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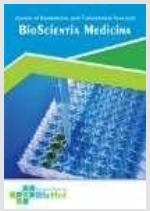

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Preventive Effects of Vitamin C on Cataracts: In Vivo Study

Andri Yosrizal[1*], Rinda Wati[1], Muhammad Hidayat[1]

[1]Division of Ophthalmology, Faculty of Medicine, Universitas Andalas/Dr. M. Djamil General Hospital, Padang, Indonesia

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***Corresponding author:**
Andri Yosrizal

E-mail address:
andriyosrizal26@gmail.com

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A B S T R A C T

Background: A cataract is a disease caused by various factors, one of which is the aging process. With increasing age, the formation of free radicals will cause pathological reactions in the lens and other toxic compounds, resulting in oxidative reactions. Administration of vitamin C injection as an antioxidant can reduce the reactivity of free radicals caused by oxidative reactions in cataract lenses induced by sodium selenite. This study aims to determine the preventive effect of vitamin C administration on selenite-induced cataracts based on the value of malondialdehyde (MDA) levels. **Methods:** The experimental study used 30 rats which were then divided into 5 treatment groups (P1, P2, P3, P4, and P5). Lenses were extracted for the measurement of malondialdehyde. The value of MDA levels was then analyzed using the One-way ANOVA statistical test. **Results:** Statistically, there was no significant difference in MDA levels in each treatment group. Analysis of the difference in mean MDA between the 2 groups showed that the average MDA levels of mice in groups P4 and P5 were relatively lower than P2 and P3, but higher than P1. **Conclusion:** Giving vitamin C was able to reduce MDA levels (a marker of oxidative stress) better than the group that did not receive vitamin C, but not statistically different.

1. Introduction

A cataract is a degenerative and multifactorial disease caused by various changes in conditions, such as the aging process, complications of eye disease, trauma, heredity, intrauterine infection, exposure to ultraviolet light, systemic disease, and due to long-term use of drugs such as corticosteroids.[1] Of these causes, oxidative stress, is the basic mechanism of cataract formation. Along with increasing age, the formation of free radicals will cause pathological reactions in the lens and other toxic compounds, resulting in oxidative reactions. Cataracts are the main cause of visual impairment and vision loss in the world, where 33% of the world's population has decreased vision due to cataracts. The World Health Organization (WHO) estimates that 18 million people are blind in both eyes due to cataracts, and this condition constitutes 48% of blindness cases worldwide. Excess free radicals cannot be destroyed, so they gradually leave the accumulation of substances. The accumulation of substances in the body will cause a phenomenon called oxidative stress. The nature of free radicals is very reactive and has a very fast half-life, and can take electrons from surrounding molecules, such as proteins and unsaturated fats. Free radicals have unstable molecules that can attack and damage healthy cells.[2-5]

Vitamin C (ascorbic acid) is an antioxidant found in the lens and aqueous humor with a concentration 50 times higher than that in plasma. Physiologically

vitamin C protects the lens from oxidative damage and regenerates vitamin E and glutathione to further increase its antioxidant capacity. With age, the level of vitamin C in the lens decreases, and a decrease in vitamin C in the lens is associated with increased cloudiness of the cataract. Consumption of additional vitamin C can increase the concentration of vitamin C in the lens to inhibit the formation of cataracts.[6,7]

This study is one of the earliest studies aimed at exploring the potential of vitamin C in preventing cataract disorders in vivo. One of the biomarkers of oxidative stress that can be measured in cataract lenses is MDA. This biomarker is most widely used as an indicator of lipid peroxidation. Sodium selenite is a selenium compound that is pro-oxidant and provides ROS production, which can induce cataracts. A selenite-induced cataract is known as a model system for cataracts induced by oxidative stress, which has similarities to cataracts in humans.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was included in this study and met the inclusion criteria in the form of the male, weight between 150-200 grams, and of age 8-10 weeks first, the rats were acclimatized for 7 days, then divided into 5 groups (P1, P2, P3, P4, and P5) at random, where each group consisted of 6 rats. The P1 group was a group of rats that were not given vitamin C injection and were not induced by sodium selenite; The P2 group was a group of rats that were induced by a single dose of sodium selenite but were not given vitamin C injection; The P3 group was a group of rats that were given vitamin C injection along with sodium selenite induction (the duration of vitamin C administration was 1 week); The P4 group was a group of rats that were given vitamin C injection 1 week after sodium selenite induction (the duration of vitamin C administration was 1 week); The P5 group was a group of rats that were given vitamin C injection 2 weeks after sodium selenite induction (the duration of vitamin C administration was 1 week). This study has been approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Andalas, No. 583/UN.16.2/KEP-FK/2022.

Cataract induction was carried out by first anesthesia in rats using ketamine (dose of 0.015 mg/gBW) intramuscularly and chlorate (dose of 0.0025 mg/gBW) subcutaneously. The sodium selenite given is 4.32345 x 10^{-3} g/KgBB. Sodium selenite was injected intraperitoneally into the abdominal region of experimental rats. Mice were monitored daily for signs of distress and signs of infection. Evacuation of the eye was carried out by performing a transpalpebral enucleation (the lens that was enucleated was the cloudiest lens) followed by making a lid incision and freeing the eyeball from the surrounding tissue, tracing the back of the eyeball with tweezers until the optic nerve could be reached. Next, cut the optic nerve and remove the eyeball. Then evacuate the anterior segment with scissors. Identify the lens, remove and rinse with physiological fluids to avoid mixing with other tissues. The eyepiece of the rat was put into a *microtube* containing 0.9% NaCl liquid, one container for one sample. The samples were temporarily stored in a cooler bag (temperature $\leq 2^0$C) and immediately stored in the freezer (temperature -20^0C). Analysis of MDA levels was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA) method according to the manufacturer's instructions (CloudClone®).

After the data is collected, data cleaning, coding, and tabulation are carried out. All results were assessed by means ± standard deviation accompanied by a normality test (Shapiro Wilk) and data homogeneity test (Levene Statistic). The test used in this research is One Way ANOVA. The results are said to be meaningful if $p \leq 0.05$. Data analysis was performed using SPSS version 25 for Windows.

3. Results

Table 1 shows that the MDA levels in the cataract-induced group (P2) showed an increase compared to the normal group (P1), although there was no statistically significant difference between the groups.

The administration of antioxidant vitamin C also showed a downward trend in MDA levels when compared to the P2 group, although statistically, there was no statistical difference between groups.

Table 1. Comparison of MDA levels between groups

Groups	Lens MDA Levels (nmol/ml)	p-value
	Mean ± SD	
P1	2.04 ± 1.06	0.403*
P2	3.24 ± 1.74	
P3	3.66 ± 1.04	
P4	2.43 ± 1.64	
P5	2.56 ± 1.04	

*one-way ANOVA, p=0.05

4. Discussion

In the group of rats that were induced with a single dose of sodium selenite but not given vitamin C injection, the mean MDA value was 3.24, while the mean MDA value of the group of rats that were given vitamin C injection at the same time as induction of sodium selenite was 3.66, where there was a difference. The mean value of MDA levels in these groups, but statistically, the difference is not significant, so it cannot be concluded that the mean levels of MDA in this group are different. The effect of cataractogenesis in rats can occur after 6 days of giving a single dose of 20 μmol/kg BW of sodium selenite, so it takes 6 days for cataracts to form, which can be seen with an increase in the mean MDA value. The toxicity of sodium selenite is due to its ability to catalyze thiols that cause oxidative stress that produces ROS. This process is initiated by the bond of selenite with thiol which produces products in the form of selenide (RSe-) and selenolate anion (RS-). RSe- is formed from the reaction of selenite with Gluthatione, this reaction forms selenotrisulfide (RSSeSR). This RSSeSR undergoes further reduction to unstable selenopersulfide (RSSeH). RSSeH further undergoes ionization of the physiological PH into selenopersulfide anion (RSSe-). RSSe- will oxidize thiol via selenium anion to superoxide (O_2-).[8-11]

When sodium selenite is induced, it takes time for ROS (O_2^-, H_2O_2) to be formed. This ROS formed can react with metal ions (Fenton reaction) and form OH- ions the most reactive free radicals). The role of vitamin C here is to prevent the Fenton reaction by binding to metal ions so that pro-oxidant substances (OH^-) are not formed, and the lipid peroxidation process does not occur. This process occurs after ROS are formed so that the absorption of vitamin C increases. This is consistent with the results of a study in a group of rats that were given vitamin C injection along with sodium selenite induction, which resulted in a high mean MDA. So it can be concluded that the administration of vitamin C in this group has not played a role in the prevention of induced cataracts.[12-14]

The absorption of vitamin C increases during conditions of oxidative stress, where the nature of vitamin C is as an antioxidant that can donate electrons to prevent other compounds from being oxidized so as to reduce the reactivity of free radicals. Vitamin C is a potent reducing agent that collects free radicals in biological systems by collecting oxidized free radicals (ROS) such as O_2-, H_2O_2 and reducing the metal ions that makeup OH- through phenton reactions. The role of vitamin C as a preventive lens-induced cataract occurs after ROS is formed, it takes 6 days for oxidative stress processes that provide ROS products in cataractogenesis after induction of sodium selenite. This may explain why in the group of mice who were given vitamin C injection 1 week after sodium selenite induction there was a decrease in the average value of MDA levels. Meanwhile, the group of rats given vitamin C injection 2 weeks after induction of sodium selenite had a higher mean MDA value, presumably due to the oxidative stress process that

had lasted for a long time (2 weeks) so that there had been an accumulation of excess ROS products. This shows the effectiveness of giving vitamin C as a preventive induced cataract in the process of cataractogenesis that has been going on for a long time is not optimal.[15-17]

5. Conclusion

Giving vitamin C was able to reduce MDA levels (a marker of oxidative stress) better than the group that did not receive vitamin C, but not statistically different.

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