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Catechin Regulates Reticulum Endoplasmic Stress in Rats Cataract Model Catechin in Cataract

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Abstract :

Background : Opacity that develops in the crystalline lens of the eye is called the cataract. Decrease amounts of GRP78 involved in the pathogenesis of cataracts by reticulum endoplasmic stress of lens epithelial cells. Aim : The aim of the study was to evaluate the effects of Catechin on sodium-selenite induced cataract formation and activities of GRP78. Methods : 19 µmol/kg of sodium selenite was injection by intraperitoneal to ten day-old Wistar rats. The rats were separated randomly in five groups (n = 5 in each group): a control group, and four cataract induction groups, treated with 0, 50, 100, 200 mg / kg catechin isolates. GMB4 Clone of Green Tea. By slit-lamp bio microscopic we performed degree of lens opacity and analysis of GRP78 by Enzyme-linked immunosorbent assay (ELISA). Results : Both eyes of all rats in Group 1 no cataract formation. 20% of group 2 were found grade 3 cataracts and the remaining four of five (80%) developed grade 4 cataracts. Cataract in the lens of all rats between group 2 and any eyes from groups 3 or 4 and 5 showed a significant difference (P = 0.022, 0.001, 0.001). The degree of cataract formation in groups 3, 4 and 5 decreased. Grp78 levels in Group I, Group III, Group IV and Group V were significantly higher than the mean lens levels in Group II (P <0.01). Conclusion : Reticulum endoplasmic stress in the lens that increased following cataract formation in rats was suppressed by catechin Isolate From GMB4 Clone Green Tea.

Key Words: Cataract, reticulum endoplasmic stress, GRP78, Catechin Isolate

Introduction

Decreased visual acuity, decreased color perception, decreased contrast sensitivity, and glare defects are symptoms of cataracts that can eventually lead to blindness. 1 in line with the planet Health Organization, 25 million people are littered with cataracts, which is additionally the leading reason for 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,

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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 foremost common forms of cataracts is expounded to age. The accumulation of unfolded proteins within the ER lumen causes Endoplasmic reticulum (ER) stress [6, 7]. ER stress can restore homeostasis in the ER so that it has been shown to be a cell self-protection mechanism [8] [9-13] [14-17]. Glucoseregulated protein 78 (GRP78) are the key markers of ER stress. The mechanisms during ER stress: GRP78 is involved within the unfolding protein reaction and also the protection mechanism during ER stress. GRP78 is an indication of ER stress. The aim of this study is to investigate GRP78 in the Cataract model, the involvement of ER stress of lens epithelium cells, in rat cataract modelGreen tea, which contains catechins or polyphenolic flavonoid compounds as its main component, is widely consumed throughout Asia [18]. 100 grams of green tea contains 12 to 14% isolated catechins. The results of HPLC analysis showed that EGCG and ECG were the foremost components of isolated catechin clones of tea GMB-4 [19]. Catechins are a category 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 GRP78 in the rat cataract model.

Materials and Methods

Twenty-five Wistar-albino rat pups were housed with their mother in special wirebottom cages and in standard conditions (12-hour daylight-dark cycle, ventilated, constant room temperature). It can be seen that the base of the cage is stronger, so it is more adequate to accommodate young rats. Twenty-five pups were divided into five groups (four experimental groups and one control group) so that each of them consisted of five pups. In Group 2, sodium selenite (19 nmol/g body weight) 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 intraperitoneal (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). Group 1 is the control group that only received subcutaneous saline injection. In groups 3, 4, and 5, the procedures performed on mice were the same but with different doses of catechin isolates. Group 4 used catechin isolates 100 mg / kg of body weight and group 5 as much as 200 mg / kg of body weight.

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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	Na2SeO3(19 μmol/kg BW)
GROUP 3	Catechin 50 mg/KgBW	Na2SeO3(19 μmol/kgBW) + Catechin 50 mg/kg BW
GROUP 4	Catechin 100 mg/KgBW	Na2SeO3(19 μmol/kgBB) + Catechin 100mg/kgBW
GROUP 5	Catechin 200 mg/KgBW	Na2SeO3(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

The camera mounted on the slitlamp was used for a photo with a 25x magnification lens (Topcon, Tokyo, Japan) (Figure 1). The lens is then removed immediately after euthanasia and the eye is enucleated. Frozen lens samples were weighed and homogenized in ice-cold phosphate-buffered isosmotic solution (0.01 mol/L and pH 7.4). The Bullet Blend Tissue Homogenizer is used for the distribution of the homogenization procedure (Next Advanced Inc, Averill Park, NY, USA), in line with the manufacturer's instructions at 4 ℃. The supernatant was obtained after the homogenate was centrifuged at 10,000 g for 30 minutes at 4 ℃.

Supernatants were used for the measurement of the degree of GRP78 and Tunel assay employing a GRP78 and Tunel assay kit (ImmuchromGmbH, Hessen, Germangradey). Data are presented as mean ± variance and differences between groups were analyzed using one-way

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ANOVA with SPSS 17.0 Statistical Package. The postdoc test was employed in the ANOVA was significant. P< 0.01 was considered statistically significant. Brawijaya University Institutional / Ethical Assessment Agency has approved this research [ref: 1114-KEP-UB, April 24, 2019]. Guidelines and regulations are used as a reference for all methods.

Result

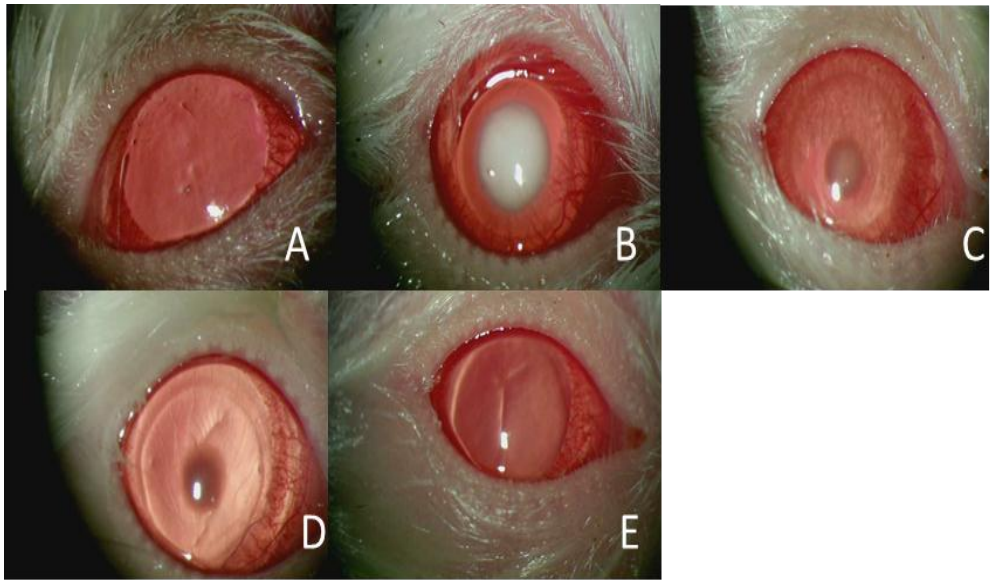

Figure 1: The slit-lamp pictures of representative lenticular opacities

(A) clear lens (grade 0) up to speed cluster, (B) grade IV in cluster of solely sodium-selenite, (C) grade III in sodium-selenite with isolate catechin fifty mg/kg weight cluster, (D) grade II in sodium-selenite with isolate catechin a hundred mg/kg weight, (E) grade I in sodium-selenite with isolate catechin two hundred mg/kg weight cluster. The comparison of the proper eye and left eye with paired samples correlations technique weren't vital (p= 0,749) and paired samples check (p=1,00).

Lenses in each eyes of all management rats remained clear (Group 1) shot of Na2SeO3 (19 μmol/kg) on postnatal day ten was comfortable to induce cataract formation, that was visible by the time the rat pups opened their eyes. scrutiny of the rat pups' eyes with a slit lamp magnifier confirmed that every animals injected solely with Na2SeO3 developed cataracts: one out of five (20%) developed grade three cataracts (Fig. 1C) and conjointly the remaining four out of five (80%) developed grade four cataracts (Fig. 1B). When compared, Na2SeO3 with Catechin five0mg/kg injections showed that the severity of cataract formation decreased; 2 rats out of 5 (40%) developed grade three cataracts (Fig. 1C), 2 rats out of five (40%) developed grade a pair of cataracts (Fig. oneD) and one out of five (20%) developed grade 1 cataract (Fig. 1E) whereas grade four cataract (Fig. 1B) wasn't based. Na2SeO3 with Catechin 100mg/kg injections ablated the severity of cataract formation; one rat out of five (20%) developed grade three cataracts, one rat out of five (20%) developed grade a pair of cataracts whereas 3 out of five (60%) did not develop any cataracts (grade 0). Na2SeO3 with Catechin 200mg/kg injections ablated the

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severity of cataract formation; just one rat out of five (20%) developed grade 1 whereas four out of five (80%) did not develop cataract (grade 0). These results indicated that Catechin particularly two hundred mg/kg pellet indefinite quantity, was flourishing in preventing cataract formation. The grading of the lens in all of the teams is tabulated in Table three, and thus the slit-lamp photos of representative biconvex opacities discovered for each cluster square measure shown in Fig. 1. No cyanogenic cyanogenic to the membrane or mucosa of the eye. This distinction was statistically vital. The comparison between cluster a pair of with cluster one, 4, and five were vital (p= zero.000, 0.000, 0.000) whereas cluster three wasn't vital (p= 0,022). The comparison between cluster three with cluster one and five were vital (p=0.001, 0.003) whereas cluster a pair of and four weren't vital (p= zero.022, 0.253).

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 3). A significant difference was also observed in GRP78 level in the lens (P<0,001) between group III and group I. GRP78 level in the lens decreased and the level of lens opacity increased in group II. Furthermore, GRP78 level decreased gradually and the opacity level decreased in accordance with the administration of catechin doses (groups III, IV, and V).

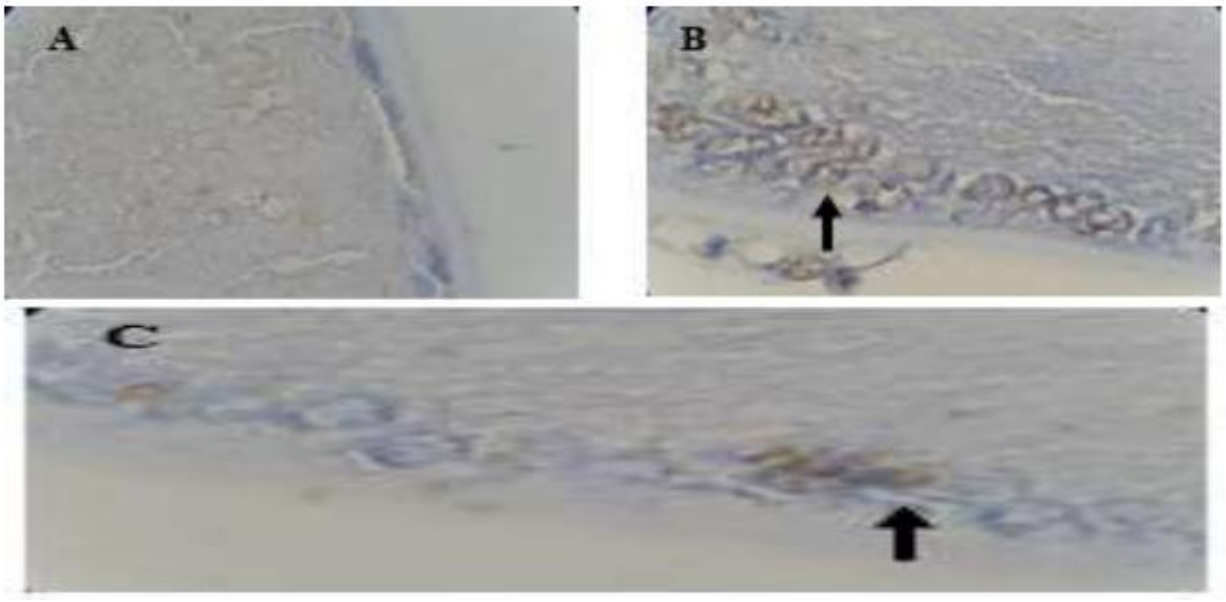

Figure 2: Effect of catechin on GRP78 in the lens-induced by cataracts. (A) control group, (B) cataract-induction group, (C) cataract-induction and 200 mg/kg BW catechin group. The sections were stained for GRP78 immunoreactivity (Brown)

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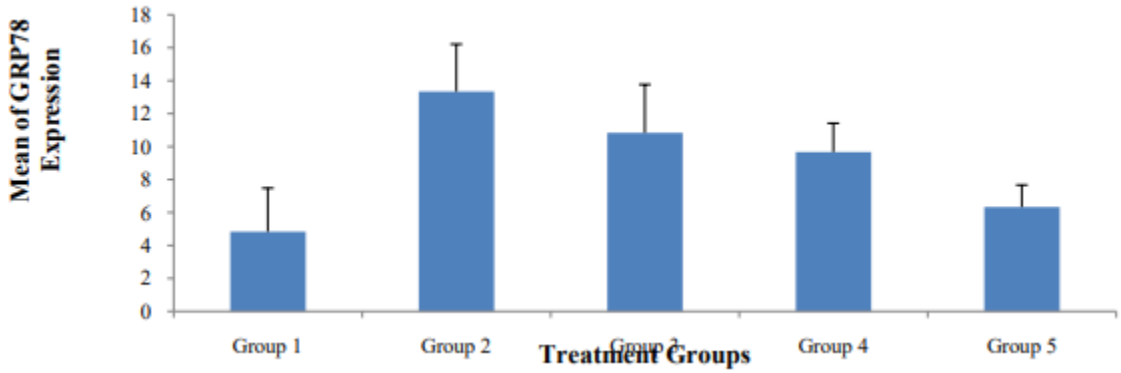

Figure 3: GRP78 in Each Treatment Group. The mean of GRP78 ± SD. *P<0, 01 vs Group 1, #P<0, 01 vs. Group 2

GRP78 in the lenses from the Na2SeO3 group were found to be significantly (p <0,001) higher than those in the control group and the catechin group. Treatment with catechins in the catechin + Na2SO3 group (Figure 3) significantly (p<0,001) decreased GRP78.

Discussion

Cataract formation is one among the foremost common causes of irreversible visual loss related to aging, thus much interest is being laid on recognition of a drug that may help to stop or treat cataractogenesis. the current investigations were undertaken to work out the efficacy of isolated catechin from tea GMB 4 to forestall the progression of cataract on in vivo animal models. Cataract could be a protein deterioration disorder characterized by irreversible modification and accumulation of lens proteins. Once cataract is made it can't be reverted back; thus the study is specializing in the prevention of lens opacity and it lacks a positive control. Selenite-induced cataract model (a single injection of sodium selenite at a dose of 19 μmol/kgBB is that the universally accepted animal model for studying oxidative stress induced experimental cataract because it shows most the events associated in human age-related cataract like membrane damage, calcium accumulation, endoplasmic reticulum stress, and proteolysis of lens proteins [22]. it's essential to test whether the anti cataractogenic potential of Catechin is by prevention of endoplasmic reticulum stress.

We found that GRP78, a stress protein on ER and a vital 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 accumulation of unfolded protein response (UPR) elevates GRP78 expression to keep the ER homoeostasis improving protein synthesis and transport [27, 28]. Recent studies revealed that UPR had a big role in neuronal degenerative disorders, like Alzheimer's disease, but few reported the role of UPR in cataracts. The existence of 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 cataract is a potential interest of study in the future.

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Several studies have shown that the inhibitory effect on cataracts may occur due to the presence of vitamins, carotenoids, caffeine, acetyl-L-carnitine, ebselene, quercetin, flavonoids, caffeic acid phenylester and curcumin [36-41]. However, nothing can completely block or delay lens opacity. Recent studies have shown that the tea leaf catechins have antioxidant, anti-inflammatory, antiangiogenic and antibacterial effects [42-46]. Such catechins bind reactive oxygen and nitrogen species and exert indirect antioxidant effects by stimulating the synthesis of endogenous antioxidant enzymes, like enzyme, glutathione reductase, glutathione-S-reductase, catalase and quinone reductase. due to those results, tea will inhibit lipid peroxidation and DNA mutation. tea leaf has high levels of catechin and shows more strongly antioxidant activity than vitamins C and E [44-48]. A recent study has shown that catechin may effectively protect individuals from corneal surface diseases, like dry eye, via its antioxidant and antiinflammatory effects [49]. Emoto et al. [51] reported that catechin prevents H2O2-induced oxidative stress within the lens epithelial cells. Chen et al. [52] reported that eye drops with catechin exhibit potent protective effects on ultraviolet B radiation induced corneal oxidative damage in mice; the consequences are likely because of increased implements of war, antioxidant activity, lipid peroxidation inhibition, and protein oxidation inhibition.

This research shows that after sodium selenite is given, lens opacity will occur and followed by an increase in GRP 78 (Figure 2). Then the opacity level decrease followed by a decrease in both GRP78 (Figure 2) in the group given catechin 50 mg / kgBW (Group 3), 100mg / kgBW (Group 4) and 200mg / KgBW (Group 5). This can be interpreted that sodium selenite causes lens opacity due to endoplasmic reticulum stress in the lens epithelium. Furthermore, catechins are given so as to reduce endoplasmic reticulum stress by decreasing GRP78 and decreasing turbidity from the lens. This can be interpreted that sodium selenite causes lens opacity due to stress endoplasmic reticulum in the lens epithelium. Furthermore, catechins are given so as to reduce endoplasmic reticulum stress by decreasing GRP78 and decreasing opacity from the lens.

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

Conclusion

Catechin isolate from GMB4 Clone Green Tea regulates reticulum endoplasmic stress in the lens in rats Cataract Model.