BACKGROUND: Aloe vera is a vitamin and mineral-rich plant with numerous health and skin-care applications. Aims of the study: To determine how Aloe vera affects the IL-6 gene expression in an adult male New Zealand rabbit.

Material and Methods: In this experiment, 48 male New Zealand rabbits were used. In each rabbit, ketamine and xylazine were administered intramuscular into the thigh muscle. In less than 5 minutes, an appropriate level of anesthesia was established. In the operating room, animals were placed on the operating table. For unipolar cauteration, electrosurgical generator cautery was used throughout the procedure. We burned 0.5 cm of the rabbits’ buccal oral mucosa with a unipolar electrocautery pen at 95.5°C. After that, the rabbits were placed into four groups, each with 12 rabbits: Group 1 (normal oral mucosa without burning), Group 2 (burning oral mucosa without medicine), Group 3 (Aloe vera plant/fresh gel), and Group 4 (Aloe vera/commercial gel). Each group’s animals were euthanized on days (3), (7), and (14) of the trial, resulting in a total of 12 rabbits slaughtered during each medication period. The IL6 gene expression in tissue was determined using Polymerase Chain Reaction (PCR).

Result: The untreated burn site had the greatest amount of IL-6 gene expression throughout the experiment, while the burn site treated with Aloe vera gel had the lowest level of IL-6 gene expression.

Conclusion: Fresh Aloe vera gel and commercial Aloe vera gel are thought to have a significant anti-inflammatory impact by lowering the amount of inflammatory mediator (IL-6) and so promoting wound healing.

Keywords: Aloe vera, Burning oral mucosa, IL6 gene expression, Rabbits, Wound healing.

Introduction
Phytomedicine or herbal medicine is the oldest form of healthcare known to human. It is based on natural resources employment as a source of potential drug substance, herbal medicine utilizes plant natural products as medication after isolation of their active constituents [1].

Aloe vera health applications include its use in wound healing, burns therapy, frost bite damage lessening, skin protection against damage from UV and x-rays, immune system improvement, and more other promising health potentials [2].

Wound healing process represents the body response to tissue injury which resulting in restoration of tissue integrity. It is a highly complicated, but well-orchestrated cascade of events, composed of three overlapping
and interrelated phases of hemostasis and inflammation, proliferation or fibroplasia, and remodeling and maturation [3].

It has been shown that many cell types’ proliferation is stimulated by *Aloe vera* extracts or other plant parts [4]. Recent research has demonstrated that using entire *Aloe vera* gel or extracts in vivo accelerated wound healing with noticeably increased angiogenesis and consequent oxygen availability [5].

The post-burn pathophysiological process has been thought to be significantly influenced by cytokines. It is understood that the local generation of proinflammatory cytokines at the site of tissue injury or infection will activate host non-specific immunity [6]. The tissue macrophages were the principal producers of the first-wave cytokines, which included interleukin-1 (IL-1) and tumor necrosis factor (TNF). Neutrophils can be produced later in addition to the second-wave cytokines, which typically have chemotactic properties like IL-6 and IL-8. Localized inflammation and its systemic effects may result from the cascade release of those secondary cytokines and hormone factors [7]. Except during infections, trauma, or other stressful situations, interleukin-6 (IL-6) is a proinflammatory cytokine that is strictly regulated and expressed at low levels. Inflammation is linked to an increase in IL-6 expression and production. If the appropriate inflammatory response is not induced, wound healing is retarded [8].

Improved endothelial-leukocyte connections and decreased IL6 gene expression are the results of active components of *Aloe vera*, such as acemannan, which can inhibit or suppress the serial sequence of TNF [9].

This study was aiming: to study the effect of application of fresh and commercial *Aloe vera* gel on the wound healing of the oral mucosa after burning and study the changes in IL6 gene expressions in the wound healing after treatment.

**Material and Methods**

**Experimental Model**

Forty eight healthy adult male white New Zealand rabbits weighing a mean of (1.75-2) kg was used in this study. The animals were routinely housed with the same food and housing conditions, at a constant room temperature of (25 ± 2) °C, and were given water and a standard diet of wheat and fresh vegetables. The animal was housed in an animal cage at the University of Mosul’s college of dentistry throughout the experiment. Before the surgery, a veterinarian checked the animals to ascertain their general health and condition. This work was conducted in accordance with the guidelines established by the institutional animal research ethical committee (UoM.Dent/A.L.45/22) [11].

**Animal Preparation**

Each animal got an intramuscular injection of 4 mg/kg xylazine and 40 mg/kg ketamine in the muscles of the rabbit’s leg [12]. To confirm that anesthetic had been provided, the weight of the rabbit was measured using electronic digital scales, and its reactions were assessed after 5–10 minutes.

**Animal Operation Procedure**

Complete anesthesia was attained in under 5 minutes. Animals were placed on the operating table. Megapower Megadyne Electrosurgical Generator cautery was used during the operation. For unipolar cauterization, the device was set on ACE (Advanced cutting effect). This mode uses 150 watts of power and operates at a frequency of 400 Hz. For burning of 0.5 cm of the cheek of the rabbit’s oral mucosa, we employed a unipolar electrocautery pen with a 2.5 inches standard blade electrode at 95.5 °C [13].

**Post-Operative Care**

The rabbits were separated after the procedure until they had recovered from the anesthetic. Following surgery, the first 24 hours were spent watching the animals’ food and activity patterns [14].

**Preparation of fresh Aloe Vera gel**

This study [15] claims that Aloe vera gel was produced. The outer layer of spotted Liliaceae Aloe vera was peeled away; the interior gel was collected with a sterile spatula. For immediate usage, the gel was kept in a clean container at room temperature.

**Experimental protocol**

Four groups of twelve rabbits each were formed from the forty-eight total. Twelve rabbits were slaughtered for each treatment period after each group was separated into three (3), seven (7), and fourteen (14) days.

1- The control negative group (normal): This group consists of 12 rabbits with normal tissue that were neither burned nor given any medication.
2- The control positive group consists of 12 rabbits that had their oral mucosal (buccal) tissue burned without any treatment.

3- Commercial Aloe Vera gel group: it involves 12 rabbits with oral mucosal tissue burn (0.5 cm) treated with commercial Aloe Vera gel, daily over (3, 7, 14 days) respectively to see its effect on wound healing.

4- Fresh Aloe Vera gel group: it involves 12 rabbits with oral mucosal tissue burn (0.5 cm) treated with fresh Aloe Vera gel, daily over (3, 7, 14 days) respectively to see its effect on wound healing.

Biochemical Examination (PCR)

Examination of IL6 gene expression in tissue by Polymerase Chain Reaction (PCR)

A- Tissue digestion

1. As PCR tubes are acquired, place a tissue biopsy measuring about 2-3 mm in each one.

2. To the PCR tube, add 100 ml of lysis buffer and 75 ml of 50 mm NaOH. Incorporate the tissue sample into the water.

3. 45 minutes at 95 °C in a thermos-cycler.

4. Tubes should be flicked right away.

5. Observe samples until they reach room temperature (RT).

6. Spin for a little while to drain liquid from caps.

7. Continue with qPCR or overnight sample storage at room temperature for use the following day.

B- mRNA-Extraction

I) Reagent preparation:

1. Before using, mix ethanol into the Wash 1 and Wash 2 solutions.

2. Verify for any precipitation in Filtration, Binding and Washing Solution 1 and any precipitate that can be dissolved by heating to 50 0C.

II) Extraction

1. Fill a 1.5 ml microcentrifuge tube with 20 µl of Proteinase K solution (20 mg/ml).

2. Use proteinase-K to transfer 200 µl of the material (tissue digest) into a 1.5 ml microcentrifuge tube.

3. 20 µl of DNase A solution (10 mg/ml, not provided) should be added.

4. 200 µl of the binding solution should be added to the sample tube. Mix thoroughly by peaking for 15 seconds.

5. 10 minutes of incubation at 56 °C.

6. 200 µl of 100% ethanol should be added, and the mixture should peak for 15 seconds. Rotate quickly downward after this action so that the drops cling to the cap.

7. Utilizing the 2.0 ml collecting tube, carefully transfer the filtrate into the upper container of the spinning column without wetting the rim.

8. Empty the flow and install the rotating column with the 2.0 ml collection tube before centrifuging at 13,000 rpm for 1 minute.

9. Centrifuge at 13,000 rpm for 1 minute after adding 500 µl of Wash Solution 1 to the revolving column with the collection tube. Install the rotating column with the 2.0 ml collection tube after emptying the stream.

10. Centrifuge at 13,000 rpm for one minute after adding 500 µl of Wash Solution 2 to the rotating column with the collection tube: Install the rotating column with the 2.0 ml collection tube after emptying the spill.

11. To get rid of any remaining ethanol in the revolving column, dry the additional rotary column at 13,000 rpm for one minute.

12. Change the 1.5 ml microcentrifuge tube with the rotary column (not supplied).

13. Utilizing a microcentrifuge tube, add 150 µl of eluate to the revolving column and allow to stand for at least one minute.

14. To elute genomic RNA, centrifuge at 13,000 rpm for 1 minute.

The Primer design

The IL6 gene primer was design using NCBI program at https://www.ncbi.nlm.nih.gov. Forward primer sequence for IL6 was ACCTTCAGGCCAAGTTCAGG and the reverse sequence was TCCTGGTTCCACCATGAGGT. The GAPDH gene was used as housekeeping gene with forward sequence ACCTTCAAGCCAAGTTCAGG and reverse sequence TCCTGTTCCACCATGAGGT.

mRNA qualification and quantification

Nano-Photometer®N60/N50 spectrophotometer was used to assess the concentration and quality of product RNA.
Quantitative polymerase chain reaction (qPCR)

The Go-Taq® qPCR master mix from Promega (A6000) (USA) was used to measure relative gene expression. Each sample underwent replication reactions for each gene of interest and common genes. To examine variations in gene expression between samples, ∆Δ Ct was computed. The difference in Ct between the target gene and the common gene was used to compute the ∆ Ct value for each sample. The difference in Δ Ct values between the experimental sample and the control sample was used to calculate ∆Δ Ct, 2^{[\Delta \Delta Ct]} , used to calculate the change in gene expression folding.

The RNA concentration was adjusted to 100 ng/ml and samples were prepared with a final volume of 2.5 µl in nuclease-free water. This volume was then put to each experimental well on a chilled 48-PCR plate from Promega and kept on ice.

12 µl of Go-Taq qPCR master mix, 2.5 µl of pertinent primers, and 3 µl of ultra-high quality DNase/RNase-free distilled water master mix were created. The plate was sealed with an optical adhesive cover (Thermo Fisher, UK) and transferred to the PCR-max-Eco 48 Real-Time qPCR System real-time thermal cycler (Ecosystem Germany) with 2 µl of enzyme added immediately before transfer to the PCR machine (Eco study software) The qPCR thermal cycle is set up as depicted in (Table1).

Results

IL6 gene expression in tissue

For IL6 gene expression measurements, the statistical analysis of the presumed data using the One-Way ANOVA test at p ≤0.05 reveals a highly significant difference between the study periods in all groups.

At the 3rd, 7th, and 14th day periods, post-hoc analysis using Duncan’s multiple range test revealed no significant change in IL6 gene expression measures in the control negative group, but there was a highly significant difference in the control positive group, fresh Aloe vera gel group and commercial Aloe vera gel group at these periods.

The highest mean of IL6 gene expression was found in the control positive group on day 3, while the lowest mean was found in the commercial Aloe vera gel group. The highest mean of IL6 gene expression was also found in the control positive group on the 7th and 14th day periods, while the lowest mean was found in the commercial Aloe vera gel group.

Except for the control negative group and the commercial Aloe vera gel group at day 14, when there were no significant differences between them, post-hoc analysis revealed a highly significant difference in IL6 gene expression measures among all groups at all research periods. (Table 2), (Fig.1).

Within the control negative group there was a slight increase in the IL6 gene expression on the 7th day after burning of the oral mucosal (buccal) tissue, while at day 14 the level of the IL6 gene expression was decreased, so the lowest mean of IL6 gene expression was obtained at 3rd day while the highest level was obtained at the 7th day periods.

Within the control positive group, the highest mean of IL6 gene expression was obtained on

<table>
<thead>
<tr>
<th>Stage</th>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerase Activation</td>
<td>Step 1</td>
<td>95</td>
<td>00:02:00</td>
<td>1</td>
</tr>
<tr>
<td>PCR Cycling</td>
<td>Step 1</td>
<td>95</td>
<td>00:00:15</td>
<td>40</td>
</tr>
<tr>
<td>PCR Cycling</td>
<td>Step 2</td>
<td>60</td>
<td>00:01:00</td>
<td>40</td>
</tr>
<tr>
<td>PCR Cycling</td>
<td>Step 3</td>
<td>60</td>
<td>00:01:00</td>
<td>40</td>
</tr>
</tbody>
</table>

Total time 1hr and 51 min / 40 cycle

TABLE 2. The mean and standard deviation of IL6 gene expression in oral mucosal between groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Control negative group (normal) (Mean ± S.D)</th>
<th>Control positive group (Mean ± S.D)</th>
<th>Fresh Aloe Vera gel group (Mean ± S.D)</th>
<th>Commercial Aloe Vera gel group (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6 gene expression (Fold change)</td>
<td>3rd day</td>
<td>0.44±0.032^a</td>
<td>26.25±1.88^b</td>
<td>22.9±1.01^c</td>
<td>17.116±1.7^d</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>0.58 ± 0.14^a</td>
<td>9.0±4.58^a</td>
<td>5.51±0.89^bc</td>
<td>4.36±0.71^c</td>
</tr>
<tr>
<td></td>
<td>14th day</td>
<td>0.47 ± 0.13^a</td>
<td>1.72± 0.24^a</td>
<td>0.7±0.21^a</td>
<td>0.476±0.055^a</td>
</tr>
</tbody>
</table>

- S.D : Stands for Standard Deviation
- The horizontal different letters mean significant difference between groups by Duncan at p<0.05
- The horizontal same letters mean non-significant difference between groups by Duncan at p<0.05

Fig.1. Showed the difference of IL6 gene expression level in oral mucosa between groups.

the 3rd day while the lowest mean of IL6 gene expression was obtained at 14th day periods. It was the same for commercial and fresh Aloe Vera gel, that the highest mean of IL6 gene expression was obtained on the 3rd day while the lowest mean of IL6 gene expression was obtained at 14th day periods within each group itself (Table 3), (Fig.2).

Discussion

The goal of this study was to assess the degree of IL6 gene expression in the oral mucosa of rabbit models after using fresh and commercial Aloe vera gel that had been burned. Rabbits have been suggested as a study model because of their high availability, simplicity of handling and treatment, and habitation [16].

In this study we also measure the differences of the IL6 gene expression after oral mucosal tissue burning and topical application of commercial and fresh Aloe vera gel, between the four groups at 3rd, 7th and 14th days to explain their effect on inflammation and wound healings.

Our findings indicate that IL6 gene expression were lower in all oral mucosal specimens treated with commercial and fresh Aloe vera gel than in control positive specimens at all research time intervals.

The highest mean of IL6 gene expression was presented in the control positive group, while the lowest mean was found in the group treated with commercial Aloe vera gel at 3rd, 7th and 14th days periods, also we found that within each group itself the highest mean of IL6 gene expression was recorded at 3rd day while the lowest mean was found at 14th day periods.

In full-thickness burn wound rabbit models, the decreased expression of the IL-6 gene may lessen the severity of the inflammatory response during the early stages of thermal injury [17].

TABLE 3. The mean and standard deviation of IL6 gene expression in oral mucosal within group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Control negative group (normal)</th>
<th>Control positive group</th>
<th>Fresh Aloe Vera gel group</th>
<th>Commercial Aloe Vera gel group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Mean ± S.D)</td>
<td>(Mean ± S.D)</td>
<td>(Mean ± S.D)</td>
<td>(Mean ± S.D)</td>
</tr>
<tr>
<td>IL6 gene expression (Fold change)</td>
<td>3rd day</td>
<td>0.44±0.032&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.25±1.88</td>
<td>22.9±1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.116±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>0.58 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0±4.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.51±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.36±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14th day</td>
<td>0.47 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.476±0.055&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

-S.D : stands for Standard Deviation
-The vertical different letters mean significant difference between groups by Duncan at p<0.05
-The vertical same letters mean non-significant difference between groups by Duncan at p<0.05

Fig.2. showed the difference of IL6 gene expression level in oral mucosa within group.

TNF-α levels may drop as a result of these impacts. Furthermore, it has been shown that the development of multiple organ failure may be significantly influenced by a sustained increase in TNF-α levels following heat injury. Consequently, a poor prognosis is predicted by transiently higher circulating TNF-α levels in burns. Both the inflammatory response and wound healing depend on transient changes in TNF-α and other cascade cytokines [18].

Acemannan from commercial and fresh Aloe vera gels have been shown to promote NF-B/DNA binding. Pre-incubating the HGFs with an anti-hTLR5 antibody resulted in less acemannan-induced IL-6 gene expression and protein secretion than the positive control group that was not treated [19].

According to our research, Aloe Vera modulates the innate immune system’s ability to produce pro-inflammatory cytokines by circulating immune cells and tissue-resident cells. By improving both healthy individuals’ and immunocompromised patients’ innate immune systems’ identification of bacterial infections, aloe vera may be helpful as an immunomodulatory drug [20].

Interestingly, our findings show that a daily treatment of fresh and commercial Aloe vera gel can considerably reduce IL-6 levels as well as the endothelial–leukocyte interaction [21].

Has also shown that Aloe vera has these effects on leukocyte recruitment [22]. It is possible
to draw the conclusion from this leukocyte recruitment study that Aloe Vera contains active substances that can stop or suppress the serial sequence of TNF-α, leading to enhanced endothelial-leukocyte interactions and decreased IL6 gene expression.

These findings come in agreement with [23] who stated that the principal carbohydrate ingredient in Aloe vera, ‘acemannan,’ is responsible for the reduction of the IL6 gene expression as well as inflammation and consequently promote wound healings in oral mucosa.

While our findings come in disagreement with Qanash et al. [24] Who stated that Aloe Vera gel has no effects on IL6 gene expression. [25]. The occurrence of diseases causes pain and suffering to the patient [26-30]. The use of herbal products can be used in the treatment of diseases due to the presence of chemical compounds such as phenols, flavonoids, tannins, anthocyanin and antioxidant substances [31-35].

Conclusion

The findings of this study demonstrated that fresh and commercial Aloe Vera gel reduced inflammation in rabbits by lowering the level of IL6 gene expression in the oral mucosal tissue. Also herbal contained many biological of chemical compounds such as phenols, flavonoids, tannins, anthocyanin and antioxidant substances that potential the biological activity of Aloe vera to accelerate a wound-healing for the oral Mucosal after Burning.

Acknowledgement
To all stuff in the department of dental basic sciences.

Conflicts of interest
The authors declared no competing interests.

Funding statements
None.

Ethical Approval
All procedures involving animals in this study followed the National Institutional Health Principles of Laboratory Animal Care guidelines. The authors disclosed that this work received institutional ethical approval REC reference no. (UoM.Dent/A.L.45/22).

References


أثر هلام الصبار الطازج والتجارب على التعبير الجيني IL6 في الأرانب بعد حرق الغشاء المخاطي للللم

مراجعات:

1. وزارة الصحة، مديرية صحة نينوى، الموصل، العراق.
2. وسم علم طب الاستناك، كلية طب الاستناك، جامعة الموصل، الموصل، العراق.
3. قسم الباثولوجيا، أمراض الدواجن، كلية الطب البيطري، جامعة الموصل، العراق.

الخلفية:


الهدف من الدراسة:

ละเอوية اللاعيبة باثولوجية تأثير هلام الصبار الطازج والتجارب على التعبير الجينيIL6 في الارناء. تم استخدام الصماني لتشخيص الارناء بالوانوي قبل حرق نسيج الفم المخاطي للللم. وتم استخدام الكيتامين و الزيلازين كحقنة في عضلة فخذ الأرنب. وتم تزويد الارناء بالقوة المتسلسلة وإعطاء القسطة فينسي مقاس 2.5 مل. وتم الفحص عند 5 دقائق لعند 35 درجة مئوية لحمرة مسألة 50 سم من سقف الغشاء المخاطي للللم للأرنب، بعد ذلك تم تقييم هذه الأرناء بتعليمات في أربع مجموعات (ملاحظة: لم تكن مواقع الحرق غير المعالجة هي الأصلية للكيسية فينسي باستخدام تفاعل البلمرة المتسلسل (PCR) لتحديد التعبير الجينيIL6 في الارناء. بالإضافة إلى ذلك، تم استنادًا إلى أداء الأرنب لتحديد التعبير الجينيIL6 في جميع فترات الدراسة الاستنتاجية: بناءً على ما سبق نستنتج أن هلام الصبار الطازج ولام الصبار التجاري لهما تأثير كبير ونافع على تطوير الجروح، وتقليل التهاب البشرة والتئام الجروح.

الكلمات المفتاحية: الصبار، حرق الغشاء المخاطي للللم، التعبير الجينيIL6، الغشاء المخاطي للللم، الأرنب، IL-6، التأهيل، التئام الجروح.