

Quercetin accelerated cutaneous wound healing in rats by increasing levels of VEGF and TGF- β 1

A Gopalakrishnan, M Ram, S Kumawat, SK Tandan & D Kumar*

Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar 243 122, Uttar Pradesh, India

Received 04 June 2014; revised 12 June 2015

Quercetin (3,3',4',5,7-penthydroxyflavone)-induced biological effects have been beneficial in various disease conditions. In this study, wound healing potential of quercetin was evaluated in a time-dependent manner in open excision wounds in adult Wistar rats. Experimentally-wounded rats were divided into two groups namely, control and quercetin-treated. Wounds were photographed and the area was measured on the day of wounding and on days 3, 7, 11 and 14 post-wounding. The granulation/healing tissue was collected on days 3, 7, 11 and 14 post-wounding for cytokine/growth factor measurements and histology/immunohistochemistry studies. There was significant time-dependent increase in wound closure in quercetin-treated rats. Vascular endothelial growth factor and transforming growth factor- β ₁ expressions were significantly upregulated in quercetin-treated rats, whereas tumor necrosis factor- α level was markedly reduced. Interleukin-10 levels and CD31 stained vessels were markedly higher on day 3 and on day 7, respectively, in quercetin-treated rats. In H & E stained sections, quercetin-treated group showed less inflammatory cells, more fibroblast proliferation, increased microvessel density, better reepithelialization and more regular collagen deposition, as compared to control. The results suggest that topical application of quercetin promotes wound healing by effectively modulating the cytokines, growth factors and cells involved in inflammatory and proliferative phases of healing.

Keywords: Cytokines, Endothelial cells, Growth factors, Interleukin

Wound healing is a complex process involving an orderly progression of highly regulated series of biological events¹. Cascades of cellular and molecular processes are set in with concomitant involvement of cytokines, growth factors and proteases to complete normal tissue repair after damage². The cytokines and growth factors are useful candidates and markers for determination of wound vitality or age³. Optimum healing of a cutaneous wound requires a well orchestrated integration of the complex biological and molecular events⁴. Significant social and physical constraints associated with wounds urge for newer drugs/formulations that accelerate the healing process. Flavonoids, a large group of phytochemicals, possess various known beneficial effects linked to health including antioxidant and free radical scavenging effects⁵⁻¹⁰. Studies have shown that topical application of compounds with free radical scavenging properties, significantly improve healing of cutaneous wounds¹¹. The wound healing

potential of several plant extracts are known to be associated with flavonoids, present in them¹².

Quercetin (3,3',4',5,7-penthydroxyflavone), the most abundant dietary flavonoid, possesses adaptogenic/antistress¹³, anticancer¹⁴, antidiabetic¹⁵, anti-inflammatory^{14,16}, antimicrobial^{6,17}, atimutagenic¹⁸, atinociceptive¹⁹, antioxidant^{5,10,15,18}, antiulcer²⁰, cardioprotective^{21,22}, cytoprotective^{23,24}, hepatoprotective^{25,26}, hypolipidemic^{10,15,25}, neuroprotective²⁷ and vasoprotective²⁸ effects and application in pain management, particularly thermal hyperalgesia and cold allodynia^{19,29}. It exhibits considerably high antiradical property³⁰. It is also known to attenuate pesticide toxicity³¹. Natural sources of quercetin ('quercus' means oak) include oak, red grapes, apples, onions, green tea, citrus fruits and other leafy green vegetables^{24,32}. Chouhan and Flora²⁴ have also, in their review, discussed the potential benefits of quercetin including arsenic and fluoride poisoning. It is regarded as a useful therapeutic agent for colitis and gastric ulcer since it potentially stimulates gastric epithelial proliferation³³ and has been suggested as an effective promising new treatment for healing common mouth ulcers³⁴. In a preliminary study, application of quercetin-incorporated collagen

*Correspondence:

Fax: +91 581 2303284

E-mail: dineshks17@ivri.res.in; dineshks17@gmail.com

increased hydroxyl-proline contents in dermal excisional wounds in rats³⁵ and in bone regeneration in rabbits³⁶. Vascular endothelial growth factor (VEGF), was upregulated by quercetin via activating the key transcription factor of VEGF gene, the hypoxia inducible factor-1 α (HIF-1 α)³⁷.

In view of the above, here we evaluated time (phase)-dependent wound healing potential of quercetin in excisional wound model in rats. Wound area was measured on different days post-wounding and major cytokines and growth factors that participate in wound healing were measured to support gross healing effects of quercetin.

Materials and Methods

Animals

Healthy adult male Wistar rats (150-170 g) were procured from the Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar (UP), India. The experimental protocols were approved by the Institutional Animal Ethics Committee (number: F.1-53/2012-13-J.D. (Res), 10th September 2012) and conforms to the guidelines for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Drug preparation

Quercetin (Q 4951-10G, $\geq 98\%$ HPLC solid) purchased from Sigma-Aldrich, USA was used to prepare 0.1% quercetin ointment in soft paraffin.

Creation of excision wound

The rats were anaesthetized using sodium pentobarbital (40 mg/kg i.p.) and a 2x2 cm full thickness wound up to the depth of loose subcutaneous tissue was created on the dorsal thoracic region of rats. The wounded rats were divided into two groups, *viz.*, control and treatment, of 20 each, and housed in individual cages. In control group, soft paraffin and in treatment group, 0.1% quercetin ointment were topically applied twice daily for 14 days. Positive control group was not used as universally accepted standard wound healing agent was not available.

Wound area measurement and photography

Margins of the wounds were traced on a transparent sheet using a fine tip marker pen. The area (mm²) within the boundaries of each tracing was determined planimetrically starting from the day of creation of wound and on days 3, 7, 11 and 14 post wounding. Along with area measurement, photograph of each wound was taken.

Tissue harvesting

Five animals from each group were sacrificed on days 3, 7, 11 and 14 with an overdose of diethyl ether and the granulation/healing tissue was carefully excised out. The tissue collected was immediately divided into three portions. Tissue homogenate was prepared with one portion in ice-cold lysis buffer and centrifuged at 12000 rpm for 10 min at 4°C. The supernatant obtained was aliquoted and stored at -80°C till further processing for Western blotting and ELISA. Second portion was stored in RNA stabilization reagent (RNAlaterTM, Qiagen, USA) at -20°C for real time-PCR studies. The third portion was preserved in 10% neutral buffer formalin for histological evaluation and for immunohistochemical studies.

mRNA expression studies (Real-time RT-PCR)

Total RNA was isolated from the granulation tissue using the standard method described by Amresco, USA with RibozolTM RNA extraction reagents and cDNA was synthesized using cDNA synthesis kit (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit), as per standard protocol. The real time PCR assay was performed by using 2x SYBR Green master mix Universal (KAPA BIOSYSTEMS, USA) in CFX96 real time PCR DET SYS (C-1000 thermal cycler, BIO-RAD laboratories India). The following thermal cycling profile was used (40 cycles): initial incubation at 95°C for 3 min followed by 95°C for 3 s, 59-62°C (depending on primer) for 30 s and 72°C for 30 s. The $\Delta\Delta CT$ method of relative quantification was used to determine fold change in gene expression and was obtained as $2^{-\Delta\Delta CT}$ ³⁸.

SDS-PAGE and Western blot analysis

The expression levels of VEGF and TGF- β_1 were determined by Western blot analysis. Equal protein concentrates of tissue homogenates were resolved by 15% SDS polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride (PVDF) membrane at 30V for 1 h 45 min. The membranes were blocked in 3% BSA for 1 h at 37°C followed by overnight incubation at 4°C with the respective primary antibodies separately: goat polyclonal antibodies of VEGF & TGF- β_1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:500 dilutions) and mouse monoclonal antibody of β -actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:1000 dilutions). After washing with Phosphate Buffered Saline with Tween 20 (PBS-T), blots were incubated with secondary antibody; horseradish

peroxidase (HRP)-conjugated chicken anti-goat IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:1000 dilutions) for VEGF & TGF- β_1 and with goat anti-mouse IgG (1:1000 dilutions) for β -actin, at 37°C for 2 h. Following successive washes, the blots were developed using the 3, 3'-Diaminobenzidine (DAB) system (GeNei, Bangalore, India). The membranes were subsequently scanned and band intensity was quantified using densitometry software (Image J, NIH). The western blot data for VEGF and TGF- β_1 were corrected for corresponding β -actin values and the results were expressed as normalised protein levels. A minimum of three such blots were performed for every protein analyzed.

ELISA for IL-10 and TNF- α

ELISA assay of the tissue lysate was conducted for TNF- α and IL-10 as per the manufacturer's instructions (Abcam UK, Catalogue Nos. ab100785 for TNF- α rat ELISA kit, and ab100764 for IL-10 rat ELISA kit).

Histological study by haematoxylin and eosin (H&E) staining

About 5 μ m thick tissue sections were stained with H & E as per standard method and visualized under light microscope at magnifications of 10x and 40x and photographed. Inflammatory cell infiltration and fibroblast proliferation in the granulation tissue sections of two groups of rats on various days were assessed by using the 0-4 numerical scale method described earlier³⁹. Ten random fields (40x) from three stained sections (at least two field from each section) of each group were scored. The scores assigned were 0 for absence, 1 for occasional presence, 2 for light scattering, 3 for abundance and 4 for confluence of cells.

Immunohistochemistry for CD31

On the 7th day, skin sections were immunolabeled for CD31 marker for angiogenesis, by incubating with primary mouse monoclonal CD31 antibody (Novus Biologicals, Littleton, CO, USA) and HRP-conjugated goat anti mouse IgG (Santa Cruz Biotechnology). The aminoethylcarbazole (AEC) was used as chromogen substrate (AEC Staining Kit; Sigma-Aldrich, USA). Microphotographs were captured under light microscope at 10x magnification. Ten random fields were observed (40x) for the semi-quantitative analysis of microvessel density (MVD).

Statistical analysis

Results are expressed as mean \pm standard error (SE) with 'n' equal to number of animals. The statistical significance between the experimental and control

values was analyzed by applying unpaired 't' test using the Graphpad Prism v4.03 software program (San Diego, CA, USA), and the differences were considered statistically significant at $P < 0.05$ or lower.

Results

The representative photographs of wounds of the two groups of rats taken on days 0, 3, 7, 11 and 14 post-wounding, are given in Fig. 1. The absolute wound surface area of two groups calculated on respective days are presented in table 1. The reduction in wound surface area was in a time-dependent manner in both the groups. On 7th day onwards, both the groups showed an increased reduction in wound area wherein wound area was significantly ($P < 0.05$) lower in quercetin-treated group (165.80 \pm 10.28) as compared to the control (203.70 \pm 11.78). The absolute wound area in quercetin-treated group remained significantly lower till 14th day post-wounding (22.14 \pm 1.59), as compared to control (33.69 \pm 2.06).

The relative VEGF mRNA expression in response to quercetin treatment is presented in Fig. 2A. The



Fig. 1—Photographs of wounds of (A) soft paraffin- (control); and (B) quercetin-treated rats on days 0, 3, 7, 11 and 14 post-wounding. Data are expressed as mean \pm SE, n= 5; * $P < 0.05$; ** $P < 0.01$ vs. control on respective day.

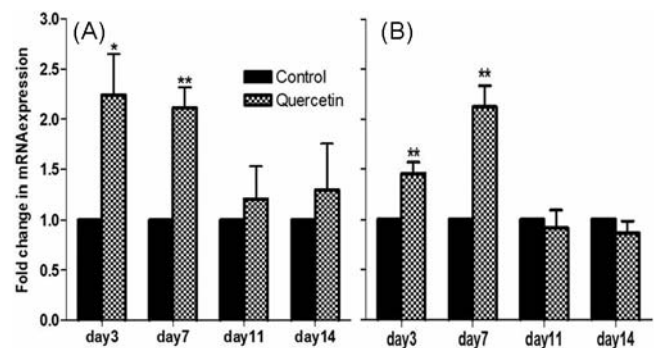


Fig. 2—Relative/fold changes in mRNA expressions of VEGF (A); and TGF- β_1 (B) in quercetin-treated wounds in comparison to control. The mRNA levels were normalized with β actin of respective samples. Data are expressed as mean \pm SE, n= 3; * $P < 0.05$; ** $P < 0.01$ vs. control on respective day.

expression of VEGF was upregulated significantly ($P < 0.05$) in quercetin group to approximately 2.24 ± 0.41 fold on 3rd day and 2.11 ± 0.20 fold on 7th day, as compared to control. The relative TGF- β_1 mRNA expression in response to quercetin treatment, as compared to the control group is presented in the Fig. 2B. There was significant upregulation of TGF- β_1 mRNA in wounds treated with quercetin, approximately 1.45 ± 0.11 and 2.12 ± 0.21 fold compared to control group on day 3 and 7, respectively.

The representative Western blots of β actin, VEGF and TGF- β_1 are presented in Fig. 3A. The band intensity of VEGF blot was more on days 3 and 7 in quercetin-treated group, as compared to that of

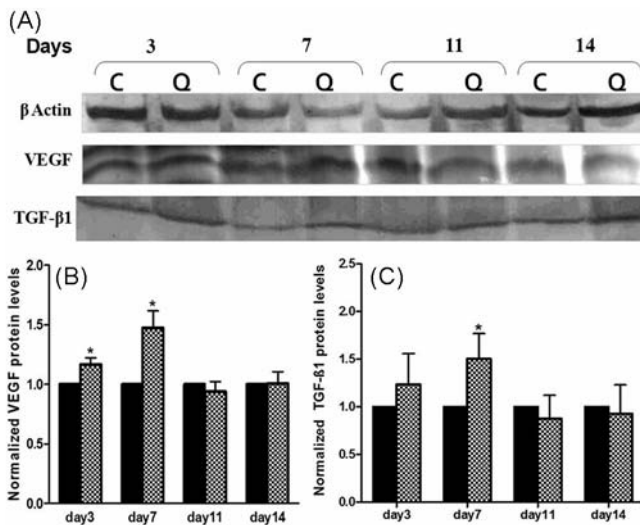


Fig. 3—(A) Representative Western blots of β actin, VEGF and TGF- β_1 showing the expression of the proteins on days 3, 7, 11 and 14 in both the groups. Relative expressions of VEGF (B); and TGF- β_1 (C) protein in quercetin-treated wounds in comparison to control. The mRNA and protein levels were normalized with β actin of respective samples. Data are expressed as mean \pm SE, $n = 3$; * $P < 0.05$; ** $P < 0.01$ vs. control on respective day.

control. The VEGF protein expression was significantly increased by 1.17 ± 0.05 fold on day 3 in quercetin treated group (Fig. 3B) and on day 7, 1.41 ± 0.14 fold increase noted in quercetin-treated group which was significantly higher, as compared to control. The intensity of TGF- β_1 protein bands was more in quercetin-treated group, as compared to control on days 3 and 7. On day 7, 1.50 ± 0.27 fold significant increase in expression was noted in treatment group as compared to control (Fig. 3C).

On all the days, TNF- α mRNA expression was found to be down-regulated in quercetin-treated group, as compared to control group (Fig. 4A). On day 3 there was significant ($P < 0.001$) reduction in expression (0.26 ± 0.05 fold) of TNF- α gene in quercetin group and on day 7 also there was 0.79 ± 0.08 fold reduction in expression noted in quercetin group, which was significantly ($P < 0.05$) less as compared to control. The relative IL-10 mRNA expression in response to quercetin treatment as compared to the control group is presented in the Fig. 4B. On day 3, IL-10 expression was increased by 1.30 ± 0.10 fold in quercetin-treated group which was significantly ($P < 0.05$) higher as compared to control.

The TNF- α protein levels remained lower during the entire experiment in the quercetin-treated group as compared to control group (Fig. 4C). The levels were significantly lower on days 3 (1940 ± 161 pg/mg protein, $P < 0.01$) and 7 (1052 ± 48 pg/mg protein, $P < 0.05$) in quercetin-treated group as compared to control. The IL-10 levels by ELISA on different days of post-wounding in both the groups are presented in Fig. 4D. IL-10 level in quercetin-treated group was significantly higher on day 3 (1154 ± 31 pg/mg protein, $P < 0.05$) as compared to control.

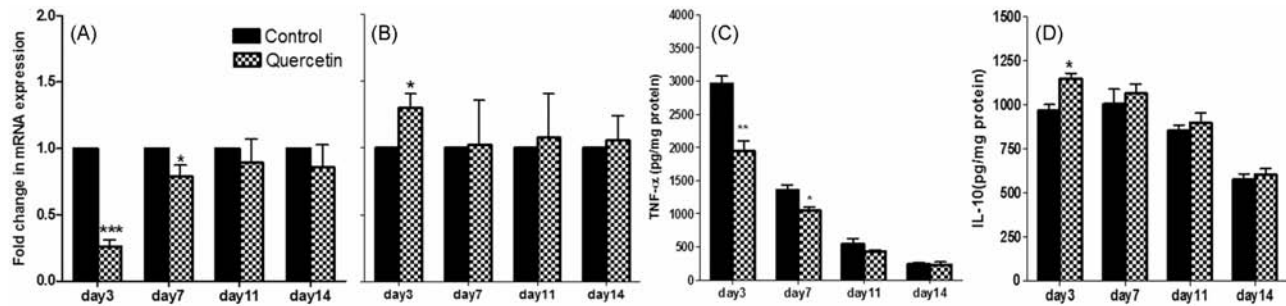


Fig. 4—Relative/fold changes in mRNA expressions of TNF- α (A); and IL-10 (B) in quercetin-treated wounds in comparison to control. The mRNA levels were normalized with β actin of respective samples. TNF- α (C) and IL-10 (D) protein levels by ELISA of granulation/healing tissue on days 3, 7, 11 and 14 in the two groups of rats. Data are expressed as mean \pm SE, $n = 3$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. control on respective day.

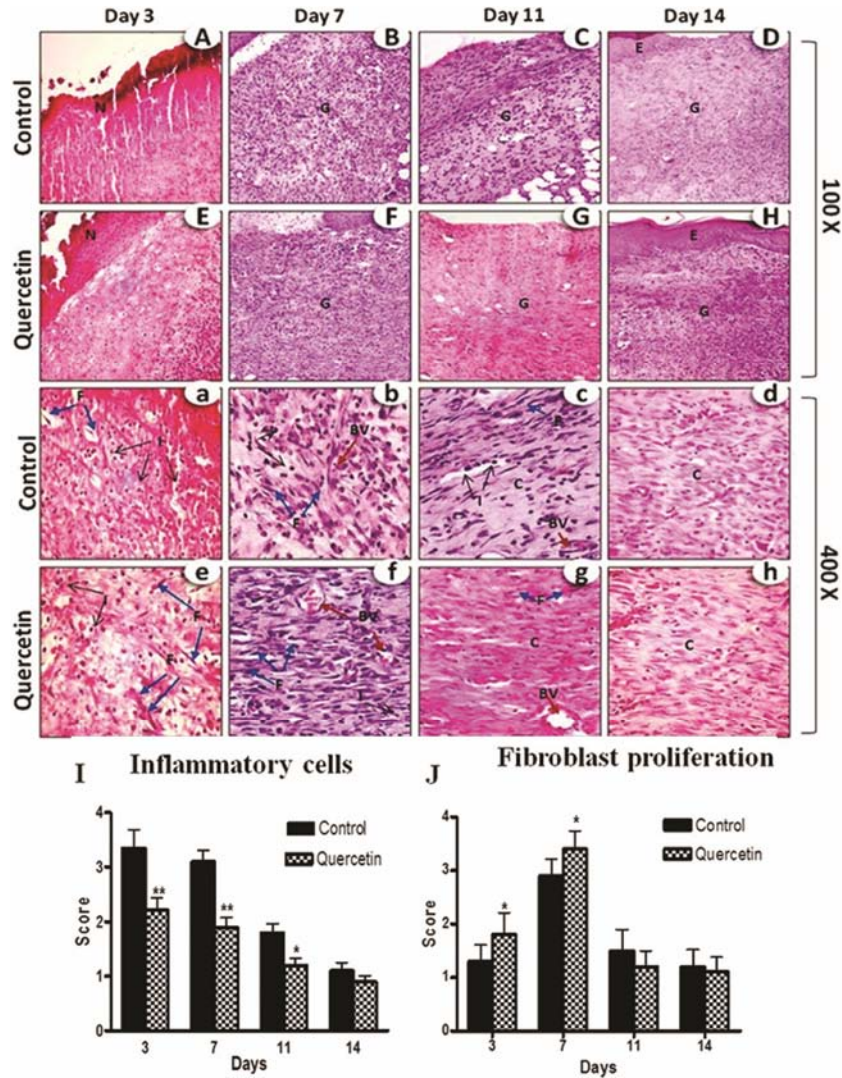


Fig. 5—Representative histological sections (H & E stained) of healing wounds of control (A–D & a–d) and quercetin-treated rats (E–H & e–h) on days 3, 7, 11 and 14. On day 3: quercetin group (e) showing less number of inflammatory cells and more fibroblasts compared to control (a). On day 7: quercetin group (f) showing more fibroblast proliferation, less inflammatory cells and more blood vessels compared to control (b). On day 11: quercetin group (G, g) showing well formed granulation tissue and regular collagen deposition compared to control (C, c). On day 14: quercetin group (H, h) showing complete superficial epithelial layer and regular collagen deposition compared to control (D, d). BV: blood vessels, C: collagen, F: fibroblasts, I: inflammatory cells, G: granulation tissue, N: necrotic tissue. (I, J) Histological scoring for inflammatory cells and fibroblast proliferation (score from 0-4). Data are expressed as mean ± SE, n= 10; **P* <0.05; ***P* <0.01 vs. control on respective day

On day 3 (Fig. 5 A,a) H & E stained sections of control group showed presence of more inflammatory cells and very few fibroblast infiltration. In quercetin-treated group, there was considerably less number of inflammatory cells and more fibroblast infiltration noticed (Fig. 5 E,e), as compared to control. The wound sections on day 7 (Fig. 5 B,F,b,f) showed appearance of granulation tissue, in both the groups. Control group still showed presence of more inflammatory cells, fibroblast proliferation was evident with few capillaries and mild collagen deposition. In

quercetin-treated group inflammatory cells were less in number and more fibroblast proliferation observed. Histological scoring of wound sections for inflammatory cells (Fig. 5I) and fibroblast proliferation (Fig. 5J) revealed significant difference between the groups on days 3 and 7. Capillary density was more in quercetin group. On day 11 (Fig. 5 C,c,G,g), collagen deposition was evident in both the groups. In quercetin-treated group deposition of collagen was regular and throughout the thickness of granulation tissue as compared to control. On 14th day (Fig. 5 D,d,H,h),

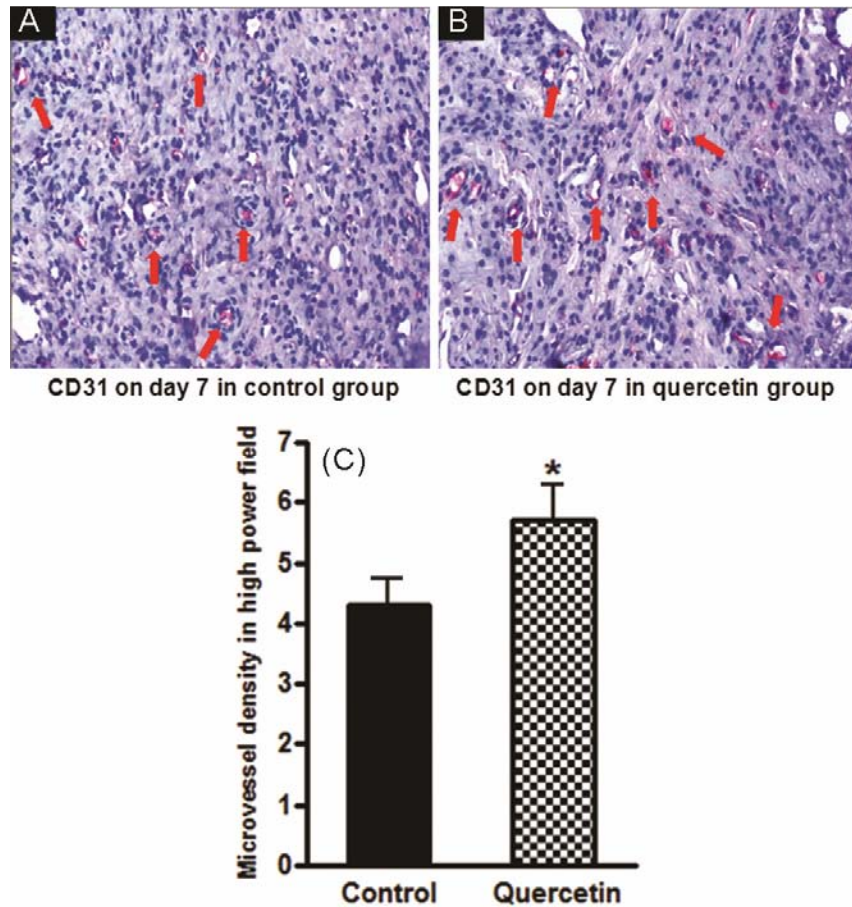


Fig. 6—Immunohistochemistry depicting for the expression of CD31 in granulation tissues of control (A) and quercetin-treated (B) rats on day 7 post-wounding. (C) Graphical representation showing semiquantitative analysis of MVD on day 7 post-wounding. The MVD was assessed by counting the number of microvessels in 10 randomly chosen high-power fields in stained wound sections of both control and quercetin-treated rats. * $P < 0.05$ vs. control ($n = 4$).

sections of both groups showed well formed granulation tissue with collagen deposition and superficial epithelialisation. Quercetin-treated group showed more compact, and regular collagen deposition and complete superficial epithelialisation as compared to control group.

The representative images of CD31-positive vessels of both groups are presented in Fig. 6A and 6B. The neovascularization was better in quercetin-treated group where well marked lumen of blood vessels with large perimeter noticed as compared to control. The MVD in quercetin-treated group was significantly ($P < 0.05$) increased on day 7 (Fig. 6C) compared to control.

Discussion

Plant derived agents have been attempted for decades to attain the goal of perfect aesthetic healing of skin wounds. In the present study, quercetin caused significant time-dependent increase in wound closure.

VEGF and TGF- β_1 expressions were significantly upregulated in quercetin-treated rats, whereas TNF- α level was markedly reduced. IL-10 level and CD31 stained vessels were markedly higher on days 3 and 7, respectively, in quercetin-treated rats. In H & E stained sections, quercetin-treated group showed less inflammatory cells, more fibroblast proliferation, increased microvessel density, better re-epithelialization and more regular collagen deposition.

In the process of healing, wound contraction occurs due to the activity of myofibroblasts⁴⁰. It begins soon after wounding and peaks at 2 weeks with significant reduction of wound area especially in loose skinned animals⁴¹. In this study, quercetin-treated wounds showed significantly higher reduction in wound area from day 7 to 14, which is suggestive of an increased myofibroblast activity, as quercetin has been reported to enhance myofibroblast activity and to increase epithelial cell growth in oral ulcers³⁴.

VEGF is the major angiogenic agent in wound that stimulates migration, proliferation and differentiation of endothelial cells⁴². Reduced expressions of VEGF-A gene or mRNA or its accelerated degradation were found to be associated with wound healing defects⁴³. Quercetin has been shown to influence the expression of some growth factors and cytokines in studies related to soft tissue healing⁴⁴. It upregulates the VEGF expression in colon epithelial cell lines and in the inflamed colonic tissue, most likely, by activating the HIF-1 α pathway³⁷. In our study, VEGF mRNA and protein levels were significantly higher in quercetin treated group on days 3 and 7, indicating prohealing effect of quercetin at the wound site. The increased MVD noted in quercetin-treated group on day 7, also suggests the angiogenic potential of quercetin in skin.

TGF- β_1 having varied functions in the healing wounds is an important growth factor modulator of cell growth and differentiation⁴⁵. It is also involved in upregulating the angiogenic growth factor VEGF⁴⁶. TGF- β_1 can be both inhibitory and stimulatory in nature⁴⁷. Several studies suggest that quercetin modulates TGF- β_1 expression and its signalling pathways and many of them emphasise that quercetin has an inhibitory effect on TGF- β_1 expression in conditions like pulmonary fibrosis⁴⁸, liver fibrosis⁴⁹ and keloids and hypertrophic scars⁵⁰, where tissue function and cosmeses are compromised by excess TGF- β_1 activity. There are also reports of quercetin enhancing TGF- β_1 expression in ovarian cancer cell line⁵¹ and in leukemic blasts, where TGF- β_1 is supposed to act selectively as a negative regulator on early hematopoietic progenitor cells and, thereby, checking angiogenesis and tumour progression⁵². However, there is a lack of information about expression pattern of TGF- β_1 in skin wounds. In the present study, TGF- β_1 levels furnish an indication that quercetin can enhance TGF- β_1 expression in healing wounds in early proliferative phase, thereby, favouring the fibroblast activity and better extracellular matrix (ECM) deposition and granulation tissue formation. We also observed a decreasing trend in TGF- β_1 expression in quercetin-treated rats by day 11 onwards, which might have balanced ECM deposition and degradation. Thus, quercetin might also reduce scar formation.

The maximum gene expression of proinflammatory cytokine TNF- α was observed at 3 h post wounding and the same was maintained until 120 h post-

wounding⁵³. Elevated levels of TNF- α and IL-1 noted in several wounds can cause increased synthesis of matrix metalloproteinase and decreased expression of tissue inhibitors of metalloproteinase and ECM proteins which can hamper smooth progress of healing⁵⁴. Throughout the course of present study, TNF- α level was lower in quercetin-treated rats and on day 3 and 7 it was significantly lower. In earlier reports, quercetin pretreatment inhibited the secretion of TNF- α and IL-1 β by almost 40% in bone marrow derived macrophages through inhibition of the NF- κ B pathway in a dose-dependent manner^{55,56}. Additionally, IL-10, the major anti-inflammatory cytokine produced in the early phases of healing by keratinocytes and infiltrating mononuclear cells, is known to limit and terminate the inflammatory responses and renders the wound to evolve into a more proliferative healing phase⁵⁷. In this study, IL-10 mRNA expression was found to be enhanced on day 3 in quercetin group and rest of the days both the groups followed almost a similar decreasing trend in expression. In an earlier study on acute pancreatitis in mice, it has been reported that quercetin treatment increased the serum IL-10 levels⁵⁸. In yet another study, quercetin stimulated the expression of the anti-inflammatory cytokine IL-10 at low concentrations⁵⁵. But some reports suggest that quercetin does not affect or might even decrease IL-10 levels^{59,60}. By analysing the results of TNF- α and IL-10 mRNA expression and protein levels, the present study proposes that quercetin might ameliorate inflammatory response in wounds mainly by downregulating the expression of proinflammatory cytokines like, TNF- α rather than by upregulating anti-inflammatory cytokines like, IL-10. Evidently, decreased inflammatory cell density in H & E stained wound sections of quercetin-treated rats strongly supports its anti-inflammatory nature. Furthermore, histological data also revealed better epithelialisation and collagen deposition in later phases of healing in quercetin-treated rats favouring better wound closure in these rats.

In conclusion, the present study suggests that quercetin has marked beneficial effects in healing of cutaneous wounds by effectively modulating the expression of cytokines and growth factors involved in multiple phases of healing.

Conflict of interest

No conflict to disclose.

References

- 1 Inan A, Sen M, Koca C, Akpinar A & Dener C, The effect of purified micronized flavonoid fraction on the healing of anastomoses in the colon in rats. *Surg Today*, 36 (2006) 818.
- 2 Singer AJ & Clark RAF, Cutaneous wound healing. *N Engl J Med*, 341 (1999) 738.
- 3 Kondo T & Ishida Y, Molecular pathology of wound healing. *Forensic Sci Int*, 203 (2010) 93.
- 4 Wu Y, Wang J, Scott PG & Tredget EE, Bone marrow-derived stem cells in wound healing: a review. *Wound Repair Regen*, 15 (2007) 18.
- 5 Bouaziz-Ketata H, Zouari N, Salah HB, Rafrafi M & Zeghal N, Flavonoid profile and antioxidant activities of methanolic extract of *Hyparrhenia hirta* (L.) Stapf. *Indian J Exp Biol*, 53 (2015) 208.
- 6 Mamta, Mehrotra S, Amitabh, Kirar V, Vats P, Nandi SP, Negi PS & Misra K, Phytochemical and antimicrobial activities of Himalayan *Cordyceps sinensis* (Berk.) Sacc. *Indian J Exp Biol*, 53 (2015) 36.
- 7 Bhatt A & Patel V, Antioxidant potential of banana: Study using simulated gastrointestinal model and conventional extraction. *Indian J Exp Biol*, 53 (2015) 457.
- 8 Chandra AK, Mondal C, Sinha S, Chakraborty A & Pearce EN, Synergic actions of polyphenols and cyanogens of peanut seed coat (*Arachis hypogaea*) on cytological, biochemical and functional changes in thyroid. *Indian J Exp Biol*, 53 (2015) 143.
- 9 Bhangale J & Acharya S, Antiarthritic activity of *Cynodon dactylon* (L.) Pers. *Indian J Exp Biol*, 52 (2014) 215.
- 10 Durkar AM, Patil RR & Naik SR, Hypolipidemic and antioxidant activity of ethanolic extract of *Symplocos racemosa* Roxb. in hyperlipidemic rats: An evidence of participation of oxidative stress in hyperlipidemia. *Indian J Exp Biol*, 52 (2014) 36.
- 11 Martin A, The use of antioxidants in healing. *Dermatol Surg*, 22 (1996) 156.
- 12 Nirmala RT, Caroline A, Lincy S, Saj M & Durai D, Evaluation of antibacterial, antioxidant and wound healing properties of seven additional medicinal plants from India in experimental animals. *Asian Pac J Trop Biomed*, 2 (2012) S1245.
- 13 Habbu PV, Mahadevan KM, Kulkarni PV, Daulatsingh C, Veerapur VP & Shastry RA, Adaptogenic and *in vitro* antioxidant activity of flavonoids and other fractions of *Argyrea speciosa* (Burm.f) Boj. in acute and chronic stress paradigms in rodents. *Indian J Exp Biol*, 48 (2010) 53.
- 14 Sharma D, Rawat I & Goel HC, Anticancer and anti-inflammatory activities of some dietary cucurbits. *Indian J Exp Biol*, 53 (2015) 216.
- 15 Veerapur VP, Prabhakar KR, Thippeswamy BS, Bansal P, Srinivasan KK & Unnikrishnan MK Antidiabetic effect of *Dodonaea viscosa* (L). Lacq. aerial parts in high fructose-fed insulin resistant rats: A mechanism based study. *Indian J Exp Biol*, 48 (2010) 800.
- 16 Preethi KC, Kuttan G & Kuttan R, Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. and its possible mechanism of action. *Indian J Exp Biol*, 47 (2009) 113.
- 17 Habbu PV, Mahadevan KM, Shastry RA & Manjunatha H, Antimicrobial activity of flavanoid sulphates and other fractions of *Argyrea speciosa* (Burm.f) Boj. *Indian J Exp Biol*, 47 (2009) 121.
- 18 Geetha T, Malhotra V, Chopra K & Kaur IP, Antimutagenic and antioxidant/prooxidant activity of quercetin. *Indian J Exp Biol*, 43 (2005) 61.
- 19 Kumar NP, Annamalai AR & Thakur RS, Antinociceptive property of *Emblica officinalis* Gaertn (Amla) in high fat diet-fed/low dose streptozotocin induced diabetic neuropathy in rats. *Indian J Exp Biol*, 47 (2009) 737.
- 20 Saranya P & Geetha A, Antiulcer activity of *Andrographis paniculata* (Burm.f.) Wall. against cysteamine-induced duodenal ulcer in rats. *Indian J Exp Biol*, 49 (2011) 525.
- 21 Yousefi K, Soraya H, Fathiazad F, Khorrami A, Hamedeyazdan S, Maleki-Dizaji N & Garjani A, Cardioprotective effect of methanolic extract of *Marrubium vulgare* L. on isoproterenol-induced acute myocardial infarction in rats. *Indian J Exp Biol*, 51 (2013) 653.
- 22 Challa SR, Akula A, Metla S & Gopal PNV, Partial role of nitric oxide in infarct size limiting effect of quercetin and rutin against ischemia-reperfusion injury in normal and diabetic rats. *Indian J Exp Biol*, 49 (2011) 207.
- 23 Middleton E, Kandaswami C & Theoharides TC, The effect of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev*, 52 (2000) 673.
- 24 Chouhan S & Flora SJS, Arsenic and fluoride: Two major ground water pollutants. *Indian J Exp Biol*, 48 (2010) 666.
- 25 Sikder K, Das N, Kesh SB & Dey S, Quercetin and β -sitosterol prevent high fat diet induced dyslipidemia and hepatotoxicity in Swiss albino mice. *Indian J Exp Biol*, 52 (2014) 60.
- 26 Kumar SV, Suresh & Mishra SH, Hepatoprotective effect of *Pergularia daemia* (Forsk.) ethanol extract and its fraction. *Indian J Exp Biol*, 46 (2008) 447.
- 27 Pujari RR, Vyawahare NS & Kagathara VG, Evaluation of antioxidant and neuroprotective effect of date palm (*Phoenix dactylifera* L.) against bilateral common carotid artery occlusion in rats. *Indian J Exp Biol*, 49 (2011) 627.
- 28 Atalık KE, Keleş B, Uyar Y, DüNDAR MA, Öz M & Esen HH, Vasoprotection by melatonin and quercetin in rats treated with cisplatin. *Indian J Exp Biol*, 48 (2010) 1188.
- 29 Anjaneyulu M & Chopra K, Quercetin attenuates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats. *Indian J Exp Biol*, 42 (2004) 766.
- 30 Morel I, Lescoat G, Cogrel P, Sergent O, Padeloup N, Brissot P, Cillard P & Cillard J, Antioxidant and iron-chelation activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem Pharmacol*, 45 (1993) 13.
- 31 Datta S, Sinha M, Das D, Ghosh S & Dhar P, Protective effect of secondary plant metabolites from *Ipomoea aquatica* Forsk. against carbofuran induced damages. *Indian J Exp Biol*, 51 (2013) 1109.
- 32 Chondrogianni N, Kapeta S, Chinou I, Vassilatou K, Papassideri I & Gonos SE, Anti-ageing and rejuvenating effects of Quercetin. *Exp Gerontol*, 45 (2010) 763.
- 33 Calvo TR, Lima ZP, Silva JS, Ballesteros KV, Pellizzon CH, Hiruma-Lima CA, Tamashiro J, Brito AR, Takahira RK & Vilegas W, Constituents and antiulcer effect of *Alchornea*

- glandulosa*: activation of cell proliferation in gastric mucosa during the healing process. *Biol Pharm Bull*, 30 (2007) 451.
- 34 Mostafa MAA & Ibrahim MAM, Management of aphthous ulceration with topical Quercetin. *Cairo Dental J*, 25 (2009) 9.
 - 35 Gomathi K, Gopinath D, Ahmed RM & Jayakumar R, Quercetin incorporated collagen matrices for dermal wound healing processes in rat. *Biomaterials*, 24 (2003) 2767.
 - 36 Wong RWK & Rabie ABM, Effect of quercetin on bone formation. *J Orthop Res*, 26 (2008) 1061.
 - 37 Jeon H, Kim H, Choi D, Kim D, Park SY, Kim YJ, Kim YM & Jung Y, Quercetin activates an angiogenic pathway, hypoxia inducible factor (HIF)-1-vascular endothelial growth factor, by inhibiting HIF-prolyl hydroxylase: a structural analysis of quercetin for inhibiting HIF-prolyl hydroxylase. *Mol Pharmacol*, 71 (2007) 1676.
 - 38 Livak KJ & Schmittgen TD, Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta CT). *Methods*, 25 (2001) 402.
 - 39 Hunt T & Mueller R, Wound healing. In: *Current surgical diagnosis and treatment*, (Ed. LW Way & Paramus NJ, Appleton and Lange, USA), 1994, 80.
 - 40 Conrad PA, Giuliano KA, Fisher G, Collins K, Matsudaria PT & Taylor DL, Relative distribution of actin, myosin I, and myosin II during the wound healing response of fibroblasts. *J Cell Biol*, 120 (1993) 1381.
 - 41 Welch MP, Odland GF & Clark R AF, Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *J Cell Biol*, 110 (1990) 133.
 - 42 Romano DI, Peppe S, Mangoni A, Zambruno G, Spinetti G, Melillo G, Napolitano M & Capogrossi MC, Adenovirus-mediated VEGF (165) gene transfer enhances wound healing by promoting angiogenesis in CD1 diabetic mice. *Gene Ther*, 9 (2002) 1271.
 - 43 Swift ME, Kleinman HK & Dipietro LA, Impaired wound repair and delayed angiogenesis in aged mice. *Lab Invest*, 79 (1999) 1479.
 - 44 Pastore S, Lulli D, Fidanza P, Potapovich AI, Kostyuk VA, De Luca C, Mikhail'chik E & Korkina LG, Plant polyphenols regulate chemokine expression and tissue repair in human keratinocytes through interaction with cytoplasmic and nuclear components of epidermal growth factor receptor system. *Antioxid Redox Signal*, 16 (2012) 314.
 - 45 Sporn MB, Roberts AB, Wakefield LM & Assoian RK, Transforming growth factor- β Biological function and chemical structure. *Science*, 233 (1986) 532.
 - 46 Riedel K, Riedel F, Goessler UR, Germann G & Sauerbier M, TGF-beta antisense therapy increases angiogenic potential in human keratinocytes *in vitro*. *Arch Med Res*, 38 (2007) 45.
 - 47 Roberts AB & Sporn MB, The transforming growth factor- β s. In: *Peptide Growth Factors and Their Receptors II, Handbook of Experimental Pharmacology*, (Eds. Sporn MB & Roberts AB, Springer Verlag, New York), 1990, 419.
 - 48 Baowen Q, Yulin Z, Xin W, Wenjing X, Hao Z, Zhizhi C, Xingmei D, Xia Z, Yuquan W & Lijuan C, A further investigation concerning correlation between anti-fibrotic effect of liposomal quercetin and inflammatory cytokines in pulmonary fibrosis. *Eur J Pharmacol*, 642 (2010) 134.
 - 49 Qi LH, Kang LP, Zhang JP, Shi N, Zhang M & Wu TM, Antifibrotic effects of genistein and quercetin *in vitro*. *Yao Xue Xue Bao*, 36 (2001) 648.
 - 50 Phan TT, Lim JJ, Chan SY, Tan EK, Lee ST & Longaker MT, Suppression of transforming growth factor beta/smad signalling in keloid derived fibroblasts by quercetin: implications for the treatment of excessive scars. *J Trauma*, 57 (2004) 1032.
 - 51 Scambia G, Panici PB, Ranelletti FO, Ferrandina G, De Vincenzo R, Piantelli M, Masciullo V, Bonanno G, Isola G & Mancuso S, Quercetin enhances transforming growth factor beta1 secretion by human ovarian cancer cells. *Int J Cancer*, 57 (1994) 211.
 - 52 Larocca LM, Teofili L, Sica S, Piantelli M, Maggiano N, Leone G & Ranelletti FO, Quercetin inhibits the growth of leukemic progenitors and induces the expression of transforming growth factor-beta 1 in these cells. *Blood*, 85 (1995) 3654.
 - 53 Bryan D, Walker KB, Ferguson M & Thorpe R, Cytokine gene expression in a murine wound healing model. *Cytokine*, 31 (2005) 429.
 - 54 Barrientos S, Stojadinovic O, Golinko SM, Brem H & Tomic-Canic M Growth factors and cytokines in wound healing. *Wound Repair Regen*, 16 (2008) 585.
 - 55 Comalada M, Ballester I, Bailón E, Sierra S, Xaus J, Gálvez J, de Medina FS & Zarzuelo A, Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. *Biochem Pharmacol*, 72 (2006) 1010.
 - 56 Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA & Kandaswami C, The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappa beta system. *Clin Vaccine Immunol*, 13 (2006) 319.
 - 57 Moore KW, deWaal Malefyt R, Coffman RL & O'garra A, Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol*, 19 (2001) 683.
 - 58 Carvalho KM, Morais TC, de Melo TS, de Castro Brito GA, de Andrade GM, Rao VS & Santos FA, The natural flavonoid quercetin ameliorates cerulein-induced acute pancreatitis in mice. *Biol Pharm Bull*, 33 (2010) 1534.
 - 59 Nieman DC, Henson DA, Davis JM, Dumke CL, Gross SJ, Jenkins DP, Murphy EA, Carmichael MD, Quindry JC, McAnulty SR, McAnulty LS, Utter AC & Mayer EP, Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. *J Interferon Cytokine Res*, 27 (2007) 1003.
 - 60 Konrad M, Nieman DC, Henson DA, Kennerly KM, Jin F & Wallner-Liebmann SJ, The acute effect of ingesting a quercetin-based supplement on exercise-induced inflammation and immune changes in runners. *Int J Sport Nutr Exerc Metab*, 21 (2011) 338.