

The Effect Of Selenium Supplementation On The Expression Of Nuclear Factor Erythroid 2-Related Factor 2(Nrf2) And Malondialdehyde (MDA) In Wistar Rats Fed AnAtherogenic Diet

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Abstract

Background: Selenium is a trace element essential for maintaining the body's redox balance. Insufficient or excessive selenium intake has been known to cause redox balance disorders and can induce endogenous antioxidants such as nuclear factor erythroid 2-related factor 2 (Nrf2). This study aimed to determine the effect of different selenium doses on Nrf2 expression and MDA levels in Wistar rats fed an atherogenic diet. **Methods:** Experimental study with Post Test Only Control Group Design were 30 male Wistar rats who were randomized into three groups and given treatment for six weeks. The control group was only assigned an atherogenic diet without adding selenium supplementation. In the treatment group, I was given additional supplementation of sodium selenite 0.0086 mg/100 g diet. The second treatment group was also assigned sodium selenite supplementation 0.1 mg/100 g diet. After treatment, Nrf2 expression and MDA levels. **Results:** The mean of Nrf2 expression in this study did not show a significant difference between the control group (29.73 ± 5.87 ng/ml) and treatment group I (29.89 ± 5.29 ng/ml), and treatment group II (28.42 ± 4.36 ng/ml) with $p = 0.807$. A similar result was also obtained for the mean level of MDA in the control group (2.17 ± 0.49), treatment group I (2.68 ± 0.40), and treatment group II (2.46 ± 0.75) with $p = 0.166$. **Conclusion:** These findings concluded that administration of low selenium supplementation, in this study, was not different in increasing Nrf2 gene expression and decreasing MDA levels in male Wistar rats fed with an atherogenic diet than high selenium supplementation.

Keywords: atherogenic diet, malondialdehyde (MDA), Nuclear factor erythroid 2-related factor 2 (Nrf2), selenium

1. Introduction

Cellular aging is a risk factor for various aging-related diseases, such as cardiovascular disease, which is a significant cause of disability and premature death globally. A systematic review and meta-analysis study stated that globally in 2020, there was an increase in the prevalence of carotid artery thickening by 27.6%, and the percentage changed by 57.46% from 2000 (Bonomini et al., 2015; Song et al., 2020).

Various hypotheses have been put forward regarding the causes of aging. Still, the most popular is the imbalance of prooxidants and antioxidants, causing oxidative stress, which further results in accelerated aging of blood vessels (Reuland et al., 2013). Excessive reactive oxygen species (ROS) can react with polyunsaturated fatty acid (PUFA) from LDL membrane phospholipids, resulting in lipid peroxidation and forming malondialdehyde (MDA) compounds. MDA compounds are toxic to cells and can be a biomarker to determine the level of oxidative stress. MDA levels can be reduced by giving antioxidants (Ayala et al., 2014; Brigelius-Flohé and Kipp, 2013; Burk et al., 2008).

Selenium is a trace element vital in producing selenoproteins that function as antioxidants (Kusmana, 2017). Inadequate selenium intake can disturb redox balance and induce activation of transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) to improve redox balance (Lennicke et al., 2017). The mechanism to induce nuclear translocation and binding of Nrf2 DNA is the modification of the thiol group

associated with the redox balance in Kelch-like ECH-associated protein 1 (Keap1), resulting in a conformational change that locks Nrf2 in Keap1, which causes Nrf2 to dissociate from Keap1. After that, Nrf2 translocation to the nucleus and binds to antioxidant response element (ARE) for the synthesis of related genes such as genes involved in antioxidant defense, regeneration of nicotinamide adenine dinucleotide phosphate (NADPH), glutathione synthesis, detoxification, and genes related to metabolic control (Chen and Maltagliati, 2018; Dodson et al., 2019; Schwarz et al., 2019)

The Nrf2 pathway can be modulated by hydrogen peroxide (H₂O₂), so selenoproteins may regulate the course. Low selenium intake reduces some selenoproteins while increasing H₂O₂. This condition makes Keap1 oxidized so that the Nrf2 pathway is activated. In contrast, high selenium concentrations induce the formation of thiol-reactive selenium and free radicals. (Brigelius-flohé and Kipp, 2013; Huang et al., 2015; Lennicke et al., 2017; Li et al., 2009). Research conducted by Muller et al., and Lennicke et al., showed that suboptimal dietary selenium (0.086 mg Se/kg diet) could induce Nrf2 activation in the duodenum and liver of rats. (Müller et al., 2010; Lennicke et al., 2017). However, Schwarz et al.'s study stated that a selenium-deficient diet (0.03 mg Se/kg diet) decreased selenoprotein expression but did not activate Nrf2 (Schwarz et al., 2019). Meanwhile, another study proves that supplementing selenium in as much as 1 mg Se/kg diet (high doses of selenium in the diet) significantly increased Nrf2 expression in diabetic rats compared to diabetic rats with selenium deficiency (Sakr et al., 2018; Tangvarasittichai, 2015; Xue and Wang, 2015).

Based on these data, research on the effect of selenium on Nrf2 expression is still controversial and inconclusive. Therefore, the basis of this study was to determine the impact of different doses of selenium on Nrf2 face and MDA levels in Wistar rats receiving an atherogenic diet

2. Methods

This study used a randomized post-test-only controlled group design. It was conducted in the Laboratorium Biomedik Terpadu of Udayana University Bali, Indonesia.

Experimental Animal

Thirty healthy male Wistar (*Rattus norvegicus*) rats (100-150 g) were taken from an animal unit of the Medical Faculty, Udayana University. Ethical permission in this study with No. B/218/UN14.2.9/PT.01.04/2021 from the Faculty of Veterinary Medicine research ethics committee, Udayana University. The adaptation period was done in a week with a standard food mixture, and drinking water was administered ad libitum. Animals were maintained at room temperature (25°C) on a 12:12 h light-dark cycle. The samples were divided randomly into three groups (n = 10). All groups were given an atherogenic diet. The control groups (K) were given an atherogenic diet without supplementation. Treatment group 1 (P1) was assigned additional supplementation of sodium selenite 0.0086 mg/100 g diet. Treatment group 2 (P2) was given sodium selenite supplementation 0.1 mg/100 g diet. After six weeks of treatment, the rats were anesthetized, and a sample of aorta tissue was taken for Nrf2 and MDA level measurement.

Selenium supplementation procedure

The chemical form of selenium supplementation used in this study is sodium selenite, obtained from P.T. Sigma Aldrich. The doses of selenium supplementation were calculated based on 20 grams of rat feed, equivalent to 0,0017 mg/20 g of the rat diet for low dose supplementation and 0,02 mg/20 g for high dose selenium supplementation. For low doses of selenium, 1 mg of sodium selenite was dissolved in 590 ml water to obtain the supplementation dose per 20 g of rat diet. For high amounts of selenium supplementation, 1 mg of sodium selenite was dissolved in 50 ml water doses of water to obtain the supplementation dose per 20 g of rat diet.

Determination of Nrf2 expression and MDA level

Before taking samples for expression of Nrf2 and MDA levels was extracted from aorta tissue samples, The experimental animals were treated by fracture of the cervical vertebrae which minimized the pain felt by the experimental animals and samples analyzed using the ELISA method from Bioassay Technology Laboratory. After protein isolation, a sample and ELISA reagent were added to each well and incubated for 1 hour at 37°C. The plate was washed five times. Substrate solutions A and B were added to the well, then set again for 10 minutes at 37°C. The last stop solution was added, and the O.D. value was read within 10 minutes.

Data analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software for Windows 26. All data were tested for normality using Shapiro-Wilk. The significance test used One-Way Anova (parametric test) for MDA level with normal distribution

3. Results

The Of 30 subjects, one from P1 and one from P2 died during the study, so those who met the requirements for analysis were 28 samples; 10 control mice, 9 P1 mice, and 9 P2 mice. The research data were then presented in descriptive form, analyzed for normality and homogeneity, and compared between groups using comparability and treatment effect analysis. The results of the study of Nrf2 and MDA expressions are obtained in table 1

Table 1				
The results of the analysis of Nrf2 expression and MDA levels				
Variable	N	Average	Standard Deviation	p ^a
Nrf2. Expression				
Control	10	29.73	±5.83	0.807
Treatment 1	9	29.89	±5.29	
Treatment 2	9	28.42	±4.36	
MDA level				
Control	10	2.17	±0.49	0.166
Treatment 1	9	2.68	±0.40	
Treatment 2	9	2.46	±0.75	

Description: p^a One Way ANOVA

In the control group, the Nrf2 expression obtained a mean result of \pm SD 29.73 \pm 5.83. In treatment group 1, the average result was \pm SD 29.89 \pm 5.29, while in treatment group 2, the average result was \pm SD 28.42 \pm 4.36. This result diagram is depicted in **Figure 1**. The results of the statistical analysis of Nrf2 expression did not show any significant difference among the three groups with the One Way ANOVA test of 0.807 ($p > 0.05$).

The mean levels of MDA results in the control group were \pm SD 2.17 \pm 0.49, treatment group 1 had an average impact of \pm SD 2.68 \pm 0.40, while the mean effect in treatment group 2 was \pm SD 2.46 \pm 0.75. This result diagram is depicted in **Figure 2**. The results of statistical analysis of MDA levels did not show any significant difference too among the three groups using the One Way ANOVA 0.166 test ($p > 0.05$)

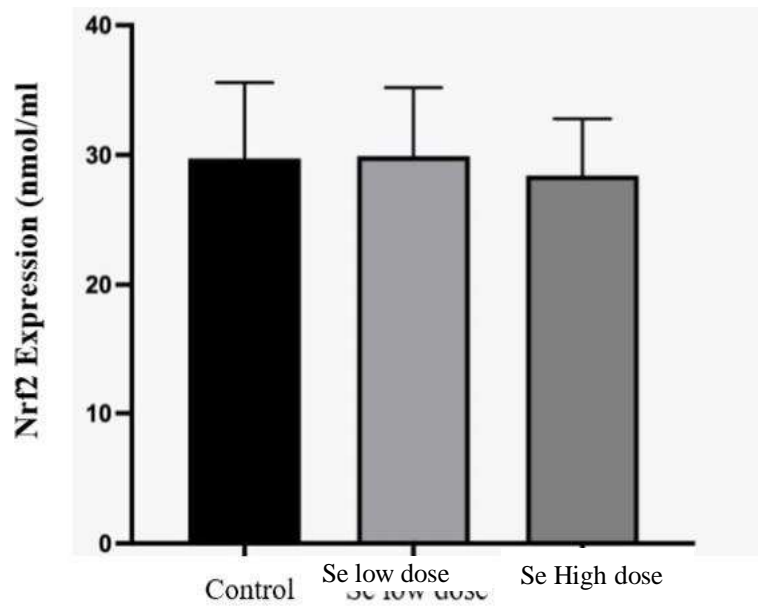


Figure 1
Nrf2 expression result

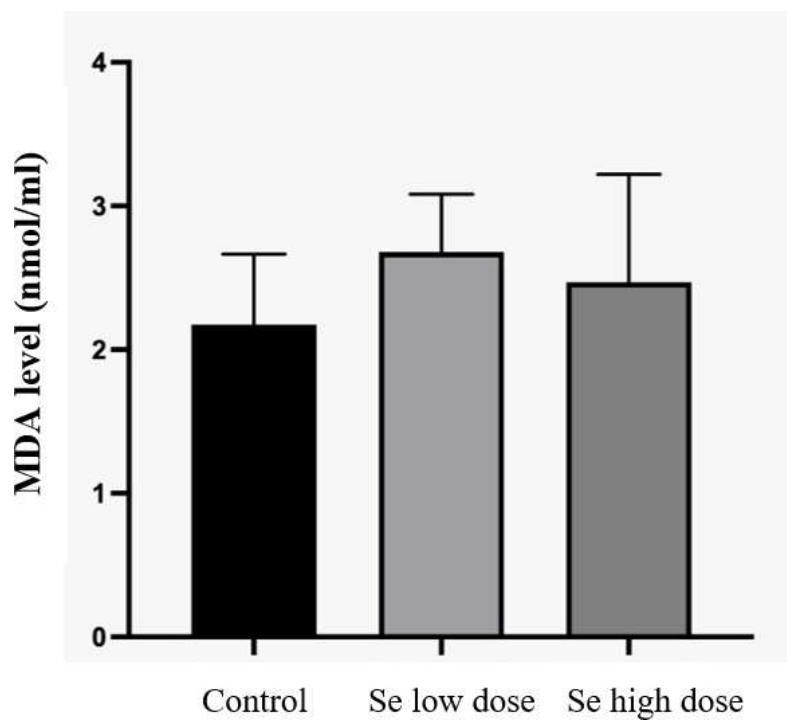


Figure 2
MDA level result

4. Discussion

Influence factors on Nrf2 levels

Nrf2 as a transcription factor plays an important role in inducing genes encoding several antioxidants and phase 2 detoxification enzymes. However, when oxidative stress increases, the cysteine residues in Keap1 are oxidized resulting in cysteine modification in Keap1 which then activates Nrf2 (Ma Q, 2013; Levonen 2007)).

The results of this study showed an increase in Nrf2 expression in the three groups of mice after 6 weeks of treatment. This can be seen from the untreated mice, the normal values of Nrf2 expression were 17.75 ng/mL and 16.76 ng/mL (Maharani NK, 2022). Although there was an increase, the difference was not significant among the three groups. The results of this study may be caused by the addition of atherogenic dietary elements in the three groups, so that oxidative stress occurs in the three groups.

This study used an atherogenic diet with a very low selenium content. The composition of the diet consisted of a mixture of standard rat feed added with 2% cholesterol, 0.2% cholic acid and 5% lard. The cholesterol used is pure cholesterol in powder form made of desmosterol, lathosterol, 2,4-Dehydrolathosterol, and 4-Methylcholest-5-en-3-ol. The addition of cholic acid aims to help the absorption of cholesterol and fat, as well as suppress the conversion of cholesterol into bile acids. Through this mechanism, cholic acid is used to change the appearance of lipoproteins to become more atherogenic, namely reducing HDL levels and increasing plasma LDL (Pellizzon, 2014; Sri Murwani, Mulyohadi Ali, 2017; Guo 2-16, Habeos, 2011)

The pork oil used is one of the recommended fats used to make a selenium deficiency diet (Burk, 1987). This is evidenced by the feed analysis that has been carried out previously at the Laboratory for Calibration and Certification Testing Services, Bogor Agricultural University, with the results that the selenium content in rat feed was <0.003 mg/100 g of feed

The dose of selenium supplementation used in this study refers to several previous studies. Some of these studies used feeds with selenium content <0.003 mg/100 g diet, 0.0086 mg/100 g diet and 0.1 mg/100 g diet (Hutchins-Wiese et al., 2013; Jenkins et al., 2020; Müller et al., 2010; Schwarz et al., 2019; Xue and Wang, 2015). In the previous study, there was no addition to an atherogenic diet, so there may be differences in results. Meanwhile, in this study, there was the addition of atherogenic dietary elements in the three groups, allowing the expression of Nrf2 to increase in all groups.

The high dietary lipid in the diet is proportional to the high synthesis of LDL, so that it affects the increase in free radicals due to lipid peroxidation in red blood cells. The continuous formation of free radicals will cause an imbalance in the number of antioxidants resulting in oxidative stress (Simanjuntak, 2021). Keap1 is the main regulator of Nrf2, where there is a cysteine residue as the main sensor of oxidative stress which will interfere with the interaction of the Keap1-Cul3 complex and cause the dissociation of Nrf2 from Keap1 resulting in Nrf2 activation (Jiang et al., 2016; Kasai et al., 2020; Lau et al., 2010; Zhao and Ewald, 2020). This can explain the results of this study where the atherogenic diet given triggers oxidative stress and causes an increase in Nrf2 expression in the three groups. Meanwhile, selenium seems to have no effect on Nrf2 expression.

In this study, the dose of selenium supplementation used depended on the amount of the rat's diet. Previous studies used the fortification technique directly into the diet of mice to obtain different doses of selenium in the diet. As a study conducted by Schwarz et al., 2019 used a torula yeast-based diet that has a low selenium content to obtain a selenium content of 0.003 mg Se/100 g diet obtained from Altromin. For adequate (0.015 mg Se/100 g diet) and high selenium (>0.06 mg Se/kg diet), obtained by mixing sodium selenite/selenomethionine into the basal diet (Schwarz et al., 2019). Then an analysis was carried out using inductively coupled plasma-mass spectrometry (ICP-MS/MS) for trace metal detection {Formatting Citation }

Fortification technique was not used in this study due to some constraints and tools to analyze the selenium content in feed, as well as constraints during the process of making selenium fortification into the diet of rats. The sonde technique was used as an alternative in this study. However, there are some disadvantages to using this technique. The supplementation dose given to each rat is very individual,

depending on the amount of diet consumed by rats per 24 hours. Furthermore, it is possible that there is a bias in the calculation of the remaining feed, which affects the dose of selenium supplementation that will be administered, potentially influencing the study results.

Influence factors on MDA levels

Malondialdehyde is a biological marker that is produced mainly under conditions of high free radicals and describes abnormalities of early cellular metabolism and disease progression. MDA is produced from the oxidation of unsaturated fatty acids, including arachidonic acid, α -linoleic acid and linoleic acid as by products of enzymatic processes (Iswari et al., 2020).

The composition of the atherogenic diet used in this study was the same as that used in the study of Muwarni et al., in which the study carried out for 8 weeks succeeded in increasing cholesterol levels significantly and there was significant foam cell formation. Pork oil can increase cholesterol levels in the blood of experimental animals because pork oil contains a lot of polyunsaturated fatty acids (Kusumastuty, 2014; Prabawati and Fajriati, 2018; Yim 2019, Yu and Xiao, 2021). The higher the cholesterol in the blood (especially LDL) and free radicals, the greater the risk of increasing lipid peroxidation and the formation of MDA (Simanjuntak, 2011).

From the results of the One Way Anova statistical test in this study, there was no significant difference in MDA levels at aortic tissue. MDA levels in the atherogenic diet group obtained an average of 2.17 ± 0.49 nmol/mL, the low selenium supplementation group was 2.68 ± 0.40 nmol/mL while the high selenium supplementation group obtained an average result of 2.46 ± 0.75 nmol. /mL. Although the differences were not significant, the results of the study showed that the mean MDA levels in the group receiving selenium supplementation were actually higher than the control group.

The addition of selenium supplementation in the chemical form of sodium selenite may have contributed to the increase in MDA levels in the treatment group. Selenium can be toxic to all animals and humans depending on the dose, duration of intake and also on its chemical form. Inorganic selenium, such as selenite used in this study, can cause an increase in malondialdehyde and a decrease in the amount of reduced glutathione (Mezes and Balogh, 2009).

There is also a trend towards potential selenium intoxication in the high selenium supplementation group. Judging from the statistical data, the excess selenium was not matched by a decrease in MDA levels, so it is possible that selenium reacts with thiols (such as glutathione) to form seleno-trisulphide and reacts with other thiols to form free radicals, such as superoxide anion (O_2^-). The resulting free radicals are involved in chain reactions that affect biomolecules, especially phospholipids and cause lipid peroxidation which is characterized by increased levels of MDA (Othman et al, 20019).

Similar results were found in the study of Lennicke et al., which stated that there was an increase in oxidized protein in the high selenium group, but no Nrf2 expression was seen in this group. This indicates that more oxidized conditions are not compensated by Nrf2 (Lennick et al., 2017). On the other hand, the shift in redox balance in the low-selenium group may have a different pathway that cannot be explained in this study.

Conclusions

1. Low selenium supplementation was not different in increasing Nrf2 gene expression in male wistar rats fed an atherogenic diet than supplementation with high selenium.
2. Low selenium supplementation was not different in reducing MDA levels in male wistar rats fed an atherogenic diet than high selenium supplementation

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Ethics In Research

Ethical permission in this study with No. B/218/UN14.2.9/PT.01.04/2021 from the Faculty of Veterinary Medicine research ethics committee, Udayana University

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