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Research Article

PROTECTIVE EFFECTS OF CYNIN ON HUMAN KERATINOCYTES (HACAT) AGAINST UV-B

[1]*Elayaperumal Natarajan, [2]Subramanian Sankarlal, [3]Raja Krishnamoorthy, [4]Rajasekaran Ramadoss and [5]Seetharaman Rathakrishnan

[1, 2,3,4] Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502
[5]Department of Botany, Faculty of Science, Annamalai University-608002

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ABSTRACT

Increased exposure to Ultraviolet-B (UV-B) radiation is the major risk for various skin injuries. Numerous studies have shown that bioproducts could demonstrate photopreventive efficacy against UV-B damage. Herein, we aimed to investigate the photoprotective effects of cynin (cynoglossus semifasciatus) on preservation of human keratinocytes,and antioxidants, reactive oxygen species (ROS) against UV-B. This study demonstrated feasibility of cynin on cosmeceutical application via ultraviolet B induced damage protective effects such as enzymatic (SOD,CAT,GPX),nonenzymatic (GSH) antioxidants, lipid peroxidation assays and decrease of apoptotic cell death. To investigate protein secondary structural characterization used Fourier transform infrared spectroscop. Secondary structure of skin epidermal mucoprotein (SEM) of C. semifasciatus was alpha-helical, henceforth so named the resultant product as cynin (cynoglossus semifasciatus). cynin pre-trated enzymatic, nonenzymatic antioxidants and lipid peroxidation significantly decreased the effects of UV-B irradiate at 15mJ/cm2 in HaCaT cells in dose dependent manner. The florescent microscopical observation revealed that the cell death was significantly reduced by pre-treated cynin in a dose dependent manner in HaCaT cells when exposed to UV-B irradiated at 15mJ/cm2. Through these results we verified that the cynin has effects on scavenging enzymatic nonenzymatic antioxidants,lipid perooxidation and protection of human keratinocytes against UV-B.

INTRODUCTION

Although ultraviolet (UV) is essential for human life, it can impair the ability of basal keratinocytes to maintain skin homeostasis against UV-induced damage, by which can be a major contributor in the development of skin cancers (Kong *et al*. 2015). Ultraviolet B (UVB), a type of UV in the wavelength ranging from 280 nm to 315 nm, has been studied in the epidermal sun-burn of keratinocytes that are destined to DNA damage and apoptosis after sun exposure. Although humans are exposed to UVA 10 to 100 folds more than UVB, UVB is known to be 1,280 folds more effective at inducing erythema, thus more than 90% of erythema induced by UV irradiation is attributed to UVB (Ryu *et al*. 2015). Furthermore, due to the ozone layer becoming thinner from anthropogenic activities, an excessive amount of UVB in the biosphere is being irradiated (**Liu *et al.* 2015**). UVB stimulates generation of reactive oxygen species (ROS), including superoxide anion radical (O2), hydrogen peroxide (H2O2), hydroxyl radical (HO·), and singlet oxygen (1O2) that may result in the destruction of keratinocytes through cellular damage and apoptosis (Kulms and Schwarz 2002, Rezvani *et al*. 2006, Kim *et al*. 2007, Gill and Tuteja 2010, Oh *et al*. 2016, Sanjeewa *et al*. 2016).

Peptides are important bioactive natural products which are present in many marine species. These marine peptides have high potential nutraceutical and medicinal values because of their broad spectra of bioactivities. Their antimicrobial, antiviral, antitumor, antioxidative, cardioprotective (antihypertensive, antiatherosclerotic and anticoagulant), immunomodulatory, analgesic, anxiolytic anti-diabetic, appetite suppressing and neuroprotective activities have attracted the attention of the pharmaceutical industry, which attempts to design them for use in the treatment or prevention of various diseases. Some marine peptides or their derivatives have high commercial values and had reached the pharmaceutical and nutraceutical markets. Bioactive peptides were first discovered and isolated in marine species as neurotoxin (Tu, 1974), cardiotonic peptide (Norton *et al*., 1976), antiviral and antitumor peptide (Rinehart *et al*., 1981)

Corresponding author:* **Elayaperumal Natarajan
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502

[5], cardiotoxin (Bemheimer *et al*., 1982) and antimicrobial peptide (Matsunanga *et al*., 1985). Since then, the investigations on marine bioactive peptides have continued with intent to also ascertain their applications.

Additionally, oxidation is believed to the major course of food deterioration because ROS-mediated oxidation can react with lipids, proteins, amino acids, vitamins, and cholesterol to produce undesirable off-flavors, and potentially toxicity during food processing, transportation, and storage (Tao *et al*., 2018, Zhao *et al*., 2018). Therefore, it is very important for pharmaceutical, health food, and food processing and preservation industries to develop efficient antioxidants (Sila *et al*., 2016). At present, some artificial antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) show stronger antioxidant activities and have been widely used in medicine and food industry for retarding oxidation in organisms and food (Gogineni and Hamann,2018, Sila *et al*., 2016). However, the side effects of synthetic antioxidants such as liver damage and carcinogenesis cause consumer anxiety and significantly affect their application (Sila *et al*., 2016, Chi *et al*., 2015). Therefore, there has been a major interest in searching for efficient antioxidants from natural sources as alternatives to synthetic antioxidants for countering these adverse effects. At present, dietary antioxidant ingredients including vitamins, carotenoids, flavonoids, phenols, saccharides, and peptides, have been continually investigated for their health benefits in terms of their scavenging potential of free radicals and low toxicity (Comert and Gokmen, 2018). Among them, bioactive peptides released from food proteins under controlled proteolysis have aroused wide public concern not only in their possibilities as natural alternatives to synthetic antioxidants, but also for their beneficial effects, lack of residual side effects, and functionality in food systems (Hu *et al*., 2015).

Since the recent research trends on marine biopeptide, there has been a process of research on anti-cancer, anti-inflammation, and anti-oxidation, but nothing about research on application of skin epidermal mucoprotein/peptide as cosmetic materials. Also, researches on skin preservation against UV and about natural substances which delay cellular senescence have been increasing continuously in these days. So it is necessary to investigate various effects of skin epidermal mucoprotein on usual action of keratinocyte in the course of dermal senescence. Therefore, this research intends to study effects of skin epidermal mucoprotein/peptide from **cyanin**, *Cynoglossus semifasciatus* such as cell preservation, anti-oxidation, and DNA repair and examine its effects to find out possibility for application of as naturalcosmetic materials which can help delaying dermal cellular senescence.Tongue sole *C. semifasciatus* is a member of cyanoglossidae family,originally native from the east and west coast of India, Sri Lanka, Phillippine, and Indonesia. This research indicate that sea food-derived protein/peptide have strong antioxidant activity and could serves as functional ingredients as pharmaceuticals agents in long-term sunshine injury prevention.

MATERIAL AND METHODS

Sample Collection, Extraction and Purification

Live specimens of the fish *cynoglossus semifasciatus* were collected from Nagapattinam as by-catch. Epithelial mucus was sampled by scraping a dull scalpel blade along the dorsal flank of live fishes, anterior to posterior. Mucus of the fish was collected from the dorsal region of the skin using blunt edged scalpel. Mucus was not collected from Ventral side of the fish to avoid urine and intestinal excreta (Chong *et al.,* 2005). The fish was placed on a flat non slippery surface with its head and eyes covered by palms to reduce the photophobic response (fear of light). Using a dull blade, mucus was gently scraped off the entire dorsal flank of fish as described by Zamzow (2004). Mucus sample was taken from the anterior section by moving from the head towards the anus using a spatula and stored in the sterile Amber bottle and stored in ice, to avoid bacterial contamination and proteins degradation during the transportation Sample Preparation: 0.1002 g of sample was weighed and dissolved in 10 ml of methanol and diluted to 25 ml with methanol (Stored at -4º C). Preparation of 0.2 % Acetic acid: 0.2 ml of Acetic acid mixed with 100 ml of distilled water, 75 ml of 0.2 % Acetic acid mixed with 25 ml of methanol and Filtered through and 0.45 µm nylon vacuum filter and sonicated. Each sample was then mixed using a sonicator in an ice bath (Unisonics) for 20 min and left to leach for 24 h at room temperature. The extracts were then centrifuged for 5 min at 18 000 × *g* and the supernatant was used for laboratory spectral UV analysis. Samples were extracted in 1.5 ml of 100% methanol and homogenized. Partial purification of tongue sole *C. semifasciatus* skin epidermal mucoprotein afte his name so called as cynin in further textual reference. Partial purification of fish mucus was carried out by Silica gel chromatography.

Charecterization of Cynin by Fourier Transform Infrared Spectroscopy (FTIR)

The cynin peptide samples were dissolved in a solution of 50 mM sodium phosphate buffer (NaPB) and trifluoroethanol (TFE, 50 % in 50 mM NaPB, (V/V) at a concentration of 2 mg/ml. Fourier-transform infrared spectra were recorded on a Perkin-Elmer spectrometer equipped with a TGS detector. A Perkin-Elmer model 4000 data station was used for acquisition, storage and analysis. Samples (volume up to 50 µl) were placed in a thermostatically controlled Beckmann FH-01CFT microcell fitted with CaF2 windows. The sample compartment was continuously purged with dry air to eliminate absorption by water vapor in the spectral region of interest. Spectral conditions (defined more fully in Haris *et al*., (1986) and Perkins *et al*., (1988) were as follows: number of scans, 200; spectral resolution, 4 cm^{-1}; sample thickness of 50 µm using a Teflon spacer; sample temperature, 20^0C. After subtraction of the background measured with ^2H_2O buffer, amide I region (1700-1600 cm^{-1}) was used for peptide structure determination.

Cell Line: Culture of Human Keratinocyte (HaCaT) Cell Line

HaCaT cells were grown in DME/HAMS F-12 medium containing 10% FBS, 10,000 IU/ml penicillin and 10,000 µg/ml streptomycin in a 25 Cm2 culture flask in a CO2 incubator at 37°C and 5% CO2 under controlled humidified atmosphere. Once the cells reached ~90% confluency, they were trypsinized using trypsin (0.05%) EDTA (0.54 mM) solution washed thoroughly with media and subcultured into a 75 Cm2 culture flask for expansion. This process was repeated twice till the cells attained a consistent growth phase. Once

after the cells attained consistent growth phase, they were trypsinized at 80% confluency and then utilized for the assay.

Experimental Design and procedure: cynin from the tongue sole *C. semifasciatus*, the FME were divided into 6 groups, 30 minutes before irradiation, test doses (3, 10, 30 and 100 µg/ml) of FME were added. Preliminary cytotoxicity studies were carried out. Group 1: Normal keratinocytes, Group 2: UV-B irradiated keratinocytes, Group 3: UV-B irradiated with cynin (100 µg/ml), Group 4: UV-B irradiated with cynin (30 µg/ml), Group 5: UV-B irradiated with cynin (10 µg/ml), and Group 6: UV-B irradiated with cynin (3 µg/ml). Irradiation procedure:

HaCaT cells were cultured in 6 well plates. Media had been removed and washed with phosphate based saline (PBS). For irradiation purpose, a broadband UV-B irradiation was applied using TL 20 W/20 fluorescent tubes served as a UV-B source in the range of 280-320 nm, peaked at 312 nm. 15 mJ/Cm^2 doses were prescribed.Sources of UVR are characterized in radiometric units. The terms dose (J/m^2) and dose rate (W/m^2) pertain to the energy and power, respectively, striking a unit surface area of an irradiated object (Jagger, 1985). UV-B radiation emitted by the narrow band TL20/W01 is 2.3 WATT. The tube emits radiation in the range of 280 nm-320 nm, peaked at 312 nm.Treatment of the cells:Thirty minutes prior to irradiation two test-doses (3 µg/ml and 10 g/ml) of cynin were added to the grouped normal Keratinocytes. Before exposure to UV light, the cell cultures were washed twice with PBS.

Evaluation of Protective effect of cynin on UV-B induced oxidative damage

The HaCaT cells were harvested by trypsinization and washed with Phosphate buffered saline (PBS). The cells were suspended in 130 mM KCl plus 50 mM PBS containing 10 µM dithiotheritol, then centrifuged at 20,000xg for 15 minutes (4ºC). The supernatant was collected and used for the biochemical estimations.

Determination of Antioxidant status

Assay of Superoxide Dismutase (SOD, EC 1.15.1.1)

SOD in the HaCaT cell suspension was assayed by the method of Kakkar *et al.* (1984).The assay is based on inhibition of the formation of NADH-Phenazine methosulphate, nitroblue tetrazolium formazan; the reaction is initiated by the addition of NADH to assay mixture. After incubation for 90 seconds, addition of glacial acetic acid stopped the reaction. The colour developed at the end of the reaction was extracted into n-butanol layer and the absorbance was measured at 520 nm.

Reagents: 1. Sodium pyrophosphate buffer: 0.025 M, pH 8.3, 2. Absolute ethanol, 3. Chloroform pH, 4. n-butanol, 5. Phenazine methosulphate (PMS). 186 µmol., 6. Nitroblue tetrazolium (NBT): 300 µmol., and 7. NADH: 780 µmol.

Procedure: The HaCaT supernatant (0.5 mL) was added to 2.5 mL of ethanol and 1.5 ml of chloroform (chilled reagents were added). This mixture was shaken for 90 sec at 4º C and then centrifuged.

The enzyme activity in the supernatant was determined as follows: The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of Phenazine methosulphate and 0.3 mL of nitroblue tetrazolium and appropriately diluted enzyme preparation in a total volume of 3 mL. The reaction was started by the addition of 0.2mL NADH. After incubation at 30º C for 90 seconds, the reaction was stopped by the addition of 1 mL of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and n-butanol layer was separated. The colour density of the chromogen in n-butanol was measured at 510 nm. A system devoid of enzyme served as control.

The enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard conditions was taken as one unit. The specific activity of the enzyme was expresses as unit/min/mg protein for HaCaT cells.

Estimation of Catalase (CAT, EC 1.11.1.6)

The activity of Catalase in the HaCaT cell suspension was determined by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H2O2. The chromic acetate formed was measured at 620 nm. The Catalase preparation was allowed to split H2O2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H2O2 as chromic acetate was determined colorimetrically.

Reagents: 1. Phosphate buffer: 0.01 M, pH 7.0., 2. Hydrogen peroxide: 0.2 M, 3. Dichromate-acetic acid reagent: 1:3 ratio of 5% potassium dichromate was mixed with glacial acetic acid. From this 1 mL was diluted again with 4 mL of acetic acid, and 4. Standard hydrogen peroxide: 0.2 mM.

Procedure: HaCaT cell suspension was prepared by using PBS. In 0.9 mL of PBS, 0.1 mL of HaCaT suspension and 0.4 mL of hydrogen peroxide were added. The reaction was arrested after 15, 30, 45 and 60 seconds by adding 2.0 mL of dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and thedeveloped was read at 620 nm. Standards in the concentration, ranged of 20-100 µmol were taken and subjected to the same and preceded as the test. The specific activity was expressed as µmol of H2O2 consumed/min/mg/protein for HaCaT cells.

Estimation of Glutathione Peroxidase (EC 1.11.1.19): The activity of glutathione peroxidase in the cell suspension was measured by the method of rotruck *et al.* (1973). A known amount of enzyme preparation was allowed to react with H2O2 in the presence of GSH for a specific time period. Then the remaining GSH content was measured.

Reagents: 1. Tris buffer: 0.4M, pH 7.0, 2. Sodium azide solution: 10 mM., 3. TCA: 10%, 4. EDTA: 0.4 mM., 5. H2O2 solution: 0.2 mM., and 6. Glutathione solution: 2 mM.

Procedure: To 0.2 mL of Tris buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.5 mL of HaCaT supernatant was added. To the mixture, 0.2 mL of GSH followed by 0.1 mL of H2O2 was added. The contents were mixed well and incubated at 37ºC for 10 minutes, along with a control containing all reagents except homogenate. After 10 minutes, the reaction was arrested by the addition of 0.5 mL of 10% TCA. The tubeswere centrifuged and the supernatant was assayed for GSH by the method of Ellman (1959). The activity was expressed as µmol of GSH consumed/min/mg protein.

Estimation of Reduced Glutathione (GSH): Reduced glutathione in the cell suspension was estimated by the method of Ellman (1959). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5-5'-dithio-bis (2-nitrobenzoic acid) (DNTB) was added to compounds containing sulphydryl groups.

Reagents: 1. Phosphate buffer: 0.1M, pH 8.0., 2. TCA: 5%, 3. Ellman's reagent: 34 mg of DTNB in 10 mL of 0.1% sodium citrate, 4. Disodium hydrogen phosphate: 0.3 M., and 5. Standard glutathione solution: 10.0 mg/100 mL.

Procedure: HaCaT supernatant (0.5 mL) was pipetted out and precipitated with 2 mL of 5% TCA. 2 mL of supernatant was taken after centrifugation and 1 mL of Ellman's reagent and 4 mL of 0.3 M disodium hydrogen phosphate were added. The yellow colour developed was measured at 412 nm. A series of standards (20-100 µg) was treated in a similar manner along with a blank containing 1mL of buffer. The amount of glutathione was expresses as mM/mg protein.

Determination of lipid peroxidation: Estimation of Thiobarbituric acid reactive substances (TBARS): The concentration of Thiobarbituric acid reactive substances (TBARS) in the cell suspension was estimated by the method of Niehaus and Samuelsson, (1968). In this method, malondialdehyde and other TBARS react with thiobarbituric acid in an acidic condition to generate a pink colour chromophore which was read at 535 nm.

Reagents: 1. TCA: 15%, 2. HCl: 0.25 N, 3. TBA: 0.375% in hot distilled water, 4. TBA-TCA-HCl reagent: 4.8 molar Solution of stock was prepared from 1, 1, 3', 3' tetra - methoxypropane purchased commercially., 5. Stock Standard: Solution1 to 3 was mixed in the ratio 0f 1:1:1 freshly prepared prior to use., and 6. Working Standard: Stock solution was diluted to get a 48 nmol/Ml.

Procedure: The HaCaT supernatant (0.5 ml) of sample was diluted with double distilled water (0.5mL) then 2.0 mL of TBA-TCA-HCl reagent was added. The mixture was kept in a boiling water bath for 15 min. After cooling, the tubes were centrifuged at 1000×g for 10 min and the supernatant was estimated for TBARS. Series of standard solutions in the concentration of 2-10 nmol were treated in a similar manner. The absorbance of the chromophore was read at 535 nm against a reagent blank. The values were expressed as nmol/mg protein for HaCaT.

RESULTS

Structural Characterization of the Purified cynin antioxidants

The secondary structure of cynin was estimated using a FTIR spectrometer in the presence of sodium phosphate buffer (NaPB). The contents of ß-turn, ß-sheet, random coils, glutamine and Histidine amino acid side chains were calculated according to **Barth, 2007**. The FTIR spectra in NaPB were characteristic of ß-sheet and random coils structure with glutamine and histidine as amino acid side chains (**Fig.1A)**. The secondary structure of cyanoglossin was further estimated by FTIR in the presence of TFE. Under the hydrophobic condition of 50% TFE solution, the contents of ß-sheet and random coils structure changed to α-helix (**Fig.1B)**.

FTIR spectra peak location and assignments for *Cynoglossus semifasciatus (Day, 1877)* cyanin (Barth, 2007)

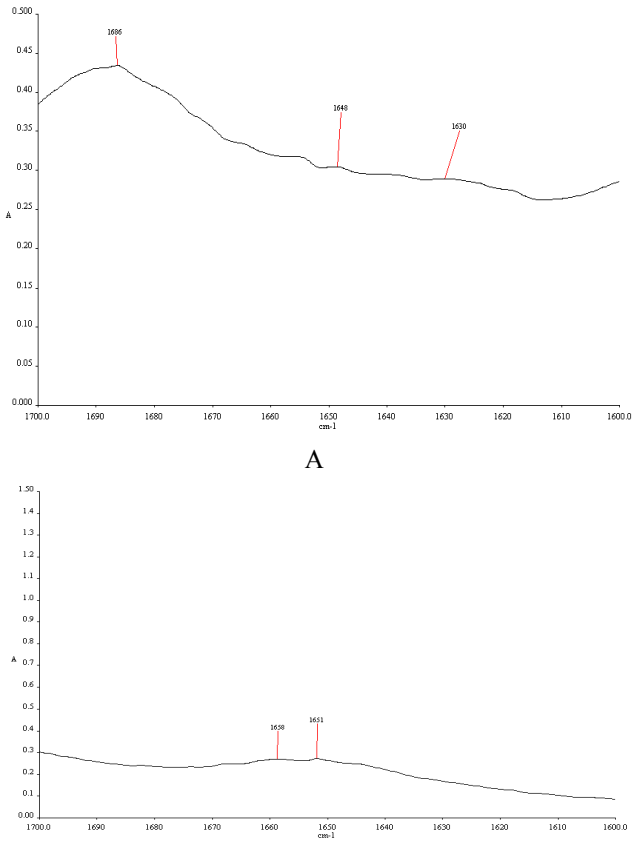

Fig 1 FTIR spectra (A) and (B) of skin epidermal mucprotein, cynin extract of *C.semifasciatus.* The FTIR spectrum of active fraction was measured in 50 mM NaPB (A) and 50% TFE in 50 mM NaPB (B).

Effect of cynin on enzymatic antioxidant activity in UV-B radiation induced HaCaT Cells.

To investigate whether the radical scavenging activity of SEM was mediated by antioxidant enzymes, the levels of SOD, CAT and GPX in HaCaT cells were analyzed. The results showed that the UV-B-irradiated HaCaT cells at 15m J/Cm2 decreased the antioxidant enzymes level when compared to control (**Fig 2, 3.4**). Cynin pre-treated HaCaT cells significantly restored the effect of UV-B radiation at 15m J/ Cm2 in HaCaT cells in dose dependent manner (**Fig. 2, 3, 4**).

Effect of cynin on non-enzymatic antioxidant activity in UV-B radiation induced HaCaT Cells.

To explore whether the radical scavenging activity of cynin was mediated by non-antioxidant enzyme, the level of GSH in HaCaT cells were analyzed. The results showed that the UV-B-irradiated HaCaT cells at 15m J/Cm2 decreased the non-antioxidant enzyme level whencompared to control (**Fig. 5**). Cynin pre-treated HaCaT cells significantly restored the effect of UV-B radiation at 15m J/Cm2 in HaCaT cells particularly 3mg/ml and 10 mg/ml in dose dependent manner (**Fig. 5**).

Effect of cynin on UV-B radiation induced lipid peroxidation in HaCaT Cells.

The results showed that the UV-B-irradiated HaCaT cells at 15m J/Cm^2 increased the TBARS level. SEM pre-treated significantly decreased the effect of UV-B radiation at 15m J/Cm2 in HaCaT cells in dose dependent manner. The values of the scavenging activity of free radicals by cynin were shown in **Fig. 6**. The figure declared that the UV-B-irradiated HaCaT cells at 15m J/Cm^2 increased the TBARS level the antioxidant activity of cynin using DPPH assay when compared to control. The activity increased by the upsurge of exposure duration. The highest increment was after one hour, it was found to be 47.54 ± 0.69% while the radical scavenging activity of control (zero time) was 29.07 ± 0.42% at the same concentration (3 mg/ mL and 10 mg/ml). This means that the UV exposure for one hour led to about 61.15% increase more than control. Cynin pre-treated HaCaT cells significantly restored the effect of UV-B radiation at 15m J/Cm2 in HaCaT cells particularly 3mg/ml and 10 mg/ml in dose dependent manner (**Fig. 6**).

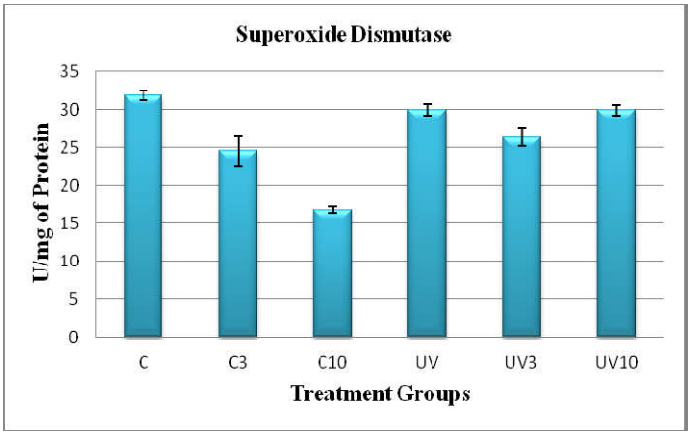

Fig 2 Antioxidant enzyme Superoxide Dismutase activity of alone cynin and UV-B irradiation with pre- treated cynin

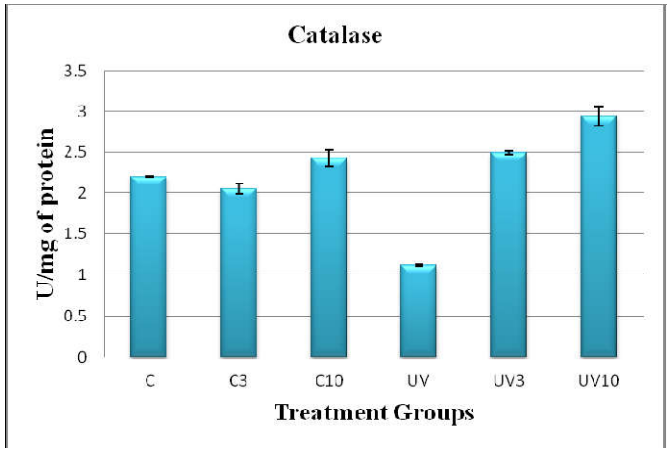

Fig 3 Antioxidant enzyme Catalase activity of alone cynin and UV-B irradiation with pre- treated cyanin

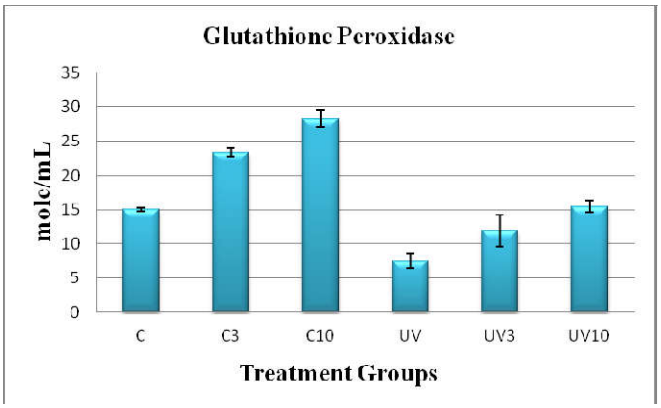

Fig 4 Antioxidant enzyme Glutathione peroxidase activity of alone cynin and UV-B irradiation with pre- treated cynin

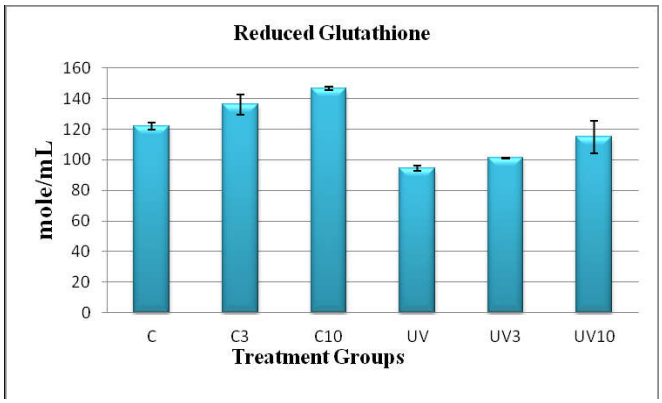

Fig 5 Non-enzymatic Antioxidant Reduced Glutathione activity of alone cynin and UV-B irradiation with pre- treated cynin

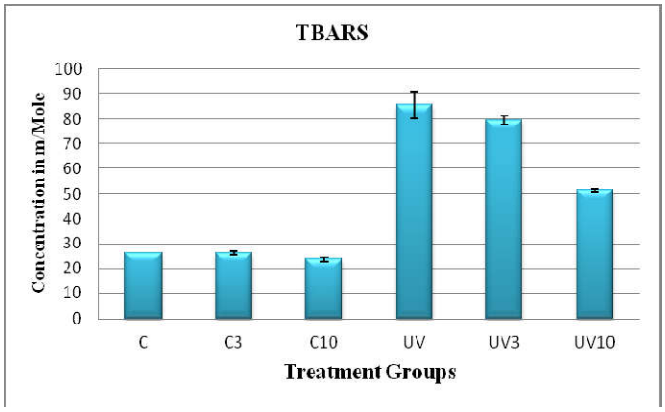

Fig 6 Lipid peroxidation activity of alone cynin and UV-B irradiation with pre-treated cynin

DISCUSSION

UVR (200–400 nm), particularly UV-A and UV-B (280–400 nm) emitted from the sun permeates the atmosphere and penetrates deep into the epidermis and dermis layer, which then influence the immune system and lead to chronic skin cancer. UV-B induced ROS induces different hazardous effects on skin, including sunburn, photoaging and skin cancer (Tomaino *et al.,* 2006). To protect against oxidative damage, skin cells have evolved a complex antioxidant defence system which includes enzymatic antioxidants, such as SOD, CAT and GPx (Cajkova *et al.,* 2000) and several non-enzymatic antioxidants such as GSH (Shindo *et al.,* 1994). Previous studies described that enhanced oxidative stress induced by UV-B radiation is accompanied with decreases in activities of SOD, CAT and

GPx (Shindo *et al.,* 1994 and Steenvoorden *et al.,* 1997). The present study clearly portrayed that there was a significant decrease in the SOD, CAT and GPx activities in Human Keratinocytes, exposed to UV-B irradiation. There are at least three ways of affecting antioxidant enzymes by UV irradiation, [1] direct absorbance of light, [2] interaction with ROS generated by UV light and [3] antioxidant-recycling mechanisms, whereby one antioxidant can be spared at an expense of another (Shindo *et al.,* 1994). Moreover, methanol extract of *Sargassum thunbergii* protects against hydrogen peroxide-induced cytotoxicity and oxidative stress by increasing the expression of antioxidative enzymes including SOD, CAT and GPx (Kim *et al.,* 2010). In our study, methanolic extracts of fish mucus from tongue sole *cynoglossus semifasciatus* provided protection against the UV-B induced oxidative stress by increasing the enzymatic antioxidants that includes SOD, CAT and GPx.

Pre-treatment with sesamol increases the activities of antioxidant enzymes in UV-B-irradiated fibroblasts, and thus, sesamol can exert a beneficial action against pathologic alterations caused by the UV-B radiation (Ravanat *et al.,* 2001). Our result also showed that there was an increase in the enzymatic antioxidants that contained SOD, CAT and GPx. GSH is considered to be a free radical-scavenger or a cofactor for protective enzymes, which plays a pivotal role in the cellular defence against oxidative damage (Moysan *et al.,* 1993). UV-B irradiation leads to decreased levels of GSH due to leakage and oxidation of GSH (Merwald *et al.,* 2005). GSH depletion of cultured human skin cells makes them sensitive to UV-B induced mutations and cell death (Punnonen *et al.,* 1991). Glutathione metabolism is important in quenching the reactive intermediates and radical species generated during oxidative toxicity (Pamela Maher, 2005). Our study also showed that UV-B irradiation caused a significant decrease in the levels of GSH, when compared with the normal HaCaT cells, SEM pretreated groups showed significantly increased levels of GSH, when compared with corresponding UV-B irradiated groups.

Overexposure to UV causes oxidative stress as evidenced by increased lipid peroxidation and by the depletion of cutaneous antioxidants (Soter, 1990; Afaq and Mukhtar, 2001). If the ROS remain, without being scavenged in the biological system, they can induce biochemical alterations, including inflammation, oxidation of lipids, proteins, DNA damage and activation or inactivation of certain enzymes (Punnonen *et al*., 1991). Lipid components in the membranes are highly susceptible to radiation damage (Bhattacharya *et al.,* 2009). The occurrence of TBARS in the biological membrane is a free radical-mediated event. Lipid peroxidation induced by UV-B radiation is known to be due to the attack of free radicals on the fatty acid component of membrane lipids (Bhattacharya *et al.,* 2009). In this study result showed an increased TBARS production in UV-B irradiated cells. Lipid peroxidation generated by oxidative stress of UV-B light in the skin has been known to be potentially deleterious for cellular function, having cytotoxic effects, stimulatory or inhibitory effects on enzymes, and cell membrane damage and carcinogenic effects (Shindo *et al.,* 1994). Lipid peroxidation is a complex multistep progression wherein the primarily formed lipid radicals get converted to TBARS. In the present study 1] The UV-B induced oxidation of lipids as TBARS was analyzed as a marker of oxidative stress, 2] The level of TBARS significantly increased in UV-B irradiated cells, and 3] cynin renders protection against UV-B-radiation induced TBARS.

UV-B generated ROS cause damage to lipid membranes. Rastogi *et al.* (2010) also show that increased lipid peroxidation in HaCaT cells, exposed to UV-B. Lipid peroxidation generated by oxidative stress of UV-B light in the skin has been known to be potentially deleterious for cellular function, having cytotoxic effects, stimulatory or inhibitory effects on enzymes, and cell membrane damage and carcinogenic effects (Shindo *et al.,* 1994). Our result showed that cynin rendered protection against UV-B-radiation induced lipid peroxidation.

Many studies report that UV-B radiation induces ROS in epidermal keratinocytes (Heck *et al.,* 2003). UV-B exposure generates ROS, which accounts for UV-B induced photocarcinogenesis (Kang *et al.,* 2003). UV-B exposure promotes the intracellular ROS production in skin, which impairs the cellular defense against oxidative stress and causes various cellular changes such as DNA damage and alters intracellular signaling (Sander *et al.,* 2004; Kundu *et al.,* 2008). Increase of ROS production was observed in UV-B induced HaCaT cells. However, the presence of cynin on UV-B exposed cells significantly reduced ROS generation in a dose dependent manner. cynin pre-treatment reduced ROS generation in UV-B irradiated cells. This might be due to ROS scavenging property of cynin. Cynin of tongue sole acted as effective molecule to protect the cells from UV-B induced oxidative damage by means of increasing antioxidant enzymes also kept the cell wall membrane protective from lipid peroxidation. This research indicate that sea food-derived protein/peptide have strong antioxidant activity and could serves as functional ingredients as pharmaceuticals agents in long-term sunshine injury prevention. Therefore, more detailed study should be designed for clarifying the relationship between the activities and structures of the isolated peptides. Nevertheless, the biological efficacy of cynin *in vivo* and the underlying mechanisms of cynin action require further exploration.

CONCLUSION

In conclusion, the results of this study demonstrate that cynin suppresses the deleterious effects of UVB irradiation in human keratinocytes, including excessive intracellular ROS generation, oxidative damage to DNA, lipids, and proteins, and mitochondrial dysfunction. Moreover, cynin improves cell viability and inhibits apoptosis in UVB-exposed human keratinocytes. These data support the hypothesis that cynin might be utilized as a novel antioxidant agent to treat , antioxidants, ROS-related skin disorders.

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References

1. Kang N, Lee JH, Lee W, Ko JY, Kim EA, Kim JS, Heu MS, Kim GH, and Jeon YJ. Gallic acid isolated from *Spirogyra* sp. improves cardiovascular disease through a vasorelaxant and antihypertensive effect. Environ. Toxicol. Pharmacol. 2015; 39:764-772.
2. Ryu B, Ahn BN, Kang KH, Kim YS, Li YX, Kong CS, Kim SK, and Kim DG. Dioxinodehydroeckol protects human keratinocyte cells from UVB-induced apoptosis modulated by related genes Bax/Bcl-2 and caspase pathway. J. Photochem. Photobiol. B Biol. 2015; 153:352-357.
3. Liu M, Li X, Liu Y, Shi Y, and Ma X. Analysis of differentially expressed genes under UV-B radiation in the desert plant *Reaumuria soongorica*. Gene. 2015; 574:265-272.
4. Kulms D & Schwarz T. Mechanisms of UV-induced signal transduction. J. Dermatol. 2002; 29:189-196.
5. Rezvan, HR, Mazurier F, Cario-André M, Pain C, Ged C, Taïeb A & de Verneuil H. Protective effects of catalase overexpression on UVB-induced apoptosis in normal human keratinocytes. J. Biol. Chem. 2006; 281:17999- 18007.
6. Kim JK, Kim Y, Na KM, Surh YJ & Kim TY. [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression *in vitro* and *in vivo*. Free Radic. Res. 2007; 41:603-614.
7. Gill SS & Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 2010; 48:909-930.
8. Oh JY, Fernando IPS. & Jeon YJ. Potential applications of radioprotective phytochemicals from marine algae. Algae. 2016; 31: 403-414.
9. Sanjeewa KKA, Fernando IPS, Samarakoon KW, Lakmal HHC, Kim EA, Kwon ON, Dilshara MG, Lee JB. & Jeon YJ. Anti-inflammatory and anti-cancer activities of sterol rich fraction of cultured marine microalga *Nannochloropsis oculata*. Algae. 2016; 31:277- 287.
10. Rinehart KL, Gloer JB, Cook JC, Mizsak SA, Scahill TA. Structures of the didemnins, antiviral and cytotoxic depsipeptides from a Caribbean tunicate. *J. Am. Chem. Soc.* 1981; *103*, 1857–1859.
11. Bernheimer AW, Avigad LS, Lai CY. Purification and properties of a toxin from the sea anemone *Condylactis gigantea*. *Arch. Biochem. Biophys.* 1982; *214*, 840–845.
12. Matsunaga S, Fusetani N, Konosu S. Bioactive marine metabolites, IV. Isolation and the amino acid composition of discodermin A, an antimicrobial peptide, from the marine sponge *Discodermia kiiensis*. *J. Nat. Prod.* 1985; *48*, 236–241.
13. Tao J, Zhao YQ, Chi CF, Wang B. Bioactive peptides from cartilage protein hydrolysate of spotless smoothhound and their antioxidant activity In vitro. Mar. Drugs 2018;16, 100. [CrossRef] [PubMed]
14. Gogineni V, Hamann MT. Marine natural product peptides with therapeutic potential: Chemistry, biosynthesis, and pharmacology. BBA—Gen. Subj. 2018; 1862, 81–196. [CrossRef] [PubMed]
15. Carocho M, Morales P, Ferreira ICFR. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci. Technol. 2018; 71, 107–120. [CrossRef]
16. Zhao WH, Luo QB, Pan X, Chi CF, Sun KL, Wang B. Preparation, identification, and activity evaluation of ten antioxidant peptides from protein hydrolysate of swim bladders of miiuy croaker (Miichthys miiuy). J. Funct. Foods. 2018; 47, 503–511. [CrossRef]
17. Sila A, Bougatef A. Antioxidant peptides from marine by-products: Isolation, identification and application in food systems. A review. J. Funct. Foods 2016; 21, 10–26. [CrossRef]
18. Chi CF, Wang B, Wang YM, Zhang B, Deng SG. Isolation and characterization of three antioxidant peptides from protein hydrolysate of bluefin leatherjacket (Navodon septentrionalis) heads. J. Funct. Foods. 2015; 12, 1–10. [CrossRef]
19. Cömert ED, Gökmen V. Evolution of food antioxidants as a core topic of food science for a century. Food Res. Int. 2018,105, 76–93. [CrossRef]
20. Hu FY, Chi CF,Wang B, Deng SG. Two novel antioxidant nonapeptides from protein hydrolysate of skate (Raja porosa). Muscle. Mar. Drugs. 2015;13, 1993–2009. [CrossRef]
21. Shindo Y E, Witt D, Han and Packer L. Dose-response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. *J. Invest. Dermatol*. 1994; 102(4): 470-475.
22. Shindo YE, Witt D, Han B, Tzeng T, Aziz L, Nguyen and L. Packer. Recovery of antioxidants and reduction in lipid hydroperoxides in murine epidermis and dermis after acute ultraviolet radiation exposure. *Photodermatol. Photoimmunol. Photomed*. 1994; 10: 183-191.
23. Shindo Y, E Witt, D Han, W Epstein and L Packer. Enzymic and nonenzymic antioxidants in epidermis and dermis of human skin. *J. Invest. Dermatol*. 1994; 102: 122-124.
24. Cejkova J, S Stipek, J Crkovska and T Ardan. Changes of superoxide dismutase, catalase and glutathione peroxidase in the corneal epithelium after UVB rays: histochemical and biochemical study. *Histol. Histopathol*. 2000; 15: 1043-1050.
25. Steenvoorden DPT, MJ Gerard and BV Henegouwen. Cysteine derivatives protect against UV-induced reactive intermediates in human keratinocytes: the role of glutathione synthesis. *Photochem. Photobiol*. 1997; 66(5): 665-671.
26. Steenvoorden DPT, MJ Gerard and BV Henegouwen. The use of endogenous antioxidants to improve photoprotection. *J. Photochem. Photobiol. B*. 1997; 41: 1-10.
27. Kim JA, CS Kong and SK Kim. Effect of *Sargassum thunbergii* on ROS mediated oxidative damage and identification of polyunsaturated fatty acid components. *Food and Chemical Toxicology*. 2010; 48(5): 1243-1249.
28. Ravanat JL, T Douki and J Cadet. Direct and indirect effect of UV radiation on DNA and its components. *J. Photochem. Photobiol. B. Biol*. 2001; 63: 88-102.
29. Moysan A, I Marquis, F Gaboriau, R Santus, L Dubertret and P Morliere. Ultraviolet A-induced lipid peroxidation and antioxidant defense systems in cultured

human skin fibroblasts. *J. Invest. Dermatol*. 1993; 100(5): 692-698.
30. Merwald H, G Klosner, C Kokesch, M Der-Petrossian, H Honigsmann and F Trautinger. UVA-induced oxidative damage and cytotoxicity depend on the mode of exposure. *J. Photochem. Photobiol. B. Biol.* 2005; 79(3): 197-207.
31. Punnonen K, P Autio, U Kiistala and M Ahotupa. In vivo effects of solar simulated ultraviolet irradiation on antioxidant enzymes and lipid peroxidation in human epidermis. *Br. J. Dermatol*.1991; 125: 18–20.
32. Pamela Maher. The effects of stress and aging on glutathione metabolism. (Review). *Ageing Res. Rev*. 2005; 4: 288-314.
33. Soter NA. Acute effects of ultraviolet radiation on the skin, *Seminars in Dermatology*.1990; 9(1): 11-15.
34. Afaq F and H Mukhtar. Effects of solar radiation on cutaneous detoxification pathways. *Journal of Photochemistry and Photobiology*. 2001; 63(1-3): 61-69.
35. Bhattacharya S, JP Kamat, SK Bandyopadhyay and S Chattopadhyay. Comparative inhibitory properties of some Indian medicinal plant extracts against photosensitization-induced lipid damage. *Food Chem*. 2009; 113: 975-979.
36. Rastogi RP, A Richa Kumar, MB Tyagi and RP Sinha. Molecular mechanisms of ultraviolet radiation induced DNA damage and repair. *J. Nucleic Acids*. 2010;1-32.
37. Kang S, JH Chung, JH Lee, GJ Fisher, YS Wan, EA Duell and JJ Voorhees.Topical N-acetyl cysteine and genistein prevent ultraviolet light induced signaling that leads to photoaging in human skin *in vivo*. *J. Invest. Dermatol*., 2003; 120: 835-841.
38. Kundu JK, KS Choi, H Fujii, B Sun and YJ Surh. Oligonol inhibits UVB-induced COX-2 expression in HR-1 hairless mouse skin-AP-1 and C/EBP as potential upstream targets. *J. Funct. Foods.* 2008; 84(2): 399-406.
39. Tu, A.T. Sea snake venoms and neurotoxins. *J. Agric. Food Chem.* 1974, *22*, 36–43.
40. Norton TR, Shibata S, Kashiwagi M, Bentley J. Isolation and characterization of the cardiotonic polypeptide anthopleurin-A from the sea anemone *Anthopleura xanthogrammica*. *J. Pharm. Sci.* 1976; *65*, 1368–1374.
41. Tomaino A, M Cristani, F Cimino, A Speciale, D Trombetta, F Bonina and A Saija.*In vitro* protective effect of a *Jacquez grapes* wine extract on UVB induced skin damage. *Toxicol. In Vitro*. 2006; 20: 1395-1402.

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