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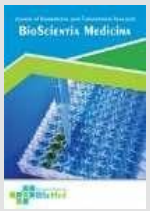

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The Effect of Topical Glutathione on Malondialdehyde Levels in Rat with Cataract-Induced Sodium Selenite

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A B S T R A C T

Background: Oxidative damage plays a key role in the lens opacity process. Reducing glutathione (GSH) is a tripeptide protein capable of preventing the accumulation of dangerous levels of oxidation products such as hydrogen peroxide and protein disulfide bonds. Human cataract lenses contain reduced levels of reduced glutathione, so by maintaining high levels of reduced glutathione in vivo, it may be possible to prevent opacification of the lens. This study aimed to determine the effect of topical glutathione on sodium selenite-induced cataracts based on the MDA level of the rat lens. **Methods**: This was an experimental study using 30 rats grouped into 3 groups (K+, P1, and P2). At the end of the study, the lens was dripped with Mydriaticum, then assessed for lens opacity. The more opacity lens was extracted, and subsequently, malondialdehyde level was measured. MDA level was analyzed using the One Way ANOVA test. **Results**: The average values of rat MDA Lens levels in the K+, P1, and P2 groups were 1.91, 1.38, and 1.2 nmol/mL. Statistically, there was a significant difference in MDA levels in each treatment group with p = 0.001 (p <0.05). Analysis of the average difference in MDA between each group showed that the average MDA level of rats in the P2 group was lower than P1, the MDA levels in the P1 and P2 groups were lower than K+, and there were statistically and clinically significant differences. **Conclusion:** Topical glutathione can effectively reduce the progression of cataracts in the lens of rats induced by sodium selenite which is identical to senile cataracts in humans in its mechanism of oxidative stress.

1. Introduction

Senile cataract presents a huge socioeconomic impact due to its high prevalence. Its etiologies are multifactorial and remain largely unknown. Several suggested mechanisms for the cause of cataracts include oxidative stress, fluid and ion imbalances, protein modifications, and metabolic disorders. Of these causes, oxidative stress is the base mechanism for cataract formation. Oxidative stress is a condition of cellular injury due to the exposure of reactive oxygen species (ROS). As people age, the formation of free radicals will lead to pathological reactions on the lens and other toxic substances, leading to an oxidative reaction. As in the aging process of the body, long-term accumulated oxidative stress on the lens is considered a key factor in causing senile cataracts.[1-4]

ROS is formed intracellularly as a consequence of induced endogenous and exogenous oxidative stress, including cellular respiratory changes, viral infections, and immunology activities. ROS consists of superoxide (O_2•-), hydroxyl (OH•), peroxyl (ROO-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide (NO-), peroxynitrite (ONOO-) and hypochlorous acid (HOCl). The endogenous fundamental source of ROS is produced in the mitochondria through an electron transport chain, in which inefficient conjugated electrons lead to superoxide formation (O_2•-). It is estimated that about 2% of oxygen is converted to O_2•-

under normal respiratory conditions. O_2•- is converted by superoxide dismutase into H_2O_2. H_2O_2 is then converted by the antioxidant glutathione into water (H_2O). Unconverted H_2O_2 can cause lipid peroxidation reactions in cell membranes.[1,5-7]

Lipid peroxidation initially occurs when free radicals attack and abstract hydrogen from methylene (CH_2) groups in fatty acids (LH), resulting in the formation of lipid radicals (LOO•). Through cyclical reactions, lipid peroxyl radicals (LOO•) form endoperoxides, which ultimately yield malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNA) as toxic end products.[7,9,10] Glutathione (GSH), along with the enzyme glutathione peroxidase (GPx), catalyzes the reduction of lipid hydroperoxides to alcohols and hydrogen peroxide to water. During this catalytic process, the disulfide bond of GSH forms oxidized glutathione (GSSG), and the enzyme glutathione reductase recycles GSSG back to GSH by oxidizing NADPH.[11-13]

This study is one of the initial studies aimed at exploring the potential of topical glutathione in preventing cataract disorders in vivo. One of the oxidative stress biomarkers measurable in cataract lenses is MDA, which is widely used as an indicator of lipid peroxidation. Sodium selenite is a pro-oxidant selenium compound that induces the production of reactive oxygen species (ROS), which can induce cataracts. Selenite-induced cataracts are known as a model system for oxidative stress-induced cataracts, which share similarities with cataracts in humans.

2. Methods

This study is an experimental research with a posttest-only with a control group design approach. A total of 30 Wistar strain rats (*Rattus norvegicus*) were included in this study, meeting the inclusion criteria of 14-day-old Wistar rats, no gender differences, visibly healthy and active, with an average body weight of 25-30 grams. The rats were acclimatized for 7 days, then randomly divided into 3 groups (K+, P1, P2), with each group consisting of 10 rats. Group K+ received an intraperitoneal injection of sodium selenite without topical glutathione, group P1 received an intraperitoneal injection of sodium selenite and topical glutathione one drop per day for 1 week, and group P2 received an intraperitoneal injection of sodium selenite and topical glutathione one drop per day for 3 weeks. This study has been approved by the research ethics committee of the Faculty of Medicine, Universitas Andalas (No. 30/UN.16.2/KEP-FK/2023).

Cataract induction was performed by a single intraperitoneal injection of sodium selenite at a dose of 20 µmol/kg body weight. The rats were monitored daily until they were terminated. One day after sodium selenite injection, topical glutathione 5 mg was administered once daily as one drop per application for 7 days in group 2 and for 21 days in group 3.

Topical glutathione 5 mg was prepared from a vial of glutathione powder containing 600 mg/5 ml of glutathione per vial, which was dissolved in a sodium phosphate buffer solution to achieve a dose of 5 mg in 5 ml. The pH level of the solution was measured using litmus paper. The pH value of the GSH solution was adjusted to 7.2-7.4 before use. The topical solution was instilled once daily as one drop. This glutathione solution was prepared daily. On day 7 for groups K+ and P1, and day 21 for group P2, mydriatic eye drops were administered to both eyes to achieve mydriasis, then the lens opacity was examined using a loupe and penlight. The more opaque eye was selected for lens extraction, and lens samples were taken to measure MDA levels.

The lens extraction procedure was performed by the researcher and a pharmaceutical analyst. Anesthesia was administered using ether. The lens was identified, extracted completely, and rinsed with physiological saline to prevent tissue contamination. The rats' lenses were placed in a capped microtube containing 0.9% NaCl solution, with one sample per container. The samples were temporarily stored in a cooler bag (temperature ≤ 2°C) and immediately stored in a freezer (temperature -20°C) before being analyzed at the biochemistry laboratory, Faculty of Medicine, Universitas Andalas, Padang.

The lens was homogenized and centrifuged at 10,000 rpm for 10 minutes at 40°C. Then, MDA analysis was performed using the TBA reagent. Data analysis was carried out using SPSS software version

25. Univariate analysis was conducted to present the frequency distribution of the test variable. Bivariate analysis was performed to assess the mean differences between the test groups, with $p<0.05$.

3. Results

Table 1 shows a difference in MDA levels among the three treatment groups. The mean MDA level in the group that did not receive topical glutathione (K) was higher (1.91 nmol/ml) compared to the group that received glutathione eye drops for 1 week (P1) with a level of 1.38 nmol/ml, and the group that received glutathione eye drops for 3 weeks (P2) with a level of 1.2 nmol/ml. The MDA level in the lens of rats in the group that received glutathione eye drops for 1 week (P1) was higher than that in the group that received glutathione eye drops for 3 weeks (P2). These differences were statistically significant, with a p-value less than 0.05.

Table 1. The differences in MDA levels between groups.

Groups	N	Lens MDA levels (nmol/ml)	P-value
		Mean ± SD	
K+	10	1.91 ± 0.24	0.001*
P1	10	1.38 ± 0.074	
P2	10	1.20 ± 0.13	

*One-Way ANOVA, P<0.05.

4. Discussion

The source of oxidative stress in cells is the formation of reactive oxygen species (ROS). Most oxidants that lead to oxidative stress in the lens are activated by ROS, which are one-electron reduction products of molecular oxygen, such as superoxide, hydrogen peroxide, hydroxyl radicals, and activated oxygen species called singlet oxygen. Free radicals or singlet oxygen molecules react with unsaturated fatty acids, leading to lipid peroxidation, which is referred to as the auto-oxidation process. Lipid peroxidation is involved in the pathogenesis of cataracts because peroxidation products induce soluble protein fragments in the lens, damage membrane structure and epithelial cell apoptosis, and are associated with increased lens opacity and refractive changes. One of the most commonly used biomarkers to indicate the level of lipid peroxidation is the concentration of malondialdehyde (MDA). Malondialdehyde is a degradation product or byproduct of lipid peroxidation and is tested calorimetrically after reacting with thiobarbituric acid.[14-16]

Lens damage caused by sodium selenite induction and the protective effects of topical antioxidant glutathione were assessed by the increase in average lens MDA levels compared to the positive control group. Thirty experimental rats were divided into three groups. In this study, a hypothesis test was conducted to compare whether there were differences in the average levels of MDA in the lenses of cataract-induced experimental rats with or without topical glutathione administration. The statistical test used to determine the significance level (95%) was one-way ANOVA for three variables.[14,16,17]

Based on the table of average lens MDA levels in this study, there was an increase in the average MDA levels in the lenses of experimental rats injected with sodium selenite. The mean MDA level in the group that did not receive topical glutathione (K+) was 1.91 nmol/ml, which was higher than the group that received glutathione eye drops for 1 week (P1) with a level of 1.38 nmol/ml, and the MDA level in the lenses of rats in the group that received glutathione eye drops for 1 week (P1) was higher than the group that received glutathione eye drops for 3 weeks (P2) with a level of 1.2 nmol/ml. There was a statistically significant difference in the average MDA levels between groups K+, P1, and P2, with a p-value less than 0.05.

The MDA levels in the positive control group were slightly higher in sample no. 1 (2.22 nmol/ml) and sample no. 7 (2.31 nmol/ml) compared to the average

MDA level of the positive control group (1.91 ± 0.24 nmol/ml). The MDA levels in group P1 were slightly higher in sample no. 2 (1.48 nmol/ml), sample no. 5 (1.50 nmol/ml), and sample no. 7 (1.47 nmol/ml) compared to the average MDA level of group P1 (1.38 ± 0.074 nmol/ml). The MDA levels in group P2 were slightly higher in sample no. 4 (1.43 nmol/ml) and sample no. 6 (1.38 nmol/ml) compared to the average MDA level of group P2 (1.2 ± 0.13 nmol/ml), and sample no. 9 (0.96 nmol/ml) had a lower MDA level than the average MDA level of group P2. However, after analyzing the data using boxplots for each group, no outliers or extreme values were found.

The differences in MDA values in each sample may be influenced by internal factors, such as endogenous ROS in the mitochondria of each sample, including the metabolic rate of each cell. ROS is formed in cells as a consequence of endogenous and exogenous oxidative stress triggers, including changes in cellular respiration, viral infections, and immune activity. Endogenous ROS is the result of normal cellular metabolism (carbohydrates and proteins) within the cell. Thus, the degree of stress, physical activity level, and nutrition of each experimental rat can also affect the level of oxidative stress in their own cells.[6,14,18]

The reaction of free radicals with polyunsaturated fatty acids (PUFAs) present in the lens cell membrane can cause lens protein degradation, damage to the lens membrane structure, and increased lens opacity through the process of lipid peroxidation (LPO) that generates malondialdehyde (MDA) compounds. MDA is the end product of lipid peroxidation in cell membranes induced by oxygen free radicals. Lipid peroxidation represents oxidative tissue damage caused by ROS, which can lead to changes in membrane structure. Lipid peroxidation occurs when ROS extract unpaired electrons from membrane lipids, ultimately resulting in lipid degradation. MDA is more stable compared to other lipid oxidation products. Many studies have shown an increase in MDA levels in cataract lenses, both in human and animal models.[6,14]

Normal lenses are equipped with natural antioxidant protection mechanisms, but as age increases and continuous exposure to oxidative stress occurs, the natural antioxidant defense mechanism of the lens can be disrupted. Glutathione plays a key role in oxidative stress protection. GSH has a short half-life of 28 hours, and within 48 hours, GSH is already eliminated from the lens.[16-18]

Glutathione is a tripeptide compound formed from glutamate, glycine, and cysteine. Glutathione is found in various tissues of the human body, with the highest concentration in the liver and lens. The lens is rich in glutathione. The decrease in GSH levels has been shown to occur in the lenses of aging humans and in cataract formation. GSH depletion has also been observed in normal aging rabbit lenses, while a slight decrease in GSH levels in rabbit lenses was found after hyperbaric oxygen treatment. Decreased GSH concentration or a shift from GSH to GSSG in the lens is believed to increase the rate of post-translational modification of crystallins and perpetuate damage to key proteins containing thiol groups, including Na/K-ATPase, cytoskeletal proteins, and membrane permeability-related proteins.[19-21]

Two types of antioxidant systems (general detoxification and enzymatic) usually detoxify reactive oxygen species to maintain lens homeostasis. These include GSH, Manganese superoxide dismutase (MnSOD), CuZnSOD, catalase, peroxiredoxins, and glutathione peroxidase. Simultaneously, metallothionein and ferritin function to limit access to free metals. Failure of these antioxidant systems leads to protein oxidation, loss of protein function, and protein aggregation. Specific repair systems include methionine sulfoxide reductases and thioredoxin, which can repair proteins and restore their normal function. Chaperone proteins such as α-crystallin, β-crystallin, and Hsp27 can eliminate toxic protein aggregates. Failure of the defense and repair antioxidant systems results in irreversible protein aggregation, loss of lens cell function, and ultimately cataract formation.[17,19,22]

The lens is equipped with several enzymes that work together to scavenge superoxide anion (O_2–) to protect against free radicals or oxidative damage. These enzymes include superoxide dismutase, which catalyzes the breakdown of superoxide anion (O_2–). Catalase, which breaks down hydrogen peroxide

produced by superoxide dismutase, and glutathione peroxidase, which catalyzes reactions resulting in the formation of glutathione disulfide (GSSG), which is then converted back to glutathione (GSH) by glutathione reductase using the pyridine nucleotide NADPH. The main source of NADPH in red blood cells is the HMP shunt, which provides NADPH as a reducing agent. Thus, glutathione indirectly acts as the main eliminator of free radicals in the lens.[10,19,23]

5. Conclusion

Topical glutathione can reduce the oxidative stress level on the lens, as evaluated by the lens' MDA levels.

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