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EFFECTS OF RED LIGHT PHOTOBIOMODULATION IN MITIGATING DNA INDUCED DAMAGE CAUSED BY ULTRAVIOLET C IN RAT EMBRYONIC FIBROBLASTS

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Abstract
Ultraviolet C radiation is a potent genotoxic agent that induces DNA strand breaks and cyclobutane pyrimidine dimers. Red light photobiomodulation (PBM), delivered via light-emitting diodes (LEDs) at 633–655 nm, has emerged as a promising non-invasive strategy to stimulate DNA repair. This study investigated and compared the cytoprotective efficacy of red LED irradiation applied after UVC exposure in rat embryonic fibroblast (REF) cells, using the alkaline comet assay as the primary quantitative endpoint. UVC irradiation (10 mJ/cm2) induced a significant increase in medium- and high-damage comets ($p < 0.0001$) relative to untreated controls. treatment strategy attenuated DNA strand breaks; however, post-irradiation PBM (UV+Red) produced a statistically significant increase in the undamaged-cell population ($p = 0.0246$), indicating active repair rather than mere damage mitigation. Red LED PBM holds translational potential as a safe and effective phototherapy intervention against UVC-induced genotoxicity.

1. Introduction

Ultraviolet C (UVC) radiation (200–280 nm) is one of the most potent environmental genotoxic agents known, capable of inducing severe DNA strand breaks, cyclobutane pyrimidine dimers (CPDs), and 6–4 photoproducts within exposed cells (Brash, 2015; Sinha & Häder, 2002). Although UVC is largely filtered by the atmospheric ozone layer under natural conditions, it is increasingly encountered in occupational and clinical settings through germicidal lamps and sterilisation devices, posing a significant risk to dermal and mucosal cells (Buonanno et al., 2020). Photobiomodulation (PBM), the therapeutic application of red and near-infrared light at non-thermal irradiances, has emerged as a promising non-invasive modality capable of stimulating cellular repair and survival pathways (Ucci et al., 2025; Hamblin et al., 2018). Unlike UV radiation, red light does not directly damage DNA; rather, it acts through mitochondrial photoacceptors to enhance ATP synthesis, modulate reactive oxygen species (ROS), and activate cytoprotective gene expression networks (Karu, 2008). Despite growing evidence supporting PBM efficacy, the comparative effectiveness of post-irradiation versus pre-irradiation red LED treatment in UVC-damaged rat embryonic fibroblasts (REF) remains poorly characterised. This study aims to evaluate and compare both treatment strategies using the alkaline comet assay as the primary quantitative endpoint of genotoxicity. Buonanno et al. established that exposure to short-wavelength UVC light induces strand and double-strand breaks in mammalian cells, with wavelengths up to 275 nm producing similar lesions at lower fluences (Buonanno et al., 2020). At the molecular level, unrepaired UVC-induced DNA damage activates p53-dependent apoptotic cascades; when the genotoxic burden exceeds repair capacity, cells undergo irreversible death (Chen et al., 2024). A critical determinant of PBM efficacy is the biphasic dose–response relationship. Calabrese and Mattson (2017) established that cytoprotective benefits occur within a defined fluence window (typically 0.5–5.0 J/cm2 for red light), beyond which excessive ROS generation overwhelms endogenous antioxidant defences (Calabrese & Mattson, 2017; Calabrese & Baldwin, 2001). The most direct evidence for PBM-mediated genoprotection comes from alkaline comet assay studies. Dube et al. (2001) demonstrated that He-Ne laser

irradiation of B-lymphoblasts prior to UVA exposure reduced DNA strand breaks, with protection contingent on a 30–60 minute interval between laser and UV exposure, indicating a protein-synthesis-dependent mechanism (Dube et al., 2001). Niu et al. (2014) further showed that red PBM increases SIRT1 expression in dermal fibroblasts, stabilising telomere integrity and conferring cellular resistance to photoageing (Niu et al., 2014). While early PBM studies focused on pre-irradiation conditioning, emerging evidence favours post-irradiation treatment as a superior strategy for active DNA repair. Ridha et al. (2011) observed that pre-irradiation He-Ne laser treatment of lymphocytes enhanced survival primarily through antioxidant modulation rather than direct DNA repair (Ridha et al., n.d). By contrast, post-irradiation PBM provides energetic and signalling support precisely when ATP-dependent repair machinery (NER, BER) is most active. Kato et al. (2025) confirmed that 655 nm red LED irradiation of endothelial cells significantly elevated mitochondrial ATP production for at least three hours post-irradiation a window directly aligned with peak NER activity (Kato et al., 2025). Taken together, this evidence provides the mechanistic rationale for the hypothesis that post-irradiation red LED treatment will more effectively reduce UVC-induced DNA strand breaks in REF cells than pre-irradiation conditioning.

2. Method

2.1 Cell Line and Culture Conditions

Rat embryonic fibroblast (REF) cells were obtained as a certified ready-to-use cell preparation from the Biotechnology Research Centre, Al-Nahrain University, Baghdad. Upon receipt, cells were maintained in complete culture medium supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 1% penicillin/streptomycin, and incubated at 37°C in a humidified atmosphere of 5% CO2. Cells were subcultured at 70–80% confluency using 0.25% trypsin-EDTA, and viability was confirmed by trypan blue exclusion (≥90%) prior to all experimental procedures. Cells were used between passages 3–8 to ensure consistent biological behaviour throughout the study.

2.2 Electromagnetic Radiation Sources

UV Radiation Source (Damage Induction): A UV light source emitting at 275 nm (UVC range) was used to induce DNA damage. This wavelength falls within the UVC spectrum (200–280 nm), which is strongly absorbed by DNA and effective in inducing CPDs and strand breaks. Red Light LED Source (Treatment): A LED device emitting in the red spectrum was used for PBM. Irradiance (mW/cm2) was monitored to calculate the precise energy density (J/cm2) delivered to the cells.

2.3 Irradiation Protocol

For irradiation, cells were harvested and resuspended in Phosphate Buffered Saline (PBS) to prevent UV absorption by culture medium components such as phenol red (Niu et al., 2014). Experimental groups were defined as follows: (i) Control Group: maintained in the dark without exposure to red LED or UV; (ii) UV Group (275 nm): exposed to 10 mJ/cm2 UV radiation; (iii) Red Only Group: exposed to 840 mJ/cm2 red LED light only; (iv) UV+Red Group: exposed to 10 mJ/cm2 UV radiation followed by 840 mJ/cm2 red LED treatment (post-irradiation strategy).

2.4 DNA Damage Assessment: Alkaline Comet Assay

DNA strand breaks were quantified using the alkaline single-cell gel electrophoresis (comet) assay, which detects single- and double-strand breaks and alkali-labile sites at the single-cell level (Olive et al., 1990). Cell viability was confirmed by trypan blue exclusion (>90%) and concentration adjusted to 1 × 105 cells/mL prior to each run (Jewell et al., 2004). The assay was performed using an OxiSelect Comet Assay Kit (Trevigen, USA) following the protocol of Olive et al. (1990). Briefly, cells were mixed with low-melting-point agarose (37°C) at 1:10 (v/v), spread onto pre-coated slides, and solidified at 4°C for 10 min. Slides were lysed at 4°C for 60 min, subjected to alkaline unwinding in 200 mM NaOH / 1 mM EDTA (pH > 13) for 20 min, and electrophoresed at 21 V for 30 min in fresh alkaline buffer at 4°C. Slides were neutralised in dH2O (×2, 5 min), fixed in 70% ethanol (5 min), dried at 37°C, and stained with 1× SYBR Green I for 30 min in the dark. A minimum of 50 randomly selected nucleoids per sample were scored by fluorescence microscopy using Comet Score software (Tri Tek Corp., USA) (Collins et al., 2008). Tail DNA (%) was the primary endpoint (Olive et al., 1990):

Tail DNA (%) = 100 × (Tail DNA Intensity / Total Cell DNA Intensity)

Damage was classified as: low (<10%), medium (10–30%), or high (>30%) per Collins et al. (2008).

3. Result and Discussion

The following subsections discuss the DNA damage assessment by the alkaline comet assay.

3.1 UVC Irradiation Induces Significant DNA Strand Breaks in REF Cells

Comparison between the UVC-irradiated and untreated control groups confirmed robust genotoxicity at the dose employed (Table 1, Figure 1). Medium-damage comets increased significantly from 9.40 ± 2.14 (control) to 35.80 ± 2.14 (UV only; $p < 0.0001$), and high-damage comets from 11.00 ± 2.61 to 34.20 ± 2.61 ($p < 0.0001$). These results are consistent with the genotoxic mechanism of UVC, which directly excites DNA bases to form CPDs and (6–4) photoproducts, activating the ATM/ATR damage-signalling cascade (Brash, 2015). A partial reduction in undamaged cells was observed ($p = 0.0124$), while the low-damage category was non-significant ($p = 0.0803$), suggesting heterogeneous intrinsic repair capacity across the cell population. Trypan blue exclusion confirmed >90% pre-assay viability, validating the sub-lethal experimental design required for PBM rescue experiments.

Table 1. Comparative analysis of DNA damage categories: UV only vs. Control.

Category	UV Only (Mean ± SE)	Control (Mean ± SE)	p-value
No comet	56.20 ± 3.79	67.40 ± 3.79	0.0124 *
Low comet	62.40 ± 4.25	69.00 ± 4.25	0.0803 NS
Medium comet	35.80 ± 2.14	9.40 ± 2.14	p < 0.0001 ****
High comet	34.20 ± 2.61	11.00 ± 2.61	p < 0.0001 ****

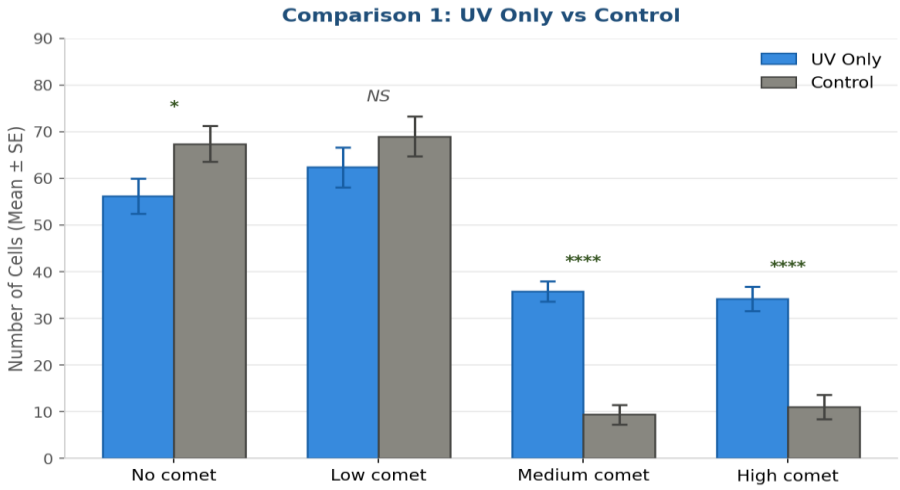

Figure 1. Graphical representation of DNA damage levels in UV and Control groups based on Comet Assay results.

3.2 Red LED Post-Treatment Achieves Superior DNA Repair Relative to Pre-Treatment

Post-irradiation red LED treatment (UV+Red group) produced a more pronounced reduction in DNA damage across all categories compared to pre-treatment (Table 2, Figure 2). Medium-damage comets decreased to 18.80 ± 2.44 ($p = 0.0002$ vs. UV only) and high-damage comets to 19.80 ± 2.73 ($p = 0.0008$ vs. UV only). Critically, the proportion of undamaged cells increased significantly relative to the UV-only group (64.20 ± 3.19 vs. 56.20 ± 3.79; $p = 0.0246$) an improvement not observed with pre-treatment consistent with the hypothesis that post-irradiation PBM directly fuels ATP-dependent DNA repair machinery at the point of maximum demand. Post-irradiation red LED application yielded superior repair outcomes, underpinned by the ATP-dependence of nucleotide excision repair (NER), which requires sustained energy for XPC–RAD23B recognition, TFIIH/XPD unwinding, dual incision, and polymerase δ/ε resynthesis (Sancar et al., 2004). Evidence indicates that 660 nm PBM sustains elevated cellular ATP for ≥24 hours with corresponding COX-1 upregulation in fibroblasts (Fuchs et al., 2021), and that this energy-replenishing effect is greatest

under high metabolic demand precisely the post-genotoxic state (Hoh Kam & Mitrofanis, 2023). These findings provide novel evidence that the temporal placement of PBM relative to the genotoxic insult is a critical determinant of therapeutic efficacy.

Table 2. Effect of red LED post-treatment on UV-induced DNA damage (UV+Red vs. UV only).

Category	UV Only (Mean ± SE)	UV+Red (Mean ± SE)	p-value
No comet	56.20 ± 3.79	64.20 ± 3.19	0.0246 *
Low comet	62.40 ± 4.25	57.20 ± 3.13	0.0683 NS
Medium comet	35.80 ± 2.14	18.80 ± 2.44	0.0002 ***
High comet	34.20 ± 2.61	19.80 ± 2.73	0.0008 ***

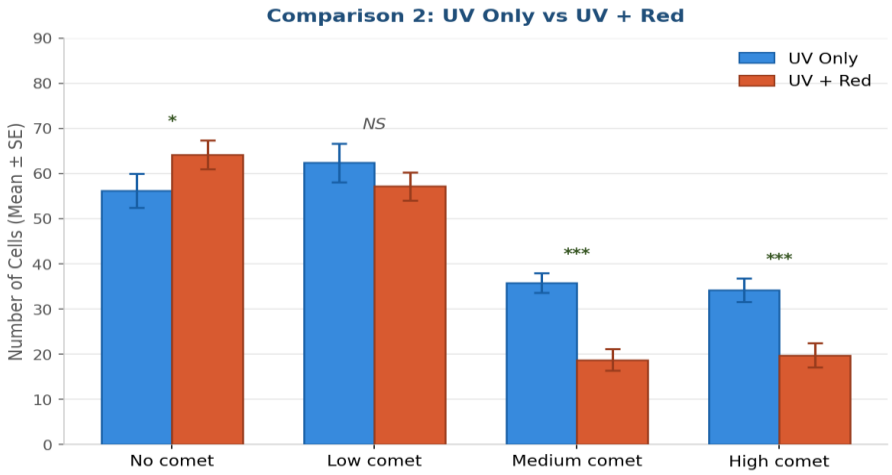

Figure 2. Distribution of comet categories across experimental groups with statistical significance.

3.3 Red LED Irradiation Alone Does Not Induce Significant DNA Damage

In the red-only group, no significant differences were observed in undamaged or low-damage cell frequencies relative to control (both p = 0.3138; Table 4, Figure 4). Minor elevations in medium- and high-damage comets reached statistical significance (both p = 0.0033); however, absolute values remained markedly lower than UV-irradiated groups (medium: 13.0 ± 0.87; high: 14.0 ± 0.86), confirming the absence of meaningful genotoxic activity from red LED irradiation at the therapeutic fluences employed. Given the low absolute comet frequencies in comparison to UV-exposed groups, the minor elevations most likely represent background variation in baseline DNA strand-break frequencies rather than significant genotoxicity.

Table 3. DNA damage comparison: Red LED only vs. Control.

Category	Red Only (Mean ± SE)	Control (Mean ± SE)	p-value
No comet	64.60 ± 3.83	67.40 ± 3.83	0.3138 NS
Low comet	66.00 ± 5.84	69.00 ± 5.84	0.3138 NS
Medium comet	13.00 ± 0.87	9.40 ± 0.87	0.0033 **
High comet	14.00 ± 0.86	11.00 ± 0.86	0.0033 **

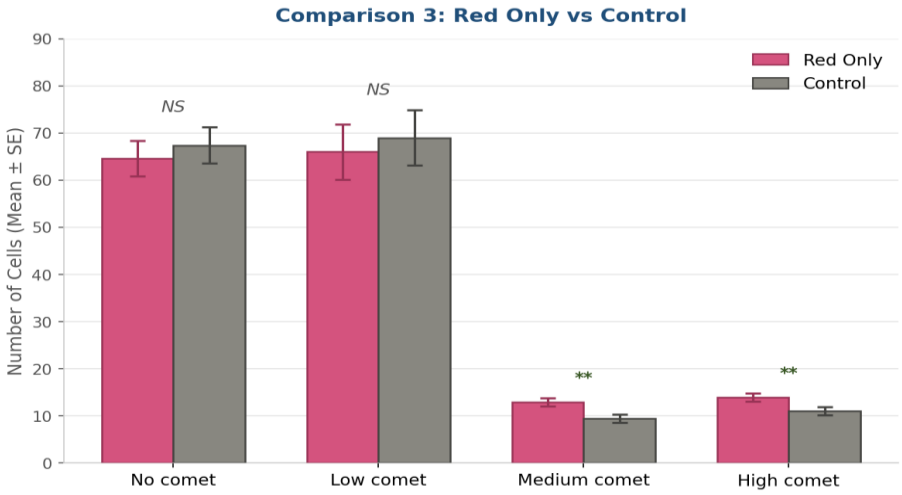

Figure 3. Graphical representation of DNA damage distribution in Red Light-Only and Control groups.

4. Conclusion

The alkaline comet assay data presented herein consistently demonstrate that: (i) the UVC dose employed (10 mJ/cm2, 275 nm) induces significant, measurable genotoxic damage in REF cells, characterised by marked elevations in medium- and high-damage comet frequencies; (ii) red LED irradiation at therapeutic fluences (840 mJ/cm2) is inherently safe and does not produce biologically meaningful DNA strand breaks; (iii) post-irradiation PBM provide statistically significant, albeit incomplete, protection against UVC-induced DNA damage; and it is the superior therapeutic modality, uniquely restoring the population of undamaged cells ($p = 0.0246$) — an effect absent in the pre-treatment group. These results align with the mechanistic hypothesis that PBM operates principally by supplying energetic and signalling support to ATP-dependent DNA repair machinery (NER, BER) rather than by preventing primary photoproduct formation. The findings contribute to the expanding body of evidence supporting red light PBM as a biologically rational, non-invasive intervention for mitigating UVC-induced cellular genotoxicity (Hamblin, 2017; Maghfour et al., 2024; Glass, 2023). Future work should characterise specific repair enzyme expression (XPC, XPA, PCNA) and correlate ATP dynamics with comet tail-DNA reduction kinetics to fully elucidate the mechanistic basis of the temporal PBM advantage demonstrated here.

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