Hypertrophic Scar Management with a Flavonoid Fraction of Cyphomandra betacea

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\section*{ABSTRACT}

\textit{Cyphomandra betacea} (Solanaceae) grows wild in India. The ethanolic fraction of the fruits of \textit{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 \textit{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 \textit{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 antihistaminic activity, this flavonoid fraction could be used as a preventive or adjutant curative treatment for hypertrophic scars.

\section*{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).

HSSs 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). HSSs 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 \textit{C. betacea} (3.47\% quercetin) on an HS animal model. Scars developed in all sample-treated and placebo-treated wounds at 4 weeks.
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 *Cyphomandra betacea* 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 humidity 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* ≤ 0.05) (Hill 1971). All data were expressed as mean ± standard deviation (SD).

RESULTS

Table 1

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Unwounded skin</th>
<th>Preventive group (n=10)</th>
<th>Curative group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>Sample-treated</td>
<td>Placebo-treated</td>
</tr>
<tr>
<td>Hydroxyproline (µg/mg tissue)</td>
<td>3.65 ± 0.41</td>
<td>5.20 ± 0.28 ab</td>
<td>7.12 ± 0.32 a</td>
</tr>
<tr>
<td>Histamine (µg/mg tissue)</td>
<td>2.21 ± 0.22</td>
<td>4.01 ± 0.26 ab</td>
<td>6.42 ± 0.16 a</td>
</tr>
</tbody>
</table>

a: Significant compared to unwounded skin (*P*≤0.001)
b: Significant compared to placebo-treated group (*P*≤0.001)

Histamine (μg/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

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* ≤ 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* > 0.01) (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 tissue). 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 consequent 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 formation and accumulation of secretory granules in rat basophilic leukemia cells.

**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.

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