The antimicrobial and wound healing effect of Aloe Vera in induced diabetic rats

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Abstract

The study was conducted on sixteen streptozotocin induced diabetic rats to investigate whether the topical application of Aloe Vera gel and alcoholic Aloe Vera leaf extract could improve the wound healing in diabetic rats. The rats were weight, matched, and placed in to four groups 4 rats of each group. Full thickness circular skin wound (2cm in diameter) was experimentally created dorsal to the neck area of each rat. Animals in group (1) left without dressing as a control group. Animals in group (2 and 3) were treated topically with Aloe Vera gel (100%) and alcoholic Aloe Vera leaf extracts (100%) respectively, while the animals of group (4) were treated with povidone iodine (4%). Treated groups were dresses twice daily for 14 successive days. The treated wound in groups 2, and 3 were significantly healed faster in comparison to the wound of control group. The effect produced by the Aloe Vera with reference to the wound contraction, wound closure, decreased in surfaces area of wound, and tissue regeneration at the wound site. Histologically wounds treated with Aloe Vera show increase in macrophages, fibroblast migration, collagen regeneration and epithelialization compared with the control group. The wet, dry granulation tissue weight, and hydroxyproline content increased significantly when compared to control. Aloe Vera was seen exhibited antimicrobial activity against some pathogenic microorganism. In conclusion Aloe Vera have strong wound healing property and effective as topical preparation and formulated as fairly economical therapeutic agent for wound management.

Key words: Diabetic, excision wound model, Aloe Vera, wound healing.

الفعالية المضادة للميكروبات والمحفزة على التئام الجروح لنبات الصبار Aloe Vera في الجرذان المستحثة الإصابة بداء السكري

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الخلاصة

أجريت الدراسة على ستة عشر من الجرذان المستحثة الاصابة بداء السكري بمادة الستربتوزوتوسين للتحري عن تأثير الاستخدام الموضعي (للهلام وللمستخلص الكحولي) لنبات الصبار Aloe Vera في تحسين شفاء الجروح في الجرذان المصابة بداء السكري .تم اخذ أوزان الجرذان ومن ثم تقسيمها إلى أربع مجاميع للمعاملة (أربع جرذان في كل مجموعة)، تم عمل جرح جلدي دائري بقطر 2 سم يشمل كل طبقات الجلد في منطقة الجزء الظهري لرقبة الجرذ. الحيوانات في المجموعة (1) تركت بدون معاملة كمجموعة سيطرة، والحيوانات في المجموعة (2 و 3) تم علاجها موضعيا بهلامي نبات الصبار بتركيز (100٪) والمستخلص الكحولي لأوراق نبات الصبار بتركيز (100٪) أيضا بينما حيوانات المجموعة (4) عوملت باليود بتركيز (4٪)، مجاميع المعالجة كانت تعالج مرتين باليوم ولمدة 14 يوم متتالية .وجد من خلال الدراسة وجود فروق معنوية في النسب المئوية والمدة الزمنية اللازمة لالتئام الجروح حيث إن التئام الجرح في المجموعة (2 و 3) كان بشكل أسرع مقارنة بمجموعة السيطرة والمجموعة (4)المعاملة باليود. إن التأثير الناتج من استخدام نبات الصبار يؤدي إلى انكماش الجرح ، وإغلاقه ، وانخفاض المساحة السطحية للجرح وإعادة تجديد الأنسجة في موقع الجرح وأظهرت نتائج الفحص النسيجي للجرح المعامل بهلامي نبات الصبار أو المعامل بالمستخلص الكحولي لأوراق نبات الصبار زيادة في الخلايا البلعمية (macrophage)، هجرة الخلايا الليفية، وإعادة تجدد الكولاجين وتكوين النسيج الندبي مقارنة مع مجموعة السيطرة .كما اظهرت الدراسة وجود فروق معنوية في نسب النسيج الحبيبي الجاف والرطب ومحتوى الهيدروكسي برولين بشكل ملحوظ بالمقارنة مع مجموعة السيطرة .وجد من خلال الدراسة إن لنبات الصبار فعالية عالية في تثبيط نمو وقتل بعض الكائنات الحية الدقيقة المسببة للأمراض .نستنتج من خلال الدراسة إن لنبات الصبار تأثير فعال في شفاء و التئام الجروح، لذا نوصي باستخدامه كمادة علاجية موضعية فعالة في التئام الجروح واقتصادية.

الكلمات المفتاحية: السكري ، التئام الجروح ، الصبار.

Introduction

Wounds are physical injuries which lead to a break in epithelial integrity of the skin and may be accompanied by alteration of the structure and function of the under lying normal tissue. It can be caused by contusion lacerations, abrasions or hematoma [(1)].Wound healing is a dynamic process starting from the time of injury and continue to a varying periods of time depending on the type of injury and the presence of other factors that affect healing as, wound infections, preexisting skin conditions, immune suppression, or diabetes mellitus[(2)]. It consist of three phases the first phase is inflammation , hyperemia and leukocyte infiltration , the second phase consist of removal of the dead tissues and the third phase consist of epithelial regeneration and formation of collagen and fibrous tissue which provide strength and integrity to the dermis[(3)].Diabetes mellitus is a condition known to be associated with a variety of connective tissue abnormalities and this lead to the impaired wound healing observed in diabetic patients. The collagen content of the skin is deceased as a result of decreased biosynthesis or increased destruction of the newly formed collagen[(4)], as a result of the impair healing of diabetic wounds lead to significant morbidity, prolonged hospitalization, and enormous health care expenses[(5)] . Surgical treatment of diabetic wounds remains difficult and insufficient leading to high morbidity among those patients [(6)]. This necessitate finding other way of therapeutic strategies that help wound healing in such patients, one of these ways is the use of herbal plants. Among various plant herbs, Aloe Vera is very popular for both ayurvedic and traditional medicine for its vast medicinal properties [(7)]; it is used internally as laxative, hemorrhoid remedy, anthelminthic and menstrual regulator. Also Aloe Vera has been used for centuries in the treatment of wound because of its excellent wound healing and antimicrobial properties [(8)] , and is often called the “Natural healer". It enhances wound healing mechanism by increasing microcirculation through increasing oxygenation [(9)]. Also it block the action of catecholamine and thus increase epithelialization and increase vascularization of the wound and help to remove dead tissue and make the wound healthy [(10)]. Aloe Vera may also increase cross linking of collagen and collagenation by stimulating macrophage cytokine production and block some wound healing inhibitor like sterols and amino acid, the ascorbic acid which is found in Aloe Vera enhances the synthesis of collagen [(11)].This study is aimed to determine the antimicrobial and wound healing properties of Aloe Vera in streptozotocin – induced diabetic rats.

Materials and methods

1-Plant sample collection :-The plant of Aloe Vera was collected from herbal garden in Al-Samawa city.

2-Collection Aloe Vera gel and leaves plant extraction :-The gel was extracted from leaves using traditional hand filtering and put it in a clean container to keep it. While the leaves from which the gel have been drained were chopped in to pieces and kept at temperature 20 – 28c°. All the dried parts of the leaves were grinded in powdered form using mortar and pestle, a 25g of the whole leaf powder were dissolved in 250 ml of ethanol for extraction. This process was allowed to soak one to three days at room temperature for proper extraction of the leave active ingredients [(12)]. The mixture then filtered using Whitman filter paper (0.22mm Millipore).The solvents were evaporated using water bath at a maintained temperature to ensure proper concentration. Then weight the powder taken from ethanol and dissolved it in sterile water to prepare for use.

3-Experimental animals :- Adult male rats were obtained from the experimental animal house, faculty of veterinary medicine, university of Baghdad. The rats were divided in to 4 groups 4 rats of each. Rats weight between 200 – 220 g. The animals were controlled on standard diet and tap water.

4-Diabetics animals:- Diabetes mellitus induced in all groups by a single injection of streptozotocin (STZ, 50mg /kg I.P) prepared in citrate buffer, after overnight fasting [(13)]. Blood was taken from the tail of the rat (24 h) after the injection and the glucose level was estimated using glucose meter.

5-Experimentally induced excision wounds :- Wounds were created on the 7th day after induction of diabetes in all rats. The animals were anesthetized by diethyl ether. The skin dorsal to the neck was shaved by electrical clipper, disinfected with 70% alcohol and injected with 1ml of Lignocaine HCL 2%. An equal uniform circular area of skin 2 cm in diameter (3.14 cm^2) was marking by circular stamp and full thickness of the nape skin was excised as described by Morton and Malone [14], with the avoiding incision of the muscular layer, and tension of skin was kept constant during the procedure. The wound area was measured immediately under light diethyl ether anesthesia by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on $1mm^2$ graph sheet and traced out. The squares were counted and the area was recorded as described by Chah [15] with slight modification.

6-Topical application of vehicles:- Wounds in G1 remain without dressing as a control group. Wounds in treated groups (G2, G3, and G4) were dresses topically twice daily for 14 successive days with 0.2 ml of (100%) Aloe Vera gel in G2, 0.2 ml of (100%) alcoholic extract of Aloe Vera leaf in G3, while in G4 with 0.2 ml of (4%) povidone Iodine.

7-Histopathological Studies:- Cross sectional full thickness skin biopsy specimens from each group was collected on day 15 and the histological evaluation was carried out during the study. The tissues were fixed in 10% buffered formalin and passed through different grades of alcohol and were embedded in paraffin wax. Samples were Sectioned (5µ) 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 counted for neovascularization and fibroblast proliferation.

8-Dead space wound model:- Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on either side in the groin and axilla on the ventral surface of each rat by using of D'Arcy *et al* technique. [16]. On the 10th post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the wet weight of the granulation tissue, the tissue was dried at 60 °C for 12 hr., the dry granulation tissue weight was recorded. 6N HCL was added to the dried tissue and kept at 110°C for 24 hr. the neutralized acid hydrolysate of the dry tissue was used for determination of hydroxyproline [17]. Additional piece of wet granulation tissue was preserved in 10% formalin for histological studies.

9- Antimicrobial activity :- *Pseudomonas aeruginosa, proteus murabilis, Enterobacter agglumerans* and *Staphylococcus aureus* were the organism tested. The bacterial strains were obtained from fresh colonies grown on MacConkey blood agar plates. Sensitivity testing was done using Muller Hinton Agar plates . Known volume of bacterial suspension was transferred to each micro plate well. Alcohol leaf extract and gel of aloe Vera were added to the micro plate wells and incubated at 37° C for 18-20 hr. results were determined by visual inspection of zones of growth inhibition [18] .

Results

Significant increase in the wound healing activity was observed in Aloe Vera leaf alcoholic extract and gel. In excision wound model, animals of groups 2 and 3 showed a better healing pattern with complete wound closure was observed in rats treated with 14 days for Aloe Vera gel and leaf alcoholic extract. There was significant reduction in the epithelialization period and increased percentage of wound contraction and closure rate when compared with animals of groups 1 and 4 (Table 1), (Figure.1a,1b) with reference to the percentage of wound closure , The results indicated that Aloe Vera gel showed (99.19%) wound closure followed by Aloe Vera leaf alcoholic extract which recorded (98.66%), (Figure2a;2b).In the dead space wound model (Table 2), The treated animals in groups 2 and 3 showed significantly higher levels of hydroxyproline compared with another group ($P< 0.001$) . A significant

increase was also observed in the dry and wet weight of the granulation tissue in the animals treated with Aloe Vera gel (P<0.001) . In animals that did not receive the Aloe Vera treatment, the wounds appeared to be hard and crusty with undermined margins. In contrast the wounds in the animals treated with the Aloe Vera were clean and showed bright red healthy granulation tissue. Wound treated with Aloe Vera compare favorably with the standard drug (povidone iodine) on the wound closure

Table1 : The percentage of wound healing activity of Aloe Vera in expressed as (mean ± SE) . P values = ** < 0.01 , *<0.001**

Animal groups	Type of dressing D.W	No of animals	Healing time (days) (mean±SE)	Percentage of wound healing on day post-surgery			Epithelializati on period
				5 day	10 day	15 day	
Group 1	Without dressing	4	15.17± 0.6	26.45	46.10	66.94	18.1±0.16
Group 2	100% Aloe Vera gel	4	6.17 ± 0.6	(77.91)***	(89.33)***	(99.10)***	15.3 ± 1.5
Group 3	100% Aloe Vera alcoho - lic leaf extract	4	8.12 ± 0.5	(57.04)**	(78.03)**	(97.09)**	13.1 ± 0.3
Group 4	(povidone iodine) (4%)	4	7.06 ± 0.5	(34.05)	(66.07)	(70.15)	16.2 ± 00

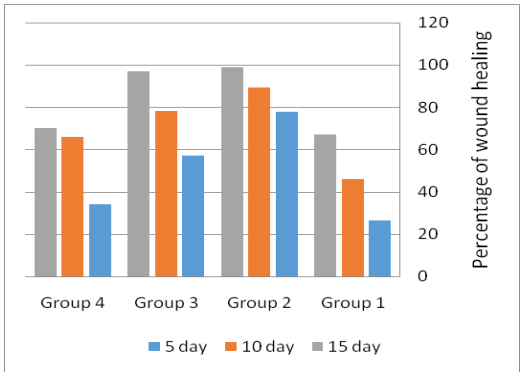

Fig. 1a: The percentage of wound healing on day post surgery

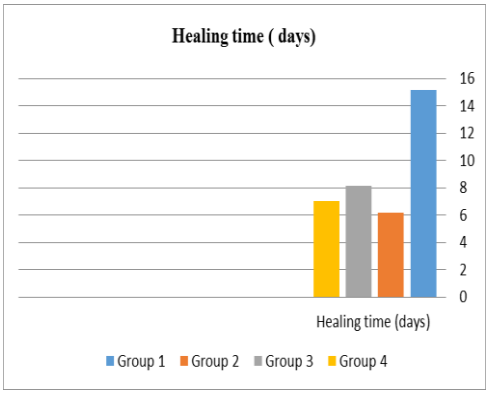

Fig.1b:The healing time in days for different groups

Table 2 : The activity of Aloe Vera gel and leaf extract on dead space wound (Values are mean ± SE)

Group of animals	No of animals	dead space wound		
		Wet granulation weight (mg)	Dry granulation weight (mg)	Hydroxyproline (mg / gl tissue)
Group 1	4	103.17 ± 5.15**	33.60 ± 1.77	106.10 ± 21.20
Group 2	4	175.33 ± 20.90	56.40 ± 5.50**	198.01± 306***
Group 3	4	150.30 ± 3.32	43.23 ± 3.50*	184.0 ± 121
Group 4	4	134.3 ± 5.2	35.34 ± 4.9	199.0 ± 13.9

The histological evaluation in group2 and group 3 showed that there were more dense collagen fibers and fibroblasts with few neovascularization and inflammatory cells, Appearance of skin appendages can also be visualized (Table.3) (Fig.3). While in control tissues showed that there were dense collagen fibers and fibroblasts with more inflammatory cells showing in complete healing (Fig.4).

Table 3: The histological finding of wound after 14 days treatment with Aloe Vera gel and leaf extract in diabetic rats.

Group	Collagen fibers	Edema	Neovascularisation	Proliferation of fibroblast
Group 1	+ +	+ + +	+	+ +
Group 2	+ + +	+	+ + +	+ + +
Group 3	+ ++	+	+ +	+ + +
Group 4	+ +	+	+ + +	+ + +

+ Slight , ++ Moderate , +++ Marked

The Aloe Vera gel showed more antimicrobial activity than Aloe Vera leaf extract against selected organism such as: *Pseudomonas aeruginosa, Proteus murabilis, Enterobacter agglumerans, and Staphylococcus aureus* (Table. 4) (Fig. 5).

Table 4 : Antimicrobial activity of Aloe Vera gel and leaf extract against different bacterial strains

Tested organism	Different group of the study			
	Group 1	Group 2	Group 3	Group 4
Pseudomonas aeruginosa	-	+ +	++	+ +
Proteus murabilis	-	+ +	+	+
Enterobacter agglumerans	-	+ +	+	+
Staphylococcus aureus	-	++	+ +	+ +

Antimicrobial activity : - No inhibition ; + Zone of inhibition ≤ 8mm in diameter ; ++ Zone of inhibition > 8mm diameter

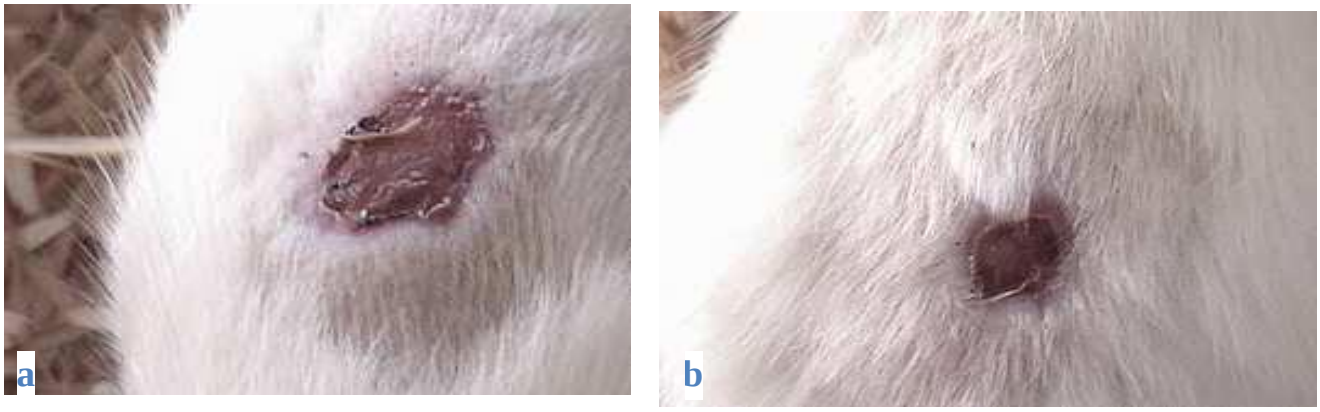

Fig.2. Gross appearance of wound healing on day 15 post surgery. a)- Wound healing without dressing in control group. b)- Wound healing dressed with Aloe Vera gel.

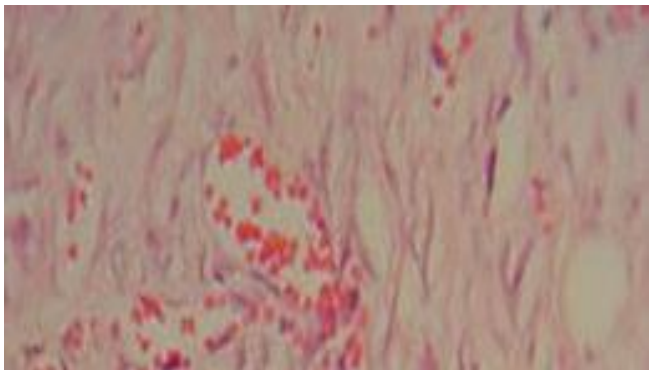

Fig.3.Histological section of healed wound (H & E stain X 40): Wound healing dressed with Aloe Vera . Granulation tissue contains comparatively more collagen, fibroblast and blood capillaries, and few inflammatory cells.

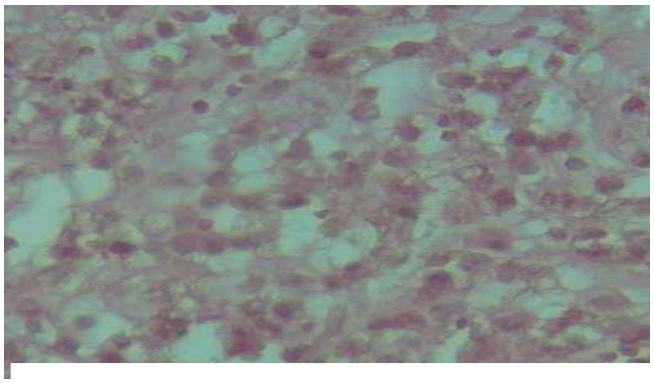

Fig.4. Histological section of healed wound (H & E stain X40): Wound without dressing in control group. Granulation tissue contains comparatively less collagen, fibroblast and blood capillaries, and more inflammatory cells.

Fig.5 .Show the antibacterial effect of A- Aloe Vera gel ; B-Aloe Vera leaf extraction in comparison with C- control group

Discussion

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state [(19)]. According to [(20)] in his study reported that wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage where by the area of the wound undergoes shrinkage. He also noticed that in the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. Wound healing deficits in diabetes are diverse, complex and inter related [(21)]. This defect is believed to be caused by impaired blood flow and oxygen release from increased blood sugar, decreased collagen and fibronectin synthesis from protein malnutrition, impaired local immune and cell defenses, and decreased anabolic activity with decreased insulin and growth hormone. Collagen, fibrin and keratin accumulate advanced glycation and this effect on binding of regulatory molecules, susceptibility to proteolysis and finally decreased the ability for protein cross – linkage [(22)]. Di Girloame et al [(23)]. Postulated that defects in wound healing are caused by the hyperglycosylation of the locally synthesized cellular fibronection. However hyperglycemia affects the whole range of neutrophil function, which includes migration, chemotaxis, adherence and phagocytes and bacterial activity [(24)]. Results of the present investigation clearly indicated the wound healing properties of Aloe Vera, after its topical application in diabetic induced rats. The wound size reduced as early as day 5 in animals with induced diabetes compared with wounds in control animals and those that were treated with

topical Ionosphere the observed increase in hydroxyproline, a specific marker of collagen and an important component of extracellular granulation tissue matrix in the wound treated with Aloe Vera this increased rate of wound contraction. Subramanian et al [25] reported that application of Aloe Vera gel to full thickness and infected pediatric burns provided effective de sloughing of necrotic tissue and sufficient wound granulation which was suitable for a spilt thickness skin graft. Tsuchiya et al [26] who reported that Aloe Vera leaf extract and gel promote the wound healing mechanism and mainly due to their antimicrobial properties and proteolysis enzyme growth factor, minerals and vitamins which appear to be responsible for wound contraction and increased rate of epithelialization. The histological studies of Aloe Vera leaf extract and (100%) of Aloe Vera gel exhibited marked dryness of wound margins with tissue regeneration in the 14th days of the experimental period. The histological evaluation showed that increased cellular infiltration in treated cases may be due to chemotactic effect enhanced by crude extract which might have attracted inflammatory cells towards the wound site[27] , or due to the antioxidant property of the extract of Aloe Vera leaves[28], and the increase of cellular proliferation may be due to the mitogenic activity of the plant , which might have significantly contributed to healing early dermal and epidermal regeneration, increased proliferation, granular tissue formation and epithelialization in treated rats, all this give good support that Aloe Vera have good wound healing properties[29].

Conclusion

The present study demonstrates that Aloe Vera leaf extract and gel applied topically promotes healing of wounds, with enhanced rate of collagen turnover and wound contraction in the late stage of wound healing mechanism as evidenced by the histological studies. Hence it may be concluded that Aloe Vera leaf extract and gel may be used as a cheap, effective topical preparation and formulated as fairly economical therapeutic agent for wound management as a pro-healer.

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