# Therapeutic Efficacy of Herbal formulations for Recurrent Aphthous Ulcer. Correlation with Salivary Epidermal Growth Factor

Maha Galal[1] ; Sherine Adel Nasry[2] ; Dina M. Mostafa[3] and Nagwa M. Ammar[4]

[1] College of Oral & Dental Surgery, Misr University for Science & Technology (MUST), Cairo, Egypt,
[2]Surgery and Oral Medicine Department, National Research Center, Cairo, Egypt
[3]Pharmaceutical Technology Department, Pharmaceutical and Drug Industries Division, National Research Center, Cairo, Egypt
[4]Department of Pharmacognosy, Pharmaceutical and Drug Industries Division, National Research Center, Cairo, Egypt
nasrysherine@yahoo.com

**Abstract: Background:** Recurrent aphthous ulceration (RAU) is one of the most common oral mucosal lesions seen in primary care. Epidermal growth factor(EGF) in saliva is cytoprotective against injuries and plays a role in maintaining the mucosal integrity and promoting wound healing. This study compared the efficacy of three herbal components in the management of RAU and correlated this effect with salivary epidermal growth factor levels. **Methods:** Forty patients with minor aphthae were selected and randomly divided into four groups. The first three groups received topical preparations of *Acacia nilotica* (A), Glycyrrhiz glabra orLicorice (L) and a mixture of *Acacia nilotica* and Licorice (A&L).The fourth group (negative control) used a placebo. Ulcer size, pain score and salivary EGF level were recorded on treatment days 0, 2 and 5. **Results:** At day zero, there was no statistically significant difference between the four groups regarding pain score, ulcer size and salivary EGF level. At days 2 and 5, there was no significant difference between Group A and control groups; both showed the highest mean pain scores($P \leq 0.05$). Group (A & L) showed the lowest mean pain score and ulcer size, followed by group L. At the same observational period, both of (A & L) and (L) groups showed the highest mean EGF values. This was followed by Group (A). Control group showed the lowest mean EGF value..**Conclusion:** Treatment of minor aphthae using a mixture of Licorice and *Acacia nilotica* extracts revealed improved pain reduction and healing potential than each substance alone. These results correlated positively with salivary EGF levels measured during the same observational periods.

[Maha Galal; Sherine Adel Nasry; Dina M. Mostafa and Nagwa M. Ammar. **Therapeutic Efficacy of Herbal formulations for Recurrent Aphthous Ulcer. Correlation with Salivary Epidermal Growth Factor.** *Life Sci J* 2012;9(3):2398-2406] (ISSN:1097-8135). http://www.lifesciencesite.com. 345

**Key words:** Medicinal plants; *Acacia nilotica*; Licorice; Aphthous ulceration; Epidermal growth factor**.**

## 1. Introduction

Recurrent aphthous ulceration (RAU) is the most common oral disease affecting 5-25% of the general population worldwide and is amongst the most prevalent and complicated disorders of the oral cavity (1). Several initiating factors have been implicated in the pathogenesis of RAU (2), however their role as the main etiological factors in the pathogenesis remains to be elucidated. Furthermore, with the dramatic worldwide increase in patients with immuno-suppression caused by medical treatments, systemic diseases, or both, the prevalence of RAU may be increasing (3).

The treatment of RAU still remains nonspecific and is based primarily on empirical data. The goals of therapy include the management of pain and functional impairment by suppressing inflammatory responses, as well as reducing the frequency of recurrences or avoiding the onset of new aphthae (4). Antibacterial, anti-inflammatory and anti-histaminic agents, analgesics, local anesthetics, and glucocorticoids have been used topically to manage RAU. Most of these therapies are associated with side effects or unwanted reactions (5).

Epidermal growth factor (EGF), also known as epithelial growth factor is the founding member of the EGF family of proteins. It is an amino acid peptide that is present in a variety of biologic fluids including saliva, and is also present in the mucosa lining the whole human digestive tract (6). EGF stimulates the division and proliferation of cells of various tissues including the oral epithelial cells (7). It plays an important physiological role in the maintenance of esophageal and gastric tissue integrity by aiding in the healing of oral and gastroesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis as well as protection of the mucosa from injurious factors (8). Preliminary studies assessing the role of EGF on mucosal healing have been conducted. Low salivary EGF levels have been observed in patients with various forms of oral mucosal disease (9),

suggesting an important role for salivary EGF in maintaining the integrity of oral epithelium (10).

There has been a substantial increase in the use of natural products including medicinal plants in primary health care. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals essential for the development of novel therapeutic agents that are safer and could be effective in the treatment of human diseases (11). Few medicinal herbs are listed as anti-aphthous agents. Quercetin, a plant flavonoid, has been shown to be effective in the treatment of RAU due to its structural similarity and functionalities with disodium cromoglycate (12). Zulene compounds found in several plant species, such as Anthemis nobilis and Matricaria recutita are reported to possess anti-allergic, anti-inflammatory and mild antibacterial activities. *Anthemis nobilis* is used for washing the mouth wounds and external use of Matricariarecutita is approved for mucous membrane inflammations including those of the oral cavity and gums (13).

*Acacia nilotica* willd L (subsp. Nilotica) belongs to Family Fabaceae, subfamily Mimosoideae. Previous phytochemical investigation of the genus Acacia resulted in the isolation of several types of chemical constituents including alkaloids, cyanogenic glycosides, gums, amino acids, terpenes, flavonoids saponins, mucilage and tannins (14). *Acacia nilotica* (A nilotica) has a potent antimicrobial activity, as it was demonstrated that the MDR strains of bacteria and fungi were sensitive to the antimicrobial activity of *Acacia nilotica*, whereas they exhibited strong resistance to the other tested plant extracts (15). *Acacia nilotica* mouth rinse showed effective antibacterial effect against halitosis inducing bacteria on the tongue (16), and is also used in the treatment of gingival bleeding and mouth ulcers (17).

*Glycyrrhiz glabra* (Licorice) also known sweet wood, family Leguminosae, contains several bioactive compounds including a water-soluble biologically active complex that accounts for 40-50 % of the total dry material weight. The beneficial effects of licorice can be attributed to a number of mechanisms. Glycyrrhizin and glycyrrhizic acid have been shown to inhibit growth and cytopathology of numerous RNA and DNA viruses, including herpes zoster and herpes simplex (19, 20). Licorice constituents also exhibit steroid like anti-inflammatory activity, similar to the action of hydrocortisone. This is due, in part, to inhibition of phospholipase A2 activity, an enzyme critical to numerous inflammatory processes. (21) *In vitro* research has also demonstrated glycyrrhizic acid to inhibit cyclooxygenase activity and prostaglandin formation (specifically prostaglandin E2),as well as indirectly inhibit platelet aggregation and all factors in the inflammatory process (21).

Glycyrrhizin has been used systemically for the treatment of chronic hepatitis C and oral lichen planus (OLP). In an open clinical trial, 17 hepatitis C-positive patients with OLP were given either routine dental care or 40 ml IV glycyrrhizin daily for one month. Among nine patients taking glycyrrhizin 66.7% noted improved clinical symptoms, such as decreased redness, fewer white papules, and less erosion of the mucosa. In the non-glycyrrhizin group of eight patients, only one (14.3 %) reported any improvement (22).

Concerning its topical use, a case report demonstrated a 2 % topical glycyrrhizic acid cream (carbenoxolone sodium) applied six times daily in 12 patients with acute oral herpetic infections resolved pain and dysphagia within 24-48 hours. Moreover, the accompanying ulceration and lymphadenopathy gradually healed within 24-72 hours (23). When Glycyrrhiz glabra was used as a mouth rinse, it had a significant effect on reducing plaque and gingivitis (24). As regards the effect on RAU, the use of the oral licorice mouthwash significantly reduced the average number of ulcers per day, pain scores, and the development of new ulcers (25). In another study of 20 RAU patients who were instructed to use de-glycyrrhizinated licorice mouthwash four times daily, fifteen patients experienced 50-75 % clinical improvement after only one day of using the mouth wash, with complete healing of canker sores after three days (26).

## 2. Materials and methods

### Plant material

### 1.Extraction of herbal actives from *Glycyrrhiz glabra* and *Acacia nilotica*

Medicinal plants were selected on the basis of their bioactive constituents, supportive reports and traditional uses. Leaves and pods of *A. nilotica* willd L and roots of Licorice were collected from Upper Egypt. Identification of the plant was confirmed by Prof. Dr. Laila Boulos, National Research Center, El-Tahrir Str., Dokki, Cairo, Egypt and compared with reference to herbarium specimens. Voucher Specimens were kept in the herbarium of the National Research Center.

Dried roots and rhizomes of *Glycyrrhiz glabra* and *Acacia nilotica* were extracted separately by the continuous extraction technique in succession using solvents with increasing polarity. The polar extracts were collected and investigated, using chromatographic and spectral methods; PC, CC, TLC, Preparative HPLC, LC/MS, High field NMR, 1H-NMR, (13) C-NMR (24), HMBC, HMQC, H1H1-COSY, ESI-MS (27), then lyophilized and saved for pharmaceutical preparation. The powdered air-dried

pods of *Acacia nilotica* and roots of licorice were defatted with CHC13 (3 x 3 L) and extracted with MeOH-H2O (7:3, 5 x3 L) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (200 g). Twenty grams of the dry residue were used for the biological study.

**Preparation and characterization of herbal adhesive pastes**

The pastes were prepared previously by heating weighed mixtures of polyethylene glycol, extract, glycerine and distilled water at 70 □ C then adding these aqueous components to the oily component made of sodium carboxymethyl cellulose, pectin and liquid paraffin heated to the same temperature. This was followed by the addition of tween 20 and mixing till obtaining a smooth homogeneous texture. The contents of glycyrrhizic acid and condensed tannins in liquorice and Acacia containing pastes, respectively, and in the pastes containing mixture of both extracts, were determined by spectrophotometric analyses (28). The herbal extracts were included in 2% composition of the formulations regarding single herb formulae and 1% of each herbal extract in the mix formula. The prepared pastes were exposed to storage at room temperature and at 40 °C for 2 months, where visual examination of physical aspects together with chemical analysis of active ingredients in the herbal pastes were conducted at zero time and after 2-months storage.

**Patients and methods:**

A total of 40 patients, 13 females (43.3%) and 17 males (56.7%) with mean age 26.5 years (18-35 years) and a current history of RAU were recruited from the patients attending at the Oral Medicine Clinic, College of Oral & Dental Surgery, Misr University for Science & Technology (MUST). The eligible subjects were informed regarding the purpose of this study. Before entering the study, each of them provided a signed consent to participate in this study. The protocol was approved by National Research Center Ethical Committee.

Only RAU cases with a history of at least two confirmed episodes of RAU during a 3-6 months non-treated baseline period were included in the present study. Exclusion criteria included patients with iron deficiency, inflammatory and allergic conditions, psychological disturbance, history of medication, smoking, pregnancy, wearing dentures, receiving antibiotics for RAU or those with special syndrome where aphthous ulcer is one of its symptoms (e.g. Behcet's syndrome); those with aphthous lesions older than 4 days, patients subjected to any other treatment for at least 4 weeks before the beginning of the study.

**Study design**

Subjects were instructed to contact the Research Study Coordinator at the first signs of an aphthous ulcer, at which point they were scheduled immediately for a screening session. Subjects satisfying the inclusion criteria were assigned randomly to one of the four-treatment groups namely Acacia nilotica (A), Licorice (L), Acacia &Licorice (A&L), and control group. Patients of each groups received herbal preparations formulated as muco-adhesive pastes and were instructed to apply the medication four times on the lesions after drying the tissues with a small sterile cotton pad and refrain from eating at least for 30 min after the drug application. Patients were examined for ulcer size, pain score and salivary EGF levels on treatment days 0, 2 and 5. Pain was recorded using the visual analog scale (VAS), which consists of a 10 cm line anchored by two extremes: no pain and pain that could not be more severe. Patients were asked to make a mark on the line representing their level of perceived pain (29).The size of the ulcer was measured with a Williams graduated periodontal probe that was held close to the photographed ulcer to calibrate measurements and determine the dimensions of the ulcer. A colored print of the image was used for subsequent analysis of size. Lesion dimensions were determined by a single oral medicine specialist who was blinded to the subject's status. The white ulcerous region of the lesion was outlined using a 0.5 mm medium lead pencil. The long axis of an ellipse across the ulcer was designated as the major axis length, while the minor axis was designated as the widest spot of the lesion perpendicular to the major axis. The length of each axis was measured on the photograph with a digital caliper and the length was corrected (to the nearest 0.5 mm) against the image of the periodontal probe (30).

**Saliva collection**

About 5 ml sample of whole unstimulated saliva (drool) was collected from each participant by the simple drooling method while sitting, where saliva was allowed to drip off the lower lip into calibrated tubes according to Wu-Wang *et al.* (40). Each sample was chilled was ice and then transferred to the laboratory, where saliva was thawed, centrifuged at 3000 rpm to remove any debris, diluted with phosphate buffer saline and stored at -70° C until required for analysis(41).

**Measurement of EGF**

Quantitative analysis of EGF was done using the DRG Human EGF ELISA (hEGF) kit (DRG International Inc., USA) which employs a competitive protein binding technique in which a biotinylated –hEGF competes with unlabeled h EGF for a limited number of specific antibody binding sites immobilized to the polystyrene wells. The percentage of antibody bound biotinylated –hEGF decreases as a function of

increasing unlabeled hEGF. The biotin groups are then determined by incubation with a streptavidin-horseradish peroxidase and subsequent color development. Absorbance which is inversely proportional to hEGF concentration, is measured with a suitable spectrophotometer. H EGF in samples is determined by comparison with a standard curve prepared with a series of h EGF samples of known concentration.

**Statistical analysis**

Data were presented as mean and standard deviation (SD) values. One-way Analysis of Variance (ANOVA) was used to compare between mean EGF and ulcer size in the four groups. **Tukey's** test for pair-wise comparisons was used to determine significant differences between groups when ANOVA test is significant.

Kruskal-Wallis test was used to compare between pain scale scores in the four groups. This test is the non-parametric alternative to one-way ANOVA test. Mann-Whitney U test was used in the procedure of pair-wise comparisons when Kruskal-Wallis test was significant.

**3. Results:**

**1. EGF level**

At day zero, there was no statistically significant difference between the four groups ($P$ = 0.06). At day 2 and at day 5, there was no statistically significant difference between (A & L) and (L) groups; both showed the highest mean EGF values, where the mean EGF level at day 2 was 66.8±5.1pg/ml in group (A& L) and 60.2± 4.8 pg/ml in the (L) group, and at day 5 the mean EGF value was 98.7± 13.9pg/ml for the (A& L) group and 85.1±9.9 pg/ml in the (L) group. This was followed by Group (A) with a mean value of 42.5 ±5.3pg/ml and 49.1±4.9 pg/ml at day 2 and day 5 respectively. The Control group showed the statistically significantly lowest mean EGF value, being 30.2±5.0 pg/ml at day 2 and 33±5.5pg/ml at day 5 (Figure 1).

As regards the % increase in EGF, there was no statistically significant difference between (A & L) and (L) groups; both showed the statistically significantly highest mean % increase in both days ($P$<0.001), where the mean % increase was 108.8±35.7 pg/ml in group (A&L), and 90.5±24 pg/ml in group (L) on day 2, and 221.9±56.5pg/ml in group (A&L), and 178.3±67.9 pg/ml in group (L) on day 5. This was followed by Group (A) with a mean increase if 22.8±8.3pg/ml and 42.4±13.1pg/ml on days 2 and 5 respectively. The control group showed the statistically significantly lowest mean % increase on both days being 20.3±6.8pg/ml on day 2 and 31.5 ± 9.5pg/ml on day 5(Figure 2).

**2. Pain score**

At day zero, there was no statistically significant difference between the four groups ($P$= 0.174).

At day 2and at day5, there was no statistically significant difference between Group (A) and control groups; both showed the statistically significantly highest mean pain scores (4.8+ 0.6 , $P$= 0.007 on day 2 and 4.7 + 0.5, $\boldsymbol{P}$ = 0.001 on day 5).This was followed by group (L) with a mean pain score of 3.5± 0.6 on day 2 and 3.1 ±1.6 on day 5. Group (A & L) showed the statistically significantly lowest mean pain score on both days ($P$ < 0.001), where their mean score read 2±0.6 on day 2 and 1.1 ±1.7 on day 5(Figure 3).

As regards the % decrease in pain score, Group (A & L) showed the statistically significantly highest mean % decrease in pain score on both days being 60 ± 24.6 on day 2 and 77.1 ± 33.5 on day 5. This was followed by group (L) with a mean score of 14.6 ± 10.2 and 22.9 ± 40.3 on days 2 and 5 respectively. The mean % pain reduction values on day 2were 2 ± 1.5 and 4± 0.7 for group (A) and the control group respectively, while on day 5 they showed a mean % of reduction of 2.1 ±5.2 for group (A) and 6 ±1.2 for the control group. Statistical comparison between both groups yielded the significantly lowest mean % decrease in pain score on both days ($P$< 0.001).(Figure 4).

**3. Ulcer size:**

At day zero, there was no statistically significant difference between the four groups. ($P$= 0.574). At day 2 and at day 5, there was no statistically significant difference between Group (A) and control groups; both showed the statistically significantly highest mean ulcer size ($P$ = 0.023 on day 2 and $P$ < 0.001on day 5), where the mean ulcer size at day 2 was 5.7±1.2 mm for group (A), and 6.5±1mm for the control group, and 5±0.8 mm for group (A) and 6.3±1.1 for the control group on day 5. Similarly no significant difference was found between the mean values of (A & L) and (L) groups on day2 ($P$ = 0.023), and day 5 ($P$ <0.001), both showed the statistically significantly lowest mean size, where the mean ulcer size of group (A &L) was 3.9±1.2 mm, and 1.1 ±0.5mm and that of group (L) was 4.3±1.4 mm and 2.6±1.4mm on day 2 and 5 respectively (Figure 5).

As regards the % decrease in size at day 2, there was no statistically significant difference between (A & L) and (L) groups; both showed the statistically significantly highest mean % decrease (53 ± 10.1, 43.4 ± 9.4 respectively. This was followed by Group (A) with a mean % reduction of 29.4 ±9.5. Control group showed the statistically significantly lowest mean % decrease (7.1 ± 5.3),( $P$ = 0.001).

As regards the % decrease in size at day 5, (A & L) group showed the statistically significantly

highest mean % decrease (87.4 ± 15.9). This was followed by group (L) with a % decrease of 43.4 ± 9.4, then group (A) with a mean % decrease of 38.1 ± 5. The control group showed the statistically significantly lowest mean % decrease (10 ± 7.3) (Figure 6).

**Table 1. Comparison between different parameters in the four groups**

| Variable \ Group | | A | A & L | L | Control | *P-value* |
|---|---|---|---|---|---|---|
| **EGF level (pg/ml)** | Day 0 | 34.6 ± 6.2 | 32 ± 4.8 | 31.6 ± 5.5 | 25.1 ± 4.3 | **0.060** |
| | Day **2** | 42.5 ± 5.3 [b] | 66.8 ± 5.1 [a] | 60.2 ± 4.8 [a] | 30.2 ± 5 [c] | **<0.001*** |
| | Day **5** | 49.1 ± 4.9 [b] | 98.7 ± 13.9 [a] | 85.1 ± 9.9 [a] | 33 ± 5.5 [c] | **<0.001*** |
| | **% increase** Day **2** | 22.8 ± 8.3 [b] | 108.8 ± 35.7 [a] | 90.5 ± 24 [a] | 20.3 ± 6.8 [c] | **<0.001*** |
| | **% increase** Day **5** | 42.4 ± 13.1 [b] | 221.9 ± 56.5 [a] | 178.3 ± 67.9 [a] | 31.5 ± 9.5 [c] | **<0.001*** |
| **Pain Score** | Day 0 | 4.9 ± 0.4 | 5 ± 0 | 4.1 ± 0.9 | 5 ± 0 | **0.174** |
| | Day **2** | 4.8 ± 0.6 [a] | 2 ± 0.6 [c] | 3.5 ± 0.6 [b] | 4.8 ± 0.6 [a] | **0.007*** |
| | Day **5** | 4.7 ± 0.5 [a] | 1.1 ± 1.7 [c] | 3.1 ± 1.6 [b] | 4.7 ± 0.5 [a] | **0.001*** |
| | **% reduction** Day **2** | 2 ± 1.5 [c] | 60 ± 24.6 [a] | 14.6 ± 10.2 [b] | 4 ± 0.7 [c] | **<0.001*** |
| | **% reduction** Day **5** | 2.1 ± 5.2 [c] | 77.1 ± 33.5 [a] | 22.9 ± 40.3 [b] | 6 ± 1.2 [c] | **<0.001*** |
| **Ulcer Size (mm)** | Day **0** | 8.1 ± 1.6 | 8.1 ± 1.3 | 7.4 ± 1.9 | 7 ± 1.3 | **0.574** |
| | Day **2** | 5.7 ± 1.2 [a] | 3.9 ± 1.2 [b] | 4.3 ± 1.4 [b] | 6.5 ± 1 [a] | **0.023*** |
| | Day **5** | 5 ± 0.8 [a] | 1.1 ± 0.5 [b] | 2.6 ± 1.4 [b] | 6.3 ± 1.1 [a] | **<0.001*** |
| | **% reduction** Day **2** | 29.4 ± 9.5 [b] | 53 ± 10.1 [a] | 43.4 ± 9.4 [a] | 7.1 ± 5.3 [c] | **0.001*** |
| | **% reduction** Day **5** | 38.1 ± 5 [c] | 87.4 ± 15.9 [a] | 67.5 ± 15.1 [b] | 10 ± 7.3 [d] | **<0.001*** |

Values are mean ± SD, *: Significant at $P \leq 0.05$Values, Different letters in the same row are statistically significantly different

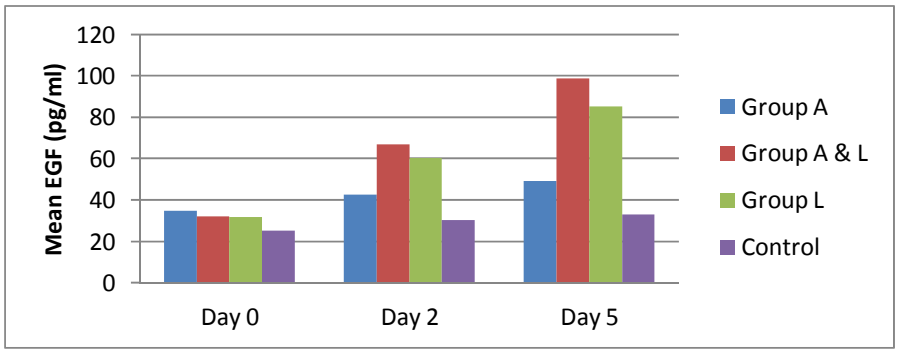


**Figure 1. Bar chart representing the mean EGF level before and after treatment in the four groups**

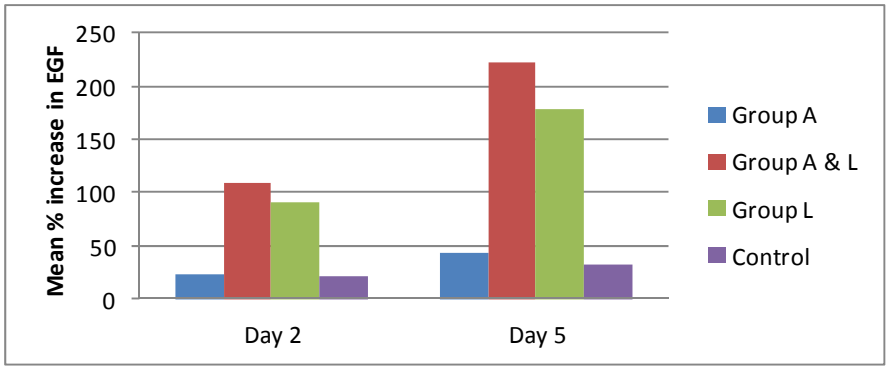


**Figure 2.Bar chart representing mean % increase in EGF after treatment in the four groups**

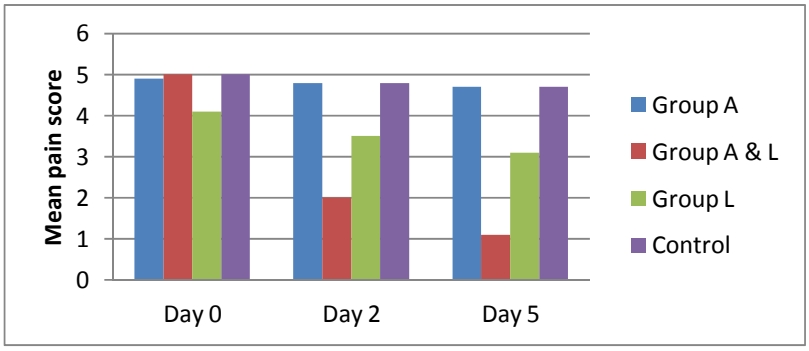


**Figure 3.Bar chart representing mean pain score before and after treatment in the four groups**

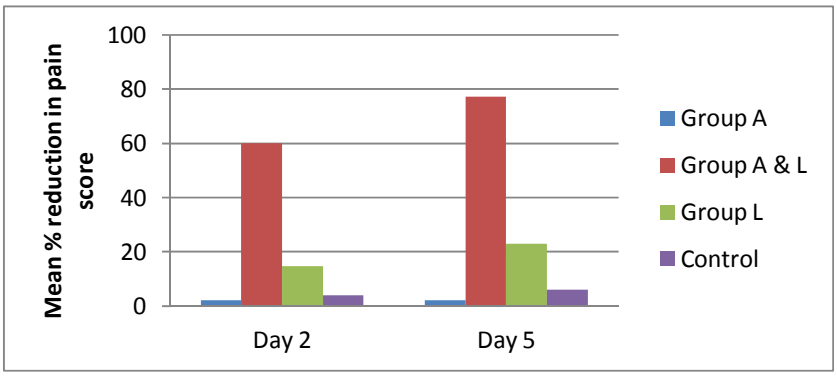


**Figure 4. Bar chart representing mean % reduction in pain score after treatment in the four groups**

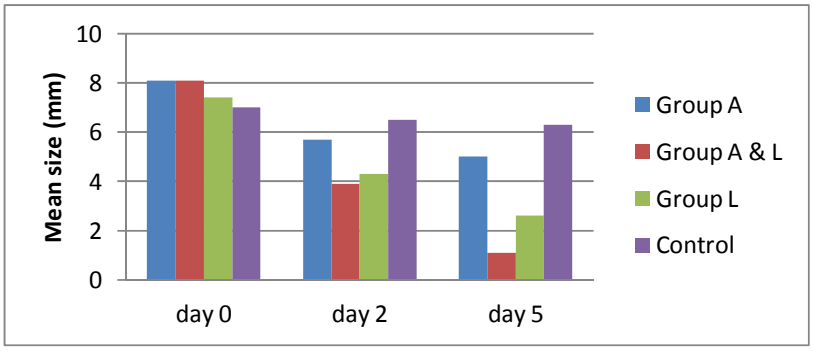


**Figure 5.Bar chart representing mean ulcer size before and after treatment in the four groups**

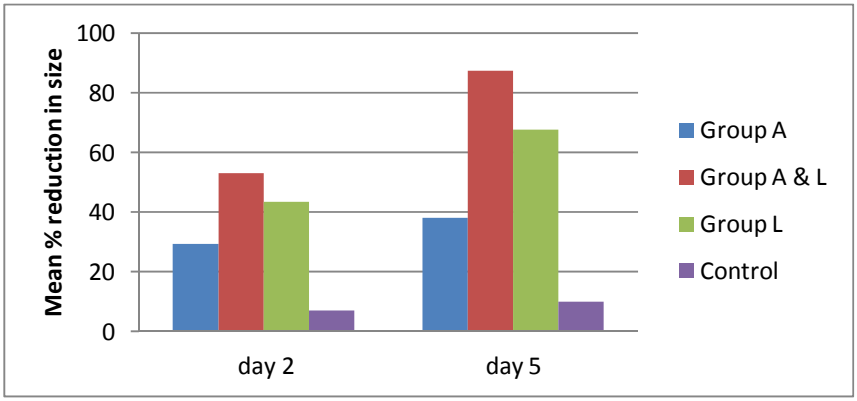


**Figure 6.Bar chart representing mean % decrease in ulcer size after treatment in the four groups**

**4. Discussion**

Recurrent aphthous stomatitis is the most common oral ulcerative condition affecting the general population, yet it is an important condition since it can be distressing and cause suffering and pain. In addition, it interferes with normal life activities by affecting eating and swallowing of the patients (1).

The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Many pharmacognostical and pharmacological investigations are carried out, to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of human diseases (32). The present investigation has been attempted as a step toward this goal.

Growth factors are mediators with essential importance in the normal repair process after wounding. It has been suggested that oral or juxtaoral injury stimulates increased synthesis and secretion of growth factors in the saliva (33). EGF in saliva is cytoprotective against injuries and contributes to the maintenance of the integrity of gastrointestinal mucosa(8). Low salivary EGF levels have been observed in patients with various forms of oral mucosal disease (9).

The purpose of this study was to compare the efficacy of three herbal mucoadhesive pastes comprising either *Acacia nilotica* (A.nilotica) pod extract or *Glycyrrhiza glabra* (Licorice) root extract or a mixture of both, as active herbal components in the management of RAU and to correlate this effect with salivary epidermal growth factors level through the same observation periods.

The main problems related to the use of herbal medicines in treatment of RAU are the low documentation of the use of these medicines, lack of standardized preparation, low acceptance by the medical communities and low patient's compliance. In the present study a complete literature search was carried out and medicinal plants were selected according to their existing scientific data.

Our plan of work was based on the preparation of therapeutic agents derived from economic medicinal plants cultivated in Egypt and the performance of the suitable pharmaceutical preparations producing low cost effective products useful in management of some oral diseases. Achievement of these goals was gained through collection and taxonomical identification of plants under investigation, preparation and phytochemical investigation of successive extracts using different chemical and chromatographic techniques, studying the biological activity of the different successive extracts and preparing the biologically active ingredients in the suitable pharmaceutical dosage form after being tested for toxicity.

Semisolid preparations including pastes are advantageous over liquid preparations regarding oral lesions because of longer retention time on the oral mucosa, thus allowing extended contact between the diseased area and the active ingredients of the preparations (34). Owing to this fact, the herbal extracts in the current study were formulated as adhesive pastes allowing lower frequency of application, more patient's compliance and relief, in addition to a sustained therapeutic effect.

Patients were instructed to use the drug locally four times daily and to retain the formulation on the lesion for 30 min. This duration is considered sufficient for the tested agents to exert their pharmacological action on the ulcerated tissue and the results of the study were in support of the applied localized manner. Assessment of the therapeutic effect was done at days 0,2 and 5 representing the different clinical stages of RAU (pre ulcerative, ulcerative and healing stages (35).

To our knowledge, no studies have been carried out on the efficacy of *A. nilotica* and Licorice mixture extract on the management of aphthous ulcers except what reported on the efficacy of Licorice extract or antibacterial effect of *A. nilotica* individually. Hence the discussion is originally focused on the results of this study.

Findings of this study revealed that at day zero, there was no statistically significant difference between the four groups as regards pain score, ulcer size and salivary EGF level. Regarding the results of pain and ulcer size, the study demonstrated that at days 2 and 5, there was no statistically significant difference between group (A) and control group; both showed the statistically highest mean pain scores and ulcer size. At the same observational periods, (L) group showed lower pain scores than (A) and control groups. The (A&L) group showed the statistically significantly lowest mean pain score. However the results of ulcer size at day 2 and 5, showed no significant difference between (A & L) and (L) groups; both showed the lowest mean size.

Investigating the individual effects, it was observed that Licorice extract showed better healing and pain reduction results when compared to the *A. nilotica* results. However, administrating a combination of both resulted in a superior and more significant healing and pain reduction effect when compared to each individually with a remarkable effect of licorice on pain reduction even when applied individually This could be explained by the fact that local anti-inflammatory agents create a helpful environment to speed up healing and relief symptoms in the management of RAU (36,37). The abundant

anti-inflammatory property of licorice in the present study created an aiding role in the healing process and pain relief when compared to other groups of the study(21). Moreover, de-glycyrrhizinated licorice formulations that were used in the treatment of gastric ulcers, promoted healing by increasing mucous production and blood supply to the damaged stomach mucosa, thereby enhancing mucosal healing (38). Accordingly, similar mechanism of action could be assumed in the healing of oral ulcers. On the other hand, Moghadamnia *et al.* reported a significant reduction in the severity of RAU following the application of bio-adhesive patches containing Licorice extract (39).The mechanical protection of bio-patches however is a very considerable factor for reducing symptoms of RAU and could exert an overlapping action on the effect of licorice in this investigation.

In agreement with the results of the present study, a previous study used a mouth wash form of de glycerinized licorice and showed a significant anti-aphthous effect (26). Nevertheless, the noticeable effect of *A. nilotica* in the current study could not be obscured. This effect may be either attributed to the antimicrobial effect reported for *A. nilotica* or alteration of the microbial flora of the mouth, which resulted in less immunologic damage as reported for the effect of some antibacterial mouth rinses against RAU (3,39). Combination of both plant extracts may present a probable synergism reported for some other combinations (40), allowing a measurable benefit from the use of both, the antibacterial effect of *A. nilotica* and the anti-inflammatory effect of Licorice. This combination enhanced the healing process with an additional improvement effect on aphthous ulcers in comparison with the other groups of the study resulting in better healing and favorable pain reduction.

As an evidence for the effect of the formulations on healing acceleration, the present investigation has also attempted to correlate between reduction of ulcer size and pain and salivary EGF level during different stages of RAU. Different studies clarified that salivary EGF levels diminished in the ulcerative stage than in healing or pre ulcerative stage (31,35). Our results were in accordance with those studies. At days 2 and 5, both of (A & L) and (L) groups showed the statistically significantly highest mean EGF values. This was followed by Group (A). Control group showed the statistically significantly lowest mean EGF value. Salivary EGF level was reduced during the active stage of RAU, that was associated with the development of the stomatitis. As the oral mucosa is constantly bathed in saliva, a diminished salivary secretion of EGF in sufferers from aphthae may weaken the cytoprotective functions of saliva on the oral epithelium (31). We also observed a differential, stage-dependent alteration of EGF levels in saliva in patients with RAU during the course of this disorder; the precise mechanism for these differential alterations is not clear. It is suggested that the synthetic mechanism for EGF in the salivary glands was impaired temporarily during the onset of aphthous ulceration and the onset might activate a self-defense mechanism by which the EGF synthesis recovered after remission (10, 31). It is reasonable to assume that the higher EGF mean values in group (A&L) than group (A) and control group, might be related to the combined anti-bacterial and anti-inflammatory effect of both Acacia and licorice respectively used by group (A&L). This combination created a field with diminished microbial and inflammatory challenge allowing enhanced activity of recovered EGF leading to accelerated healing of oral aphthous ulcers. On the other hand, the anti-inflammatory effect of Licorice either individually or mixed had a greater impact than the antimicrobial effect of *Acacia nilotica* on both the healing process and pain relief.

In conclusion, treatment of minor RAU using a mixture of Licorice and *Acacia nilotica* extracts revealed improved pain reduction and healing potential than each substance alone. These results correlated positively with salivary EGF levels measured during the same observational periods suggesting its potency as an effective product for treatment of minor aphthae.

**Recommendation**

Further studies are required to elucidate the probable mode of action of these plants with different concentrations and standardization of the products in comparison to other herbal pharmaceutical formulations. If similar results are confirmed in clinical trials, these plant extracts constitute a natural alternative to the conventional synthetic formulas utilized in current medications.

**Corresponding author:**
Sherine Adel Nasry,
Surgery and Oral Medicine Department, National Research Center, 33 El Bohouth St.(Ex El-Tahrir), Dokki, Cairo 12311, Egypt
Email: nasrysherine@yahoo.com

**References**

1. Porter SR, Scully C, Pedersen A. Recurrent aphthous stomatitis. *Crit Rev Food Sci Nutr.*, 1998; 9: 306-21.
2. Miles DA, Bricke SL, Razmus TF, Potter RH. Triamcinolone acetonide versuschlorhexidine for treatment of recurrent stomatitis. *Oral Surg Oral Med Oral Pathol., 1993*; 75: 397-402.
3. Ship JA. Recurrentaphthous stomatitis. *Oral Surg Oral Med Oral Pathol.*, 1996;81: 141-7.

4. Scully C, Gorsky M, Lozad F. The diagnosis and management of recurrent aphthous stomatitis. A consensus approach. *JADA* 2003; 134: 200-7.
5. Eisen D, Lynch DP. Selecting topical and systemic agents for recurrent aphthous stomatitis. *Cutis* 2001; 68: 201-6.
6. Weaver LT, Gonnella PA, Israel EJ, Walker WA. Uptake and transport of epidermal growth factor by the small intestinal epithelium of the fetal rat. *Gastroenterology* 1990; 98:828-37.
7. Waterfield MD. Epidermal growth factor and related molecules. *Lancet* 1989; 1:1243-6.
8. Tunio AM, Hobsley M. Epidermal growth factor in saliva and gastric juice: response to histamine. *Gut* 1995; 37:335-9.
9. Girdler NM, McGurk M, Aqual S, Prince M. The effect of epidermal growth factor mouthwash on cytotoxic-induced oral ulceration. A phase I clinical trial. *Am J Clin Oncol* 1995; 18:403-6.
10. Epstein JB, Gorsky M, Guglietta A, Le N, Sonis ST. The correlation between epidermal growth factor levels in saliva and the severity of oral mucositis during oropharyngeal radiation therapy. *Cancer* 2000;89: 2258-65.
11. Anne D, Haffajee BDS, Tina Y, Askell BS, Sigmund S, Socransky DDS. Antimicrobial effectiveness of an herbal mouth rinse compared with an essential oil and a chlorhexidine mouth rinse. JADA 2008; 139: 606-11.
12. Murray M T, Pizzorno JE. Aphthous stomatitis: Textbook of Natural Medicine, In:Pizzorno, J.E., Murray, M.T., (Eds.), 2ed edn, Churchill Livingston, Edinburgh, 1999;Vol. 2, p: 1085-7.
13. Wichtl M. (2001) Herbal Drug and Phytopharmaceuticals, English edition (N.G. Bisset,translated and edited), 2nd ed., Boca Raton, Fl, CRC Press, Boca Raton, F1, pp: 140-2.
14. Seigler DS. Phytochemistry of *Acacia sensu* Lato. *Biochemical systematics and Ecology* 2003, 31: 845-73
15. Khan R, Islam B, Akram M, Shakil S, Ahmed A, Ali M, Siddiqui M and Khan AU: Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 2009; 14:586-97
16. Galal M, El-Khouli A, El-Menoufy H, Afifi MS, Ammar NM. The efficacy of a new mouth rinse containing pod extract of *Acacia nilotica* L in the treatment of oral halitosis (clinical and microbiological study). Egyptian Dental Journal 2010; 56:2445-62.
17. Dabur R, Gupta A, Mandel T K, Singh DD,Bajpai V,Guray AM. Antimicrobial activity of some medicinal plants Afr J Trad CAM 2007; 4: 313 -8.
18. Obolentseva GV, Litvinenko VI, Ammosov AS, *et al.* Pharmacological and therapeutic properties of licorice preparations (a review). *Pharm Chem J* 1999;33:24-31.
19. Baba M, Shigeta S. Antiviral activity of glycyrrhizin against Varicella-zoster virus in vitro.*Antiviral Res* 1987;7:99-107.
20. Partridge M, Poswillo DE. Topical carbenoxolone sodium in the management of herpes simplex infection. *Br J Oral Maxillofac Surg* 1984;22:138-45.
21. Wang CY, Kao TC, Lo WH, Yen GC.Glycyrrhizic acid and 18β-glycyrrhetinic acid modulate lipopolysaccharide- induced inflammatory response by suppression of NF-KB through P13K p110σ and P110γ inhibitors, *J Agric Food Med* 201; 59: 7726- 33.
22. Da Nagao Y, Sata M, Suzuki H, *et al.* Effectiveness of glycyrrhizin for oral lichen planus in patients with chronic HCV infection. *J Gastroenterol.,* 1996;31: 691-5.
23. Partridge M, Poswillo DE. Topical carbenoxolone sodium in the management of herpes simplex infection. *Br J Oral Maxillofac Surg* 1984; 22:138-45.
24. Hassan AH. Applied pharmacognostical studies of Glycyrrhizaglabra, Plantago major and Chrysanthemum frutescens, useful in treatment of some oral diseases, MSci Thesis,Pharmacognosy, Faculty of Pharmacy, Cairo University 2010.
25. Poswillo D, Partridge M. Management of recurrent aphthous ulcers. *Br Dent J* 1984; 157: 55-7.
26. Das SK, Das V, Gulati AK, Singh VP.Deglycyrrhizinatedliquorice in apthous ulcers. *JAssoc Physicians India* 1989;37:647.
27. WasselG, Abdel Wahab SM, Aboutabl EA, Ammar NM, El-Fiki NM, Afifi MS. Phytochemical examination and biological studies of *Acacia nilotica* L. Willd and *Acacia farnesiana* L.Willd, Egypt. *J Pharm Sci* 1992; 33: 327-40.
28. Raja MS, Khan I, Perumal P, Srikakolapu SR, Gotteti SD. Quantitative analysis of glycyrrhizic acid in crude drug and its herbal formulation of UV spectrometry. *Arch ApplSci Res* 2010 ; 2: 184-189.
29. Rashwan WA. The Efficacy of acetaminophen–caffeine compared to ibuprofen in the control of postoperative pain after periodontal surgery: a crossover pilot study. J Periodontol 2009; 80:945-52.
30. Martin MD, Sherman J, Van der Ven P, Burgess J.A controlled trial of a dissolving oral patch concerning glycyrrhiza (licorice) herbal extract for the treatment of aphthous ulcers. *Gen Dent* 2008; 56:206-10
31. Wu-Wang Y, Patel M, Femg J, Milles M and Wang S L: Decreased levels of salivary prostaglandin E2 and epidermal growth factor in recurrent aphthous stomatitis *Archs oral BioL*1995; 40: 1093-8
32. Mothana RA, lindequist U, Gruenert R, Bednarksi P. Studies of the invitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island soqotra. *BMC complementary and Alternative medicine* 2009; 9:7.
33. Coflkun, Gönül, Acartürk, Take, Erdogan . The effect of EGF-PEG Bead Implantation in Oral Mucosal Wound on the Rabbit Salivary Gland ,Trace Element Levels and EGF Receptor Immunoreactivity.FABAD J. Pharm. Sci.
34. Bhalodia R, Basu B, Garala K, Joshi B, Mehta K. Bucco adhesive drug delivery systems: A review. *Int J Pharm Bio Sci.,* 2010; 1:1-32.
35. Adişen E, Aral A, Aybay C, Gürer MA. Salivary epidermal growth factor levels in Behçet's disease and recurrent aphthous stomatitis. *Dermatology* 2008; 217:235-40.
36. David and Mcbride .Management of Aphthous Ulcers.*Am FaPhysician* 2000; 62:149-54.
37. Moghadamnia A, Motallebnejad M, Khanian M. The Efficacy of the Bioadhesive Patches Containing Licorice Extract in the Managementof Recurrent Aphthous Stomatitis. *Phytother Res 2009;* 23: 246-50.
38. Goso Y, Ogata Y, Ishihara K, Hotta K. Effects of traditional herbal medicine on gastric mucin against ethanol-induced gastric injury in rats. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1996;113:17-21.
39. Meiller TF, Kutcher MJ, Overholser CD, Niehaus C, DePaola LG, Siegel MA. Effect of an antimicrobial mouth-rinse on recurrent aphthous ulcerations. Oral Surg. Oral Med Oral Pathol 1991; 72: 425-9.
40. Wilt TJ, Ishani A, Stark G, MacDonald R, Lau J, Mulrow C. Saw palmetto extract for treatment of benign prostatic hyperplasia: a systematic review. *JAMA 1998;* 280: 1606-9.