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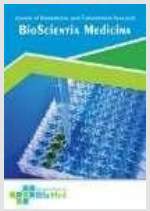

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Efficacy of N-Acetylcysteine (NAC) Against Malondialdehyde (MDA) Levels in Diabetic Cataracts: In vivo Study

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A B S T R A C T

Background: Diabetes mellitus is a systemic condition that affects various organs, including the eyes. N-acetylcysteine (NAC) functions as an antioxidant because it belongs to a thiol group synthesis glutathione. However, the availability of cysteine in the body is only 15%, so cysteine supplementation can help with oxidative stress in diabetic cataracts. That it will prevent the reaction of lipid peroxidase and the formation of PUFA in the lens membrane, which causes damage to lens cells and is characterized by an increase in malondialdehyde. This study aims to determine the comparison of malondialdehyde levels in the lenses of diabetic cataract rats given or without topical NAC. **Methods:** 36 rats were divided into the control group (received streptozotocin) and treatment group (received streptozotocin and topical NAC. Intraperitoneal injection of streptozotocin was given 90 mg/kg BW and performed once a week for 3 weeks. GDP examination was performed the next day after the rats fasted for 16 hours from the vein. In the tail, GDP > 110 mg/dl categorized as diabetes. Topical NAC was given 4 times per day for 3 weeks. At the end of the study, the lens was extracted for the measurement of malondialdehyde. **Results:** The mean MDA levels in the control group were higher (2.90±0.71nmol/ml) than in the treatment group (2.33±0.38nmol/ml), $p<0.05$. **Conclusion:** NAC was effective in reducing oxidative stress levels in diabetic cataract lenses by lowering MDA levels better than the group that did not receive MDA in vivo.

1. Introduction

A cataract is a clouding in the lens that can occur due to the hydration process (increase in fluid) of the lens, the process of lens protein denaturation, or both. Cataracts are the leading cause of blindness in the world. It is estimated that worldwide there are 50 million people with blindness, and almost half of them are caused by cataracts. By 2050, the number of cataracts in the United States is expected to double from 24.4 million to 50 million. Data from the WHO in 2015 in the United States showed that as many as 24,409,978 people suffered from cataracts. This figure had increased when compared to data from 2005 when there were 20,476,040 cataract sufferers. This figure shows that there has been an increase of almost 4 million people in the span of 10 years. A cataract is also the main cause of blindness in Indonesia, which is 70-80%. According to the Rapid Assessment of Avoidable Blindness (RAAB) survey, the prevalence of blindness in Indonesia is 3%, with the largest number caused by cataracts (0.78%), glaucoma (0.20%), and followed by refractive errors (0.14%). Meanwhile, the prevalence of blindness in the Indonesian population aged 50 years and over, based on the results of the RAAB in the 15 provinces, is around 1.7% in West Sumatra.[1]

The lens of the eye is an avascular organ that is

located in the back of the eye and is surrounded front by aqueous fluid. This aqueous fluid is a source of nutrition for the lens and also functions as a reservoir for metabolites excreted by the surrounding tissue. The mechanism of glucose toxicity in diabetes mellitus that causes diabetic cataracts can basically go through three pathways, 1. Due to increased activity of the enzyme aldose reductase, which causes the formation of sugar alcohol, sorbitol, and galactitol in the crystalline lens. 2. Through a non-enzymatic glycation process where glucose which has a reactive carbonyl compound (C=O), will bind to the amino group of the lens crystalline protein (-NH_2). This reaction will cause a decrease in the level of protein solubility. 3. At high blood glucose levels, there will be a process of glycol oxidation which causes oxidative stress conditions. A normal eye lens is actually equipped with a natural antioxidant protection system, but age and continuous exposure to oxidative stress can cause disruption of the natural antioxidant protection mechanism of the eye lens. The reaction of free radicals with polyunsaturated fatty acids (PUFA) found in the lens cell membrane will cause lens protein degradation, damage the lens membrane structure, and increase lens opacity through the lipid peroxidase (LPO) process, which produces malondialdehyde compounds. (MDA). Oxidative reactions on the lens membrane also cause disruption of the active transport mechanism of nutrients and electrolytes from the aqueous humor to the lens, disruption of the composition of intracellular components of the lens, and disruption of the electrolyte balance of potassium, sodium, and calcium of the lens that plays a role in the pathogenesis of cataracts.[2,3]

N-acetylcysteine (NAC) is a form of the amino acid cysteine. Basically, NAC functions as an antioxidant. NAC belongs to the thiol group as well as glutathione sulfhydryl (GSH) and can be used as a substitute for GSH because it has an active sulfhydryl group (-SH) so that it can become a hydrogen donor after entering the cell and being hydrolyzed into cysteine. The –SH group is reactive to molecules with unpaired electrons (free radicals), where this cysteine will form a disulfide bond to become cystine. Cysteine can be an antioxidant by preventing the accumulation of hydroxyl radicals (OH) by catalyzing it into H_2O, and cysteine can also increase GSH levels by entering the glutathione metabolic pathway (stimulating glutathione synthesis together with glycine and glutamic acid). However, the availability of cysteine in the body is only 15-20% compared to total glycine and glutamate, so exogenous cysteine supplementation can help in chronic oxidative stress processes.[4-6] This study is one of the earliest studies aimed at exploring the potential of NAC in suppressing oxidative stress processes in diabetic cataract lenses as assessed by the expression of MDA protein in vivo.

2. Methods

The research design was an experimental study with a posttest-only approach with a control group design in vivo to determine the difference in MDA levels in the lens of white rats induced by diabetic cataracts and in the group not induced by diabetic cataracts. A total of 36 Wistar White Rats were used in this study, with the inclusion criteria being male, weighing 20-30 grams, 8 weeks old, a health condition characterized by active movement, not being alone in the corner of the cage, clean fur, no defects, and clear eyes. This study has been approved by the research ethics committee of the Faculty of Medicine, Universitas Andalas.

Experimental animals were acclimatized for 7 days before induction with streptozotocin (STZ). STZ induction was administered intraperitoneally at a dose of 90 mg/kg BW every week. Experimental animals were grouped into a control group that was induced only with STZ and a treatment group that received STZ induction and topical NAC. STZ induction was administered intraperitoneally at a dose of 90 mg/kg BW every week for 4 weeks. NAC was administered topically 4 times a day for 28 days. After 28 days, the organ was evacuated from the white rat's eye lens by first being anesthetized with ketamine at a dose of 80 mg/kg BW intraperitoneally. The evacuated lens was placed in a physiological solution of 0.9% NaCl. The

lens was homogenized and centrifuged at 10,000 rpm for 10 minutes at a temperature of 40°C.

Tools and materials needed for MDA examination; 200 µl pipettes, pipette tip, stir bar, polypropylene microcentrifugation tube, semi-micro cuvette, spectrophotometer, vortex, magnetic stirrer, water bath, 2-thiobarbituric acid, glacial acetic acid, sodium hydroxide, malondialdehyde bis, and aquabidest. Preparation of the reagent begins with making a TBA reagent by dissolving 0.67 g 2 thiobarbituric acid in 100 ml aquabidest, then adding 0.5 g sodium hydroxide and 100 ml glacial acetic acid. Next, make a standard serial solution and 125 µl MDA stock solution dissolved in aquabidest. MDA levels were checked by adding 100 µl of sample (blood plasma) or standard into a labeled centrifuge tube. In each tube added aquabidest 0.9 ml in the sample, then added TBA reagent 0.5 ml. The tube containing the solution was then heated in a water bath at a temperature of 95 degrees Celsius for 1 hour. Then it was centrifuged at 7000 rpm for 10 minutes. The absorbance obtained was measured using a spectrophotometer at a wavelength of 532 nm.

Data analysis was carried out with the help of SPSS version 25 software. Univariate analysis was carried out to present the frequency distribution of variables test. Bivariate analysis was performed to assess the mean difference between the test groups, with $p< 0.05$.

3. Results

Table 1 shows that there are differences in MDA levels between the 2 treatment groups. The mean value of MDA levels in the group that did not receive topical NAC (K) was higher (2.90 nmol/ml) than the group that received NAC eye drops (P), namely 2.33 nmol/ml. Then by statistical test, it was found that there was a significant difference between the average levels of MDA in the lenses of experimental mice that were not given topical NAC and those given topical NAC ($p<0.05$).

Table 1. Comparison of the mean lens MDA levels of diabetic cataract rats with and without topical NAC

Group	**N**	**Lens MDA levels (nmol/ml)**	***P-value***
		Mean ± SD	
Controls induced only by STZ	18	2.90 ± 0.71	0.005*
Induction of STZ + topical NAC	18	2.33 ± 0.38	

*Unpaired T-Test

4. Discussion

NAC can slow the progression of cataracts because it contains thiol/sulfur groups which act as antioxidants. The hydrogen atom in the (-SH) group contained in NAC contains many oxidants that function as electron donors to neutralize free radicals. Glutathione is the dominant antioxidant in the cell cytoplasm, which is the synthesis of the three amino acids, which are a combination of glutamic acid, glycine, and cysteine. Since NAC is a precursor of the amino acids cysteine and reduced glutathione (GSH), administration of NAC results in an increase in endogenous cysteine levels, thereby stimulating glutathione synthesis when demand increases, strengthening glutathione-dependent enzyme activity, and increasing the antioxidant activity. About 20% - 40% is in the form of oxidized glutathione (GSSG). Then GSSG will be converted into GSH by the enzyme glutathione reductase, and in this reaction, NADPH is needed. This cycle (glutathione redox cycle) is the main defense mechanism against reactive oxygen compounds in the eye lens. Due to the low NADPH in diabetes mellitus, it is suspected that the activity of the glutathione redox cycle will also decrease,

resulting in a decrease in GSH. This is where the role of NAC as an indirect antioxidant is as a precursor of GSH to increase the amount of GSH as an antioxidant. Oxidative stress conditions due to hyperglycemia are characterized by an increase in ROS, such as superoxide (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). The sulfhydryl group (-SH) in NAC also functions as a direct antioxidant that binds the ROS so that they are no longer harmful oxidants to the body.[7-14]

Lipid peroxidase (LPO) can damage cell membrane permeability, causing the release of several ions, including thiol. This results in a decrease in the amount of thiol, which is one of the forming glutathione. NAC, with its sulfhydryl group (-SH), which is a thiol group, can restore the amount of thiol that comes out due to LPO so that the free radical process does not continue. One of the processes of diabetic cataract formation is through non-enzymatic glycation, where the presence of glucose in cells will bind to amino groups of lens proteins, especially crystalline lenses, and will undergo *cross-linking* to form water-insoluble proteins. So under normal conditions, the percentage of water-insoluble protein is only 20%, but in diabetes mellitus conditions, this percentage increases. This is what causes the appearance of protein aggregation. The NAC in this prevents the cross-linking process so as to prevent the buildup of water-insoluble proteins.[15-17]

Research on the use of NAC as an antioxidant in cataracts has been carried out, although with different oxidant biomarkers from this study (malondialdehyde). A study stated that administration of topical NAC twice a day for 2 weeks in acetaminophen-induced rat cataracts resulted in an increase in thiol transferase (TTase) when compared to untreated mice. The study explained that the presence of NAC exerts an antioxidant protective effect by stimulating the formation of GSH in lens cells.[16] Another study stated that a group of naphthalene-induced cataract rats experienced glutathione and an 86.6% decrease in hepatic glutathione. Meanwhile, the group of cataract rats in that study that was cultured with glycine and cysteine for 48 hours only experienced lenticular glutathione levels of 34% and glutathione of 26% ($p = 0.02$). This shows that cysteine and glycine can help maintain glutathione levels around 2/3 of normal values.[18-20] In addition, the administration of glycine and cysteine also reduced the incidence of cataracts by 66% of the total sample of naphthalene-induced rats.[21]

5. Conclusion

NAC was effective in reducing oxidative stress levels in diabetic cataract lenses by lowering MDA levels better than the group that did not receive MDA in vivo.

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