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RESEARCH ARTICLE

Comparative Cytotoxicity and Wound Closure Effects of the Sunscreen UV Filters Bemotrizinol and Ethylhexyl Triazone in HaCaT and L929 Cell Lines

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SUMMARY

Ultraviolet (UV) filters are the main active ingredients in sunscreens, but the safety of some conventional organic filters is debated due to their potential for dermal penetration and systemic toxicity. This study aimed to evaluate the cytotoxic effects and wound closure of bemotrizinol (BEMT) and ethylhexyl triazone (EHT), new-generation triazine derivative UV filters, on human keratinocyte (HaCaT) and mouse fibroblast (L929) cell lines in vitro. HaCaT and L929 cells were cultured under standard cell culture conditions. Cell viability was assessed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) method after treatment of BEMT and EHT at concentrations of 5–800 μM for 24 and 48 hours, and IC_{50} values were calculated. Wound closure was evaluated using a scratch wound healing assay performed with one-fifth of the IC_{50} concentrations to minimize the influence of cytotoxicity on wound closure. Statistical analyses were performed using two-way analysis of variance (ANOVA) and the Bonferroni multiple comparison test ($p<0.05$). Both UV filters reduced cell viability depending on concentration and incubation time. BEMT exhibited a more pronounced cytotoxic effect than EHT in both cell lines. Furthermore, HaCaT cells were found to be more sensitive to both BEMT and EHT than L929 cells. The lowest IC_{50} value was calculated as 441.9 μM for BEMT in HaCaT cells after 48 h. Wound healing test results showed that both EHT and BEMT inhibited wound closure. This inhibition was more pronounced in the BEMT-treated group compared to EHT. The findings indicate that BEMT has a higher cytotoxic potential compared to EHT, and that HaCaT cells are more sensitive to both UV filters than L929 cells. Furthermore, both compounds were found to suppress wound closure. Overall, these findings demonstrate differences in the cytotoxicity and wound closure effects of BEMT and EHT under the experimental conditions used, with BEMT exhibiting more pronounced effects than EHT. These results provide a comparative in vitro assessment of the cellular responses to these two UV filters.

Keywords: *Bemotrizinol, ethylhexyl triazone, UV filter, cytotoxicity, HaCaT, L929, wound closure, MTS*

Güneş Kremi UV Filtreleri Bemotrizinol ve Etilheksil Triazonun HaCaT ve L929 Hücre Hatlarında Karşılaştırmalı Sitotoksisite ve Yara Kapatma Etkileri

ÖZ

Ultraviyole (UV) filtreler güneş kremlerinin ana aktif bileşenleridir, ancak bazı geleneksel organik filtrelerin güvenliği, dermal penetrasyon ve sistemik toksisite potansiyelleri nedeniyle tartışılmaktadır. Bu çalışma, yeni nesil triazin türevi UV filtreleri olan bemotrizinol (BEMT) ve etilheksil triazonun (EHT) insan keratinosit (HaCaT) ve fare fibroblast (L929) hücre hatlarında in vitro sitotoksik etkilerini ve yara kapatma özelliklerini değerlendirmeyi amaçlamıştır. HaCaT ve L929 hücreleri standart hücre kültürü koşulları altında kültüre edilmiştir. Hücre canlılığı, 24 ve 48 saat boyunca 5-800 μM konsantrasyonlarında BEMT ve EHT ile muamele edildikten sonra MTS (3-(4,5-dimetiltiyazol-2-il)-5-(3-karboksimetoksifenil)-2-(4-sülfofenil)-2H-tetrazolyum) yöntemi kullanılarak değerlendirilmiş ve IC_{50} değerleri hesaplanmıştır. Yara kapanması, sitotoksisitenin yara kapanması üzerindeki etkisini en aza indirmek için IC_{50} konsantrasyonlarının beşte biri ile gerçekleştirilen çizik yara iyileşme testi kullanılarak değerlendirildi. İstatistiksel analizler, iki yönlü varyans analizi (ANOVA) ve Bonferroni çoklu karşılaştırma testi ($p<0,05$) kullanılarak yapıldı. Her iki UV filtresi de konsantrasyona ve inkübasyon süresine bağlı olarak hücre canlılığını azalttı. BEMT, her iki hücre hattında da EHT'den daha belirgin bir sitotoksik etki gösterdi. Ayrıca, HaCaT hücrelerinin hem BEMT hem de EHT'ye L929 hücrelerinden daha duyarlı olduğu bulundu. En düşük IC_{50} değeri, 48 saat sonra HaCaT hücrelerinde BEMT için 441,9μM olarak hesaplandı. Yara iyileşme testi sonuçları, hem EHT hem de BEMT'nin yara kapanmasını inhibe ettiğini gösterdi. Bu inhibisyon, EHT ile karşılaştırıldığında BEMT ile tedavi edilen grupta daha belirgindi. Bulgular, BEMT'nin EHT'ye kıyasla daha yüksek sitotoksik potansiyele sahip olduğunu ve HaCaT hücrelerinin her iki UV filtresine de L929 hücrelerinden daha duyarlı olduğunu göstermektedir. Ayrıca, her iki bileşiğin de yara kapanmasını baskıladığı bulunmuştur. Genel olarak, bu bulgular, kullanılan deneysel koşullar altında BEMT ve EHT'nin sitotoksisite ve yara kapatma etkilerinde farklılıklar olduğunu ve BEMT'nin EHT'ye göre daha belirgin etkiler gösterdiğini ortaya koymaktadır. Bu sonuçlar, bu iki UV filtresine karşı hücresel yanıtların karşılaştırmalı bir in vitro değerlendirmesini sağlamaktadır.

Anahtar Kelimeler: *Bemotrizinol, etilheksil triazon, UV filtresi, sitotoksisite, HaCaT, L929, yara kapanması, MTS*

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INTRODUCTION

Ultraviolet (UV) radiation is an environmental factor that has both beneficial and harmful effects on human health. While it supports vitamin D synthesis at low doses, prolonged or intense exposure can increase the formation of reactive oxygen species (ROS), leading to oxidative stress, DNA damage, immunosuppression, photoaging, and skin cancer development (Juzeniene & Moan 2012; Yu et al., 2024). UVA rays, in particular, penetrate deeper layers of the skin, increasing oxidative damage, while UVB rays directly damage DNA, contributing to mutations and the carcinogenesis process. Therefore, the use of sunscreens is becoming increasingly common to protect against the harmful effects of UV radiation (Brar et al., 2025).

UV filters are classified as inorganic or organic according to their composition and photoprotective mechanisms (Breakell et al., 2024). Organic UV filters, due to their aromatic structure, absorb UV rays, converting harmful radiation into a lower-energy form and thus providing photoprotection. However, the safety of traditional organic UV filters has become a significant research topic in recent years. It has been reported that some low molecular weight filters cross the skin barrier and enter the systemic circulation. They have been detected in biological samples such as blood, urine, breast milk, and placenta (Popiół et al., 2019). Furthermore, it has been reported that some filters, such as oxybenzone, octinoxate, homosalate, and 4-methylbenzylidene camphor, can exhibit endocrine-disrupting properties, produce estrogenic or antiandrogenic effects, and affect thyroid hormone metabolism (Schneider & Lim, 2019). These findings highlight the need for a detailed evaluation of the biosafety of UV filters used in sunscreens, in addition to their effectiveness.

Among the new-generation organic UV filters approved for cosmetic use in the European Union (European Parliament and Council, 2009), ethylhexyl triazone (EHT) and bis-ethylhexyloxyphenol methoxyphenyl triazine (Bemotrizinol, BEMT) have attracted considerable attention because of their high molecular weight, superior photostability, and broad-spectrum UV protection (U.S. Food and Drug Administration, 2024). The chemical structures of BEMT and EHT are shown in Figure 1.

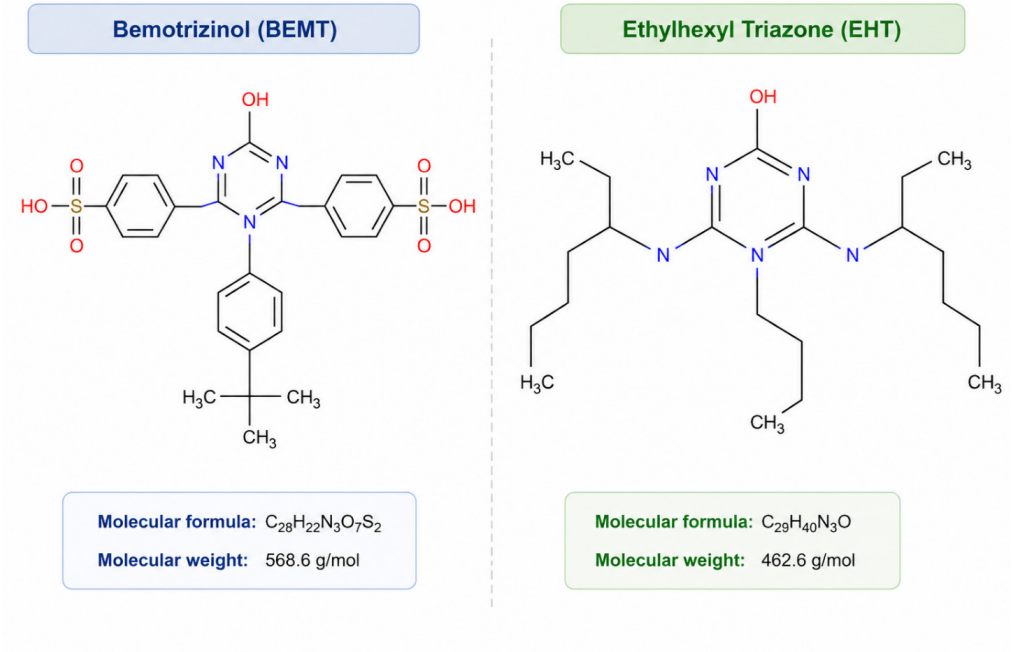

Figure 1. Chemical structures of the sunscreen UV filters BEMT and EHT

Both compounds are reported to remain on the skin surface without significantly penetrating the stratum corneum, exhibiting very low dermal penetration, and therefore having more limited systemic exposure risks compared to conventional UV filters. Furthermore, their strong photostability makes them a popular choice in sunscreen formulations (Varrella, Danovaro, & Corinaldesi, 2022). Although the photoprotective efficacy and dermal penetration properties of EHT and BEMT have been demonstrated in various studies, data on their direct cytotoxic effects on skin cells are still limited (Nitulescu, Lupuliasa, Adam-Dima, & Nitulescu, 2023; Scalia, Battaglioli, & Bianchi, 2019; Souza & Maia Campos, 2017). In particular, the number of comparative *in vitro* studies conducted on epidermal keratinocytes and dermal fibroblasts is insufficient. Therefore, it is important to evaluate the cellular safety profiles of these filters in different cell models.

Consequently, their use in modern sunscreen formulations has increased substantially. However, despite their widespread use and favorable safety profiles regarding systemic exposure, information regarding their direct effects on skin cells remains limited.

Table 1. Physicochemical properties of the sunscreen UV filters BEMT and EHT (European Parliament and Council, 2009; Jesus et al., 2022; Nitulescu et al., 2023; Pniewska & Kalinowska-Lis, 2024; U.S. Food and Drug Administration, 2021)

Property	**BEMT**	**EHT**
INCI name	Bemotrizinol	Ethylhexyl Triazone
Chemical class	Triazine derivative	Triazine derivative
Molecular formula	$C_{38}H_{49}N_3O_5$	$C_{48}H_{66}N_6O_6$
Molecular weight (g/mol)	627.8	823.1
CAS No.	187393-00-6	88122-99-0
Appearance	Off-white powder	White to pale-yellow powder
Water solubility	Practically insoluble	Practically insoluble
Oil solubility	Highly oil-soluble	Highly oil-soluble
UV absorption	UVA + UVB	UVB
λmax	310 and 340 nm	314 nm
Absorption range	~280–400 nm	~280–320 nm
Photostability	Very high	Very high
Maximum permitted concentration (EU)	10%	5%
Main application	Broad-spectrum sunscreen	UVB sunscreen
FDA OTC sunscreen status	Included in the FDA OTC sunscreen monograph	Not listed as a permitted active ingredient in the current FDA OTC sunscreen monograph

This study aimed to evaluate the cytotoxic effects of two commonly used triazine derivative UV filters, bemotrizinol (BEMT) and ethylhexyl triazone (EHT), in human keratinocytes (HaCaT) and mouse fibroblasts (L929) using the MTS assay, and to investigate their effects on wound closure using a scratch wound assay. The findings provide comparative *in vitro* data on the cellular effects of these two UV filters under the experimental conditions investigated.

MATERIALS AND METHODS

Cell culture and experimental protocol

Cell culture studies were performed under sterile conditions. Human HaCaT and mouse L929 cells were used as healthy control cells. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Lou-

is, MO, USA) and 1% penicillin/streptomycin/amphotericin B (P/S/A, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C in a humidified incubator containing 5% CO_2. Cells stored by freezing were rapidly thawed in a 37 °C water bath. The cell suspension was diluted with culture medium to remove dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA), which was used as a cryoprotectant, and then centrifuged at 1300–1500 rpm for 5 minutes. The cell pellet was resuspended in fresh culture medium, transferred to culture flasks, and cultured under appropriate conditions. Cells were passaged when they reached approximately 80–90% confluence. For this, the culture medium was removed, the cells were washed with calcium- and magnesium-free phosphate-buffered saline (PBS, Gibco, Thermo Fisher Scientific, USA), and separated from the surface with a 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA, Thermo Fisher Scientific, Waltham, MA, USA) solution. After the trypsinization process was completed, the reaction was stopped with complete culture medium. The cell suspension was centrifuged to obtain the pellet, resuspended with fresh culture medium, and plated into new culture flasks. Cell counting was performed using the Trypan Blue exclusion method. The cell suspension was mixed with Trypan Blue solution at a 1:1 ratio, and the number of viable cells was determined using a hemocytometer. Only cells with a high viability rate were used in the experiments. BEMT and EHT (TargetMol Chemicals Inc., Boston, MA, USA) were dissolved in DMSO to prepare 10 mM stock solutions. Subsequently, 1 mM intermediate stock solutions were prepared by diluting the initial stock solutions with fresh culture medium. The working concentrations used in the experiments were prepared by diluting intermediate stock solutions using fresh culture medium. Cells were incubated with different concentrations of BEMT or EHT: 5, 10, 25, 50, 100, 200, 400, 600, and 800 µM. The final DMSO concentration was standardized to 0.1% (v/v) in all treatment groups. The negative control group received culture medium only, the vehicle control group received culture medium containing 0.1% DMSO, and the positive control group was treated with 20% DMSO.

Cytotoxicity analysis

The cytotoxic effects of BEMT and EHT were evaluated using the MTS cell viability test. HaCaT and L929 cells were seeded into 96-well microplates at a concentration of 5 x 10^3 cells per well and incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2 to allow cell adhesion to the surface (Yüce, Berberoğlu, Aşkın Özek, & Ünüvar, 2026). Following incubation, the culture medium was removed, and the cells were treated with BEMT or EHT prepared at concentrations of 5-800 µM. Cells were incubated with the test compounds for 24 and 48 h. At the end of the incubation period, cell viability was assessed using MTS reagent (Sigma-Aldrich, St. Louis, MO, USA). The absorbance of the resulting formazan product was measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). All experiments were performed in four independent replicates (n = 4).

Wound healing test

A wound healing (scratch) test was performed to evaluate wound closure. HaCaT and L929 cells were seeded into 12-well plates at a concentration of 1 x 10^4 cells/mL per well and incubated for 24 h at 37°C in a humidified incubator containing 5% CO_2 to allow the cells to form a monolayer (Karakuş, Eyol, Yılmaz, & Ünüvar, 2018). Once the cells reached confluence, a linear scratch was created across the cell monolayer using a sterile 200 µL pipette tip. The wells were washed with fresh culture medium to remove cell debris (Akkutlu, Yüce, Aşkın Özek, Berberoğlu, & Ünüvar, 2025). The cells were then treated with to one-fifth of the IC_{50} concentrations of BEMT or EHT. Wound closure was visualized using an inverted phase-contrast microscope (SOPTOP ICX41, China) before treatment (0 h) and 24 to 48 h after treatment. Wound closure rates were evaluated using the obtained microscopic images. All experiments were performed in three independent replicates (n = 3).

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Graphs were generated, and IC_{50} values were calculated using GraphPad Prism version 11.0 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean ± standard deviation (SD). Wound healing assay data were analyzed using two-way repeated-measures analysis of variance (ANOVA), followed by Tukey's multiple comparisons post hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Cytotoxicity analysis

The effects of BEMT and EHT on cell viability in L929 and HaCaT cells after 24 and 48 h of incubation were evaluated using the MTS method. Both compounds caused a concentration- and time-dependent decrease in cell viability (Figure 2.).

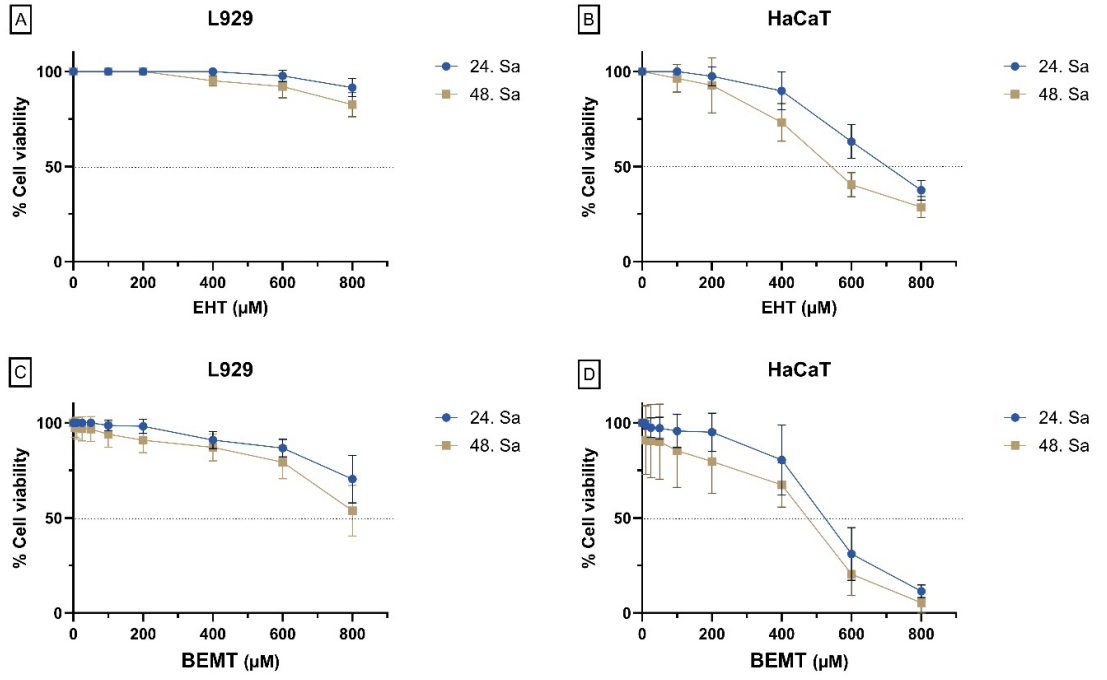

Figure 2. Effects of EHT and BEMT on the viability of L929 and HaCaT cells following 24 and 48 h of treatment, determined by the MTS assay. (A) L929 cells treated with EHT, (B) HaCaT cells treated with EHT, (C) L929 cells treated with BEMT, and (D) HaCaT cells treated with BEMT. Data are presented as mean ± SD. Cell viability is expressed as a percentage of the untreated control. IC_{50} values were calculated from the corresponding dose–response curves using nonlinear regression analysis. (n = 4)

The cytotoxic effect of BEMT on L929 and HaCaT cells increased depending on the concentration and incubation time. In L929 cells, no significant change in cell viability was observed at concentrations of 0–400 µM in 24 and 48 h incubations, while viability began to decrease at 600 µM and a significant decrease occurred at 800 µM. Furthermore, the 48 h incubation resulted in a higher cytotoxic effect compared to the 24 h incubation. In HaCaT cells, the cytotoxic effect of BEMT began at lower concentrations. A significant decrease in cell viability was observed from 400 µM onwards, and this decrease became even more pronounced at 600 and 800 µM concentrations. At the same concentrations, the 48 h incubation resulted in lower cell viability compared to the 24 h incubation. These findings indicate that HaCaT cells are more

sensitive to BEMT than L929 cells. BEMT showed a higher cytotoxic effect compared to EHT in both cell lines, and HaCaT cells were found to be more sensitive to both compounds compared to L929 cells. The 24 and 48 h IC_{50} values of BEMT and EHT in L929 and HaCaT cells are presented in Table 2.

Table 2. IC_{50} values of EHT and BEMT in L929 and HaCaT cells following 24 and 48 h of treatment, determined by the MTS assay

Cell line	Compound	Time	IC_{50} (μM)	95% CI
L929	EHT	24 h	1301	1022 to 2346
L929	EHT	48 h	1504	1111 to 2775
L929	BEMT	24 h	1165	983.8 to 1580
L929	BEMT	48 h	909.7	790.2 to 1353
HaCaT	EHT	24 h	696.7	662.2 to 737.1
HaCaT	EHT	48 h	550.2	501.4 to 602.9
HaCaT	BEMT	24 h	519.6	488.3 to 551.4
HaCaT	BEMT	48 h	441.9	331.7 to 511.7

Values represent IC_{50} (μM) with 95% confidence intervals (CI), calculated by nonlinear regression (log[inhibitor] vs. normalized response; variable slope) using GraphPad Prism. IC_{50} values exceeding 800 μM were estimated by extrapolation using nonlinear regression analysis.

Wound healing (scratch) test

The effects of EHT and BEMT on wound closure were evaluated using a wound healing test. Representative microscopic images obtained at 0, 24, and 48 h for the control group and the EHT- and BEMT-treated groups in L929 cells are shown in Figure 3.

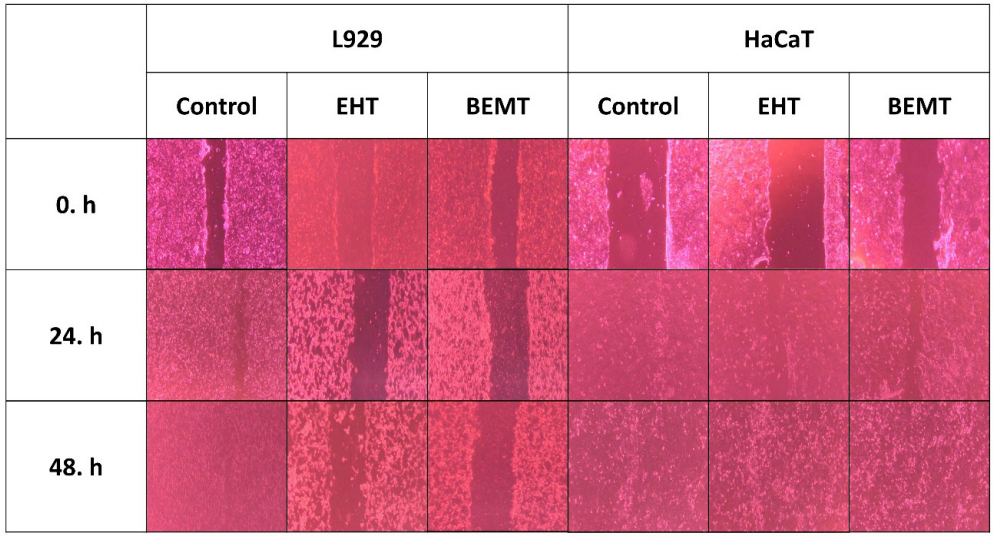

Figure 3. Representative wound healing assay images of L929 and HaCaT cells at 0, 24, and 48 h following treatment with EHT and BEMT. For the wound healing assay, cells were treated with a concentration corresponding to one-fifth of the IC_{50} value obtained from the MTS assay. Images demonstrate the effects of the treatments on wound closure over time

While the wound area closed regularly over time in the control group, both EHT and BEMT treatments reduced wound closure compared with the control group. Wound closure was more significantly suppressed in the BEMT-treated group compared to the EHT-treated group. Quantitative analysis of wound closure percentages is presented in Figure 4.

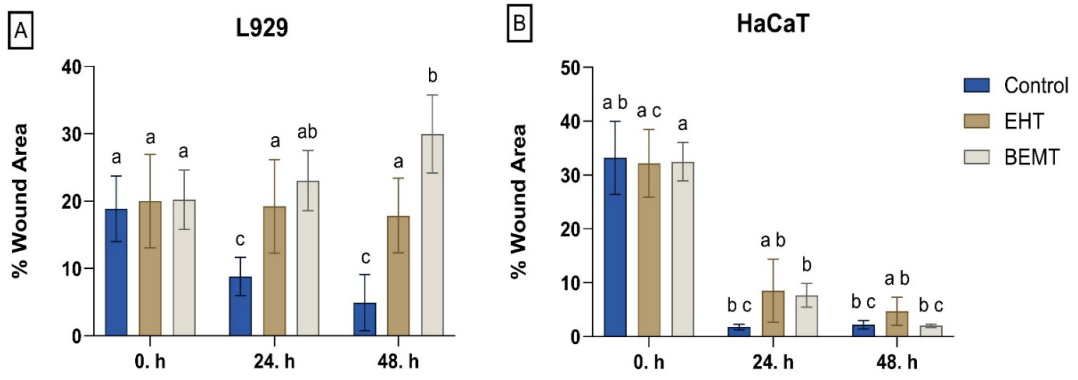

Figure 4. Effects of EHT and BEMT on wound closure in (A) L929 and (B) HaCaT cells at 0, 24, and 48 h, as determined by the wound healing assay. For the wound healing assay, cells were treated with a concentration corresponding to one-fifth of the IC_{50} value obtained from the MTS assay. Data are presented as mean ± SD. Different letters indicate statistically significant differences among groups ($p < 0.05$). (n = 3)

This study compared the effects of EHT and BEMT on cell viability and wound closure in HaCaT keratinocytes and L929 fibroblasts. Both compounds reduced cell viability at higher concentrations, with BEMT generally showing lower IC_{50} values than EHT. Both compounds also reduced wound closure, although the biological relevance of these *in vitro* findings requires further investigation.

Several conventional organic UV filters, including oxybenzone, octinoxate, and homosalate, have previously been reported to induce oxidative stress, apoptosis, and reduced cell viability in different mammalian cell lines. Compared with these conventional filters, BEMT and EHT have generally been considered safer alternatives because of their lower dermal absorption. Nevertheless, our findings indicate that these newer compounds are not completely devoid of biological activity at the cellular level. Therefore, replacing conventional UV filters with next-generation filters should not eliminate the need for comprehensive preclinical toxicological evaluation (Kryczyk-Poprawa et al., 2025). Bemotrizinol and ethylhexyl triazone are among the preferred filters in many sunscreen formulations today due to their high photostability and broad-spectrum UV protection (Turner & Torgerson, 2025). However, it is reported that these compounds have limited penetration into the stratum corneum due to their high molecular weight, and their systemic absorption is quite low (Jaskulak, Cinkusz, Franchuk, & Zorena, 2025). However, low systemic absorption does not mean they are completely safe for cells on the skin surface. The findings in this study showed that both filters significantly reduced cell viability in epidermal keratinocytes, especially at high concentrations. This suggests that low systemic exposure does not rule out the possibility of local cellular toxicity. A recent study evaluating the genotoxicity and phototoxicity of triazine UV filters reported that EHT did not exhibit significant genotoxic or phototoxic effects under the experimental conditions employed. While that study focused primarily on genetic safety rather than cytotoxicity, our results complement these findings by demonstrating that cellular viability may still be affected at high concentrations. Together, these observations indicate that different aspects of biological

safety, including cytotoxicity, genotoxicity, and phototoxicity, should be evaluated comprehensively when assessing the safety profile of next-generation UV filters (Wei et al., 2025).

The lower IC_{50} values of HaCaT cells compared to L929 fibroblast cells in our study indicate that epidermal keratinocytes are more sensitive to UV filters. Since the epidermis is the first skin layer to come into contact with sunscreens, this sensitivity observed in keratinocytes is of toxicological importance (Hayden, Cross, Anderson, Saunders, & Roberts, 2005). HaCaT cells are one of the most common *in vitro* models representing epidermal biology and are frequently used in studies of oxidative stress, phototoxicity, and cytotoxicity (D'Costa, 2020; Hofer, 2018). Our findings support the need for detailed investigation of the effects of next-generation UV filters on epidermal cells.

One of the most notable findings of the present study is that BEMT exhibited greater cytotoxicity than EHT in both HaCaT and L929 cells. Although both compounds are triazine-based UV filters, the consistently lower IC_{50} values of BEMT indicate a stronger cytotoxic potential. Since these mechanisms were not investigated in the present study, the exact reason for the higher cytotoxicity of BEMT remains unclear. Therefore, the present study provides comparative evidence demonstrating that BEMT consistently exhibited lower IC_{50} values than EHT in both keratinocyte and fibroblast cell lines.

In the wound healing assay, both UV filters reduced wound closure. However, because wound closure is influenced by both wound closure and cell proliferation, the observed effect may reflect impaired wound closure and/or reduced proliferation. Nevertheless, BEMT produced a greater reduction in wound closure than EHT, suggesting a stronger inhibitory effect on the cellular processes involved in wound repair (Abiola et al., 2021).

The study has some limitations. Experiments were conducted only in two-dimensional cell culture models, and UV irradiation was not applied. In addition, the mechanisms underlying the observed effects, including oxidative stress, apoptosis, mitochondrial dysfunction, and genotoxicity, were not investigated. The compounds were also not evaluated in finished sunscreen formulations or under exposure conditions representative of topical application. Future studies using three-dimensional human skin models, formulation-based exposure assessments, and mechanistic assays are warranted to clarify the biological relevance of these findings.

CONCLUSION

EHT and BEMT reduced cell viability at higher concentrations in HaCaT keratinocytes and L929 fibroblasts under the experimental conditions investigated. BEMT generally exhibited lower IC_{50} values than EHT, and both compounds reduced wound closure in the scratch assay. However, these *in vitro* findings cannot be directly extrapolated to normal sunscreen use or used alone to establish the biological safety of either compound. Further studies using physiologically relevant skin models and exposure conditions representative of topical application are needed to determine the relevance of these findings to sunscreen formulation safety.

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AUTHOR CONTRIBUTION STATEMENT

Concept – S.Ü., H.Y., D.A.Ö.; Design – D.A., M.Ö.; Supervision – S.Ü.; Resources – H.Y., D.A.Ö., D.A., M.Ö.; Materials – D.A., M.Ö.; Data Collection and/or Processing – H.Y., D.A.Ö.; Analysis and/or Interpretation – H.Y., D.A.Ö., D.A., M.Ö.; Literature Search – S.Ü., H.Y., D.A.Ö., D.A., M.Ö.; Writing – S.Ü., H.Y., D.A.Ö.; Critical Reviews – S.Ü.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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