Journal of Animal and Veterinary Advances 11 (14): 2445-2452, 2012
ISSN: 1680-5593

Effect of *Aloe vera* on Healing of the Experimental Skin Wounds on Rats and its Comparison with Zinc Oxide: A Geometry and Histopathologic Study

[1]Ali Rezaie, [3]Amirreza Ebadi, [2]Mehrdad Nazeri, [4]Samad Rezaie and [5]Fatemeh Elmi
[1]Department of Clinical Science, [2]Young Researchers Club,
Tabriz Branch, Islamic Azad University, Tabriz, Iran
[3]Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran
[4]Department of Dentistry, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
[5]Faculty of Dentistry, Tabriz University of Dentistry, Tabriz, Iran

Abstract: *Aloe vera* is a perennial succulent belong to the Lily (Liliaceae) family. This plant has been known as the healing plant. *Aloe vera* has been used for traditional medical purposes in several cultures for millennia. It has been demonstrated that *Aloe vera* has growth promoting activities. The objective of this study was to determination of the effect of *Aloe vera* on healing of the experimental skin wounds on rats and its comparison with zinc oxide. Zinc oxide is being used worldwide as an absorbent and protective compound. Its pharmacological properties are wide and its non-toxic material allows it to be used as a routine skin care substance. In current study, 70 female wistar rats where included in 5 groups. Full thickness incisional wound with 23 mm diameter was made with surgical scissors and scalpel. The whole operation was taking place under general anesthesia and analgesia circumstances. After making surgical wounds, rats are treated as mentioned in the text. Rats are observed for 21 days for wound closure process and inflammatory conditions taking place in wound. Biopsy intervals are 0 (the day of surgery), 3, 7, 14 and 21th day after surgery. In these certain days rats were euthanized and biopsies of wound sites were obtained. Wounds areas are also, measured by Scion Image ™ Software daily. At last, all data were analyzed using SPSS Statistics Ver.17. As a result, *Aloe vera* at the dose of 10% has significant healing properties compared to Zinc oxide. These data were validating under confidence surface of 95% ($p<0.01$).

Key words: *Aloe vera*, zinc oxide, healing, skin wounds, rats

INTRODUCTION

Polysaccharides are found in abundance in Nature and are readily available from sources such as algae (e.g., alginates), plants (e.g., pectin, guar gum and mannan) microbes (e.g., dextran and xanthan gum) and animals (e.g., chitosan and chondroitin) and they can also be produced by means of recombinant DNA techniques. Monosaccharide polymers have many favorable properties such as high stability, non-toxicity, hydrophilicity, biodegradability, gel forming properties and ease of chemical modification (Coviello *et al*., 2007; Sinha and Kumria, 2001). An enormous variety in plant polysaccharide structural composition exists which is not only associated with different plants but also with the part of the plant that they originate from such as the leaves, seeds, roots and tubers. The complexity and variety of polysaccharides can be explained by two unique structural features: firstly monosaccharides can be linked together in different ways (1→2, 1→3, 1→4, 1→5 and 1→6, in an α or β-configuration) and secondly due to the presence of branched side-chains (Ni *et al*., 2004a, b).

Complex carbohydrates obtained from natural sources such as plants have shown diverse biological activities such as wound healing, enhancement of the reticuloendothelial system, stimulation of the immune system, treatment of tumours and effects on the hematopoietic system (Talmadge *et al*., 2004). *Aloe vera* (L.) Burm.f. (*Aloe barbadensis* Miller) is a perennial succulent xerophyte which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The innermost part of the leaf is a clear, soft, moist and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of viscous mucilage (Newton, 2004). Therefore, the thick fleshy leaves of aloe plants contain not only cell wall carbohydrates such as cellulose and hemicellulose but also, storage carbohydrates such as acetylated mannans (Ni *et al*., 2004a).

Corresponding Author: Ali Rezaie, Department of Clinical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran

A. vera has been used for many centuries for its curative and therapeutic properties and although >75 active ingredients from the inner gel have been identified, therapeutic effects have not been correlated well with each individual component (Habeeb *et al*., 2007; Saka *et al*., 2011; Fei *et al*., 2002; Hasani-Ranjbar *et al*., 2011; Alqasoumi *et al*., 2008). Many of the medicinal effects of aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue (Ni and Tizard, 2004; Ni *et al*., 2004a, b) but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance (Dagne *et al*., 2000).

A. vera is the most commercialised *Aloe* species and processing of the leaf pulp has become a large worldwide industry. In the food industry, it has been used as a source of functional foods and as an ingredient in other food products for the production of gel-containing health drinks and beverages. In the cosmetic and toiletry industry, it has been used as base material for the production of creams, lotions, soaps, shampoos, facial cleansers and other products. In the pharmaceutical industry, it has been used for the manufacture of topical products such as ointments and gel preparations as well as in the production of tablets and capsules (Eshun and He, 2004; He *et al*., 2005). Important pharmaceutical properties that have recently been discovered for both the *A. vera* gel and whole leaf extract include the ability to improve the bioavailability of co-administered vitamins in human subjects (Vinson *et al*., 2005). Due to its absorption enhancing effects, *A. vera* gel may be employed to effectively deliver poorly absorbable drugs through the oral route of drug administration. Furthermore, the dried powder obtained from *A. vera* gel was successfully used to manufacture directly compressible matrix type tablets. These matrix type tablets slowly released a model compound over an extended period of time and thereby showing potential to be used as an excipient in modified release dosage forms (Jani *et al*., 2007).

Aloe vera leaf composition

Structural composition: The aloe leaf can be divided into two major parts namely the outer green rind including the vascular bundles and the inner colourless parenchyma containing the aloe gel. Description of the inner central part of the aloe leaf may sometimes be confusing due to the different terms that are used interchangeably such as inner pulp, mucilage tissue, mucilaginous gel, mucilaginous jelly, inner gel and leaf parenchyma tissue. Technically, the term pulp or parenchyma tissue refers to the intact fleshy inner part of the leaf including the cell walls and organelles while gel or mucilage refers to the viscous clear liquid within the parenchyma cells (Ni and Tizard, 2004).

The three structural components of the *Aloe vera* pulp are the cell walls, the degenerated organelles and the viscous liquid contained within the cells. These three components of the inner leaf pulp have been shown to be distinctive from each other both in terms of morphology and sugar composition as shown by Ni *et al*. (2004a, b). The raw pulp of *A. vera* contains approximately 98.5% water while the mucilage or gel consists of about 99.5% water (Eshun and He, 2004). The remaining 0.5-1% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids (Boudreau and Beland, 2006). It has been hypothesized that this heterogenous composition of the *Aloe vera* pulp may contribute to the diverse pharmacological and therapeutic activities which have been observed for aloe gel products (Talmadge *et al*., 2004).

Chemical composition: Many compounds with diverse structures have been isolated from both the central parenchyma issue of *A. vera* leaves and the exudate arising from the cells adjacent to the vascular bundles. The bitter yellow exudate contains 1, 8 dihydroxyanthraquinone derivatives and their glycosides which are mainly used for their cathartic effects (Vazquez *et al*., 1996). The aloe parenchyma tissue or pulp has been shown to contain proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and small organic compounds in addition to the different carbohydrates. Some evidence of chemotaxonomic variation in the polysaccharide composition of aloes exists (Cosmetic Ingredient Review Expert Panel, 2007; Ni and Tizard, 2004; Reynolds, 2004). The large fluctuations in polysaccharide composition of *A. vera* fillet as found in the literature has been explained by the fact that the mannosyl residues are contained in a reserve polysaccharide with a significant seasonal influence as well as large variations between cultivars in terms of the quantities of mannose-containing polysaccharides within the parenchyma cells (Femenia *et al*., 1999).

Zinc oxide is an inorganic compound with the formula ZnO. It is a white powder that is insoluble in water. Zinc is an essential trace element of which about 2 g is found in the adult human body. At least 200 enzymes in different biological systems are dependent on the presence of the zinc ion. Among these zinc-dependent enzymes, DNA and RNA polymerases are crucial during tissue repair as they affect cell proliferation and protein

synthesis. In accordance with the biochemical role of zinc a reduced synthesis of DNA, reduced deposition of granulation tissue decreased tensile strengths in skin incisions and delayed closure rates in excised wounds in zinc-deficient rats have been demonstrated (Prasad and Oberleas, 1974; Sandstead *et al*., 1970). Zinc supplementation restored to normal the tensile strengths of the incisional and healing rates of the excisional wounds (Sandstead *et al*., 1970). It has been clinically shown that the healing of leg ulcers is delayed in patients with subnormal serum-zinc levels (Haley, 1979). Zinc given as oral and topical zinc sulfate or as topical zinc oxide normalizes impaired healing ability in these patients (Golden *et al*., 1980; Haley, 1979; Stromberg and Agren, 1984).

The objective of this study was to determination of the effect of *Aloe vera* on healing of the experimental skin wounds on rats and its comparison with zinc oxide.

MATERIALS AND METHODS

Animals: This study was conducted in Islamic Azad University Research Center during summer 2011. In this study, 70 male Wistar rats weighted 210±10 g and aged 12 weeks old were selected. All animals were kept in same situation (temperature 24°C and humanity 70%) and food and water were provided *ad libitum*.

Pre-operation measures: The operation (induction wound in the skin) required general anesthesia, analgesia and muscle relaxation. In term, researchers used of Ketamine (10%, 60 mg kg^{-1}) and xylazine (2%, 10 mg kg^{-1}) through IM injection to induction of anesthesia and pre-operation drugs, respectively. To prevention of drugs side effects, liquid therapy with dextrose 5% at the dose of 50-100 mg/kg/day was exerted immediately after induction of anesthesia.

Operation measures: After preparation the dorsal skin of rats (distinct between scapula to ischial tuberosity), a wound in circle shaped with 7 mm in diameter and by biopsy punch were inducted. In this study rout of wounding was excisional wounding that in way epidermis, dermis, hypoderm and panniculus carnosus completely were removed. After wounding, rats were divided into five groups of fifteen.

- Group 1: Received high doses (20%) of herbal extract
- Group 2: Received low doses (10%) of herbal extract
- Group 3: As positive control group received zinc oxide 20%
- Group 4: As negative control group received eucerin
- Group 5: As control not received any drug

Samples were fixed in the formalin 10% and sent to pathology laboratory.

Post-operation measures: After biopsy and washing wound area with normal saline all drugs were administrated as local way by an applicator in the wound area. This administration continued for 21 days.

Sampling: On days 0, 3, 7, 14 and 21 of research samples as tissue specimens from biopsy areas were collected and sent to pathology laboratory. Sampling was done under anesthesia condition and this anesthesia was induced by Ketamine and Rampon. Sampling was exerted by scalpel. Samples were fixed into formalin 10%. In lab, after processing and staining to H and E Method slides were achieved. Slides were investigated by a light microscope.

Statistical analysis: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 17.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. The $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Geometric findings: On the 1st day of trial, ulcer size in all five groups showed a significant increase compared to day 0. On the 2nd day wound size in high dose treatment group was reduced significantly. So that, this finding was observed in low dose and zinc oxide groups, control group and eucerin group on days 3, 4 and 5, respectively. About >7 days until day 7, according to the size of the wound, the highest rate of wound shrinkage was observed in high dose, low dose, zinc oxide, eucerin and control group, respectively. On day 21st, maximum and minimum shrinkage was observed in low dose and control groups, respectively.

Histopathologic findings: On day 3 in high dose treatment group, pustule covered the wound but still retains its moisture. Re-epithelialization is seen from wound sides. Inflammatory cells also are existed. Infiltration of fibroblasts into the connective tissue was obvious. In low dose treatment group, wound was covered by pustule consist of fibrin and blood cells and purulent materials such as neutrophils and RBC remnants. Clod on the wound had more and low inflammatory cells than high dose and zinc oxide groups, respectively. In zinc oxide group wound was covered by thick and keratinous

pustule. Wound was filled with granular connective tissue and hyperemia was obvious. In eucerin group, hemorrhage in the profound layers was obvious and was not seen any pustule and healing has not been started (Fig. 1a, b).

On day 7 in high dose treatment group in some cases pustules on the wound still have not been completely dried but wound area has been filled with multicellular and vascular granular tissue. dried but wound area has been filled with multicellular and vascular granular tissue. Epithelial regeneration continues and the amount of inflammatory cells is also greatly reduced. In low dose treatment group wound surface covered by pustules and internal space of wound in the middle parts filled by fibrinous and granular connective tissue and inflammatory and purulent cells are seen between fibrin and connective tissue and granular texture is full of newly built vessels.

In zinc oxide group situation is entirely like with low dose treatment group. In eucerin treatment group wound surface is covered by pustules and regenerative epithelial cells starts to expanding on to the wound surface from sides. Inflammatory cells are purulent and infiltration of fibroblasts and existence of newly built vessels indicates formation of new granular tissue. In control group granular tissue as vascular and low filament tissue are seen. Hemorrhage and hyperemia is also seen (Fig. 2a, b).

On day 14 in high dose treatment group granular tissue is existed in the wound area and newly formed vessels are low than previous days. The intensity of inflammatory cells is reduced. Collagen fibers are more delicate and have more organization. Hydropic degeneration is also seen in some of the epithelial cells. In low dose treatment group more thickly epithelial covered wound surface. Collagen is thicker and has more organization. Coagulum is not existed. In zinc oxide group, fibroblasts start to synthesis of collagen. Inflammatory cells reduced and newly formed vessels increased. Lining tissue is seen in margin of the wound but clot is seen in some places. Blood clot on the wound contains large amounts of acute inflammatory cells were neutrophils and RBC has penetrated into the clot. In eucerin group space of wound around is occupied by young and multicellular

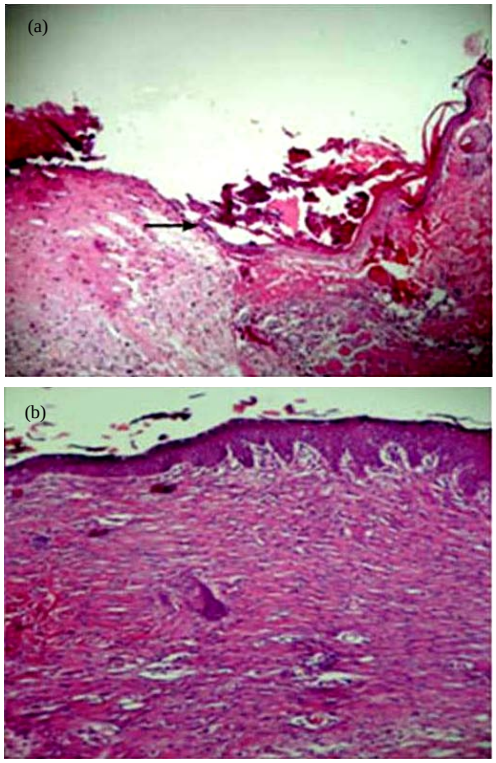

Fig. 1: a) Microscopic view from wound area in healing from high dose group on day 3. Arrow shows re-epithelialization from wound sides. b) Microscopic view from wound area in healing from low dose group on day 3. H and E 100x

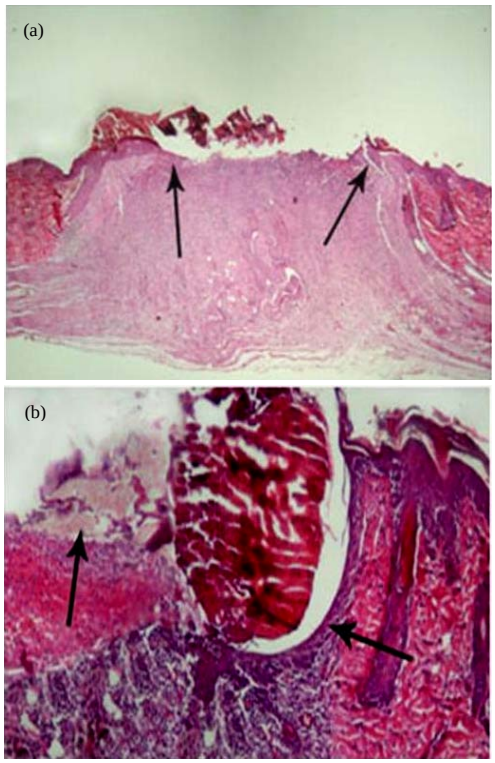

Fig. 2: a) Microscopic view from wound area in healing from high dose group on day 7. Arrows show re-epithelialization from wound sides. H and E 40x; b) Microscopic view from wound area in healing from Group 4 on day 7. Arrows shows expanding of epithelial cells into the pustule. H and E 100x

tissue and regeneration of the lining tissue starts from sides. Wound surface covered by pustules that follows contains hyperemia granular tissue. In control group, marginal parts of the wound are completely covered by epithelial tissue. Also, new and hyper cellular connective tissue covered dermal layer (Fig. 3a, b).

On day 21 in high dose treatment group in some cases wound surface is covered by lining tissue but in some others this is not occurred completely. The severity of inflammation and hydropic degeneration is reduced. In low dose treatment group, partial edema and hyperemia is still seen and collagen fibers were thicker and condensed and were more organized than day 14. In zinc oxide group, dermal accessories and hair follicles increased in the treated tissues. Collagen fibers increased and have more organization. In the eucerin and control groups, situations were same with day 14 with exception hydropic degeneration (Fig. 4a, b).

Zinc oxide ointment is among the most widely used topical ointments to treat ulcers is that content is 20% zinc oxide powder. Protect the surface being astringent, antiseptic and nontoxic relative of the outstanding characteristics that make the drug as an active ingredient in health and pharmaceutical compounds widely used. In this study were used of this ointment as positive control group too.

Normal wound healing occurs in three stages: inflammation, proliferation and remodeling. The wound healing process depends on a given provision of local circulation as well as the formation and deposition of collagen. A considerable amount of evidence has shown that *Aloe vera* improves wound and burn healing in animals and humans (Frank *et al*., 1995). Some studies found that 50% of rats showed improved wound healing >7 days (Choi *et al*., 2002, 2001). Aloe given subcutaneously showed a dose-response relationship on improvement of wounds. A similar response was recorded in diabetics whose wounds normally are characterized by poor or delayed healing (Desmouliere *et al*., 1993).

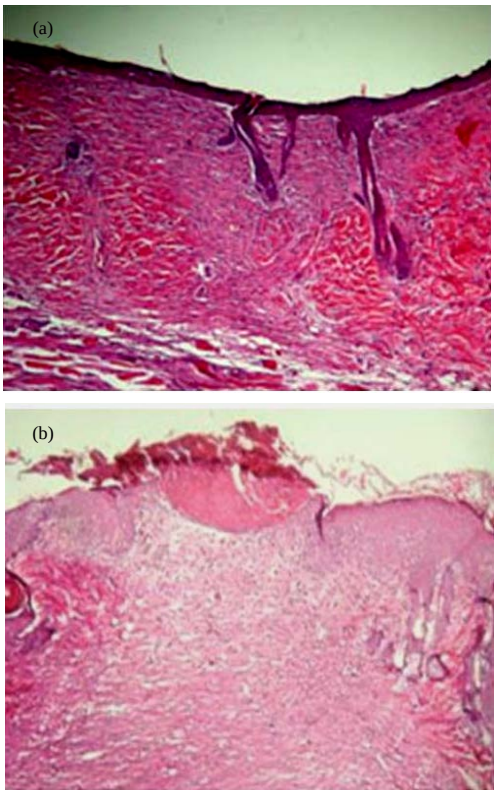

Fig. 3: a) Microscopic view from wound area in healing from Group 2 on day 14. H and E 80x; b) Microscopic view from wound area in healing from Group 3 on day 14. H and E 80x

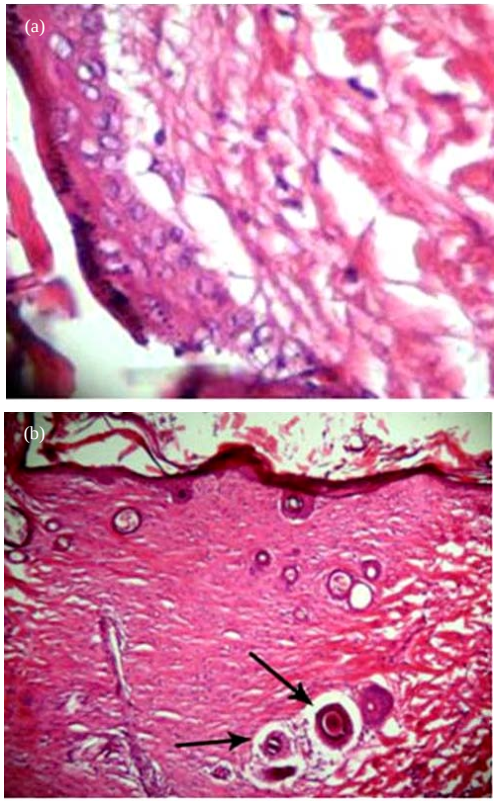

Fig. 4: a) Microscopic view from wound area in healing from Group 2 on day 21. H and E 220x; b) Microscopic view from wound area in healing from Group 3 on day 21. Arrows shows skin appendix H and E 60x

Several factors delay or reduce wound healing including bacterial infections, necrotic tissue, interference with blood supply, lymphatic blockage and diabetes mellitus. These conditions that inhibit wound healing can be combined under the classification of tissue anoxia (Liu *et al*., 2006) or reduction of oxygen in body tissue below physiologic levels. If tissue anoxia could be altered by regional superoxygenation an increased healing rate could be achieved.

It was found in the laboratory studies that *Aloe vera* was effective orally in promoting wound healing. Oral food grade *Aloe vera* (100 mg/kg/day) improved wound healing compared to the healing of control animals receiving only water. The decrease in wound diameters for the control animals was 3.5+/−0.3 mm (51.1%) whereas the Aloe-treated mice had a decrease in wound diameters by as much as 4.8+/−0.5 mm (62.5%). The difference was significant at $p<0.05$ (Davis *et al*., 1989).

Also, they found that Aloe vera administered topically also served to improve wound healing. The wounds on the mice that received 25% colorized *Aloe vera* demonstrated a 3.9 +/−0.4 mm reduction in diameter, as compared to the wound diameter reduction of 1.9 +/−0.3 mm ($p<0.001$) for the animals that received cream alone. No significant difference was observed between the untreated wounds and the wounds treated with Eucerin cream alone ($p>0.5$). Therefore, the percentages of decrease in wound diameters for the nontreatment control group, cream alone control group, and cream plus 25% *Aloe vera* group were 32.5% +/−4.8%, 25.4%+/−3.4%, and 50.8%+/−3.6%, respectively. These findings clearly suggest that 25% colorized *Aloe vera* was a significant factor in the healing of the wounds. Since, oxygen is required for the synthesis of collagen by fibroblasts (Moon *et al*., 1999), *Aloe vera* may improve the vascular supply and make more oxygen available to improve collagen formation for wound healing.

During the wound-healing process, epithelial cells proliferate, migrate from the edges of the wound and eventually cover the wound with skin. By lysing collagen with enzymes, the epithelial cells move across the wound and attach to viable tissue. The proliferation and migration of the epithelial cells are dependent on an adequate supply of oxygen. Therefore, the increased presence of oxygen caused by the *Aloe vera* improving microcirculation should greatly improve the wound-healing process (Mustoe *et al*., 1991). It is hypothesized that catecholamines (epinephrine and norepinephrine) retard epithelial cell proliferation (Pan *et al*., 2002). When a wound is sustained, the supply of catecholamines is interrupted and the barrier to mitosis is removed.

Thus, cells begin to grow and divide. Possibly some constituents of *Aloe vera* may either block catecholamines or directly stimulate epithelialization to improve wound healing. During early wound healing, the vascular and lymphatic systems are of primary importance. Failure or delay of vascular regeneration decreases oxygen transport to the wound which subsequently depresses the mobilization of excessive fluids from the wound site. The wound becomes edematous leading to further damage infection and eventually cell death. In wound healing new blood vessels sprout up from platelets or macrophages to keep the wound open-ended. Hypoxia may be a stimulant to revascularization. Aloe may thus achieve the following effects to improve tissue healing: an increased blood supply and hence an increased oxygen supply to the wound by blocking vasoconstrictive compounds (inflammation stage) greater migration of epidermal cells over moist tissue caused by factors and enzymes present in *Aloe vera* (proliferation stage) and extensive reorientation of collagen fibers caused by a stronger cross-linking (remodeling stage) (Qiu *et al*., 2000). *Aloe vera* also, provides for a clean wound free of excess exudate and contamination making it a favorable treatment for wounds.

CONCLUSION

Therefore, from these findings it could be concluded that application of Av to an open wound induces significant wound contraction and accelerates wound healing and this herbal aqueous extract may be a promising medication for open wounds. Macroscopic, microscopic and biomechanical results indicated that topically administered Av accelerated epithelialization, wound contraction, tissue alignment and tissue strength at the later stage of wound healing.

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