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# Ultraviolet A (UV-A) Radiation-Induced Damage in the Skin and Vital Organs of Albino Rat: An Indirect Correlation with Melatonin

**Rakesh Verma*, Sriparna Pal, Jitendra Kumar and Chandana Haldar**

Department of Zoology, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, India; rakeshverma2527@gmail.com

## Abstract

Ultraviolet radiation is causative of generation of reactive oxygen species (ROS) in the body that significantly affects normal physiology and disturbs homeostasis. In the present study we investigated the effect of UV-A radiation exposure on the first line of defence system such as skin and vital organs such as liver, kidney and spleen of *Rattus norvegicus*. Adult female rats were exposed to UV-A radiation for seven days at a dose of 6.36 J/cm$^2$ and the changes in the skin histoarchitecture, oxidative load of spleen, liver and kidney along with cellular ROS levels of splenocyte determined using DCFDA staining were recorded. UV-A exposure severely damaged the histoarchitecture of skin and reduced proliferating cell nuclear antigen (PCNA) expression. The lipid peroxidation (MDA) level in spleen, liver and kidney were increased to significant levels while the activities of the enzymatic antioxidants, SOD and catalase were significantly decreased. Significant decrease of glucose content and increase of LDH of both spleen and liver were found. Cellular damage of splenocyte was observed as evidenced by increase in percentage of intense DCFDA-stained cells in UV-A treated rats. Thus, our results clearly demonstrate that UV-A radiation exposure may have detrimental effects on the antioxidant defence system of the body, including melatonin, leading to disruption of physiology by affecting vital organs.

**Keywords:** Kidney, Liver, Oxidative Stress; Skin, Spleen, Splenocyte, DCFDA, PCNA, UV-A Radiation

## 1. Introduction

UV is one of the non-ionizing radiations in the electromagnetic spectrum and classified into three bands based on wavelengths of light. Ultraviolet A (UVA) spans the electromagnetic spectrum of wavelengths between 320 and 400 nm, ultraviolet B (UVB) spans the spectrum between 290 and 320 nm, and ultraviolet C (UVC) spans the spectrum between 200 and 290 nm. Ozone in the stratosphere absorbs all UVC and absorbs UVB partially; it does not absorb any UVA. Solar UV exposure is known to be associated with various skin cancers, accelerated skin aging, cataract of the lens and other eye diseases, and possibly has an adverse effect on a person's ability to resist infections (https://www.nasa.gov/topics/solarsystem/features/uv-exposure.html).

UVA causes generation of reactive oxygen species (ROS) leading to oxidative stress in the skin and other tissues, opposite to conditions of melatonin secretion.[1] ROS interfere with structure and function of the cells, making them weak and defenceless. Studies have shown that UV radiation produces a variety of adverse effects that specifically include DNA damage,[2] inflammation, immuno-suppression[3] and photo-aging. However, there are arrays of complex antioxidant defence systems to combat the deleterious effects of ROS-mediated oxidative damages. Therefore, we proposed that the UV-A exposure may inflict deleterious effects on the tissues of vital importance such as liver, kidney, and lymphoid organ (spleen) besides its direct effect on the first line of defence system viz, skin. In the present study we investigated the effect of UV-A radiation on the histoarchitectural defects of skin, PCNA expression variation in the skin, altered antioxidant capacity of liver, kidney and spleen along with the cellular ROS level of splenocyte and glucose, and LDH activity in the liver and spleen of *Rattus norvegicus*.

**Author for correspondence*
* **Phone:** 352-294-4008, **Fax:** 352-392-8908

# 2. Materials and Methods

## 2.1 Animal Maintenance and UV-A Treatment

Adult female Wistar rats, *Rattus norvegicus*, weighing 170 ± 10 g, were used for the experiment, and the experiment was approved by the Institutional Animal Ethics Committee. Rats were kept in commercial polypropylene cages of equal sizes, fed with commercially available rodent pellet feed along with soaked gram and water *ad libitum* and maintained at constant temperature (25 ± 3° C) and light/dark cycle (12L:12D). A total of ten healthy adult female rats were randomly selected and divided into two groups, Group I: Control (n = 5) and Group II: UV-A treated (n = 5). Non-ionizing ultraviolet radiation UV-A tube (Philips Actinic BL reflector UV-A) was used for the experiment. The rats were anesthetized and the ventral abdominal skin was shaved aseptically and exposed to UV-A at a dose of 6.36 J/cm$^2$.[4]

## 2.2 Sample Collection and Processing

Seven days after the exposure the rats were weighed and sacrificed under deep anaesthesia. Spleen, liver and kidney were immediately dissected out and kept at -80° C for biochemical analysis. A small portion of the shaved skin was dissected out and fixed in Bouin's fixative and processed for histological analyses. After fixation, skin was washed in running tap water to remove excess fixative and dehydrated in graded series of alcohol. After clearing in xylene, tissues were embedded in paraffin wax and cut into 5 µm sections. The sections were spread on clean glass slides pre-coated with 2% gelatine. Deparaffinised sections were stained using haematoxylin and eosin stains. Skin histoarchitecture in randomly selected sections was observed in a research microscope (Nikon, E 200, Japan).

Immunohistochemical analyses of PCNA were performed according to a published method.[5] Briefly, 5 µm thick skin sections mounted on glass slides were sequentially rehydrated. Endogenous peroxide activity was blocked using 0.1 % $H_2O_2$ in methanol for 30 min at room temperature (RT) followed by washing with phosphate-buffered saline (PBS) and incubated with horse blocking serum (1:100 in PBS; PK-6200, Vector Laboratories, Burlinghame, CA) for 2 h. Then, the sections were incubated with primary antibody against PCNA (Santa Cruz, SC-7907, dilution 1:200) overnight at 4$^0$ C followed by washing thrice with PBS, and sections were then incubated with biotinylated secondary antibody [rabbit IgG, PK-6200, Vector Laboratories, Burlinghame, CA, dilution 1:50]. After washing thrice with PBS, a pre-formed ABC reagent conjugated to the free biotin of the secondary antibody was administered. The immunoreactivity was visualized using the 0.03% peroxidase substrate 3, 3-diaminobenzidine (DAB, Sigma Chemicals, St. Louis, Mo, USA) in 0.01M Tris-Cl, pH 7.6 and 0.1% $H_2O_2$, followed by its dehydration. The slides were, mounted with DPX mountant and observed in a research microscope (Nikon, E 200, Japan).

## 2.3 Oxidative Load Parameters Assay

Spleen, liver and kidney tissues were homogenized in ice-cold PBS (50 mM, pH 7.4) to obtain 10% homogenate which was centrifuged at 12,000 x g for 30 minutes to obtain the supernatant. The protein levels in the supernatants were determined using BSA as the standard.[6] The supernatants were used for measurement of different parameters described elsewhere.[7]

### *2.3.1 Superoxide Dismutase (SOD) Activity Assay in Spleen, Liver and Kidney*

Homogenates of spleen, liver and kidney tissues were processed for assay of SOD activity adopting the following protocol. 100 µL of enzyme extract was mixed in a 1.4 mL reaction mixture containing 20 mM L-Methionine, 1% (v/v) Triton X, 10 mM Hydroxylamine hydrochloride and 50 mM EDTA. Eighty microlitres of 50 µM Riboflavin was then added and the whole mixture was incubated under 20W white light for 10 min. The reaction was stopped by adding freshly prepared Griess reagent and the OD was read at 543 nm.

### *2.3.2 Indirect Catalase Activity Assay in Spleen, Liver and Kidney*

Homogenates of spleen, liver and kidney tissues were processed for indirect catalase assay in a reaction mixture comprising 0.8 mM $H_2O_2$, PBS and Potassium dichromate in Glacial acetic acid. The reaction was stopped by heating in a water bath for 10 min and the OD was read at 570 nm and the decrease in $H_2O_2$ content was calculated. The depletion of $H_2O_2$ by enzyme present in spleen extract was depicted as catalase activity. The standard curve was calibrated with varying concentrations of 0.2 mM $H_2O_2$ in PBS.

### *2.3.3 Lipid Peroxidation (MDA) Assay in Spleen, Liver and Kidney*

Homogenates of spleen, liver and kidney were centrifuged at 3000 x g for 15 min at 4° C and the supernatants were subjected to TBA assay. The supernatant was mixed with 2.8 mM BHT, 8.1% SDS, 20% Glacial acetic acid and 0.8% TBA and boiled for 1 h at 100° C. The reaction mixture was immediately cooled in running tap water and shaken vigorously with n-butanol: pyridine (15:1). The mixture was centrifuged at 1500 x g for 10 min and the absorbance of the upper phase was checked at 534 nm.

### *2.3.4 Glucose and LDH Measurement in Liver and Spleen*

Glucose level was measured using commercially available glucose kit (Autospan, India) in tissue homogenates as per the manufacturer's instructions. LDH level was measured in tissue homogenates using commercial kit (Coral Clinical Systems, Spain).

### *2.3.5 Measurement of Cellular ROS Level (DCFH-DA Method)*

This technique uses cell permeable fluorescent and chemiluminescent probe 2',7'-Dichlorodihydrofluorescein di-acetate (DCFH-DA) for directly measuring the amount of free radicals generated and accumulated within a cell. DCFH-DA, a cell permeable, non-fluorescent precursor of DCF is used as an intracellular probe for oxidative stress. The splenocytes were mixed with the DCFH-DA dye and viewed in a fluorescent microscope (Motic BA410) at 485 nm excitation wavelength and 530 nm emission wavelength.[7]

## 2.4 Statistical Analysis

The data were analyzed using Student's *t*-test. All the data were expressed as mean + SEM. The data were considered statistically significant when $p \leq 0.05$.

# 3. Result

## 3.1 Effect of UV-A Exposure on Skin Histoarchitecture and PCNA Expression

UV-A treatment produced prominent changes in the histoarchitecture of the skin. The uppermost epidermal layer, stratum corneum, was disrupted while irregular foldings were noted in the stratum spinosum and stratum basale layers. There was significant reduction in the number of hair follicles and sebaceous glands of UV-A treated animals. Degenerative changes were noticed in dermal glands and hair follicles of UV-A treated animals. Further, the skin of control animals showed abundant highly intense PCNA immune-positive cells as compared to the skin of treated animals (Figures. 1, 2).

## 3.2 Effect of UV-A Exposure on Pro-Oxidants Load of Liver, Kidney and Spleen

A significant reduction in liver SOD and CAT were noticed in UV-A treated group of rats as compared to the control group. Further, there was significant increase in lipid peroxidation level of liver of UV-A treated animals as compared to control (Figure 3).

A significant reduction in kidney SOD and CAT activities were noted in UV-A treated rats as compared to the control group. Further, there was significant increase in kidney lipid peroxidation (LPO) level in UV-A treated group as compared to control (Figure 5).

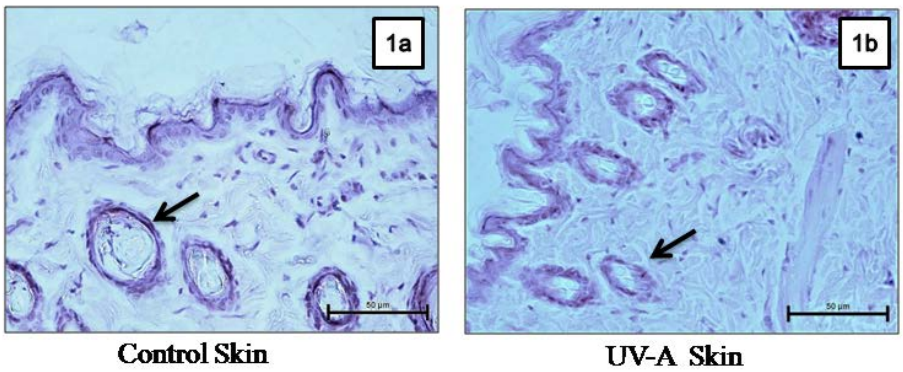


**Figure 1.** Effect of UV-A treatment on histoarchitecture of skin. (1a) Histoarchitecture of control group skin. (1b) Histoarchitecture of UV-A treated group skin. Original magnification x400; scale bar = 50µm.

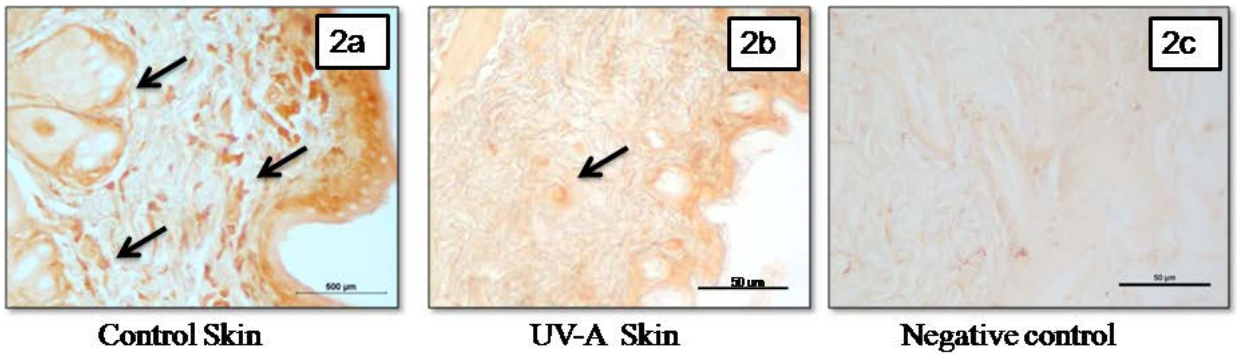


**Figure 2.** Effect of UV-A treatment on immunohistochemical expression of PCNA in skin of rats. (2a) Immunopositive cells for PCNA in control group skin. (2b) Immunopositive cells for PCNA in UV-A treated group skin. (2c) Negative control section (incubated without primary antibody). Original magnification x400; scale bar = 50µm.

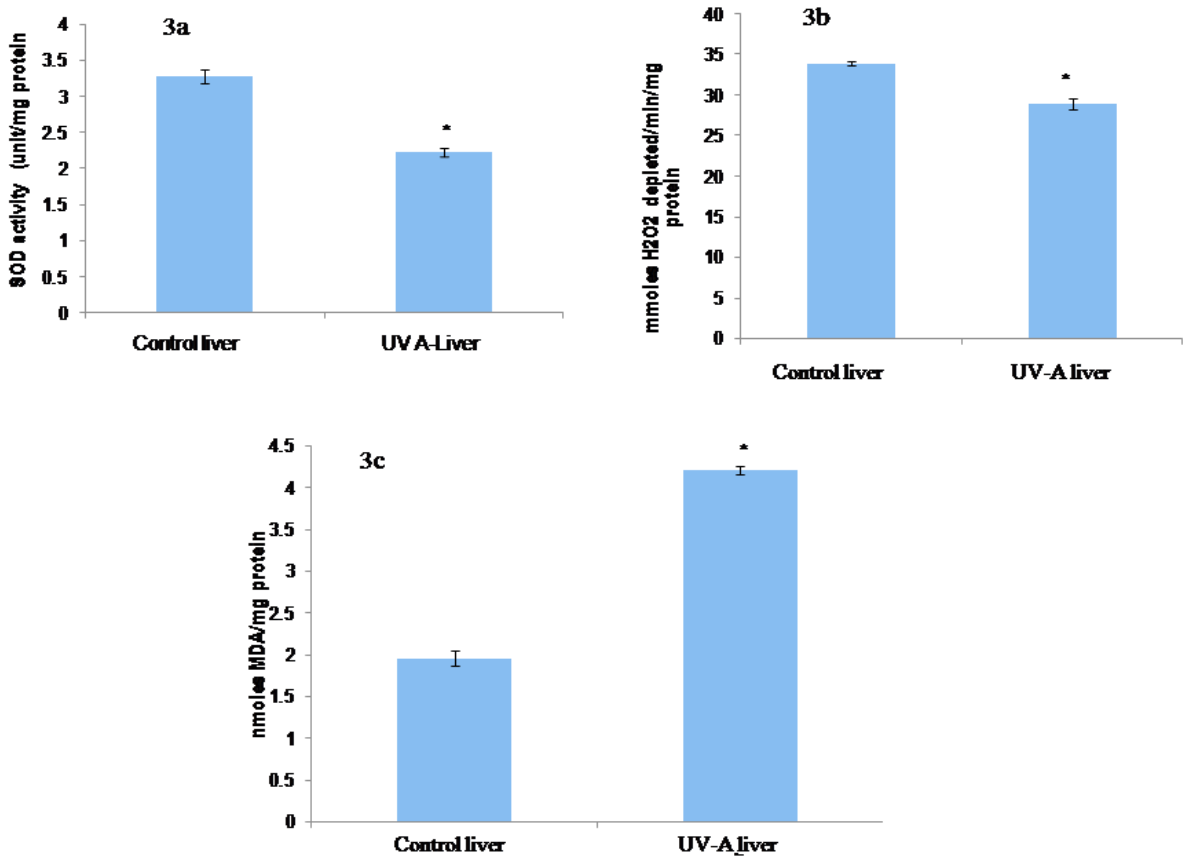


**Figure 3.** Effect of UV-A treatment on SOD, catalase activity and lipid peroxidation levels (MDA) in the liver. (3a) Histogram of SOD activity in liver. (3b) Histogram of catalase activity in liver. (3c) Histogram of MDA content in liver. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference $p<0.05$, control vs UV-A treated groups.

The SOD activity was significantly decreased in treated rats as compared to the control. The CAT activity also showed similar trend, while spleen LPO level in the UV-A treated animals was significantly increased as compared to control rats (Figure 6).

## 3.3 Effect of UV-A Exposure on Glucose and LDH Activity of Liver and Spleen

Liver and spleen glucose content was found to be significantly decreased in UV-A treated group as compared to the control. On the other hand, LDH activity was found

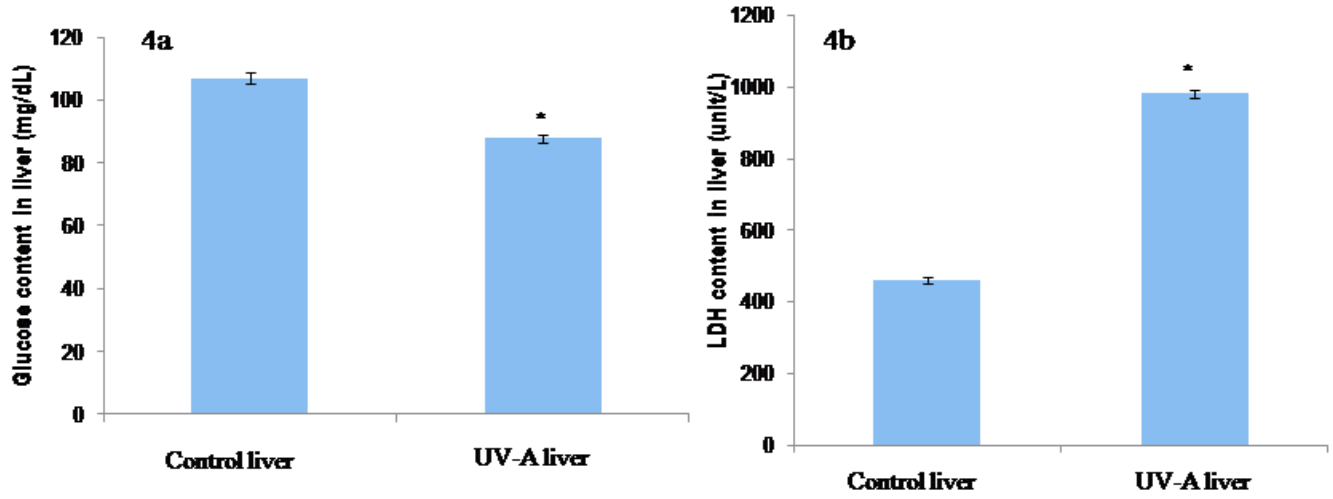


**Figure 4.** Effect of UV-A treatment on glucose content (4a) and LDH activity (4b) in liver of rats. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference p<0.05, control vs UV-A treated groups.

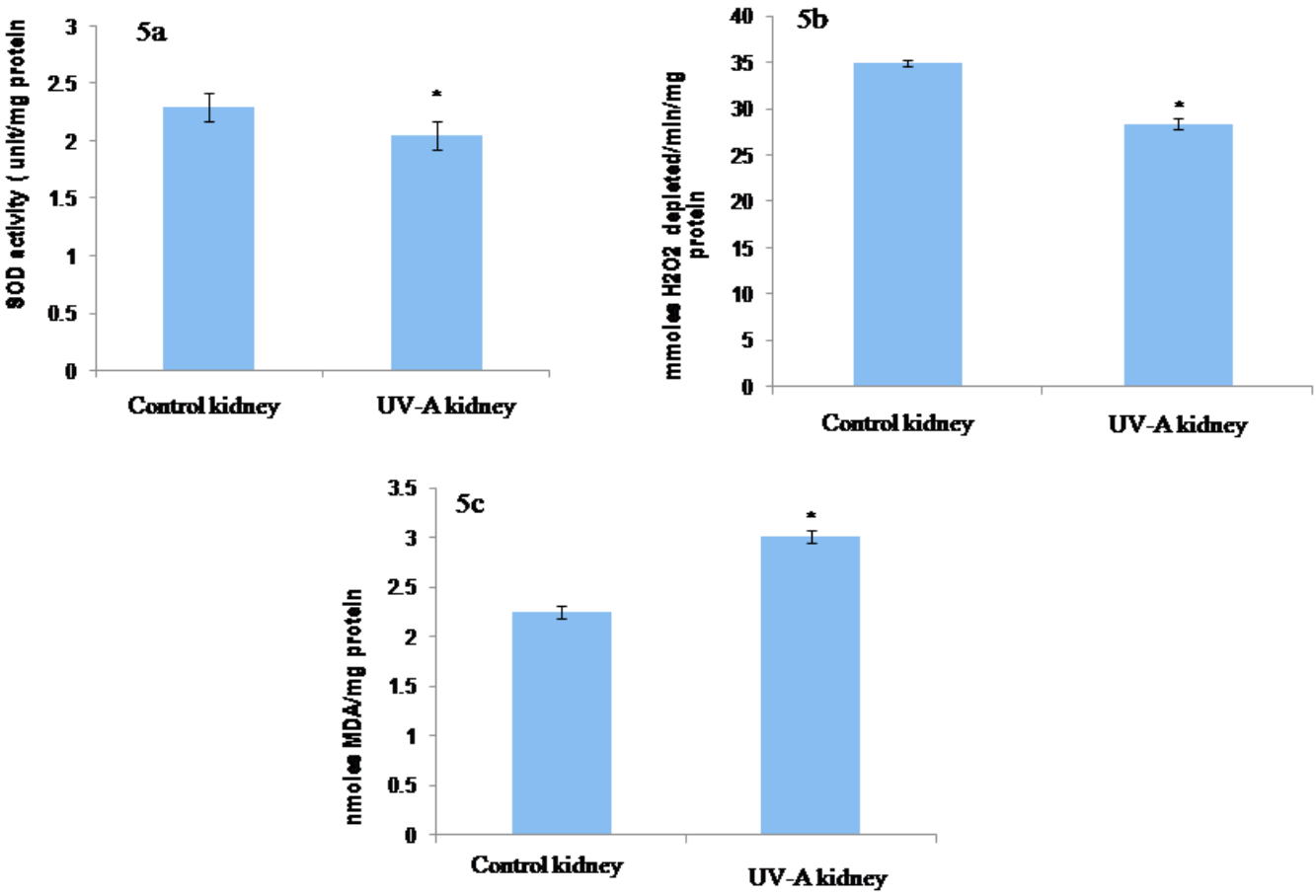


**Figure 5.** Effect of UV-A treatment on SOD, catalase activity and lipid peroxidation levels (MDA) in the kidney. (5a) Histogram of SOD activity in kidney. (5b) Histogram for catalase activity in kidney. (5c) Histogram of MDA content in kidney. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference p<0.05, control vs UV-A treated groups.

to be significantly increased in both liver and spleen in UV-A treated rats as compared to the control group (Figure 4, 7).

### 3.4 Effect of UV-A Exposure on Cellular ROS Level of Spleen

There was significant increase in the percentage of intense DCFDA stained splenocytes in case of UV-A exposed animals as compared to control group (Figure 8).

## 4. Discussion

Ultraviolet (UV) radiations are potent abiotic, ecological stress factor causing oxidative stress by augmenting the production and accumulation of reactive oxygen species (ROS) in the organism. Although at a lower level the ROS are not harmful, but rather play important role in signalling cascade whereas their over-production and accumulation leads to disturbed homeostasis as they damage cell membrane, proteins, carbohydrates and DNA, leading to their malfunctioning and ultimately cell death.[8] Organisms have evolved with antioxidant defence system where there are various kinds of enzymes such as superoxide dismutase (SOD), catalase and GPx that neutralize ROS and reduce the oxidative load and thereby increase the longevity of cells. UV radiations can damage cellular integrity and cell signalling pathways leading to toxic manifestations.[9] Further, UV-A radiations are considered important in regulating human health as they can penetrate the skin. Therefore, they are considered as the potent damaging part of the UV spectrum of solar radiation.

UV-A contributes greatly to squamous cell carcinomas, malignant melanomas and photodermatoses[10-13]. Our results show that upon seven days exposure to UV-A the normal skin histoarchitecture is disrupted. We noticed regressed dermal gland and decrease of their numbers. Also, the immune-positive cells for PCNA were less in skin of UV-A exposed rat, providing evidence for damage in the skin and their less proliferative potential as com-

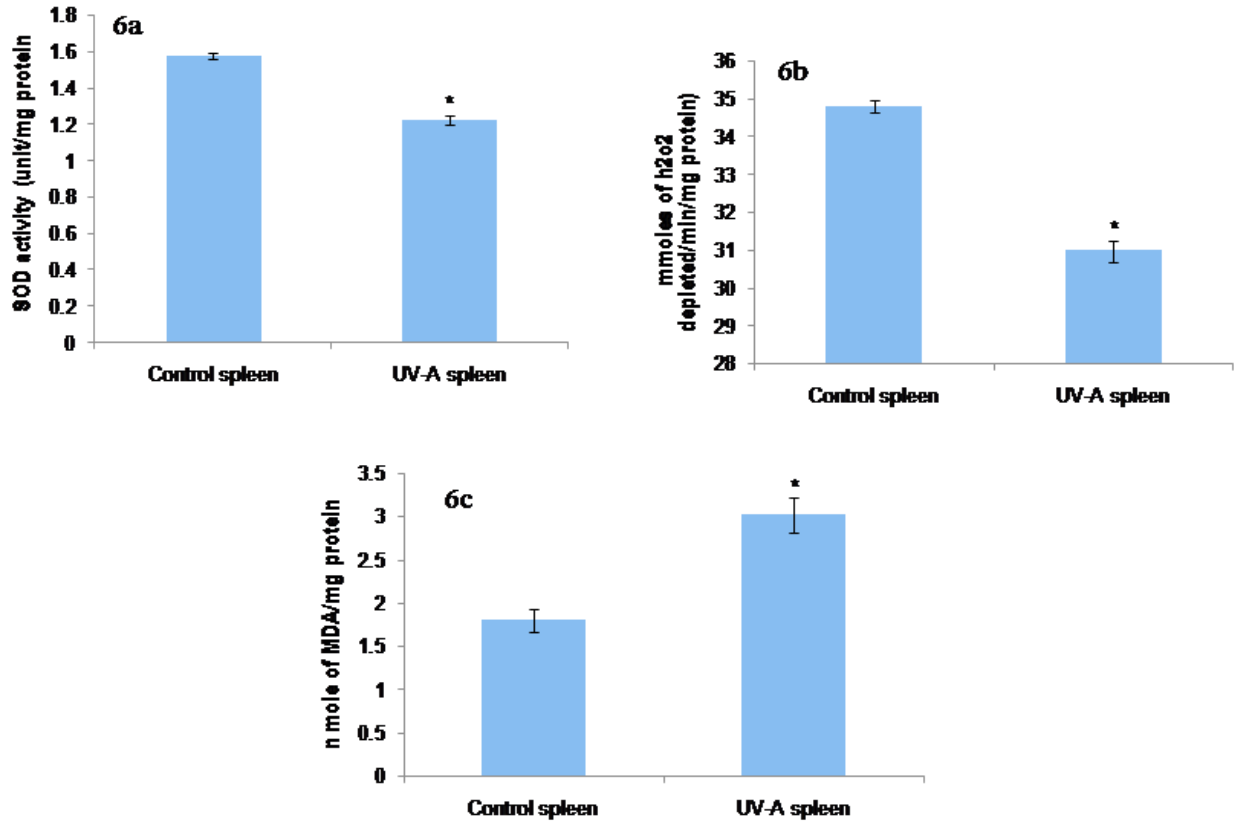


**Figure 6.** Effect of UV-A treatment on SOD, catalase activity and lipid peroxidation levels (MDA) in the spleen. (6a) Histogram of SOD activity in spleen. (6b) Histogram for catalase activity in spleen. (6c) Histogram of MDA content in spleen. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference $p<0.05$, control vs UV-A treated groups.

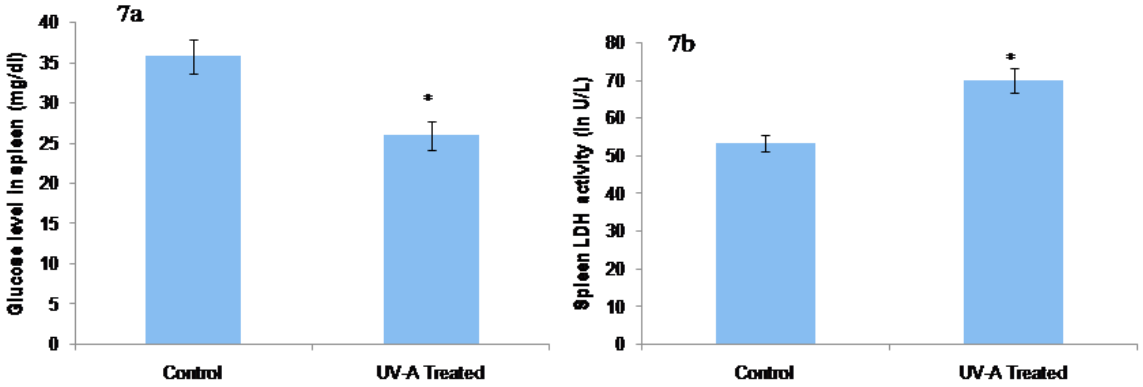


**Figure 7.** Effect of UV-A treatment on glucose content (7a) and LDH activity (7b) in spleen of rats. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference $p<0.05$, control vs UV-A treated groups.

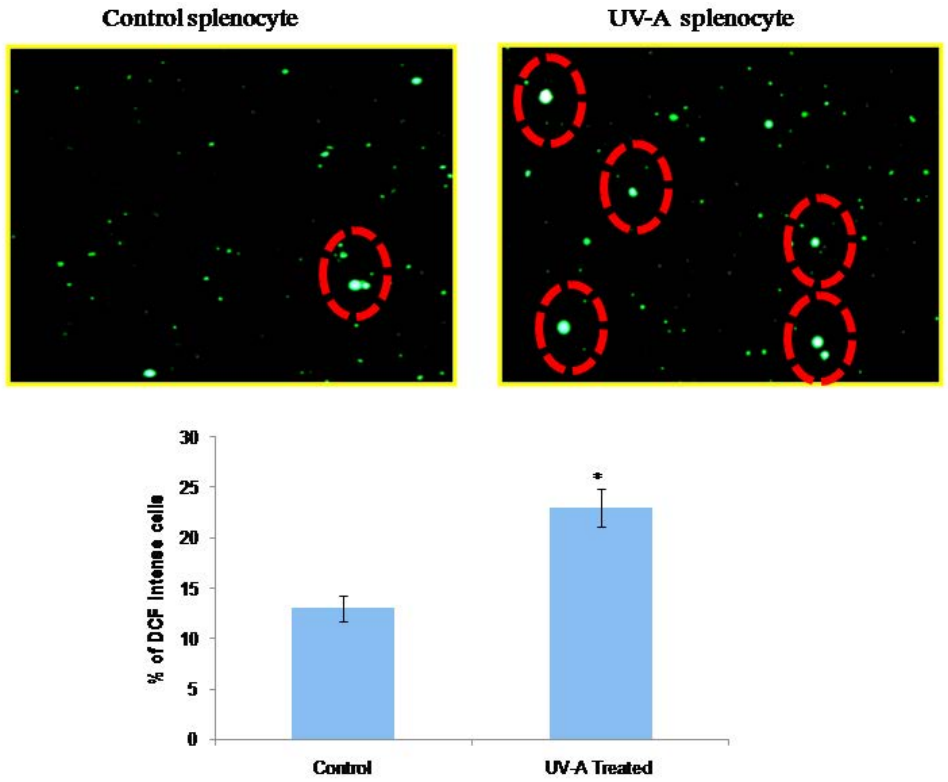


**Figure 8.** Effect of UV-A treatment on cellular ROS level of splenocyte. Histogram showing the percentage (%) of intense DCF fluorescent splenocytes. Values are expressed as Mean ± SEM, N=5 for each group. *Significance of difference $p<0.05$, control vs UV-A treated group.

pared to the skin of control group where PCNA immunopositive cells were more in both number and intensity showing normal features providing for their continuous growth and proliferation.

Cytoprotective antioxidant enzymes are generally assessed as they have ability to offer protection against the deleterious effects of ROS that leads to oxidative stress. Study regarding implication of melatonin as a potent antioxidant molecule against UV-A induced oxidative load in the seasonally breeding mammal, *Funambulus pennantii,* reveals the deleterious effect of such radiations in the skin[4]. Our result showed that exposure to UV-A caused significant decrease in the SOD and catalase activities of liver and kidney along with spleen as

compared to control group rats. This indicates reduced defence mechanism against ROS production and their accumulation. Further, significant increase in lipid peroxidation level was also noted in the liver, kidney and spleen of UV-A exposed animals as compared to control group showing severe damage to cellular integrity and membrane dynamics. Our result also showed higher cellular ROS level of splenocyte as we have noted significant increase in the percentage of intense DCFDA stained spleen cells of UV-A exposed rats as compared to control group indicating the increased level of oxidative load in the lymphoid organ. Glucose and its metabolite, lactate, plays crucial role in regulation of cellular survival. Lactate dehydrogenase (LDH) is the key enzyme that converts sugar into energy form (inter-conversion of pyruvate to lactate). Our result showed significant decrease in glucose content of liver and spleen of UV-A exposed rats as compared to control rats. Significant increase in the LDH activity of spleen and liver of UV-A exposed rats as compared to control rats, indicate detrimental effects of UV-A on vital organs such as liver, kidney spleen. In conclusion, present study clearly demonstrates that UV-A radiation exposure may have detrimental effects on the antioxidant defence system of body leading to disturbed physiology.

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## 6. References

1. Valencia A, Rajadurai A, Carle AB, Kochevar IE. 7-Dehydrocholesterol enhances ultraviolet A-induced oxidative stress in keratinocytes: roles of NADPH oxidase, mitochondria and lipid rafts. *Free Radic Biol Med*. 2006; 41: 1704–1718. https://doi.org/10.1016/j.freeradbiomed.2006.09.006 PMid:17145559 PMCid:PMC1880892
2. Douki T, Reynaud-Angelin, A, Cade, J, Sage E. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry*. 2003; 42: 9221–9226. https://doi.org/10.1021/bi034593c PMid:12885257
3. Halliday, G. M. Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis. *Mutat. Res*. 2005; 571: 107–120. https://doi.org/10.1016/j.mrfmmm.2004.09.013 PMid:15748642
4. Goswami, S, Haldar, C. Melatonin pre-treatment alleviates UVA radiation induced oxidative stress and apoptosis in the skin of a diurnal tropical rodent *Funambulus pennantii*. *J Nucl Med Radiat Ther*. 2016; 8: 318.
5. Verma R, Haldar C. Photoperiodic modulation of thyroid hormone receptor (TR-α), deiodinase-2 (Dio-2) and glucose transporters (GLUT 1 and GLUT 4) expression in testis of adult golden hamster, *Mesocricetus auratus. J Photochem Photobiol B*. 2016; 165: 351–358. https://doi.org/10.1016/j.jphotobiol.2016.10.036 PMid:27838488
6. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem*. 1976; 72: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3
7. Vishwas DK, Mukherjee A, Haldar C, Dash D, and Nayak MK. Improvement of oxidative stress and immunity by melatonin: an age dependent study in golden hamster. Exp Gerontol. 2013; 48: 168–182. https://doi.org/10.1016/j.exger.2012.11.012 https://doi.org/10.1016/j.exger.2013.05.052 PMid:23220117
8. Lijun L, Xuemei L, Yaping G, EnboM. Activity of the enzymes of the antioxidative system in cadmium-treated Oxya chinensi (Orthoptera Acridoidae). *Environ Toxicol Phar*. 2005; 20: 412–416. https://doi.org/10.1016/j.etap.2005.04.001 PMid:21783620
9. McMillan TJ, Leatherman E, Ridley A, Shorrocks J, Tobi SE, Whiteside JR. Cellular effects of long wavelength UV light (UVA) in mammalian cells. *J Pharm Pharmacol*. 2008; 60: 969–976. https://doi.org/10.1211/jpp.60.8.0004 PMid:18644190
10. Hasei K, Ichihashi M. Solar urticaria: determinations of action and inhibition spectra Arch. *Arch of Dermatol*. 1982; 118: 346–350. https://doi.org/10.1001/archderm.118.5.346 https://doi.org/10.1001/archderm.1982.01650170060026
11. Ortel B, Tanew A, Wolff K, Honisgman H. Polymorphous light eruption: action spectrum and photoprotection. *J Am Acad Dermatol*. 1986; 14: 748–753. https://doi.org/10.1016/S0190-9622(86)70088-1
12. Garland CF, Garland FC, Gorham EC. Epidemiologic evidence for different roles of ultraviolet A and B radiation in melanoma mortality rates. *Ann Epidemiol (AEP)*. 2003; 13: 395–404. https://doi.org/10.1016/S1047-2797(02)00461-1
13. Agar NS, Halliday GM, Barnetson RStC, et al. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc Natl Acad Sci USA*. 2004; 101: 4954–4959. https://doi.org/10.1073/pnas.0401141101 PMid:15041750 PMCid:PMC387355