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### Effect of Quercetin on Excessive Dermal Scarring

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#### Abstract

**Objective:** Quercetin is a bioflavonoid noted for its antihistamine release and antiproliferative effects. These properties could theoretically prove beneficial in reversing the inflammatory and proliferative responses in hypertrophic scars. The aim of this study was to evaluate both preventive and curative effect of quercetin on animal model of hypertrophic scars.

**Materials and Methods:** Full thickness four circular excisional wounds were performed on each ear of ten rabbits. Quercetin cream was applied immediately on one wound for four weeks as a preventive treatment and for eight weeks on one hypertrophic scar as a curative treatment. Placebo cream was used for the other two wounds.

**Results:** Clinically, after four weeks, hypertrophic scars were developed in all non-treated and placebo-treated wounds. On the other hand, only 40% of quercetin-treated wounds healed with hypertrophic scars. The level of histamine and hydroxyproline was significantly increased in placebo-treated wounds in the preventive group. However, their levels in quercetin-treated wounds were significantly decreased. In the curative group, after eight weeks treatment with quercetin, only 20% of hypertrophic scars were flattened. While histamine level was significantly decreased in quercetin-treated scars, hydroxyproline level was insignificantly decreased as compared to placebo-treated scars.

**Conclusion:** Due to its antihistamine effect beside its antifibrotic effect, quercetin could be an effective preventive and to lesser extent an adjuvant curative treatment for hypertrophic scars.

## Introduction

Excessive dermal scarring in the form of hypertrophic scars and keloids continues to be a clinical problem[1].

Moreover, the functional and aesthetic deformities associated with them remain a significant concern.[2, 3].

Because little is known about the pathophysiology of hypertrophic scarring, an animal model is of great importance as a mean for studying the evolution of a hypertrophic scar from early healing and for evaluating therapeutic modalities[1].

There is growing evidence that 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.[4].

Wounding results in adaptive changes in histidine decarboxylase enzyme activity and increases histamine forming capacity.[5]. Increased histamine synthesis was reported in many tissues undergoing rapid growth or repair.[6] Hydroxyproline is an amino acid formed from proline incorporated into collagen and it is a subproduct of collagen synthesis. Tissue hydroxyproline assay presents a parallel increase with tissue collagen level and it is the best indicator of collagen synthesis and wound healing.[7].

Quercetin, a bioflavonoid noted for its antiproliferative effects on both normal and malignant cells and its antihistamine release effects. These properties could theoretically prove beneficial in reversing the proliferative and inflammatory responses in hypertrophic scars [8]

The aim of this study was to assess both preventive and curative effect of quercetin on animal model of hypertrophic scars.

## Materials and methods

The study was carried out on ten age-matched healthy white male rabbits, weighing approximately 2 Kg each. Animals were housed under the same controlled environmental conditions at the animal house of the pharmacology department, Alexandria faculty of medicine, fed normal laboratory diet and they had free access to tap water.

Animals were anesthetized with thiopental sodium (2.5mg/kg I.V.). Full thickness four circular excisional wounds were performed

down to bare cartilage on the ventral surface of each ear by using a 4-mm punch biopsy. A magnifying binocular loupe C 2.3x340mm (Heine, USA) was used. Hemostasis was then obtained by applying pressure. All wounds were covered using an occlusive polyurethane dressing (Tegaderm 3M, Minneapolis, Minn.) until the entire wound appeared re-epithelialized on gross examination.

Photographs were obtained and treatment of one of four wounds per ear was begun immediately with quercetin cream three times daily for four 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 hypertrophic scar was established. After four weeks, treatment of the third wound that developed elevated scar was begun three times per day for eight more weeks. The fourth wound was treated with placebo cream to serve as control group for the curative group (n=10).

The ingredients in the cream were modified from the formula according to Katsarou et al.[9] Each 100 gm cream contain: quercetin (Carl Roth, GmbH co. 76185 Karlsruhe, Germany) 7.5 gm, white soft paraffin 9.5 gm, liquid paraffin 4.75 gm, acetyl alcohol 3.5 gm, glyceryl monostearate 2.5 gm, cremophor RH40 4.00gm, methyl paraben 0.25gm, propyl paraben 0.10gm, propylene glycol 10.00gm and water to 100gm. The placebo control was identical in composition except for quercetin.

With the animals under anesthesia, serial photographs were taken. The scars on the left ear were carefully excised and stored at -80°C for use in the biochemical measurements of: 1-Hydroxyproline concentration which is considered a reflection of collagen content as it comprises approximately ten percent of collagen.[10] 2-Histamine concentration was carried out according to the method of Shore et al.(11)

Statistical analysis[12]: All data were expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) techniques were used to examine the studied parameters. For pairwise comparisons among groups, the least significance difference test (LSD) was used. P value was calculated and statistical significance was set at ( $P \leq 0.05$ )

## Results

Clinically, after four weeks, hypertrophic scars were developed in all non-treated and placebo-treated wounds. On the other hand, only 40% of quercetin treated wounds healed with hypertrophic scars. In the curative group, after eight weeks treatment with quercetin, 20% of hypertrophic scars were flattened. At the same time,

simultaneous reduction in the prominence of the placebo-treated scars occurred in 10% of the scars. (Figure 1).

The concentration of hydroxyproline in placebo-treated scars was significantly high at four weeks as compared to unwounded skin ( $7.92 \pm 0.27$  vs  $3.44 \pm 0.14 \mu\text{g}/\text{mg}$  tissue  $P < 0.001$ ). The quercetin-treated wounds had significantly decreased hydroxyproline level than placebo-treated wounds at four weeks (preventive group) ( $4.80 \pm 0.28$  vs  $7.92 \pm 0.27 \mu\text{g}/\text{mg}$  tissue  $P < 0.001$ ). In the curative group, the concentration of hydroxyproline decreased in quercetin-treated scars. Nevertheless, there was no significant difference in the scars treated by quercetin compared to those treated by placebo ( $9.91 \pm 0.28$  vs  $10.15 \pm 0.36 \mu\text{g}/\text{mg}$  tissue  $P > 0.05$ ). (Table 1)

At four weeks, compared to unwounded skin, the placebo-treated scars in the preventive group contained triple the level of histamine ( $6.39 \pm 0.9$  vs  $2.11 \pm 0.17 \mu\text{g}/\text{g}$  tissue  $P < 0.001$ ). Moreover, histamine continued to increase after eight more weeks ( $14.01 \pm 1.22 \mu\text{g}/\text{g}$  tissue). However, it was significantly less in quercetin-treated scars than placebo-treated scars in both preventive group ( $4.49 \pm 0.52$  vs  $6.39 \pm 0.9 \mu\text{g}/\text{g}$  tissue  $P < 0.001$ ) and curative group ( $5.37 \pm 0.49$  vs  $14.01 \pm 1.22 \mu\text{g}/\text{g}$  tissue  $P < 0.001$ ). (Table 1)



**Figure 1: a-Two weeks photo showing healing with flat scar only in quercetin treated wound (preventive group); b- Four weeks photo showing flat scar in quercetin treated wound and hypertrophic scar in the rest (preventive group); c- Twelve weeks photo showing flattening of the hypertrophic quercetin treated scar and the control scar (encircled) is still hypertrophic (curative group)**

**Table1: Effect of quercetin on hydroxyproline and histamine concentration in rabbit's ear wounded skin (mean  $\pm$  SD).**

	Unwounded skin (n=10)	Preventive group (n=10)		Curative group (no=10)	
		Quercetin treated	Placebo treated	Quercetin treated	Placebo treated
Hydroxyproline ( $\mu$ g/mg tissue)	3.44 $\pm$ 0.14	4.80 $\pm$ 0.28*#	7.92 $\pm$ 0.27#	9.91 $\pm$ 0.28#	10.15 $\pm$ 0.36#
Histamine ( $\mu$ /mg tissue)	2.11 $\pm$ 0.17	4.49 $\pm$ 0.52*#	6.39 $\pm$ 0.9#	5.37 $\pm$ 0.49*#	14.01 $\pm$ 1.22#

\* Significant as compared to placebo-treated group. # Significant as compared to unwounded skin.

## Discussion

Hypertrophic scars are abnormal healing responses that develop as a result of an exaggerated proliferation of dermal fibroblasts after skin injury and are characterized by excess accumulation of collagen in the wound.[13]. 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 as a result of complications such as an infection, a foreign body within the wound, excessive tension on the wound or persistent mobilization of the wound edges[14]. However, in some cases, the pathogenesis of hypertrophic scarring is unknown.[8].

Hypertrophic scars generally 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[2, 15]. A peak in collagen synthesis at six months is followed by a decrease in synthesis and parallels the clinical changes[16]. In the present work, similar to Saulis[8] group, clinically at four weeks hypertrophic scars were developed in all non-treated and placebo treated wounds. Morris et al[1] ,in the early model of scarring in rabbits, found that these scars tended to decrease in prominence subsequent to day 22, although it has previously been observed in the laboratory that some remain elevated for months. Reduction in the prominence of scars in this study occurred in 10% of placebo treated scars at twelve weeks.

During the present study, there was a continuous increase in the amount of collagen measured as hydroxyproline. This result

supports the conclusion that the process of scar remodeling including collagen cross linking and active collagen turnover occurs from one month and continues to one year [17]. Moreover, fibrotic conditions such as keloids and hypertrophic scars have excess connective tissue with the collagen being the major contributor [18]. Mast cells are identified in both of these fibrotic conditions and are implicated as a possible contributor to their development and possibly involved in the collagen scar-like organization. They specifically influence granulation tissue organization during wound repair [4, 19]. Rothe et al [20] reported that products of mast cell degranulation (in vitro) are conspicuously implicated in these pivotal events. One such mast cell product, histamine, significantly increased fibroblast proliferation and collagen synthesis [4, 20, 21].

Reich et al [22] observed that immediately after incisional wounding in a domestic pig model, mast cell numbers increased rapidly and subsequently peaked two days after wounding, declined at a relatively constant rate from day two to four and then returned gradually to normal levels at day fourteen. This fluctuation of mast cell numbers and histamine release correlates well with the concomitant formation of granulation tissue, the hallmark of early wound healing [23]. This decline in mast cell number 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 [24]. These fibrotic lesions reportedly exhibit as much as 10 to 100 times more mast cells than normal human skin [25]. The results of the present work showed that, after four weeks, the level of histamine in placebo treated wounds was triple the normal level in the preventive group. Moreover, it continued to increase after eight more weeks.

Although many articles have been published on the management of hypertrophic scars and keloids, there is no universally accepted treatment protocol. Prevention of them remains the best strategy [17]. Quercetin is a bioflavonoid known to inhibit free radical processes in cells [9]. 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 [26]. In addition, quercetin has been shown to have an anti-inflammatory effect by stabilizing mast cell membranes and inhibit histamine release from basophils and mast cells as well as an antiproliferative effect in both normal and malignant cells of various types [8, 27]. It causes cell cycle arrest and apoptosis [28, 29].

Limiting inflammation is paramount in the control of scar growth and scar-associated symptoms [30]. The anti-inflammatory effect of quercetin, that was previously reported [31], may be a reasonable explanation of its antifibrotic effect. In the current study, hypertrophic scars 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 hydroxyproline was proved in the preventive group of this study. The decrease in histamine, as suggested by Lee et al[32], was due to inhibition of mast cell degranulation. The antioxidant effect of quercetin 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[33]. These substances, although important for eliminating infection, can damage surrounding tissue and host cells and initiate further inflammation and pro-inflammatory mediator production. The importance of reactive oxygen species (ROS) in the development of fibrosis is supported by the fact that antioxidants have been shown to be antifibrogenic.[9, 33]. However, the role of ROS in cutaneous scarring remains under investigation. Another possible mechanism for the antifibrotic action of quercetin is its apoptotic effect. It inhibits insulin-like growth factor I, a potent mitogen and inhibitor of apoptosis for cell types[29]. IGF-I has been shown to stimulate fibroblast proliferation and enhances collagen synthesis[34].

In another study [8], a significant improvement in dermal collagen organization was noted on comparing mederma-treated with untreated scars on rabbit's ear hypertrophic scar. However, there was no decrease in scar height. They suggested two possibilities, either the conversion of immature to mature collagen is accelerated in some fashion with no net decrease in overall collagen production by fibroblasts or the treatment period of four weeks was too brief to detect a significant decrease in scar hypertrophy. Although the treatment period was extended to eight weeks in the present study with significant reduction in histamine level, flattening occurred only in 20% of hypertrophic scars with insignificant decrease in hydroxyproline level. It seems that early control of inflammatory stage by starting treatment concomitantly with skin incision may decrease or block histamine release and its subsequent stimulation of fibroblast proliferation and excessive collagen synthesis. However, it may not affect collagen breakdown and degradation in mature hypertrophic scars. Furthermore, quercetin concentration may have a role. Lim et al [35] found that treatment of fibroblasts derived with quercetin in a dose-dependant manner led to a significant inhibition of fibroblast proliferation. In conclusion, due to its anti-histamine effect beside its antifibrotic effect, quercetin could be an effective preventive and to lesser extent adjuvant curative treatment for hypertrophic scars.

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