The effectiveness of Acacia oral gel on wound healing in rabbits clinically and histopathologically

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ABSTRACT

Oral mucosa considered as one of defensive mechanism of oral cavity, it is important in oral functions as eating, swallowing and even talking. Any wound or discontinuity effects on these functions so speeding the healing process with less inflammatory reactions is crucial, many herbs were used at different eras to reach this goal one of these herbs is Acacia plant. The aim of this study is to evaluate the effect of acacia oral gel extract on the wound healing. In materials and method This study include (twenty-one) male rabbits, (seven) for each group used for this study, two incisions of one centimeter length were made on buccal oral mucosa bilaterally one is treated by acacia oral gel while other left for normal healing, the follow up of the wound at twenty-four hours, three days and seven days clinically and histopathological examination were done. In result of this study, The study shows decrease in inflammatory reaction after seventy-two hours of the wound and increase in re-epithelialization rate in wound healing of acacia nilotica gel group, So it is concluded from this study that the acacia plant gel extract has anti-inflammatory effect and accelerate the wound healing of oral mucosa in rabbits.

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INTRODUCTION

Restoration of damaged tissue plays a vital role in survival of life and it is looming for basis of all surgical manipulations. Wound is defined as the commotion of cellular and anatomical continuity of tissue integrity and sometimes accompanied by loss of function. Healing occurs after any insult that causes tissue destruction and is crucial for survival of the organism. (Nayak et al., 2007). Wound care goal is to aid healing of wounds in fast duration, with nominal pain, distress and marking to the patient and should happened in a physiological situation encouraging the tissues to restore and regenerate. Impaired and aberrant healing of wound executes an enormous financial load and places a massive drain in health care resources in the industrialized world and an insurmountable issue in the developing countries too. Multiple local obstacles like contagion, shock, hypoxia and chronic diseases such as diabetes mellitus and malnutrition can result in impaired healing of wound process (Bowler et al., 2001). The pervasiveness of the chronic wounds in the civic was conveyed as 4.5 per 1000 inhabitants, where was approximately double, at 10.5 per 1000 population for acute wounds (Shukla et al., 2004). It has been detecting that one third of all traditional herbal remedies are used in the handling of wounds and skin diseases. Nowadays in evolving countries about 80% of the population hinge mainly
on therapeutic plants for their hygiene. (Valte et al., 2012). Distress in healing of the wound present a stern clinical obstacle and are usually increases in the presence of chronic disorders as diabetes, hypertension and over weightiness. Thus, plentiful animal mock-ups have been established to serve as experimental basis to regulate the mechanisms and controlling undisturbed healing course. (Abdurahim et al., 2016). The core goals of the researches in wound healing are to screen drugs, herbs or any new established materials that boost healing progression professionally and to assess the impact of numerous ways in wound management lineups on healing. (Arulpriya et al., 2013). Numerous medicinal plants have been cast-off since time for wounds management and displayed auspicious effects. Mondal et al. (2013). The therapeutic value of these plants’ deceits in their biologically active phytochemical ingredients that yield physiological exploit on the human body. These ingredients comprise innumerable chemical groups like alkaloids, essential oils, tannins, flavonoids, saponins, terpenoids, and phenolic complexes. (Edooga et al., 2005). The herbal abstracts and fractions obstruct microbial growth, stop wound bleeding professionally and hasten wound healing. (Okoli et al., 2007). Herbal harvests have been used in healing and handling of wounds over the ages. The phyto-medicines for wound curing are not only judicious and low-priced but similarly evidently harmless as hyper sensitive responses are seldomly observed with the use of these agents. (Raina et al., 2008). Thus, these products need to be recognized and framed for dealing of wounds. Acacia species are usually branded as ‘Babool’ in India have been used for the treatment of skin, stomach, sexual and tooth issues. Acacia nilotica (Acacia arabica) (Mimosaceae) Commonly known as babul, kikar or Indian gum Arabic tree has been defined internationally as a multiuse tree. Acacia arabica has been demonstrated as efficient medicine in treatment of malaria; toothache (bark), sore throat (aerial part) and have tested the anti-fertility activity of A. arabica pods and nuts. It is broadly spread in arid and semi-arid districts of the world. (Rajvaidhya et al., 2012). Acacia arabica gum is an old-style oral hygiene element which has been used for epochs by many societies in the North Africa and Middle East. It composed chiefly of arabica, a complex mixture of the magnesium, calcium and potassium salts of Arabic acid. There are also other constituents such as cyanogenic glycosides, tannins, oxidases and peroxidases and pectinases; all of which have been revealed separately to reveal antimicrobial properties. (Pradeep et al., 2010). Clark et al. has described the antiprotease and antibacterial actions of Acacia arabica. (Clark et al., 1993).

In this study we try to appraise the consequence of acacia arabica abstract as a gel form on the healing of oral mucosal tissue wounds in rabbits.

**MATERIALS AND METHODS**

**Preparation of acacia gel**

Two concentrations (5%, 10%) of acacia were chosen as the active ingredient in different gel formulas using Carbopol 934 as a gelling polymer. Carbopol 934 concentration was manipulated in order to prepare the optimum gel formula. Six different formulas were prepared. Table 1 shows the composition of each formula. Neutralization of the prepared formulas were performed using Triethanolamine (TEA) as the neutralizing agent. TEA was added drop by drop using a micro-pipette until a suitable gel consistency was obtained.

**Table 1: Composition of acacia gel**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Acacia (%)</th>
<th>Carbopol 934 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>F2</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>F3</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>F4</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>F5</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>F6</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

**Acacia gel preparation method**

Acacia powder was weighed and dissolved in D.W. The mixture was stirred for 30 minutes in order to aid the dissolution process. The resultant solution was left overnight to ensure complete dissolution. The required amount of Carbopol 934 was then added and left for 24 hours to ensure complete swelling of the polymer. TEA was added in order to transform the solution into the gel consistency. On neutralization, all acacia formulas with 5% concentration showed a very weak gel, so it was excluded from further evaluation. For the 10% acacia formulas, these showed a different behavior from the 5% acacia formulas.

On neutralization with the same neutralizing agent, F3, F4 and F5 have produced a weak gel although the concentration of Carbopol 934 was increased. But, F6 formula has resulted in a proper gel that is suitable for the proposed aim. Accordingly, one formula (namely F6) was chosen to subject for further evaluation.
The protocol and the guidelines of the present study had been permitted by Scientific Committee of Oral and Maxillofacial Department, Dentistry College, Mosul University. All procedures on animal were done with no any cruelty harming to the experimental animals.

**Animals**

(21) domestic healthy male rabbits of 7-8 months old, each weighing 2 ± 0.5 kg were used in this study. They were independently held and fed in regular diet (vegetables, grains and corn) and water in a room with normal light cycle and stable temperature (24±2°C). The College of dentistry of Mosul University Animal Care and scientific Committee has agreed on the study protocol. Prior to the treatment, the rabbit’s male was acclimatized for one week to check the general health and ensure the absence of any infectious disease.

**Surgical Procedure**

The rabbits were randomly divided into three equal groups according to the healing period (1, 3 and 7 days), each group containing seven animals. Every rabbit anesthetized using 40mg/kg ketamine injection (Paknejad *et al.*, 2007). Intramuscular in the thigh muscle mixed with xylazine 4 mg/kg body weight (Kilic, 2004). During surgery, supplemental sedation was administered when needed. Complete anesthesia had been obtained within 5min, Local anesthesis was given in the surgical area (1 ml of 2% lidocaine with epinephrine 1:80,000, New Stetic com. Colombia), two longitudinal wounds of (about 1cm length and 0.5 cm depth) made on the buccal oral mucosa of all rabbits (1) wound on left and (1) on the right side of the buccal mucosa. One of the wounds left without any treatment while the other treated by 10% acacia oral gel applied once daily for about 7 days, as shown in Figure 1.

Histopathological specimens were collected from mucosa defects of seven rabbits from each group at 1, 3 and 7 days after scarifying. After that all mucosa biopsies were conserved in newly formed 10% formalin for 48 hours. Following fixation of the tissue specimens in 10% formalin, the specimens dehydrated through ascending graded series of ethanol and xylene before being fixed in paraffin wax. Slices of 4-5 μm thickness were obtained and discolored with hematoxylin and eosin. Inspection of the slides was done by binocular light microscope (Optika, Ponteranica (BG)- Italy). The images of the histologic sections in all groups were captured by a digital camera 8 mega pixel (Aiptek, Germany) connected to same light microscope, then the images were saved on a personal computer as shown in Figures 2, 3 and 4. The histopathological examination was performed by two expert pathologists using double-blind checking manner. All slides were evaluated under a 4 x and 10 x magnification low power field (LPF) to assess the wound healing.

**Histopathological examination and scoring system**

After scarifying the rabbits histopathological specimens of all wounds were taken at different duration of (1, 3, and 7 days), Histopathological recording of inflammation and repair was done depending on the system projected by (Gupta *et al.*, 2015), (Camacho-Alonso *et al.*, 2009), This system Include inflammation, Granulation tissue and re-epithelialization as following

**Inflammatory infiltrate scoring**

(Score1 plenty, Score2 moderate, Score3 a few)

**Amount of granulation tissue**

(Score1 profound, Score2 Moderate, Score3 scanty, Score 4 absent)

**Re-epithelialization Scoring**

Score 0: Re epithelialization at the edge of wound
Score 1: Re epithelialization covering less than half of the wound
Score 2: Re epithelialization covering more than half of wound
Score 3: Re epithelialization covering the entire wound, irregular thickness.
Score 4: Re epithelialization covering the entire wound, normal thickness.

**Data Analysis**

All values are presented as mean with standard deviation of all parameters, analysis of statistical difference between groups was made by using T-test. P value less than or equal to 0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

Clinical Evaluation: For all the specimens in both groups there is no dehiscence, infection exudate, wound surfaces were normal scar formation present in control group while the acacia group no scar found.

Histopathological finding: At first week the inflammation for control group was moderate first, then increased for the first 72 hours, at 7 days the inflammatory reaction decreased to the lowest mean score which was (3), while for the acacia few inflammatory cells infiltrate present at first day with mean...
Figure 1: (incision on buccal mucosa of rabbit). (A) control. (B) 10% gel acacia extract

Figure 2: wound at 24 hours (A) control group, (B) acacia group (inflammatory cells by Hematoxylin and eosin stain at 40X power)
Figure 3: Wound at 3 days (A) control group (B) acacia group (at 10X power with Hematoxylin and eosin stain)

Figure 4: Wound at 7 days (granulation tissue and epithelialization) (A) control group (epithelium cover less than half of wounds edge), (B) acacia group (epithelium is irregular and covering entire wound) (at 10X power)
Table 2: Inflammatory reaction

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>10% Gel</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2±0</td>
<td>3±0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.57±0.534</td>
<td>2.42±0.53</td>
<td>0.005*</td>
</tr>
<tr>
<td>7</td>
<td>3±0</td>
<td>3±0</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as Mean ± Standard deviation

P-Value ≤ 0.05 was significant

Table 3: Granulation tissue

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>10% Gel</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3±0</td>
<td>3.28±0.48</td>
<td>0.07</td>
</tr>
<tr>
<td>3</td>
<td>1±0</td>
<td>2.28±0.48</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3±0</td>
<td>3±0</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as Mean ± Standard deviation

P-Value ≤ 0.05 was significant

Table 4: Re-epithelialization

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>10% Gel</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0±0</td>
<td>0±0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1±0</td>
<td>1±0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.14±0.37</td>
<td>2.71±0.48</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Data presented as Mean ± Standard deviation

P-Value ≤ 0.05 was significant

Figure 5: Inflammatory score

Figure 6: Granulation tissue scoring through 7 days

Figure 7: Re-epithelialization scoring through 7 days

score of (3) which slightly increased after 72 hours then decreased to return to same value of first day. As shown in Table 2 and Figure 5. For granulation tissue at first day the mean for control group was (3) while acacia group was (3.2), after 3 days the control group shows mean (1) and acacia group mean (2.2), after week the mean of both groups was (3). As in Table 3, Figure 6. Re-epithelialization at first day both groups show no any re-epithelialization with means of (0), but the re-epithelialization increased at 3 with mean of (1) for the control and study groups, the differences appear at 7 days almost all slides of the acacia group shows epithelium at entire wound but it is irregular with mean of (2.7) rather than control group mean which was(2.1) shown in Table 4, Figure 7. The rabbit model can be measured as a useful showing tool for testing new rehabilitation. Rabbit skin is evidently very diverse from human skin. The healing potential is high, with fast contraction of the wound and a high proliferation rate. (Lemo et al., 2010).

Diminishing of multifactorial process of healing of the wound can be occur by the insufficient or absence of harmony between many perilous aspects. Wounds can left open to restore by secondary intention owing to infection or contamination this occur in surgical theater by packing with a humble dressing, such as ribbon gauze soaked in antiseptic or normal saline, otherwise most of surgical wounds are closed during the immediate postoperative period. (Fries et al., 2005; Pukki et al., 2010). Antibacterial and homeostatic properties of Acacia Arabica can help in the accelerating wound healing. The homeostatic efficacy of the biopolymers which is active ingredients in this herb fasten the clotting of blood and decrease the duration of both the partial thromboplastin and prothrombin time. The Acacia plant gum applied to calm the skin flushes, soreness, irritation, and burns. Moreover, the antibacterial feature of this plant contrary to the organisms that usually infect the wounds helps in hasten of the restoration of the damaged tissues. (Bhatnagar et al., 2013). In our study aca-
Acacia arabica extract used as gel which applied to oral mucosa and compared to control groups to detect the activity and effectiveness of acacia in wound healing. Inflammatory reaction at first week shows variation between the acacia arabica and control groups, as generally speaking the inflammation was less in the acacia group than study group and there was a significant decrease in inflammatory reaction after 72 hours of wound healing in acacia group as it has an anti-inflammatory effect and this agree with (Dafallah et al., 1996) who stated that “Acacia nilotica extract had an inhibitory effect on carrageenan induced paw edema and yeast-induced pyrexia in rats. It also produced a significant increase in the hot plate reaction time in mice”. Another study reveals that “A. nilotica is easily accessible source of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc.” (Ali et al., 2012), and agree with (Musa et al., 2018) who stated that “The mechanism of action through which GA improves the antioxidant capacity may be due to the fact that GA contains several types of amino acid residues such as lysine, tyrosine, and histidine, which are commonly considered as antioxidants biomolecules”. About granulation tissue, as we did not found researches discuss this issue, so we depend on our analysis according to the normal physiology of wound healing and our results. There is no significant difference between the control or acacia groups, At first day both groups shows scanty granulation tissue which increased after 3 days to reach to the maximum to become profound specially to control group while is less to acacia group this may be due to the anti-inflammatory effect of the acacia which lead to decrease the amount of inflammatory cytokines and amines which activate the new blood vessels formation and aggregate and activate the microphages which in turn responsible for fibroblast activity and collagen formation, after one week as the epithelialization starts, increasing in the granulation tissue falls down to become scanty again for both groups. Re-epithelialization is the method of reestablishing an integral epithem after cutaneous injury. In general, re-epithelialization include a series of processes: the immigration of keratinocytes in the epidermis from edges of the wound; the proliferation of keratinocytes that are used to supplement the progression and epidermis drifting; differentiation of the neo-epithelium into stratified epidermis; an integral basement membrane sector renovation that connects the both dermis and epidermis; and the regeneration of specific cells that direct sensory function (Merkel’s cells), immune functions (Langerhanse cells) and pigmentation (melanocytes) (Li et al., 2005). Re-epithelialization progresses faster in oral mucosal wounds than skin. In a mucosal wound, the cells of epidermis travel straightly onto the surface of the fibrin clot that is moist and exposed contrary of under the dry exudate of the dermis (Miloro, 2004).

We came across no studies on acacia arabica effect on re-epithelialization of oral mucosa to compare with this discussion, so we shifted to other studies used this material in re-epithelialization of corneal injuries. Re-epithelialization at first day both control and study groups show no any epithelium formation, at third day re-epithelialization appears cover less than half of wound for all the specimens of both groups, but at the end of first week (seventh day) the study group shows significant increase in re-epithelialization of oral mucosal wound in comparison to control group, as some of slides shows epithelium covering whole wound but it’s thickness is uneven, while control group shows epithelium covering more than half of the wound, and this is agree with (Ker-Woon et al., 2015) who stated that “In the present study, we showed the migration of corneal epithelial cells was accelerated in the Acacia Honey-supplemented media” also stated “The activation of hydrogen peroxide in Acacia Honey-supplemented media serves as an ideal culture condition for Corneal epithelial cell, thus accelerating the migration of Corneal epithelial cell in closing the wounded area”. So, our study found that acacia gel accelerates the wound healing and this agree with (Mohammad et al., 2018) who stated that “The combination of two plant Acacia nilotica and Curcuma longa mixture preparation was found very significant in wound healing as compared with the control group”.

CONCLUSION

Acacia gel has anti-inflammatory effect on healing process as it contain active ingredients that decrease irritation and effect on inflammatory mediators, also as Acacia contain biopolymers that decrease prothrombin and bleeding times and make the coagulation faster with antibacterial activity against micro-organisms, all this properties impact and accelerate wound healing when applied topically on oral mucosa.

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Conflict of Interest
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