

# Topical Application of Curcumin Augments Healing of Deep Dermal Excision Wound of Mice Exposed To Whole-Body Gamma Radiation

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## Abstract

Exposures of wounds to ionizing radiations have been found to prolong the healing and also cause defective healing of irradiated wounds. Although, the descriptions of wound care techniques have been found in some of the oldest archeological findings, little attention has been given to capitalize on the conservative wound therapies in the treatment of irradiated wounds. Therefore, the present study was designed to enunciate the effect of topical application of 0.5, 2, 5 and 10 % curcumin ointment on wound contraction and mean wound healing time of excision skin wound in mice whole-body exposed to 6 Gy  $\gamma$ -radiations. The topical application of curcumin ointment increased the wound contraction and reduced mean wound healing time by 2.2 days in normal unirradiated wounds. The irradiation of mice to 6 Gy resulted in the retardation in healing of the wounds, whereas topical application of different concentrations of curcumin ointment resulted in a concentration dependent rise in the wound contraction at different post-irradiation times and a maximum wound contraction was detected in the wounds receiving topical application of 7% curcumin twice a day. A similar effect was observed in the mean wound healing time, which showed a reduction of 1.5 days for 5% curcumin ointment. Observations demonstrated that topical application of curcumin twice daily until complete healing of wound significantly improved contraction of irradiated wound and decreased the mean healing time.

**Keywords:** Mice,  $\gamma$ -radiation, Curcumin, Wound contraction, Mean wound healing time.

## Introduction

The ionizing radiations interact with biological tissues by producing free radicals in the aqueous milieu [1]. The free radicals produced by ionizing radiations trigger a cascade of events by interacting with various important biomolecules that could be detrimental to the exposed individuals depending on the irradiation dose [2,3]. The burst of free radicals generated by ionizing radiation has also been found to retard the progress of regenerating irradiated wounds [2,4-5]. Skin is an important tissue which protects the individuals from onslaught of external physical or chemical agents and any negative effect produced on the skin could be detrimental. The skin is the first tissue to bear the brunt of accidental or intentional exposure to ionizing radiation [6,7]. During nuclear accidents humans will be exposed not only to ionizing radiations but also to a host of other agents including heat. The exposure of skin to ionizing radiations along with other injuries lead to a synergistic interaction, which results in greater morbidity and mortality than either treatment alone [2,8-9].

The acute radiation exposure associated with combined injuries in the form of superimposed skin wounds and/or burn injury, produces

serious clinical problems, since ionizing radiation disrupts normal response to injury, leading to a protracted recovery period [4,10-12]. Exposure of wound to ionizing radiations has been reported to alter the local conditions of wound, resulting in a delay in wound contraction, which retards wound repair and regeneration and eventually have negative effect on the healing process [4,5,11,13-15].

Wound healing involves a sequence of well-orchestrated biochemical and cellular events leading to growth and regeneration of wounded tissue in a specific manner [16,17]. Wound injuries that are benign by themselves may become lethal when combined even with relatively small dosages of irradiation [4,5,14,15,18-20]. Though, the descriptions of wound care techniques have been found in some of the oldest archeological findings, little attention has been given to capitalize on conservative wound therapies in the treatment of chronic radiation wounds [3,5,14,15,21,22]. The use of herbal antioxidants to manipulate the redox environment and reconstruction of irradiated wounds is an attractive proposition, as they have wide acceptability, better tolerance, and no side effects, economical and also can be safely manipulated for human use [23].

Curcumin or diferuloylmethane (1,7-bis (4-hydroxy-3-methoxy-phenyl) hepta-1, 6-diene-3, 5-dione), is an active phenol compound

present in the curry spice, the turmeric (*Curcuma longa* Linn). It is a well-known culinary agent, which is extensively used as food additive to impart yellow color. Curcumin has been reported to be effective in wound repair in normal and diabetic impaired wound healing, both orally and topically [24-26]. Topical application of curcumin differentially regulated TGF-beta1, its receptors and nitric oxide synthase in dexamethasone-impaired cutaneous healing in a full thickness punch wound model in rats [25]. Curcumin has been demonstrated to offer cytoprotection against oxidative stress [26].

Phan et al. demonstrated that curcumin possess inhibitory activity against hydrogen peroxide induced oxidative damage in human keratinocytes and fibroblasts [27]. Curcumin has been ascertained to have potent antioxidant and free radical scavenging activity [28-32]. Curcumin possesses antibacterial, anti-inflammatory, anticancer, antiviral, chemo preventive, wound healing, and radio sensitizing activities [4,19,33-37]. Curcumin treatment has also been reported to confer protection against radiation-induced damage in vitro and in vivo [33,35,38-40]. The other attractive feature of curcumin to explore, as a vulnerary agent is that it is non-toxic and being consumed daily for centuries in Asian countries and it has been found to be non-toxic up to a dose of 8-12 g curcumin/day in phase-I human clinical trials [41,42]. Earlier studies from this laboratory have shown that curcumin helps to accelerate healing of irradiated wounds [4,19]. However, the effect of topical application of curcumin on irradiated wound has not been studied. Therefore, the present study was designed to enunciate the effect of topical application of curcumin on wound contraction and mean healing time in excision skin wound in mice whole-body exposed to 6 Gy  $\gamma$ -radiations.

## Materials and Methods

The handling and care of animals were carried out according to the guidelines issued by the World Health Organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi). Eight to ten-weeks old Swiss albino mice of both sexes (1:1 ratio) weighing 30 to 36 g were selected from an inbred colony maintained under the controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and light (14 and 10 hrs of light and dark, respectively). The animals were allowed free access to sterile food and water. The food consisted of 70% cracked wheat, 40% Bengal gram, 4% milk powder, 4% yeast powder, 0.75% sesame oil, 0.25% cod liver oil, and 1% salt. Four animals were housed in a sterile polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The study was approved by the institutional animal ethical committee of Kasturba Medical College, Manipal, India.

## Preparation of Drug and Mode of Administration

Various concentrations of curcumin ointment were prepared by Hospital Pharmacy of Kasturba Medical College, Manipal. The animals were applied topically with different concentrations of the curcumin ointment, twice a day, on the deep dermal excision wound after exposure to 0 or 6 Gy radiations until complete healing of the wounds.

## Experimental Protocol

**Effect of various concentrations of curcumin ointment on wound contraction:** The animals were divided into following groups: **ONT+ Sham-irradiation:** The animals of this group received only ointment base (ONT) topically on the wound, twice a day, until complete healing of wounds, after sham-irradiation (0 Gy).

**CUM+ Sham-irradiation:** The animals of this group received 0.5, 2, 5 and 10 % of curcumin ointment (CUM) topically on the wound, twice a day, until complete healing of wounds, after sham-irradiation (0 Gy).

**ONT+ Irradiation:** The animals of this group received only ointment base topically on the wound, twice daily, after exposure to 6 Gy radiation until complete healing of wounds.

**CUM+ Irradiation:** The animals of this group received 0.5, 2, 5 and 10 % of curcumin ointment topically on the wound, twice daily, after exposure to 6 Gy radiation until complete healing of wounds.

## Irradiation

Each prostrate and unanesthetized animal was placed into the individual chamber of a specially designed well-ventilated acrylic restrainer and whole-body of the animals were exposed to 0 or 6 Gy, given at a dose rate of 1.35 Gy/min from a  $^{60}\text{Co}$  Teletherapy source (Theratron, Atomic Energy Agency, Ontario, Canada).

## Production of Full-Thickness Skin Wound

The fur of the dorsum of each animal was removed with a cordless electric mouse clipper (Wahl Clipper Corporation, Illinois, USA) before exposure to  $\gamma$ -radiation and a full-thickness skin wound was produced on the dorsum (below the rib cage) as described earlier [14]. Briefly, the animals were anesthetized and the entire body was cleaned and decontaminated by wiping with sterillum (Bode Chemie, Hamburg, Germany) disinfectant solution. The cleared dorsal surface of skin was marked with a sterile circular (15-mm-diameter) stainless steel stencil. A full-thickness wound was created by excising the skin including *panniculus carnosus* in an aseptic environment using sterile scissors and forceps. Each wounded animal was housed in a separate sterile polypropylene cage. Immediately after irradiation of wound, animals were applied twice daily with either ointment base or various concentrations of curcumin ointment, depending upon the experimental group.

## Wound Contraction

Wound contraction was monitored by capturing the video images of each full-thickness wound with a CCD camera connected to a computer. The first image of each wound from different groups was obtained one day after wounding, and that day was considered as day one. The subsequent images were captured on days 3, 6, 9, 12 and 15 post-wounding. The wound area was calculated using Auto CAD R14 (Autodesk Inc., San Rafael, CA) software. Eight animals were used in each group.

## Mean wound healing time

A separate experiment was performed to evaluate the effect of topical application of curcumin ointment on mean healing time after exposure to 0 or 6 Gy whole-body  $\gamma$ -radiations, where grouping and other conditions were similar to that described above. All animals of each group were monitored until complete healing of wounds and the day at which each wound healed was recorded. Mean of all healed wounds was determined and has been expressed as mean wound healing time in days. Eight animals were used in each group at each exposure dose.

## Analysis of Data

Statistical significance between the treatments was determined using one way ANOVA. The Solo 4 Statistical Package (BMDP Statistical Software Inc., Los Angeles, CA, USA) was used for data analysis. All data are expressed as mean  $\pm$  SEM (Standard error of mean).

## Results

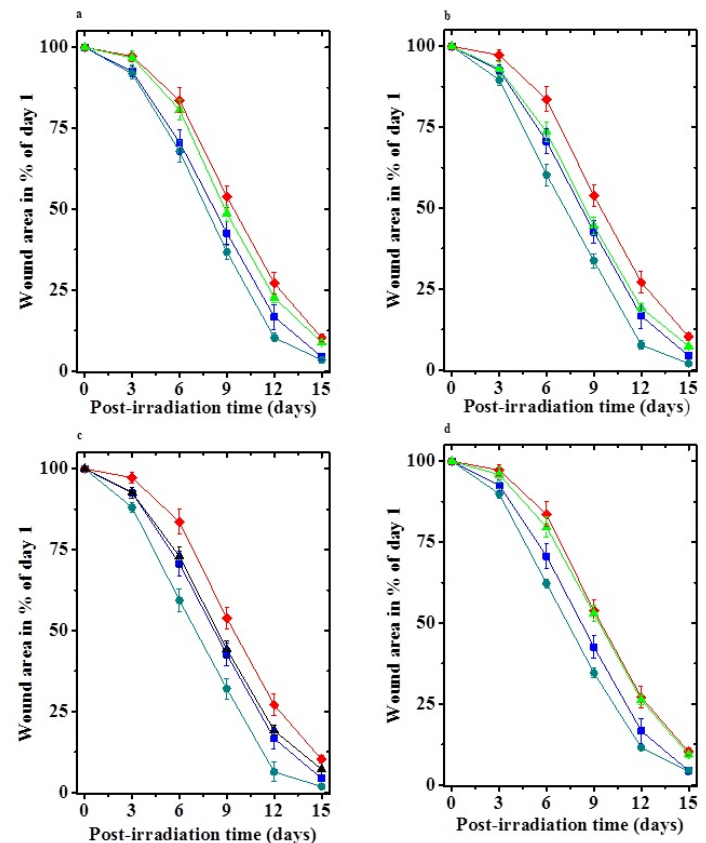
The results obtained from the experiments are depicted in Figure 1 and 2.

### Effect of various concentrations of curcumin ointment on wound contraction

Treatment of mice with ONT or various concentration of curcumin base resulted in a steady contraction of excision wounds with time in both ONT+ Sham-irradiation and CUM+ Sham-irradiation groups (0 Gy) (Figure 1). The topical application of 0.5, 2, 5 and 10 % of curcumin enhanced the wound contraction in a concentration dependent manner in CUM+ Sham-irradiation group at various post-irradiation times (Figure 1), and a significant enhancement of wound contraction was recorded at day 3 ( $p < 0.05$ ), 6 ( $p < 0.05$ ) and 9 ( $p < 0.05$ ) post-irradiation only after application of 5% curcumin ointment, when compared with ONT+ Sham-irradiation group (Figure 1c). Curcumin treatment also showed a progressive reduction in the scab formation with increasing dose of curcumin and the scab formation was almost absent in the wound receiving the topical application of 5% curcumin ointment in CUM+ sham-irradiation group.

The whole body irradiation of mice to 6 Gy resulted in a thick scab formation in ONT+ Irradiation group accompanied by a significant delay in wound contraction when compared with ONT+ Sham-irradiation group at all post-irradiation days (Figure 1). Topical application of animals with various doses of curcumin ointment led to a thin scab formation and early fall of scab. The scab formation was relatively less in the animals treated with 5 % curcumin ointment twice daily after 6 Gy irradiation, until complete healing of wounds in CUM+ Irradiation group. The topical application of different concentrations of curcumin ointment resulted in a dose dependent elevation in the wound contraction up to 5%, where a maximum wound contraction was observed in the CUM+ Irradiation group when compared to ONT+ Irradiation group (Figure 1). The wound contraction was significantly enhanced at 3 ( $p < 0.05$ ), 6 ( $p < 0.05$ ) and 9 ( $p < 0.05$ ) day post-irradiation in the animals treated with 5 % curcumin (Figure 1c). While 0.5, 2 and

10 % curcumin did not significantly alter the contraction of wound when compared to ONT+ irradiation group (Figure 1).



**Figure 1:** Effect of various concentrations of topical application of curcumin on the contraction of excision wound in mice exposed to 6 Gy whole-body  $\gamma$  radiation. a) 0.5, b) 2, c) 5 and d) 10 % of curcumin applied twice daily. Squares: Ointment base +Sham-irradiation; Circles: Curcumin+ Sham-irradiation; Diamonds: Ointment base +Irradiation and Triangles: Curcumin+ Irradiation.

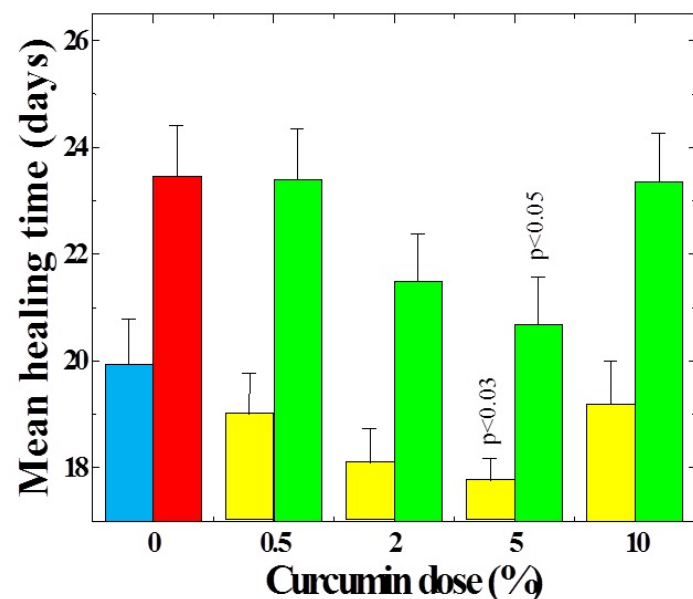
## Mean wound healing time

The complete closure of wounds was observed by day 20 post-irradiation in ONT+ Sham-irradiation group (0 Gy). However, treatment of mice with 0.5 and 2 % curcumin concentration did not alter the mean healing time significantly when compared with the ONT+ Sham-irradiation group (Figure 2). The mean healing time was shortest (17.8 day post-irradiation) for 5 % curcumin concentration when compared with other doses in the CUM+ Sham-irradiation group, whereas further increase in drug dose did not alter this pattern significantly (Figure 2).

The whole-body exposure of mice to 6 Gy  $\gamma$ -radiation resulted in a significant delay in the complete closure of wounds, which led to a rise in the mean wound healing time by 3.5 days (23.5 day post-irradiation) in ONT+ Irradiation group when compared with the ONT+ Sham-irradiation group (20 day post-irradiation) (Figure 2). The treatment of animals with 5 % curcumin resulted in an early healing of wound and the complete healing of wound was effected by 21 day post-irradiation in CUM+ Irradiation



group when compared with Ointment+Irradiation, whereas topical application of 0.5, 2 and 10 % curcumin did not alter the mean wound healing time significantly (Figure 2).



**Figure 2:** Effect of topical application of various concentrations of curcumin on the mean wound healing time in mice exposed to 6 Gy whole-body  $\gamma$ -radiation.  $p < 0.05$  when Ointment base + Sham-irradiation group are compared to curcumin groups. **Blue Colour:** Ointment base + Sham-irradiation; **Yellow colour:** Curcumin+ Sham-irradiation; **Red colour:** Ointment base + Irradiation and **Green colour:** Curcumin + Irradiation.

## Discussion

Polyphenolic phytochemicals are synthesized by plants as secondary metabolites for various purposes [43]. The secondary metabolites derived from plants have attracted the attention of researchers and many medicinal aspects have been investigated. However, little attention has been given to the effects of economic, non-toxic herbal products/active principles on the radiation response of healing wounds, which accentuate a need for continued research in the area of medical management of radiation-impaired wounds. Curcumin was isolated from turmeric as early as 1815 [44]. However it has been used in India for various purposes since Vedic times in the form of turmeric. It is commonly used to treat wounds, cough and cold and several other conditions in India and other Asian countries. Curcumin has been reported to be effective in wound repair in normal and diabetic impaired healing, both orally and topically [24-26]. The topical application of curcumin has been also reported to heal burn wounds in rats [45]. Its non-toxic nature, pleiotropic action and the crucial practical importance of combined injuries in healthcare stimulated us to conceptualize the present study and investigate the efficacy of topical application of various concentrations of curcumin in the skin of mice exposed to 6 Gy whole-body  $\gamma$ - radiation and stressed with an additional trauma as an open deep dermal excision wound.

The wound contraction measurement in experimental conditions is the best parameter to measure the progress of healing of regenerating wounds. This method allows the precise evaluation

of regeneration in healing wounds due to development of new tissues that contribute for shrinkage of wound leading to its complete closure [4,14,15,19]. The capturing of video images periodically allows monitoring wound contraction of regenerating wounds accurately [4,19]. The wound contraction is the centripetal movement of the edges of a full thickness wound that leads to attrition in the size of wound and closure of the wound [46,47]. Irradiation of excision wound to 6 Gy retarded the closure of regenerating wound in the present study. Likewise, irradiation has been known to delay the closure of wound due to reduced contraction and increase the wound healing time significantly in mice earlier [4,5,9,11,14,15,18,19]. The wound contraction is directly related to the function of contractile fibroblasts, the myofibroblasts as they secrete and organize extracellular matrix in regenerating wounds [48-50]. The delay in wound contraction may be due to the radiation-induced inhibition of inflammatory reactions, fibroblast and connective tissue proliferation, formation and maturation of granulation tissue in the present study [2,5,12,19,51]. Other possibility includes a delay in fixation of wound edge to the underlying tissue after irradiation, due to the lack of fibroblast proliferation and a decrease in fibroblast synthetic function in the granulation bed. The irradiation has been reported to alleviate DNA synthesis and vasculature in the granulation tissue [4,5,9,19]. Irradiation is thought to impair wound healing in skin through its cytotoxic effect on fibroblasts [4,9,19,52]. This impairment may be due to the delay in the progression of cells through the cell cycle induced by radiation [51,53]. Radiation has adverse effects on fibroblasts and endothelial cells through bone marrow depression, since radiation has been reported to diminish hematopoiesis in a dose- and time-dependent manner [54,55]. This may have delayed wound contraction and increased mean wound healing time.

Numerous earlier investigations have reported beneficial effects of curcumin on wound healing [24-26]. Our earlier studies reported that oral administration of curcumin increased wound contraction, collagen deposition and improved fibroblast and vascular densities in mice exposed to different doses of whole-body, hemi-body or fractionated irradiation [4,9,19]. Similarly, topical application of curcumin twice daily may also have provided strength to the regenerating wound by increasing cellular proliferation, collagen deposition and vascular supply, causing early closure of the wound in the CUM+ irradiation group. Numerous studies have reported the beneficial effect of curcumin on wound healing through changes in cell regeneration, collagen and DNA synthesis [4,9,19,24-26]. Topical application of low molecular weight hyaluronic acid has been reported to prevent oxygen free radical damage to granulation tissue during wound healing [56]. Similarly, inhibition of the delay in contraction of irradiated wounds by curcumin may be due to its antioxidant, free radical scavenging and radio protective activities [28-30,34,35,38-40].

The exact mechanism of acceleration of wound repair and regeneration after topical application of curcumin is not known. However, there are a multitude of possibilities by which curcumin may have enhanced the regeneration of irradiated wound. The

curcumin may have reduced radiation-induced free radicals and lipid per oxidation in the granulation bed of the irradiated wound inhibiting the deleterious changes triggered by the increased oxidative stress. Our earlier study has shown that curcumin reduced radiation-induced lipid per oxidation accompanied by a rise in the activities of glutathione peroxides and superoxide dismutase and increased the glutathione concentration in the irradiated wounds [4,33]. The increased wound contraction may be due to stimulation of collagen synthesis in the irradiated wound as reported earlier [4,9,19]. The curcumin has been reported to increase nitric oxide, which may have neutralized the negative effect of radiation on inflammatory responses, essential for wound healing [4,9,19]. The deposition of fibrin stimulated in response to injury immediately after wounding and its degradation is indispensable for migration of various cells in the wound bed for proper wound repair. The application of curcumin may have hastened the fibrinolytic activity in the irradiated wound causing enhanced repair and reduced healing time when compared to non-curcumin treated irradiated wounds. A similar action of curcumin has been reported during wound healing earlier [57]. Curcumin may have stimulated molecular mechanisms to enhance wound contraction. The irradiation and wounding have been known to trigger activation of NF- $\kappa$ B, COX-II and LOX, which have negative effect on wound healing [2,58-60]. The topical application of curcumin may have suppressed the activation of these genes leading to enhanced repair of the irradiated wound. The vasculoendothelial growth factor (VEGF) plays a crucial role during early periods of wound healing and radiation is known to suppress angiogenesis and VEGF that may be also one of the reasons of delayed wound healing [61]. Curcumin application may have up regulated the VEGF promoting angiogenesis and reduced the radiation-induced retardation in wound healing. Our earlier studies have shown that curcumin treatment increased blood vessel formation in the irradiated wounds [4,9,19]. The curcumin has been reported to block the activation of NF- $\kappa$ B, TNF- $\alpha$ , COX-II and LOX, which have negative effect of wound healing [62]. The curcumin stimulates antioxidant mechanisms by up regulating Nrf2 thereby reducing the radiation-induced oxidative stress, which may have led to early repair of excision wound in the present study [63].

Life-threatening doses of acute total body radiation are so infrequently encountered that management policies must be derived in part from different but analogous clinical situations and/or from studies in experimental animals. Therefore, present study attempted to explore the usefulness of curcumin on the healing of full thickness skin wounds in mice, which were whole-body exposed to  $\gamma$ - radiation. Observations demonstrated that topical application of curcumin twice daily until complete healing of wound significantly improved contraction of irradiated wound and decreased the mean healing time. The possible mechanisms of action of curcumin may be free radical scavenging, increased antioxidant status, inhibition of NF- $\kappa$ B, TNF- $\alpha$ , COX-II and LOX and up regulation of Nrf2.

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