



Research Article

Anatomical Changes in the Skin of *Rattus norvegicus* after Artificial UV Exposure

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Abstract: Acute and subacute artificial UV exposure to albino rats exhibit morphological and histo-anatomical changes in the skin of albino rat. The anatomical changes include hyperplasia, hyperkeratosis, hypergranulosis and acanthosis beside numerical changes in keratinocytes, Langerhans, melanocytes and fibroblast seen after acute and subacute artificial UVB exposure.

Keywords: UVB exposure, Albino rats, Hyperplasia, Epidermal and dermal cell, Morphological and Histological changes.

1. Introduction

The invisible ultraviolet spectrum makes up one specific portion of sunlight. This unique portion accounts for three percent of all solar radiation reaching the earth. UV light is measured at wavelengths of light in units of nanometers (nm). Three types of UV light have been identified that are UVC, UVB and UVA. UVC is the wavelength in the C range of the ultraviolet solar spectrum, between 190 and 280nm. UVC is most carcinogenic. The ozone layer absorbs most UVC rays. However, recent research indicates that the ozone layer may be depleted in certain areas by chlorofluorocarbons (CFCs, chiefly used in refrigerators, air conditioners, and Styrofoam insulation). UVB is the wavelength in the B range of the ultraviolet solar spectrum, measured at between 280 and 315nm. UVB is the primary cause of sunburn and skin cancers. UVA is the wavelength in the A range of the ultraviolet solar spectrum, between 315 and 400nm. UVA may be up to 1,000 times more intense than UVB. It can penetrate into underlying tissues of the skin and cause photoaging, or long-term skin damage.

UV radiation is the principal etiological factor responsible for the majority of skin cancers. Exposure to UVR causes inflammation, erythema and edema.

In humans, prolonged exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye, and immune system. UVC rays are the highest energy, most dangerous type of ultraviolet light. Little attention has been given to UVC rays in the past

since they are filtered out by the atmosphere. However, their use in equipment such as pond sterilization units may pose an exposure risk, if the lamp is switched on outside of its enclosed pond sterilization unit.

The skin is one of the larger and protective organs of the body. It covers the surface area of about 2m² in man and about 0.008m² in the mouse and weighing about 2.1kg and 3.3g respectively. UVB radiations are absorbed by the skin, producing specific symptoms and eventual skin cancer, which increase skin roughness and loss of skin elasticity after prolonged exposure. One study showed that UVA-induced erythema is oxygen dependent (as are many UVA responses) and that UVB-induced erythema is oxygen independent.

Epidermis consists of various layers: stratum corneum, stratum granulosum, stratum spinosum and stratum malpighii. Stratum malpighii is also known as stratum basale or stratum germinativum. The stratum corneum is responsible for the skin barrier that protects the skin, preventing water loss, maintaining satisfactory hydration of the skin, and preventing overhydration in addition to many other protective functions. The back skin of mouse contains up to 10-15 cell layers with the total thickness of <15-20µm. These cell layers contain 14,200 basal cells, 87-92% keratoblasts, 300-400 (2-3%) melanocytes and 800-1400 (6-10%) Langerhans cells. Keratinocytes or keratoblasts are keratin producing cells, which are vast majority cells in the epidermis. Langerhans cells are involved in the immune system. Melanocytes are responsible for pigmentation.

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Merkel cells are sensitive to mechanical stimuli, especially pressure.

Dermis consists of fibroblast, nerve fibers, vascular tissue (endothelial cells in capillaries). The dermis and its blood supply are responsible for delivering nutrients and circulatory support because the epidermis has no blood supply of its own. Fibroblasts produce most of the dermal proteins as collagen, elastin and reticulin. Hypodermis is an adipose layer which serves temperature regulation.

All electromagnetic radiations cause damage to the skin anatomically too. Various cell populations as keratinocytes, Langerhans cells, melanocytes and fibroblasts etc. get damaged by UVB radiation.

The present investigation highlights morpho-anatomical changes under artificial UV exposure. The morphological changes have been assessed in terms of surface colour responses along with inflammation and morphological connectivity besides anatomical changes unveil the limitations of the most sensitive organ, the skin with regard to alterations in epidermal and dermal dexterity in albino rats.

2. Materials and Methods

2.1 Mice

The experimental albino rats of 100-140gms from inbred colony were selected for exposure due to their easy handling and care and acclimated for a photoperiod of 12 hours/day at temperature $22 \pm 3^{\circ}\text{C}$ & relative humidity $50\% \pm 5$ with proper hygiene. Albino rats were kept in polypropylene cages and fed with standard laboratory diet; the Goldmohar rat feed and had water *ad libitum*.

2.2 Experimentation

2.2.1 Radiation chamber

Albino rats were placed in a rectangular chamber of size $90 \times 45 \times 30\text{cms}$, made of glass of 3mm thickness fitted with Philips F30T8 fluorescent tube light as artificial source of UVB for exposure to albino rats [PLATE - 1(b)].

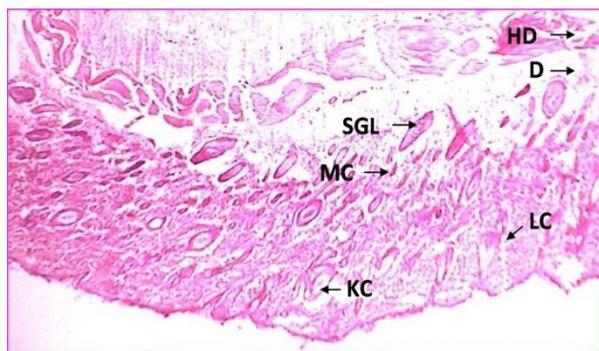


Plate 1(a). Photograph of rat skin showing keratinocytes (KC), Langerhans cell (LC), melanocytes (MC), sweat gland (SGL), dermis (D), hypodermis (HD) in control [4X].

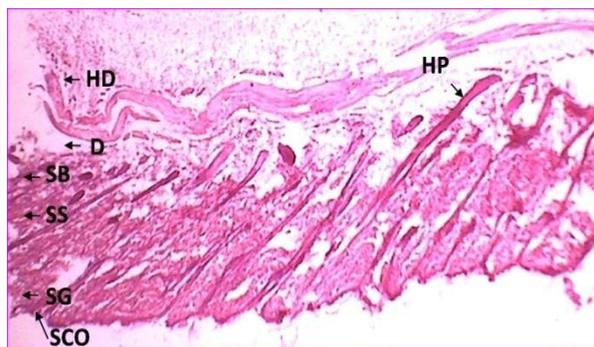


Plate 1(b). Photograph of rat skin showing stratum corneum (SCO), stratum granulosum (SG), stratum spinosum (SS), stratum basale (SB), dermis (D), hypodermis (HD) and hair papilla (HP) in control [4X].

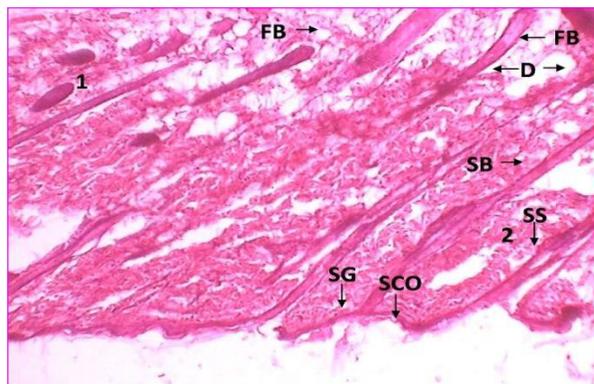


Plate 1(c). Photograph of rat skin showing stratum corneum (SCO), stratum granulosum (SG), stratum spinosum (SS), stratum basale (SB), dermis (D), hypodermis (HD), fibroblast (FB) and hair papilla (HP) in control [10X].

2.2.2 Depilatory material

The hair remover cream as depilatory material was applied to rectangular patch made on dorsal side of rats to be exposed to UVB.

2.2.3 Artificial UVB exposure

a. Preparatory phase

The hairs of the experimental albino rats were cut from the dorsal surface and depilatory material was then applied for removing the hairs neatly. Animals were exposed to a F30T8 fluorescent UVB tube light (300nm) fitted at a distance of 45cm in a rectangular chamber.

b. Exposure phase

The exposed albino rats were categorized into following sets:

- Set A- Served as control without UVB exposure.
- Set B- Albino rats were exposed to $0.44\text{J}/\text{cm}^2$ of UVB radiation continuously for 10 hours for one day.

- Set C- Albino rats were exposed to 0.088J/cm² of UVB radiation continuously for 2 hours/day for 5 days consecutively.
- Set D- Albino rats were exposed to 0.04419J/cm² of UVB radiation continuously for 1 hour/day for 10 days consecutively.
- Set E- Albino rats were exposed to 0.03314J/cm² of UVB radiation continuously for 45 minutes/day for 15 days consecutively.

2.2.4 Anatomical procedure

The albino rats from the control and exposed sets were etherized and the skin of dorsal side was cut open by an incision and then peeled off with the help of forceps. Separated skin cut down into small pieces of a size of 2-3mm. skin pieces were fixed into Bouin's fixative for 24 hours and were then dehydrated through of alcoholic series (30%→50%→70%→90%→100%). Skin pieces were cleared with xylol and infiltrated with xylol + wax and finally embedded in wax (60^oC M.P.). The wax blocks were made and trimmed. The paraffin-embedded skin was cut into 5µm sections placed on slides already covered with Mayer's albumin, deparaffinized, dehydrated, stained with Haematoxylin and eosin and were mounted with Canada balsam (Humason, 1979).

The histomorphological data were accessed to statistics after Fisher and Yates (1963). Mean & ANOVA of histoarchitectural irregularities were assessed in different exposed rats.

3. Results

Various cell populations have been observed in the epidermis, the primary cell of which is the keratinocyte besides additional cells like melanocytes and Langerhans cells. Langerhans cells, melanocytes and keratinocytes have been observed in the stratum spinosum, stratum basale and throughout epidermis respectively.

The junction between the epidermis and dermis undulates; the epidermis protrudes more deeply into the dermis, alternating with a more shallow protrusion into the epidermis. The final outcome causes the epidermis and dermis to interdigitate like fingers coming in-between each other to form, the rete pegs *via* invagination. Fibroblasts have been observed in the dermis.

The epidermis is comprised of various layers anatomically. The epidermal regenerative layer contains the basal keratinocyte where mitosis (regeneration of cells) occurs. The developing keratinocytes mature by becoming the stratum spinosum, the stratum granulosum, and finally the stratum corneum. The stratum corneum is responsible for the skin barrier that protects the skin, preventing water loss, maintaining satisfactory hydration of the skin, and preventing

overhydration, in addition to many other protective functions.

The controls did not reveal anatomical changes in the skin layers as all the layers of skin have been observed to be fully intact since were not subjected to UVB exposure [PLATE - 2(a-c)].

The anatomical changes even after 15 days have not been observed in the skin layers of control set *vide supra*. Epidermal and dermal layers of skin retained normalcy after 5, 10, and 15 days *vide supra* [PLATE-2(a-c)].

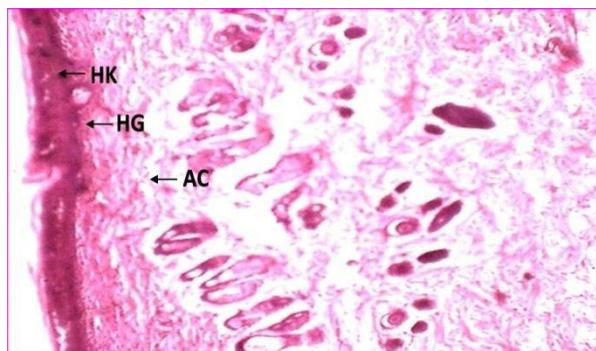


Plate 2(a). Photograph of rat skin showing mild acanthosis (AC), mild hypergranulosis (HG) and moderate hyperkeratosis (HK) in 1 day [10X].

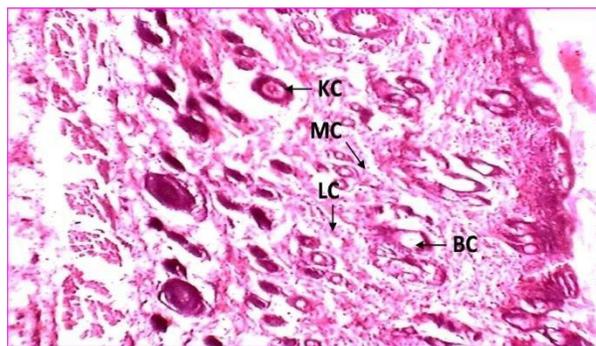


Plate 2(b). Photograph of rat skin showing burnt cell (BC), keratinocyte (KC), melanocyte (MC) and Langerhans cell (LC) in 1 day [10X].

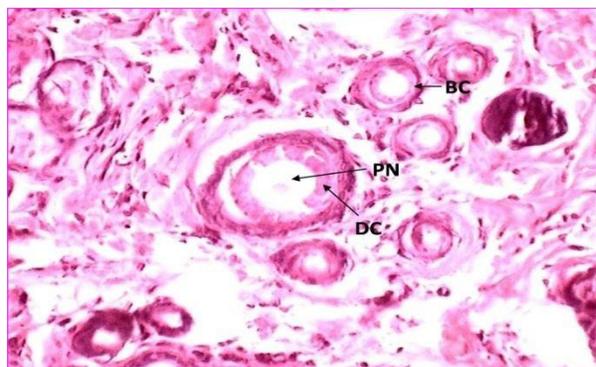


Plate 2(c). Photograph of rat skin showing burnt cell (BC), pyknotic nucleus (PN) and dense cytoplasm (DC) in 1 day [40X].

Moderate hyperkeratosis, mild hypergranulosis and mild acanthosis have been observed. UVB burnt cells have also been observed with pyknotic nucleus and dense cytoplasm. Besides Langerhans cells have been found reduced in number and mitotic keratinocytes have been observed with intracellular vacuolization. No change in the number of melanocytes has been observed. Fibroblasts of dermal layer did not reveal any change [TABLES - 1 & 2]; [PLATE - 3(a-c)].

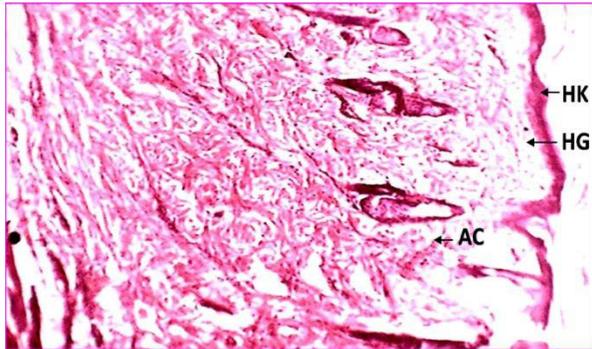


Plate 3(a). Photograph of rat skin showing moderate hyperkeratosis (HK), mild hypergranulosis (HG) and mild acanthosis (AC) in 5 days [10X].

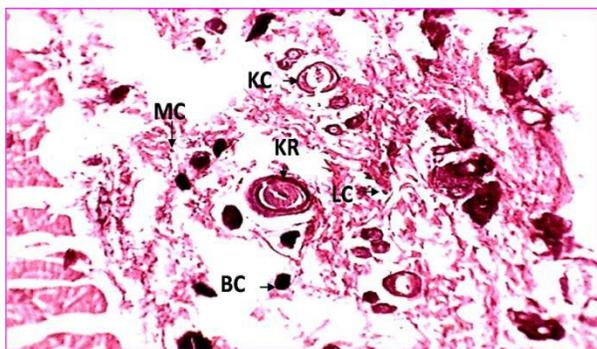


Plate 3(b). Photograph of rat skin showing damaged keratinocyte (KC), Langerhans cell (LC), melanocyte (MC), karyorrhexis (KR) and burnt cell (BC) in 5 days [10X].

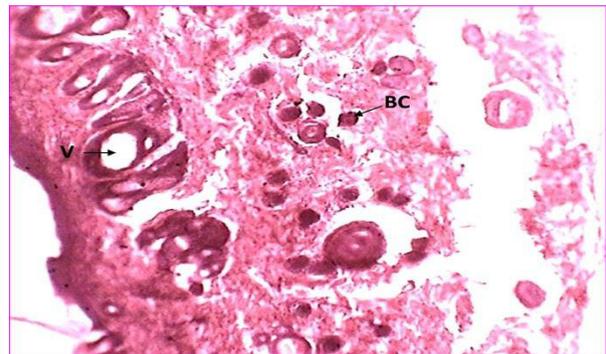


Plate 3(c). Photograph of rat skin showing burnt cell (BC) and vacuolization (V) in 5 days [10X].

Moderate hyperkeratosis, mild hypergranulosis and mild acanthosis have been recorded after 5 days exposure and moderate hypergranulosis, acanthosis and mild hyperkeratosis with the chap formation after 10 days exposure, while mild hyperkeratosis with severe chap formation and severe hypergranulosis and acanthosis have been observed after 15 days exposure. Number of UVB burnt cells increased after exposure from 5 to 15 days. Cell membrane blebbing have been found with pyknotic nucleus and dense cytoplasm after 5 days exposure, which tends towards severity on days 10 to 15 with pustule formation after 10 days exposure and intracellular vacuolization in epidermal layers after 15 days exposure. No change has been noticed in the number of melanocytes after 5 to 15 days exposure. Langerhans cells were found to reduce in number after 5 days exposure when compared with controls, while gradually reduced from 10 to 15 days exposure. Langerhans cells became round and slightly larger after 10 days exposure, while short dendritic processes have been recorded after 15 days exposure. Mild fibroblast proliferation in dermis could be found only after 15 days exposure [TABLES - 1 & 2]; [PLATE - 4(a-c)]; [PLATE - 5(a-c)].

Table 1. Hyperplasia, Anatomical anomaly after UVB exposure.

S. No.	No. of rats	No. of days	Hyperplasia		
			Hyperkeratosis	Hypergranulosis	Acanthosis
1.	5	1	■ ■	■	■
2.	5	5	■ ■	■	■
3.	5	10	■	■ ■	■ ■
4.	5	15	■	■ ■ ■	■ ■ ■

■ = Mild response; ■ ■ = Moderate response; ■ ■ ■ = Severe response

Table 2. Epidermal and dermal cell changes, Anatomical anomalies after UVB exposure.

S. No.	No. of rats	No. of days	Epidermal cell changes			Dermal cell changes
			Keratinocyte	Langerhans	Melanocyte	Fibroblast
1.	5	1	■ ■	■ ■	—	—
2.	5	5	■ ■	■ ■ ■	—	—
3.	5	10	■	■ ■	—	—
4.	5	15	■	■	—	■

— = Normal number; ■ = Minimal number; ■ ■ = Moderate number; ■ ■ ■ = Marked number

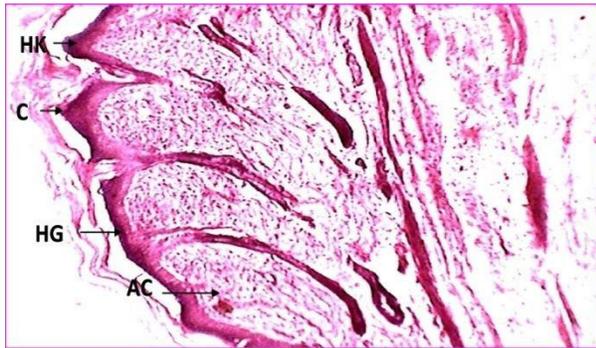


Plate 4(a). Photograph of rat skin showing mild hyperkeratosis (HK) with chap formation (C), moderate hypergranulosis (HG) and moderate acanthosis (AC) in 10 days [10X].

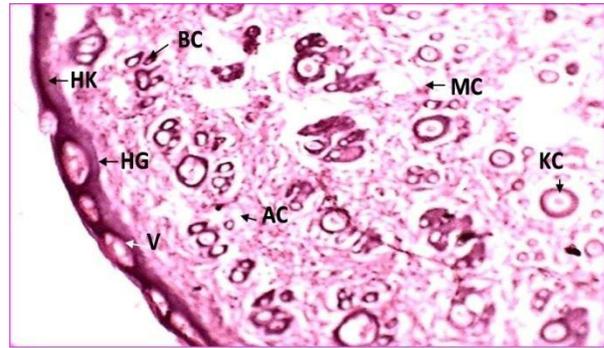


Plate 5(a). Photograph of rat skin showing mild hyperkeratosis (HK), severe hypergranulosis (HG), severe acanthosis (AC), vacuolization (V), melanocyte (MC), keratinocyte (KC) and burnt cell (BC) in 15 days [10X].

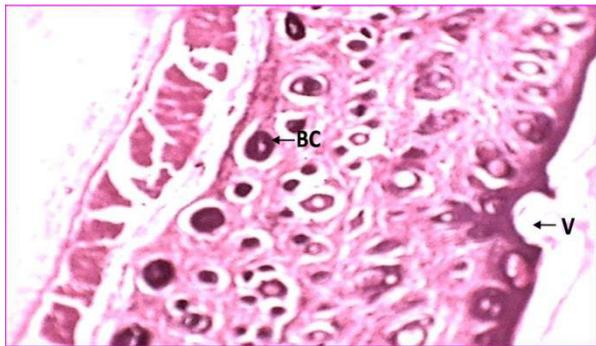


Plate 4(b). Photograph of rat skin showing burnt cell (BC) and vacuolization (V) in 10 days [4X].

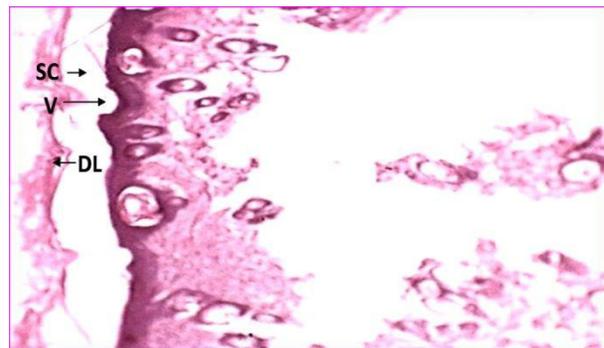


Plate 5(b). Photograph of rat skin showing severe chap formation (SC), dead layer (DL) and vacuolization (V) in 15 days [4X].

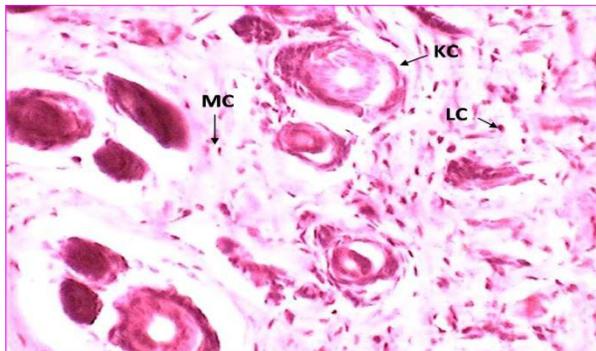


Plate 4(c). Photograph of rat skin showing rounded langerhans cell (LC), keratinocyte (KC) and melanocyte (MC) in 10 days [40X].

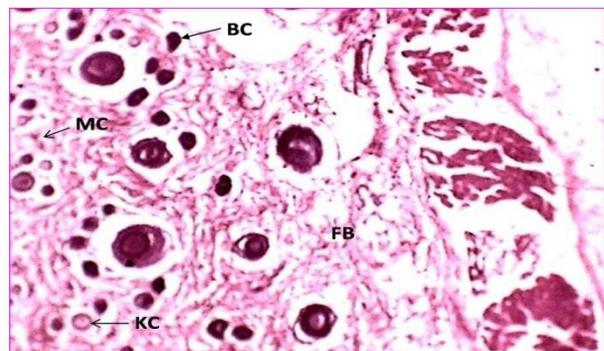


Plate 5(c). Photograph of rat skin showing burnt cell (BC), melanocyte (MC), keratinocyte (KC) and fibroblast (FB) in 15 days [10X].

4. Discussion

Skin is most important organ of the body. It provides a continuous protective outer sheath to whole body. It protects the body from various types of bacterial, viral and fungal infection, which may alter the metabolism of the body. Skin serves as a physical barrier to protect against physical factors, chemical factors, UV rays and mechanical damages and also helps in thermoregulation, insulation, excretion and specialized secretion. Above all, skin is a sensory organ.

The toxic effects of UV from natural sunlight and therapeutic artificial lamps are a major concern for human health. UVB from natural sunlight causes remarkable pigmentation while artificial UVB exposure causes severe apoptotic reactions in the skin.

Present study highlights that acute and subacute UVB exposures are less toxic than long-term chronic UVB exposure. The observations of one way analysis of variance exhibit that skin toxicity is dose dependent and exposure duration dependent. Acute UVB exposure causes moderate erythema and dermatitis, but mild

pustule formation and exudation. Squamation and desquamation have not been exhibited. Whereas albino rats exposed to 5, 10 and 15 days cause mild to severe pustule formation, exudation, squamation and desquamation, while moderate to mild erythema and dermatitis.

It could happen due to more of the radiant energy given to the albino rats for 10 hours continuously in 1 day because phototoxicity is the amount and exposure time dependent, which has been analysed by variance analysis. When time duration of UVB exposure decreased from 5 to 15 days as 2 hours for 5 days, 1 hour for 10 days and 45 minutes for 15 days, the morphological changes exhibited an increasing trend. The morphological changes were maximum during first 3 days of UVB exposure and gradually declined to day 5. Similar changes could be seen on days 10 and 15.

In the present investigation, anatomical changes hyperkeratosis, hypergranulosis, acanthosis, reduction in the numbers of epidermal and dermal cells and UVB burnt cell (apoptotic cell) formations have been predominantly observed after UVB exposure.

Anatomical changes lead moderate to mild hyperkeratosis and mild to severe hypergranulosis and acanthosis. Besides the moderate to minimal number of keratinocytes, marked to the minimal number of langerhans cells, normal numbers of melanocytes and normal to the minimal number of fibroblasts have been recognized after 5 to 15 days exposure.

In to normal mouse epidermis, cells are constantly turning over. Thus, differentiated cells are constantly replaced by proliferating cells from the basal layer.

UVB exposed mouse skin results in induction of p53 which cause apoptotic cells. In addition, UVB causes severe hyperplasia in the skin to replace the dead cells. Hence, the process of apoptosis and proliferation are closely linked and tightly regulated. UVB radiation is a physiologic inducer of apoptosis in keratinocytes on the other hand melanocytes is markedly resistant to UVB induced apoptosis. Long-term UVB exposure leads to decline in the regulation of the hyaluronic acid synthase (HAS) enzyme, which causes damage to fibroblast.

UVB radiation also activates the epidermal growth factor receptor (EGFR) which cause proliferation of keratinocytes and may induce proliferation of various epidermal layers as hyperkeratosis, hypergranulosis and acanthosis. UVB because of its low penetration may influence the epidermis more than the deeper region of skin (Potten, 1986).

Histologically, UVB radiation causes damage to the basal cells, Langerhans cells, melanocytes, keratoblasts and other epidermal and dermal cells, etc. The Nomenclature Committee on Cell Death (NCCD, 2005) proposes unified criteria for the definition of cell death and of its different morphologies. Cell death classified according to its morphological appearance which may be apoptosis, cornification, autophagy and

necrosis. Apoptosis contains the events like reduction of cellular and nuclear volume (pyknosis), nuclear fragmentation (karyorrhexis) etc. Cornification contains the events like modifications of plasma membrane, desquamation (loss of corneocyte) etc. Autophagy contains the events like lack of chromatin condensation, massive vacuolization of the cytoplasm etc. Necrosis contains the events like rupture of plasma membrane, swelling of cytoplasmic organelles, cytoplasmic swelling (oncosis) etc. (Kroemer *et al.*, 2009).

UVB is a causative factor of apoptosis in the epidermal cells, and that apoptotic cell is formed as a result of the apoptosis (Bayerl *et al.*, 1995; Baba *et al.*, 1996; Murphy *et al.*, 2001).

In anatomical studies, it is evident that UVB induces significant thickening (proliferation) of the epidermis as hyperplasia, thickening of the stratum corneum as hyperkeratosis, thickening of the stratum granulosum as hypergranulosis and thickening of stratum spinosum as acanthosis. Gambichler *et al.*, (2005) introduced optical coherence tomography (OCT) *in vivo* for the investigation of changes of epidermal thickness (ET) following UVA1, and UVB irradiation and evaluated the kinetics of acute UVB as well as UVA1 induced skin alterations by means of OCT, and correlated the results obtained with routine histology. The results suggest that, cells highly responsive to UVB exist in the epidermal layers and that their hyperproliferation leads to elongation of the epidermal layers (Baba *et al.*, 2005). UVB irradiation of mouse skin induces apoptosis and is mediated by the p53/p21/E2F-1/bax pathway and that the dead cells are replaced by hyperproliferative cells, leading to epidermal hyperplasia (Lee *et al.*, 2003).

Programmed cell death and induction of hyperplasia were examined in UV irradiated mouse skin (Ouhtit *et al.*, 2000). UVB radiation causes reduction in the number and morphologic alterations of Langerhans cells *in vivo* which are accompanied by an influx of monocytoid cells into the epidermis (Bacci *et al.*, 1998).

Ultraviolet irradiation induces keratinocyte proliferation and epidermal hyperplasia through the activation of the epidermal growth factor receptor (El-Abaseri *et al.*, 2006). UVB radiation decreases LC density and alters their morphology and immunological function in the present investigation and is in accordance to Seit  *et al.*, (2003). UV-irradiated mouse embryonic fibroblast and keratinocytes in skin die by apoptosis (Naik *et al.*, 2007).

The deterioration of keratin intermediate filaments (KIFs) in the stratum corneum caused by repetitive UVB irradiation decreases the elastic properties of the stratum corneum, resulting in the formation of wrinkles (Sano *et al.*, 2005).

It is thus evident that UV radiation induces various acute and chronic reactions in animal skin. Direct and indirect injuries result in a number of harmful effects

such as disrupted cell metabolism, morphological and ultrastructural changes, attack on the regulation pathway and alterations in the differentiation, proliferation and apoptosis of skin cells. Process like these leads to erythema, sunburn, inflammation, immunosuppression, photoaging, gene mutation and development of cutaneous malignancies (Svobodova et al., 2006).

Artificial UV irradiated skin shows less pigmentation and severe sunburn reaction, while solar exposure provoked remarkable pigmentation in the dorsal skin of hairless dogs (Kimura et al., 1995). Early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice are time course dependent (Ping Lu et al., 1999).

These events are held by the strike of radiant energy onto the skin and this energy is produced by excitation of electrons. Radiant energy induces the damage to the cells of the skin and to the layers of the skin which further leads erythema, dermatitis, pustule formation, exudation, squamation and desquamation. These morphological (biometrically supported) and anatomical changes to the skin could be expected in other higher mammalian groups too. In order to avoid such changes, exposure to UVB light should be minimized.

It is thus obvious that UVB has substantial potential to inflict injury to the outermost covering of the body which is evident both morphologically and anatomically.

References

- [1]. Baba, H., Yoshida, M., Yokota, T., Uchiwa, H. and Watanabe, S. (2005). Human epidermal basal cell responses to ultraviolet-B differ according to their location in the undulating epidermis. *J. Dermatol. Sci.*, 38(1):41-6.
- [2]. Baba, T., Hanada, K. and Hashimoto, I. (1996). The study of ultraviolet B-induced apoptosis in cultured mouse keratinocytes and in mouse skin. *J. Dermatol. Sci.*, 12(1):18-23.
- [3]. Bacci, S., Romagnoli, P., and Streilein, J.W. (1998). Reduction in number and morphologic alterations of Langerhans cells after UVB irradiation *in vivo* are accompanied by an influx of monocytoïd cells into the epidermis. *J. Invest. Dermatol.*, 111:1134-1139.
- [4]. Bayerl, C., Taake, S., Moll, I. and Jung, E.G. (1995). Characterization of sunburn cells after exposure to ultraviolet light. *Photodermatol. Photoimmunol. Photomed.*, 11(4):149-54.
- [5]. Deliconstantinos, G., Villiotou, V. and Stravrides, J.C. (1995). Release by ultraviolet B (u.v.B) radiation of nitric oxide (NO) from human keratinocytes: a potential role for nitric oxide in erythema production. *Br. J. Pharmacol.*, 114:1257-1265.
- [6]. El-Abaseri, T.B., Putta, S. and Hansen, L.A. (2006). Ultraviolet irradiation induces keratinocyte proliferation and epidermal hyperplasia through the activation of the epidermal growth factor receptor. *Carcinogenesis*, 27(2):225-231.
- [7]. Gambichler, T., Boms, S., Stucker, M., Moussa, G., Kreuter, A., Sand, M., Sand, D., Altmeyer, P. and Hoffmann, K. (2005). Acute skin alterations following ultraviolet radiation investigated by optical coherence tomography and histology. *Arch. Dermatol. Res.*, 297(5):218-25.
- [8]. Gambichler, T., Kunzlberger, B., Paech, V., Kreuter, A., Boms, S., Bader, A., Moussa, G., Sand, M., Altmeyer, P. and Hoffmann, K. (2005). UVA1 and UVB irradiated skin investigated by optical coherence tomography *in vivo*: a preliminary study. *Clin. Exp. Dermatol.*, 30(1):79-82.
- [9]. Kimura, T. and Doi, K. (1995). Dorsal skin reactions to sunlight and artificial ultraviolet light in hairless descendants of Mexican hairless dogs. *Exp. Anim.*, 44(4):293-299.
- [10]. Kroemer, G., Galluzzi, L., Vandenabeele, P., Abrams, J., Alnemri, E.S., Baehrecke, E.H., Blagosklonny, M.V. et al., (2009). Classification of cell death: Recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.*, 16:3-11.
- [11]. Lee, J.K., Kim, J.H., Nam, K.T. and Lee, S.H. (2003). Molecular events associated with apoptosis and proliferation induced by ultraviolet-B radiation in the skin of hairless mice. *J. Dermatol. Sci.*, 32(3):171-9.
- [12]. Lowe, N.J., Meyers, D.P., Wieder, J.M., Luftman, D., Borget, T., Lehman, M.D., Johnson, A.W., and Scott, I.R. (1995). Low doses of repetitive ultraviolet A induce morphologic changes in human skin. *J. Invest. Dermatol.*, 105:739-743.
- [13]. Lu, Yao-Ping., Lou, You-Rong., Yen, P., Mitchell, D., Huang, Mou-Tuan and Conney, A.H. (1999). Time course for early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice. *Cancer Res.*, 59:4591-4602.
- [14]. Murphy, G., Young, A.R., Wulf, H.C., Kulms, D. and Schwarz, T. (2001). The molecular determinants of sunburn cell formation. *Exp. Dermatol.*, 10(3):155-60.
- [15]. Naik, E., Michalak, E.M., Villunger, A., Adams, J.M., Strasser, A. (2007). Ultraviolet radiation triggers apoptosis of fibroblasts and skin keratinocytes mainly via the BH3-only protein noxa. *J. Cell Biol.*, 176 (4):415-424.
- [16]. Ouhtit, A., Muller, H.K., Davis, D.W., Ullrich, S.E., McConkey, D., Ananthaswamy, H.N. (2000). Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. *Am. J. Pathol.*, 156 (1):201-207.

- [17]. Papazoglou, E., Sunkari, C., Neidrauer, M., Klement, J.F. and Uitto, J. (2010). Noninvasive assessment of UV-induced skin damage: comparison of optical measurements to histology and MMP expression. *Photochem. Photobiol.*, 86(1):138-45.
- [18]. Sano, T., Kume, T., Fujimura, T., Kawada, H., Moriwaki, S. and Takema, Y. (2005). The formation of wrinkles caused by transition of keratin intermediate filaments after repetitive UVB exposure. *Arch. Dermatol. Res.*, 296(8):359-65.
- [19]. Seit , S., Zucchi, H., Moyal, D., Tison, S., Compan, D., Christiaens, F., Gueniche, A. and Fourtanier, A. (2003). Alterations in human epidermal Langerhans cells by ultraviolet radiation: quantitative and morphological study. *Br. J. Dermatol.*, 148(2):291-9.
- [20]. Svobodova, A., Walterova, D., and Vostalova, J. (2006). Ultraviolet light induced alteration to the skin. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.*, 150(1):25-38.
- [21]. Takema, O., Nishijima, A., Ohsu, H., Fujimura, T. and Hattori, M., (1997). Skin morphology at the time of UV irradiation is important for wrinkle formation. *J. Soc. Cosmet. Chem.*, 48:297-306.
- [22]. Tronnier, M., Smolle, J. and Wolff, H.H. (1995). Ultraviolet irradiation induces acute changes in melanocytic nevi. *J. Invest. Dermatol.*, 104(4):475-8.
- [23]. Viac, J., Goujon, C., Misery, L., Staniek, V., Faure, M., Schmitt, D. and Claudy, A. (1997). Effect of UVB 311 nm irradiation on normal human skin. *Photodermatol. Photoimmunol. Photomed.*, 13(3):103-8.
- [24]. World Meteorological Organization. (2003). Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project, Report No. 47. Geneva.