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RESEARCH ARTICLE

Effect of Isolate Catechin GMB4 in Expression of GRP78 and Tunel Assay in Rat Cataract Model

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Abstract

This research is to explore glucose-regulated protein 78 (GRP78) expression and Tunel assay, signs of stress of endoplasmic reticulum (ER) and apoptosis in rat cataract model. Cataract was formed by inducing a subcutaneous injection of 19 µmol/kg sodium selenite to ten-day-old Wistar rats. The neonatal rats were divided into five groups randomly (n=5 in each group): a control group, and four cataract-induction groups, treated with either0, 50, 100, 200 mg/kg catechin Isolate From GMB4 Clone Green Tea. We performed slit-lamp biomicroscopic analysis, immunohistochemistry for GRP78 and Tunel. Both eyes of all rats in Group 1 did not exhibit cataract formation. In Group 2, one out of five (20%) developed grade 3 cataracts and the remaining four out of five (80%) developed grade 4 cataracts. The grade of cataract formation decreased in groups 3, 4 and 5. The diff erence in exhibited cataract in the lens of all rats between Group 2 and any eyes of groups 3 or 4 and 5 were significant (P = 0.022, 0,001, 0,001). The mean amount of Tunel cells and GRP78 of group II rats were significantly ($P < 0.01$) higher than the levels in Group I, Group III, Group IV and Group V. Stress of Endoplasmic reticulum and apoptosis in the lens that increased following cataract formation in rats was suppressed by catechin Isolate From GMB4 Clone Green Tea.

Keywords: *Cataract, Endoplasmic reticulum stress, Apoptosis, Catechin.*

Introduction

A cataract is defined as any opacification of the lens that causes symptoms, including decreased visual acuity, decreased color perception, decreased contrast sensitivity, and glare disability, which eventually result in blindness. 1 According to the World Health Organization, 25 million people are affected by cataracts, which is also the leading cause of blindness worldwide. 2.3 Cataract is a major health problem and the major cause of blindness throughout the world caused by opacity that develops in the crystalline lens of the eye, [1-3].

Although surgery is the best treatment, it is not free of complications. Attempts to prevent cataract formation, or at least significantly retard the onset of the disease would be of great value [4, 5]. Pathogenesis of Cataracts is multifactorial, such as metabolic disorders, malnutrition, age-related changes or other pathways. Several risk factors, such as radiation and toxic damage to the lens, oxidative damage, impaired glucose metabolism, also play an important role in the pathogenesis of cataracts.

One of the most common types of cataracts is related to age. The accumulation of unfolded proteins in the ER lumen causes Endoplasmic reticulum (ER) stress [6, 7]. ER stress has been proven as a self-protection mechanism of cells; it can restore the homeostasis within the ER. However, ER stress that isn't treated and ongoing will eventually cause apoptosis namely ER stress-mediated cell death [8].

Recently, ER stress-mediated apoptosis pathway has been proven to play a crucial role in the degenerative pathophysiology of neurodegenerative disorders [9-13] retinitis pigments, and another eye disease [14-17]. Glucose-regulated protein 78 (*GRP78*) are the key markers of ER stress. The mechanisms during ER stress: GRP78 is involved in the unfolding protein reaction and the protection mechanism during ER stress.

The expression of GRP78 is an indication of ER stress, while apoptosis is detected by Tunel assay. The aim of this study is to investigate the expression of GRP78 and Tunel Assay in the Cataract model, the involvement of ER stress and its association with the apoptosis of lens epithelium cells, in rat cataract model.

Green tea, which contains polyphenol flavonoid compounds called catechins as its main component, is widely consumed throughout Asia [18]. One hundred grams of green tea contained 12 to 14% isolated catechins. The result of HPLC analysis showed that EGCG and ECG were main components from catechins isolation of green tea GMB-4 clone [19]. Catechins are a class of catechin compound group consisting of epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), catechin, gallocatechin, catechin gallate, and gallocatechin gallate. EGCG is predominant catechin which has content from 48% up to 55% in total polyphenols of green tea leaves [20]. Neither in-vivo nor in-vitro research on catechins isolated from green tea GMB4 clone in relation to cataract has ever been performed. As such, this study was needed to understand the effects of catechins isolated from GMB4 clone on the expression of GRP78 and Tunel Assay in the rat cataract model.

Materials and Methods

This study was performed in Biosains Laboratory of Brawijaya University. Twenty-five Wistar-albino rat pups were housed with their mother in special wire-bottom cages and in standard conditions (12-hour daylight-dark cycle, ventilated, constant room temperature). It has been considered that solid-bottom cages are more adequate for the housing of the rat pups. The rat pups were divided into five groups (four experimental and one control), each consisting of five pups. Group 1 received only a subcutaneous saline injection and was the control group. In Group 2, sodium-selenite (19 nmol/g body weight, Sigma Chem.

Co., St Louis, USA) was injected subcutaneously on a postpartum Day 10. In Group 3, subcutaneous sodium-selenite (19 nmol/g body weight) was injected on a postpartum Day 10 and injection of isolate catechin intraperitoneally (50 mg/kg body weight), starting one day before sodium-selenite injection (on postpartum Day 9) and was continued for 5 days (till postpartum Day 13). The procedures performed on Group 3 rats were also performed on Group 4 and Group 5, the diff erence being the dosage of isolate catechin. Group 4 had used 100 mg/kg body weight of isolate catechin and Group 5 was 200 mg/kg body weight.

Table 1: Treatment groups studied

GROUPS (*N* = 5)	INJECTIONS (DAY 9,10,11,12, 13)	INJECTION (DAY 10)
GROUP 1	Saline	Saline
GROUP 2	Saline	Na_2SeO_3(19 µmol/kg BW)
GROUP 3	Catechin 50 mg/KgBW	Na_2SeO_3(19 µmol/kgBW) + Catechin 50 mg/kg BW
GROUP 4	Catechin 100 mg/KgBW	Na_2SeO_3(19 µmol/kgBB) + Catechin 100mg/kgBW
GROUP 5	Catechin 200 mg/KgBW	Na_2SeO_3(19 µmol/kgBB) + Catechin 200mg/kgBW

On a postpartum day 17, all rats were anesthetized with intraperitoneal ketamine injection (80 mg/kg BW) and xylazine (15 mg/kg BW). The rat pups were taken out and the pupils were dilated with tropicamide 0.5% every 30min for

two hours. All lenses were evaluated and were morphologically staged for cataract development and grading was performed by slit-lamp biomicroscopy on a scale of 0 to 4 as follows in Table 2 [26]:

Table 2: Grade of Lenticular Opacification

GRADE	
GRADE 0	Normal transparent lens
GRADE 1	The lens with a subcapsular opacity
GRADE 2	Was a nuclear cataract
GRADE 3	Was a strong nuclear cataract with opacity in the perinuclear area
GRADE 4	Was a mature dense opacity involving the entire lens

Lens photos ×25 magnifications were taken using a camera attached to slit-lamp (Topcon, Tokyo, Japan) (Figure 1). The lens was then taken immediately after euthanasia, the eyes were enucleated. Frozen lens samples were weighed and homogenized in ice-cold phosphate-buffered saline solution (0.01 mol/L and pH 7.4). Homogenization procedures were carried out using Bullet Blend tissue Homogenizer (Next Advanced Inc, Averill Park, NY, USA), according to the manufacturer's instructions at 4 ℃.

These homogenates were centrifuged at 10 000 g for 30min at 4 ℃, and supernatants were obtained. Supernatants were used for the measurement of the levels of GRP78 and Tunel assay using a GRP78 and Tunel assay kit (ImmuchromGmbH, Hessen, Germangradey). Data are presented as mean ± standard deviation and differences between groups were analyzed using one-way ANOVA with SPSS 17.0 Statistical Package. The post-doc test was used in the ANOVA was significant. P< 0.01 was considered statistically significant. This study was approved by the Institutional Review/ Ethics Board of Brawijaya University [ref: 1114-KEP-UB, dated 24th April 2019]. All methods were performed in accordance with guidelines and regulations.

Results

The mean GRP78 levels lenses (13, 33 ± 2,875) of Group II rats were significantly (P <0,001) higher than the levels in Group I lens I (4,833 ± 2,639), Group III (10,833 ± 2,926), Group IV (9,667 ± 1,751), and Group V (6,333 ± 1,329) (Figure 2). A significant difference was also observed in the GRP78 level in the lens (P <0.001) between group III and group I. The GRP78 level in the lens decreased and the level of lens opacity increased in group II. Furthermore, the GRP78 level decreased gradually and the opacity level decreased in accordance with the administration of catechin doses (groups III, IV, and V).

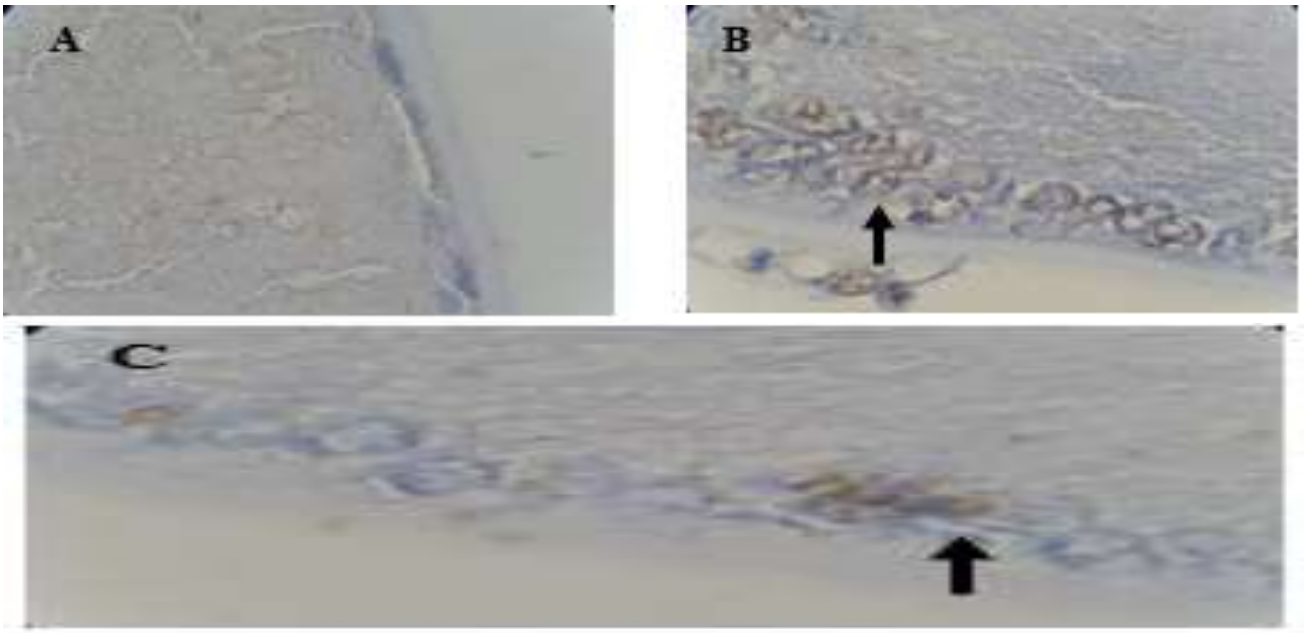

Figure 1: Effect of catechin on GRP78 in the lens-induced by cataracts. (A) control group, (B) cataract-induction group, (C) cataract-induction and 200 mg/kg BW catechin group. The sections were stained for GRP78 immunoreactivity (Brown)

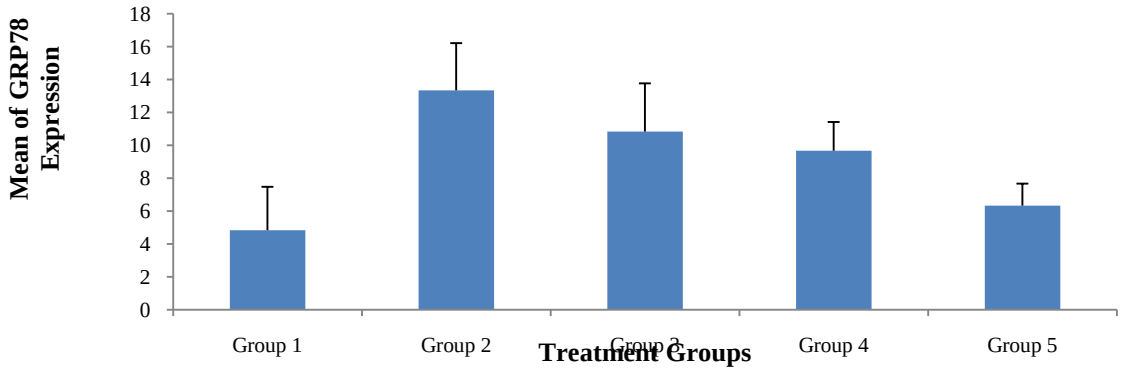

Figure 2: Expression of GRP78 in Each Treatment Group. Results are expressed as the mean ± SD. *P<0, 01 vs Group 1, #P<0, 01 vs. Group 2

GRP78 levels in the lenses from the Na2SeO3 group were found to be significantly (p <0.01) higher than those in the control group and the catechin group. Treatment with catechins in the catechin + Na2SO3 group (Figure 2) significantly (p <0.01) decreased GRP78 levels.

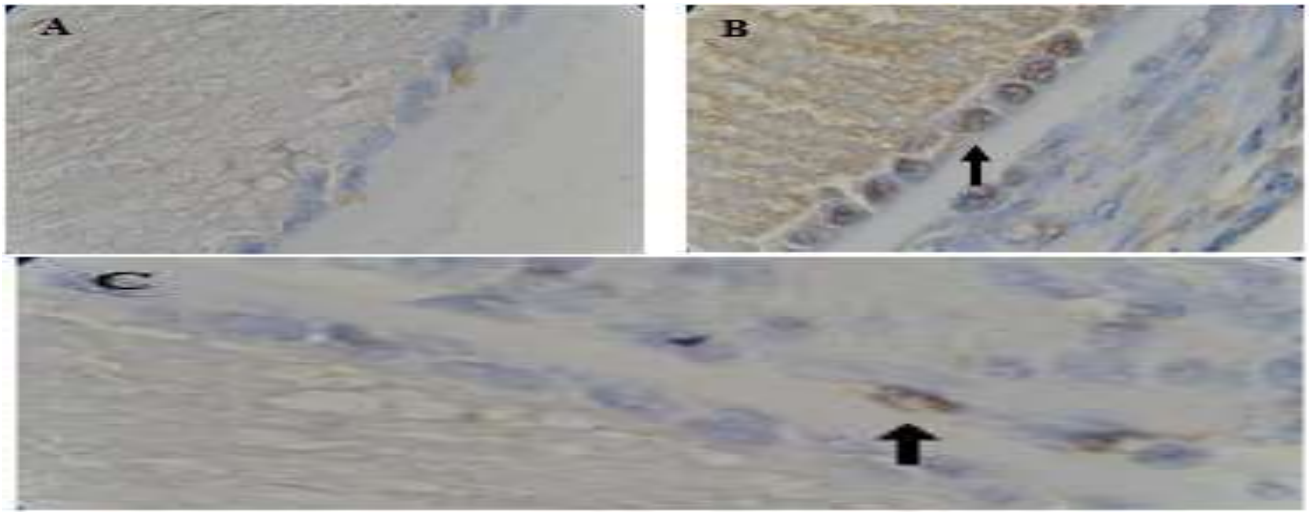

Figure 3: Effect of catechin on DNA fragmentation in the lens epithelium induced by cataract. Photomicrographs of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) positive cells in the lens epithelium. (A) control group, (B) cataract-induction group, (C) cataract-induction and 200 mg/kg BW catechin group

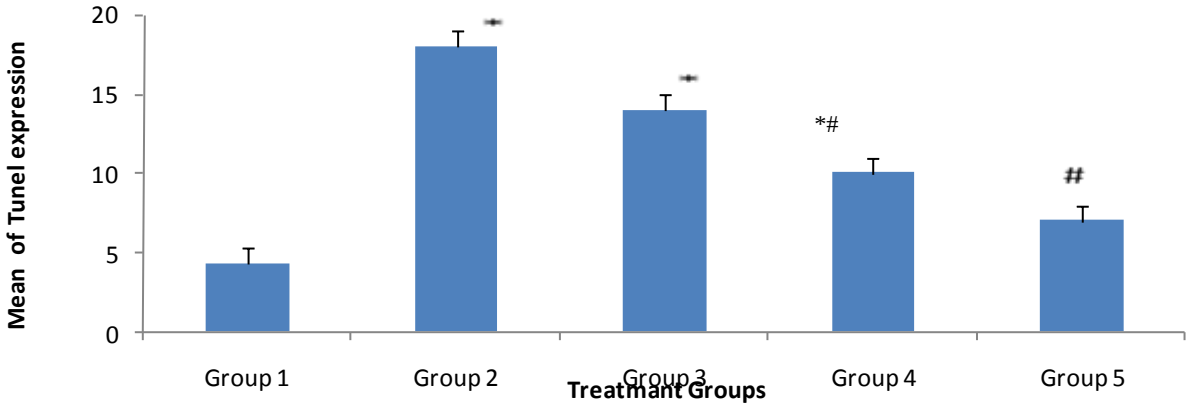

Figure 4: Expression of Tunel in Each Treatment Group. Results are expressed as the mean ± SD. *P<0, 01 vs Group 1, #P<0, 01 vs. Group 2

The mean number of Tunel cells in Group II mice (18 ± 1,264) was significantly (P <0.001) higher than the number of Tunel cells in Group I (4.3 ± 2.136), Group III (14 ± 2.562), Group IV (10 ± 2,136), and Group V (7 ± 2,097) (Figure. 4). A significant difference was also observed in the number of Tunel cells in the lens (P <0.001) between group III and group IV. The number of Tunel cells in the lens decreased and the level of lens opacity increased in Group II. Furthermore, the number of Tunel cells decreases gradually and the level of opacity decreases according to the administration of catechin

doses (groups III, IV, and V).

Discussion

Radiation, galactose, streptozocin, and selenite are used in most experimental cataract models to cause cataracts [21-23]. Selenite cataract is similar in many respects to human cataracts. In 1978, Ostadalova *et al.* first used selenite, which is one of the most widely used pharmacological agents in experimental cataract studies, in such a sample [23].

The basic mechanism of selenite in the formation of cataracts is that it acts as an oxidant, thereby causing damage to the lens [23-27]. It also causes lipid peroxidation in the crystalline lens, induces hydrogen peroxide, and lowers GSH in the lens [27]. Effects of sodium selenite towards retinal cells have been researched and resulted in increased expression of GRP78 that shows reticulum endoplasmic stress in the retina but research on the effect on lens epithelium has not yet been conducted.

We found that GRP78, a stress protein on ER and an important marker for the protection mechanism of ER stress, was elevated after Cataract. Stresses like oxygen deficiency, low glucose, and low Ca2+ can lead to disruption of protein metabolism in cells; then the accumulation of unfolded protein response (UPR) elevates GRP78 expression to keep the ER homeostasis improving protein synthesis and transport [29, 30].

Recent studies revealed that UPR had a significant role in neuronal degenerative disorders, such as Alzheimer's disease, but few reported the role of UPR in cataracts. The existence of a protective mechanism against apoptosis during ER stress after Cataract provides us a window of opportunity for recovery of visual function before the occurrence of irreversible widespread damage.

How to promote the protection mechanism of ER stress and attenuate injury mechanism after the cataract is a potential interest of study in the future. Cataract formation is one of the most common causes of irreversible visual loss associated with aging, thus much interest is being laid on recognition of a drug that will help to prevent or treat cataractogenesis. The present investigations were undertaken to determine the efficacy of isolated catechin from green tea GMB 4 to prevent the progression of cataract on in vivo animal models. A cataract is a protein deterioration disorder characterized by irreversible modification and accumulation of lens proteins. Once the cataract is formed it cannot be reverted back; thus the study is focusing on the prevention of lens opacity and it lacks a positive control.

Selenite-induced cataract model (a single subcutaneous injection of sodium selenite at a dose of 19 µmol/kg BB is the universally accepted animal model for studying oxidative stress induced experimental cataract as it shows almost all the events associated in human age-related cataracts such as membrane damage, calcium accumulation, endoplasmic reticulum stress, lenticular apoptosis, and proteolysis of lens proteins [17].

It is essential to check whether the anti cataractogenic potential of Catechin is by the prevention of endoplasmic reticulum stress or lenticular apoptosis. Several studies have shown that the presence of significant amounts of vitamins, carotenoids, caffeine, acetyl-L-carnitine, ebselen, quercetin, flavonoids, phenyl ester of caffeic acid and curcumin exerts inhibitory effects on cataracts [30-35].

Nevertheless, no agent can completely block or delay lens opacification. Recent studies have shown that the green tea catechins have antioxidant, anti-inflammatory, antiangiogenic and antibacterial effects [36-40]. Such catechins bind reactive oxygen and nitrogen species and exert indirect antioxidant effects by stimulating the synthesis of endogenous antioxidant enzymes, such as superoxide dismutase, glutathione reductase, glutathione-S-reductase, catalase, and quinone reductase. Because of those results, green tea will inhibit lipid peroxidation and DNA mutation. Green tea has high levels of catechin and shows more strongly antioxidant activity than vitamins C and E [38-42].

A recent study has shown that catechin may effectively protect individuals from corneal surface diseases, such as dry eye, via its antioxidant and anti-inflammatory effects [43]. Emoto *et al* [44].Indicated that green tea extracts suppresses N-methyl-N-nitrosourea-induced photoreceptor apoptosis in Sprague-Dawley rats, and Cia *et. Al* [45].

Reported that catechin prevents H2O2-induced oxidative stress in the lens epithelial cells. Chen *et al* [46].Reported that eye drops with catechin exhibit potent protective effects on ultraviolet B radiation-induced corneal oxidative damage in mice; the effects are likely due to increased defense system, antioxidant activity, lipid peroxidation inhibition, and protein oxidation inhibition. Recent studies have demonstrated that catechin also protects human γB-crystallin from UV-induced damage and cultured human lens epithelial cells from hyperglycemia-induced damage [47, 49].

Catechin prevents tryptophan oxidation in cataractous human lens γ-crystallin in the presence of H2O2 [47].Heo *et al*. [50-59].Showed that catechin increased cell count and cell viability after the UV irradiation of cultured human lens epithelial cells, indicating that catechin can protect lens epithelium against UV damage.

No studies have thus far been conducted on the preventive effects of catechin in experimental cataract models. This research shows that after sodium selenite is given, lens opacity will occur and followed by an increase in GRP 78 (Figure 1) and cells with positive Tunel (Figure 3). Then the opacity level decrease followed by a decrease in both GRP78 (Figure 2) and cells with positive Tunel (Figure 4) in the group given catechin 50 mg/kg BW (Group 3), 100mg / kg BW (Group 4) and 200mg / KgBW (Group 5). This can be interpreted that sodium selenite causes lens opacity due to endoplasmic reticulum stress and apoptosis in the lens epithelium. Furthermore, catechins are given so as to reduce endoplasmic reticulum stress and apoptosis by decreasing GRP78 and positive Tunel cells and decreasing turbidity from the lens.

This can be interpreted that sodium selenite causes lens opacity due to stress endoplasmic reticulum and apoptosis in the lens epithelium. Furthermore, catechins are given so as to reduce endoplasmic reticulum stress and apoptosis by decreasing GRP78 and positive Tunel cells and decreasing opacity from the lens.

Conclusion

The present research demonstrated that catechin significantly inhibits the development of cataracts by inhibiting reticulum endoplasmic stress and apoptosis.

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