Comparative study of the antioxidant effects of lavender and flax oils in recurrent aphthous ulceration treatment

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ABSTRACT

Background: Recurrent aphthous ulceration (RAU) is an inflammatory condition of unknown etiology characterized by painful recurrent (single or multiple) ulcerations of the oral mucosa. It is one of the most common and poorly understood mucosal disorders. It occurs more frequently in times of stress. Local and systemic conditions, genetic, immunologic, microbial factors, and oxidative stress may play a role in the pathogenesis of RAU. The objective of this study was to evaluate the free radical metabolism and antioxidant activity of RAU patients treated by lavender or flax oil paint.

Methods: Sixty-six RAU patients were enrolled in this prospective randomized double-blind placebo-controlled study. Analysis of the plasma levels of malondialdehyde (MDA), glutathione (GSH), Nitric oxide (NO), myeloperoxidase (MPO), Vit. E, Vit. C, and Trolox equivalent antioxidant capacity (TEAC) were determined. The ulcer size, healing time, and healing process were correlated to the plasma levels of the studied parameters for the assessment of the antioxidant mechanism of action of lavender and flax oil paint in the treatment of RAU.

Results: Statistical analysis showed that lavender antioxidant action was higher than flax oil paint compared to placebo. The decreased oxidative stress and the damage caused by free radicals was significant in the lavender group than both flax group and placebo (P<0.001).

Conclusion: This study proves the significant antioxidant mechanism of both lavender and flax in the treatment of RAU patients.

Keywords: Recurrent aphthous ulceration (RAU), oxidative stress, antioxidants, lavender, flax. (Received: 15/11/2019; Accepted: 30/12/2019).

INTRODUCTION

Recurrent aphthous ulceration (RAU) is a chronic inflammatory disease of oral mucosa categorized by single or multiple painful ulcers in non-keratinized mucosa. (1,2) The main factors linked to RAU include genetic factor, hematologic deficiencies, immunologic abnormalities and other factors such as trauma, stress, and food allergy. (3,4) All those conditions can lead to oxidative stress by disturbing oxidant–antioxidant balance of organism and accelerate the formation of free radicals.

The cytotoxic effects of free radicals are harmful for mammalian cells that lead to different diseases such as inflammatory condition, ulcers, cancer, and aging. (5,7) Mucosa membranes are protected by natural free radical scavengers which include the antioxidant vitamins, superoxide dismutase, glutathione peroxidase (GSH), reductase, and catalase.

Malondialdehyde (MDA) is a stable end-product of peroxidation of membrane lipids by free radicals and it is an indicator of increased lipid peroxidation, MDA caused the disturbance of structure and function of cell membranes. (8,9) Antioxidants commonly neutralize radicals via a hydrogen atom transfer (HAT) or single electron transfer (SET) mechanism. Although the products of ROS- induced oxidative stress are extensively used to monitor their biological effects, it is also important to evaluate the antioxidant capacity of biological fluids, cells, and extracts. Trolox Equivalent Antioxidant Capacity (TEAC) Assay measures the total antioxidant capacity of biomolecules from a variety of hydrophilic or lipophilic samples. (10) The TEAC Assay is based on the conversion of oxidized 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS·+) radical to ABTS via SET or HAT antioxidant mechanisms. (10)

Lavender has been used for centuries as a herbal remedy. Lavender oil has in vitro antimicrobial activity against bacteria, fungi and some insects. Lavender’s essential oil exerts spasmylic activity in smooth muscle in vivo, supporting its historical use as a digestive aid. Topical lavender oil displayed the stronger antioxidant activity in a linoleic acid model system and good antibacterial activity. The broad antibacterial and analgesic activities of lavender oil may explain its efficacy.

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in treating and healing of RAU. Lavender oil may have another action such as anti-inflammatory and immune enhancer; these may explain its ability in treating RAU.

Consumption of Flaxseed exhibits potential health benefits in reducing the risk of cardiovascular disease, cancer and diabetes mellitus. Flax paint reduced the time to repair mucosal tissue (ulcer healing), and pain persistence, it has both anti-inflammatory and analgesic effect.

The aim of the present study was to assess the healing process and free radical metabolism status in RAU patients treated with lavender or flax oils compared with placebo therapy, in relation to the size and type of ulcer.

MATERIALS AND METHODS

Patients: A prospective randomized double-blind placebo-controlled study was carried out at the Oral Diagnosis Department, College of Dentistry, Hawler Medical University, Iraq. This study was approved by the Institutional review board and the ethics committee. Written consent was given by all participants according to the Declaration of Helsinki (2018). The study group composed of sixty-six patients with an age ranges 18-46 years, who were diagnosed with recurrent aphthous ulceration. All were selected on the bases of history and clinical features. Patients with any systemic disease, who received any therapeutic regimen during the past three months, were excluded from the study.

Data collection: Demographic details of the enrolled patients such as name, age, gender, occupation and address were recorded. RAU is classified with respect to frequency and duration of ulcers according to the history and presentation. Duration in days of the current ulcer and the number of years that they had experienced similar ulcers were recorded. They were examined clinically, and their RAU status was recorded. The classification of RAU presentation was based on the WHO classification with respect to size, shape, site and healing status as minor, major ulcers. The number of present ulcers and their location was also recorded.

Formulation of paints:

Lavender oil paint is composed of standard lavender oil in glycerin (glycerol). Both constituents are authorized by European Pharmacopoeia V (EU) for the oral use. Placebo is free from active constituents.

The product is presented in white plastic 10 mL bottle containing approximately 9 mL of paint.

The same procedure was used for flax oil paint preparation.

Plasma preparation: The blood samples were collected from all enrolled patients under aseptic protocol by vein puncture. Venous blood (3 cc) was withdrawn with a 5 ml disposable syringe with a 24-gauge disposable needle in to a plain vacutainer containing heparin as anticoagulant. The blood was centrifuged at 1000 g for 10 minutes at 40°C to obtain plasma, which was stored in small aliquots at -200°C.

The patients were asked to participate after a routine dental examination and explanation of the study aims. The patients’ group was selected following a detailed case history and clinical examination. All participants, who were not currently taking any medications, were nonsmokers, and free from any periodontal disease.

Randomly, the patients group distributed as acute, minor RAU lesions diagnosed by a dentist during the collection of plasma samples and had experienced oral ulcer attacks at least three times a year.

The patients randomly were divided into three groups:

Group I: Patients administered Lavender oil (n=23).
Group II: Patients administered Flax oil (n=23)
Group III: Placebo control group (n=20)

The treatment was applied topically to the ulcerated area three times daily.

Free radical metabolism status & Antioxidant Assay

Malondialdehyde (MDA) Assay

Assessment of MDA level, which used a derivatization step that protein-bound MDA was hydrolyzed (60 min at 95°C) and converted into a fluorescent probe, this fluorescent probe was cooled to 2-8°C, centrifuged at 10,000 g for 5 min, mixed with a reaction solution and injected into the HPLC (High-performance liquid chromatography) system (Agilent Technologies 1200 series, USA; kits: Immuchrom GmbH, Germany). The isocratic separation via HPLC at 30°C, using a reversed-phase column (Bischoff Prontoisil Eurobond, 5 mm, 125 mm x 4 mm; Germany), lasted for 4 min for each sample. The chromatograms were recorded by a fluorescence detector. Quantification was performed with the calibrator from the kit.

Glutathione (GSH) Assay

Measurement of GSH levels, during the derivatization reaction, glutathione was converted
into a fluorescent probe. A subsequent precipitation step removed high molecular weight substances, after centrifugation at 10,000 g for 5 min, the fluorescent probe was cooled to 2-8°C and injected into the HPLC system. The isotropic separation via HPLC at 30°C was performed with a reversed-phase column (M2 Inertsil ODS, 5 mm, 125 mm x 4 mm) in 2 runs. Each of these runs lasted 4 min. The chromatograms were recorded by a fluorescence detector. The quantification was performed with the delivered EDTA-blood calibrator; the concentration was calculated using the internal standard method.

Nitric Oxide (NO) Assay

The NO values were given as the sum of nitrite and nitrate, which are the stable end products of NO. The nitrate levels in the samples were spectrophotometrically determined based on the reduction of nitrate to nitrite by VaCl3. Nitrite levels were measured by the Griess reaction. Measurement of NO was done by a modified spectrophotometric method used by Green et al. Sodium nitrite and nitrate solutions (1, 10, 50, and 100 mM) were used as standards.

Myeloperoxidase (MPO) Assay

Assessment of MPO activity is made by measuring the H2O2-dependent oxidation of o-dianisidine. The MPO activities in the samples were determined with a modified spectrophotometric method (Shimadzu UV mini-1240, Japan) used by Colowick and Kaplan. One unit of enzymatic activity was defined as the amount of MPO that caused a change in the absorbance of 1.0/min at 410 nm at 37°C.

Vitamne E and Vitamne C Assay

The level of vitamins E, and C were determined according to the manufacturer kit method procedure of enzyme-linked immunosorbent assay (ELISA).

TEAC Assay

Plasma TEAC was measured according to the Rice-Evans and Miller method. TEAC Assay Kit measures the total antioxidant capacity within a sample. Samples were compared to the known concentrations of Trolox standards within a 96-well microtiter plate format. Samples and standards were added to the microplate well, and upon the addition of the primed ABTS probe, the reaction proceeds for a few minutes. The reaction was read with a standard 96-well spectrophotometric microplate reader at 405-415 nm. Antioxidant capacity was determined by comparison with the Trolox standards.

Statistical analysis

All data were reported as means ± SD. All statistical analyses were performed using the SPSS version 18.0 statistical software package (SPSS Inc., USA). The data were analyzed with the Mann-Whitney U-test, Chi-square test, and independent t-test to find the significance of the difference in levels of antioxidants between the study and placebo groups in plasma and the significance of the different levels of antioxidants in patients having single and multiple ulcers and it was performed to compare mean values of antioxidant levels between minor and major type of ulcers among the study group. Pearson correlation was performed to analyze the correlation of antioxidant levels with respect to the duration of current ulcers in days and recurrence of ulcers in years. The level of significance was set at P<0.05.

RESULTS

Demographic data: The enrolled study groups (I & II) comprised 46 patients of which females were twenty-five (n=25), and males were twenty-one (n=21). The 20 patients in placebo group (III) consisted of 11 females and 9 males. The minimum age was 18 years in both groups and the maximum age was 46 years. The most common patients were in the age group of 22-35 years for both groups. The mean age was found to be 24.5, 21.2, and 26.3 years for groups I, II, and III, respectively (Table 1).

Clinical features: In the study groups, 48 (73%) out of 66 patients were with single ulcer, while 18 (27%) patients were with multiple ulcers. Ten Patients with multiple ulcers were seen with (56%) (of the two study groups) with two ulcers and eight (44%) were seen with three or more ulcers.

Out of the 66 patients, 49 (74%) had minor RAU, and 17 (26%) had major RAU (Table 1).
Table 1: Demographic and clinical features of patients with recurrent aphthous ulceration

| Demographic data: | Group I Lavender oil | Group II Flax oil | Group III Placebo control |
|-------------------|----------------------|-------------------|---------------------------|
| Mean Age (years)  | 24.5                 | 21.2              | 26.3                      |
| Gender            | Female: N=25          | Male: N=21        |                           |
| Age group (years) | 22-35                | 21-40             |                           |

Clinical features:

|                      | Pre-treatment | Post-treatment |
|----------------------|---------------|---------------|
| Single ulcer         | 73%           | 51%           |
| Multiple ulcer       | 27%           | 25%           |
| Type of ulcer        | Minor: 74%    | Major: 26%    |

Assessment of inflammation and ulceration: Both ulceration and inflammation decreased significantly post-treatment by lavender and flax oil paint (P=0.001), from 4 to 0 (severe to none) or healed ulcers, as shown in figure (1).

Table 2: Plasma values of antioxidant parameters of enrolled patients’ groups.

| Patients’ groups | MDA (µmol/L) | GSH (U/mg protein) | NO (µmol/L) | MPO (U/mg protein) | Vit. E (µg/ml) | Vit. C (µg/ml) |
|------------------|--------------|--------------------|-------------|--------------------|----------------|----------------|
| G I: Pre-Tx      | 1.4±0.85     | 21.23±1.84         | 15.94±2.24  | 17.94±2.88         | 13.07±0.43     | 1.23±0.05      |
| G II: Pre-Tx     | 1.49±0.76    | 20.42±1.6          | 16.1±1.84   | 17.96±2.01         | 13.16±0.32     | 1.28±0.04      |
| G III: Pre-Tx    | 1.47±0.75    | 21.43±1.66         | 15.98±2.01  | 17.97±2.67         | 13.1±0.23      | 1.33±0.03      |
| G IV: Post-Tx    | 1.49±0.73    | 20.03±1.36         | 14.98±2.09  | 18.27±2.18         | 13.9±0.21      | 1.43±0.04      |

Group I: Patients administered Lavender oil, Group II: Patients administered Flax oil, Group III: Placebo control group. Pre-Tx: Pre-treatment, Post-Tx: Post-treatment. MDA: malondialdehyde; GSH: glutathione; NO: nitric oxide; MPO: myeloperoxidase; vitamin E: Vit. E, vitamin C: Vit. C, Trolox equivalent antioxidant capacity: TEAC. * P<0.05, compared to control (Mann-Whitney U-test, Levene test, and t-test).

Assessment of Trolox equivalent antioxidant capacity (TEAC) showed an elevation in lavender oil group (I) more than flax oil group (II) compared to placebo group (III), the plasma levels were 0.85, 0.9, 0.87 pre-treatment, and 2.6, 2.1, 0.76 post-treatment in the groups I, II, III, respectively, (P=0.001) (Figure 2).
Comparative Study of

Figure 2: Trolox equivalent antioxidant capacity (TEAC) plasma values (µmol Trolox Eq/L) of tested groups I, II, and III, pre- and post-treatment. Group I: Patients administered Lavender oil, Group II: Patients administered Flax oil, Group III: Placebo control group.

Correlation of antioxidants with respect to duration of ulcers: Pearson correlation was performed to analyze the correlation of antioxidants with respect to the duration of the current ulcers in days (Figure 3).

Figure 3: Antioxidant plasma levels according to the duration of current ulcer in the tested groups. MDA: malondialdehyde (µmol/L); GSH: glutathione (U/mg protein); NO: nitric oxide (µmol/L); MPO: myeloperoxidase (U/mg protein); Vit. E: vitamin E (µg/ml), Vit. C: vitamin C (µg/ml). TEAC: Trolox equivalent antioxidant capacity (µmol Trolox Eq/L).

There was a positive correlation of antioxidant parameters MDA, GSH, NO, MPO, Vit. E, Vit. C, TEAC and the duration of current ulcer in the tested groups (Figure 4).
Comparison of antioxidants among the types of recurrent aphthous ulcers:
Analysis of MDA, GSH, NO, MPO, Vit. E, and Vit. C in comparison to the type of ulcer showed no significant difference; 1.4±0.85, 21.23±1.84, 15.94±2.24, 17.94±2.88, 13.07±0.43, 1.23±0.05, 0.90±0.04 of minor ulcers, respectively, while the values of same parameter in major aphthous ulcers were as follows; 1.48±0.54, 21.87±1.44, 16.33±2.18, 18.21±2.21, 13.42±0.18, 1.38±0.08, 0.94±0.08, respectively (Table 3).

Table (3): Plasma values of tested parameters to the type of recurrent aphthous ulcers.

| Type of ulcer | MDA (µmol/L) | GSH (U/µg protein) | NO (µmol/L) | MPO (U/µg protein) | Vit. E (µg/ml) | Vit. C (µg/ml) | TEAC (µmol Trolox Eq/L) |
|---------------|--------------|--------------------|-------------|--------------------|----------------|----------------|------------------------|
| Minor         | 1.4±0.85     | 21.23±1.84         | 15.94±2.24  | 17.94±2.88         | 13.07±0.43     | 1.23±0.05      | 0.90±0.04              |
| Major         | 1.48±0.54    | 21.87±1.44         | 16.33±2.18  | 18.21±2.21         | 13.42±0.18     | 1.38±0.08      | 0.94±0.08              |

MDA: malondialdehyde; GSH: glutathione; NO: nitric oxide; MPO: myeloperoxidase; Vit. E: Vit. E, Vit. C: Vit. C; Trolox equivalent antioxidant capacity: TEAC. * P<0.05, compared to control (Mann-Whitney U-test, Levene test, and t-test).

DISCUSSION
The cellular damage caused by reactive oxygen species (ROS) has been emboled in the development of many disease states, such as cancer, cardiovascular disease, and neurodegenerative diseases. In normal physiological status, the cellular ROS generation is counterbalanced by the action of cellular antioxidant enzymes, macro or micro molecules, as well as other redox molecules. Antioxidants include both hydrophilic and lipophilic molecules for metabolizing ROS. These may be localized transiently within different tissues or cells. Due to their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms. Oral ulcers are recognized as the most common oral mucosal diseases worldwide. The aim of this study was to assess and to evaluate the free radical metabolism status & Antioxidant action in plasma samples from Lavender or flax treated oral ulcer patients in comparison with placebo (control). There have been different studies about impaired oxidant–antioxidant balance in RAU patient. Cimen et al. (2003) mentioned that the increased MDA level and decreased antioxidant enzymatic system (SOD, GSH, and CAT) in RAU patients.
Yet, Arikan et al. (2009) recorded a significant increase in plasma MDA and decreased GSH levels, and vitamin E levels in patients with RAS.\(^{(18-22)}\)

This study agrees with those researches in which the MDA (an indicator of lipid peroxidation) levels were elevated significantly pre-treatment in enrolled RAU patients and declined significantly post-treatment. GSA was decreased significantly pre-treatment and increased significantly post-treatment in lavender users more than flax oil paint patients, which support the antioxidant action of lavender and flax in size reduction of ulcer and healing, in addition to decline in healing time.

The imbalance between free radicals and antioxidant system causes more inflammatory oral pathologies.\(^{(23,24)}\)

The plasma levels of NO were found to decrease in RAU patients and elevated after treatment by lavender or flax oil paint compared to placebo, this study disagrees with the research made by Gunduz et al.\(^{(25)}\) who reported no significant change in plasma NO levels in RAU and Bechet’s disease.

Myeloperoxidase (MPO) plasma level was elevated in RAU patients and after treatment by lavender and flax oil paint, which explains the antioxidant activity and free radical scavenger effects of the tested materials.

In this study, the authors found that the plasma levels of some nonenzymatic indicators of the antioxidant protective system (vitamins E, and C) have a significant difference after treatment by lavender or flax in RAU patients compared to the placebo control group (\(P=0.01\)).

This study showed that plasma levels of vitamins E, and C were significantly higher pre-treatment and significantly lowered post-treatment by lavender more than flax oil paint compared to placebo with RAU.

The acquired and environmental situation rather than genetic and inheritance factors have a role to influence the level of oxidant and antioxidants status and these factors may explain the difference.

Trolox antioxidant capacity level was measured in plasma from RAU patient treated by lavender or flax oil paint in this research, showed that the level of TEAC elevated significantly in lavender group more than flax group compared to placebo, which proves the antioxidant mechanism of action in the process of ulcer healing. Lavender has been used for centuries as an herbal remedy. Its major clinical benefits are on the central nervous system. Many studies support its use as a sedative, anxiolytic and mood modulator.\(^{(11)}\)

This study proved the antioxidant activity of lavender and flax oil paint in the healing process of ulceration by reduction of ulcer size and healing time more than flax oil paint regarding its antioxidant mechanism of action.

Flax is the richest source of α-Linolenic acid (ALA) in the North American diet. It’s converted to two major metabolites in vivo, the long-chain &-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) after elongation and desaturation.\(^{(26,27)}\) Many studies supported the hypothesis that -3 fatty acid intake, especially in the form of eicosapentaenoic acid (EPA) and DHA, have anti-inflammatory properties effects.\(^{(28-30,31)}\) Omega-3 fatty acids have been shown to suppress oxygen-free radicals from neutrophils and monocytes, as well as the production of interleukin-1, tumor necrosis factor, and leukotriene B4.\(^{(32)}\) This explains the antioxidant activity of flax oil.

The comparison of antioxidant levels between the single and multiple ulcers as well as between the types of ulcers showed a positive significant correlation. The comparison of antioxidant levels between minor and major ulcers reveals that there is a difference between them, the decline level of GSH, NO, and TEAC and elevation of plasma levels of MDA, MPO, Vit. E and C in major ulcer more than minor aphthous ulcer plays a significant role in ulceration and oxidative damage, the usage of lavender or flax oil paint back the parameters value to the normal due to its characteristics as powerful antioxidants to fight against and scavenge the free radicals and oxidative stress that lead to ulceration, and finally promote healing process, reduce ulcer size and decrease the healing time.

Oxidative stress, occurring because of imbalance between the formation of free oxygen radicals and inactivation of these species by antioxidant defense system, which cause damage to various cellular and extracellular constituents; this harmful effect of increased oxidative stress is called oxidative damage that appear after exposure to a relatively high concentration of reactive oxygen species (ROS) and/or a decrease in antioxidant defense system against ROS.

The usage of drugs or materials have antioxidant ability to either increase the internal antioxidant or decrease the oxidants by scavenging the free radicals leading to decline in the harmful oxidative damage, which explain the impact of lavender and flax oil in decreasing the harmful oxidant free radicals and elevation of antioxidant parameters or scavenge the harmful oxidative damage free radicals.
CONCLUSION
This study provides information about the activity of free radical metabolism and antioxidant levels in plasma of RAU patients treated with lavender or flax oil paint. It can be concluded that the antioxidant action of lavender and flax has an important role in the healing process of oral ulcers.

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الخلاصة
الخلفية: التقرح القلاعي المتكرر (RAU) هو حالة التهابية لمسببات مرضية غير معروفة تتميز بفرح متكررة مؤلمة (مفردة أو متعددة) في الغشاء المخاطي لللم. إنها واحدة من اضطرابات الغشاء المخاطي الأكثر شيوعا. يحدث بشكل متكرر في أوقات التوتر. قد تلعب الظروف المحلية والجهائية والعوامل الوراثية والمادية والبيئية والأدوات التأكسدية دورًا في النسب، كان الهدف من هذه الدراسة هو تقييم عملية التمثيل الغذائي للجذور الحرة ومضادات الأكسدة لمرضى التقرح القلاعي المتكرر الذين عولجوا بواسطة زيت اللافندر أو الالافندر.
الطريقة: تم تسجيل ستة وستين مريضة بالقرح القلاعي المتكرر في هذه الدراسة العشوائية التي تسيطر عليها وهمي. تحليل مستويات البلازما من مالوديايلد (MDA)، الجلوتاثيون (GSH)، أكسيد الأنيتيك (NO)، أكسيد البيروكسيديز (MPO)، فيتامين C، فيتامين E، فيتامين C، فيتامين E. تم تحديد مضادات الأكسدة عادل مطلاق ترولك (TEAC). ارتبط حجم القرحة، ووقت الشفاء، وعملية الشفاء بمستويات البلازما للمعايير المدروسة لتقييم آلية عمل مضادات الأكسدة لعمل طلاء زيت اللافندر والكان في علاج RAU.
النتائج: أظهر التحليل الإحصائي أن علاج الخزامى يكون له تأثير مضاد للأكسدة أعلى من طلاء زيت الالافندر بمقارنة مع الدواء الهمي. كان تقليل الضغط التأكسدي والضرر الناجم عن الجذور الحرة معنوي في مجموعة الخزامى أكثر من مجموعة الالافندر، p <0.001.
الاستنتاج: هذه الدراسة تثبت الآلية المضادة للأكسدة الهامة لعمل الخزامى والكان في علاج مرضى التقرح القلاعي المتكرر.