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# Hypertrophic Scar Management with a Flavonoid Fraction of *Cyphomandra betacea*

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## ABSTRACT

*Cyphomandra betacea* (Solanaceae) grows wild in India. The ethanolic fraction of the fruits of *C. betacea* has a rich content of the bioflavonoid quercetin, which has antihistaminic and antiproliferative activities. A study was carried out to evaluate the preventive and curative properties of a flavonoid fraction of *C. betacea* containing 3.47% quercetin on an animal model of hypertrophic scarring. Four circular excisional wounds were produced on each ear of 10 rabbits. Sample cream containing the flavonoid fraction of the ripe fruits of *C. betacea* was applied to one wound immediately and then three times a day thereafter for four weeks as a preventive treatment and three times a day for eight weeks on one hypertrophic scar as a curative treatment. Placebo cream was used on two of the other wounds and one wound was left untreated. Hypertrophic scars developed in all untreated and placebo-treated wounds after four weeks and 60% of sample-treated wounds healed with hypertrophic scars. The level of histamine and hydroxyproline increased significantly in placebo-treated wounds in the preventive group and their levels in sample-treated wounds decreased significantly. In the curative group all the hypertrophic scars were flattened after eight weeks' treatment with sample and the histamine level was decreased significantly in sample-treated scars with a slight decrease in hydroxyproline level compared to placebo-treated scars. Due to its antihistamine activity, this flavonoid fraction could be used as a preventive or adjuvant curative treatment for hypertrophic scars.

**Keywords:** antihistamine, hydroxyproline, quercetin, rutin
**Abbreviations: HP**, hydroxyproline; **HS**, hypertrophic scars

## INTRODUCTION

Mast cells play a fundamental role in tissue homeostasis, remodeling and repair. Mast cells store and release various potent mediators, in particular histamine, proteases, lipid mediators and cytokines through which they can influence different stages of cutaneous wound healing (Crivellato *et al.* 2010).

Wounding results in adaptive changes in histidine decarboxylase enzyme activity and increases histamine forming capacity (Fitzpatrick *et al.* 1982; Artuc *et al.* 2002). Increased histamine synthesis was reported in many tissues undergoing rapid growth or repair (Singer *et al.* 1999; Mohammad *et al.* 2008). Hydroxyproline (HP) is an amino acid formed from proline incorporated into collagen and it is a byproduct of collagen synthesis. Assay for tissue HP shows an increase in parallel with tissue collagen levels and HP is the best indicator of collagen synthesis and wound healing (Kahlson *et al.* 1960; Lodhi *et al.* 2010).

Bioflavonoids are known for their antiproliferative effects on both normal and malignant cells and for their ability to block histamine release. These properties could theoretically prove beneficial in reversing the proliferative and inflammatory responses in hypertrophic scars (HSs) (Saulis *et al.* 2002).

HSs are abnormal healing responses that develop because of an exaggerated proliferation of dermal fibroblasts after skin injury and are characterized by excess accumulation of collagen at the wound site (Clark 1993). The development of this abnormal pattern of healing has been associated with an extended period between wounding and re-epithelialization of the wound, resulting in a prolonged inflammatory phase. This may occur because of complications such as an infection, a foreign body within the wound, excessive tension on the wound or persistent mobilization of the wound edges (Muir 1990). HSs appear within four weeks after trauma, enlarge for three to six months, remain static for several months and gradually regress in terms of erythema, size, and irritability over approximately one year (Niessen *et al.* 1999). A peak in collagen synthesis at six months is followed by a decrease in synthesis that parallels the clinical changes (Uppal *et al.* 2001).

Excessive dermal scarring in the formation of an HS continues to be a clinical problem. Considering the ethical problems involved in studies of HS pathophysiology in humans, an animal model is of great usefulness for studying the stages in hypertrophic scarring from early healing to mature HSs and for evaluating therapeutic modalities (Morris *et al.* 1997). One study using a domestic pig model reported that immediately after incisional wounding, mast cell numbers increased rapidly and subsequently peaked two days after wounding then declined at a relatively constant rate from day two to day four and gradually returned to normal by day 14 (Reich *et al.* 1991). This fluctuation in mast cell numbers and histamine release correlated well with the concomitant formation of granulation tissue, the hallmark of early wound healing (Sasaki *et al.* 2003). The decline in mast cell numbers is in contrast to the cellular events seen in hypertrophic scars, in which increased numbers of mast cells persist indefinitely with an associated elevation of tissue histamine (Hermes *et al.* 2000). These fibrotic lesions reportedly exhibit as much as 10 to 100 times more mast cells than normal human skin (Tharp 1987; Mohammad *et al.* 2008).

The aim of this study was to assess the preventive and curative effects of the flavonoid fraction of *C. betacea* (3.47% quercetin) on an HS animal model. Scars developed in all sample-treated and placebo-treated wounds at 4 weeks.

Received: 23 February, 2010. Accepted: 28 August, 2010.

Short Communication

**Table 1** Effect of flavonoid fraction of *Cyphomandra betacea* on hydroxyproline and histamine concentration in wounded skin of the rabbit's ear. (Mean ± SD).

| Compound | Unwounded skin (n=10) | Preventive group (n=10) | | Curative group (n=10) | |
|---|---|---|---|---|---|
| | | Sample-treated | Placebo-treated | Sample-treated | Placebo-treated |
| Hydroxyproline (µg/mg tissue) | 3.65 ± 0.41 | 5.20 ± 0.28 ab | 7.12 ± 0.32 a | 10.14 ± 0.21 a | 10.45 ± 0.47 a |
| Histamine (µ/mg tissue) | 2.21 ± 0.22 | 4.01 ± 0.26 ab | 6.42 ± 0.16 a | 8.13 ± 0.32 ab | 15.04 ± 0.22 a |

a: Significant compared to unwounded skin.($P \leq 0.001$)
b: Significant compared to placebo-treated group. ($P \leq 0.001$)

These scars tended to decrease in prominence within 8 weeks in sample-treated wounds, while a reduction in the prominence of scars in this study occurred in only 10% of placebo-treated animals at 16 weeks.

## MATERIALS AND METHODS

### Plant material

The fruits of *C. betaceae* were collected from the campus herbal garden in May, 2008. They were authenticated by the Field Botanist, Survey of Medicinal Plants and Collection Unit, Nilgiris District and the voucher specimens were deposited in the Department of Pharmacognosy, JSS College of Pharmacy, Ooty, Nilgiris, India.

### Preparation of extract

Fully ripened fruits were selected and sliced into small pieces. The sliced fruits (100 g) were placed in 500 ml of distilled water and heated at 60°C with occasional stirring for 2 hrs. This solution was adjusted to pH 4.5 with tartaric acid, transferred to a round-bottom flask fitted with a condenser and boiled for 1 hr with continuous stirring. This solution was filtered while hot. The filtrate was cooled and gradually transferred into a beaker containing 600 ml of acetone with continuous stirring, to precipitate the pectin. The solution was fractionated with 500 ml of ethanol and chloroform, and the ethanolic fraction was subjected to freeze drying (relative yield on a dry weight basis: 16.3%). Phytochemical screening using HPTLC and HPLC techniques, revealed the presence of the bioflavonoid, quercetin (relative yield on a dry weight basis 3.47%).

### Animals

The study was carried out on ten age-matched healthy male white rabbits, weighing 1.8-2.2 kg each. Animals were housed under the same controlled environmental conditions at the animal house of the pharmacology department, JSS College of Pharmacy, Ootacamund, India. The animals were fed a normal laboratory diet with free access to drinking water. The experiments were carried out with the approval of the Institutional Animal Ethics Committee following CPCSEA guidelines (Winter *et al* 1962) (Approval no: JSSCP/IAEC/ M.PHARM/ Ph.Cog/05/2008-2009).

### Cream formulation

Each 100 g of cream contained 30 g of *C. betacea* flavonoid fraction (3.47% quercetin), 20 g of soft, white paraffin, 10 g liquid paraffin, 10 g acetyl alcohol, 5 g glyceryl monostearate, 1 g of methyl paraben, 0.50 g of propyl paraben, 10 g of propylene glycol and water up to 100 g. The placebo control was identical in composition except for omission of the flavonoid fraction of *C. betacea*. The sample cream was prepared according to the formula of (Katsarou *et al.* 2000).

### Evaluation of hydroxyproline and histamine concentration in wounded skin

Animals were anesthetized with thiopental sodium (2.5 mg/kg iv). Four circular full-thickness excisional wounds were performed down to bare cartilage on the ventral surface of each ear by using a 4-mm biopsy punch. A magnifying binocular loupe C 2.3 × 340 mm was used. Hemostasis was then obtained by applying pressure. All wounds were covered with an occlusive polyurethane dressing until the entire wound appeared re-epithelialized on gross examination. Photographs were taken and treatment of one of four wounds per ear was begun immediately with sample cream three times daily for 4 weeks. The second wound was treated with placebo cream at the same time to serve as control for the preventive group (n = 10). The remaining two wounds per ear remained untreated during this period till a HS was established. After 4 weeks, treatment of the third wound that developed an elevated scar was begun and continued at three times per day for 8 more weeks. The fourth wound was treated with placebo cream to serve as control for the curative group (n = 10).

At the end of the treatment period, the scars on the rabbits' ears were carefully excised and stored for use in the biochemical measurement of HP concentration. HP comprises approximately 10% percent of collagen and the HP level is a good surrogate for the collagen content (Cheng 1969). Histamine concentration was determined by a fluorometric method (Shore *et al.* 1959). One-way analysis of variance (ANOVA) techniques were used to examine the study parameters. For pair-wise comparisons among groups, the least significance difference test (LSD) was used. *P* value was calculated and statistical significance was set at ($P \leq 0.05$) (Hill 1971). All data were expressed as mean ± standard deviation (SD).

## RESULTS

HS developed in all non-treated and placebo-treated wounds after 4 weeks, and 60% of sample-treated wounds healed with HS. All the HS were flattened in the curative group, after 8 weeks' treatment with sample and simultaneous reduction in the prominence of the placebo-treated scars occurred in 10% of the scars in a period of 16 weeks.

The concentration of HP in placebo-treated scars was significantly higher at 4 weeks than in unwounded skin (7.12 ± 0.32 vs 3.65 ± 0.41 µg/mg tissue; $P < 0.001$). HP levels in the sample-treated wounds were significantly lower than in placebo-treated wounds at four weeks in the preventive group (5.20 ± 0.28 vs 7.12 ± 0.32 µg/mg tissue; $P < 0.001$). In the curative group, the concentration of HP decreased in sample-treated scars. Nevertheless, there was no significant difference in the scars treated by sample compared to those treated by placebo (10.14 ± 0.21 vs 10.45 ± 0.47 µg/mg tissue; $P > 0.001$) (**Table 1**).

When compared to unwounded skin after 4 weeks, the placebo-treated scars in the preventive group contained triple the level of histamine (6.42 ± 0.16 vs 2.21 ± 0.22 µg/mg tissue; $P < 0.001$). Moreover, histamine continued to increase for 8 more weeks (15.04 ± 0.22 µg/mg tissues). However, it was significantly less in sample-treated scars than placebo-treated scars in both the preventive group (4.01 ± 0.26 vs 6.42 ± 0.65 µg/mg tissue; $P < 0.001$) and the curative group (8.13 ± 0.32 vs 15.04 ± 0.22 µg/mg tissue; $P < 0.001$) (**Table 1**).

The results of the present work showed that after 4 weeks, the level of histamine in placebo-treated wounds was three times the normal level in the preventive group. Although the treatment period was extended to 8 weeks in the present study with significant reduction in histamine level, flattening occurred in only 20% of HS with insignificant decrease in HP. It seems that early control of the inflammatory stage by starting treatment at the same time as skin incision may decrease or block histamine release and its subsequent stimulation of fibroblast proliferation and excessive collagen synthesis (Rothe *et al*. 1990; Garbuzenko *et al*. 2000). It was found that treatment of fibroblasts with

quercetin led to a significant inhibition of fibroblast proliferation in a dose-dependent manner (Lim *et al.* 2003).

## DISCUSSION

The results of the present study indicate that there was a continuous increase in the amount of collagen measured as HP. This supports the conclusion that the process of scar remodeling including collagen cross-linking and active collagen turnover takes place over a period extending from about one month to at least one year. Fibrotic conditions such as HS have excess connective tissue with collagen being the major contributor (Alster *et al.* 2003; Rahban *et al.* 2003). Mast cells have been identified in this fibrotic condition and are implicated in their development and also possibly in the scar-like collagen organization. Mast cells specifically influence granulation tissue organization during wound repair (Moyer *et al.* 2004).

Quercetin is a bioflavonoid known to inhibit free radical processes in cells. It is able to protect cutaneous tissue-type cell populations, fibroblasts/keratinocytes and endothelial cells of human origin from cytotoxic oxidative stress induced by protracted depletion of cellular glutathione (Skaper *et al.* 1997). In addition, quercetin has been shown to have an anti-inflammatory effect by stabilizing mast cell membranes and inhibiting histamine release from basophils and mast cells as well as an antiproliferative effect in various normal and malignant cells (Alexandrakis *et al* 1999). Quercetin treatment also leads to cell cycle arrest and apoptosis (Yoshida *et al.* 1992; Wei *et al.* 1994).

Limiting inflammation is paramount in the control of scar growth and scar-associated symptoms. The reported anti-inflammatory effects of quercetin, may account for its antifibrotic activity (Danielson *et al.* 2004). In the current study, HSs developed in less than half of quercetin-treated wounds. Inhibition of excess collagen formation could be a consequence of early control of histamine release as a significant decrease in the level of histamine and HP was found in the preventive group of this study. The antioxidant effect of quercetin's antioxidant activity might be another explanation for its antifibrotic action. Free radicals are likely to contribute to progressive fibrosis and excessive scar formation in abnormal wound healing. A role for reactive oxygen species (ROS) in the development of fibrosis is supported by the fact that antioxidants have been shown to be antifibrogenic (Phan *et al.* 2003). However, a causal relationship between ROS and cutaneous scarring remains under investigation. Another possible mechanism for the antifibrotic action of quercetin is its apoptotic effect (Wei *et al.* 1994).

To conclude, a flavonoid fraction of *Cyphomandra betacea* containing 3.47% quercetin could be an effective preventive and, to a considerable extent, an adjuvant curative treatment for HS due to its antihistamine effect.

## ACKNOWLEDGEMENTS

The authors would like to thank the management of JSS University, India, for providing the facilities and help for the study. The authors are also thankful to the Indian Pharmaceutical Association (IPA), Nilgiris branch, for providing financial support.

## REFERENCES

**Alexandrakis M, Singh L, Boucher W, Letourneau R, Theofilopoulos P, Theoharides T** (1999) Differential effect of flavonoids on inhibition of secretion and accumulation of secretory granules in rat basophilic leukemia cells. *International Journal of Immunopharmacology* **21**, 379-390

**Alster T, Tanzi E** (2003) Hypertrophic scars and keloids: etiology and management. *American Journal of Clinical Dermatology* **4**, 235-243

**Artuc M, Steckelings M, Henz B** (2002) Mast cell-fibroblast interactions: Human mast cells as source and inducers of fibroblast and epithelial growth factors. *Journal of Investigational Dermatology* **118**, 391-395

**Cheng P** (1969) An improved method for the determination of hydroxyproline in rat skin. *Journal of Investigational Dermatol*ogy **53**, 112-115

**Clark RAF** (1993) Basics of cutaneous wound repair. *Journal of Dermatology, Surgery and Oncology* **19**, 693-706

**Crivellato E, Travan L, Ribatti D** (2010) Mast cells and basophils: a potential link in promoting angiogenesis during allergic inflammation. *International Archives in Allergy and Immunology* **151**, 89-97

**Danielson J, Walter R** (2004) Salicylic acid may be useful in limiting scar formation. *Plastic Reconstruction Surgery* **114**, 1359-1361

**Fitzpatrick D, Fisher H** (1982) Histamine synthesis, imidazole dipeptides and wound healing. *Surgery* **91**, 430-438

**Garbuzenko E, Nagler A, Pickholtz D, Gillery P, Maquart F, Levi-Schaffer F** (2002) Human mast cells stimulate fibroblast proliferation, collagen synthesis and lattice contraction: A direct role for mast cells in skin fibrosis. *Clinical and Experimental Allergy* **32**, 237-246

**Hermes B, Feldmann I, Welker P, Algermissen B, Steckelings MJ, Henz B** (2000) Altered expression of mast cell chymase and tryptase and of c-kit in human cutaneous scar tissue. *Journal of Investigational Dermatolology* **114**, 51-55

**Hill B** (1971) *Principles of Medical Statistics*, Lancet Ltd., London, **9 (147)**, 383-386

**Kahlson G, Nilsson K, Rosengren E, Zenderfeldt B** (1960) Wound healing as dependent on the rate of histamine formation. *Lancet* **2**, 230-234

**Katsarou A, Davoy E, Xenos K, Theoharides T** (2000) Effect of an antioxidant (quercetin) on sodiumlauryl-sulfate-induced skin irritation. *Contact Dermatitis* **42**, 85-89

**Lim I, Phan T, Lee S, Huynh T, Longaker M** (2003) Quercetin inhibits keloid and hypertrophic scar fibroblast proliferation and collagen production. *ANZ Journal of Surgery* **73**, 286

**Lodhi S, Pawar RS, Jain AP, Jain A, Singhai A** (2010) Effect of *Tephrosia purpurea* (L) Pers. on partial thickness and full thickness burn wounds in rats. *Journal of Complementary and Integrative Medicine* **7 (I)**, 3-6

**Mohammad MVi, Mohammad B, Fatemesadat R, Aghdas B, Mojtaba K** (2008) Effect of low-level laser therapy on mast cells in second-degree burns in rats. *Photomedicine and Laser Surgery* **26**, 1-5

**Morris D, Wu L, Zhao L, Bolton L, Roth S, Ladin D, Mustoe T** (1997) Acute and chronic animal models for excessive dermal scarring: Quantitative studies. *Plastic Reconstruction Surgery* **100**, 674-681

**Moyer K, Saggers G, Ehrlich P** (2004) Mast cells promote fibroblast populated collagen lattice contraction through gap junction intercellular communication. *Wound Repair and Regeneration* **12**, 269-275

**Muir IF** (1990) On the nature of keloid and hypertrophic scars. *British Journal of Plastic Surgery* **43**, 61-69

**Niessen FB, Spauwen PH, Schalkwijk J, Kon M** (1999) On the nature of hypertrophic scars and keloids: A review. *Plastic Reconstructive Surgery* **104**, 1435-1458

**Phan T, Sun L, Bay B, Chan S, Lee S** (2003) Dietary compounds inhibit proliferation and contraction of keloid and hypertrophic scar-derived fibroblasts *in vitro*: Therapeutic implication for excessive scarring. *Journal of Trauma* **54**, 1212-1224

**Rahban S, Garner W** (2003) Fibroproliferative scars. *Clinical Plastic Surgery* **30**, 77-89

**Reich J, Cazzaniga A, Mertz P, Kerdel F, Eaglstein W** (1991) The effect of electrical stimulation on the number of mast cells in healing wounds. *American Academy of Dermatology* **25**, 40-46

**Rothe M, Nowalk M, Kerdel F** (1990) The mast cell in health and disease: A greater role. *Journal of the American Academy of Dermatology* **23**, 615-624

**Sasaki A, Mueller R, Xi G, Sipe R, Buck D, Hollinger J** (2003) Mast cells: An unexpected finding in the modulation of cutaneous wound repair by charged beads. *Plastic Reconstructive Surgery* **14**, 1446-1453

**Saulis AS, Mogford Jon H, Mustoe TA** (2002) Effect of edema on hypertrophic scarring in the rabbit ear model. *Plastic Reconstructive Surgery* **110**, 177-183

**Singer A, Clark R** (1999) Cutaneous wound healing. *English Journal of Medicine* **341**, 738-746

**Skaper S, Fabris M, Ferrari V, Carbonare M, Leon A** (1997) Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: Cooperative effects of ascorbic acid. *Free Radical Biological Medicine* **22**, 669-678

**Shore P, Burkhalter A, Cohn V** (1959) A method for fluorometric assay of histamine in tissues. *Journal of Pharmacological and Experimental Therapeutics* **127**, 182-186

**Tharp M** (1987) The mast cell and its role in human cutaneous diseases. *Progress in Dermatology* **21**, 1-5

**Uppal R, Khan U, Kakar S, Talas G, McGrouther A** (2001) The effects of a single dose of 5-fluorouracil on keloid scars: A clinical trial of timed wound irrigation after extralesional excision. *Plastic Reconstructive Surgery* **108**, 1218-1224

**Wei Y, Zhao X, Kariya Y** (1994) Induction of apoptosis by quercetin: Involvement of heat shock protein. *Cancer Research* **54**, 4952-4957

**Winter CA, Nuss GW** (1962) Carrageenan-induced edema in the hindpaw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine* **111**, 544-547

**Yoshida M, Yamamoto M, Nikaido T** (1992) Quercetin arrests human leukemic T-cells in late G1 phase of the cell cycle. *Cancer Research* **52**, 6676-6681