Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

Ultrasonic Enhancement of Aloe Vera Gel Effect on Full-thickness Wound Healing in Diabetic Rats

Hesham Galal Mahran[1] Mohamed A.S. Aborehab[2,3]

[1]Department of Physical Therapy for Surgery, Faculty of Physical Therapy, Cairo University, Egypt.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia, Egypt.
[3]Department of Pharmaceutics, Faculty of Pharmacy, Umm Al-Qura University, Makkah, KSA

Abstract

Objective: To investigate the ultrasound-enhanced Aloe Vera gel efficacy on diabetic wound healing in rats. **Methods:** Sixty male, rats were used and classified randomly into three groups; (A, B, and C.) **Interventions**: Streptozotocin (STZ) was intraperitoneally injected for induction of diabetic model. A diabetic wound area of 6 cm^2 on the back of each rat was made after anesthesia. In group (A): Twenty rats received combined treatment of topical Aloe Vera gel and ultrasonic waves with a frequency of one MHz, an intensity of a half-watt per centimeter square and 40% on-off cycle. In groups (B): Twenty rats received ultrasonic waves with the same parameters as in group A then followed by topical Aloe Vera gel application while the last 20 rats in group (C) received the same combined treatment in group (A) while the ultrasound device was in off mode. Session time for A and C groups was 5 minutes while for the group (B), the time was totally 10 minutes; 5 minutes for ultrasonic and 5 minutes for topical Aloe Vera gel. All groups received 3 sessions/week for two weeks. **Outcome measures:** Wound area and its reducing rate were measured 3 times; at 0 day, and at the end of 1^{st} and 2^{nd} weeks. **Results:** Wound healing in group A was significantly enhanced compared with that in the other two groups. **Conclusion:** Ultrasound waves significantly enhance the effect of topical Aloe Vera gel on thefull-thickness wound model in diabetic animals.

Keywords: Full-thickness Wound, Diabetic Rat, Ultrasonic, Aloe Vera gel.

INTRODUCTION

Wound is a break in the integrity of the skin epithelial, it is usually characterized by obevious changes in the underlying normal tissue structure and function (1). Healing of the wound is a highly orchestrated, but complicated series of events which can be diversified into three overlapping phases including hemostasis and inflammation, proliferation, and remodeling (2; 3). At the stage of inflammation; the phagocytic cells produce clots that have a role in the debridement of the injured tissues. The second phase is a proliferative phase at which; Epithelialization, fibroplasia, and angiogenesis occur and also the formation of mature granulation tissue and contraction of the wound begin (4), and finally, the last one is maturation phase where the strong links between collagens and proteins are formed so that increasing scar tissue strength (5-7). Normal cell repair, at the cellular level, is a complex multifactorial of replacing devitalized and missing cellular structures and tissue layers (8). The healing process is directly affected by several interlocated factors, which may hemper the wound healing, especially

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

in diabetic conditionss (9). The diabetes prevalence is continuosly increasing globally, where, the estimated world prevalence of diabetes in 2013 was 8.3% (10). And, diabetic wounds, represent a major challenges for all health care providers, as these cases necessiate extended medication consumption with an increased burden on the patient and health care system. Retardation of wound healing is the most common serious problem in diabetic patients, where, the percentage of diabetic individuals who may have foot ulcerations is about 15% (11). Belated diabetic wound healing is mainly attributed to various biological events, including mainly the decreased angiogenesis (12), which is directly coupled to hyperglycemia, over-expression of inflammatory mediators (cytokines), free radicals (oxidative stress), delayed collagen synthesis, and reduced angiogenesis in addition to the microbial infections (13-15). Elevated blood glucose levels, may directly contribute to impaired wound healing, as, glucose undergoes nonenzymatic reactions with different cell long-lived tissue proteins, lipoproteins, and nucleic acids to form reversible, early glycation products (16-18). Several mechanisms have been proposed for wound healing impairment in diabetes cases, most of these mechanisms are based on defects in the inflammatory response (19).

A wide range of medicines and natural materials in different researches have been used to speed up open wound healing. Despite its considerable laboratory or clinical value, no therapy has been confirmed its significant value in the treatment of different types of wounds, therefore, delayed wound healing still to be one of the causes of significant morbidity and mortality (20). Several physical therapeutic methods have been used in many types of research as adjunctive tools to enhance and speed up healing of wounds they imply; low power lasers, electrical stimulators, negative pressure devices, hyperbaric oxygen and ultrasonic (21).

Ultrasound has a range of frequency between 0.75 to 3.00 MHz and most ultrasonic devices introduce their effects while the frequency was set at one or three MHz. Ultrasound biophysical and thermal effects were elicited where ultrasonic was in continuous mode whereas the cyclical interruption of energy emitted in the pulsed mode decreases the thermal effect while the biological effect is maintained (22). Ultrasound-activated drugs penetrate deeply through the skin, a technique which is known as sonophoresis (23). Ultrasound waves stimulate tiny vibrations inside the epidermal skin layer, they also raise the molecular kinetic energy of topical agents (24), which directed to speed up the absorption of drugs through the transcellular, intercellular and appendageal pathways (25).

Aloe vera plant is one of the most potent healer plants used for centuries in the folk medicine (26). Its glutinous gel is commonly utilized in many cosmetic preparations and alternative medicine field for wound healing and other dermatological disorders (27). Aloe vera has been proven to possess numerous pharmacological activities including, immune modulatory, anti-inflammatory, antiprotozoal, and wound and burn healing (28-31).

Chemically, the A. vera leaves have been reported to contain phytochemical components, which are mainly responsible for the anti-inf lammatory, immune modulating activities, macrophage stimulation and cytokine synthesis (33-34). Several clinical studies revealed that Aloe vera gel increased collagen amount within the healed wound in addition to alteration of the collagen composition with mofification of the collagen cross-linking degree (35). Other studies concluded that Aloe vera oral or topical administration resulted in significant enhancement of both angiogenesis and hyaluronic acid synthesis in the granulation tissue of a healing wound (36-37).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

Transdermal Drug delivery (TDD) is a convenient, noninvasive, localized method of administering drugs to the skin. The technique of applying ultrasound to assist drug delivery through the different dermal layrs of skin is recognized as sonophoresis (38). It is suggested that sonophoresis significantly enhances the penetration of different drug categories through the topical route (39-41).

Deep or full-thickness wound healing provides a challenging in its treatment particularly in diabetic patients, accordingly, synergestic actions of various treatments may be useful for enhancing healing in this complicated wound model.

No previous studies had been carried out to evaluate the potential effects of combined treatment of ultrasonic therapy with topical formulations of Aloe vera on wound in streptozotocin-induced diabetic rats, therefore, the present work aimed not only to investigate combined treatment of ultrasonic therapy with powerful antibacterial and anti-inflammatory Aloe vera plant which still not fully covered yet in wound studies but also to examine the ability ultrasonic to magnify and enhance Aloe vera effect on diabetic wound in rats.

Materials and Methods

Materials:

Streptozotocin (STZ); PEG 400, Methyl Paraben and Propyl Paraben was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Carbopol 934 was purchased from Sisco Research Laboratories (SRL Pvt Ltd., Maharashtra, India). Other materials and solvents are all of analytical grades and used without further purification.

Animals

Sixty adult male albino rats, 6 weaks of age and weighing 200-250 gm. Animals were received from the Central Animal House of Faculty of Medicine, Umm Al Qura University. Animals were individually housed in stainless steel cages with wire-mesh flooring in a controlled environment at 23-25°C and 50% humidity with a 12 h artificial light cycle on a 12:12-h dark-light cycle (07.00-19.00 lights on). Food and water were maintained on a pellet diet and tap water *ad libitum* during the entire period of the study.

Ethical committee approval:

The experiment was carried in compliance with the Guidelines and Policies approved by the Institutional Animal Ethics Committee in Umm Al-Qura University, KSA. The study started after getting approval and permission from the organization's ethical committee.

Experimental procedure

I- Collection and Preparation of A. vera gel ethanol extract

The Aloe vera leaves were identified and collected locally from Makkah Region. The full-size mature leaves were separated from the main plant, fully washed with distilled water to remove superficial impurities and the rind removed. Parenchyma was homogenized in a mechanical blender and then the mixture was centrifuged at 10,000 rpm for 30 min at 4°C to get rid of the remaining fibers. The obtained clear supernatant was then freeze-dryed for complete

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

dryness and kept in air-tight closed vials at room temperature till further use. Then, the lyophilized mass was dissolved in 95% ethanol, then filtered, and the filtrate was then collected and evaporated in a rotary evaporator under reduced pressure to dryness. The formed solid mass residue was collected and stored in well-closed air-tight dry vials at 4 °C till further use (42).

II- Method for gel preparation

Formulation of Aloe Vera gel:

Carbopol 934	1 Gm.
PEG 400	13 Gm.
Aloe vera extract	20 Gm.
Methyl paraben	0.18
Propyl paraben	0.02
Triethanolamine	q.s
Purified Water to	100 Gm

The weighed amount of carbopol (1 gm) was sprinkled in 80 ml of distilled water with continuous stirring using a magnetic stirrer at 800 rpm for 1 h. In another container Aloe vera extract (20 gm) were mixed with PEG 400; methyl paraben and propryl paraben, then this mixture was added to carbopol solution with continuous stirring. Then triethanolamine was added to the mixture for neutralization with slow stirring for 15 min. Then, water was added to complete the required volume with constant rapid mixing till a uniform transparent gel was formed. The gel formed was transferred to aluminiun tube and sealed.

III- Dermal Irritation Study:

It is an evaluation test for the potency of the substance to induce erythema/eschar and/or edema following a single topical application (43). In this study, four (4) rats were randomly chosen from the test animals, the hair of the dorsal and trunk area was removed with asuitable animal clipper. Then animals were carefully examined for any skin abnormalities. A suitable amount of the formula was applied to 6 cm^2 intact area on each animal, then were caged separately. After 4 h of gel application, the amount of gel was removed and the sites of gel application were carefully cleaned. The application area was visually inspected for any allergical reactions, edema or erythema (44). Individual inspection of the test was done and scored after removing the formulation at 1, 24, 48 and 72 hours. Animals that showed preexisting skin irritation or abnormalities was excluded from the study.

IV- Induction of diabetic animal model

Animals were kept in lab for one week before study as an acclimatation period to accommodate the laboratory conditions applied during the study. Experimental diabetes was induced in animals chemically. Animals were overnight fasted and a single intraperitoneal (i.p) Streptozotocin (STZ) in a dose of 65 mg/kg body weight dissolved in 0.1 M citrate buffer (pH 4.5) as freshly prepared was administered to induce diabetes (45). To avoid hypoglycemic lethal effect which may be resulted from initial STZ dose, 5% dextrose solution were given to animals for 24 h following diabetes induction (46). Blood plasma glucose level was monitored using an automated glucose analyzer device; Glucometer (One Touch Basic, USA) to confirm the diabetic condition after 48 h of STZ injection (47). Normal rats (Control non diabetic group) were treated

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

in the same manner as diabetic rats using the same volume of 0.1 M citrate buffer solution. Rats have blood glucose levels ≥ 300 mg/dL were recognized diabetic and consequently subjected to the subsequent experiments (48; 49).

V- Level of blood glucose

To maintain the plasma glucose level stable at 200 mg/dL during the experiment period, intermittent-acting insulin (1–2 U/kg; Montards Novo Nordisk A/S, Frankfurt, Germany) was subcutaneously administered to the rats once a day (48); and to confirm consistency, the blood glucoselevels were measured before surgery, and at the end of each week. Plasma glucose levels were estimated using a glucometer, blood samples collected from the rat tail. The rats were permitted to remain to become fed on their own diet routine (50)**.**

VI- **Wound Surgery Procedures:**

For all experiment rats, the upper dorsal skin was shaved using electrical clipper, cleaned, disinfected with 70% alcohol, then, rats were anesthetized by inhalation (diethyl ether). After anesthesia, the area for wound was measured (2x3 cm), a full-thickness excisional wound was performed as a 6 cm^2 wound area was excised from the dorsal aspect of all rats extending up to the adipose tissue. To maintain the consistency of the experiment, the same researcher performed all surgical procedures (51).

Experiment design:

According to the experimental design, the day for creation the wound was considered to be day zero (0), then, after wound creation, rats were randomly divided into three equal groups of twenty animals each; A, B and C. Treatment started for animals in each group within 2 hours of the wound creation procedure.

The group (A): Twenty rats received combined treatment of both topical Aloe Vera gel (20%) and ultrasonic waves (model pulson 200. Manufacturer; GymnaUniphy N.V. Belgium.) with a frequency of one Mega Hz, an intensity of a half-watt per centimeter square and a 40% on-off cycle. The group (B): Twenty rats received ultrasonic waves with the same conditions as in group A followed by topical application of Aloe Vera gel. The group (C) (sham phonophoresis): Twenty rats received combined treatment of topical Aloe Vera gel and ultrasonic while te ultrasonic device in off mode

Interventions

A)-Treatment:

- Standard cleaning of wound area by alcohol.

- As wound dimensions were approximately (20mm x 3mm x 2mm) so the approximate amount of Aloe Vera gel required to cover the wound cavity was about 1.2 cm^3 and applied by using a disposable sterile syringe. This amount (1.2 cm^3) was fixed during all the experiment period.

- In all groups, prepared gel was added to the complete wound area and filled the wound cavity and maintained for 5 minutes.
- To inhibit, or decrease cross-contamination, a sterile plastic drape was applied to the wounds and surrounding tissues (17).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

- For animals in groups (A) and (B), the pulsed ultrasonic therapy was in **ON/mode** with the following specifications: pulsed duty cycle 40% (4 ms on, 6 ms off) and Power density (0.5 W/cm^2).
- While for group C., ultrasound therapy was in **OFF/mode** as treatment was applied with no current (sham method) in order to control any effect of handling or movement of ultrasonic head over the wound.
- At the end of each session, gel and plastic drape was removed and, the wound was dried, cleaned and perfectly disinfected.

B-Measurement:

1- Wounded area

The wound area of individual animal was measured using tracing paper method, where a transparent tracing graph paper was placed over the wound and tracing the wound out, then the tracing paper fixed on a standard 1 mm^2 graph sheet and traced out. Count the squares and consequently, the area will be calculated. Measurement of wound area for each rat was repeated three times at the end of; (0) day, 1^{st} week and 2^{nd} week in all groups.

2- Percent wound contraction rate

The contraction rate of the wound at a session (x) was calculated from the wound area (A0) at (0) day and unhealed area (Ax) **a**t the session (x) by the following equation:

Percent wound contraction rate (%) = (A0-Ax / A0) x100.

Statistical procedure:

Results were collected and expressed as means ± SD for animals in each group. Statistical analysis was performed using repeated measures one-way analysis of variance (ANOVA) test to compare mean values within a group and one way ANOVA to compare mean values between groups. Data were analyzed using SPSS program version 16.0 software (SPSS® Inc., USA). Values were considered statistically significant when *p*-value <0.05.

RESULTS

Dermal Irritation Study:

The dermal irritation study showed negative signs of reaction towards the applied formulation, where, none of the used animals showed any undesirable irritation effects of the prepared Aloe vera gel formulation, , as, very mild erythema was observed only after 24 hrs of application, and this erythema was disappeared after 48 hrs. No edema was observed in any treated rats, therefore, the formula was used safely for the experiment.

Wound healing is an integrated dynamic and normal biological process consists of precise consequent steps, starting by fibroblast activation and migration, followed by re-epithelization, proliferation of endothelial cells, and finally angiogenesis within the damaged tissues. The innate immune system is immediately activated just after the injury occurs; as the process of wound healing process triggered by interactions between different growth factors and extracellular matrix and cytokines, in addition to the inflammatory response (52).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

A) Compare within each group:

Results obtained from repeated measures ANOVA test showed a significant decrease in wound area throughout treatment phases (0 day, 1st week, and 2nd week) in all three groups as ***p***-value < 0.05 as shown in Table (1) and Figure (1).

(B) Compare between groups:

At (0) day of treatment, wound areas mean value for the group (A) was (6.2±0.35) cm^2 and it was (6.06±0.27) cm^2 for (B) group wile it was (6.03±0.31) cm^2 for (C) group. Between-groups results as analyzed by One way ANOVA test shows that no differences between groups at (0) day as p-value was (0.68).

Measuring of wounded areas at the end of 1st week, founded that the wound areas reduced as wound areas mean value for group (A) was (2.23±0.29) cm^2 with percentage of shrinking of % (64.03±7.4) and it was (2.48±0.51) cm^2 for (B) group wit percentage of shrinking of %(59.07 ±5.3) while it was (2.61±0.53) cm^2 for (C) group and wit percentage of shrinking of % (56.7±6.5) . Analysis of these results using one way ANOVA test showed that no significant differences between groups as p-value was (0.087) and further analysis using Post-hoc test confirmed that; no differences between group (A) and group(B) as well as between group (B) and group(C) regarding wound area mean values and percentage of shrinking as p-value > 0.05. While a significant difference between group (A) and group (C) was noticed as p-value was (0.041).
At the end of 2nd week, te wound areas sowed more reduction as wound areas mean value for group (A) was (0.1±0.07) cm^2 wit percentage of shrinking of % (98.3±0.52) and it was (0.19±0.09) cm^2 for (B) group wit percentage of shrinking of %(96.8±0.51) while it was (0.25±0.2) cm^2 for (C) group and with percentage of shrinking of % (95.8±0.36) .Analysis of these results using one way ANOVA test showed that a highly significant differences between groups as p-value was (0.0001) and further analysis using Post-hoc test confirmed that significant differences between group (A) and group (B), group (B) and group (C) and group (A) and group (C) regarding wound area mean values and percentage of shrinking as p-value were (0.019), (0.03) and (0.0001) respectively. Between-groups results are shown in table (1) and figure (2).

Table I: Comparisons of wounded areas within the group and between groups.

	Group A		Group B		Group C		P value	Post-Hoc test
	WSA	Healing %	WSA	Healing %	WSA	Healing %		
0 day	6.2±0.35		6.06±0.27		6.03±0.31		0.68	$(0.54)^{ab}$ $(0.93)^{bc}$ $(0.49)^{ac}$
1st week	2.23±0.29	64.03±7.4	2.48±0.51	59.07 ±5.3	2.61±0.53	56.7±6.5	0.087	$(0.1)^{ab}$ $(0.64)^{bc}$ $(0.041)^{ac}$

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

2nd week	0.1±0.07	98.3±0.52	0.19±0.09	96.8±0.51	0.25±0.2	95.8±0.36	0.0001	$(0.019)^{ab}$ $(0.03)^{bc}$ $(0.0001)^{ac}$
P value	0.0001		0.0001		0.0001			
Repeated measures	$(0.0001)^{1vs3}$ $(0.0001)^{1vs6}$ $(0.0001)^{3vs6}$ for all groups							

WSA: Wound Surface Area , (P value) ^{ab}: Group A vs Group B, (P value) ^{ac}: Group A vs Group C, (P value) ^{bc}: Group B vs Group C, (P value) ^{1vs3}: 1st session vs 3rd session, (P value) ^{1vs6}:1st session vs 6th session, (P value) ^{3vs6}:3rd session vs 6th session.

Figure (1): Comparison of means of wound surface area (W.S.A) among different measurements within each group

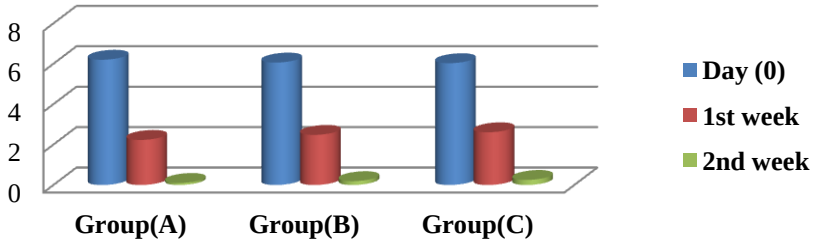
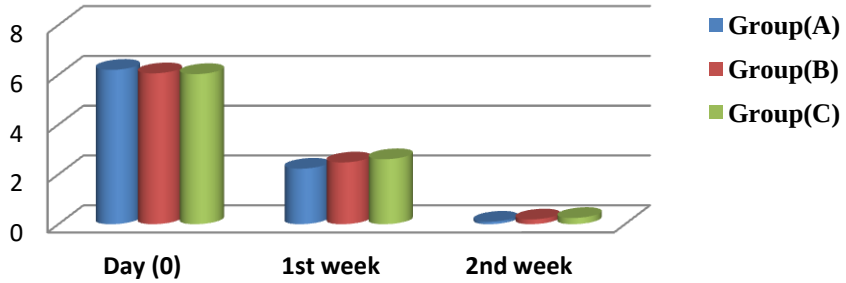


Figure (2):Comparison of means of wound surface area (W.S.A) among different groups at each measurement.



Study results demonstraed that there was significant (reduction) difference between; groups A versus C as p-value (0.0001), this result may attribute to transdermal aloe vera gel enhancement by ultrasonic as explain above, in addition to ultrasonic effect on healing which can be also considered when explain the result between group B versus group C at the end of 2nd week as there was significant (reduction) difference between both groups p-value (0.03).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

DISCUSSION

Impaired wound healing is a serious microvascular complications of diabetes, which results in a significant clinical problems including long hospitalization time, ambutations and morbidity. The exact mechanism underlying the delayed diabetic wound healing is completely unkown, as several parameters and interlocking factors may participate in this action but has been attributed to the impaired cellular infiltration. Elevated blood glucose level, a decrease in growth factors levels and prevention of fibroblast proliferation all of these factors may synergisticaaly contribute in impairment of diabetic wound healing phenomenon.

In this study, an excision wound in a diabetic rat model was employed to investigate the ultrasonic enhancement effect of aloe vera gel on diabetic wounds. The results of this study provided evidence for the ability of ultrasonic to improve and augment the healing effect of aloe vera gel on diabetic wound and to shorten the healing period in rats; there was significant (reduction) difference between both groups A and B at the end of 2^{nd} week as p-value (0.019).

Ultrasound may enhance the drug delivery via skin by three main effects: cavitational, microstreaming and thermal. Increased membrane permeability induced by ultrasound depends mainly on connective tissue loosening and hyaluronic acid depolymerization. Intracellular microflows, which enhances the sensitivity of cells to both physical and chemical stimulators, are the key-factors for the mechanism of the action of biological activities induced by ultrasound (53)**.**

It has been demonstrated that introduction of a suitable blood flow within the injured tissues, significantly increased the number of endothelial cells, macrophages and fibroblasts, which is coupled with superior maturation of the fibroblasts, lead to enhanced collagen formation (54).

Several studies demonstrated that ultrasound significantly improved the percutaneous absorption of different drug moieties (53). When utilizing ultrasound for TDD, increased blood flow in target tissues can decrease the ability of the desired medication to reach target tissues. As the medication passes the stratum corneum of the skin, increased blood flow can cause the drug to be taken into the bloodstream more rapidly before reaching target tissues (24). During the 'proliferative' stage of wound healing, cells migrate to the site of injury and start to divide, and fibroblasts begin to produce collagen, and it has been proven that ultrasound significantly accelerates collagen synthesis by fibroblasts and consequently enhances repairing of the epithelium (55). Several studies have been carried out to precisely clarify the mechanism of action of ultrasound on wound healing; these studies lead to the findings that its effects include mainly, collagen synthesis, enhanced collagen tensile strength, in aadition to promotion of the proliferative phase of healing (56).

The healing activity of the Aloe Vera may be mainly attributed to the glucomannan compounds, which stimulates the fibroblast growth factor, that directly affects fibroblast cell proliferation, accelerates the formation of collagen and increasing its tensile strength, and speeds up wound healing process (32). Also, it has been reported that Aloe Vera stimulates the fibroblasts and building up the extracellular matrix at the site of the wound (12). Oryan et al demonstrated that the aloe vera gel significantly accelerates the rate of wound contraction, epithelialization, and then maturation (7).

It has been also reported that, aloe vera stimulated the stimulating growth factor SGF-β1 which is a main part in the fibroblast mitosis mechanism and extracellular matrix formation (57, 58).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

Another study demonstrated that, aloe vera stimulated macrophages cytokines production and cellular proliferation at specific stages of wound healing process (59). Antioxidant activity and anti-inflammatory properties of aloe vera also play an important role for accelerating the healing activities through neutralizing the free radicals within the affected wound area (60, 61).

CONCLUSIONS

It could be concluded that ultrasonic therapy may enhance and maximize the healing effect of aloe vera gel on the diabetic wound in rats.

Acknowledgment

Authors would like to express their special thanks of gratitude to IOI OlEOCHEM Co. (IOI Oleo GmbH; Hamburg, Germany) for their support through generous gifts of material required to complete the research.

REFERENCES

(1) Enoch S, John Leaper D. Basic Science of Wound Healing. Surgery (Oxford). 2008 Feb; 26 (2): 31-37.
(2) Kondo T, Ishida Y. Molecular pathology of wound healing. Forensic Sci Int. 2010; 203:93-98.
(3) YADAV K.C.H, KUMAR J.R., BASHA S.I., DESHMUKH G.R., SANTHAMMA R.G.B. Wound Healing Activity of Topical Application of Aloe Vera Gel in Experimental Animal Models. International Journal of Pharma and Bio Sciences. 2012; 3(2): 63-72.
(4) Desmouliere A. Chaponnier C.Gabbiani G. Tissue repair, contraction and the myofibroblast. Wound Repair Regen. 2005; 13(1):7–12. doi.org/10.1111/j.1067-1927.2005.1304S1.x.
(5) Ishida Y., Gao J., Murphy P.M. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. J Immunol. 2008; 180:569–579.
(6) Altavilla, D., Saitta, A., Cucinotta, D., Galeano, M., Deodato, B., Colonna, M., Torre, V., Russo, G., Sardella, A., Urna, G., Campo, G. M., Cavallari, V., Squadrito, G. and Squadrito, F. Inhibition of lipid peroxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. Diabetes 2001; 50: 667–674.
(7) Oryan A, Mohammadalipour A, Moshiri A, and Tabandeh M.R. Topical Application of Aloe vera Accelerated Wound Healing, Modeling, and Remodeling An Experimental Study. Annals of Plastic Surgery. 2016; 77(1): 37-46.
(8) Wang, W., Lin, S., Xiao, Y., Huang, Y., Tan, Y., Cai, L. and Li, X. 2008. Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant acidic fibroblast growth factor in healing-impaired STZ diabetic rats. Life Sci. 82: 190–204.
(9) Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014; 103(2):137-49.
(10) Chattopadhyay D., Arunachalam G., Mandal A.B., Sur T.K., Mandal S.C., Bhattacharya S.K. Antimicrobial and anti-inflammatory activity of folklore:Mellotuspeltatus leaf extract. Journal of Ethnopharmacology. 2002; 82: 229–237.
(11) Rafehi H, A. El-Osta T. C. Karagiannis B."Genetic and epigenetic events in diabetic wound healing," International Wound Journal, 2011. 8(1):12-21; doi.org/10.1111/j.1742-481X.2010.00745.x
(12) Ayman ATIBA, Hiroshi UENO and Yuji UZUKA. The Effect of Aloe Vera Oral Administration on Cutaneous Wound Healing in Type 2 Diabetic Rats. J. Vet. Med. Sci. 73(5): 583–589, 2011
(13) Gautam M.K. and Goel R.K.. Wound Healing Effect of Ocimum sanctum Leaves Extract in Diabetic Rats. International Journal of Pharmacological and Pharmaceutical Sciences. 2013; 7 (9): 538-541.

(14) Christman AL, Selvin E, Margolis DJ, Lazarus GS, Garza LA. Hemoglobin A1c Predicts Healing Rate in Diabetic Wounds. J Invest Dermatol 2011.
(15) Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA, Kirsner RS. Diabetic foot ulcers: Part I. Pathophysiology and prevention. J Am Acad Dermatol 2014; 70(1):1.e1-1.e18.
(16) Yamagishi S, Nakamura N, Suematsu M, Kaseda K, Matsui T. Advanced Glycation End Products: A Molecular Target for Vascular Complications in Diabetes. Mol Med 2015; 21 Suppl 1:S32-S40.
(17) Mustoe, T.A., Pierce, G.F., Morishima, C. and Deuel, T.F. 1991. Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. J. Clin. Invest. 87: 694–703.
(18) Lee, P. Y., Chesnoy, S. and Huang, L. 2004. Electroporatic delivery of TGF-□1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice. J. Invest. Dermatol. 123: 791–798.
(19) Hawksworth, J.S., Stojadinovic, A., Gage, F.A. et al. Inflammatory Biomarkers in Combat Wound Healing, Ann. Surg., 2009; 250: 1002–1007.
(20) Low J, Reed A. Laser therapy. In: Low J, Reed A. editors. Electrotherapy explained: principle and practice. London: Butterworth Heinemann Ltd; 1993. p. 299–313.
(21) A Ghamsari SM, Taguchi K, et al. Evaluation of low level laser therapy on pri mary healing of experimentally induced full thickness teat wounds in dairy cattle. Vet Surg. 1997;26:114–20; doi.org/10.1111/j.1532-950X.1997.tb01473.x.
(22) Watson T. Ultrasound in contemporary physiotherapy practice. Ultrasonics 2008; 48:321–9;
(23) Mitragotri, S., 2005. Healing sound: the use of ultrasound in drug delivery and other therapeutic applications. Nat. Rev.: Drug Discovery 4, 255–260; doi.org/10.1038/nrd1662.
(24) Mitragotri S, Blankschtein D, Langer R, Transdermal drug delivery using low-frequency sonophoresis, Pharm Res. 13:411-420, 1996; doi.org/10.1023/A:1016096626810.
(25) Mitragotri, S., Ultrasound-mediated transdermal drug delivery. Ph.D. Thesis, Massachusetts Institute of Technology, U.S.A, (1996).
(26) Eshun K, Qian H. Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries. A review. Crit Rev Food Sci Nutr. 2004;44:91-96.
(27) Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian J Dermatol. 2008;53(4):163-166; doi.org/10.4103/0019-5154.44785.
(28) Jia Y, Zhao G, Jia J. Preliminary evaluation: the effects of Aloe ferox Miller and Aloe arborescens Miller on wound healing. J Ethnopharmacol 2008; 120: 181-189.
(29) Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, et al. The efficacy of aloe vera used for burn wound healing: a systematic review. Burns. 2007; 33: 713-718.
(30) Tai-Nin Chow J,Williamson DA, Yates KM, et al. Chemical characterization of the immunomodulating polysaccharide of Aloe vera L. Carbohydr Res. 2005;340:1131-1142.
(31) Vijayalakshmia D, Dhandapanib R, Jayavenia S, et al. In vitro anti inflammatory activity of Aloe vera by down regulation of MMP-9 in peripheral blood mononuclear cells. J Ethnopharmacol. 2012; 141: 542-546.
(32) Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2006; 24(1):103-154; doi.org/10.1080/10590500600614303.
(33) Kima J, Leea IS, Parkb S, et al. Effects of Scutellariae radix and Aloe vera gel extracts on immunoglobulin E and cytokine levels in atopic dermatitis NC/Nga mice. J Ethnopharmacol. 2010; 132: 529-532.

(34) Pugh N, Ross SA, ElSohly MA, et al. Characterization of Aloeride, a new highmolecular-weight polysaccharide from Aloe vera with potent immunostimulatory activity. J Agric Food Chem. 2001; 49: 1030-1034.
(35) Oryan A, Tabatabaeinaeini A, Nikahval B, et al. Effects of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat. Vet Arh. 2010;80: 509-522.
(36) Chithra P, Sajithlal G, Chandrakasan G. Influence of Aloe vera on the glycosaminoglycans in the matrix of healing dermal wounds in rats. J Ethnopharmacol. 1998;59:179-186.
(37) Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol. 2007; 25: 9-18.
(38) Escobar-Chavez JJ, Bonilla-Martinez D, Villegas-Gonzalez MA, Rodriguez-Cruz IM, Dominguez-Delgado CL. The use of sonophoresis in the administration of drugs throughout the skin. J Pharm Pharm Sci. 2009; 12: 88-115; doi.org/10.18433/J3C30D.
(39) Hippius M, Uhlemann C, Smolenski U, Schreiber U, Reissig S, Hoffmann A. In vitro investigations of drug release and penetration-enhancingeffect of ultrasound on transmembrane transport of flufenamic acid. Int J Clin Pharmacol Ther. 1998; 36: 107-111.
(40) Singer AJ, Homan CS, Church AL, McClain SA. Low-frequency sonophoresis: Pathologic and thermal effects in dogs. Acad Emerg Med. 1998; 5: 35-40; doi.org/10.1111/j.1553-2712.1998.tb02572.x.
(41) Ueda H, Mutoh M, Seki T, Kobayashi D, Morimoto Y. Acoustic cavitation as an enhancing mechanism of low-frequency sonophoresis for transdermal drug delivery. Biol Pharm Bull. 2009; 32: 916- 920; doi.org/10.1248/bpb.32.916.
(42) Mohan Daburkar, Vikram Lohar, Arvind Singh Rathore, Pravin Bhutada, and Shrikant Tangadpaliwar. An in vivo and in vitro investigation of the effect of Aloe vera gel ethanolic extract using animal model with diabetic foot ulcer. J Pharm Bioallied Sci. 2014 Jul-Sep; 6(3): 205–212.
(43) Priyanka Chhabra, Lalita Mehra, Gaurav Mittal, Amit Kumar. A Comparative Study on the Efficacy of Chitosan Gel Formulation and Conventional Silver Sulfadiazine Treatment in Healing Burn Wound Injury at Molecular Level. Asian Journal of Pharmaceutics. 2017; 11 (3): 489-496.
(44) Wardah Abdullah Alasmari, Naser A. ElSawy, Mohammed A.S. Abourehab, Eman Mohamed Faruk, Ashwaq Abdullah Alasmari. Effect of Topical Resveratrol Formulation on Healing of Experimental Full Thickness Wound in Diabetic Male Albino Rats: (Histological and Immunohistochemical study). Life Science Journal. 2018; 15(10).
(45) Mahmoud Y.K., Saleh S.Y., El Ghannam A.E.R.A. and Ibrahim I.A. Biochemical Efficacy of Nigella Sativa Oil and Metformin on Induced Diabetic Male Rats. American Journal of Animal and Veterinary Sciences. 2014; 9: 277-284.
(46) Postic, C., Dentin, R. and Girard, J. (2004) Role of the Liver in the Control of Carbohydrate and Lipid Homeostasis. Diabetes & Metabolism. 2004; 30: 398-408.
(47) Pushparaj, P., Tan, C.H. and Tan, B. Effects of Averrhoa bilimbi Leaf Extract on Blood Glucose and Lipids in Streptozotocin-Diabetic Rats. Journal of Ethnopharmacology. 2000; 72: 69-76.
(48) Kuo, Y.-R., Wang, C.-T., Wang, F.-S., Chiang, Y.-C., and Wang, C.-J. Extracorporeal shock-wave therapy enhanced wound healing via increasing topical blood perfusion and tissue regeneration in a rat model of STZ-induced diabetes. Wound Repair and Regeneration. 2009; 17(4), 522–530; doi:10.1111/j.1524-475x.2009.00504.x.
(49) Wu, K.-L., Chiu, Y.-C., Yao, C.-C., Tsai, C.-E., Hu, M.-L., Kuo, C.-M., Hsiao, C.-C. Effect of extracorporeal low-energy shock wave on diabetic gastroparesis in a rat model. J Gastroenterol Hepatol. 2019 Apr; 34(4): 720-727. doi: 10.1111/jgh.14368.
(50) Reed MJ.Meszaros K.Entes LJ.Claypool MD,Pinkett JG, Gadbois TM, et al. A new rat model of type 2 diabetes, the fat-fed, streptozotocin-treatedrat. Metabolism 2000; 49: 1390-1394; https://doi.org/10.1053/meta.2000.17721.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9169 – 9181
Received 25 April 2021; Accepted 08 May 2021.

(51) Chithra, P., Sajithlal, G. B. and Chandrakasan, G. Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. Mol. Cell Biochem. 1998; 181: 71–76.
(52) Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol 2016; 91: 614-20. doi: 10.1590/abd1806-4841.20164741.
(53) Pahade A., Jadhav V. M. and Kadam V. J.. Sonophoresis: An Overview. 2010; 3 (2): 24-32.
(54) Wahba E. S. Aloe vera gel phonophoresis versus silver nanoparticles phonophoresis on burn wound: a prospective randomized double-blinded trial. Bulletin of Faculty of Physical Therapy. 2018; 23: 30-35; doi:10.4103/bfpt.bfpt_16_17.
(55) Zhou S., Schmelz A., Seufferlein T., Li Y., Zhao J. and Bachem MG. Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts J Biol Chem. 2004; 279(52): 54463-9; doi.org/10.1074/jbc.M404786200.
(56) Ter Riet G, Kessels AG, Knipschild P. A randomized clinical trial of ultrasound in the treatment of pressure ulcers. Phys Ther 1996;76:1301-1311; doi.org/10.1093/ptj/76.12.1301
(57) Massagué J. The transforming growth factor-beta family. Annual Review of Cell Biology. 1990; 6:597-64; doi.org/10.1146/annurev.cb.06.110190.003121.
(58) Sánchez-Elsner T., Botella L. M., Velasco B., Corbí A., Attisano L., et al. Synergistic cooperation between hypoxia and transforming growth factor-β pathways on human vascular endothelial growth factor gene expression. Journal of Biological Chemistry.2001 ;276 (42): 38527-38535; doi.org/10.1074/jbc.M104536200.
(59) Liu C., Leung M. Y. K., Koon J. C. M. et al. Macrophage activation by polysaccharide biological response modifier isolated from Aloe vera L. var. chinensis (Haw.) Berg. International Immunopharmacology. 2006; 6(11): 1634-1641 ;doi.org/10.1016/j.intimp.2006.04.013.
(60) Somboonwong J., Thanamittramanee S., Jariyapongskul A., and Patumraj S.. Therapeutic effects of aloe vera on cutaneous microcirculation and wound healing in second degree burn model in rats. Journal of the Medical Association of Thailand.2000; 83(4): 417-425.
(61) V. Hajhashemi, A. Ghannadi, and A. H. Heidari. Anti-inflammatory and wound healing activities of Aloe littoralis in rats.Research in Pharmaceutical Sciences.2012;7(2):73-78.