

PROTECTIVE EFFECT OF SILK LUTEIN EXTRACT ON UV-B INDUCED RETINAL PIGMENT EPITHELIAL CELL DAMAGE

SATHID AIMJONGJUN

A Thesis Submitted to the Graduate School of Naresuan University in Partial Fulfillment of the Requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) May 2012 Copyright 2012 by Naresuan University b0025593b

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#### SATHID AIMJONGJUN

A Thesis Submitted to the Graduate School of Naresuan University in Partial Fulfillment of the Requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) May 2012 Copyright 2012 by Naresuan University This thesis entitled "Protective effect of silk lutein extract on UV-B induced retinal pigment epithelial cell damage" submitted by Sathid Aimjongjun in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) is hereby approved.

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#### ABSTRACT

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UV-B induced oxidative stress of retinal pigment epithelial cells plays an important role in the development of age-related macular degeneration (AMD). Although, lutein has been shown to protect cultured retinal cells from various oxidative insults, there was no direct evident showing its protective effect against UV-B irradiation. Besides marigold flower, yellow silk cocoon is an interesting source of lutein. The aim of this study was to investigate the effect of lutein extracted from silk yellow cocoon, on UV-B induced retinal epithelial cell damage. ARPE-19 cells line was used in this study. The results showed that UV-B irradiation decreased cell viability via caspase 3 dependent cell apoptosis, increased production of intracellular reactive oxygen species (ROS) and lipid peroxidation as well as altered activity of antioxidant enzymes including catalase and superoxide dismutase and glutathione peroxidase. These effects of UV-B could be inhibited by pre-treatment cells with 50 µM of lutein. Silk lutein extract, and standard lutein (marigold flower) similarly exhibited the protective effect against UV-B induced oxidative stress in ARPE-19 cells. In addition, the combination of both luteins and vitamin E (25 µM each) showed the protective effect in the same degree as individual compound at 50 µM. This synergistic effect might be because vitamin E prevented the degradation of lutein. Taken all data together, silk lutein extract could prevent retinal pigment epithelial cells

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damage mediated by UV-B irradiation and its combination with vitamin E could be useful for development of lutein preparation as dietary supplement for AMD prevention.

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### **ABBREVIATIONS**

3-D	=	three dimensional
α	=	alpha
β	=	beta
λ	=	lambda
μm	=	micrometer
μl	=	micro liter
kDa	=	kilodalton
°C	=	degree celsius
Abs.	=	absorbance
ACE	=	angiotensin converting enzyme
AMD	=	age-related macular degeneration
CAT	=	catalase
CaCl <sub>2</sub>	==	calcium chloride
cm <sup>-1</sup>	=	inverse centimeters
$cm^2$		centimeter square
Cont.	=	continued
$CO_2$	==_	carbon dioxide
Cu	=	copper
DAB	=	3, 3'- diaminobenzidine
DI	=	deionized
DMEM	=	Dulbecco's Modified Eagle Medium
DMSO	=	dimethyl sulfoxide
DNA	=	Deoxyribonucleic acid
EDTA	=	ethylenediaminetetraacetic acid
EtOH	=	ethanol
FBS	=	fetal bovine serum

## **ABBREVIATIONS (CONT.)**

g	=	gram
GPx	=	glutathione peroxidase
Gly	=	glycine
h	=	hour(s)
Н	=	hematoxylin
$H_2O$	=	hydrogen oxide
$H_2O_2$	=	hydrogen peroxide
HDL	=	high density lipoproteins
HPLC	=	High-performance liquid chromatography
М	=	molarities
MDA	=	malondialdehyde
mJ/cm <sup>2</sup>	=	millijoule per centimeter
mg	=	milligram
min	=	minute
ml		milliliter
mm	=	millimeter
MW	=	molecular weight
MTT	=	methylthiozol tetrazolium
MWCO	=	molecular weight cut-off
nm	=	nanometer
$O_2$	=	superoxide anion
OH	=	hydroxyl radical
р	=	probability values
Р	=	passage
pAb	=	polyclonal antibodies
PBS	=	phosphate buffer saline
pAb	=	polyclonal antibodies

# ABBREVIATIONS (CONT.)

рН	=	power of hydrogen ion concentration
PDT	=	photodynamic therapy
ROS	=	reactive oxygen species
RPE		retinal pigment epithelial
SD	=	standard deviation
SEM	=	standard error of mean
SOD	=	superoxide dismutase
std.	=	standard
Т	=	temperature
TBS	=	tris buffered saline
TE	=	tissue engineering
TE U	=	tissue engineering unit
U	=	unit