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EFFECT OF AQUEOUS EXTRACT OF *Aloe vera* ON EXPERIMENTAL CUTANEOUS WOUND HEALING IN RABBITS

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Abstract

The present study was designed to evaluate the effectiveness the *Aloe vera* gel and leaf extract on wound healing. Eight adult male rabbit with age between 12 - 15 weeks were used. The animals were divided into two groups namely, control and treated 5 m full-thickness skin wound experimental induced on gluteal region by sharp dissection. The treated group was healed faster in comparison with control group. The effect produced by *Aloe vera* with references to wound healing contraction, wound closure and regeneration of tissue at the site of wound, clear in histopathological

Key words: Wound , *Aloe vera*, Coetaneous wound and Wound healing.

1. Introduction

Aloe vera is a succulent plant that is found only in cultivation, having no naturally occurring population, although closely related aloes do occur in Northern Africa. The species was frequently cited as being used in herbal medicine since the beginning of the first century AD. Extract from *Aloe vera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing or soothing properties. There is however, little scientific evidence of the effectiveness or safety of *Aloe vera* extract for either cosmetic or medicinal purposes, and that positive evidence was frequently contradicted by other studies (Rama and Srini, 2008).

The flowers are produced in summer on a spike upto 90 cm (35 inches) tall, each flower being pendulous, with a yellow tubular corolla 2 - 3 cm (0.8 - 1.2 inches) long. Like other aloe species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (James *et al.*, 2004). *Aloe vera* leaves contain phyotochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, such as emodin and various lectins (James *et al.*, 2004).

In pathology, it specifically refers to a sharp injury which damages the dermis of the skin (Eliyam and Banda, 2011). The classic model of wound healing comprises three or four sequential, yet overlapping, phases (1) homeostasis (not considered a phase by some authors), (2) inflammation, (3) proliferation and (4) remodeling upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage (Nguyen *et al.*, 2009). The present study was conducted to evaluate the wound healing capacity of *Aloe vera*.

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2. Materials and Methods

The plant of *Aloe vera* was collected from College of Agriculture, Basra University, Iraq. The gel was extracted from leaves using tradition hand filtering and put in a clean container keep it. While the leaves from which the gel have been drained were chopped in to pieces and kept at room temperature. All the dried parts of the leaves were grinded in powdered from mortar and pestle, 40 g of the whole leaf powder were dissolved in 300 ml of ethanol for extraction. This process was allowed to soak one – three day proper extraction of the action ingredients at room temperature. The mixture then filtered using Whitman No 0.22 mm Millipore filter paper. The solvents were evaporated using water bath at a maintained temperature to ensure proper concentration. Then, the powder weighed, taken from ethanol and dissolved in sterile water to prepare for use.

The animals were anesthetized by intra muscular injection (3 mg/kg/ B.W) of xylazine hydrochloride and (22 mg/kg/ B.W) of ketamine hydrochloride (Fig 1 and Fig 3). The skin shaved by ordinary clipper, disinfected with 70 % alcohol. An area of uniform wounds (5 cm in diameter) was incised in the thigh region. Avoiding incision of the muscle layer and tension skin was kept constant during the procedure. The wound was measured immediately under general anesthesia by placing a measurement ruler (Fig 1 and Fig 2). Wounds in group 1 rabbits remain without dressing as a control group, rabbits in group 2 were dresses topically once daily with 0.5 ml of 100 % alcoholic extract of *Aloe vera* leaf (Fig 3). Cross sectional full thickness skin biopsy specimens from each group were collected on days 3, 5, 7 and 10 and the histological evaluation was carried out during the study. The tissue were fixed in 10 % buffered formalin and passed during through different grades of alcohol and were embedded in paraffin wax.

Samples were sectioned (3 – 5 mm) and stained with hematoxylin and eosin. For collagen deposition studies, traces of staining reaction, hyalinization and irregular arrangement of collagen bundles were considered as positive. Two areas in each section were control for neovascularization and fibroblast proliferation.

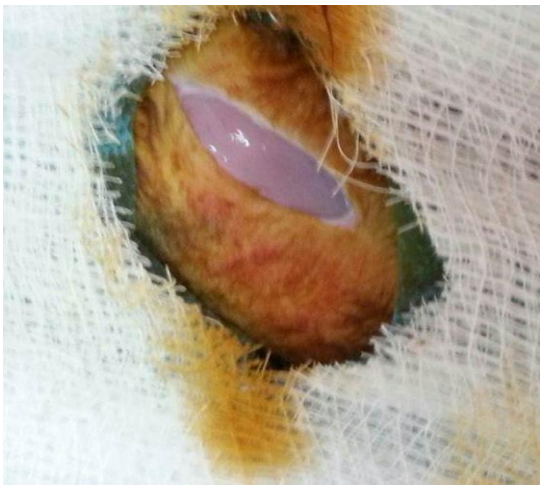

Fig - 1: Skin in the gluteal region

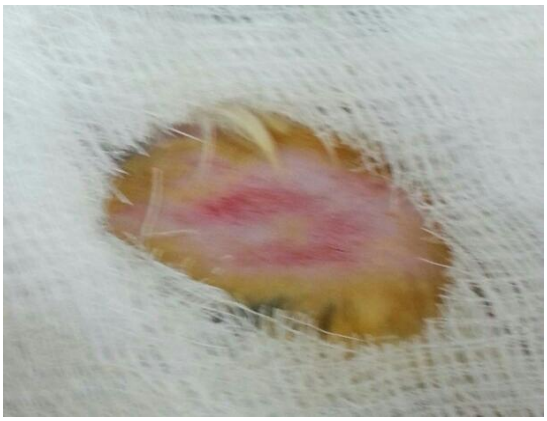

Fig - 2: Wound incision in the gluteal region

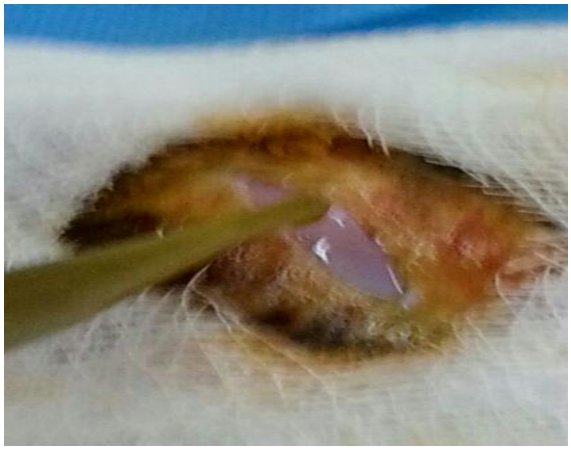

Fig - 3: Topical application of aqueous extract of *Aloe vera* on wound

3. Results

On three days in control group, show ulcer associated with heavy infiltration of inflammatory cells (Fig. 4) but in show treated group in this period that new epidermal growth thin layers of cells covering the area of wound and also presence of scab formation consist mostly of neutrophil (Fig. 5). On five day in control group, show ulcer with heavy infiltration of inflammatory cell (Fig. 7), but in treated group in this period that explain thicken epidermis with slight hyperkeratosis (Fig. 6).

On seven day in control group, show ulcer with heavy infiltration of inflammatory cell (Fig. 9), but in treated group in this period that explain thicken epidermis and scab formation with slight hyperkeratosis (Fig. 8). On ten day in control group show ulcer on one side and thicken epidermis and scab formation on other side (Fig. 11) but in treated group in this period that explain thicken epidermis and scab formation, dermal fibrosis and increase number of hair follicles (Fig. 10).

4. Discussion

During the early healing, epithelial cell proliferate and migrate from the edges of the wound and eventually cover it. Proliferation and migration of the epithelial cells and fibroblast is dependent on an adequate oxygen supply. This amount of oxygen was supplied either by increasing the rate of blood flow in the existing blood vessels or the granulation tissue received its oxygen requirement through newly formed blood vessels, increase in the blood flow by this reagent was very unlikely and the angiogenic activity of this extract possibly was responsible for providing more blood and oxygen supply and therefore an enhanced wound healing outcome (Rubin, 1984).

In addition to an improved alignment and reduced inflammatory cell in filtration of the treated lesions compared to those of the untreated ones, the treated lesion after both 10 and 20 day pl in the present study were more vascular. It has been said that *Aloe vera* speeds up the healing of damaged epithelial tissue in wound by providing essential micronutrients by its angiogenic behavior, eliciting an anti-inflammatory effect and stimulation of skin fibroblasts (Danhoff and Mcanally, 1983). Moghbel *et al*. (2007) reported that the rate of found healing of burn wounds treated with *Aloe vera* gel was 50 % faster than routine treatment with silver sulfadiazine. They were not able to propose the exact mechanisms of the action of *Aloe vera* natural gel on burn wounds but suggested that the mannose-6-phosphate present in *Aloe vera*, which contains glucose and mannose chains may be effective in improving the healing rate (Moghbel *et al*., 2007). Mannose 6-phosphates has been introduction as the active part of Av responsible for wound healing (Davis *et al*., 1994). This substance also contains enzymes, glycoprotein, growth factors, vitamins and minerals (Davis, 1991) that have been shown to improve healing with enhanced epitheliazation and rapid, formation and maturation of granulation tissue in burn wounds (Visuthikosol *et al*, 1995).

It has been stated that insulin-like growth factor all and mannose-6-phosphate bind to the same receptor on the fibroblast (Westlund *et al*., 1991). These two ligands activate the fibroblast to enhance the wound healing process. An important criterion that might make (Surbamanian *et al*., 2006) and Moghbel *et al*. (2007) findings different from the finding of the present study was that they applied the gel for longer time. Subramanian *et al*. (2006) applied the gel twice a day for a period of 14 days and Moghbel *et al*. (2007) applied the gel twice a day for 18 days, while in the present experiment the *Aloe vera* was applied once a day for ten days.

Higher rate of wound contraction on days 15 and 20 pl together with significant improvement of biomechanical and histopatological finding in the treated lesions on day 20 pl compared to those of the untreated ones in the present study are in accordance with findings of most of the previous investigators (Chitha *et al.*,1998; Supramanian *et al.*, 2006; Moghbel *et al.*, 2007; Feily and Namazl, 2009). The enhanced rate of wound contraction and reduction in healing time in treated rats might be due to the anti-inflammatory effects of this

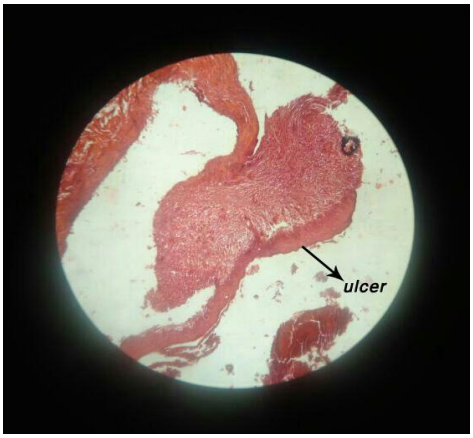

Fig – 4: Control wound lesion on third day show ulcer associated with heavy infiltration of inflammatory cells. H and E Stain 10X

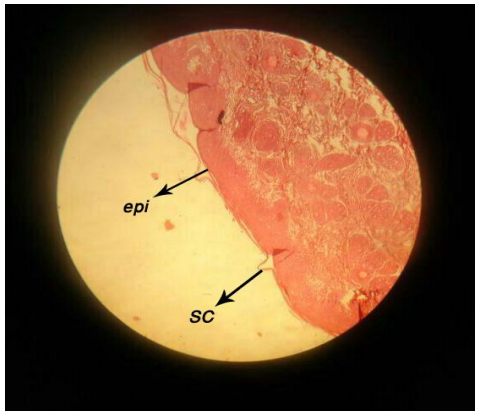

Fig – 5: Treated lesion on third day show new epidermal (epi) growth thin on one to two layers of cell covering the area of wound and also presence of scab (sc) formation consist mostly of neutrophil with heavy infiltration of inflammatory cells E Stain 10X

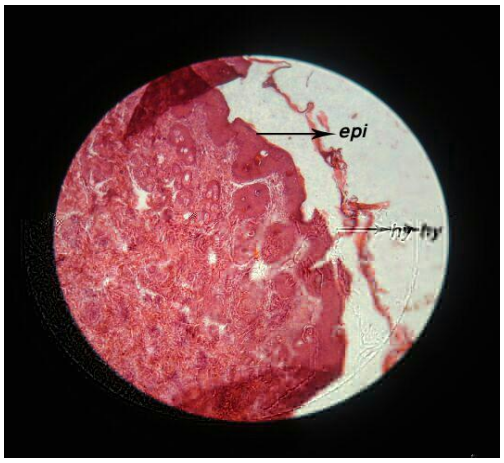

Fig - 6: Treated wound lesion of fifth day show thicken epidermis (epi) with slight hyperkeratosis (hy) H and E Stain 10 X

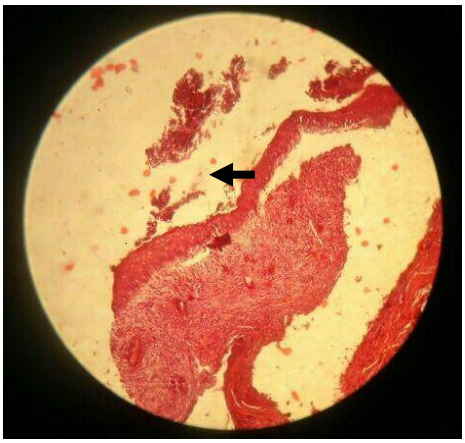

Fig - 7: Control wound lesion on fifth day show ulcer with heavy infiltration of inflammatory cells H and E Stain 10X (Arrow mark represents the ulcer cells)

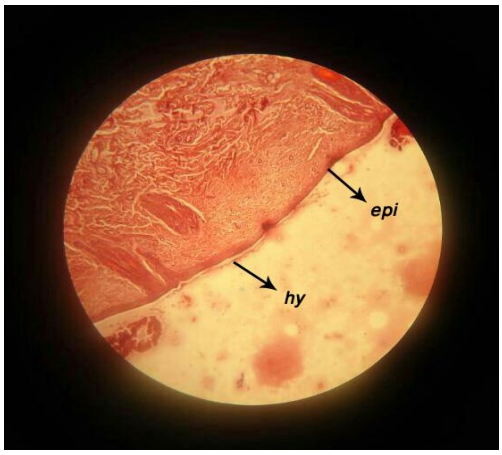

Fig - 8: Treated wound lesion seventh day shows thicken epidermis (epi) and scab formation with hyperkeratosis (hy). H and E Stain 10X

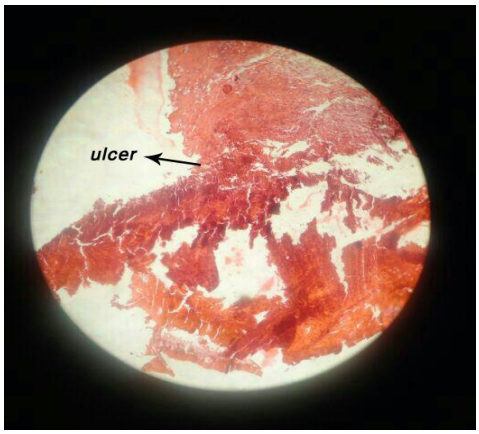

Fig - 9: Control wound lesion on seventh day show ulcer with heavy infiltration of inflammatory cells. H and E Stain 10X

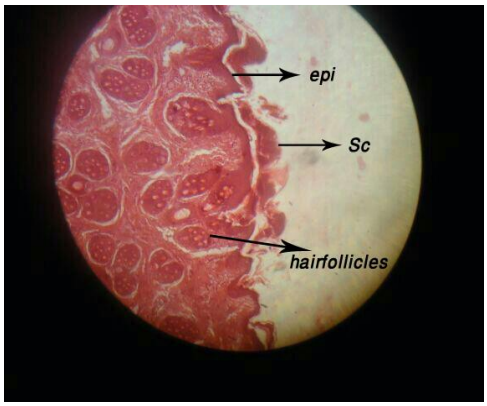

Fig - 10: Treated wound lesion on Ten day show thickens epidermis (epi), scab (sc) formation, dermal fibrosis and increase number of hair follicles. H and E Stain 10X

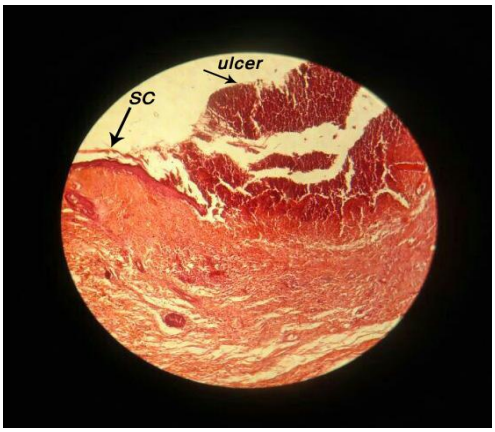

Fig - 11: Control wound lesion on Ten day show ulcer on one side and thicken epidermis and scab (sc) formation on other side. H and E Stain 10X

material together with its effect on maturation and organization of the granulation tissue.

Another and perhaps significant feature in the treated group was that their newly formed collagen fibers were aligned and were not randomly distributed as in the untreated lesions. The histological appearance indicated a greater degree of organization of the collagen orientation in the treated lesions and a more normal alignment of new collagen, which was strikingly similar to that of the normal undamaged skins. It was possible that this was brought about by a modification of the reaction or organization of the fibrin network in the tissue spaces at early stages of inflammatory phase of healing by the *Aloe vera* extract, which may act as a scaffold or template for fibroblast activity. It also appears that *Aloe vera* may have enhanced the return of cellularity to within normal level. This could merely reflect the anti- inflammatory effect of the drug in reducing the number of adventitious phagocytic cells in the area.

The conclusion of this study was the application of *Aloe vera* to an open wound induces significant wound contraction and accelerates wound healing and this herbal aqueous extract may be a promising medication for open wound and microscopic examination indicated that topically administered *Aloe vera* accelerated

wound contraction, tissue alignment and tissue strength at the later stage of wound healing.

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