ABSTRACT

Background and objective: Recurrent aphthous ulceration is the most commonly known oral mucosal disease. It presents as three types: minor (most prevalent), major and herpetiform. Quercetin is a useful therapeutic agent for the treatment of colitis and gastric ulcer. The objective of this study was to determine the effect of topical application of quercetin in the treatment of the most prevalent form of aphthous ulcers.

Methods: Forty male patients (aged 18-41 years, with mean age 24.8 years) presented with minor aphthous ulcer were enrolled for this study. The patients were systemically free and no specific pathology of the oral mucosa other than minor aphthous ulcers. Patients were randomized divided into two groups, each consisting of 20 patients. Group I (control group) received benzydamine hydrochloride mouth wash 3 times daily. Group II received 2-3 drops of quercetin 3 times daily directed on the ulcer. Clinical evaluation of patients includes assessment of ulcer size, pain measure, and questioner about the topical application of quercetin in terms of texture, taste, local tolerability, and ease of application.

Results: Topical application 3 times daily of quercetin cream to minor mouth ulcers relieved pain and produced complete healing in the majority of patients (35%) between 2 to 4 days and in 90% between 4 to 7 days, compared with no ulcer healing within 7 days in control group. On comparing the mean of ulcer size obtained > 7 days between the two groups, there was statistically a highly significant difference (<0.004). Most patients (90%) appreciated the quercetin’s ease of application, taste, texture, and well tolerated locally.

Conclusion: Quercetin is a safe, well tolerated and highly effective promising new treatment for healing common mouth ulcers.
INTRODUCTION

Recurrent aphthous ulceration or recurrent aphthous stomatitis is the most commonly known oral mucosal disease with a prevalence of up to 25% in the general population and 3-month recurrence rates as high as 50% (1-3). Popularly referred to as mouth ulcers or canker sores, aphthous ulcers are round or oval with a yellow or grey floor surrounded by an erythematous halo of inflamed mucosa. They can cause considerable pain and may interfere with eating, talking and swallowing (4,5).

Aphthous ulcers can be classified into three different types: minor, major and herpetiform. Minor mouth ulcers are by far the most common representing ulcers (an estimated 80–87% of all aphthous). They have a diameter of <1cm, usually occur on the non-keratinized oral mucosa, have a good prognosis, and generally do not persist for >2 weeks (spontaneous healing within 7–10 days is usual). Major aphthous, also termed Sutton disease, constitute an estimated 10–15% of all aphthous, are larger (>1cm in diameter) and involve deeper ulceration that heals slowly over weeks or months, often with scarring. Herpetiform ulcers constitute only 5–10% of all aphthous, consist of clusters of multiple pinpoint-type ulcers that are 1–3mm in diameter and the whole cycle may take only few days (3-4 days), but the new crops may develop during this time with the cycle pattern of these ulcers reach about 1 month (5-8).

The pathophysiology of aphthous ulcers is still poorly understood (5,7,8). Aphthous more commonly affect young adults; some cases have a familial and genetic basis, but most patients seem to be otherwise well (9,10). Attacks may be precipitated by local trauma, stress, food intake, drugs, hormonal changes, GIT diseases, immunological factors, and vitamin and trace element deficiencies. In HIV-seropositive persons, mouth ulcers occur more frequently, may be larger, take longer to heal spontaneously and produce more painful symptoms than in immunocompetent persons (2,7,8-11).

Treatment for oral aphthous can be included antibacterial, anti-inflammatory and analgesic mouth rinse, immunomodulatory, hormones, and CO₂ laser. In most patients, topical agents, including over-the-counter preparations such as antiseptic mouth- washes, are recommended. In patients with frequent exacerbations or more severe forms of aphthous that are unresponsive to topical treatments, systemic agents such as corticosteroids, colchicines, dapsone, or antibacterials are indicated (10,11). However, the treatment of aphthous ulcers remains unsatisfactory, since both topical and systemic therapies are palliative, reducing the severity of the ulceration; none result in permanent remission. Moreover, the lack of predictability of the efficacy of a particular treatment reflects the aetiology of the condition. A substantial need exists for an effective and well tolerated agent that can promote complete ulcer healing within a short period of time (11,12).

The ideal treatment of aphthous ulcers would improve ulcer healing by stimulating mucosal cell growth and removing bacterial cells that otherwise retard the healing process (13,14). Phytochemical agents (phytochemicals from fruits and vegetables) have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcer (15). Quercetin is an important dietary flavonoid and from whole onion and apple extracts (16-19). The uptake of quercetin aglycon and quercetin 3-glucoside were recently found to accelerate cutaneous lesion healing in rats when applied topically once daily. Calvo et al (2007) (20) showed that single oral administration of quercetin (250mg/kg/once daily) potently stimulates gastric epithelial cell proliferation that contributes to the accelerated healing of gastric ulcers. The antioxidant activity of quercetin was mentioned by Suzuki et al (1998), Janisch et al (2004), and Hamalainen (2007) (16,21-22). They reviewed that quercetin had free radical-scavenging activities through inhibition iNOS expression and NO production and also modulated LDL oxidation.

The healing properties of quercetin were found to be associated with enhanced myofibroblast and epithelial cell growth, two key processes involved in granulation tissue formation (16). Since the formation of granulation tissue
is a key process in healing aphthous ulcers, the present study was undertaken to determine the potential healing properties and tolerability of a quercetin in otherwise healthy patients presenting with minor mouth ulcers.

**PATIENTS AND METHODS**

Forty male patients ranging in age from 18 to 41 years with mean age 24.8 years were selected from outpatient clinic of Oral Medicine, Periodontology, and Oral Diagnosis Department, Faculty of Dentistry, Minia University. Patients presented with minor aphthous ulcers; no specific pathology of the oral mucosa other than minor aphthous ulcers. Patients were informed of the nature and objectives of the study and willingness to apply topical treatment from the beginning of the study until complete ulcer healing. Patients with major or herpetiform aphthous ulcers were excluded. The patients were systemically free according to Cornell medical index. Patients were randomized divided into two groups, each consisting of twenty patients. Group I (control group) received benzydamine hydrochloride mouth wash three times daily. Group II received quercetin (Rootage skin cream “quercetin, glycol, EBC, Zn, Myristica” by Elsi-Si) in which patients were instructed to apply 2-3 drops of quercetin cream three times daily directed on the ulcer. Patients of both groups were asked to continue daily treatment until complete healing had occurred.

**Clinical evaluation of both groups**

### Pain measure using Visual Analog Scale

- Score 0 = no pain
- Score 1 = pain with rough aggravation
- Score 2 = pain with moderate aggravation
- Score 3 = pain with slight aggravation
- Score 4 = constant pain
- Score 5 = severe pain

Patients were told that aggravation was anything that moved or touched the ulcer area and that pain associated with gentle touching was greater than that of rough handling.

1. **Assessment of the ulcers size:**
   
   For accurate evaluation of affected areas the patients were subjected to the following:
   
   i) With the patient sitting upright in good lighting, the ulcer area on the cheek was dried carefully.
   
   ii) A transparent plastic sheet was cut to suit its introduction intraorally and its direct application on the ulcer.
   
   iii) Using a faber-cast pen (multimark 1513 permanent water proof) the all around the ulcer were traced on the plastic sheet.
   
   iv) The tracing was then placed on graph paper and the numbers of mm² units included inside the drawn areas were counted.
   
   v) Ulcers were assessed 5 times, pre-treatment, 2 days, 4 days, 7 days, and > 7 days.
   
   vi) The sizes of the lesions were plotted in a table and compared statistically using paired t test within the group and ANOVA test between two groups.

2. **Patients were asked through questioner about the topical application of quercetin in terms of texture (excellent, good, unsatisfactory), taste (excellent, good, unsatisfactory), local tolerability (none, slight, moderate), and ease of application (very easy, easy, difficult).**

**RESULTS**

Forty patients with aphthous ulcers were enrolled in the study. The patients were randomly classified into two groups each consisting of 20 patients. Patients of group one (control group) were treated by topical benzydamine hydrochloride mouth wash while patients of group two were treated by topical quercetin. The lesions were evaluated clinically for the size of the total area, pain measure by the patients using Visual Analog Scale, and through questioner about assessments of the topical application of quercetin.
Of 20 patients (groups two) treated with topical quercetin, 7 (35%) indicated complete ulcer healing between 2 and 4 days, 18 (90%) between 4 and 7 days, and 20 (100%) in >7 days. This compared with patients treated with topical benzydamine hydrochloride mouth wash (group one), no ulcer healed before 7 days and 12 (60%) experienced complete ulcer healing in >7 days.

Patients’ assessments of the topical application of quercetin in terms of texture, taste, local tolerability, and ease of application were reported that the majority of patients (90%) found quercetin very easy or easy to apply, non irritant, and good or moderate taste and texture.

**TABLE (1) Mean and Standard deviation of size of ulcer (mm) and pain score in both patients’ groups**

<table>
<thead>
<tr>
<th></th>
<th>Pre-therapy</th>
<th>2 days</th>
<th>2-4 days</th>
<th>4-7 days</th>
<th>&gt;7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of ulcer</td>
<td>59.6 ±11.69</td>
<td>43.9 ±12.47</td>
<td>20.55 ±6.74</td>
<td>9.45 ±2.92</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Pain score</td>
<td>3.2 ±0.61</td>
<td>1.7 ±0.57</td>
<td>1.2 ±0.72</td>
<td>0.3 ±0.47</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

|                  | Group 2      |        |          |          |        |
| Size of ulcer    | 61.85 ±13.58| 20.35 ±6.74 | 3.65 ±3.54 | 0.2 ±0.69 | 1.8 ±2.46 |
| Pain score       | 3.2 ±0.61   | 1.7 ±0.57  | 1.2 ±0.72 | 0.3 ±0.47 | 0 ± 0  |

**TABLE (2) Analysis of variance of size of ulcer in both patients’ groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (pre-therapy Vs &gt;7 days)</th>
<th>Group 2 (pre-therapy Vs &gt;7 days)</th>
<th>Group 1 Vs group 2 &gt; 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>t- value</td>
<td>26.563</td>
<td>20.366</td>
<td>3.269</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>significance</td>
<td>significance</td>
<td>high significance</td>
<td>high significance</td>
</tr>
</tbody>
</table>

**TABLE (3) Analysis of variance of pain score in both patients’ groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (pre-therapy Vs &gt;7 days)</th>
<th>Group 2 (pre-therapy Vs &gt;7 days)</th>
<th>Group 1 Vs group 2 &gt; 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>t- value</td>
<td>20.241</td>
<td>23.247</td>
<td>2.853</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>significance</td>
<td>significance</td>
<td>high significance</td>
<td>moderate significance</td>
</tr>
</tbody>
</table>
Aphthous ulcers are a common and painful problem. Their pathophysiology is still not fully understood and this is reflected by the lack of reliable efficacy of currently used topical or systemic antibacterial, anti-inflammatory, immunomodulatory or symptomatic treatments for the condition\(^6\). Most patients with aphthous ulcers are likely to obtain over-the-counter treatments that are chiefly antiseptic mouthwashes\(^3,8\). There is currently no single well established treatment for common mouth ulcers\(^6,8,11\) and none of the existing treatments accelerates the healing process. The aim of the present study was therefore to determine the potential healing properties quercetin.

Topical application of quercetin to minor mouth ulcers produced complete healing in 35% of patients between 2 and 4 days, 90% between 4 and 7 days, and all patients treated with quercetin experienced complete ulcer healing in >7 days. Compared with patients treated by topical benzydamine hydrochloride mouth wash, no ulcer healed before 7 days and only 60% of patients showed complete ulcer healing in >7 days. On comparing the mean of ulcer size obtained > 7 days between the two groups, there was statistically a highly significant difference (<0.004). As regard to pain score, there was a moderate significant difference (<0.01) between two groups > 7 days. Taken together, the results of this study suggest

**Table (4) Correlations between the mean sizes of ulcer and the mean pain scores in both patients’ groups**

<table>
<thead>
<tr>
<th>Mean Size of Ulcers</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-therapy</td>
<td>R 0.653</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>After 2 days</td>
<td>R 0.519</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>From 2 – 4 days</td>
<td>R 0.386</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>From 4 – 7 days</td>
<td>R 0.305</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>&gt;7 days</td>
<td>R 0.207</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>P &gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**FIG (1) The correlations between the mean sizes of the ulcers and mean scores of pain; 1,2,3,4 and 5 represents the examination periods of the patients.**

**DISCUSSION**

Aphthous ulcers are a common and painful problem. Their pathophysiology is still not fully understood and this is reflected by the lack of reliable efficacy of currently used topical or systemic antibacterial, anti-inflammatory, immunomodulatory or symptomatic
promising beneficial effects of quercetin on mouth ulcer healing. Accelerated healing of aphthous ulcers provides relief from pain and discomfort. Importantly, quercetin facilitated complete ulcer healing from 2 to 4 days in 35% of the patients in this study, compared with a period <7 days for the healing in patients taking topical benzydamine hydrochloride mouth wash.

The rapidity of lesion healing in the mucous membrane is dependent upon competition between bacterial multiplication, which retards healing, and the growth of myofibroblast and epithelial cells, which are fundamental for tissue repair to occur. The healing properties of quercetin on mouth ulcers include enhancement of myofibroblast and epithelial cell growth, which are vital to the tissue repair process, and removal of bacterial contamination (25). Jeon et al (2007) used quercetin in treatment of colon ulcers and found that quercetin up-regulated vascular endothelial growth factor (VEGF), an ulcer healing factor, not only in colon epithelial cell lines but also in the inflamed colonic tissue. VEGF derived from quercetin treated colon epithelial cells promoted tube formation. Kahraman et al (2003) added that the anti-ulcerative effect of quercetin could be attributed to its anti-peroxidative, anti-oxidant, anti-inflammatory and anti-histaminic activity favouring cutaneous wound healing. Prevention of dermal enzyme degradation, cutaneous lipid peroxidation and enhanced wound healing properties have been described for quercetin (15). Accelerated ulcer healing also requires removal of bacterial contamination from the wound to provide favourable grounds for mucosal cell growth and repair. The combination of quercetin and glycol would appear to be particularly adapted to topical treatment of minor oral aphthous. Glycol is commonly employed for the storage and preservation of biological materials such as skin. Although glycol is not generally considered to be antisepsics, a physicochemical role for glycol in removing bacterial contamination from the wound is likely. Hypertonic glycol solution may create an osmotic gradient on the mucosal surface of the ulcer that favours plasma exudation from inside the ulcer down its osmotic gradient and into the buccal cavity, thus extruding contaminating bacteria, and consequently favouring wound healing (14). Newly formed cells occupy space in the wound, leading to extracellular matrix deposition and neovascularisation. Quercetin has been reported to accelerate intercellular matrix regeneration and experimental cutaneous wound healing in rats (14).

CONCLUSION

Topical application of quercetin to minor mouth ulcers in otherwise healthy patients produced complete healing in 35% of cases between 2 to 4 days and the majority of cases (90%) from 4 to 7 days compared to patients received topical benzydamine hydrochloride mouth wash. Most patients appreciated the ease of quercetin application, and approved its taste and texture.

REFERENCES


