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*Research article*

# The Effect Salvia Hispanica L Seed Extract on Stres Oxidatif in Rats with Overtraining

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**Abstract:** The purpose of this study was to determine: Increased oxidative stress as seen from malondialdehyde (MDA) and superoxide dismutase (SOD) levels after overtraining and the effect of chia seeds (Salvia Hispanica L) can reduce oxidative stress as seen from malondialdehyde (MDA) and superoxide dismutase (SOD) levels after overtraining. This study used a true experimental design method with a post-test only control group design. The number of samples in this study was 18 rats, then divided into 3 groups. The Control Group was only given standard feed, group I was given overtraining swimming start at 09.00 am - 12.00 noon every day for 28 days. and standard feed. Group KII was given overtraining swimming start at 09.00 am - 12.00 noon every day for 28 days and chia seed extract at a dose of 4.5 mg / kgBW. Blood serum samples were taken on the 29th day. Then the samples were examined by The Research Center of Biology and Chemistry Laboratory of IBL FK Unissula for SOD and MDA analysis. The results of this study indicate that SOD levels in the control group (4.83), group I (1.45), group II (3.11). The results of the ANOVA test were $p<0.05$. MDA levels in the control group (1.67), group I (1.91), group II (1.28) with ANOVA test results $p<0.05$. Thus overtraining can decrease SOD and increase MDA. Giving chia seed extract at a dose of 4.5 mg/kgBW can significantly overcome the decrease in SOD and increase MDA.

**Keywords:** chia seeds; oxidative stres; overtraining

## 1. Introduction

The use of herbal supplements by athletes has increased significantly over the past decade. Herbal products are extracted from seeds, sap, roots, leaves, bark, fruits, or flowers, and contain a variety of phytochemical compounds such as carotenoids and polyphenols, including phenolic acids, alkaloids, flavonoids, glycosides, saponins, and lignans that are believed to provide health benefits. The use of herbal products is regulated by the Food and Drug Administration (FDA) as a special category of food and is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (1).

One of the food ingredients used as a source of energy and nutrition in formulating sports drinks is chia seeds (*Salvia hispanica L.*). Chia seeds have a slightly oval shape, usually black, gray, or brown with small white spots. In water, chia seeds can expand and produce clear white mucus. Chia seeds are also often used as a component in the manufacture of several food products and function (2). Chia seeds have high fiber and protein content, are gluten-free, rich in omega-3 fatty acids, rich in vitamins and minerals, and contain antioxidants (polyphenols, tocopherols, and isoflavones)(3). The addition of chia seeds as a component in sports drinks can increase energy content and reduce the amount of fluid in sports drinks, because athletes are expected to consume high-calorie drinks with little fluid. Athletes who do high-intensity endurance training are restricted from consuming excessive fluids during exercise, because it can cause discomfort to the digestive system and can produce oxidative stress (4).

Oxidation-reduction (redox) reactions occur widely in biology and are required to maintain an optimal environment for cell signaling and function. These redox reactions involve reactive oxygen species (ROS) such as superoxide (O2.-) and hydrogen peroxide (H2O2) that regulate several physiological and pathophysiological processes ranging from apoptosis to cell death(5). In humans, some of the most well-known stimuli to induce ROS production include postprandial substrate oxidation and exercise-induced mechanical and physiological stress(6).

To maintain cellular redox homeostasis, many enzymatic and non-enzymatic antioxidant defenses have evolved to regulate ROS levels in cells(7). Examples of enzymatic antioxidant sources include superoxide dismutase, glutathione peroxidase, and catalase, while examples of non-enzymatic sources involve glutathione (GSH) and vitamins C and E. Increased ROS or decreased antioxidant activity can lead to a cellular redox environment commonly referred to as "oxidative stress."

During the period of overtraining will release ROS exceeding the protective capacity of the endogenous antioxidant system and causing dysregulation in the inflammatory system, oxidative phosphorylation and neuroendocrine. Increased exercise intensity has been shown through several studies to increase oxidative stress and free radical production in cells(5).

Overtraining through several studies on experimental animals has proven changes in the form of increased heart rate and at the end of the overtraining period there was a decrease in stroke volume of the heart(8). The physiological function of the kidney organ has also been proven through studies on experimental animals with exhaustive exercise treatment experiencing pathological changes in the form of proteinuria and acute kidney injury. Acute exhaustive exercise is illustrated as short-term exercise with an intensity that exceeds the body's recovery ability(9). In addition to being experienced by athletes, overtraining also causes muscle damage that usually occurs in individuals who rarely exercise who then experience exercise overloading(10).

In relation to overtraining, several biomarkers are thought to be able to indicate the process of oxidative stress, inflammation, proliferation and infarction as well as other processes that may occur in the heart organ in overtraining conditions(11). Several biomarkers used in this study are Malondialdehyde (MDA), Superoxide Dismutase (SOD). There are several biological markers (biomarkers) that are commonly used as a measure of oxidative stress, including superoxide dismutase (SOD) and malondialdehyde (MDA). Malondialdehyde (MDA) is the end result of the lipid peroxidation process in the aerobic process (9). During the lipid peroxidation process, superoxide dismutase (SOD) is the leading defense mechanism against the lipid peroxidation process so that it does not occur excessively. During this process, its metabolism depends on mineral metals such as copper (Cu) and manganese (Mn) so that it can work optimally(12).

Based on this description, it is possible that giving chia seed extract as an exogenous antioxidant can be used as additional intake for athletes to prevent oxidative stress due to overtraining.The parts of this book are organized as follows: the first part deals with the revision of the introduction. Section 2 reviews the overall findings and methods, Section 3 presents the results, Section 4 presents the discussion of the study and finally Section 5 concludes the results of the study. Based on this background, it is necessary to conduct in-depth research related to the protective study of chia seed extract (Salvia hispanica L.) against oxidative stress (SOD & MDA) in rats with overtraining.

## 2. Results

The research was conducted for three months at FK UNISSULA Semarang Integrated Biomedical Laboratory. During treatment, no rats died and all met the inclusion criteria. No mice stopped until the end of the study. Then, on day 30, SOD and MDA levels were monitored in male wistar rats that had been post overtraining. In this study, there are 3 groups that make up the P0 group, i.e. the control group that received only the standard diet, group P1, Experimental group 2 consisted of 5 rats given standard feed, forced to do physical exercise for 28 days and after physical exercise given chia seed extract dose of 4.5mg/kgBW. On the 29th day blood samples were taken. Group P3, Experimental group 1 consisted of 5 rats given standard feed, forced to do physical exercise for 28 days. On the 29th day blood samples were taken.

Research has been conducted by administering chia seed extract to SOD (superoxide dismutase), MDA (malondialdehyde) levels in male Wistar rats with overtraining in the form of swimming activities.

2.1.1. Results of Normality Analysis and Homogeneity Analysis

In table 1, the results of the statistical analysis of the normality and homogeneity tests that have been carried out using the Shapiro Wilk test and Levene test on all SOD and MDA variable data, the results of the data normality test were obtained with a significance value of p>0.05, which means that the data is normally distributed and homogeneous.

**Table 1.** Results of Normality Analysis

| Variabel | K0 | K1 | K2 |
|---|---|---|---|
| SOD Level | 0,429* | 0,942* | 0,992* |
| MDA Level | 0,395* | 0,747* | 0,395* |

Description: *Normal>0.05

**Table 2**. Results of Homogen Analysis

| Variabel | Sig. (p) | Description |
|---|---|---|
| SOD Level | 0,547 | Homogen |
| MDA Level | 0,100 | Homogen |

2.1.2. Average SOD levels

The research data showed that the highest average SOD levels were in the control group, followed by group 2, and group 1. The control group had an average SOD level of 4.83 U/ml. Group 1 had the lowest SOD level with an average SOD level of 1.45 U/ml. Then Group 2 had an average SOD level of 2.86 U/ml. Data on superoxide dismutase levels can be seen in the figure 1.

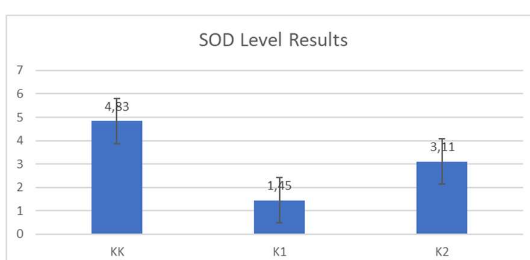


**Figure 1.** Average SOD levels

2.1.3. Average MDA levels

The results of the research data showed that the lowest MDA levels were in group 2, followed by group 1 and the control group. The control group had an average MDA level of 1.67 nmol/ml. Group 1 had the highest MDA levels with an average MDA level of 1.91 nmol/ml. Group 2 had an average MDA level of 1.28 nmol/ml. Data on MDA levels can be seen in the figure 2.

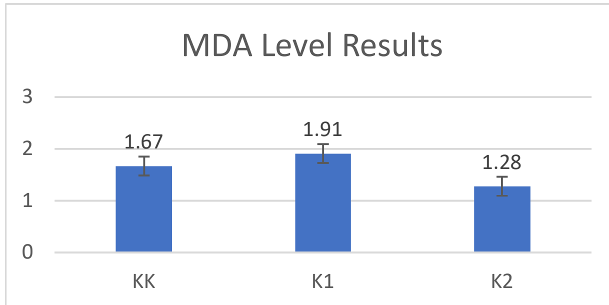


**Figure 2.** Avarage MDA Levels

2.1.4. Data Anova SOD levels and MDA levels

The data is analyzed using parametric statistics, namely the Anova test as the data distribution is normal and uniform. The results of the analysis using the ANOVA test obtained a p-value of 0.000, which means that there is a significant difference in the SOD levels and MDA levels in the three groups. To identify significantly different groups, post hoc LSD tests were performed as described below.

The results of the LSD post hoc test in Table 3 show that there is a significant difference in the mean SOD levels in the P0 and P1 groups (p=0.000); P0 and P2 (p=0.000); P1 and P0 (p=0.000); P1 and P2 (p=0.000); P2 and P0 (p=0.000); P2 and P1 (p=0.000). Based on the above data, it can be concluded that the administration of chia seeds and overtraining affects the reduction of SOD in Wistar rats with overtraining.

The results of the LSD post hoc test in Table 4 show that there is a significant difference in the mean MDA levels in the P0 and P1 groups (p=0.000); P0 and P2 (p=0.000); P1 and P0 (p=0.000); P1 and P2 (p=0.000); P2 and P0 (p=0.000); P2 and P1 (p=0.000). Based on the above data, it can be concluded that the administration of chia seeds and overtraining affects the reduction of MDA in Wistar rats with overtraining.

| Variable | KK | K1 | K2 |
|---|---|---|---|
| | *Sig.(p)* | *Sig.(p)* | *Sig.(p)* |
| **K0** | - | 0.000 | 0.000 |
| **K1** | 0.000 | - | 0,000 |
| **K2** | 0.000 | 0.000 | - |

**Table 3. Anova SOD Levels**

| Variable | KK | K1 | K2 |
|---|---|---|---|
| | *Sig.(p)* | *Sig.(p)* | *Sig.(p)* |
| **K0** | - | 0.000 | 0.000 |
| **K1** | 0.000 | - | 0.000 |
| **K2** | 0.000 | 0.000 | - |

**Table 4. Anova MDA Levels**

## 3. Discussion

The results showed that the group given overtraining had lower SOD levels, higher MDA, 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 so as not to be damaged. Cell damage, especially at the muscle level, is associated with the inflammatory process that occurs during the repair of damaged tissue, and this inflammation can cause further production of pro-oxidants by neutrophils and macrophages. On the other hand, nitric oxide and reactive oxygen species play an important role in regulating immune function, affecting almost every step in the development of inflammation(6). ROS production comes from mitochondria with 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 reactive oxygen species (ROS)(13).

ROS occur when oxygen is insufficient to complete the reduction, resulting in the production of free radicals(14). Free radicals are highly reactive due to the unpaired electrons in their outermost orbitals(15). Due to their reactivity, high concentrations of free radicals can cause damage to lipids, proteins, and DNA. During exercise, increased oxygen consumption leads to increased ROS production(16). Increased ROS production during exercise can lead to muscle damage, impaired immunity, and fatigue. Muscle damage, including lipid peroxidation, due to ROS during acute exercise has been suggested as a cause of delayed onset muscle soreness (DOMS) and decreased exercise performance(17). Apart from the damage they cause, free radicals are also important for proper physiological function and act as intracellular messengers. The high levels of these reactive oxygen species cause the body to mount a defense. These antioxidants are the first line of defense aimed at suppressing the formation of free radicals(18).

Antioxidants are compounds that help protect cellular organs from oxidative damage caused by free radicals. There are many different antioxidants that can be classified as endogenous (produced within the body) or exogenous or dietary (taken from external sources). Antioxidants can also be classified as enzymatic (eliminate free radicals catalytically) or non-enzymatic (eliminate free radicals by means other than catalytic reactions). Antioxidants protect against oxidative stress by converting free radicals to non-radicals thereby reducing their reactivity, or by preventing the conversion of inactive radicals to more damaging species (2).

Endogenous antioxidants are proteins produced by the body and can be enzymatic or nonenzymatic. Endogenous enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxide (GPX). The main non-enzymatic endogenous antioxidant is glutathione (GSH). Endogenous antioxidant production increases after exercise and plays a role in protecting cells from oxidative damage. Most fruits and vegetables contain a variety of exogenous antioxidants. However, humans can also obtain exogenous antioxidants through other food sources such as nuts and seeds. Important exogenous antioxidants that play a role in protection from free radicals include vitamin E, vitamin C, vitamin A, polyphenols, and several minerals (Zinc, Manganese, Cupper, Selenium)(19).

In this study, the SOD levels in the overtraining group were lower than the control group. The control group had an average SOD level of 4.83 U/ml. The overtraining treatment group had the lowest SOD levels with an average SOD level of 1.45 U/ml. This is due to the use of quite a lot of SOD to prevent free radicals. SOD breaks down reactive oxygen species (ROS) into hydrogen peroxide (H2O2) and oxygen(20). Proteins, unsaturated fatty acids, lipoproteins, and DNA elements such as carbohydrates and RNA are the main targets of free radicals(21). The most vulnerable target to ROS attack is unsaturated fatty acids. The high concentration of unsaturated fatty acids in phospholipids in each cell membrane makes them the main target for oxidant reactions and allows them to participate in long-chain free radical reactions(22). Lipid peroxidation is the name for this process. Malondialdehyde is the end product of lipid peroxidation (MDA). The higher the level of MDA in the body, the more free radical damage it causes(23). The results of this study showed that the overtraining treatment group had significantly higher MDA levels than the control group ($p < 0.05$). The control group had an average MDA level of 1.67 nmol/ml. The group that was only given overtraining had MDA levels of 1.81 nmol/ml.

In this study, SOD levels in the group given chia seed extract at a dose of 4.5 mg/kgBW after overtraining had higher levels compared to the group given overtraining alone. Group 1 had the lowest SOD levels with an average SOD level of 1.45 U/ml. Group 2 had an average SOD level of 3.11 U/ml. Group 3 had an average SOD level of 2.86 U/ml. Group 3 had higher SOD levels due to the administration of chia seed extract at a high dose. The administration of chia seed extract also significantly reduced MDA levels after overtraining when compared to the group given overtraining without chia seed extract. Group 1 had the highest MDA levels with an average MDA level of 1.91 nmol/ml. Group 2 had an average MDA level of 1.28 nmol/ml. Group 2 had lower MDA levels due to the administration of chia seed extract at a dose of 4.5 mg/kgBW.

Chia seeds contain quercetin, a very powerful antioxidant in preventing oxidation of fat, protein and DNA and is the most effective of the flavonoid compounds. Caffeic acid and chlorogenic acid work against free radicals by inhibiting fat peroxidation. Anthocyanins can neutralize free radicals hydroxyl (OH *), peroxyl (ROO *), and singlet oxygen (O2 *) produced by reactive oxygen (Trisnadi, 2023b). In this case, flavonoids work to inhibit CETP so that there is an increase in HDL cholesterol levels and a decrease in LDL levels51. Flavonoids also have anti-inflammatory effects by inhibiting cytokines such as tumor necrosis factor $\alpha$ (TNF-$\alpha$). The author conducted preliminary research by testing the antioxidant levels contained in chia seed extract of 87 with the IC50 method, which is said to be a strong antioxidant of 50-100.

Reducing TNF-$\alpha$ will increase insulin sensitivity, increase fatty acid oxidation in the liver, inhibit cholesterol synthesis by cells In addition to containing essential fatty acids, according to Campos (2014), the behavior of chia seeds is 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, caffeic acid(24). These compounds are primary and synergistic antioxidants that provide a high proportion of antioxidant activity from chia seeds(25).

There are many obstacles that must be overcome when conducting research, so there are many shortcomings in this study. The following are some limitations of the study:

1. Researchers did not conduct nutritional checks and toxicity tests on the chia seeds used for the study.

2. There are still biomarkers that have not been studied to see oxidative stress (glutathione peroxidase, catalase, il-10, tnf-a) and muscle cell damage.

## 4. Materials and Methods

This research is an true experimental study using a post-test control group design. The Research Center of Biology and Chemistry Laboratory of IBL FK Unissula The research period is conducted for 3 months from May 2024 to July 2024. Independent change of chia seeds at a dose of 4.5 mg / kg body weight in the macerated method, the change depends on SOD and MDA. The number of groups in this study is 3 groups, group 1 is the control group, K1: Experimental group 1 consisted of 5 rats fed a standard diet and subjected to forced physical exercise for 28 days. Blood samples were taken on day 29. K2: Experimental group 2 consisted of 5 rats fed a standard diet and subjected to forced physical exercise for 28 days. After the physical exercise, chia seed extract was administered at a dose of 4.5 mg/kg body weight. Blood samples were taken on day 29. It exclusion and inclusion criteria. 1. Inclusion criteria: The research mice were in good health, the mice were male, the mice weighed 200-250 grams, and the mice were 8 weeks old. 2. Exclusion criteria: The mice were sick or injured. 3. Dropout criteria: The mice were sick or injured during the study, and the mice died during the study. A sample of 15 rats was reconditioned for one week before treatment. All eligible rats will be included in the study sample. 15 Wistar rats were recruited and randomly divided into three groups, each group containing six rats. Group 1 was a control group, group 2 was a treatment group with 4,5 mg/kg body weight of chia seeds after overtraining, and group 3 was a treatment overtraining only. This research has received approval from Unissula School of Medicine Bioethics Commission Ethics Clearance Number 219/VI/2024/Bioethics Commission. Look for a method :

1. Selection of 15 white rats of the Wistar breed (Rattus Norvegicus) aged 3-4 months and weighing about 180-200 grams.
2. Rats were acclimated for approximately one week and fed a standard diet in the form of HBS pellets ad libitum.
3. The mice are placed in the cage with the requirements as a research study, that is, the mice are placed in a plastic cage, measuring 30 x 20 x 20 cm and covered sand and wire insulation. The holes are placed in an air-conditioned room with natural ventilation. In 1 point put 6 limits.
4. Extraction process of Chia seeds: The process of ethanolic extraction of Chia seeds is carried out in the chemical laboratory of FK UNISSULA Semarang. Simplicia was dried in a 400 °C oven for 7 hours to 1500 grams of crushed and processed chia seeds, which were extracted in 7.5 liters (1:5) of 70% ethanol using the maceration method and agitated with an agitator for 1 hour. , and leave for 24 hours.
5. Overtraining: the experimental animals were given a 100 x 80 x 80 aquarium swimming treatment with partitions. The aquarium water was set at 36°C, and the mice undergoing swimming would show signs of fatigue, such as starting to sink and increasingly weak movements. This swimming activity treatment was started at 09.00 am – 12.00 noon every day for 28 days.
6. Treatment given: P0 treatment and control group only with standard food. Group P1 received an overtraining swimming activity treatment started at 09.00 am – 12.00 noon every day for 28 days. Then 4.5 mg/kg of chia seed extract for 7 days. P2 received an overtraining This swimming activity treatment started at 09.00 am – 12.00 noon every day for 28 days.
7. After treatment, check the SOD and MDA level as post-test.
8. MDA levels were measured from blood samples taken through the medial cantus of the orbital sinus on day 29 using the TBARS method and read on a spectrophotometer at a wavelength of 532 nm in nmol/ml.
9. SOD levels were measured from blood samples taken through the medial cantus of the orbital sinus on day 29 using the ELISA method in ng/ml.
10. Take 1 cc of blood from the medial canthus of the orbital sinus and a mixture of ketamine - xylazine anesthesia 40 - 80 mgkgBB (k) intraperitoneal and 5 - 10 mg / kgBB (x) intraperitoneal.

Data was tested using the IBM SPSS Statistics program version 21.0 on a Windows device. The prerequisite test was carried out using the Shapiro Wilk normality test and the homogeneity test using the Levene Test. To test the potential of chia seed extract (Salvia Hispanica L.) in overcoming oxidative stress with indicators of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) levels, in overtraining model mice, ANOVA was used comparing K1, K2, and K3 followed by the Post Hoc LSD test.

**5. Conclusions**

The conclusion of this study is that administration of chia seed extract (Salvia Hispanica L.) at a dose of 4.5 mg/kgBW can reduce oxidative stress which is characterized by increased levels of superoxide dismutase (SOD) and decreased production of malondialdehyde (MDA).

**Author Contributions:** This research involves several authors and requires all authors to be experts in their respective fields. The following is the division of writing and research conducted. Reza Adityas Trisnadi : Study Design, Statistical Analysis & Manuscript final preparation, BM. Wara Kushartanti : Study design & manuscript preparation, Rachmah Laksmi Ambardini : Statistical Analysis, Amran Amran : Statistical Analysis, Danarstuti Utami : Data Collection & Statistical Analysis, Setyo Trisnadi : Data Collection, Statistical Analysis and Etichal Clearance, Syafinda Meisari Trisnani : Data Collection & Manuscript Preparation, Nur Ulayatilmiladiyyah : making chia seed extract & animal care experiment, Mahendra Wahyu Dewangga : Experimental animal treatment of overtraining dose.

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**Informed Consent Statement:** This research uses laboratory animals so it does not use informed consent, it only requires ethical clearance.

**Data Availability Statement:** Not applicable.

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