Ultraviolet Radiation Protection Potentials of Methylene Blue for Human Skin and Coral Reef Health

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Abstract

Methylene blue (MB) is a century-old medicine, a laboratory dye, and recently shown as a premier antioxidant that combats ROS-induced cellular aging in human skins. Given MB's molecular structure and light absorption properties, we hypothesize that MB has the potential to be considered as a sunscreen active for UV radiation protection. In this study, we tested the effects of MB on UVB ray-induced DNA double strand breaks in primary human keratinocytes. We found that MB treatment reduced DNA damages caused by UVB irradiation and subsequent cell death. Next, we compared MB with Oxybenzone, which is the most commonly used chemical active ingredient in sunscreens but recently proven to be hazardous to aquatic ecosystems, in particular to coral reefs. At the same concentrations, MB showed similar broad-spectrum UV light absorption ability as Oxybenzone but significantly outperformed Oxybenzone in the prevention of UVB-induced DNA damage and the clearance of UVA-induced cellular ROS. Furthermore, unlike Oxybenzone, MB-containing seawater did not affect the growth of the coral species *Xenia umbellata*. Altogether, our study suggests that MB has the potential to be a coral reef-friendly sunscreen active ingredient that can provide broad-spectrum protection against UVA and UVB.

Introduction

Methylene blue (MB) is a common laboratory dye and widely used medicine¹. Since its first synthesis in 1876, MB has been reported to have broad applications in clinical treatments of methemoglobinemia, malaria, vasoplegia, septic shock, and Alzheimer's disease, etc^{2,3,4}. As a diaminophenothiazine, MB has a low redox potential of 11 mV. This antioxidant property allows for efficient cycling between the oxidized form (MB) and the reduced form leucomethylene blue (MBH₂), which facilitates electron transport in the mitochondrial inner membrane and reduces mitochondrial superoxide production^{5,6}. The use of MB in skincare helps combat ROS-induced cellular aging. We have previously demonstrated that MB outperforms other leading antioxidants (e.g., N-Acetyl-L-Cysteine (NAC), MitoQ, and mitoTEMPO) in neutralizing free radicals and improving skin longevity⁷. MB also shows promising effects in stimulating the production of healthy skin fibers (e.g., Collagen and Elastin), improving skin hydration, and promoting wound healing⁷. Additionally, MB is environmentally friendly and is highly compatible with marine ecosystems. It is commonly used as an aqua culture supplement to treat injured or sick fish⁸. The properties of MB as an oxygen transporter make it useful in the treatment of known cyanide and nitrite poisoning of aquarium fish⁹.

The two types of UV radiation that reach our skin are UVA and UVB waves, which cause different types of skin damage. UVA waves are longer in wavelength (320-400nm) and penetrate deeper into the skin, generating high levels of ROS in the dermis and cause photoaging. UVB waves are shorter (290-320nm) in wavelength and carry higher energy—they cause erythema (i.e., sunburn), and DNA damage/mutations within skin cells (e.g., melanocytes, keratinocytes) that may lead to skin cancer.

Oxybenzone (BP-3) is one of the most commonly used chemical blockers in sunscreen products as a chemical blocker—over 80% of chemical sunscreens contain Oxybenzone or its derivatives¹⁰. However, multiple studies have shown that Oxybenzone is a significant hazard for aquatic ecosystems and expedites the destruction of coral reefs^{11,12}. These compounds have been found entering the ocean environment through wastewater effluent or directly from swimmers wearing sunscreens. In particular, a study published recently showed four major toxic effects of Oxybenzone in early, developing coral: increased susceptibility to bleaching; DNA damage; abnormal skeleton growth (via endocrine disruption); and gross deformities of coral nubbins¹¹. Thus, searching for nontoxic Oxybenzone alternatives is critical for protecting reefs and the exacerbating effects posed by climate change and bleaching. In May 2018, Hawaii became the first state to ban chemical sunscreens containing Oxybenzone¹³. Since then, Florida, California, the US Virgin Islands, Australia, Mexico Bonaire, Palau, and Aruba have joined the ever-growing list of concerned states and countries.

Given MB's phenothiazine ring structure, solubility, and antioxidant property¹⁴, we speculate that MB can be served as a potential Oxybenzone alternative. In this study, we measured the UV absorption properties of MB in comparison with Oxybenzone and tested the effects of MB on DNA damage and cell death induced by UVB in primary human keratinocytes. To determine the effects of MB on UVA-induced photoaging, we compared the cellular ROS and cell proliferation in skin cells treated with MB, Oxybenzone, or as well as two popular antioxidants in skincare, Vitamin A and Vitamin C. Finally, we compared the effects of MB exposure on a soft coral species *Xenia umbellata* with Oxybenzone.

Results

MB, but not Oxybenzone (BP-3), mitigates DNA double-strand breaks induced by UVB irradiation in human keratinocytes.

Our previous study demonstrated that MB could stimulate the DNA repair process by upregulating DNA damage repair pathway factors, such as PARP1, which potentially can undo the DNA damage caused by UVB radiation¹⁵. To directly analyze the impact of MB on DNA damage caused by UVB irradiation, we conducted a dosage escalation study. Primary human skin keratinocytes were pre-treated with 100 nM MB for two weeks and then exposed to 0, 5, 10, and 20 seconds of UVB rays at 1W/cm², which resulted in irradiation dosages of 0, 5, 10 and 20 J/cm². This range of UVB irradiation intensity was chosen empirically based on the UVB intensities detected on hot summer days at the University of Maryland campus (Supplemental Figure S1A-C).

To quantify the levels of DNA double-strand breaks, we used a well-established DNA damage marker γ H2AX¹⁶. The histone H2AX is phosphorylated to a gamma form (γ H2AX) upon DNA double-strand breakage, and the levels of γ H2AX positively correlate with the presence of DNA breaks, which is commonly generated by UVB¹⁷. β -actin was also probed as an internal loading control of each sample and the total expression of H2AX was measured as well. The DNA damage could be detected as early as

two hours post UVB exposure, indicated by elevated γ H2AX levels (Fig. 1A). Interestingly, compared to non-treated controls, skin keratinocytes that were pre-treated with MB showed reduced γ H2AX expression upon UVB irradiation at each dosage (Fig. 1B), suggesting that MB provide protection of the skin cells from the UVB damage.

Furthermore, to determine the long-term recovery potential in these irradiated cells, we measured γ H2AX levels at 2 and 24 hours after UVB irradiation (Fig. 1C-F). We found that under a lower dosage (5 J/cm^2), it was possible for the control cells (without MB pre-treatment) to recover and express relatively normal level of γ H2AX after 24 hours (Fig. 1C-D). Under the higher dosages (10 J/cm^2 or 20 J/cm^2) of UVB irradiation, the expression of γ H2AX continued to increase until at least 24 hours after UVB exposure (Fig. 1E-F and Supplemental Figure S2). Interestingly, in MB pre-treated cells, the magnitude of DNA damage was significantly reduced under all these dosages of UVB radiation (Fig. 1C-F and Supplemental Figure S2).

Next, we compared MB with Oxybenzone (BP-3), the leading sunscreen ingredient, to examine the potential mitigation of UVB-induced DNA damage. Primary human keratinocytes were pre-treated with 100 nM of MB or BP-3 for one week before exposure to UVB irradiation at 5 J/cm^2 . Again, relative γ H2AX expression was used as an indicator of DNA damage and was measured at 2, 6, and 24 hours after UVB exposure. Surprisingly, at the tested concentration, BP-3 did not provide any beneficial effects on lessening the DNA damage in skin keratinocytes when compared to the solvent controls (Supplemental Figure S3). In fact, the γ H2AX level at 24 hours post UVB exposure in the BP-3 sample was even higher than that of the control. In contrast to BP-3, MB protected the cells from UVB-induced DNA damage, indicated by milder γ H2AX elevation and better recovery in the MB-pretreated cells than control samples (Supplemental Figure S3).

MB prevents human keratinocytes from UVB-induced cell death.

Following the induction of unfixable amounts of DNA damage by UVB, prominent routes of cell inactivation are either cell senescence or cell death¹⁸. To further evaluate MB's ability to protect skin cells from UVB damage, we examined the cell senescence and viability by senescence-associated β -galactosidase (SA- β -Gal) staining and AlamarBlue cell viability assay, respectively. Both control and MB-treated keratinocyte samples were subjected to 0, 5, 10, or 20 J/cm^2 of UVB irradiation and allowed to recover for five days. At each UVB dosage, we found that there was an overall healthier appearance and reduced β -Gal staining in the MB-treated cells than in the control. A higher percentage of dead keratinocytes were found in the untreated controls, as indicated by the dark staining of the cells (Fig. 2A). Consistently, the MB-treated groups showed significantly higher survival rates after five days of recovery (Fig. 2B).

Together, these results indicate that MB treatment effectively reduces the extent of DNA damage induced by UVB in primary human keratinocytes, which further protects these skin cells from premature senescence and death.

MB shows a broad UV absorption capability comparable to Oxybenzone (BP-3).

One of the major characteristics of a chemical sunscreen blocker is its ability to absorb UV rays and convert the rays into heat. Thus, we next measured the absorption characteristics of MB using spectrophotometric analysis and compared the results with BP-3 at the same concentrations of 100uM and 1mM (Fig. 3A). The result indicated that MB has a broad-spectrum absorption range across both UVA and UVB wavelengths, and its absorption capacity is comparable to that of BP-3, especially at the high energy, short wavelength UVB range.

MB is a more robust ROS scavenger than BP-3, Vitamin A, and Vitamin C in skin cells.

UVA rays are longer in wavelength and penetrate deeper into the skin, generating high levels of ROS, leading to skin cell aging and death. To compare the MB's and BP-3's protective effects on UVA radiations, next, we conducted the cell proliferation and ROS assays in keratinocytes treated with the same concentrations of either MB or BP-3 (Fig. 3B-C). We observed an increase in cell proliferation in MB-treated human primary keratinocytes, indicating MB is beneficial to overall skin cell longevity. Although BP-3 has an EWG hazard score of 8 (toxic) on the human body¹⁹, the proliferation study did not reveal any significant inhibitory effects of BP-3 (Fig. 3B), which suggests that at the 100nM and 1µM concentrations, BP-3 is probably safe to human skin cells. Moreover, consistent with our previous study⁷, an evident dosage-dependent reduction in ROS levels in MB-treated cells was found (Fig. 3C). In contrast, no significant changes in ROS levels were observed in the BP-3 treated cells compared to Dimethyl Sulfoxide (DMSO)-treated controls, indicating BP-3 is not a ROS scavenger.

To further investigate MB's capability as a ROS scavenger and UVA protective agent for the skin, we obtained four human skin fibroblast cell lines from young male, old male, young female and old female donors (Detailed information in Supplemental Table S1). We evaluated the effectiveness of MB in comparison with Vitamin A and Vitamin C, both of which are popular antioxidants in skincare products²⁰. We first monitored the cell proliferation for a two-week time frame. In this experiment, we observed that MB enhanced the cell proliferation greatly in all four lines with comparable effectiveness to Vitamin C (Fig. 4A-B). Vitamin A, conversely, did not affect the cell proliferation rates (Fig. 4A-B). Interestingly, the combination of MB and Vitamin C synergistically boosted the cell proliferation capacities even higher in all four lines (Fig. 4A-B). Next, we measured the mitochondrial ROS after a four-week treatment period. Surprisingly, MB was the only treatment that consistently reduced the mitochondrial ROS in all four cell lines. The combination of MB and Vitamin C worked more effectively in old fibroblasts in ROS clearance, while in young cell lines, this combination has less of an effect (Fig. 4C-D).

Together, these results indicated that MB is a more robust ROS scavenger than Vitamin A and Vitamin C, and the combination of MB and Vitamin C has unexpected synergy in terms of promoting skin cell proliferation and scavenging cellular ROS, particularly in aged skin cells.

MB is non-toxic to coral health as compared to Oxybenzone (BP-3)

The results of the previous experiments support that MB's potential as an effective protectant for both UVA and UVB. To determine MB's effects on coral reefs, we grew the soft coral species *Xenia umbellata* in seawater containing either 1 μ M MB or 1 μ M Oxybenzone for one week (Fig. 5A). We observed that by Day 7 in the Oxybenzone-treated coral, tissue debris was found at the bottom of the coral incubating bag (Figured 5B). Also, following the collection of the tissue debris, we observed the lack of pulsing from the treated coral. Pulsing in the *Xenia* coral completely ceased (Supplementary videos). The coral colony appeared limp, disintegrated, and fell away from the light above (Fig. 5B), possibly indicating an expulsion of symbiotic zooxanthellae species from within the coral to the treated seawater²¹. None of these observations were found within the MB-treated corals (Supplementary videos).

Discussion

The two types of sunscreens on the market include physical blockers that scatter UV rays, i.e., Zinc Oxide, Titanium Dioxide, and chemical absorbers, such as Oxybenzone, that absorb UVA and UVB rays; both are designed to prevent UV rays from penetrating the skin. Although this traditional method for sun protection is mostly effective, even the sunscreens with the highest SPFs can only stop up to 98% of UVB rays from penetrating the skin²². The remaining UV waves that penetrate the skin can still cause damage. Moreover, penetration of UV waves into the skin can have positive health benefits, such as the generation of Vitamin D. Thus, we argue that new sun protection products should also be geared towards reversing the negative effects of UV radiations in addition to blocking UV waves.

MB is the first man-made compound that was synthesized in 1876²³. It has been approved for medical use by the FDA, and it is also on the WHO list of safe and essential medicines. Our previous work indicated that MB activates the DNA damage repair pathway inside the cell, possibly through activating DNA damage repair pathways¹⁵. In this study, we tested the ability of MB on mitigating UVB-induced DNA damages in primary human keratinocytes. We found that MB treatment reduced the overall levels of DNA double strand breaks caused by UVB irradiation and increased cell survival rate (Figs. 1 and 2). Absorption spectroscopic analysis revealed that MB has a broad-spectrum light absorption range covering from UVA, UVB to UVC wavelength (Fig. 3A). Thus, MB has the desired abilities of both UVB blocking and DNA damage mitigation.

We further evaluated the potential of MB as a sunscreen agent in comparison with Oxybenzone. Not only did MB show a broad UV absorption capability comparable to BP-3 (Fig. 3A), but MB also outperformed BP-3 in the alleviation of UVB-induced DNA damage (Supplemental Figure S3).

UVA radiation significantly increases levels of ROS inside the skin. These high levels of ROS result in damage to skin cells and their surrounding connective tissue, which leads to the physical signs (e.g., wrinkles, reductions in skin elasticity) of premature photoaging. Endogenous antioxidants within the skin are depleted during elevated levels of UVA generated ROS. As a result, many anti-aging skincare creams deliver exogenous antioxidants to help combat ROS. Vitamin A (commonly known as Retinol in skincare) and Vitamin C are popular active antioxidants used in skincare. In the comparative studies demonstrated

in Figs. 3B-C and 4, both MB and Vitamin C promoted cell proliferation, but BP-3 and Vitamin A had little effects at the tested concentrations (Fig. 3B and Fig. 4A-B). Among these four compounds, MB is no doubt the most effective ROS scavenger in human skin cells (Fig. 3C and Fig. 4C-D). Interestingly, we found that when MB is combined with Vitamin C, a positive synergistic benefit was observed. The MB plus Vitamin C treated cells showed the highest skin cell proliferation rate and lowest cellular stress as determined by mitochondrial ROS assay. Furthermore, this synergistic effect on combating ROS is more prominent in old skin cells than in young cells (Fig. 4C-D). These results indicate the combination of MB and Vitamin C could be used in skincare to deliver optimal anti-aging effects.

Besides MB's UVA and UVB blocking and mitigating effects, the soft coral experiment indicated that at a high concentration (1uM), MB posed no negative effects on coral health and growth (Fig. 5), which was not a surprise as MB has been commonly used by fish hobbyists as an aquaculture supplement. In contrast, Oxybenzone, which was reported to pollute the marine ecosystem previously^{11,12}, impaired the growth of coral reefs and caused coral bleaching and death in our experiments.

Altogether, these results suggest that MB has great potential to serve as a coral reef-friendly, Oxybenzone alternative in sunscreen products. It will bring many benefits that outweigh most of the current sunscreen actives, particularly relative to Oxybenzone. Firstly, as a potent antioxidant, the MB-based sunscreen may protect skin from ROS and photoaging caused by UVA rays⁷, which is lacking in current sunscreen products²⁴. Secondly, MB has a broad-spectrum absorption covering UVA and UVB and is able to lessen the UVB-induced DNA damages and protect cells from cell death, possibly by activating DNA repair pathways. Therefore, MB has the abilities of both UVB blocking and UVA/B damage mitigation. Lastly and most importantly, the MB-based sunscreen is safe for marine life and ecosystems, especially for the already endangered coral reefs²⁵.

Materials And Methods

Cell culture and drug treatment

The normal human primary epidermal keratinocyte line (PCS-200-010) was obtained from ATCC. Keratinocytes were thawed and cultured in Dermal Cell Basal Medium (PCS200030, ATCC) supplemented with Keratinocyte Growth Kit (PCS200040, ATCC) at 37°C with 5% CO₂. Four of normal human skin fibroblasts were purchased from Coriell Institute (detailed information described in Table S1). All fibroblast cells were cultured in MEM (Life Sciences) supplemented with 15% FBS (Gemini Bio-Products) and 2 mM L-glutamine (Life Sciences) at 37°C with 5% CO₂.

Methylene blue (1%, Akron) and L-Ascorbic acid (Vitamin C, A4544, Sigma-Aldrich) were prepared in PBS. Retinoid acid (Vitamin A, R2500, Sigma-Aldrich), Oxybenzone (BP-3, H36206, Sigma-Aldrich), and Octinoxate (PHR1080, Sigma-Aldrich) were dissolved in DMSO (final concentration 0.01% in culture medium or 0.005% in seawater).

UVB irradiation

UVB treatment on human keratinocytes was conducted in a tissue culture hood with a handheld UV lamp emitting a peak wavelength of 302 nm (UVM-57, Analytik Jena). The UVB intensity was measured each time before irradiation with a digital Radiometer (UVP UVX™, Analytik Jena) that is attached to a radiometer sensor (UVX-31 300NM, Analytik Jena). The cells were rinsed out with PBS then exposed to UVB at the intensity of 1 W/cm² for 5 sec., 10 sec., or 20 sec. respectively, which is equal to various doses at 5 J/cm², 10 J/cm², and 20 J/cm², respectively.

AlamarBlue cell viability assay

Cells growing in a culture plate were incubated in complete medium containing 10% alamarBlue reagent (DAL1025, ThermoFisher Scientific) for 3 hours and the plates were read by the fluorescence spectrophotometer using 570/590 nm (excitation/emission) filter settings (Molecular Devices Spectramax M5).

Flow cytometry analysis for mitochondrial ROS

Mitochondrial superoxide was measured following the same protocol as described in Xiong et al.²⁶ Briefly, cells cultured on 60-mm dishes were incubated with fresh complete medium containing 5 µM MitoSOX Red (Life Technologies, M36008) at 37°C. After 30 minutes, stained cells were harvested by trypsin digestion and rinsed twice with PBS. Single cell suspensions in 400 µl PBS were prepared for FACS analysis (FACS Canto II; BD). MitoSOX Red was excited by a laser at 488 nm, and the data was collected at 582 ± 21 nm.

Western blotting

Western Blotting was done following the same protocol as described in Xiong et al.²⁶ Antibodies used in this study included: γH2AX (Ab11174, Abcam), β-actin (A3854, Sigma-Aldrich), H2AX (600201, Biolegend).

Senescence associated β-Galactosidase activity assay

We followed the manufacturer's protocol (#9860; Cell Signaling). Briefly, keratinocytes cells grown on six-well plate were fixed in 1X fixative solution for 10 minutes, and then stained overnight at 37°C with the β-galactosidase staining solution at pH 6.0 for 15 hours. Bright field and phase contrast images were taken with a Zeiss AX10 microscope and a SPOT PURSUIT camera.

UV Spectra measurement

Absorbance of MB or BP-3 to broad UV-VIS spectrum was measured in triplicate using NanoDrop 2000 spectrophotometer (ThermoFisher Scientific). Briefly, 2 µL of MB or BP-3 was pipetted onto the pedestal for measurement after establishment of a blank using PBS under the setting of UV-Vis application. The

solvent of BP-3, DMSO, at a final concentration of 0.01% in PBS, was also measured for adjustment of BP-3 absorbance.

Coral growth and treatment

Soft Corals (*Xenia umbellata*) frags were purchased from ORA. The corals were cultivated in a 12 gallon NanoCube aquarium with a 12 hour light cycle (JBJ). Reef Salts (Instant Ocean) were used to bring the specific gravity of the aquarium and treatment water to 1.024. Corals were fed with liquid and dry feeds (Polyp Lab) and supplemented with potassium iodide (SeaChem). Water temperature was held constant at 25.5°C by submersible HT10 thermometer (Tetra). Chemical tests on coral health started after one month acclimation period. On day 0, each coral colony was gently transferred into a 2mil thick resealable bag (VWR) containing 600 mL seawater. The bag was then magnetically mounted on the wall of the aquarium (Siam). The coral colony was imaged two hours after settlement and various chemicals were administered directly to the bag water on day 0 and day 4.

Statistical Analysis

Results are presented as the mean $\pm$ standard deviation. Data was analyzed using 2-tailed Student's t-test, and a *p* value less than 0.05 was considered significant.

Declarations

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Figures

Figure 1

Figure 1

MB alleviates DNA damage induced by UVB irradiation in human keratinocytes (A) Western blotting analysis with anti- γ H2AX, H2AX and β -actin antibodies showing DNA damage response after 2 hours of non-UVB (designated as “□”) or UVB irradiation at different dosages in human keratinocytes pretreated with PBS (Control) or 100 nM MB for two weeks. (B) Quantitative band intensity of γ H2AX normalized to H2AX for (A). (C&E) Western blotting analysis with anti- γ H2AX, H2AX and β -actin antibodies in human keratinocytes without UVB irradiation (designated as “□”), and after 2 or 24 hours of UVB irradiation at 5 J/cm² (C) or 10 J/cm² (E). Quantitative band intensity of γ H2AX normalized to H2AX for (C&E) was analyzed accordingly (D&F). (* $p < 0.05$ MB vs Control at the same dose for B or at the same time points for D & F)

Figure 2

MB prevents human keratinocytes from UVB-induced cell death (A) Representative images of senescence-associated β -galactosidase (SA- β -gal) staining in human keratinocytes five days after UVB irradiation at 0, 5, 10, and 20 J/cm². The cells were pretreated with Control (PBS) or 100 nM MB for two weeks. Scale bar = 200 μ m. (B) AlarmaBlue cell viability assay in Control and MB-treated human keratinocytes five

days after UVB exposure at various dosages. The cells were pretreated with Control (PBS) or 100 nM MB for two weeks. (* $p < 0.05$ MB vs Control)

Figure 3

MB is a potent ROS scavenger with stronger UVB absorption than Oxybenzone (BP-3). (A) Absorbance comparisons of MB and BP-3 for UVC (200 nm ~ 280 nm), UVB (280 nm ~ 320 nm), and UVA (320 nm ~ 400 nm). (B) Cell proliferation after MB and BP-3 treatment. Human keratinocytes were maintained in medium containing PBS (solvent for MB), 100 nM or 1 mM MB, 0.01% DMSO (solvent for BP-3), 100 nM or 1 mM BP-3, respectively. (C) Comparison of mitochondrial specific superoxide (MitoSOX) levels in human keratinocytes pretreated with PBS, 100 nM MB, 1 mM MB, 0.01% DMSO, 100 nM BP-3 and 1 mM BP-3 for two weeks. (* $p < 0.05$; ** $p < 0.01$ vs PBS at day 10; ## $p < 0.01$ vs. PBS)

Figure 4

Figure 4

MB is a more effective ROS scavenger than Vitamin A and Vitamin C in human fibroblasts (A) Young (Left) and old (Right) male fibroblasts proliferation during the fourteen-day treatment with control PBS, 0.01% DMSO, 100 nM MB, 100 nM Vitamin A, 100 mM Vitamin C, and MB + Vitamin C, respectively. (B) Young (Left) and old (Right) female fibroblasts proliferation during the fourteen-day treatment with control PBS, 0.01% DMSO, 100 nM MB, 100 nM Vitamin A, 100 mM Vitamin C, and MB + Vitamin C, respectively. (C) Comparison of mitochondrial specific superoxide (MitoSOX) levels in young and old male fibroblasts pretreated with control PBS, 0.01% DMSO, 100 nM MB, 100 nM Vitamin A, 100 μ M Vitamin C, and MB + Vitamin C for four weeks. (D) Comparison of mitochondrial specific superoxide (MitoSOX) levels in young and old female fibroblasts pretreated with PBS control, 0.01% DMSO, 100 nM MB, 100 nM Vitamin A, 100 μ M Vitamin C, and MB + Vitamin C for four weeks. (* $p < 0.05$, ** $p < 0.01$ vs Control; # $p < 0.05$ or ## $p < 0.01$ vs MB + Vitamin C-treated cells).

Figure 5

Figure 5

MB is non-toxic to coral health compared to oxybenzone (BP-3) (A) Schematic experimental timeline showing the two administrations of PBS control, DMSO 0.005%, 1 μ M MB, and 1 μ M Oxybenzone on day 1 and day 4, respectively. (B) Representative images of coral colonies growing on the stone base in the seawater containing different chemicals on day 0 (before adding chemicals), day 1, day 3, and day 7. Red arrows indicate the death of corals in the Oxybenzone-treated experiment on day 7.

Supplementary Files

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