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PHOTOPROTECTIVE EFFECTS OF CARRAGEENANS AGAINST ULTRAVIOLETB-INDUCED EXTRACELLULAR MATRIX (ECM) DAMAGE IN KERATINOCYTES

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Abstract: Carrageenans, the polysaccharide from red seaweeds, are widely used in food, medicine and as an excipient in cosmetics and skincare products. Carrageenans have shown a prospective photoprotective effect against ultraviolet B (UVB) irradiation on immortalised normal human keratinocyte (HaCaT) cells. This research evaluated the photoprotective effect of iota (ι), kappa (κ)-carrageenans and their combination with vitamin E against UVB-induced extracellular matrix (ECM) damage in HaCaT cells. The study also assessed the superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes in UVB-exposed pre-treated and non-pre-treated cells. The parameters assessed were biological mediators important for the structural integrity of ECM, comprising of epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), activating protein- (AP-1) and matrix metalloproteinases (MMPs), liberated by the UVB exposed cells. Enzyme-linked immunosorbent assay was used to evaluate the release of these mediators. Carrageenans enhanced the activities of the antioxidant enzymes SOD and CAT, which acts as a defence mechanism against oxidative stress. The levels of the biological mediators were also reduced in cells pre-treated with carrageenans, suggesting that the polysaccharide has the potential to retain skin's structure by reducing the damage to ECM upon UVB exposure. In conclusion, the results imply that carrageenans possess photoprotective effects against UVB-induced ECM damage and stimulate antioxidant enzymes.

Keywords: *ultraviolet B (UVB), photoaging, extracellular matrix, antioxidant enzymes, carrageenan*

1. Introduction

Ultraviolet radiation (UVR) causes not only skin cancer but also photoaging (Rabe et al.,2006), a cumulative process characterised by clinical, histological and biochemical changes in skin that makes one appear aged (Berneburg et al.,2000). The extent of damage to the extracellular matrix (ECM) following UVB exposure could be understood by the initiation of epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), activating protein-1 (AP-1) and matrix metalloproteinases (MMPs) liberated by the cells. These biological mediators are important in a cell's structural integrity and help keep a cell in shape.

Ingredients with anti-inflammatory, antihistamine or antioxidant properties such as vitamin E, vitamin A, green tea extract and polyphenols are incorporated for photoprotection (Davis & Callender,2010) as topical skin care products or sunscreens. However, not all sunscreens grant an antioxidant effect, and not all antioxidants produce a sunscreen effect (Lin et al.,2005). Algae and their extracts are increasingly recognised in skin care regimes. They are used in cosmetics either as an excipient or therapeutic agent (Agatonovic-Kustrin & Morton,2013). Carrageenans are used in cosmetics as an excipient to improve lubricity and softness. They are high molecular weight polysaccharides extracted from red seaweeds (Rhodophyceae) cell wall and intercellular matrix (Kalitnik et al.,2013), mostly of the genera Chondrus, Eucheuma, Gigartina and Iridea with 15% to 40% ester-sulphate content (Necas & Bartosikova,2013). Due to the thickening water-binding nature, carrageenans are incorporated into sunscreens, anti-ageing creams, facial creams, and soap (Campo et al.,2009). Although extensively used as an excipient, the value of carrageenans as a photoprotective or therapeutic agent for skin has not been explored, especially in their protective effect against UV damage.

The photoprotective property of carrageenans is expected to be contributed by their sulphur moieties, polysaccharide nature, mineral content and gelling property. Due to their sulphated characteristic (Zhang et al.,2010),

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carrageenans possess antioxidant activity by quenching free radicals induced by UVR (Yuan et al.,2006). Photoprotective effects of carrageenans on HaCaT and Mouse Embryonic Fibroblasts (MEF) cells (Ren et al.,2010) and antioxidative (Sokolova et al.,2011) activities have been reported. Our previous study showed the ability of carrageenans to act as an antioxidant against reactive oxygen species (ROS) in UVB-induced HaCaT cells, along with their photoprotective and anti-inflammatory properties (Thevanayagam et al.,2014). Incorporating photoprotective agent into cosmetic products is desirable. Combining several antioxidants is a therapeutic approach to reducing photoaging and skin cancer incidence. Hence, the potential use of carrageenans as the main compound for photoprotection is worth exploring.

Pathologically, photoaging is characterised by the abnormal structure and alteration of the dermal connective tissue and ECM (Hwang et.al.,2011). Such effects impair skin function, causing it to look aged (Quan et al.,2009). Extreme change of biochemical mediators causes degradation of ECM. A complex cascade of biochemical reactions in the human skin is triggered by UVR. These biological mediators are of importance to be studied since photoprotection could be achieved if the release of these mediators could be manipulated via effective treatment.

In UVB-exposed skin, keratinocytes absorb the radiation, generates ROS along with impaired natural antioxidants, leads to a flow of events starting with the stimulation of EGFRs (Saeed et al.,2012) followed by MAPK signalling (Rabe et al.,2006) that induces AP-1 (Wen et al.,2012) and leads to the transcription of MMPs, (Pandel et al.,2013) enzymes responsible for ECM and collagen degradation that promotes wrinkle and metastases (Brenneisen et al.,2002). Dermal fibroblasts and epidermal keratinocytes have MMP expression (Yasui & Sakurai, 2003). The MMPs include MMP-1 (collagenase), which degrades collagen types I and III; MMP-9 (gelatinase B) further breakdowns collagen and MMP-3 (stromelysin) degrades collagen type IV (Herouy, 2001; Sachs et al., 2009). The ability of carrageenans and/or vitamin E treatment to suppress these mediators in UVB-exposed HaCaT is expected to protect the ECM and integrity of the skin, hence shielding against photoaging.

This study aims to assess the photoprotective action of carrageenans against UVB-induced damage in keratinocytes. The assessment was based on the quantification of biological mediators liberated by UVB-induced cells. The effect of carrageenans on superoxide dismutase (SOD) and catalase (CAT) was also assessed. This study has incorporated a novel use of carrageenans in protecting UVB-induced ECM degradation in keratinocytes. Apart from being an excipient, carrageenan is a potentially photoprotective agent. These findings will be a breakthrough in the field of dermatology and cosmeceutical, and our results will create a paradigm for future studies in the evolution of carrageenans for photodamage prevention and treatment.

2. Materials and Methods

2.1 Cell culture

Immortalised normal human keratinocyte (HaCaT) cells from cell line services (CLS) (Germany) were cultured and maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO Life Technologies, USA), 1% penicillin-streptomycin (10,000 units of penicillin (base) and 10,000 µg of streptomycin (base) ml−1, GIBCO Life Technologies), and stored at 37°C in an atmosphere of 5% CO2 and 99% humidity incubator.

2.2 Carrageenans

Two isomers of purified food-grade carrageenans, namely iota (ι)-carrageenan from Eucheuma denticulatum and kappa (κ)-carrageenan from Eucheuma cottonii (PT. Wahyu Putra Bimasakti, Indonesia) were used. Vitamin E (Vit-E) (α-tocopherol) of 96% purity from Sigma Aldrich, USA, was positive control. Dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific, USA) was used to dissolve stock solutions of ι, κ and vit-E. For subsequent experiments, compounds were taken from stock solutions and diluted to the desired concentrations using complete DMEM.

2.3 Setup for UVB

The UV source was a UVB lamp (Uvitec, UK) emitting UV radiation at 312 nm. The UVB intensity was measured using a UVA/B light meter (Sper Scientific, USA), and a constant intensity of 1.17 mW was maintained throughout the study. The time of exposure was varied to obtain the different UVB fluences.

2.4 Treatment

Four sets of cells used are as below:

a) Control (cells alone): Cells without treatment or irradiation.
b) Cells pre-treated with carrageenans and/or vit-E without UVB irradiation.
c) Cells subjected to UVB irradiation without pre-treatment.
d) Cells pre-treated before UVB irradiation.

For all the tests, ι, κ, vit-E and the combination of ι and vit-E, and κ and vit-E at a final concentration of 12.5 µg/mL were added in triplicate into microplate wells containing 2x104 cells/mL unless stated otherwise. For the combination solution, equal volume and concentration of individual

chemicals were added to a final concentration of 12.5 µg/mL. After treatment, the samples were incubated for 24 hours as pre-treatment time. After 24 hours, the culture medium was replaced with fresh medium before being irradiated with UVB at 100 and 300 mJ/cm². The plates were incubated for 24 hours as a recovery period. All experiments were carried out in triplicate unless stated otherwise.

2.5 Effect of UVB on biochemical mediators and antioxidant enzymes

2.5.1 SOD and CAT

SOD and CAT were detected using The OxiSelect Superoxide Dismutase Activity Assay and The OxiSelect Catalase Activity Assay kit (Cell Biolabs', USA) according to the manufacturer's protocols. The extracellular SOD activity was determined based on the inhibition of the superoxide anion by each test group. The percentage of inhibition following equation 1 was calculated by comparing the optical density (OD) of the blank and samples. A catalase standard curve was plotted, a trend line of a second order polynomial was established, and the chart equation was used to calculate CAT activity.

$$\textbf{SOD Activity (inhibition } \%) = \frac{\textbf{ODblank} - \textbf{ODsample}}{\textbf{ODblank}} \textbf{ X 100} \quad (1)$$

2.5.2 EGFR and phosphorylated EGFR

The intracellular EGFR and its activation (Phosphorylated EGFR) form were detected using the Pierce In-Cell Colorimetric ELISA Kit (Thermo Fisher Scientific, Illinois, USA) per the manufacturer's protocols. The HaCaT cells were cultured in a 96-well microplate at a density of 1x104 cells per well and incubated for 24 hours and proceeded with the treatment protocol mentioned above. The readings at 450 nm were averaged for each experimental condition, and the target protein modification was assessed by calculating the fold change as a ratio of the treated and non-pre-treated modified protein. The values were then presented as a percentage of EGFR and Phosphorylated EGFR.

2.5.3 MAPK

Endogenous levels of MAPK were evaluated using the PathScan®MAP Kinase Multi-Target Kit (Cell Signalling Technologies, Massachusetts, USA), which detects six different target proteins per the manufacturer's protocols. The experiment was carried out in duplicate. The magnitude of absorbance is proportional to the number of bound target proteins. Each protein released was compared to the untreated group and converted to a percentage.

2.5.4 AP-1

After the treatment and irradiation period, DNA from the desired cells was extracted using the Genomic DNA Mini Kit (Geneaid Biotech Ltd, Taiwan). The amount of DNA was standardised to 0.6 µg/µL using the Nuclear Extract Dilution Buffer. The AP-1 levels were determined using the TF ELISA target-specific kit (Panomics, USA) per the manufacturer's protocols. The absorbance was read at 450 nm and converted to a percentage based on the comparison with the blank and positive reading (supplied in the kit) for each sample.

2.5.5 MMPs

The extracellular release of MMP 1, 3 and 9 were determined using the Human MMP-1, 3, 9 enzyme-linked immunosorbent assay (ELISA) kit (RayBio®, Inc, Norcross, Georgia, USA). The amount of MMPs was determined in each sample by using the linear interpolation equation from the standard curve that was generated based on the standard control given in the kit.

2.5.6 Statistical analysis

Data were analysed using one-way ANOVA followed by Bonferroni post hoc multiple comparisons using SPSS Version 18. A p value <0.05 between the control and test groups was considered significant. Data presented as the mean of triplicate of each group ± SD (n=3).

3. Results

3.1 SOD and CAT

The control cells inhibited superoxide anion the highest (65.79%), showing functional SOD activity. Carrageenan and vit-E pre-treated but nonirradiated cells showed moderate inhibition of superoxide anions. Cells exposed to UVB without treatment had a weaker ability to inhibit superoxide anion, with only 20.77% inhibition after 100 mJ/cm², which reduced to 13.94% after 300 mJ/cm². In contrast, pre-treated groups significantly elevated inhibition against superoxide anions by 32.51-44.47% after 100 mJ/cm² UVB, the highest being κ and vit-E combination (65.24%). At 300 mJ/cm², cells treated with κ showed the highest percentage (61.42%) of superoxide anion inhibition. The combination treatment showed better SOD activity compared to vit-E alone but not against carrageenan by itself (Figure 1).

Catalase in control cells was the highest, 78.63 Units/ml (U/ml). Non-pre-treated cells had 14.95 U/ml CAT after 100 mJ/cm², which decreased by almost half after 300 mJ/cm². However, pre-treated cells showed significantly

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higher ($p < 0.05$) CAT. After 100 mJ/cm² and 300 mJ/cm², the highest CAT was in cells treated with κ, 48.53 U/ml, 68.39 U/ml, respectively and combination treatment induced higher CAT compared to vit-E alone. (Figure 2).

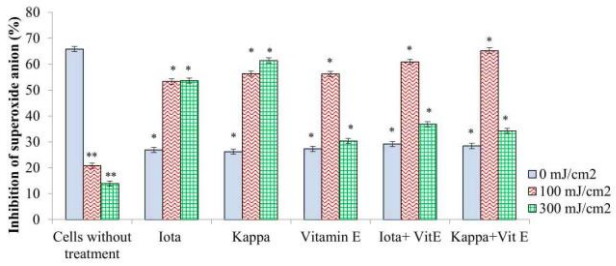

Figure 1. Percentage of superoxide anion inhibition. *Significant difference between non-pre-treated irradiated cells at p<0.05 with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at p<0.05 with non-pre-treated and nonirradiated cells.

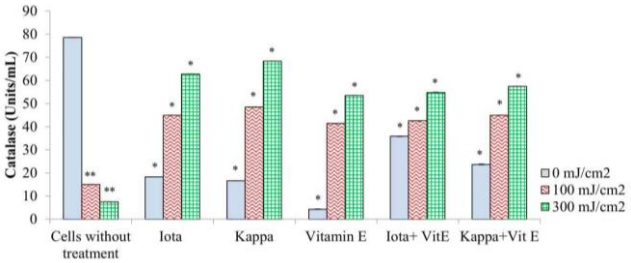

Figure 2. Catalase production. *Significant difference between non-pre-treated irradiated cells at p<0.05 with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at p<0.05 with non-pre-treated and nonirradiated cells.

3.2 EGFR and Phosphorylated EGFR

The level of both EGFR and its activated form (phospho-EGFR) in control cells was taken as 100% as the starting point. A reduction in EGFR was found in cells treated with carrageenans and vit-E without irradiation (Figure 3a), with a 3.00-4.00% increase in phospho-EGFR level (Figure 3b).

After 100 mJ/cm² UVB, the EGFR level in all irradiated groups increased by 31.00% compared to the control cells. However, the increase in pre-treated groups was only 1.00-12.00%, indicating the treatment was able to suppress EGFR. The phospho-EGFR in non-pre-treated cells was higher than the treated groups. After 300 mJ/cm² significantly ($p < 0.05$) higher level of EGFR was observed, with a 150% increase compared to the control cells and phospho-EGFR with a 226% increase in cells without treatment.

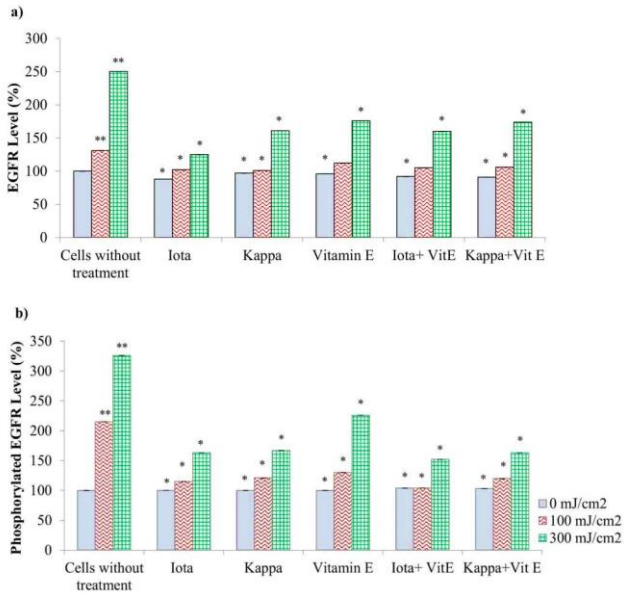

Figure 3. Levels of a) EGFR and b) Phospho-EGFR. * Significant difference between non-pre-treated irradiated cells at p<0.05 with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at p<0.05 with non-pre-treated and nonirradiated cells.

3.3 MAP kinase

Activated EGFRs lead to an increase in MAP kinase signalling. In this study, six target proteins, namely the Phospho-p44/42 MAPK (Thr202/Tyr204), Phospho-p38 MAPK (Thr180/Tyr182), MEK1, Phospho-MEK1/2 (Ser217/221), SAPK/JNK and Phospho-SAPK/JNK(Thr183/Tyr185) were evaluated to determine the overall MAPkinase action (Table 1).

In comparison to the control cells, protein concentrations of the lysates in cells treated with carrageenans and/or vit-E without UVB irradiation showed either a decrease or increase in the release of Phospho-p44/42 MAPK (Thr202 /Tyr204), Phospho-p38 MAPK (Thr180/Tyr182), MEK1 and SAPK / JNK. After UVB, all six protein lysates increased in a fluence-dependent manner in comparison to the control. The most pronounced increase was Phospho-p44/42 MAPK (Thr202/Tyr204), which increased by 183.33% and 289.58% after 100 mJ/cm² and 300 mJ/cm², respectively. Carrageenans and/or vit E pre-treated cells showed a lower percentage of MAPK proteins after UVB irradiation compared to the untreated cells.

Protein concentrates in the pre-treated cells compared to the control ranged from 16.67-139.58% for Phospho-p44/42 MAPK (Thr202/ Tyr204), 7.58-48.48% for Phospho-p38 MAPK (Thr180/Tyr182) and 12.50-143.75% for MEK1. In cells irradiated with 300 mJ/cm2, treatment of carrageenans and/or vit-E suppressed the release of Phospho-MEK1/2 (Ser217/221) and SAPK/JNK compared to the control.

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MAPK Proteins	UVB Fluence (mJ/cm^2)	Cells without treatment	Iota	Kappa	Vit E	Iota +Vit E	Kappa +VitE
Phospho-p44/42 MAPK(Thr202/Tyr204)	0	0±0.00	2.08±0.04*	12.5±0.08*	-10.42±0.13*	6.25±0.25*	-2.08±0.18*
	100	183.33±0.06**	85.42±0.08*	118.75±0.08*	100±0.11*	116.67±0.07*	139.58±0.09*
	300	289.58±0.05**	43.7±0.05*	27.08±0.09*	87.5±0.10*	102.08±0.14*	16.67±0.05*
Phospho-p38 MAPK (Thr180/Tyr182)	0	0±0.00	-3.03±0.09*	7.58±0.11*	0±0.10*	-16.67±0.05*	-24.24±0.07*
	100	69.7±0.09**	25.76±0.05	36.36±0.09	40.91±0.12*	27.27±0.07*	45.45±0.10*
	300	183.33±0.08**	36.36±0.11*	31.82±0.13*	48.48±0.18*	56.06±0.20*	7.58±0.04*
MEK1	0	0±0.00	-13.39±0.14*	10.71±0.08*	-7.14±0.16*	-8.93±0.17*	-14.29±0.17*
	100	144.64±0.09**	35.71±0.07	42.86±0.07*	143.75±0.12*	83.04±0.04*	118.75±0.14*
	300	158.04±0.07**	26.79±0.10	12.5±0.08*	29.46±0.18*	26.79±0.11*	14.39±0.09*
Phospho-MEK1/2 (Ser217/221)	0	0±0.00	43.24±0.16*	31.08±0.13*	37.84±0.08*	8.11±0.07*	31.08±0.13*
	100	135.14±0.07**	56.76±0.21	60.81±0.14*	127.03±0.07*	40.54±0.17*	167.57±0.13*
	300	235.14±0.06**	1.35±0.15	-39.19±0.20*	-10.81±0.34*	-33.78±0.03*	9.46±0.05*
SAPK/JNK	0	0±0.00	54.45±0.21*	20.45±0.14*	-36.36±0.16*	-54.55±0.12*	18.18±0.12*
	100	204.55±0.10**	72.72±0.21*	163.64±0.10	195.45±0.06*	179.55±0.10*	143.18±0.10*
	300	215.91±0.06**	-10±0.06*	-47.730.05*	6.38±0.25*	102.27±0.13*	-2.27±0.04*
Phospho-SAPK/JNK(Thr183/Tyr185)	0	0±0.00	-68.18±0.14*	-98.86±0.01*	-84.09±0.14*	-94.32±0.05*	-93.18±0.06*
	100	72.72±0.09**	-67.05±0.14*	-67.05±0.16*	-57.95±0.11*	-69.32±0.27*	-79.55±0.18*
	300	89.77±0.09**	-89.77±0.09*	-54.55±0.09*	-51.14±0.11*	-52.27±0.42*	-72.73±0.24*

TABLE 1. Percentage of MAPK proteins. *Significant difference between non-pre-treated irradiated cells at p<0.05 with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at p<0.05 compared with non-pre-treated and nonirradiated cells.

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3.4 Transcription factor (AP-1)

The control cells had 0.22% AP-1. An increase between 2.12-4.21% was seen in pre-treated cells without UVB. A UVB fluence-dependent pattern was observed in the percentage of AP-1 after 100 and 300 mJ/cm2 UVB in both treated and non-pre-treated groups, with the latter being the highest. In general, the protection against the formation of AP-1 in DNA was significantly observed after carrageenans treatment compared to nontreated cells. Although the combination group exhibited protection, it was not significantly ($p>0.05$) better than those treated with carrageenans alone. However, it exhibited a stronger protective effect compared to vit-E alone (Figure 4).

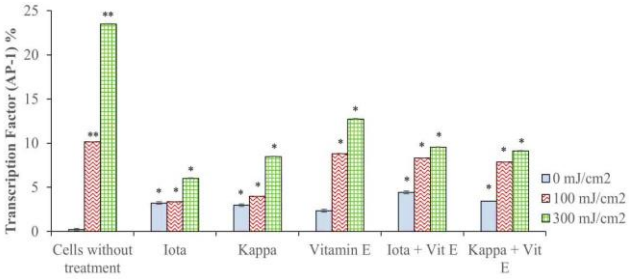

Figure 4. Percentage of transcription factor (AP-1) in 6 ug DNA. *Significant difference between non-pre-treated irradiated cells at $p<0.05$ with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at $p<0.05$ with non-pre-treated and nonirradiated cells.

3.5 MMPs

The MMP-1 in nonirradiated cells increased significantly ($p < 0.05$) after pre-treatment with carrageenans and vit-E. After subjecting to UVB, the level of MMP-1 in non-pre-treated cells increased significantly but decreased significantly in pre-treated cells. The reduction in MMP-1 was more pronounced in cells treated with the combination of carrageenans and vit-E compared to vit-E alone (Figure 5a).

Extracellular quantification of MMP-3 in nonirradiated cells remained almost constant when treated with the test compounds without UVR irradiation. The highest increase (2.79 ng) was observed in the non-pre-treated cells after 300 mJ/cm² irradiation, followed by 100 mJ/cm² (1.56 ng). All pre-treated groups showed lower amounts of the MMP compared to those without treatment after irradiation with UVB. The lowest was the κ- pre-treated group (0.32 ng) after 100 mJ/cm² UVB (Figure 5b).

The levels of MMP-9 increased significantly in nonirradiated pre-treated cells compared to the control cells (1295.83 pg). Likewise, the levels increased after UVB irradiation, with the highest level in non-pre-treated groups after 100 and 300 mJ/cm², respectively (Figure 5c).

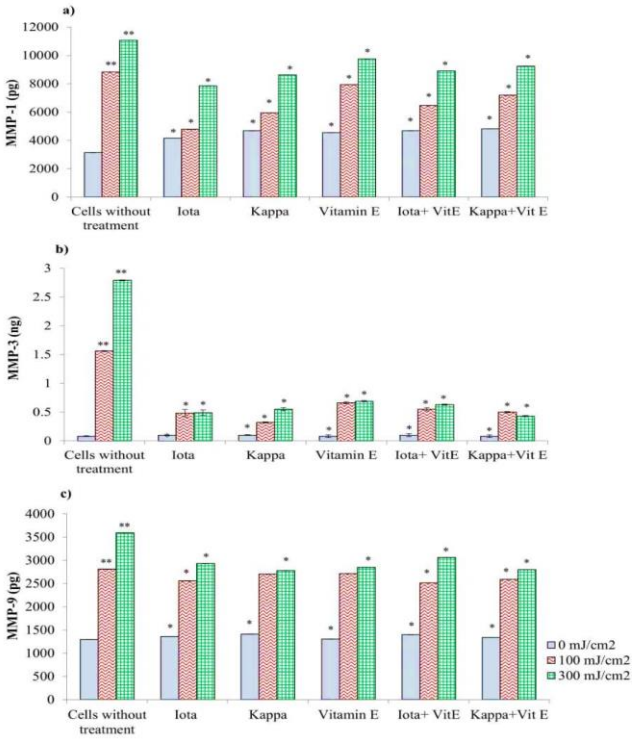

Figure 5. Level of a) MMP-1 b) MMP-3 c) MMP-9. *Significant difference between non-pre-treated irradiated cells at $p<0.05$ with pre-treated irradiated groups. **Significant difference between non-pre-treated irradiated cells at $p<0.05$ with non-pre-treated and nonirradiated cells.

4. Discussion

Superoxide dismutase and CAT protects human keratinocytes against UVB-induced cell damage (Rezvani et al.,2006), and a decrease in these enzymes causes excessive ROS damage. In this study, it was noticeable that the activity of SOD was higher in cells without irradiation than in those subjected to UVB, showing that normal cells spontaneously inhibit superoxide anions, but the tendency reduced after UVB.

Nevertheless, carrageenans enhanced SOD in irradiated cells by effectively removing superoxide anions after UVB compared to the non-pre-treated cells. A similar trend was observed with CAT. Sasaki et al., (1997) showed that SOD in HaCaT cells peaked following a single exposure to UVB, followed by a decline, which suggested the increase occurs as a cutaneous antioxidant defence against UVB. In the present study, higher inhibition of superoxide anion and CAT release was observed after exposure to 300 mJ/cm2 UVB compared to 100 mJ/cm2, which could be a defence mechanism against UVB, as postulated by Sasaki. The antioxidant defence in UVB irradiated cells was enhanced by carrageenans pre-treatment.

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The same study mentioned that treatment with high calcium following UVB exposure in keratinocytes induced higher SOD activity and cell viability (Sasaki et al.,1997). This further explains the possibility of carrageenans in stimulating SOD and protecting cells since carrageenan has high calcium content along with sodium, potassium and magnesium (Cian et al.,2015). As reviewed by Pandel et al., (2013), natural photoprotection agent exhibits their effect as an antioxidant and their capacity to increase SOD, CAT, GPx and other antioxidant enzymes, which collectively provides anti-inflammatory, anti-carcinogenic and anti-ageing properties. Similarly, we postulate carrageenans can enhance SOD and CAT activities as part of the antioxidant defence against UVB, thus, providing an overall photoprotective effect.

An increase in EGFR happens within minutes after UVR in cultured keratinocytes and mouse skin (El-Abaseri et al.,2005). He et al., (1997) found that EGFR was inhibited in breast cancer cells treated with kappa-selenocarrageenan preventing cell proliferation. The findings were like the present study, where carrageenans were able to inhibit EGFR and phospho-EGFR, and is postulated to be due to carrageenans' potential antioxidant mechanism whereby once ROS is attenuated, the actions of receptor ligands that activate EGFR are blocked (Meves et al.,2001). In a study using green tea, it was said that the green tea polyphenols repealed the UVB-induced proinflammatory signalling by binding with EGFR and suppressing extracellular signalling, which inhibited cell proliferation and led to apoptosis (Bowden, 2004). There is a possibility that carrageenans could have bonded to the EGFR, thus, suppressing its activation. In contrast, the high levels of EGFR in non-pre-treated cells could have led to the activation of other signalling pathways.

Activation of EGFR leads to increased signalling of MAPK proteins and pathways. The present study showed an increase in all the six target proteins after UVB, especially in the non-pre-treated groups, which could be due to the phosphorylation of EGFR. Phosphorylation of protein kinases can be regulated by UVR-induced oxidative stress via MAPKs and AP-1 signalling pathways (Mantena & Katiyar,2006). Mutations or alterations in the proteins regulating MAPK signalling, such as EGFR overexpression, mutations of rat sarcoma (RAS), or rapidly accelerated fibrosarcoma (RAF), can cause changes in MAPK regulation (Ouwens,2001).

Ultraviolet radiation activates all three MAPKs (p38, JNK, ERK) in skin cells (Dent et al.,2003). The activity of p38 due to ionising radiation is variable, showing either no activation (Kim et al.,2002), weak activation (Taher et al.,2000) or strong activation (Narang et al.,2009), whereas activation of ERK (MEK1/2) and SAPK/JNK pathways has been observed in varied cell types, whether to low or high radiation (Munshi & Ramesh, 2013). UVB raised p38 and ERK phosphorylation between two and ten hours after exposure. Although p38 activation was constant, a gradual increase was seen in the phosphorylation of ERK (Chen & Bowden, 1999). A similar pattern was observed in this study, where the highest was phospho-MEK1/2, phospho-SAPK/JNK, followed by phospho-p38 and phospho-p42/44. It is also proven that UVR, cytokines, DNA-damaging agents, and ROS strongly activate the SAPK/JNK pathway (Ouwens et al.,2001), as in this study. Moreover, ERK pathways are mostly associated with growth factor receptors, whereas activation of cytokine receptors regulates JNK and p38 pathways (Fisher et al.,1998); hence, in this study, it is surmised that the activation of ERK pathway is more likely compared to JNK and phospho-p38.

It was observed that UVB-induced JNK activation only lasted about an hour (Chen & Bowden,1999). Similarly, after 300 mJ/cm², SAPK/JNK activation was lower than phospho-p38 and phospho-p42/44. Hence, results suggest that SAPK/JNK activation could be reduced after 24 hours, in comparison to phospho-p38 and phospho-p44/42, which gradually increased proportionally to UVB fluence. Antioxidants attenuate the activation of MAPK signalling (Afaq & Mukhtar,2006); thus, this study suggests that MAPK suppression could be due to carrageenans being sulphated polysaccharides that act as antioxidants (Zhang et al.,2010).

Activation of AP-1 is directly related to phosphorylation of MAPK proteins, especially ERK and JNK (López-Camarillo et al.,2011), as observed in this study, especially in the non-pre-treated cells. A decrease of AP-1 in pre-treated groups could be due to the antioxidant capacity of carrageenans, which attenuated the MAPK proteins and failed to activate AP-1 in large quantities. AP-1 increases collagen breakdown and remains elevated within 24 hours after UVB exposure (Fisher et.al.,1996). Similarly, AP-1 remained elevated in non-pre-treated cells, but the reduction was observed in pre-treated groups.

Liebel et al., (2012) demonstrated that human epidermal keratinocytes generate a dose-dependent increase in proinflammatory cytokines and expression of MMPs after UVB, as seen in this study. Initiation of MMPs can be due to AP-1 activation (Berneburg et al.,2000). The potential antioxidant and immunomodulatory activities of carrageenans could have suppressed MMPs in treated cells, as supported by Sun et al., (2017), who showed that MMPs in keratinocytes could be prevented by antioxidant and immunomodulatory effects.

Studies proved that seaweeds or their extracts could inhibit MMPs (Chung et al.,2001; Kang J et al.,2008) due to their high phenolic content (Kim et al.,2006) by impeding the ERK pathways (Moon et al.,2008) or MMP-1 promoter (Moon et al.,2009). After UVA exposure, MMP-1 was directly stimulated in the dermal fibroblasts; however, MMP-1

release due to UVB required the presence of epidermis (Battie et al.,2014). Thus, it is appropriate to use keratinocytes in this study to assess the release of MMPs after UVB. The ability of carrageenans to induce SOD and CAT could have played a role in inhibiting MMPs.

Vitamin E protects cellular membranes and lipoproteins against oxidative damage by quenching singlet oxygen and superoxide anions (Agatonovic-Kustrin & Morton, 2013). It prevents the breakdown of collagen and reduces MMP-1; therefore, the use of α-tocopherol help protects the dermis from degrading, provided it is in high concentrations (Zussman et al.,2010). This corroborates with the present finding, which showed the lack of protection when keratinocytes were treated with vit-E at low concentrations. However, the combination of carrageenans and vit-E could provide stronger protection than vit-E alone.

5. Conclusion

This study showed that carrageenans have a potential photoprotective effect against UVB in keratinocytes, possibly through the antioxidant and anti-inflammatory properties that attenuated ROS and inflammatory cytokines, potentially halting the activation of other mediators responsible for photoaging. The outcome of this research would highlight the prospective value of carrageenans in enhancing or promoting photoprotection and photocarcinogenesis against UVB. However, further studies would have to be conducted to uncover the best formulation.

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