



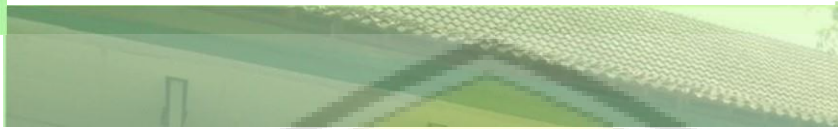
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# The 3rd ICOLIB

International Conference on Life Sciences and Biotechnology

Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember  
(ICOLIB 2919)

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## ABSTRACT BOOK

Dafam Lotus Hotel Jember  
East Java, Indonesia  
November 26, 2019

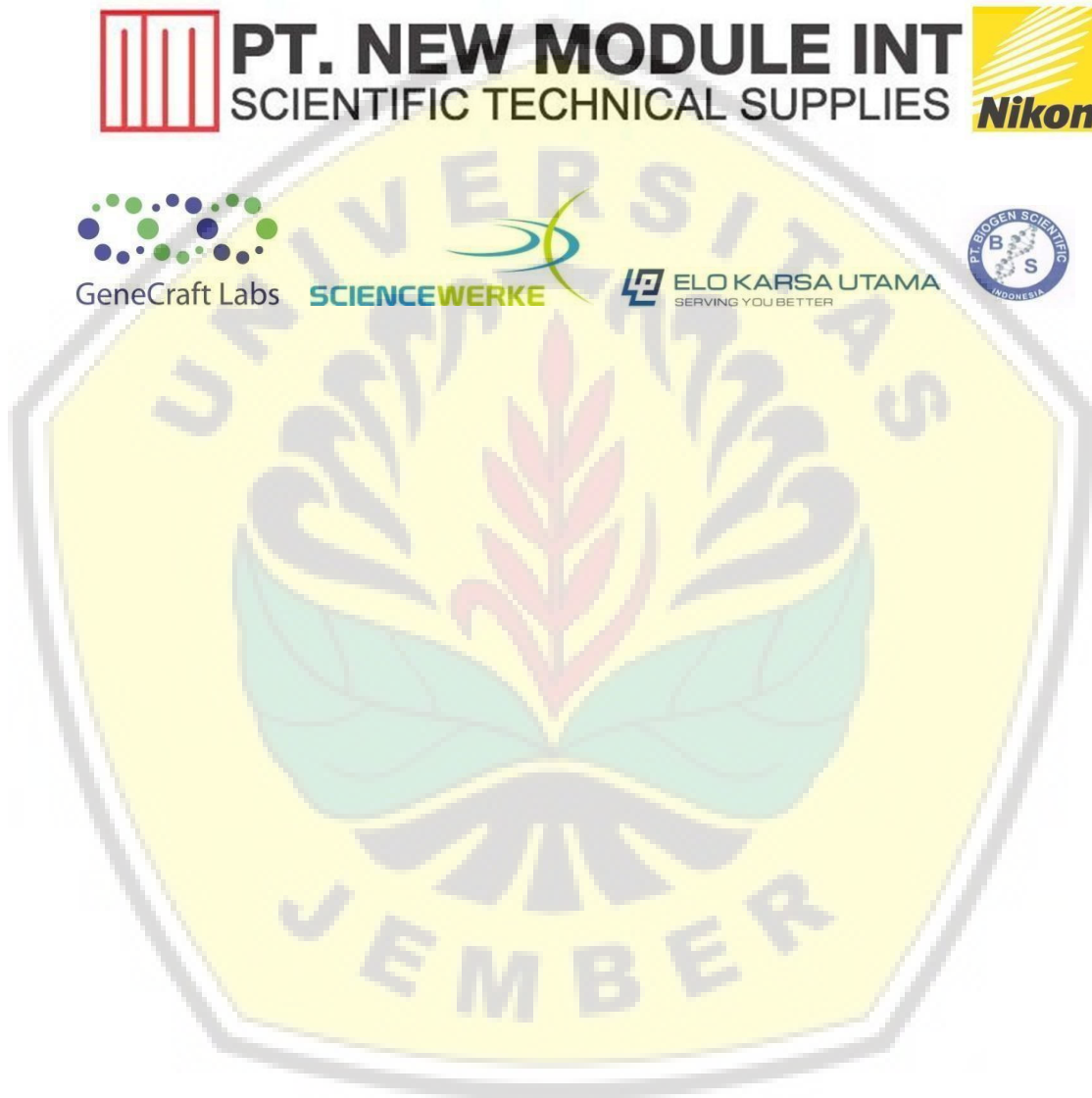
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## Acknowledgements

The organizers 3<sup>rd</sup> ICOLIB 2019 express sincere appreciation and grateful thanks to all those who have contributed their kind support to facilitate this conference



## Welcome Message

It is my great pleasure to welcome all of the speakers and participants to the 2019 International Conference on Life Sciences and Biotechnology (3rd ICOLIB), which is held from the 25th to 26th of November, located in Dafam Lotus Hotel Jember, Indonesia. The theme of the 3rd ICOLIB 2019 "BIODIVERSITY Molecules to Biosphere" provide a platform for researchers, academics, professionals, industries, and policy makers to exchange ideas, sharing the recent advanced and development on life sciences, and can be a valuable place for starting fruitful collaboration, especially in uncovering the potential of biodiversity at the molecular level to biosphere.

The conference is organized by the Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Jember. This conference covers all subjects in Life Sciences and Technology including cell Biophysical and Biological Science biology, Mathematics, Statistics, and Modeling, Health And Medicine, Horticulture, Molecular Medicine Bioinformatics, Breeding, Food Science, System Biology, Genomics, Biodiversity and Conservation Biology. I am very much excited that this conference has been well recognized by researchers and academic communities. In addition, more than hundreds scientists send their research titles to present in this conference. Furthermore, all of the article submitted to 3rd ICOLIB 2019 will be peer reviewed by expert, and the selected one will be published in Scopus-indexed journals or proceedings.

On behalf of organizing committee, I would like to thank all of distinguished invited speakers and presenters for participating in the 3rd ICOLIB 2019. In particular, I want to express my sincerely gratitude to Rector of University of Jember and Dean of Faculty of Mathematics and Natural Sciences, and also a deep appreciation of the member of the organizing committee for excellent team work that bring to the success of this conference.

Finally, I wish you have a fruitful meeting, and I hope that you will have a plentiful benefit in this conference, and wonderful memories after visiting Jember.

Thank you.

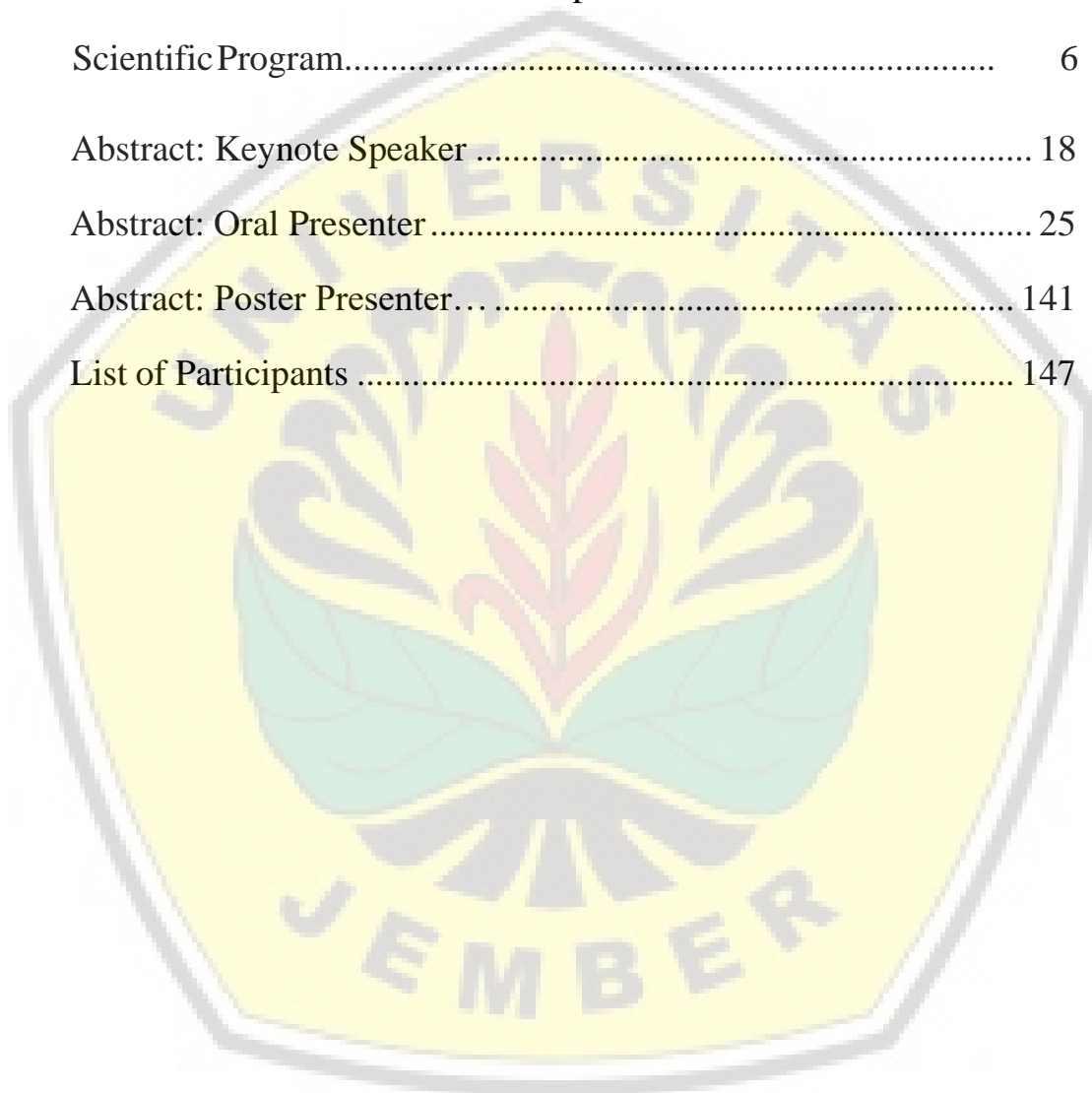
Jember, November 2019

Mukhamad Su'udi

Chairman of The 3<sup>rd</sup> ICOLIB 2019

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## General Information for the Participant

### Registration Information

#### Conference Venue

- The venue for the conference is the Dafam Hotel, Jember, East Java, Indonesia

#### Registration

- Registration includes:
  - ❖ Seminar kit
  - ❖ ID Card
  - ❖ Refreshment (coffee & tea) during the conference day
  - ❖ Buffet Lunch

#### ID Card

- Participants are requested to display their ID Card during the conference for entry to scientific sessions, melas and the wellcome reseption. Please also show the ID Card to committee before transportation to the conference venue.

### Instruction for the Moderator

- Please ensure that the sessions and speaker presentations are kept stricly ontime

### Instruction for Speakers (Keynote Speaker and Oral Presenter)

- Speaker are requestes to submit their presentation to staff in the audio-visual room at the least 1 hours before each presentation, then upload and ensure that the proper presentation is in the computer provided
- 45 minutes have been allocated for each keynote speakers (please allowtime within this period for answering the questions)
- Free oral presenter will last 10 minutes only (please allow time within this period for answering questions)
- Please be aware that the above times must be strictly adhered to

### Instructions for PosterPresenter

- Poster presentations will be located in the front of the conference space along the second floor.
- Poster will be display throughout the conference, and presenters are responsible for putting them and removing them.

## 3<sup>rd</sup> ICOLIB Committes

Mukhamad Su'udi, Ph.D, Indonesia	msuudi.fmipa@unej.ac.id
Dr. Sattya Arimurti, S.P, M.Si, Indonesia	arimurti30@gmail.com
Purwatiningsih, Ph.D, Indonesia	purwatiningsih2000@gmail.com
Dr. Retno Wimbaningrum, Indonesia	wimbaningrum.fmipa@unej.ac.id
Dra Mahriani, M.Si, Indonesia	yani_hendro.fmipa@unej.ac.id
Dr. Rike Oktarianti, Indonesia	oktarianti@gmail.com
Hari Sulistiyowati, Ph.D, Indonesia	sulistiyowati.fmipa@unej.ac.id
Dwi Setyati, M.Si, Indonesia	setyatidwi.fmipa@unej.ac.id
Esti Utarti, S.P, M.Si, Indonesia	esti.fmipa@unej.ac.id
Dra. Susantin Fajariyah, M.Si, Indonesia	susantin.mipa@gmail.com
Rendy Setiawan, S.Si, M.Si, Indonesia	rendy.fmipa@unej.ac.id
Syubbanul Wathon, S.Si, M.Si, Indonesia	syubbanulwathon@unej.ac.id
Arif Mohammad Siddiq, S.Si, M.Si, Indonesia	arifsiddiq.fmipa@unej.ac.id
Yoyok Yulianto, Indonesia	yoyok@unej.ac.id
Eva Tyas Utami, S.Si, M.Si, Indonesia	evatyas.utami@gmail.com
Husnatun Nihayah, S.Si, M.Si, Indonesia	husnatunnihayah@unej.ac.id
Drs. Rudju Winarsa, M.Kes, Indonesia	rudju_win@yahoo.co.id
Drs. Siswanto, M.Si, Indonesia	siswanto.fmipa@unej.ac.id
Dr. H. Teguh W. M.Pd, Indonesia	teguh.unej@gmail.com

## 3<sup>rd</sup> ICOLIB Scientific Committes

Prof. Ir. Bambang Sugiharto, Ph.D, Indonesia	sugiharto.fmipa@unej.ac.id
Prof. Drs. Sudarmadji, Ph.D, Indonesia	sudarmadji.fmipa@unej.ac.id
Kahar Muzakhar, Ph.D, Indonesia	kaharmzk@unej.ac.id
Merites Buot, Philipines	mmbuot@up.edu.ph
Prof. Fahrul Huyop, Malaysia	fahrul@utm.my
Palaksha K.J., India	palakshakj1@gmail.com
Dr. rer. nat. Kartika Senjarini, Indonesia	senjarini@unej.ac.id
Deirdre Conroy, USA	Dconroy1@vt.edu
Prof. Inocencio E. Buot Jr, Philipines	Inocencio.buot@upou.edu.ph

## Programme Chair

Mukhamad Su'udi, Ph.D, Indonesia

## Finance and Fund-Raising Committee

Dr. Rike Oktarianti, M.Si, Indonesia

Dra. Susantin Fajariyah, M.Si, Indonesia

## Secretary

Dr. Sattya Arimurti, S.P, M.Si, Indonesia

## SCIENTIFIC PROGRAM

**Monday, November 25<sup>th</sup> 2019**

**TIME VENUE: HALLROOM PAGE**

07.00-08.00 Registration

08.05-08.15 Indonesia Raya Anthem and Hymne UNEJ

08.20-08.20 Speech: Chairman 3<sup>rd</sup> ICOLIB : **Mukhamad Su'udi, Ph.D**

08.20-08.30 Speech: Rector of Jember University : **Moh. Hasan, Ph.D**

08.30-08.40 Token of Appreciation

08.40-08.45 Pray (**Kosala, S.Si, M.Si**)

08.45-09.00 Gandrung Dance

09.00-09.15 Opening ceremony

09.15-09.45 Coffea Break

09.45-10.30 **Speaker 1.** Bioengineering Challenges Toward Regenerative Medicine

**Prof. Koichi Kato**, Department of Biomaterials, Graduate School of Biomedical and Health Science, Hiroshima University

**Chairperson: Prof. Dr. Ir. Bambang Sugiharto, M.Agr.Sc**

10.30-11.15 **Speaker 2.** The Dynamics and Evolution of Plant Viruses in Indonesia Influenced by Climate Change

**Prof. Budi Setiadi Daryono**, Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta

**Chairperson: Prof. Dr. Ir. Bambang Sugiharto, M.Agr.Sc**

11.15-12.00 **Speaker 2.** Regulation of Aliphatic Glucosinolates Biosynthesis in Radish (*Raphanus sativus L.*) Plant

**Prof. Kim Jongkee**, Department of Plant Science and Technology, Chung-Ang University, Anseong, South Korea

**Chairperson: Prof. Dr. Ir. Bambang Sugiharto, M.Agr.Sc**

**12.00-13.30 ISHOMA and Poster Session**

**VENUE: HALLROOM**

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**Tuesday, November 26<sup>th</sup> 2019**

**TIME VENUE: HALLROOM PAGE**

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08.45-09.30	<b>Speaker 5.</b> The Need to Appraise Biodiversity: A Challenge for Ecological Valuation Perspective  <b>Hari Sulistiyowati, Ph.D</b> , Associate professor of Biology Department, MIPA Faculty-University of Jember  <b>Chairperson: Dr. Ir. Siswoyo, MSc.</b>	

**09.30-10.00 Coffea Break**

10.00-10.45	<b>Speaker 6.</b> Characterisation and Exploring Biotechnological Potential of Halophilic Bacterium Isolated from Hypersaline Environment for Bioremediation?  <b>Prof. Dr. Fahrul Huyop</b> , Biosciences Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia  <b>Chairperson: : Drs. Asmoro Lelono, MSc., Ph.D</b>	
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**VENUE: HALLROOM**

10.45-12.00	<b>Parallel Oral Session I</b> Chairperson: Dr. rer. nat Yunus Effendi, MSc <b>1. Virtual Screening the Interaction of Various Compound from Indonesian Plants with the HGXPRT Enzyme As a Novel Antimalarial Drug</b>	
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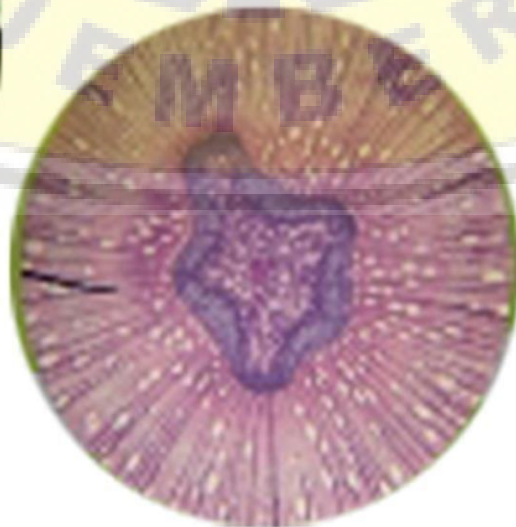
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**KEYNOTE  
SPEAKER**





**Effects of Bangle Methanol (*Zingiber cassumunar* Roxb.)  
Fraction on Mice Brain Histopathology Study Infected by  
*Plasmodium berghei***

**Bagus Hermansyah<sup>1</sup>, Wiwien Sugih Utami<sup>2</sup>, Pudyo Kriswardani<sup>3</sup>, Rosita Dewi<sup>4</sup>**

<sup>1,2</sup>Laboratory of Parasitology, Faculty of Medicine, University of Jember

<sup>3</sup>Medical Faculty Undergraduate Program, University of Jember

<sup>4</sup>Laboratory of Histology, Faculty of Medicine, University of Jember

correspondence email: bagus\_hermansyah@unej.ac.id

**Abstract**

Methods of preventing severe malaria such as cerebral malarial complications by using adjuvant therapy being developed. One of the bioactive materials developed today is curcumin. Bangle (*Zingiber cassumunar* Roxb.) is one of the spices that have medicinal properties. The purpose of this research is to know the effect of fraction methanol bangle (*Zingiber cassumunar* roxb.) to the histopathology's changing of the brain of mice infected with *Plasmodium berghei*. The treatment was in the form of stimulation of Bangle methanol fraction in treatment group 1 and 2 for 14 days orally, followed by malaria induction with *Plasmodium berghei*. After the mice were infected and had a rapid murine coma behavior scale score of less than 13, treatment group 1 received complementary therapy in the form of ACT for 48 hours and treatment group 2 obtained only Bangle methanol fraction at the same dose. The positive control group was administered Artemisinin alone, and the negative control group infected without any treatment. The effect of Bangle (*Zingiber cassumunar* Roxb.) Methanol fraction on the prevention of intracerebral hemorrhage obtained by examining the number and the area of intracerebral hemorrhage in mice brain tissue preparations. Data on the number of intracerebral hemorrhages tested with non-parametric Kruskal Wallis test ( $p = 0.083$ ) and the area of intracerebral hemorrhage tested with non-parametric Kruskal Wallis test ( $p = 0.089$ ). The conclusion of this study is the fraction of methanol Bangle (*Zingiber cassumunar* Roxb.) can not prevent the occurrence of intracerebral hemorrhage.

**Keywords:** malaria, bangle, intracerebral hemorrhage

## Preventive Effect of Catechin Isolate From GMB4 Clone Green Tea in Selenite Induced Cataract

Nugraha Wahyu Cahyana<sup>1,2</sup>, Edi Widjajanto<sup>3</sup>, Umi Kalsum<sup>4</sup>, Seskoati Prayitnaningsih<sup>5</sup>, Hidayat Sujuti<sup>6</sup>

<sup>1</sup>Doctoral Program of Medical Science, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>2</sup>Department of Ophthalmology, Faculty of Medicine, Jember University, Jember, East Java, Indonesia

<sup>3</sup>Department of Clinical Pathology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>4</sup>Doctoral Program of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>5</sup>Department of Ophthalmology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>6</sup>Department of Biochemistry and Molecular Biology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

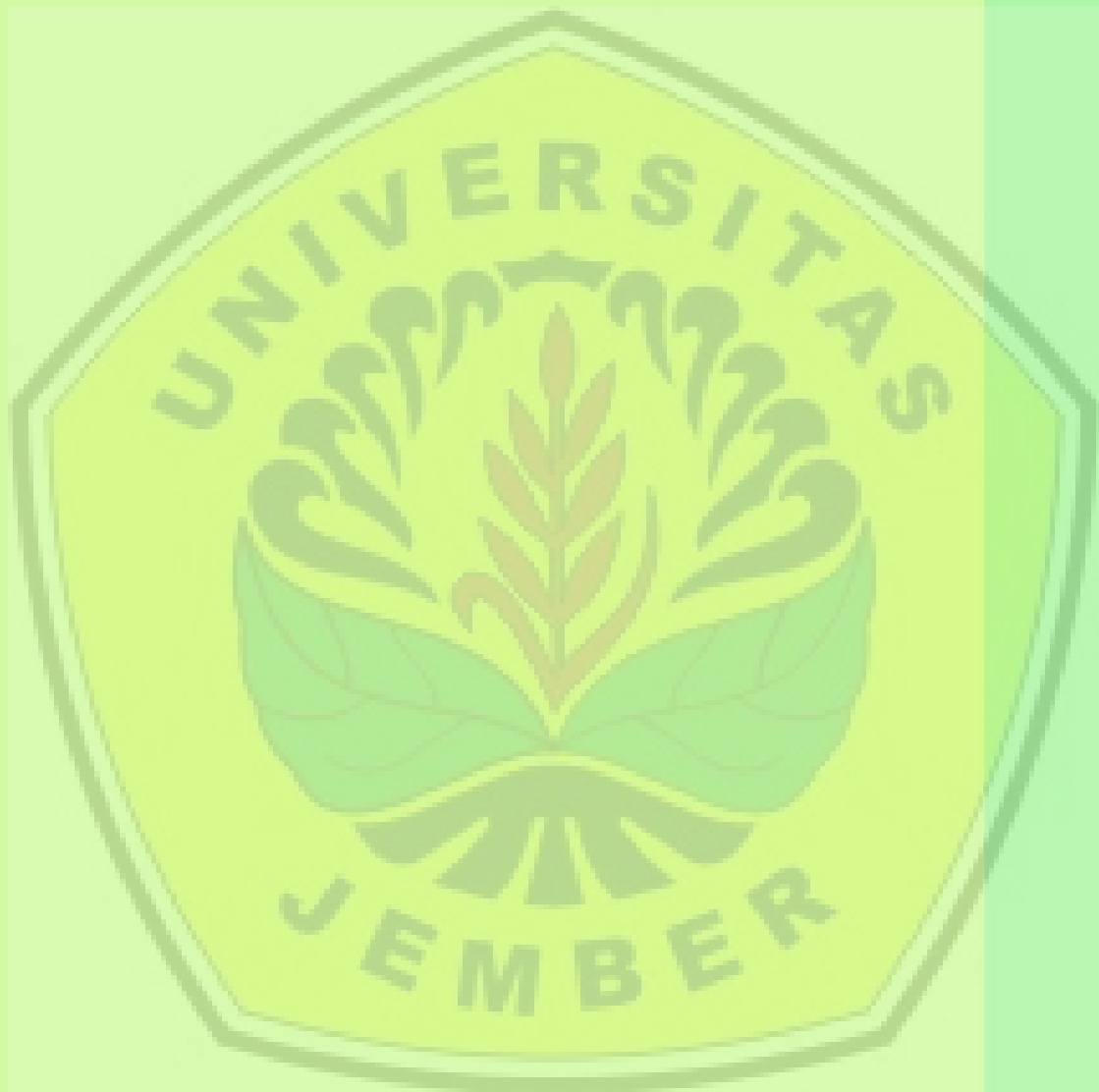
### Abstract

**Purpose:** To evaluate the effects of Catechin Isolate From GMB4 Clone Green Tea on sodium-selenite induced cataract formation. **Methods:** Cataract Isolate From GMB4 Clone Green Tea was induced by intraperitoneal injection of 19  $\mu\text{mol/kg}$  sodium selenite to ten day- old Wistar rats. The neonatal rats were randomly divided into five groups ( $n=5$  in each group): a control group, and four cataract- induction groups, treated with either 0, 50, 100, 200 mg/kg catechin Isolate From GMB4 Clone Green Tea. We performed slit-lamp bio microscopic analysis, level of GSH and GR. **Results:** Both eyes of all rats in Group 1 did not exhibit cataract formation. In Group 2, all rats developed Grade 3 cataract in the lenses of both eyes. The difference in exhibited cataract in the lens of the right eyes in all rats between Group 2 and any eyes of groups 3 or 4 were significant ( $P = 0.001$ ). The mean activities of GSH and GR in Group 2 rat lenses were significantly lower than the values in lenses of all rats in Group 1 ( $P = 0.001, 0.003, 0.001$ ), and in the lenses of the right eyes of rats in Groups 3 and 4 ( $P = 0.001, 0.020, 0.001$ ). **Conclusion:** Isolate catechin can effectively prevent selenite-induced cataract formation. This effect was associated with increased level of opacity, GSH and GR activities in the lens.

**Key words:** antioxidants, cataract, catechin isolate







## Preventive Effect of Catechin Isolate From GMB4 Clone Green Tea Green Tea in Selenite Induced Cataract

Nugraha Wahyu Cahyana<sup>1,2\*</sup>, Edi Widjajanto<sup>3</sup>, Umi Kalsum<sup>4</sup>, Seskoati Prayitnaningsih<sup>5</sup>

<sup>1</sup>Doctoral Program of Medical Science, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>2</sup>Department of Ophthalmology, Faculty of Medicine, Jember University, Jember, East Java, Indonesia

<sup>3</sup>Department of Clinical Pathology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>4</sup>Doctoral Program of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

<sup>5</sup>Department of Ophthalmology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

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### Abstract

**Purpose:** To evaluate the effects of Catechin Isolate From GMB4 Clone Green Tea on sodium-selenite induced cataract formation. **Methods:** Cataract Isolate From GMB4 Clone Green Tea was induced by intraperitoneal injection of 19  $\mu\text{mol/kg}$  sodium selenite to ten day-old Wistar rats. The neonatal rats were randomly divided into five groups (n=5 in each group): a control group, and four cataract- induction groups, treated with either 0, 50, 100, 200 mg/kg catechin Isolate From GMB4 Clone Green Tea. We performed slit-lamp bio microscopic analysis, level of GSH and GR. **Results:** Both eyes of all rats in Group 1 did not exhibit cataract formation. In Group 2, all rats developed Grade 3 cataract in the lenses of both eyes. The difference in exhibited cataract in the lens of the right eyes in all rats between Group 2 and any eyes of groups 3 or 4 were significant ( $P = 0.001$ ). The mean activities of GSH and GR in Group 2 rat lenses were significantly lower than the values in lenses of all rats in Group 1 ( $P = 0.001, 0.003, 0.001$ ), and in the lenses of the right eyes of rats in Groups 3 and 4 ( $P = 0.001, 0.020, 0.001$ ). **Conclusion:** Isolate catechin can effectively prevent selenite-induced cataract formation. This effect was associated with increased level of opacity, GSH and GR activities in the lens.

**KEYWORDS:** Antioksidants, cataract, catechin Isolate

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### INTRODUCTION:

The cataract is an opacity that develops in the crystalline lens of the eye; it varies in degree from slight to completely opaque, obstructing the passage of light. The lens epithelium covers the anterior surface of the lens. Epithelial cells near the lens equator divide and differentiated into the lens fibers. This process continues at a constant, slow rate throughout adult life, resulting in the steady growth of the lens fiber mass [1]. Damage of the lens epithelium has been a major focus in the identification of causes of cataract formation [2].

Pathogenesis of cataracts is multifactorial, with the disease developing as a result of heredity, trauma, inflammation, metabolic disorders, malnutrition and age-related changes, amongst other pathways. Some risk factors, such as oxidative damage, impaired glucose metabolism, radiation damage and toxic damage to the lens, also play an important role in the pathogenesis of cataracts. One of the most common types of cataracts is that related to age. Although the exact mechanism of age-related cataract formation is unknown, the increase in free oxygen radicals and the reduction in antioxidant enzymes in the lens have been identified as possible mechanisms. According to the theory of oxidative damage, free oxidant radicals lead to cataract formation by cross-linking and aggregation of lens proteins, the peroxidation of membrane lipids and by apoptosis of epithelial cells in the lens [3-5].

Increased amounts of oxidative substances and reduced levels of antioxidants in the lens such as glutathione were proposed to be involved in the pathogenesis of cataracts [6-8]. Researchers have uncovered the importance of increased oxidative substances and reduced levels of antioxidants in the pathogenesis of cataracts [9-11].

Glutathione is the most important antioxidant in the lens and is synthesized the lens epithelium. The reduced glutathione (GSH) exists in high concentration in the lens. GSH provides maintenance of the lens transparency by

scavenging reactive oxygen species and protecting protein thiols. It has been reported that the GSH level in the lens is decreased in age-related cataract [12-15].

Apoptosis, also known as programmed cell death, is a form of cell death that serves to eliminate dying cells in proliferating or differentiating cell populations. Thus, apoptosis plays a crucial role in normal development and tissue homeostasis. Previous studies have shown that apoptosis of lens epithelial cells plays an important role in the development of several types of cataracts. These studies have suggested that apoptosis of lens epithelial cells appears as a common cellular mechanism mediating stress-induced non-congenital cataractogenesis [16].

Apoptosis can be detected using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, a measure of DNA fragmentation in tissue sections, and by observation of a DNA ladder, a measure of fragmentation in DNA extracted from cells or tissues. In human cataract research, TUNEL-positive cells indicate apoptotic cell death in the lens epithelium. Another important characteristic of apoptosis is caspase activation. Caspase-3 is one of the most widely studied caspases, and it is a key executor of apoptosis [17].

Cataract is a major health problem and the major cause of blindness throughout the world [18]. Currently, the only available treatment for the disease is the surgical extraction of the cataractous lens followed by replacement with a synthetic implant. Although such a surgical replacement of the natural lens with an artificial lens is significantly effective in restoring vision to most patients, it is not free of complications. Attempts to reverse cataract formation, or at least significantly retard the onset of the disease would be of great value [19, 20, 21].

The functional roles of catechin have been well documented, but its effect on the lens epithelium following cataract formation remains poorly understood [22]. Accordingly, research is needed to prove the effect of GMB4 clone green tea catechin isolates that can protect lens epithelial cells against oxidative stress and apoptosis so as to delay the onset of cataracts. The aim of the study was to evaluate the effects of Catechin Isolate from GMB4 Clone Green Tea on sodium-selenite induced cataract formation and activities of the enzymes glutathione (GSH), and Caspase-3.

## **MATERIAL 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 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 postpartum Day 10. In Group 3, subcutaneous sodium-selenite (19 nmol/g body weight) was injected on postpartum Day 10 and injection of isolate catechin (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 difference 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.

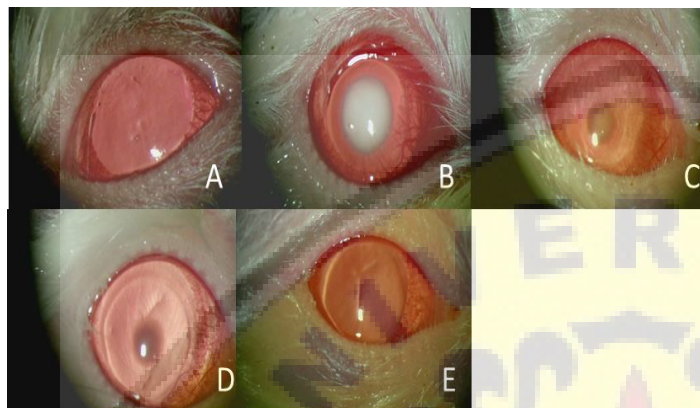
On postpartum Day 17 all rats were anesthetized with intraperitoneal ketamine injection (80 mg/kg) and xylazine (15 mg/kg). The rat pups were taken out and the pupils were dilated with tropicamide 0.5% every 30min for two hours. All lenses were evaluated and were morphologically staged for cataract development and staging was performed by slit-lamp bio microscopy on a scale of 0 to 4; Grade 0 was a normal clear lens, Grade 1 was a sub capsular opacity, Grade 2 was a nuclear cataract, Grade 3 was a strong nuclear cataract with an opacity in the perinuclear area, and Grade 4 was a mature dense opacity involving the entire lens [23]. Lens photos  $\times 25$  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 °C. These homogenates were centrifuged at 10 000 g for 30min at 4 °C, and supernatants were obtained. Supernatants were used for the measurement of the levels of GSH and caspase-3.

The GSH measurements were carried out using a GSH kit (ImmuchromGmbH, Hessen, Germany) with high-performance liquid chromatography. During the reaction of derivatisation glutathione is converted into a fluorescent probe. The precipitation step removes high molecular substances. After centrifugation, the fluorescent probe is cooled (2°C -8°C) and 20  $\mu$ L samples are injected into the HPLC system. Measurements were carried out on the HPLC system with a fluorescence detector at 385 nm (excitation) and 515 nm (emission). Results were expressed as micromoles per liter. To visualize caspase-3 expression, we performed caspase-3 immunohistochemistry using a previously described method [24]. Sections were drawn from each lens and incubated overnight with mouse anti-caspase-3 antibody (1:500; Santa Cruz Biotech) and then for another 1 h with biotinylated mouse secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA). Bound secondary antibodies were then amplified with a Vector Elite ABC Kit® (1:100; Vector Laboratories). The antibody-biotin-avidin-peroxidase complexes were

visualized using 0.03% DAB, and the sections were finally mounted onto gelatin-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

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-hoc test was used if the ANOVA was significant.  $P < 0.01$  was considered as statistically significant.

A 0.45 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.



**Fig.1: The Slit-lamp Pictures of Representative Lenticular Opacities**

(A) clear lens (grade 0) in control group, (B) grade IV in group of only sodium-selenite, (C) grade III in sodium-selenite with isolate catechin 50 mg/kg body weight group, (D) grade II in sodium-selenite with isolate catechin 100 mg/kg body weight, (E) grade I in sodium-selenite with isolate catechin 200 mg/kg body weight group.

The comparison of the right eye and left eye with paired samples correlations method were not significant ( $p = 0,749$ ) and paired samples test ( $p = 1,00$ ).

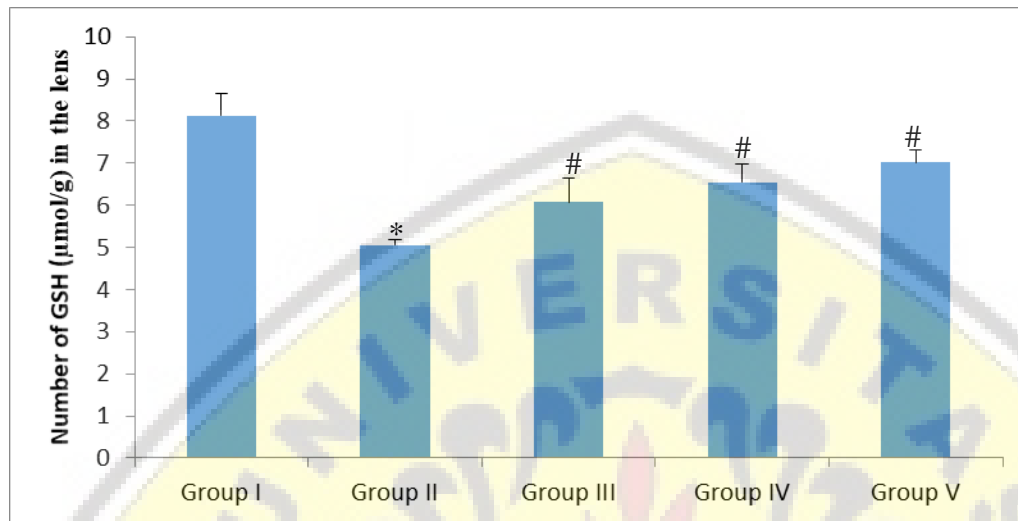
**Table 1: The Opacity Grading of the Lens in All of the Groups**

Experimental Groups	Number of Wistar Rats	Number of pups with different degrees of lenticular opacification					Number of pups in which lenticular opacification occurred
		0	+	++	+++	++++	
Group 1 (Normal)	5	5	-	-	-	-	0
Group II (Sodium Selenite only)	5	-	-	-	1	4	5 (100%)
Group III (Sodium Selenite + catechin 50 mg/kg) body weight group	5	-	1	2	2	-	5 (100%)
Group IV (Sodium Selenite + catechin 100 mg/kg) body weight group	5	2	1	1	1	-	3 (60%)
Group V (Sodium Selenite + catechin 200 mg/kg) body weight group	5	4	1	-	-	-	1 (20%)

Lenses in both eyes of all control rats (Group 1) remained clear [Fig. 1A]. Subcutaneous injection of Na<sub>2</sub>SeO<sub>3</sub> (19 µmol/kg) on postpartum day 10 was sufficient to induce cataract formation, which was visible by the time the rat pups opened their eyes. Inspection of the rat pups' eyes with a slit lamp microscope confirmed that all animals injected only with Na<sub>2</sub>SeO<sub>3</sub> developed cataracts: one out of five (20%) developed grade 3 cataracts (Fig. 1C) and the remaining four out of five (80%) developed grade 4 cataracts (Fig. 1B). In comparison, Na<sub>2</sub>SeO<sub>3</sub> with Catechin 50mg/kg injections showed that the severity of cataract formation decreased; two rats out of 5 (40%) developed grade 3 cataracts (Fig. 1C), two rats out of 5 (40%) developed grade 2 cataracts (Fig. 1D) and one out of five (20%) developed grade 1 cataract (Fig. 1E) while grade 4 cataract (Fig. 1B) was not founded. Na<sub>2</sub>SeO<sub>3</sub> with Catechin 100mg/kg injections decreased the severity of cataract formation; one rat out of 5 (20%) developed grade 3 cataracts, one rat out of 5 (20%) developed grade 2 cataracts while three out of five (60%) did not develop any cataracts (grade 0). Na<sub>2</sub>SeO<sub>3</sub> with Catechin 200mg/kg injections decreased the severity of cataract formation; only one rat out of five (20%) developed grade 1 while four out of five (80%) did not develop cataract (grade 0). These results indicated that Catechin especially 200 mg/kg BB dosage, was successful in preventing cataract formation.



The grading of the lens in all of the groups is tabulated in Table 1, and the slit-lamp pictures of representative lenticular opacities observed for each group are shown in Fig. 1. No toxic effects to the cornea or conjunctiva of the eye. This difference was statistically significant. The comparison between group 2 with group 1, 4, and 5 were significant ( $p= 0.000, 0.000, 0.000$ ) while group 3 was not significant ( $p= 0,022$ ). The comparison between group 3 with group 1 and 5 were significant ( $p=0.001, 0.003$ ) while group 2 and 4 were not significant ( $p= 0.022, 0.253$ ).



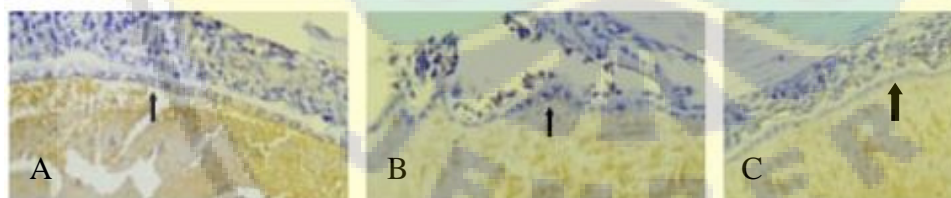
**Fig.2: Mean GSH Level 7 Days after Injection in Five Experimental Groups**

The scale bar represents 50 mm number of GSH expression in each group. (1) group 1, (2) group 2, (3) group 3, (4) group 4, (5) group 5.

\* $p < 0.01$  - compared to the control group (group 1); # $p < 0.01$  - compared to the cataract induced group (group 2).

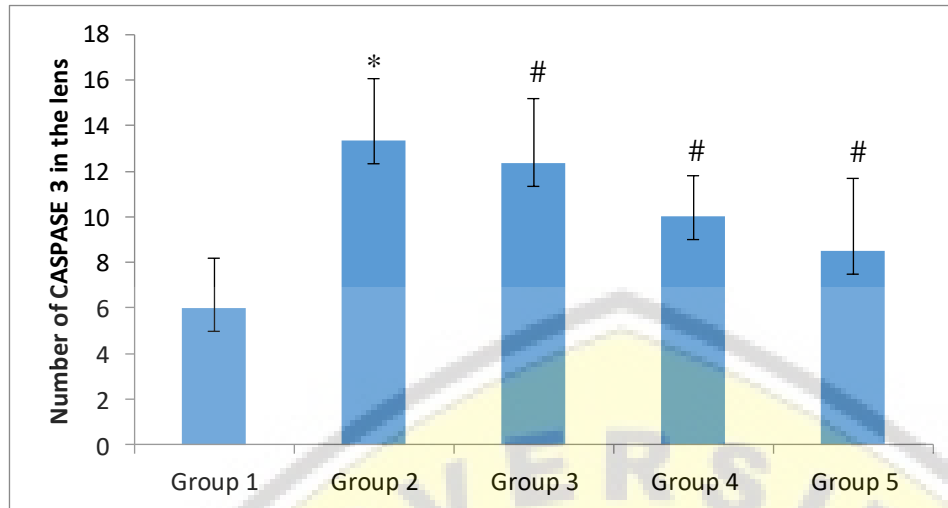
The mean GSH levels lenses ( $5.06 \pm 0.13$ ) of group II rats were significantly ( $P < 0.001$ ) lower than the levels in Group I lens ( $8.36 \pm 0.51$ ), Group III lens ( $6.08 \pm 0.56$ ), Group IV ( $6.54 \pm 0.45$ ), and Group V ( $7.02 \pm 0.29$ ) (Figure 2). Significant differences were also observed in levels of GSH in lenses ( $P < 0.001$ ) between group III and group I. The lens GSH level decreased gradually with increase in the stages of lens opacity (that is, with increasing opacification) in group II and group III.

GSH levels in lenses from the  $\text{Na}_2\text{SeO}_3$  group were found to be significantly ( $p < 0.01$ ) lower than those of the lenses from the control and Catechin groups. Treatment with Catechin in the Catechin +  $\text{Na}_2\text{SeO}_3$  group (Fig. 2) significantly ( $p < 0.01$ ) increased GSH levels.



**Fig.3: Caspase-3 (A) Group 1 Results, (B) Group 2, (C) Group 5**

Effect of catechin on caspase-3-expression in the lens epithelium induced by cataracts. Photomicrographs of caspase-3-positive cells in the lens epithelium. (A) control group, (B) cataract-induction group, (C) cataract-induction and 200 mg/kg catechin group. The sections were stained for caspase-3 immunoreactivity (brown).



**Fig.4: Effect of Catechin on Caspase-3-Expression in the Lens Epithelium Induced by Cataracts**

The scale bar represents 50 mm number of caspase-3-positive cells in each group. (1) group 1, (2) group 2, (3) group 3, (4) group 4, (5) group 5. \* $p < 0.01$  - compared to the control group (group 1); # $p < 0.01$  - compared to the cataract induced group (group 2).

## DISCUSSION:

Cataract is the leading cause of blindness worldwide, and surgical replacement of the opacified lens with an artificial lens is currently the only way to remedy vision loss. Although cataract surgery is considered to be very successful in terms of visual outcome, the cost, need for trained personnel, and postsurgical complications limit the worldwide availability and accessibility to this procedure. Hence, development of alternatives to surgical intervention is warranted [23].

Oxidative stress is an imbalance between the rate of oxidant production and degradation [25]. Substantial supporting evidence suggests that reactive oxygen species (ROS) and oxidative damage are involved in the development of cataracts [26, 27]. Cataract formation has a multifactorial etiology. Oxidative stress, resulting in the depletion of antioxidant defense systems in the lens, is considered to be a major factor in the formation of cataracts. Lens transparency is dependent on the preservation of a favorable redox balance, which is, in part, maintained by its high GSH content [28, 29]. De-creased levels of GSH in the lens can lead to free radical accumulation, resulting in lipid peroxidation and de-creased antioxidant enzyme activity [30, 31], all of which lead to cataract development. Therefore, an alternative method to prevent or treat cataracts would be the use of antioxidant. Based on this, we have investigated the effects of antioxidant, Catechin in selenite-induced cataracts. Results from morphological observation indicate that Catechin was able to prevent the formation of cataracts in the Catechin + Na<sub>2</sub>SeO<sub>3</sub> group (Table 1).

As discussed earlier, GSH is the most important anti-oxidant in the lens; it is the first line of defense against oxidative stress [32]. Our results show a significant decrease in GSH levels in the lenses of the Na<sub>2</sub>SeO<sub>3</sub> group (Fig. 2) when compared to those of the control group indicating oxidative stress. Treatment with Catechin significantly increased the GSH levels in the Catechin-treated group. This suggests that Catechin was able to prevent oxidative stress by restoring GSH levels [33].

However, significant improvement in the GSH was observed in the Catechin + Na<sub>2</sub>SeO<sub>3</sub> group (Fig. 2). Furthermore, changes in the levels of GSH was seen to affect the activity of GR. This enzyme regenerates GSH from its oxidized form and is imperative to GSH homeostasis. Increased activity of GR in the lenses of the Na<sub>2</sub>SeO<sub>3</sub> group could be attributed to the activation of the lens antioxidant defense network against a change in the redox status. Furthermore, Catechin treatment increased the levels of GSH and restored GR activity.

Several animal species experience spontaneously occurring cataract of known inheritance and offer valuable model for studying human cataract [34]. Various chemicals are known to contribute to the development of cataract in animals. Among these chemicals, catechin, a direct-acting alkylating agent that does not require metabolic activation, is known as a cataractogenic agent in rats [35]. In addition, young animals are reported to be more susceptible to catechin than are adult animals. Therefore, in this study, a cataract model was constructed using a single intraperitoneal injection of catechin in rats at postnatal day 10.

Division of the lens epithelial cells is confined to the periphery of the lens. These cells move toward the equator and then differentiate into lens fibers. Apoptosis of lens epithelial cells can occur during this differentiation process [36, 37]. It is well known that apoptotic death of lens epithelial cells induces lens opacification. Lens epithelial cells play a vital role in the metabolic homeostasis and maintenance of transparency in the lens [38], and damage to lens epithelial cells potently contributes to cataractogenesis. Moreover, apoptosis of lens epithelial cells has been reported to be the earliest event in the experimental formation of cataracts, such as those induced by hydrogen peroxide and catechin [39]. In human studies, caspase-3 is up-regulated and activated in the early stages of apoptosis following cataractogenesis [40].

We found that the numbers of caspase 3-positive cells in the lens epithelium were significantly higher following cataract induction (Fig.3). Opacification in the eye- ball was also greater following cataract induction. These findings indicate that catechin injection-induced cataracts increased apoptosis in the lens epithelium.

We observed that catechin significantly suppressed both cataract-induced increases in DNA fragmentation and caspase-3 expression in the lens epithelium in dose-dependent manners. In addition, catechin alleviated the degree of opacity induced by cataract formation.

In summary, our data indicate that oxidative stress and apoptosis plays a role in cataract formation, particularly in glutathione and caspase-3 maintenance and suppression of apoptotic cell death in the lens epithelium. The data support our hypothesis that Catechin especially with 200mg/kg body weight protects the lens by increase number of GSH and decrease number of caspase-3. Our present and future studies may eventually help prevent cataract formation in high-risk populations and treat early-stage cataracts without need for surgical intervention. Catechin could potentially be used to delay cataractogenesis through the suppression of apoptotic cell death and oxidative stress in the lens epithelium.

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## CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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