose of 4.5 mg/kgBW. Analysis was performed on myocardial histopathology. Data processing used descriptive statistics and ANOVA with a significance level of 0.05. This study showed that chia seed extract could potentially interfere with myocardial muscle thickness in the control group (171.56±0.5), group II (209.53±1.4), and group III (188.86±0.8). ANOVA results obtained a P value of 0.000, and the results of the LSD post hoc test between groups obtained a P value of 0.000 (<0.05). Administration of chia seed extract significantly reduced myocardial muscle thickness.

Keywords: chia seeds, myocard, overtraining

1. Introduction

Regular exercise provides health benefits. However, unconventional or excessively strenuous exercise can lead to adverse health effects such as muscle damage, inflammation, and oxidative stress. Specifically, repeated muscle contractions involve the accumulation of reactive oxygen species (ROS)(1). The World Health Organization (WHO), the American Heart Association (AHA), the American College of Sports Medicine (ACSM), and the United Kingdom Department of Health recommend 150 minutes of moderate-intensity aerobic exercise per week for health-promoting benefits, or 30 minutes per day, five days a week(2).

Overtraining syndrome is detrimental to the health of athletes, and its negative effects can also damage careers(3). Athletes experiencing overtraining syndrome will not reach their previous performance levels(4). High-intensity exercisers produce large amounts of free radicals. Continuous physical exercise to the point of fatigue can induce oxidative stress in skeletal muscle(5). During overtraining, the release of reactive oxygen species (ROS) exceeds the protective capacity of the endogenous antioxidant system and causes dysregulation in the inflammatory, oxidative phosphorylation, and neuroendocrine systems. Several studies have shown that increasing exercise intensity can increase oxidative stress and free radical production in cells(6). Several studies in

experimental animals have shown changes in overtraining, including increased heart rate and decreased stroke volume at the end of the overtraining period(7). The physiological function of the kidney has also been demonstrated through animal studies that undergo pathological changes in the form of proteinuria and acute kidney injury. Acute exhaustive exercise is defined as short-term exercise with an intensity exceeding the body's recovery capacity(8). Besides affecting athletes, overtraining also causes muscle damage, typically occurring in individuals who rarely exercise and subsequently experience training overload(9).

In relation to overtraining, several biomarkers are suspected of indicating oxidative stress, inflammation, proliferation, infarction, and other processes that may occur in the heart during overtraining(10). Animal studies began with cases of sudden cardiac death in athletes, the cause of which, to date, has been attributed solely to hypertrophic cardiomyopathy.

Hypertrophic cardiomyopathy is a congenital disorder that, through autopsy results in several cases of sudden cardiac death, has been concluded as the cause of death in athletes. The phenomenon of sudden cardiac death in athletes is another aspect of sports that has not been well documented. Sudden cardiac death cases are stated as the main cause of death due to medical conditions in cases of athlete deaths. Several studies describe the incidence of sudden cardiac death. The limitations of these studies are stated that the incidence of sudden cardiac death in athletes worldwide is not well documented because the study sample age group is very wide (ages 8-40 years) and underestimates the actual number of cases because the data sources obtained are limited to media reports, insurance claims reports, and records of sudden cardiac death cases that have been autopsied only. The best estimate of the incidence of sudden cardiac death in athletes in the United States varies widely, ranging from 1 in 23,000 to 1 in 300,000 athletes annually. In Italy, the incidence of sudden cardiac death is obtained from autopsy reports of athlete deaths. The incidence rate of sudden cardiac death in young athletes in Italy was reported to be 2.3 - 4.4/100,000 young athletes per year(11).

The use of herbal supplements by athletes has increased significantly over the past decade. Herbal products are extracted from seeds, sap, roots, leaves, bark, fruit, or flowers and contain various phytochemical compounds such as carotenoids and polyphenols, including phenolic acids, alkaloids, flavonoids, glycosides, saponins, and lignans, believed to provide health benefits. The use of herbal products is regulated by the Food and Drug Administration (BPOM) as a special category of food and classified as a "dietary supplement" under the Dietary Supplement Health and Education Act (DSHEA) of 2024(12).

One food ingredient used as a source of energy and nutrients in formulating sports drinks is chia seeds (*Salvia hispanica* L.). Chia seeds are slightly oval in shape, typically black, gray, or brown in color with small white spots. In water, chia seeds can swell and produce a clear, white slime. Chia seeds are also often used as a component in the manufacture of several food products and function as a thickener, emulsifier, or stabilizer(13).

Chia seeds are high in fiber and protein, gluten-free, rich in omega-3 fatty acids, rich in vitamins and minerals, and contain antioxidants (polyphenols, tocopherols, and isoflavones)(14). Adding chia seeds as a component to sports drinks can increase energy content and reduce the amount of fluid in sports drinks, as athletes are expected to consume high-calorie drinks with little fluid. Athletes engaging in high-intensity endurance training are advised to limit excessive fluid consumption during exercise, as this can cause digestive discomfort and lead to oxidative stress(Bermejo & Munné-Bosch, 2023). Increasing the consumption of natural antioxidants found in fruits, vegetables, flowers, and other plant parts can prevent degenerative diseases. Micronutrients in fruits, vegetables, and other plants, such as vitamins A, C, and E, folic acid, anthocyanins, phenolic compounds, and flavonoids, can be used as substitutes for synthetic antioxidants. This can be recommended for consumption by athletes(15).

Chia seeds are a readily available food rich in antioxidants. This plant originates from Central America, specifically Mexico and Guatemala. Chia seeds contain protein (15–25 percent), fat (30–33 percent), carbohydrates (26–41 percent), fiber (18–30 percent), and minerals (4–5 percent). Chia seeds also contain 17.83 percent omega-3 fatty acids (linolenic acid)(16). Chia seeds also contain vitamin C, thiamin, riboflavin, and vitamin A, as well as phenolic acid components (myricetin, quercetin, kaempferol, and caffeic acid). These compounds are primary and synergistic antioxidants that contribute to the high antioxidant activity of chia seeds(17). Analysis at the Udayana University Food Laboratory showed that methanol extract of chia seeds contained 18.20 mg/100g of flavonoids with an antioxidant capacity of 8307.98 mg/L GAEAC.

A study conducted by Vuksan in Canada on the effect of chia seeds on oxidative stress concluded that administering chia seed extract at a dose of 36g/1000 calories for 6 months can reduce CRP levels by 1.1 ± 0.5 mg/L in rats with diabetes mellitus(18). Furthermore, another study conducted by Trisnadi concluded that administering chia seed extract at a dose of 13.5 mg/kgBW for 7 days can prevent the effects of oxidative stress and reduce TNF α and IL-6 levels in mice with a high cholesterol diet. The author conducted preliminary research by testing the antioxidant content of chia seed extract at 87, using the IC50 method, which indicated a strong antioxidant of 50-100(19).

Another study conducted by Marinelli in Spain on the benefits of chia seeds concluded that administering chia seed extract at a dose of 133 grams/day for 6-12 weeks in 36 obese mice resulted in reduced oxidative stress and lipid levels(20). Furthermore, research in Spain showed that chia seeds extracted into chia oil can significantly lower blood glucose levels(13).

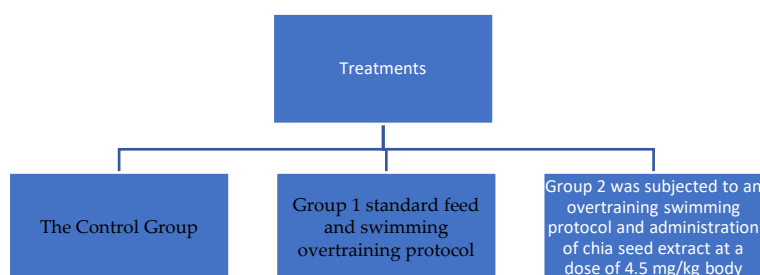
2. Materials and Methods

This research is a true experimental study using a post-test control group design. The Biology and Chemistry Laboratory Research Center, IBL FK Unissula The research period was carried out for 3 months from May 2024 to July 2024. The replacement of chia seeds was independently at a dose of 4.5 mg/kg body weight using the maceration method, the replacement depended on. The number of groups in this study was 3 groups, group 1 was the control group, groups 1 and 2 were groups that received chia seeds and received overtraining in each group, there was a limit of 5 remaining and were selected based on that. exclusion and inclusion criteria. A sample of 15 rats was reconditioned for one week before treatment. All eligible rats will be included in the research sample. A total of 15 Wistar rats were recruited and randomly divided into three groups, each group containing five rats. Group 1 was the control group, group 2 was the treatment group with 4.5 mg/kg body weight of chia seeds after overtraining, and group 3 was only given overtraining treatment. This research has received approval from the Bioethics Commission of the Faculty of Medicine, Unissula, with Ethics Permit Number 219/VI/2024/Bioethics Commission. Find the method:

1. This study utilized male Wistar rats, eight weeks of age, weighing between 200 and 250 grams. The test animals were required to be healthy and exhibit normal activity levels. Initially, the animals underwent a seven-day acclimatization period to allow them to adapt to the laboratory environment; during this time, they were provided with standard feed and distilled water. Fifteen white Wistar rats (*Rattus norvegicus*) aged 3-4 months.
2. The rats were acclimatized for approximately one week and fed standard HBS pellets ad libitum.
3. The rats were housed in cages according to research requirements: 30 x 20 x 20 cm plastic cages lined with sand and insulating wire. The holes were placed in an air-conditioned room with natural ventilation. Six boundaries were marked at each point.
4. Chia seed extraction process: The ethanol extraction process of chia seeds was carried out in the chemistry laboratory of the Faculty of Medicine, UNISSULA Semarang. The crude drug was dried in a 400°C oven for 7 hours, resulting in 1,500

grams of crushed and processed chia seeds. The extract was then macerated in 7.5 liters (1:5) of 70% ethanol, stirring with an agitator for 1 hour, and allowed to stand for 24 hours.

5. Overtraining: The experimental animals were subjected to swimming in a 100 x 80 x 80 aquarium with partitions. The aquarium water temperature was set at 36°C, and the swimming mice showed signs of fatigue, such as sinking and increasingly weak movements. This swimming activity began at 9:00 AM to 12:00 PM daily for 28 days. Signs that a test animal is experiencing overtraining include it sinking and attempting to surface to take a breath.
6. Rats were randomly assigned to treatment and control groups using a simple randomization method. Blinding was implemented during the staining and microscopic examination of histopathology slides, ensuring the evaluator was unaware of the group identity of the tissue slides being examined.
7. Treatments: The Control Group and Group 1 were provided exclusively with standard feed and swimming overtraining protocol. Group 2 was subjected to an overtraining swimming protocol (daily from 9:00 AM to 12:00 PM) for 28 days, followed by the administration of chia seed extract at a dose of 4.5 mg/kg body weight for 7 days.



8. After treatment, histopathology slides were prepared as a post-test.
9. This study implemented humane endpoints, whereby animals were euthanized prematurely if they exhibited >20% weight loss, severe respiratory distress, or an inability to move. The animals were continuously monitored during the prolonged swimming protocol. At the end of the treatment period, the animals were euthanized under intraperitoneal anesthesia using a combination of Ketamine (80 mg/kg body weight) and Xylazine (10 mg/kg body weight) to minimize pain, followed by cervical dislocation prior to the excision of the heart for histological analysis.
10. Histology slides were prepared by making sections of the heart muscle using a microtome.
11. The slides were then stained with Hematoxylin-Eosin (HE) to facilitate observation using a light microscope at 40x magnification to assess Myocardial Thickness
12. The slides were stained with Hematoxylin-Eosin (HE) and mounted with Permount:
 - Xylol I for 5 minutes
 - Xylol II for 5 minutes
 - Xylol III for 5 minutes
 - 100% Alcohol I for 5 minutes
 - 100% Alcohol II for 5 minutes
 - Several dips of distilled water, Harris-Hematoxylin for 15 minutes
 - Dips of distilled water for 1 minute, dipping up and down
 - 1% acid alcohol for 5-7 dips, making sure the color is not pale
 - Dips of distilled water for 1 minute
 - Dips of distilled water for 15 minutes

- Eosin for 2 minutes
 - Alcohol 96% Alcohol I for 3 minutes
 - 96% Alcohol II for 3 minutes
 - 100% Alcohol I for 3 minutes
 - 100% Alcohol II for 3 minutes
 - Xylol IV for 5 minutes
 - Xylol V for 5 minutes.
 - Measurement of necrosis in the slides is the visible necrosis image from five fields of view in one slide under a microscope.
13. After the slides were prepared, the mice were euthanized. Average data are presented in tables or graphs.
14. The study examined the anatomical structure of myocardial muscle thickness in the experimental animals, comparing muscle width between the overtraining and chia seed extract groups. Markers for fibrosis and necrosis were not assessed in this study. The anatomical pathology examination adhered to standard operating procedures, utilizing a single section in accordance with INHAND guidelines, which recommend one transverse (cross-sectional) slice taken from the mid-ventricular region. This section is typically cut at the level of the papillary muscles, as this area provides a view of the left ventricular (LV) wall thickness, right ventricular (RV) wall thickness, and the interventricular septum.

The data were tested using the IBM SPSS Statistics program version 21.0 on a Windows device. The data were tested with the Kolmogorov-Smirnoff test to demonstrate normality and the Levene test to demonstrate homogeneity. If the results are normal in one group, we continue with the one-way ANOVA test. If the result of the one-way ANOVA test is $p < 0.05$, this means there is a difference from one group to another and the other, followed by Post Hoc. Try the LSD test. If the data is not normal in one way or another, the one-way ANOVA test does not meet the requirements and the data are tested using the Kruskal Wallis test (non-parametric test) if the result of the Kruskal Wallis test is $p < 0.05$, it means there is a difference between the groups and to see the difference they are tested with a different test Mann Whitney.

3. Results

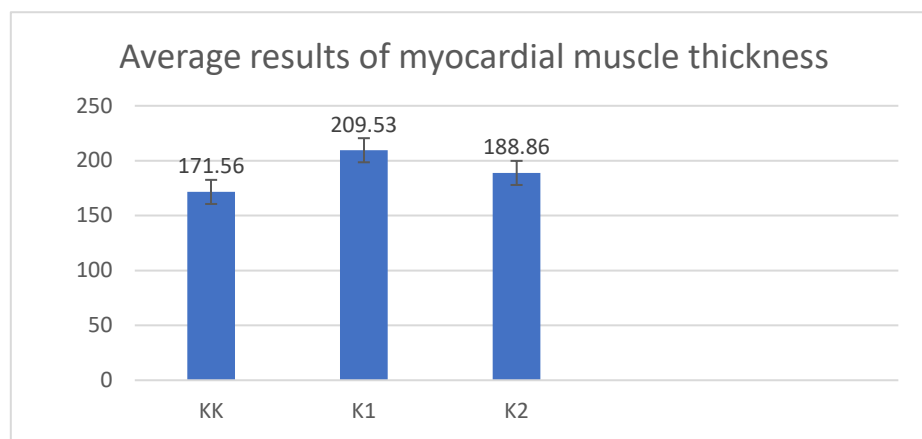
This study was conducted for one month at the Integrated Biomedical Laboratory of the Faculty of Medicine, University of Islamic Sultan Agung Semarang. No rats died during the treatment, and all met the inclusion criteria. No rats dropped out until the end of the study. Then, on day 29, myocardial histopathology was performed on male Wistar rats that had been overtrained. The following descriptive data for all groups is presented in Table 1.

Table 1. Analysis of Mean Myocardial Thickness

Variable Group	Group Control N=5 Mean±SD	Group 1 N=5 Mean±SD	Group 2 N=5 Mean±SD	Sig.(p)
Myocardial thickness (µm)	171,56±0,5	209,53±1,4	188,86±0,8	
<i>Shapiro wilk</i>	0,906*	0,956*	0,704*	
<i>Levene test</i>				0.330**
<i>Anova</i>				0,000***
	Description:			
	Normal			*
	Homogen			**
	There is a significant difference between groups			***
				>0,05
				>0,05
				<0,05

The results of the research data on the lowest myocardial muscle thickness were in group control, then followed by group 2, and group 1. Group 1 had the highest average. The control group had an average result of 171.56. Group 1 had the highest average with a myocardial muscle thickness of 209.53. Group 2 had an average result of myocardial muscle thickness of 188.86. The results of the average myocardial muscle thickness can be seen below.

Figure 1. Average Thickness of Myocardial Muscle



Hypothesis testing in this study was conducted using ANOVA. Hypothesis testing was tailored to the research hypotheses and variables as shown below.

Tabel 2. Results of ANOVA Test Analysis

Variabel	Sig. (p)	Information
Myocardial muscle thickness	0,000	There is an Influence

Based on the ANOVA test, all analyzed variables yielded $p < 0.05$, thus concluding that "at least two groups have significantly different mean myocardial histopathology." To further examine the differences in the mean of each variable between groups, a further post-hoc LSD test was conducted.

The results of the post-hoc LSD analysis of myocardial muscle thickness, based on the Shapiro-Wilk test, showed that myocardial muscle thickness was normally distributed ($p > 0.05$). The homogeneity test using the Levene test showed that the data had a homogeneous variance ($p = 0.330$). Furthermore, the hypothesis test using one-way ANOVA statistically showed $p = 0.000$ ($p < 0.05$). Because $p < 0.05$, it can be concluded that "at least two groups have significantly different mean myocardial muscle thickness." Clinically, at least two groups contribute to the reduction in myocardial muscle thickness. Furthermore, to find out which groups were different and meaningful, further tests were carried out, namely multiple comparison analysis with the LSD post hoc test.

Based on the LSD post hoc test in Table 3, it shows that there is a statistical difference in the average thickness of the myocardium muscle between group 1 and group 2 because $p = 0.000$. Clinically, there is an effect of administering chia seed extract at a dose of 4.5 mg/kgBW on reducing the thickness of the myocardium muscle in the group that was only given overtraining. In group 1 and group 3, there is a statistical difference in the average thickness of the myocardium muscle because $p = 0.000$. The significance of the difference in the average thickness of the myocardium muscle between groups in the study is shown in Table 3.

Tabel 3. Post Hoc LSD Test of Myocardial Muscle Thickness

Groups	Control	Group 1	Group 2
	Sig.(p)	Sig.(p)	Sig.(p)
Control	-	0,000	0,000
Group 1	0,000	-	0,000
Group 2	0,000	0,000	-

The following are the results of making histological preparations of heart muscle in mice.

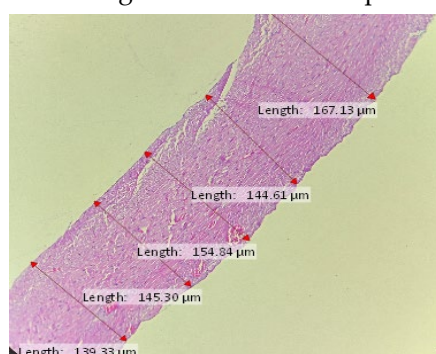
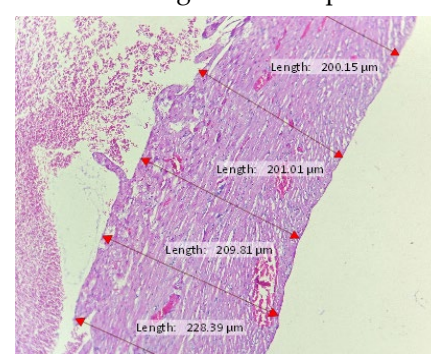
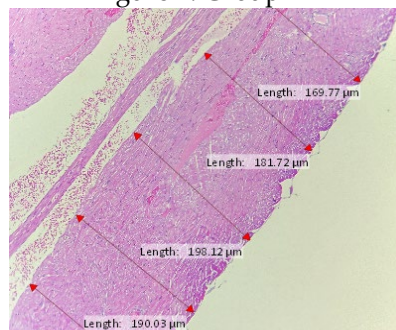
Figure 2. Control Group**A****Figure 3. Group 1****B****Figure 4. Group 2****C**

Image caption: second image : There were no obvious signs of tissue damage. There were no large areas of necrosis (tissue death), massive infiltration of inflammatory cells, or scar tissue formation (fibrosis). Third image: The most prominent finding is myocardial wall thickening. The visible measuring lines (ranging in length from approximately 200 µm to 228 µm) represent wall thickness measurements at various points. Compared with the thinner control image (approximately 140-167 µm), this thickening indicates myocardial hypertrophy, in which the heart muscle cells (cardiomyocytes) increase in size. Fourth image: Moderate Myocardial Hypertrophy: When comparing this measurement value with the control group, which ranged from ~139 to 167 µm, the heart walls in Group 2 experienced thickening. Comparison of Severity: The thickening that occurred in Group 2 was at a moderate level. The walls were thicker than the control group, but tended to be thinner when compared to Group 1 (which measured over 200 µm) all of which were the effects of the antioxidants from chia seeds.

4. Discussion

Research in the health sciences generally begins with *in vitro* studies. If research results are to be used for human benefit, further *in vivo* studies are required, such as using human blood, cell cultures, or tissue. However, to observe, study, and draw conclusions about all events in living organisms, experimental animals are needed. For example, in this study, which required myocardial tissue levels and myopathological images, ethical constraints for direct human analysis are certainly present(21). The results showed that the overtraining group had higher myocardial muscle thickness compared to the control group ($p < 0.05$).

Overtraining can increase oxidative stress because it increases blood circulation in the body to meet the needs of cells to prevent damage. Cell damage, particularly at the muscle level, is associated with the inflammatory process that occurs during tissue repair, and this inflammation can lead to further pro-oxidant production by neutrophils and macrophages. On the other hand, nitric oxide and reactive oxygen species play a crucial role in regulating immune function, influencing nearly every step in the development of inflammation(22). ROS production originates in mitochondria through the role of the oxidative enzymes NADPH (nicotinamide adenine dinucleotide phosphooxidase) and XO (xanthine oxidase). The sarcoplasmic reticulum, T-tubules, and sarcolemma all contain NADPH. The XO enzyme is found in the plasma membrane of skeletal muscle and can convert hypoxanthine to xanthine, forming superoxide. By releasing electrons, skeletal muscle can increase ROS production. Superoxide, hydrogen peroxide, hydroxyl radicals, hydroxyl ions, and nitric oxide are all reactive oxygen species (ROS)(23).

Considering left ventricular hypertrophy, an important discussion concerns its role in the body's hemodynamics. Hemodynamic function is related to the heart's ability to pump blood into the pulmonary and aortic circulations. Cardiac output is the volume of blood pumped by each ventricle per minute. Factors that determine cardiac output are the heart rate per minute and the volume of blood pumped by the heart per beat (stroke volume)(24). Stroke volume is influenced by preload, afterload, and myocardial contractility. Preload is the degree of stretching of the myocardial fibers immediately before ventricular contraction. Myocardial fiber stretching depends on the volume of blood stretching the ventricles at the end of diastolic contraction. Venous return to the heart determines the end-diastolic volume of the ventricles. Increased venous return increases the end-diastolic volume of the ventricles, which in turn increases the stretching of the myocardial fibers. The Frank-Starling mechanism states that within physiological limits, the greater the stretching of myocardial fibers at the end of diastole, the greater the force of contraction during systole. Referring to the Frank-Starling law, left ventricular hypertrophy in overtraining exercise in this study explains the failure of the stretching mechanism of myocardial muscle fibers which is thought to have implications for the force of contraction during systole(25). This study proved that overtraining can significantly increase myocardial muscle thickening ($p < 0.05$). The control group had an average myocardial muscle thickness of 171.56 per field of view. Group 1 had the highest average myocardial muscle thickness with 209.53 per field of view.

Hypertrophy causes the left ventricular wall thickness to increase, which leads to decreased muscle fiber stretch. The ventricular cavity narrows due to thickening of the myocardial wall. This mechanism explains why hypertrophy during overtraining is a pathological phenomenon that is highly detrimental and dangerous in high-intensity exercise because it can lead to decreased stroke volume. The next mechanism is explained through another determinant of stroke volume, afterload. Afterload can be defined as the tension in myocardial fibers that must be created for contraction and blood pumping(26). The results showed that the group given chia seed extract after overtraining had lower myocardial muscle thickness compared to the group given overtraining alone (K1) ($p < 0.05$).

Chia seeds contain quercetin, a powerful antioxidant that prevents the oxidation of fats, proteins, and DNA and is the most effective of the flavonoid compounds. Caffeic acid and chlorogenic acid work against free radicals by inhibiting lipid peroxidation. Flavonoids can neutralize hydroxyl (OH^*), peroxy (ROO^*), and singlet oxygen (O_2^*) free radicals produced by reactive oxygen species (Firsta Yosika et al., 2020). In this case, flavonoids work by inhibiting CETP, resulting in increased HDL cholesterol levels and decreased LDL levels (19). Flavonoids also have anti-inflammatory effects by inhibiting cytokines such as tumor necrosis factor α ($\text{TNF-}\alpha$). Reducing $\text{TNF-}\alpha$ will increase insulin sensitivity, increase fatty acid oxidation in the liver, and inhibit cholesterol synthesis by cells. In addition to containing essential fatty acids, according to Campos, chia seeds are also reported to contain phenolic compounds. These phenolic compounds are bioactive components that contribute to the health benefits of chia seeds. Among the phenolic acid components (myricetin, quercetin, kaempferol, and caffeic acid) are compounds (20). These compounds are primary and synergistic antioxidants that contribute a high proportion of the antioxidant activity of chia seeds (27). The author conducted preliminary research on the antioxidant content of chia seeds, determining it to be 85 mg/L GAEAC using the IC50 method.

Administration of chia seed extract also significantly reduced the risk of muscle tissue damage, indicated by a decrease in myocardial muscle thickness compared to the overtraining group without chia seed extract. Group 1 had the highest myocardial muscle thickness, with an average of 209.17 per field of view. Group 2 had an average of 188.86 per field of view. Group 3 had an average of 157.46 per field of view. Myocardial thickening is a manifestation of an increase in cardiomyocyte size (hypertrophy). In the early stages, this process is adaptive, serving to normalize cardiac wall stress in accordance with the Law of Laplace in response to an increased vascular pressure load. Based on fundamental literature, the primary distinction lies in the initial stimulus and reversibility. Physiological hypertrophy is an adaptation to intermittent hemodynamic workloads such as those resulting from physical exercise accompanied by adequate rest. Cardiac structures enlarge proportionally (balanced eccentric or concentric hypertrophy) without associated fibrosis. Crucially, ventricular systolic and diastolic functions remain normal or even improve, and the condition is reversible (the heart can return to its original size if training ceases). In contrast, pathological hypertrophy is triggered by chronic, constant pressure or volume overload. This response is maladaptive, inducing interstitial collagen accumulation (fibrosis) and, over time, leading to a decline in cardiac pumping function that culminates in heart failure.

The reduction in inflammation due to muscle tissue damage is due to the caffeic acid and chlorogenic acid in chia seeds, which are important antioxidants that have a protective effect against DNA damage caused by reactive oxygen species (ROS). Caffeic acid attenuates hydrogen peroxide (H_2O_2)-mediated cell viability decline and reduces the level of H_2O_2 -induced DNA double-strand breaks. Caffeic acid also activates the extracellular signal-regulated kinase signaling pathway. Extracellular RNA inhibition blocks the caffeic acid-induced increase in cell viability and the protective effect against H_2O_2 -mediated DNA damage, thus possibly having an anti-inflammatory effect (28). This inhibition can reduce inflammation and muscle wasting (29). Reports regarding the effects of caffeic and chlorogenic acids may contribute to preventing muscle protein breakdown. This reduction in myocardial thickness and degeneration is also due to Caffeic and chlorogenic acids can improve fatty acid metabolism, mitochondrial biosynthesis through increased PGC-1 γ , and suppress inflammatory cytokines, which can reduce fatigue levels and improve physical performance. Caffeic and chlorogenic acids can also increase maximal oxygen consumption and antioxidant capacity, as well as reduce inflammation and lipid peroxidation, aiding muscle cell recovery (30).

5. Conclusions

In conclusion, this study provides preliminary evidence that the administration of chia seed (*Salvia hispanica* L.) extract is associated with a lower myocardial wall thickness profile following physical intervention, compared to a group of animals subjected solely to overtraining. Given the limited sample size and a study design focused on basic morphometry, these findings cannot yet be interpreted as demonstrating a definitive cardioprotective effect. Further confirmation is essential through future research employing larger sample sizes, standardized histopathological endpoint measurements, analyses of biochemical and molecular markers (such as oxidative stress markers and inflammatory cytokines), and direct assessment of cardiac hemodynamic function (via echocardiography).

Supplementary Materials: Not applicable.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, Reza Adityas Trisnadi ; methodology, Reza Adityas Trisnadi; software, Nur Ulayatilmiladiyyah; validation, Reza Adityas Trisnadi., Setyo Trisnadi. ; formal analysis, Nur Ulayatilmiladiyyah; investigation, Reza Adityas Trisnadi; resources, Syafinda Meisari Trisnani; data curation, Syafinda Meisari Trisnani; writing—original draft preparation, Reza Adityas Trisnadi; writing—review and editing, Nur Ulayatilmiladiyyah; visualization, Syafinda Meisari Trisnani; supervision, Setyi Trisnadi; project administration, Reza Adityas Trisnadi ; funding acquisition, Reza Adityas Trisnadi.

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