

# The Role of Vitamin 'C' on the Thickness of the Epidermis after X-Irradiation of the Guinea Pigs a Morphological Study under Light Microscope

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## ABSTRACT

**Objective:** To study the role of vitamin 'C' on X-irradiated epidermis of Guinea pigs under light microscope.

**Study Design:** A prospective experimental study.

**Place and Duration of Study:** This study was conducted at the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi 2008 to 2009.

**Materials and Methods:** Twenty seven animals were taken and were divided in to three groups. Each group was further subdivided into three subgroups containing three animals each according to the time of sacrifice i.e. 48 hours, 15<sup>th</sup> day and 45<sup>th</sup> day respectively. A single whole body x-irradiation in a dose of 5 Gy was given. Group C animals were also given injection of vitamin-C intraperitoneally in the dose of 1 mg/G/day. Animals were sacrificed under ether anaesthesia after completion of their respective periods. Tissues were processed and 4-5 micron thick paraffin embedded sections were cut and stained with Hematoxylin and eosin

**Results:** Decrease in epidermal thickness was observed in Group B1 animals. Which is highly significant ( $P < 0.001$ ) when compared to control. Results of animals of Group B2, and B3 were non significant ( $P > 0.05$ ) when compared to control. Non significant results were obtained when Group C was compared to control group.

**Conclusion:** X-irradiation damages the epidermis and vitamin 'C' minimizes the damaging effects.

**Key Words:** X-ray radiation, Gy, Epidermis, Vitamin 'C'.

## INTRODUCTION

Life on earth has evolved in the presence of ionizing radiation. The natural or background radiation is derived from three major sources, cosmic rays from sun and outer space, radium and other radioactive elements contained in the earth crust and Potassium-40 and other naturally occurring radionuclides (Anderson, 1990). The ever increasing use of radioactive substances both in industry and medicine has made the study of radiation damages, of great practical importance. On human body its effects vary from local tissue necrosis to genetic damage, cancer and death (Walter and Talbot, 1996). In humans (first of all skin) is exposed to the radiation during medical examination (Andronov et al 1988; Bardychev et al, 1982).

Use of nutritional factors in the reconstruction of radiation induced cell injury is an attractive proposition (Jagetia et al., 2003). Vitamin-A supplementation has been reported to ameliorate the acute radiation induced skin injuries (Gavenson et al., 1984). Vitamin-E treatment has also been reported to normalize the breaking strength of wounds that receive pre-operative radiation (Taren et al., 1987). Vitamin-C (a reactive oxygen scavenger) is considered to be a dietary radio-protective agent and acts as an anti-oxidant to protect DNA damage from ionizing radiation (Cai et al., 2001). L-ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>) is the trivial name of vitamin-C. The chemical name is 2-oxo-L-threo-Hexano-1, 4-lactone-2, 3-enediol L-ascorbic and

dehydro-ascorbic acid are the major dietary forms of vitamin-C (Moser and Bendian, 1990) The radio-protective effects of ascorbic acid seen to result from the interaction of ascorbic acid with radiation-induced free radicals (Duschesne et al., 1975).

The pre-treatment of mice with ascorbic acid improved the collagen synthesis, and increased vascularity and fibroblast density in radiated animals (Naidu, 2003). Furthermore, ascorbic acid has found to be a good anti-oxidant and radio-protective agent (Frie et al., 1989; Navas et al., 1994). Ascorbic acid treatment has been reported to confer protection against radiation in vitro and in vivo (Barerstock, 1979) and it has beneficial effects on the course of radiation-induced skin injuries (Decosse, 1988). Keeping in mind the above facts this study was planed to observe protective role vitamin C in relation to x-irradiation.

## MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 27 adult male Guinea pigs weighing 400 to 450 G were taken from Animal House. The animals were divided into three groups, A, B and C containing nine animals each and were further sub-divided into three sub-groups containing three animals each according to time of sacrifice, i.e. 48 hours, 15<sup>th</sup> day and 45<sup>th</sup> day. Group-A served as control. Group-B received whole body X-radiation in dose of 5 Gy at Karachi Institute of

Radiotherapy and Nuclear Medicine Karachi. Group-C received whole body X-radiation in dose of 5 Gy and injection of vitamin-C intraperitoneally in the dose of 1 mg/G/day. [Injection vitamin-C manufactured by Shanghai Medicines and Health Products Shanghai, China].

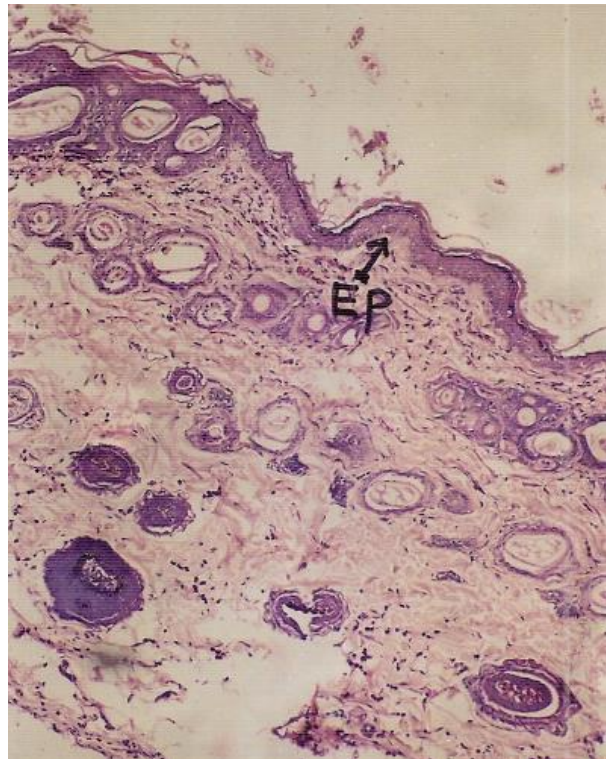
The animals (Guinea pigs) were sacrificed at the end of their respective period of treatment under the ether anaesthesia. The skins of guinea pigs were shaved and skin fragment (size one centimeter square in shape) from face, back and abdomen were collected. Skin fragment from each side was fixed in 10% formalin for 12-18 hours. After that tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin. Five micron thick vertical sections were cut at rotatory microtome and floated in hot water bath and placed on glass slide and stained with Hematoxylin and eosin for morphology of skin. The thickness of epidermis was measured with the help of ocular micrometer scale in 10x objective and 8x ocular under light microscope. The statistical significance of difference of various quantitative changes between treated and control Guinea pigs were evaluated by student's t' test. The difference was regarded as statistically significant, if the P-value was equal to or less than 0.05. All calculations were done by utilizing computer software SPSS through Microsoft Excel in Windows 2000 XP.

## RESULTS

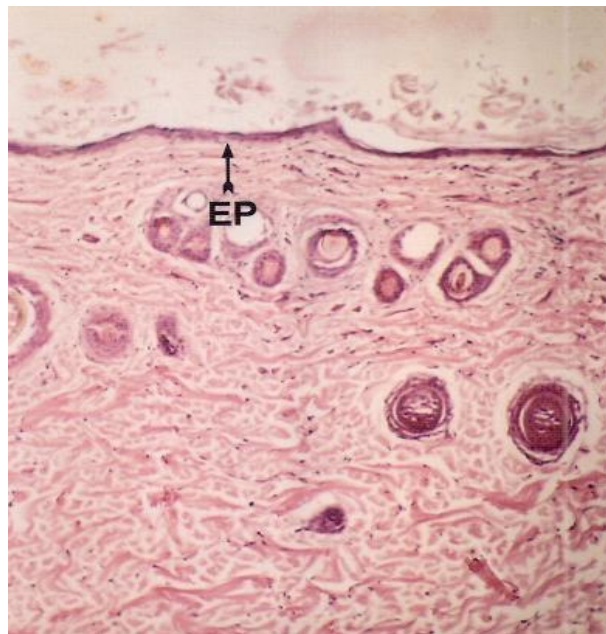
The epidermis of control group showed 5-6 varying layers of cells in addition to stratum corneum. All layer cells and their nuclei were visualized-Fig: 1. Mean thickness of epidermis was  $40.32 \pm 0.32 \mu\text{m}$ ,  $49.08 \pm 0.17 \mu\text{m}$  and  $58.04 \pm 0.29 \mu\text{m}$  in face, abdomen and back respectively.

The Group-B1 animals were ill looking. The skin appeared thin, dehydrated and hyperemic showing the patches of alopecia and cell layer were reduced to half with desquamation of surface epithelium Fig: 2 and 3. Mean thickness of epidermis was  $21.36 \pm 0.32 \mu\text{m}$ ,  $29.08 \pm 0.38 \mu\text{m}$  and  $29.80 \pm 0.38 \mu\text{m}$  in face, abdomen and back respectively. When compared with control results were highly significant ( $P < 0.001$ ). The skin of Group-B2 appeared thin and dehydrated with wide patches of alopecia. Epidermis showed reduced number of cell layers to half, but thickness of epidermis was near to control (due to hyperkeratinization) Fig.4, 5 and 6. Mean thickness of epidermis was  $39.92 \pm 0.26$ ,  $49.36 \pm 0.47$  and  $57.76 \pm 0.26$  in face, abdomen and back respectively. When compared with control results were insignificant ( $P > 0.05$ ). In Group B3 the hairs were very sparse but hyperemia of skin was diminished and numbers of cell layers were as near to control and keratinization was decreased. Mean thickness of epidermis was  $40.28 \pm 0.47 \mu\text{m}$ ,  $48.94 \pm 0.73 \mu\text{m}$  and  $57.76 \pm 0.26 \mu\text{m}$  in face, abdomen and back respectively.

When compared with control results were insignificant ( $P > 0.05$ ).



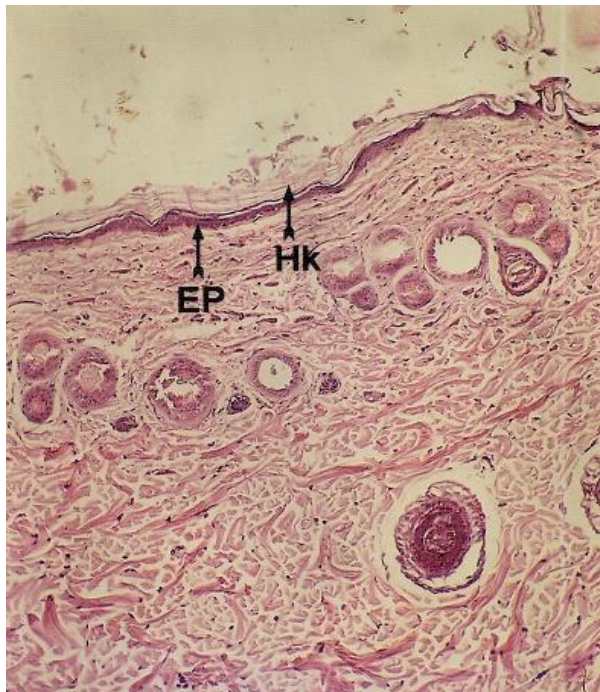
**Figure No. 1:** H&E stained 5µm thick longitudinal section of abdomen skin showing epidermal thickness (Ep) in control guinea pig. Photomicrograph X 100.



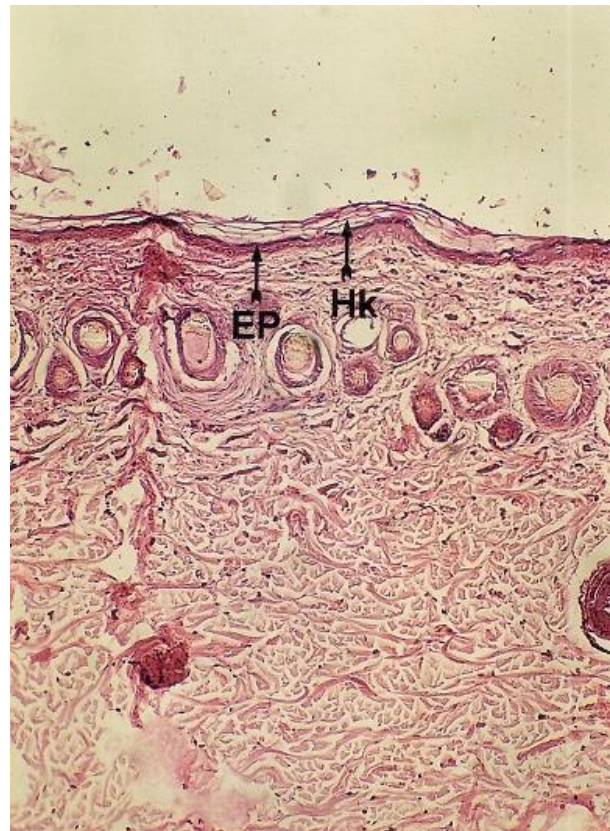
**Figure No.2:** H&E stained 5µm thick longitudinal section of abdomen skin showing desquamation and decreased thickness of epidermis (Ep) 48 hours after treatment with x-radiation in Guinea pig. Photomicrograph X 100.



**Figure No.3:** H&E stained 5µm thick longitudinal section of back skin showing desquamation and decreased thickness of epidermis (Ep) 48 hours after treatment with x-radiation in Guinea pig. Photomicrograph X 100.



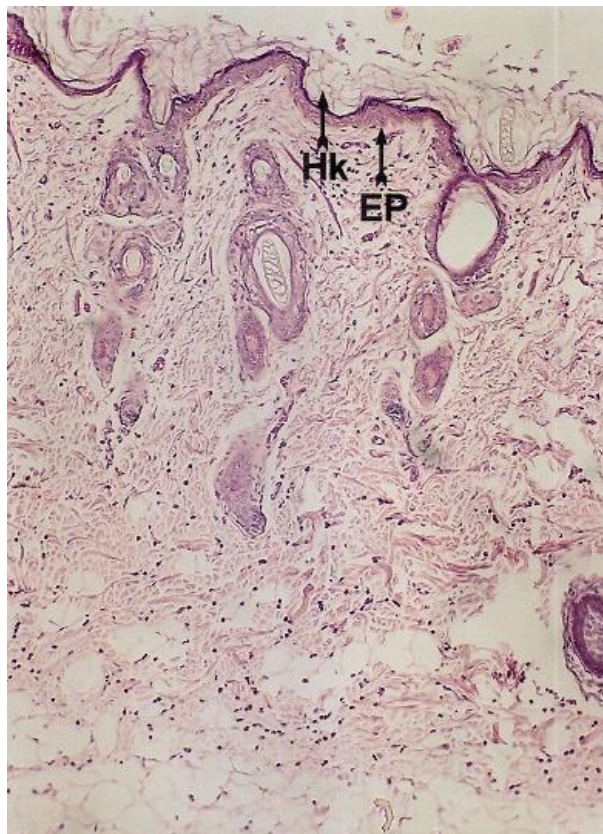
**Figure No.4:** H&E stained 5µm thick longitudinal section of face skin showing increase in thickness of epidermis (Ep) to hyperkeratinization (Hk) after 15 days treatment with x-radiation in Guinea pig. Photomicrograph X 100.



**Figure No.5:** H&E stained 5µm thick longitudinal section of abdomen skin showing increase in thickness of epidermis (Ep) due to hyperkeratinization (Hk) 15 days after treatment with x-radiation in Guinea pig. Photomicrograph X 100.

The Group C1 animals were ill looking, skin appeared thin and dehydrated showing patches of alopecia and hyperemia, number of cell layers were reduced to half and there was desquamation of surface epithelium. Mean thickness of epidermis was  $21.00 \pm 0.27$ ,  $29.68 \pm 0.42$  and  $38.04 \pm 0.52$  in face, abdomen and back respectively. When thickness of face and abdomen was compared to the control group the results are highly significant ( $P < 0.001$ ) and, insignificant ( $P > 0.05$ ) when compared with subgroup B1 but in case of thickness of epidermis of the back the results are highly significant when compared to control and subgroup B1. In gross examination of skin of Group C2 animals appeared thin and dry and hairs were very sparse. And epidermis showed increased number of cells layer near to control. Mean thickness of epidermis in face was  $40.00 \pm 0.64$ ,  $48.86 \pm 0.84$  and  $57.00 \pm 0.32$ . When compared to control Group and B2 insignificant ( $P > 0.05$ ) results were found. In gross examination of the skin of Group C3 animals at three sites was found thicker and dry with diminished hyperemia. Number of cell layers was increased near to control. The mean thickness of epidermis was  $39.88 \pm 0.66$ ,  $49.60 \pm 0.51$  and  $58.20 \pm 0.80$  in face, abdomen and back respectively. When

compared with control Group and B3 insignificant ( $P>0.05$ ) results were found.



**Figure No.6: H&E stained 5µm thick longitudinal section of back skin showing increase in thickness of epidermis (Ep) due to hyperkeratinization (Hk) 15 days after treatment with x-radiation in Guinea pig. Photomicrograph X 100.**

## DISCUSSION

The ever increasing use of radioactive substances both in industry and medicine has made the study of radiation damages of great practical importance. On human body its effects vary from local tissue necrosis to genetic damage, cancer and death (Walter and Talbot, 1996). X-ray irradiation (XRI) can affect both normal and neoplastic cell especially the rapidly growing one such as epidermal cells (Hussein et al., 2005).

Ascorbic acid is one of the important water soluble vitamin and natural anti-oxidant compound. It is expected to help in restricting the propagation of chain reaction initiated by free radicals which are produced either naturally or as a result of environmental factors (Shamberger et al., 1979). The previous studies are based on morphological x-rays induced skin changes. The present study was designed to observe the morphometric changes in skin of different locations induced by x-rays with role of vitamin-C in Guinea pigs.

In the present study, in gross examination in all the treated animals of groups-B and C, hair loss was seen in all three sites. The above findings were in agreement with Hopewell (1990) and Malkinson and Prasad (1995). They observed that loss of hair occurs after treatment with relatively low doses of radiation, indicating the hair follicles are highly sensitive to ionizing radiation.

Animals of groups-B1 and C1 with dose of 5 Gy radiation the thickness of epidermis in face, abdomen and back was reduced to half as compared with the control. This finding was in agreement with observation of Hussein et al (2005) who observe in their study that in x-ray irradiated skin the stratification of epidermis was reduced to one layer each of basal, spinous, and granular as well as few layer of corneocytes.

Increase in thickness of epidermis in face, abdomen and back of C2 and C3 was in agreement with the observation of Shamber (1979) who explained that vitamin-C, a natural anti-oxidant compound being anti-oxidant, it is expected to help in restricting the propagation of chain reaction initiated by free radicals.

## CONCLUSION

The study concludes that X-radiation produces damaging effects on the epidermis of skin and vitamin-C treatment has beneficial effects on irradiated epidermis of skin.

## REFERENCES

1. Anderson RE, Berthrong M, Fajardo LF. Radiation injury in Anderson's pathology. 10<sup>th</sup> ed. St. Louis: the CV Mosby Company; 1990.p.484-512.
2. Walter JB, Talbot IC. The effects of ionizing radiation. In: Walter and Israel general pathology. 7<sup>th</sup> ed. New York: Churchill Livingstone; 1996.p 557-68.
3. Jagetia GC, Rajanikant GK, Rao MK. Modulation of radiation induced delay in wound healing by ascorbic acid in mice exposed to different doses of hemi body gamma radiation. <http://www.medscape/viewarticle/464469>.2003. 10: 324-38.
4. Gavenson SM Gruber CA, Ruttura G, et al. Supplemental vitamin A prevents the acute radiation induced defects in wound healing. *Ann S* 1984; 200: 494-512.
5. Tarven DL, Chvapil M Weber CW. Increasing the breaking strength of wounds exposed to pre-operative irradiation using vitamin E supplementation. *Int J Vitam Nutr Res* 1987; 57:133-7.
6. Cai L, Koropathic J, Cherian MG. Roles of vitamin-C in DNA damage in presence and absence of copper. *Chem Biol Interaction* 2001; 137: 75-88. Moser U and Bendian A. Vitamin-C.

- In: LJ Maclin, editor. Hand book of vitamins. New York: Marcel Dekker;1990.
7. Duschesne J, Gillies R, Mosora F. Effect of antioxidant substances on the level of free organic radicals naturally present in the rat diaphragm. *Ascad Sci* 1975; 945-47.
  8. Naidu KA. Vitamin-C in human health and disease is a mystery? An overview. *Nutrition J* 2003;1-27.
  9. Frie B, England L, Ames BN. Ascorbic acid is an outstanding antioxidant in human plasma. *Proc Natl Acad Sci* 1989; 86: 6377-81.
  10. Navas P, Villalba JM, Corboda F. Ascorbate function as plasma membrane. *Biochem Biophys Acta* 1994; 1197: 3-14.
  11. Andronov AV, Krymskii VA, Labanov GV. *Med Radiol Besopasn* 1998; 43: 20-23.
  12. Bardychev MS, Peteric VD, Krasnov AS. *Med Radiol* 1982; 6: 7-11.
  13. Hussein MR, Eman E, Dief-Abu, Mohammad H, Raheem AE, Abdel-Rehman. Ultrastructural evaluation of the radioprotective effects of melatonin against x-ray induced skin damage in albino rats. *Int J Exp Path* 2005; 86: 45-55.
  14. Shamberger RJ, Corlitt CL, Beman KD, Kasten BL. Antioxidants reduce mutagenic effects melanoldehyde and B-propectone. *Mutation Res* 1979; 66: 349-55.
  15. Hopewell JW. The skin in its structure and response ionizing radiation. *Int J Radiat Biol* 1990; 57: 751-73.
  16. Malkinson FD. Some principles of radiology: A selective review. *J Invest Dermatol* 1981;77:32-38.
  17. Prasad KN. Hand book of radiology 2<sup>nd</sup> ed. New York: CRC Press; 1995.
  18. Baverstack KF. Radioprotection by vitamin-C. *Br J Radiol* 1979; 52: 593-3.
  19. Decosse JJ. Antioxidants. *Prog Clin Biol Res* 1988; 279: 131-4.

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