Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Oxidative stress mediated DNA damage and cytotoxicity by para-phenylenediamine (PPD) under ambient environmental intensities of UV radiation

Taiyab Shamshad Naqvi*, Naushad Ahmad, Syed Faiz Mujtaba
Department of Zoology, Faculty of Science, Shia P.G. College, Lucknow
E-mail: tsnaqvi8@gmail.com

Abstract

The popularity of hair dyes use has been increasingly regularly throughout the world as per the demand of hair coloring fashion trends and other cosmetic products. Para-phenylenediamine (PPD) is widely used as a hair dye ingredient around the world. The generation of $^1O_2$, O_2^-, and $^{\cdot}OH$ were studied under the sunlight exposure. Photodegradation of 2-deoxyguanosine (2'-dGuO) and linoleic acid peroxidation showed that ROS were mainly responsible for ppd phototoxicity. Photocytotoxicity of PPD in human keratinocytes (HaCaT) was measured by mitochondrial (MTT) and Lysosomal (NRU) assay which showed significant decrease in cell viability.Involvement of was substantiated by the quenching with specific free radical quenchers. Significant intracellular ROS generation was measured through DCF fluorescence intensity and increased in a concentration dependent manner. MTT assay showed decrease in cell viability upto 70% which is validated through NRU assay. The result of single cell gel electrophoresis showed the highest DNA damage, in the form of tail length (30.6%) by PPD (1.0 µg/ml) under sunlight exposure. Thus, the study suggests that PPD generates reactive oxygen species ($^1O_2$, O_2^-, and $^{\cdot}OH$) induces photogenotoxicity, phototoxicity and cell death under ambient UV exposure. Therefore, the long term use of PPD dye and sunlight exposure jointly increased the oxidative stress mediated phototoxicity in skin cells.

Key words- PPD, DNA damage, Oxidative stress, Phototoxicity.

Introduction

Paraphenylenediamine (PPD) is an intermediate oxidative dye used in hair dye formulation. Almost all of the major hair color brands contain PPD, because it works so well at covering gray hair. During the past fifty years, p-phenylenediamine (PPD) has frequently been used as a

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

primary precursor in the process of manufacturing oxidative hair dye products. PPD is associated with multiple cases of dermatitis and allergies (Shakoor Z., et.al., 2017).PPD is now one of the most common causes of allergic skin reactions in the United States.PPD containing hair dye have been associated with cancer and mutagenicity. PPD has been reported to increase the formation of liver tumors in mice (J.M. Sontag., 1981). Currently, PPD is present in more than 1000 hair dye formulations marketed all over the world (L.A. Stanley., 2005). The concentration of PPD used in hair dye is 2%.

The skin is more or less exposed to sunlight as part of daily life, in which the effects of ultraviolet radiation are not negligible. It is generally accepted that UVA in sunlight damages DNA through generation of singlet oxygen or through oxidative stress (J. Cadet., 2005).

Sunlight exposure and coloring are the most common and important factors that can lead to oxidative stress mediated phototoxicity. UV induced damage changes the structure of keratin through the photo oxidative stress mediated DNA damage, lipid peroxidation and cytotoxicity (A. Roddick-Lanzilotta., 2004;Z.D. Draelos., 2006). Previous studies showed higher risk of leukemia, lymphoma and bladder cancer among user of hair dye containing PPD (L. David., 2016).

Thus, the current study suggest that applying hair coloring agents like PPD and sunlight exposure may lead oxidative stress mediated DNA damage, lipid peroxidation which results in cytotoxicity of skin cells.

Materials and methods

Radiation exposure

Radiation exposure was carried out in a temperature controlled conditions. Sunlight exposure was carried out during clear sunny days between 11.00 AM. to 2.00 P.M . Petri dishes / culture plates were kept on a platform surrounded by ice packs (Polar tech Industries, Genova IL, Los Angeles) to prevent thermal effect.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Chemical and culture wares

Paraphenylenediamine (PPD), fetal bovine serum, Dulbecco's modified eagle's medium(DMEM F-12 HAM), antibiotic and antimycotic solution, trypsin (0.25%), 3-(4,5- dimethylthiozoly-2)-2,5-diphenyl-2H tetrazolium bromide (MTT), Neutral red uptake (NRU), Hank's Balanced Salt solution (HBSS), sodium chloride (NaCl), ascorbic acid, carbonate and phosphate buffers and 2-dGuO were procured from Sigma (St.Louis, MO). Linoleic acid and Tween-20 were obtained from M.P.Biomedicals Inc. (Solon), All plastic wares including 48-well plates and 25 cm^2 (polystyrene coated) culture flasks were purchased from Nunc (USA). Cell lines were procured from National Centre for Cell Sciences, Pune, India, since then it was maintained in our laboratory.

Photochemical assays

Determination of singlet oxygen **($^1O_2$):** The generation of $^1O_2$ under aerobic condition was measured in aqueous solution as per the method of (S.F.Mujtaba., 2011). RNO solution (0.35–0.4 x 10^{-5} M) was prepared in 0.025 M potassium phosphate buffer (pH = 7.0) and L-histidine (10^{-2} M) was added as a selective acceptor of $^1O_2$. A 10 ml assay solution in a petri dish with or without compound was irradiated under sunlight. The production of $^1O_2$ was monitored by measuring the decrease in RNO absorbance at 440 nm. The generation of $^1O_2$ was further substantiated by the administration of DABCO (25 and 50 mM) as a specific quencher (Foote C.S., 1972; Ouannes C., 1968).

Determination of superoxide anion radical (O_2^-): The generation of O_2^- was monitored by recording the photosensitized reduction of nitrobluetetrazolium (NBT) to nitrobluediformazan (NBF) spectrophotometrically(Ray R.S., 2006). NBT solution (1.67 x10^{-4}M) was prepared in 0.01 M sodium carbonate buffer (pH = 10). A 10 mL assay system containing the compound from 0.1,0.5 and 1 ug/ ml was irradiated under sunlight (20 min). The production of NBF was monitored by measuring the increase in absorbance at 560 nm. The generation of O_2^- was further confirmed by carrying out quenching with SOD (10 and 25 U/ ml).

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Determination of hydroxyl radical (·OH)-The ·OH generation was measured by ascorbic acid-iron-EDTA system proposed by Cohen (24). The iron-catalyzed oxidation of ascorbic acid at 37ºC was used. The standard reaction mixture contains 100 mM potassium phosphate buffer pH 7.4, 167 µM iron-EDTA (1:2 mixture), 0.1 mM EDTA, 2 mM ascorbic acid and 33 mM dimethyl sulfoxide in a final volume of 3.0 mL and irradiated. Ascorbic acid was replaced by the PPD. After the completion of irradiation, 1.0 mL of TCA (17.5%, wt / vol) was added. The samples were then assayed for formaldehyde formation by the method proposed by Nash (Nash, T., 1953).

Ammonium acetate acetylacetone reagent was prepared by 2.0 M ammonium acetate, 0.05 M acetic acid and 0.02 M analytical grade redistilled acetylacetone. Equal volumes of aliquot (1.5 mL) and reagent (1.5 mL) were mixed and kept at 37ºC for 40 min. Theproduction of formaldehyde was monitored at 412 nm. Further the quenching of ·OH was performed by adding mannitol (0.5 M) and sodium benzoate (0.5 M) as specific quenchers.

Linoleic acid photoperoxidation

Linoleic acid solution was freshly prepared in phosphate buffer saline (0.01M, pH= 7.2) using 0.05% tween-20 as an emulsive agent. Solution containing 0.8 mM linoleic acid and variable concentrations of PPD were irradiated under sunlight. Light-induced peroxidation of linoleic acid was measured through the increase in absorbance at 233 nm (Castell J.V., 1994). linoleic acid peroxidation quenching was carried out by SOD(25 and 50 U ml).

Cell culture- The human keratinocyte (HaCat) cell line were grown in DMEM F-12 HAM culture medium supplemented with 10% FBS and antibiotic-antimycotic solution (1.5%) at 5% CO_2 and 95% relative humidity at 37^0C.

Cell viability assay by MTT- In brief, cells (2 x 10^4) were seeded per well in 96-well plates and kept in the CO_2 incubator for 48 h at 37^0C prior to experiment for the proper attachment of the cells. The medium was replaced by HBSS containing compound for exposure purpose. At the

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612
ISSN: 2582-2004
Volume 06, Issue 02

end of irradiation, HBSS was replaced by complete medium containing 20 µl MTT (5 mg/ml) with 200 µl complete medium. The culture plates were kept in the CO_2 incubator for 4 h. After incubation, the culture plates were washed twice with HBSS and 200 µl of DMSO was added to each well by pipetting up and down to dissolve the content. The absorbance was recorded at 530 nm by using multiwellmicroplate reader (T. Mosmann., 1983).

Neutral red uptake (NRU) assay-

Briefly, after irradiation, the cells containing PPD were washed with HBSS. The culture well plates were allowed to incubate for 3 h in complete medium (DMEM F-12 HAM) containing NR dye (50 µg mL) followed by a quick wash with fixative (1% wt / vol $CaCl_2$; 0.5% vol / vol formaldehyde) to remove the unbounded dye. The accumulated dye in cells was extracted with 50% ethanol containing 1% (vol / vol) acetic acid and plates were kept for 20 min on a shaker. The absorbance was recorded at 540 nm. (N.Agrawal 2007).

Generation of intracellular ROS-

Cells were grown in 35 mm petri plates at 2 x 104 cells/well. Cells were incubated with non-fluorescent dye carboxy H2-DCFDA (2'7 dichlorodihydrofluoresceindiacetate, 5µM) for 30 min at 370C, then treated with PPD and exposed to sunlight. After irradiation cells were washed twice with HBSS. Fluorescence of DCF in the cells was observed under the fluorescence microscope and photographs were acquired (K. Zhao., 2005).

Single cell gel electrophoresis (SCGE) assay-

The alkali single cell gel electrophoresis (SCGE) was performed as a three layer procedure (D. Ali., 2011). The parameters used to measure DNA damage in cells were % tail DNA and Olive tail moment (OTM). Images from 50 random cells (25 from each replicate slide) were analysed for each experiment.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Statistical analysis- Group means ± SE were compared by one way analysis of variance (ANOVA) followed by Tukey's*post hoc* test. Correlation and simple linear regression analysis were used to assess the ROS generation, 2'dGuO photodegradation, lipid peroxidation and cell viability against different PPD concentrations. A two tailed (α) probability $P<0.05$ was considered statistically significant.

Results

1O2 generation

Fig.1 (a) shows absorption spectra of PPD having maximum absorption (γmax) in UV-A (334 nm). Mean photochemical generation of 1O2 by PPD (0.1 to 1 µg/ml) under sunlight (30 min), UV-A (2.16 J/cm2) and UV-B (0.72 J/cm2) exposures. PPD at 1µg/ml generates the highest amount of 1O2 under sunlight followed by UV-A and UV-B exposure (Fig. 1 b). In an attempt to evaluate the possible role of ROS in PPD phototoxicity, specific 1O2 quencher was used (Fig. 1 c). Results showed maximum quenching of 1O2 by NaN3 (10 mM) with 1µg/ml PPD under UV-A (2.16 J/cm2) exposure.

O2•- generation

Generation of O2•- is summarized in (Fig. 2 a). Results showed that mean generation of O2•- by PPD was comparatively higher in sunlight (30 min) followed by UV-A (2.16 J/cm2) and UV-B (0.72 J/cm2). The order of O2•- generation was following: sunlight>UV-A>UV-B. Additional evidence of the production of O2•- was obtained by examining the O2•- quenching study with SOD (10 and 25 Units/ ml) under UV-A (2.16 J/cm2) irradiation. 80% quenching of O2•- was measured by 25 Units/ml of SOD (Fig. 2 b).

˙OH generation

Generation of ˙OH is summarized in (Fig. 3 a). Results showed that mean ˙OH generation was comparatively higher in sunlight (30 min) followed by UV-A (2.16 J/cm2) and UV-B (0.72

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

J/cm2). (Fig.3 b) shows the percent quenching of ˙OH under UV-A (2.16 J/cm2) at 1µg/ml of PPD by mannitol (0.5 M) and sodium benzoate (0.5 M) and caused 40% and 70% quenching of ˙OH respectively, highest quenching was observed by sodium benzoate in comparison to mannitol.

Intracellular ROS generation

Fig. 4 (a) Showed the DCF-fluorescence intensity at different concentrations of PPD under sunlight (30 min), UV-A (2.16 J/cm2) and UV-B (0.72 J/cm2) exposure. As compared with control, a significant (<0.05 or p<0.01) increase in DCF intensity was observed in all treated cells in a concentration dependent manner. Maximum DCF intensity was recorded under sunlight (30 min) at 1 µg/ml of PPD followed by UV-A and UV-B. Result indicates that PPD facilitates UV-induced oxidative stress in HaCaT cell line. In the presence of an antioxidant, N-acetyl-cysteine (NAC) inhibited DCF fluorescence intensity in a dose-dependent manner under sunlight (30 min) exposure (Fig. 4 b).

Photocytotoxicity analysis

Fig. 5 (a & b) showed the PPD phototoxicity on human keratinocyte cell line (HaCaT) through MTT and NRU assays. Photosensitizing effect of PPD (0.1- 1 µg/ml) was recorded as percent cell viability. The phototoxicity was assessed under sunlight (30 min), UV-A (2.16 J/cm2) and UV-B (0.72 J/cm2) exposures. Cytotoxicity was also assessed with different PPD concentrations without irradiation. Chlorpromazine (1 µg/ml) and L-histidine (10 µg/ml) were used as positive and negative controls, respectively. The highest decrease ($p<0.01$) in percent cell viability by PPD (1 µg/ml) was observed under sunlight (80%) followed by UV-A (65%) and UV-B (50%) exposure. It indicates that UV-B irradiated PPD (1 µg/ml) was least phototoxic, while PPD (1 µg/ml) under sunlight was highly phototoxic. Phototoxicity of PPD was observed in the following order: Sunlight > UV-A > UV-B. In all three exposures as concentration increase % cell viability reduced significantly ($p<0.05$ or $p<0.01$) when compared to negative control (L-Histidine). The results of MTT assay accorded the NRU assay.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Single strand breakage

The single strand breakage of DNA was measured as % tail DNA (Fig.6 a) and olive tail moment (OTM) (Fig 6 b) in the control as well as exposed cells. During electrophoresis, the cell DNA was observed to migrate more rapidly towards the anode at the highest concentration than the lowest concentration. The cell exposed to PPD (0.1 to 1 µg/ml) under sunlight (30 min), UV-A (2.16 J/cm2) and UV-B (0.72 J/cm2) showed significant ($p<0.05$ or $p<0.01$) higher DNA damage than control. The gradual increase in DNA damage was observed in cells as concentration of PPD increases and highest damage was recorded at (1 µg/ml) under sunlight followed by UV-A and UV-B irradiation.

Cyclobutane pyrimidine dimers (CPDs) formation

PPD (0.1 to 1 µg/ml) under sunlight/UV-R exposure induced cyclobutane pyrimidine dimers (CPDs) formation. Maximum CPDs formation were detected at1 µg/ml under sunlight (30 min) exposure. There was no significant CPDs formation under dark control cells in presence of different concentrations of PPD (Fig. 7).

Analysis of mitochondrial transmembrane potential (MMP)

Fig. 8 Mitochondrial depolarization and DNA damage were compared after treatment with PPD (1 µg/ml) under sunlight (30 min) exposure.

The results suggest that PPD (1 µg/ml) under sunlight exposure decreased mitochondrial membrane potential concomitantly increase in DNA damage. There was no significant decrease in mitochondrial membrane potential as well as increase in DNA damage under dark control and sunlight exposure.

Apoptosis

Our results suggest that PPD induced apoptotic cell death under sunlight exposure. The translocation of phosphatidylserine from inner leaflet of the plasma membrane to the outer leaflet

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612
ISSN: 2582-2004
Volume 06, Issue 02

is observed early in the initiation of apoptosis without disruption of other membrane components. Using Annexin-V as specific marker for PS translocation in addition to propidium iodide, which identifies permeabilized cells. Our results suggest that photosensitized PPD (1 µg/ml) has induced apoptosis with marked difference of early and late apoptotic cells, as compair to dark control and sunlight exposure. In presence of NAC (10 µM) photosensitized PPD (1 µg/ml) reduced apoptotic cells significantly which advocates the involvement of ROS in PPD phototoxicity (Fig. 9).

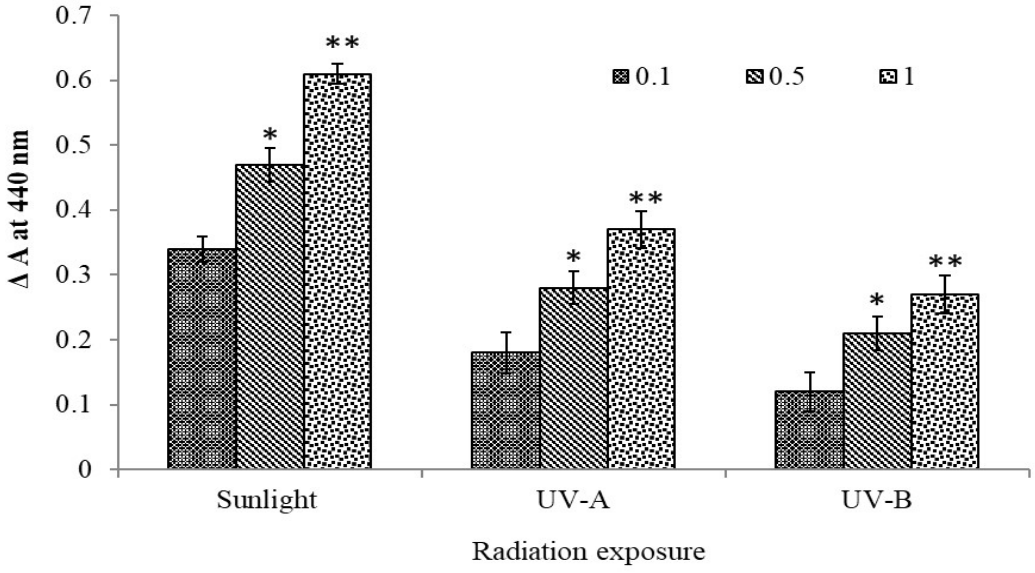
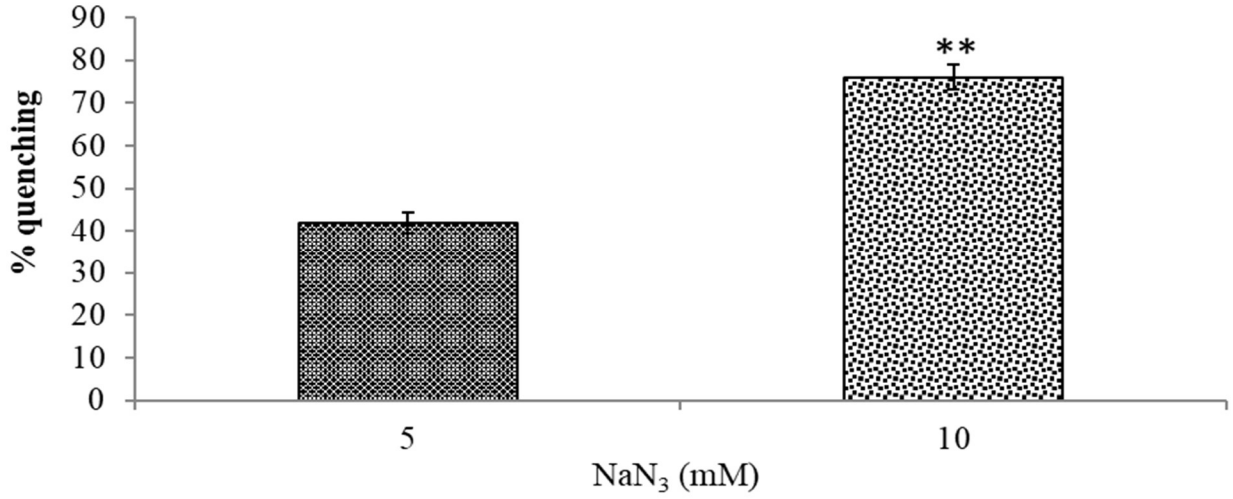
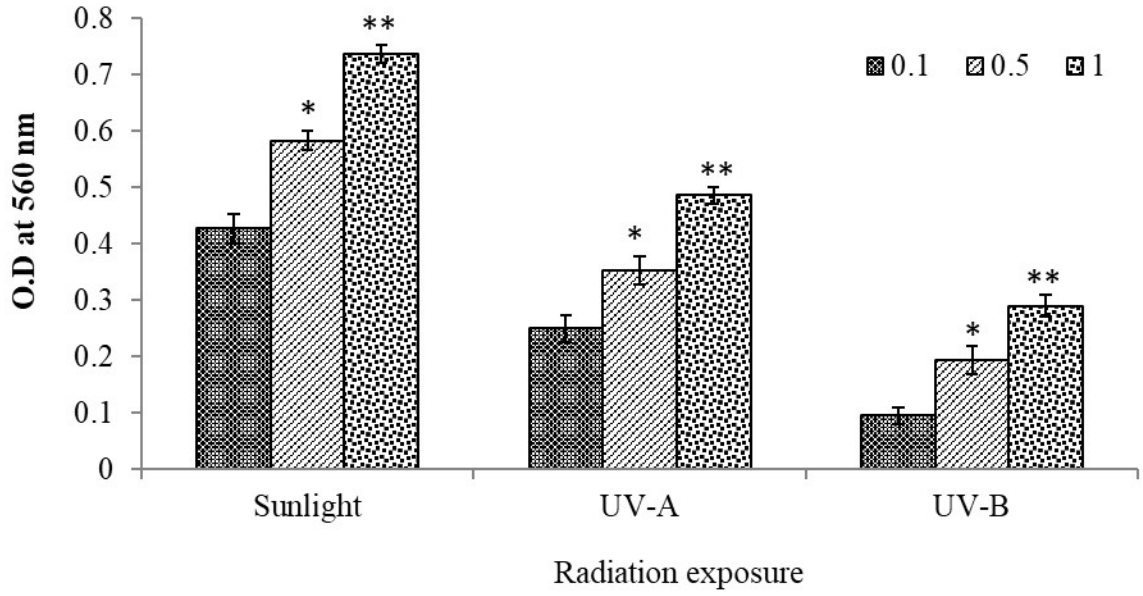


Fig. 1 (a) Photochemical generation of $^1O_2$ by PPD (µg/ml) at different concentrations under sunlight (30 min), UV-A (2.16 J/cm^2) and UV-B (0.72 J/cm^2) exposure. Values presented are mean of three observations ±SE. (*p<0.05 or **p<0.01- as compared to 0.1 µg/ml). **(b)** Percent photochemical quenching of $^1O_2$ generated by PPD (1 µg/ml) through NaN_3 under UV-A (2.16 J/cm^2) exposure (**p<0.01- as compared to 5 mM NaN_3). Each value presented is mean of three observations ±SE.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612





Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

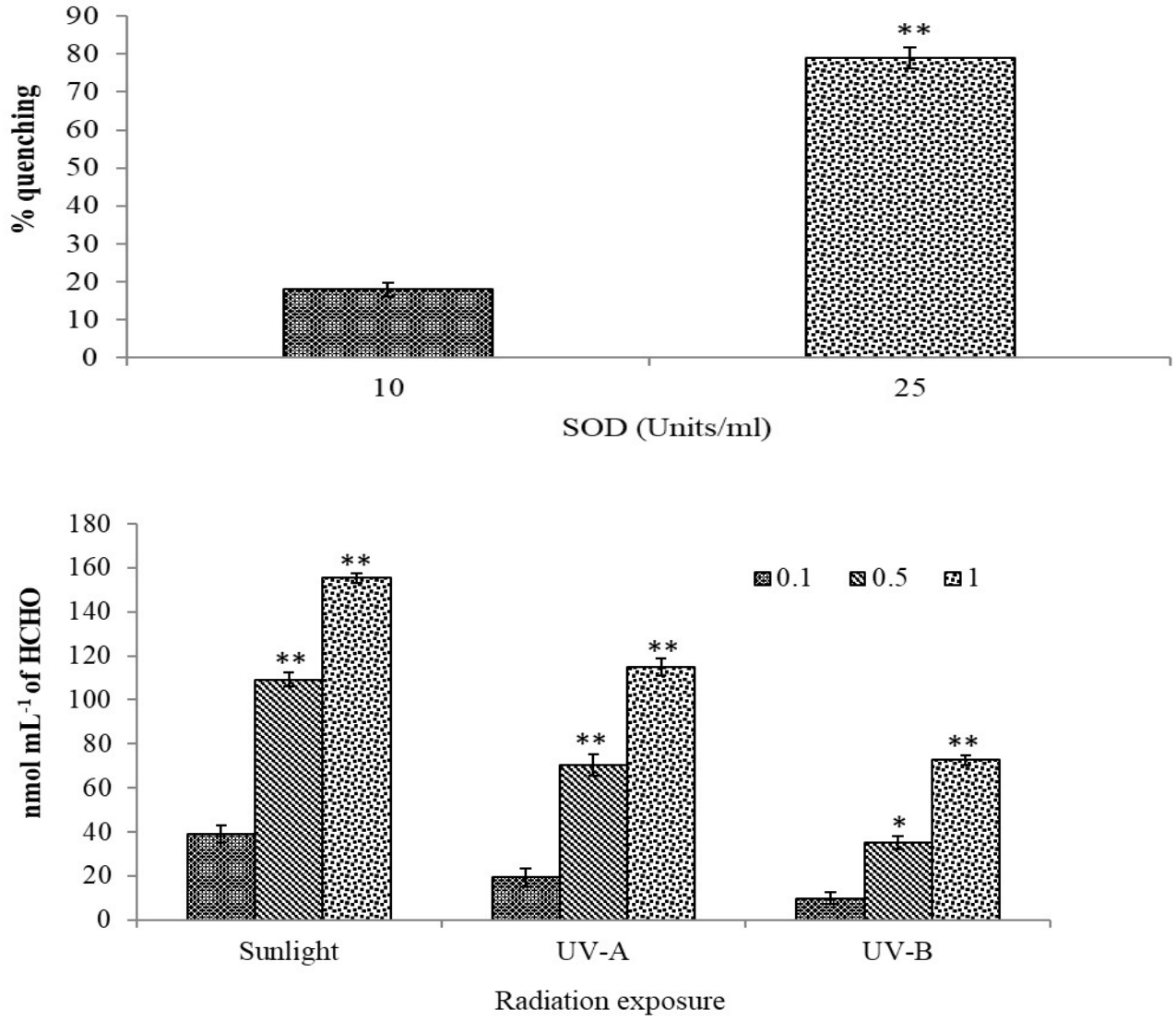


Fig. 2 (a) Photochemical generation of $O_2^{\cdot -}$ by PPD (µg/ml) at various concentrations under Sunlight (30 min), UV-A (2.16 J/cm^2), and UV-B (0.72 J/cm^2). Values presented are mean of three observations ±SE. (*p< 0.05 or **p<0.01- as compared to 0.1 µg/ml). **(b)** Photochemical quenching of $O_2^{\cdot -}$ generated by PPD (1 µg/ml) through SOD (10 and 25) under UV-A (2.16 J/cm^2). Values presented are mean of three observations ±SE. (**p<0.01- as compared to 10 Units/ml).

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

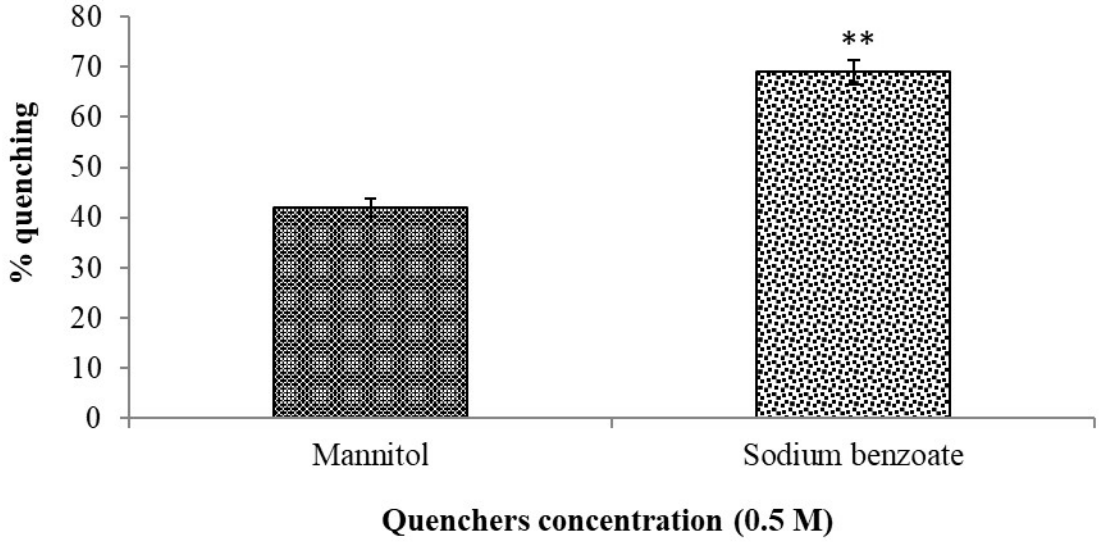
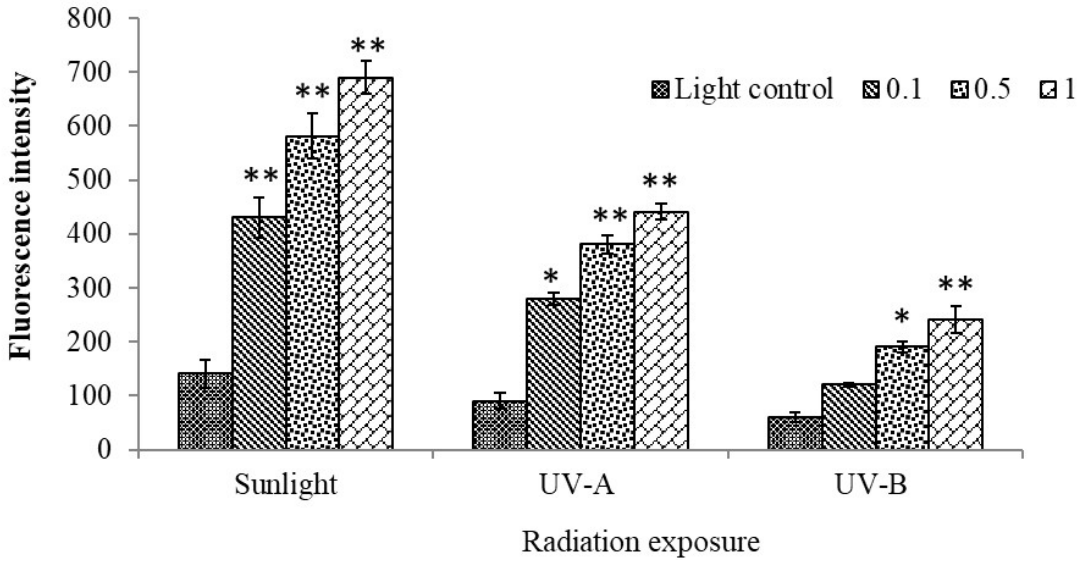


Fig. 3 (a) Photochemical generation of ·OH at various concentrations of PPD under sunlight (30 min), UV-A (2.16 J/cm^2) and UV-B (0.72 J/cm^2). Values presented are mean of three observations ±SE. (*p<0.05 or **p<0.01- as compared to 0.1 µg/ml). **(b)** Percent quenching of ·OH at 1 µg/ml of PPD by mannitol and sodium benzoate under UV-A (2.16 J/cm^2). Values presented are mean of three observations ±SE. (**p<0.01- as compared to 0.5 M mannitol).



Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

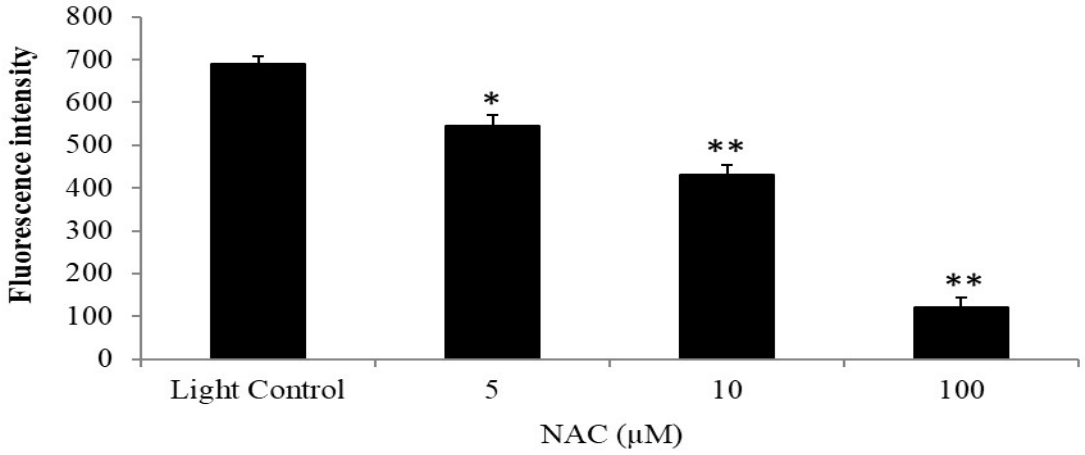
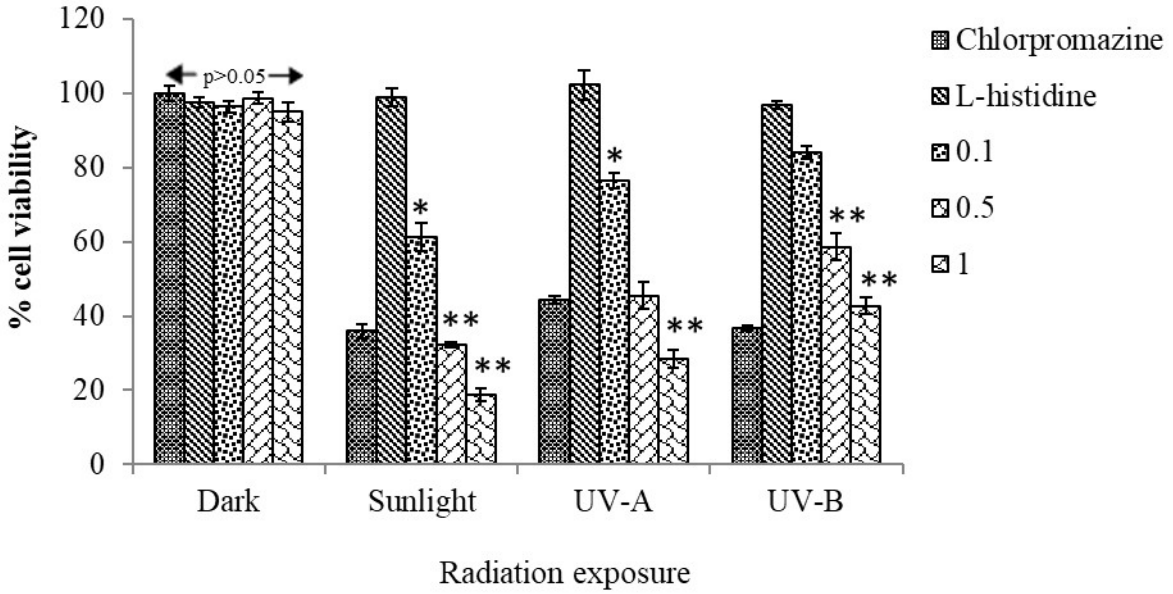


Fig. 4 (a) DCF-Fluorescence intensity at different concentrations of PPD (µg/ml) under sunlight (30 min), UV-A (2.16 J/cm^2) and UV-B (0.72 J/cm^2) exposure. Values presented are mean of three observations ±SE. (*p<0.05 or **p<0.01- as compared to Light control). **(b)** Intracellular quenching of DCF fluorescence by NAC under sunlight (30 min) exposure. Values presented are mean of three observations ±SE. (*p<0.05 or **p<0.01- as compared to Light control).



Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

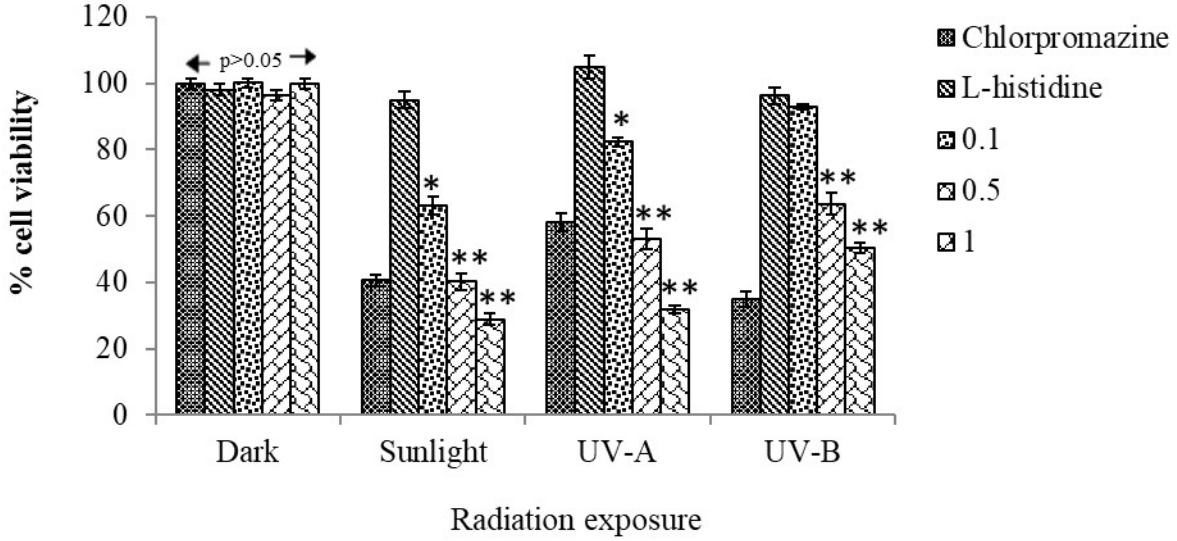
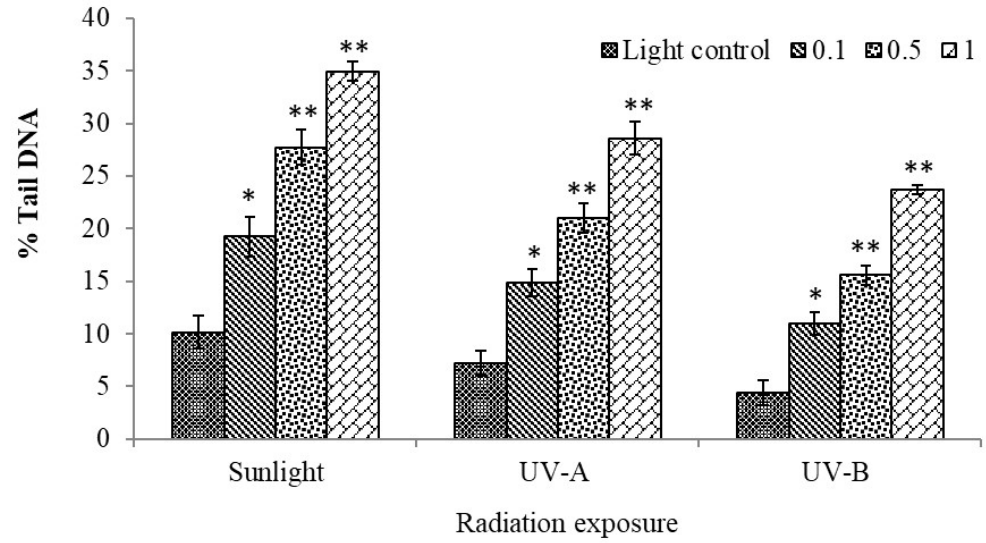


Fig. 5 Photosensitizing potential of PPD (µg/ml) on human keratinocyte as percent cell viability by recording MTT **(a)** and NRU **(b)** assay under dark, sunlight (30 min), UV-A (2.16 J/cm^2), and UV-B (0.72 J/cm^2) exposures. Chlorpromazine (1µg/ml) and L-histidine (10 µg/ml) were used as positive and negative controls, respectively. Values presented are mean of three observations ± SE. (*p<0.05 or **p<0.01- as compared to L-Histidine).



Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612
ISSN: 2582-2004
Volume 06, Issue 02

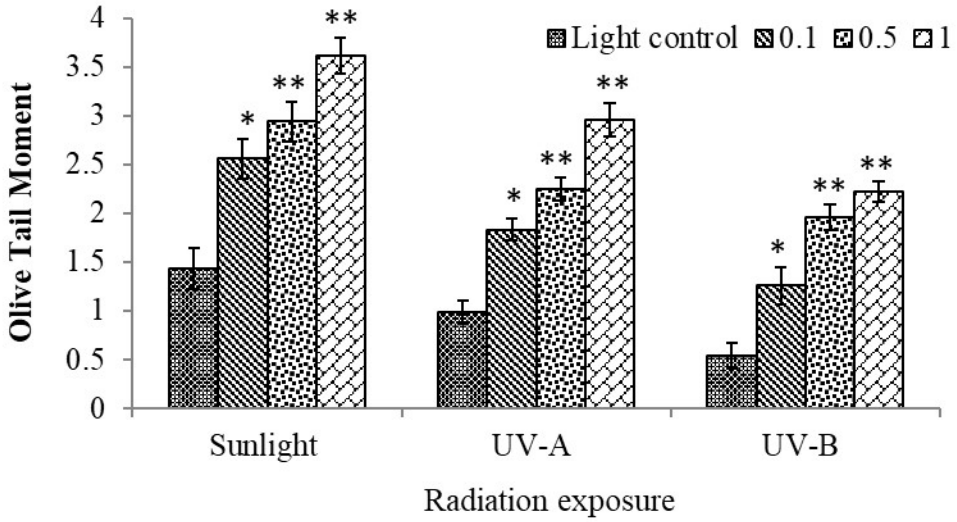


Fig. 6 DNA damage in keratinocytes after exposure of PPD under Sunlight (30 min), UV-A (2.16 J/cm^2), and UV-B (0.72 J/cm^2) exposure **(a)** % Tail DNA **(b)** Olive tail moment. Values presented are mean of three observations ± SE. (*p<0.05 or **p<0.01- as compared to Light control).

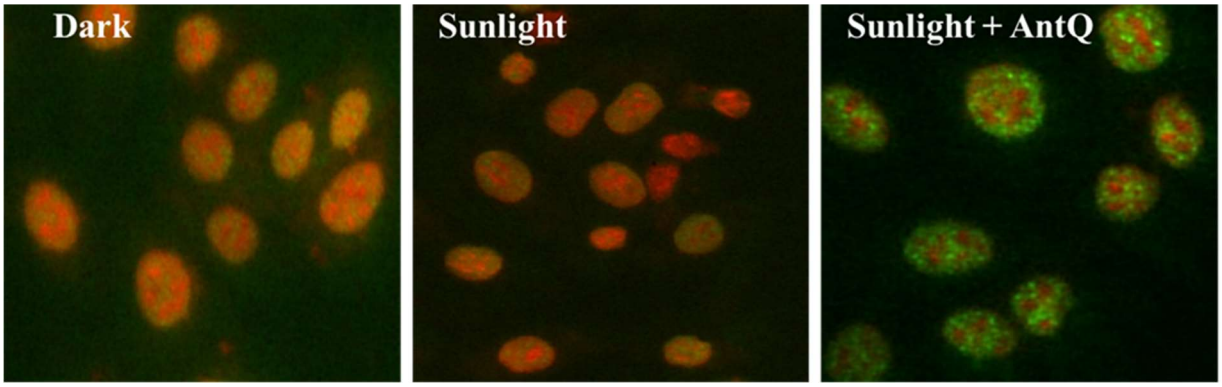


Fig. 7 Genotoxic evaluation of PPD (1µg/ml) under sunlight (30 min) exposure. Maximum CPDs formation was under joint exposure of PPD under sunlight exposure. No significant CPDs were detected under sunlight exposure alone and dark control cells.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612
ISSN: 2582-2004
Volume 06, Issue 02

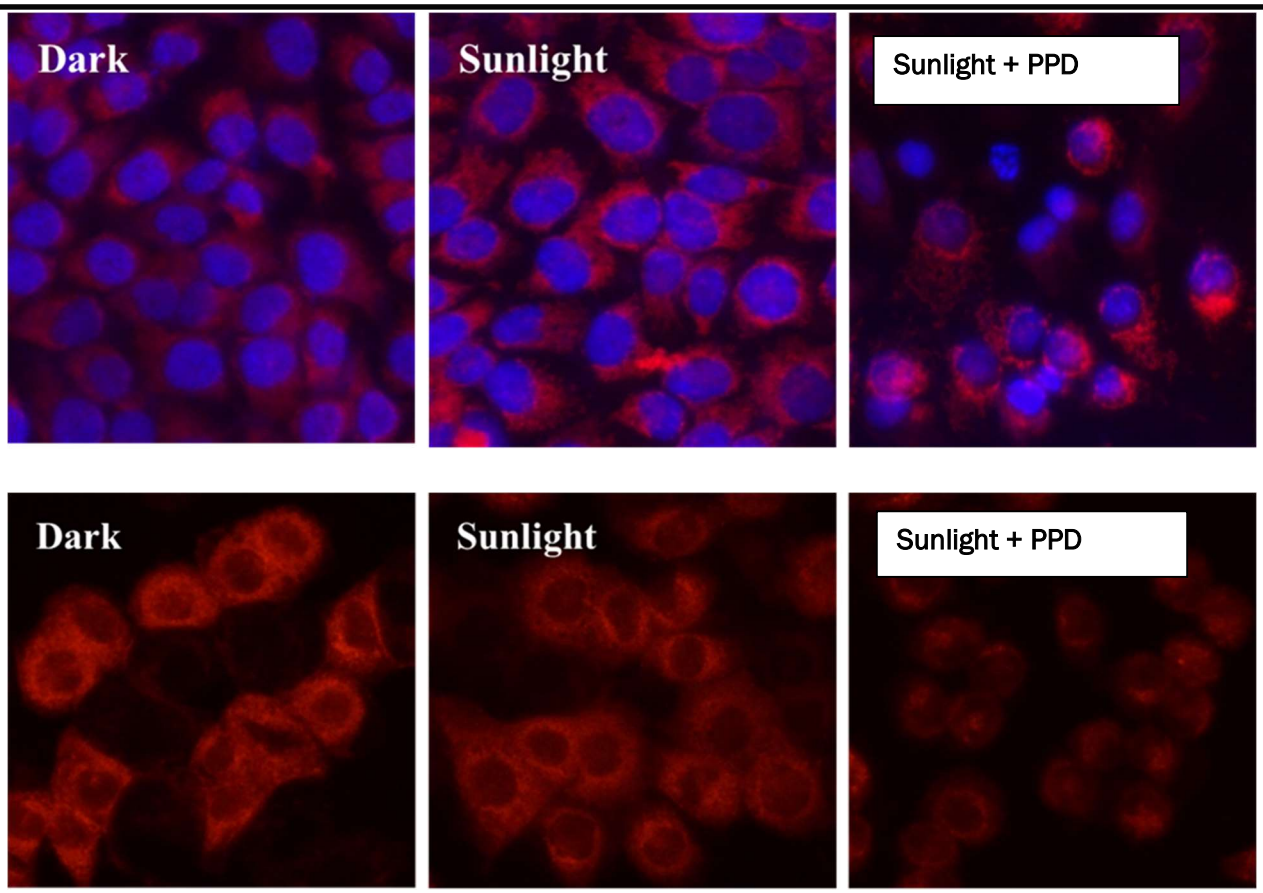


Fig. 8 Analysis of Mitochondrial membrane and its relation with DNA damage after treatment with PPD under sunlight exposure. Fluorescence micrographs of keratinocytes showing decrease in mitochondrial membrane potential after treatment of PPD under sunlight (30 min) exposure. MitoRed and DAPI double staining showed the relation of mitochondrial depolarization and DNA damage as well as Rhodamine staining to assess mitochondrial membrane potential.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

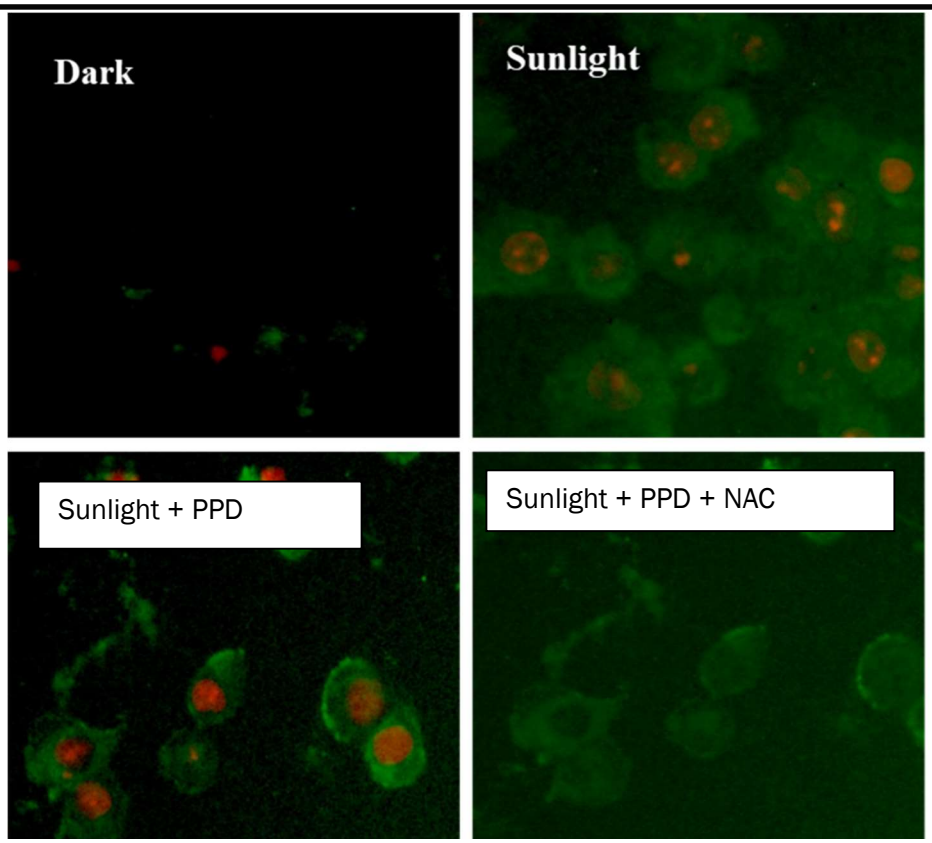


Fig. 9 Analysis of apoptotic cell death. Fluorescence images of Annexin V/PI double staining, showing maximum apoptotic cells after treatment with PPD (1 µg/ml) under sunlight (30 min) exposure. There was no significant apoptotic cells detected in dark control and sunlight exposure alone. NAC (10 µM) reduced the apoptotic cell death in keratinocytes after treatment with PPD (1 µgml) under sunlight (30 min) exposure.

Discussion

As a tropical country India, most of the human activities (agricultural, commercial, sports, etc.) are accomplished in bright sunlight; therefore the use of photosensitive sunscreens ingredients may lead to skin phototoxicity. Currently used sunscreens are unable to provide protection from photoaging, photo-immunosuppression, and photo-carcinogenesis instead reported a role in DNA damage. Inadequate protection of sunscreens may be associated with the lack of durability of the

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

application, the lack of inadequacy of UVA filters in sunscreens, and the photo-instability of UV filters which result in less protection (Haywood et al., 2003). The UV radiation on earth's surface is mainly of UVA (95%) and only 5% of UVB. Thus, it is essential to find efficient UVA photoprotective agent and to develop the broad-spectrum sunscreen. UVA radia tion is highly abundant and has highest penetration properties that are reaching at earth surface. UVA exposure plays an essential role in UVR-induced skin toxicity and essential factor in the development of skin cancer, which is more prevalent. The new cases of skin cancers diagnosed each year are equivalent to the combined incidence of malignancies of other organs (Katiyar, 2007). Chronic exposure of PPD to UV leads to photocytoxicity in HaCaT cell line. The oxidative stress induced by PPD under UV irradiation is primarily mediated through reactive oxygen intermediates and has been found to induce the expression or activation of several key signal pathways which are responsible for apoptotic cell death. Thus, our study was focused on the impact of PPD on human skin cells (HaCaT) under UV radiation.

ROS can cause severe stress in cells, leading to the oxidation of cellular components including lipids, proteins and DNA and in some cases the cleavage of DNA. PPD induced ROS mediated single strand breakage of DNA in keratinocytes under UV radiation. Maximum DNA damage was seen under sunlight alone.

PPD reduced keratinocyte viability significantly under sunlight/UVR exposure. Maximum reduction in cell viability was seen under sunlight followed by UV-A and UV-B exposures. There was no significant reduction in viability in dark control cells (Mukherjee et.al., 2013).

To further confirm the apoptotic cell death PS translocation were detected through annexin-V and PI staining (Zhao et.al., 2005). Maximum apoptotic cells were detected under joint exposure of PPD and sunlight exposure. There was no significant apoptotic cells were detected under joint exposure of PPD sunlight exposure. To elucidate the ROS mediated DNA damage induced apoptotic cell death, NAC was used as a quencher to prevent ROS mediated apoptosis.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
ISSN: 2582-2004
Volume 06, Issue 02
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612

Conclusion

The study conclude that the PPD generates ROS mediated apoptosis in keratinocytes through DNA damage. Hence, it is important to look the environment concentration and its toxicity impact on human beings due to stability of PPD in the environment.

References

1. Shakoor Z, Al-mutairi, A.S., Al- Shenaifi, A.M., Al- Abdulsalam, A.M., Al- Shirah, B.Z., Al- Harbi S.A. (2017). Screening for skin sensitizing allergens among patients with clinically suspected allergic contact dermatitis. *Saud. Med. J*. 38, 922-927.
2. J.M. Sontag, Carcinogenicity of substituted-benzenediamine (phenylenedaimine) on rats and mice, J. Natl. Cancer Inst. 66 (1981) 591–602.
3. L.A. Stanley, J.A. Skare, E. Doyle, R. Powrie, D. D'Angelo, C.R. Elcombe, Lack of evidence for metabolism of p-phenylenediamine by human hepatic cytochrome P450 enzymes, Toxicology 210 (2005) 147–157.
4. J. Cadet, E. Sage, T. Douki, Ultraviolet radiation-mediated damage to cellular DNA, Mutat. Res. 571 (2005) 3–17.
5. A. Roddick-Lanzilotta, R. Kelly, Measurement and prevention of hair photoaging, J. Cosmet. Sci. 55 (2004) S113–S121.
6. Z.D. Draelos, Sunscreens and hair photoprotection, Dermatol. Clin. 2 (2006) 81–84.
7. L. David, M. John, H. Jamie, A review of aspects of oxidative hair dye chemistry with special reference to N-nitrosamine formation, Materials 6 (2013) 517–534.
8. S.F. Mujtaba, A. Dwivedi, M.K.R. Mudiam, D. Ali, N. Yadav, R.S. Ray, Productionof ROS by photosensitized anthracene under sunlight and UV-R atambientenvironmental intensities, Photochem. Photobiol. 87 (2011)1067–1076.
9. Foote CS, Fugimoto TT and Chang YC (1972).Chemistry of singlet oxygen XV. Irrelevance of azide trapping to mechanism of the ene reaction. *Tetrahedron letters*1,45-48.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023

Journal of Advance Research in Science and Social Science (JARSSC)
Publisher: Indian Mental Health & Research Centre
DOI: 10.46523/jarssc.06.02.10
Multidisciplinary, Open Access
Impact Factor: 3.612
ISSN: 2582-2004
Volume 06, Issue 02

10. Ouannes C and Wilson T (1968).Quenching of $^1O_2$ by tertiary aliphatic amines.Effects of DABCO.*Journal of the American Chemical Society*90,6527-6528.
11. Ray RS, Agrawal N, Misra RB, Farooq M and Hans RK (2006). Radiation-induced *in vitro* phototoxic potential of some fluoroquinolones.*Drug and Chemical Toxicology* 29, 25-38.
12. Nash, T. (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55, 416–421.
13. Castell JV, Gomez-lechon MJ, Grassa C, Martinez LA, Miranda MA and Tarrega P (1994). Photodynamic lipid peroxidation by the photosensitizing nonsteroidalantiinflammaory drugs suprofen and tiaprofenic acid.*Photochemistry and Photobiology* 59, 35-39.
14. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
15. AgrawalN,RayRS,FarooqM,PantAB,Hans RK. (2007).Photosenstizing potential of ciprofloxacin at ambient level of UV radiation.Photochemistry and Photobiology.83.1226-1236.
16. K. Zhao, G. Luo, S. Giannelli, H.H. Szeto, Mitochondria-targeted peptideprevents mitochondrial depolarization and apoptosis induced by tert-butylhydroperoxide in neuronal cell lines, Biochem. Pharmacol. 70 (2005) 1796–1806.
17. D. Ali, A. Verma, F. Mujtaba, A. Dwivedi, R.K. Hans, R.S. Ray, UV B inducedapoptosis and DNA damaging potential of chrysene via reactive oxygen speciesin human keratinocytes, Toxicol. Lett. 204 (2011) 199–207.
18. Mukherjee A., N. Boujedaini, A. R. K.Bukhsh (2013). Homeopathic thuja 30C ameliorates benzo(a)pyrene- induced DNA damage, stress and viability of perfused lung cells of mice in vitro. Journal of Integrative Msedicine, 11(6); 397-404.

Received: 03.10.2023 **Accepted:** 10.11.2023 **Published**: 11.11.2023