Aloe vera stimulate cell proliferation, cell migration, expression of vascular endothelial growth factor-A (VEGF-A), and c-Jun N-terminal kinase-1 (JNK-1) on fibroblast of diabetic rat models

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ABSTRACT

The disturbance of cell migration and cell proliferation, diminished production of vascular endothelial growth factor-A (VEGF-A) and c-Jun N-terminal kinase-1 (JNK-1) are important factors in wound healing process. Aloe vera contains active compounds which can help in the wound healing process. The study aimed to investigate the effect of ethanol extract of A. vera on cell proliferation, cell migration, VEGF-A and JNK-1 expression of skin fibroblast cells of diabetic rats. The primary skin fibroblast cells were isolated from diabetic Wistar rat and incubated with the A. vera extract in various concentrations i.e. 500 (AV500), 250 (AV250), and 125 µg/Ml (AV125) for 24, 48 and 72 h. The cell proliferation was examined visually by counting the cells number, the cell migration was observed using in vitro scratch assay, whereas VEGF-A and JNK-1 expression were examined using RT-PCR. In 24 and 48 h incubation, the cell proliferation of AV500 and AV250 groups had higher number of cells than negative control group, but there was no significant difference (p>0.05). However, in 72 h incubation, the cell proliferation of AV500 group (29.33±1.28x10⁴ cells/mL) was significantly different compared to negative control group (22.91±3.21x10⁴ cells/mL) (p<0.05). In 24 h incubation, the cell migration of AV500 (78.13±7.18%), AV250 (73.88±4.75%) and AV125 (68.80±17.11%) groups were significantly higher than that of negative control group (53.91±2.74%) (p<0.05). In contrast in 48 and 72 h incubation, there were no significantly different in cell migration (p>0.05). The expression of VEGF-A and JNK-1 after incubation with the AV500 for 48 h were significantly higher than those of negative control group (p<0.05). In conclusion, A. vera increases cell proliferation, cell migration, VEGF-A and JNK-1 expression on fibroblast of diabetic rat skin.

ABSTRAK

Gangguan migrasi dan proliferasi sel, juga pengurangan produksi VEGF dan JNK-1 yang merupakan faktor penting dalam proses penyembuhan luka. Lidah buaya (Aloe vera) mengandung berbagai senyawa aktif yang dapat membantu dalam proses penyembuhan luka. Penelitian ini bertujuan untuk mengkaji efek ekstrak etanol A. vera terhadap proliferasi sel, migrasi sel, ekspresi VEGF-A dan JNK-1 dalam kultur fibroblast kulit tikus diabetes. Kultur sel primerfibroblast diisolasi dari kulit tikus Wistar diabetic dan diinkubasikan dengan ekstrak A. vera dengan konsentrasi 500 (AV500), 250 (AV250), dan 125 µg/Ml (AV125). Proliferasi diperlihatkan secara visual dengan menghitung jumlah sel, migrasi sel diamati dengan metode uji gores in vitro, dan ekspresi VEGF-A dan JNK-1 diperlihatkan dengan RT-PCR. Pada inkubasi 24 dan 48 jam, proliferasi sel kelompok V500 dan AV250 lebih tinggi dibanding kelompok kontrol negatif, tetapi tidak bermakna secara nyata (p>0.05). Namun pada inkubasi 72 jam, proliferasi sel kelompok AV500 (29,33±1,28x10⁴cells/mL) berbeda nyata dengan

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INTRODUCTION

Patients with diabetes mellitus (DM) can develop neuropathy which can cause diabetic foot ulcer. The neuropathy caused by disruption of vascular system and sensory nerve damage in extremities area leads to loss of sensation and skin integrity damage. This is a port of the entry for microbe to cause a chronic wound, which is difficult to cure. Moreover patients with severe diabetic foot ulcer can be amputated. Hyperglycemia condition changes metabolism of tissue connection because of disturbance in synthesis process and increased the speed of collagen degradation, which results to delayed wound healing process. Delayed diabetic wound healing is related to low number of vascular endothelial growth factor (VEGF) expression and other growth factors which are the main factors of angiogenesis, cell proliferation, and cell migration on wound healing process. Cell migration activity decreased in hyperglycemia due to the excessive production of reactive oxygen species (ROS) that causes oxidative stress and DNA damage, also failure in cell function. Hyperglycemia obstructs c-Jun N-terminal kinase (JNK) phosphorylation which is the downstream of VEGF, as a result there are disturbance on migration activity and cell proliferation. 

Aloe vera is nontoxic and can maintain or increase epithelial cell and fibroblast cell viability. Aloe vera contains variety of active compounds such as aloe emodin, β-sitosterol, and acemannan which can stimulate VEGF production in ischemia reperfusion damaged brain of Mongolian gerbil and fibroblast cell culture of gingival rats. Aloe vera oral administration significantly stimulates production of TGF-β1 and bFGF on wound radiation and increases wound contraction, and increase fibroblast migration, also endothelial cells. This study aimed to investigate the effect of A. vera ethanol extract on cell proliferation, cell migration, VEGF-A and JNK-1 expression of fibroblast cell culture of diabetic rat skin.

MATERIALS AND METHODS

Preparation of A. vera ethanolic extract

Aloe vera barbadensis Miller was collected from Unit Pelaksana Teknis Daerah (UPTD) Agribisnis Pontianak, West Borneo, Indonesia. The extract was prepared according to Moniruzzaman et al. after modification. The leaves of A. vera were washed in several times then chopped and homogenized by using blender. About one kg of A. vera was macerated using two L of 80% ethanol for 24 h. The macerates were then evaporated under reduced pressure using a rotary evaporator. The semi-dried ethanol extract was then freeze-dried at -55°C and transferred to a reagent bottle to be stored in a freezer at -8°C.
Phytochemical screening

The ethanolic extract of *A. vera* was subjected to various qualitative tests for identification of its constituents using thin-layer chromatography (TLC). Separation was performed using silica gel GF\textsubscript{254} as the stationary phase and mixture of chloroform : methanol : ethyl acetate (5:1:1, v/v/v) as mobile phases. The chromatogram was observed under UV light at wave length 254 and 366 nm and sprayed with citroborate to detect flavonoids in the extract. Mannose examination was performed using high performance liquid chromatography (HPLC). Sample was added one g to volumetric flask and added 10 mL H\textsubscript{2}O, furthermore vortexed and centrifuged at 10,000 rpm for 5 min. The product was filtered by Millex 0.45 µm and injected to HPLC as much 50 µL. The chromatographic separation was conducted with MetaCarb 87P column kept in column oven at 85 °C, H\textsubscript{2}O as mobile phase at a flow rate of 0.6 mL/min and RID detector for detection.

Animal preparation

Protocol of the study has been approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (ref. KE/FK/422/EC/2016). Male albino rats of the *Wistar* strain weighing ±200 g obtained from the Department of Pharmacology and Therapy, Public Health and Nursing, Universitas Gadjah Mada were maintained on standard rat feed and water *ad libitum*. The animals were fasted for 12 h but had been allowed free access to water. After fasting, DM was induced by intraperitoneal injection of streptozotocin 60 mg/kg body weight (BW) and nicotinamide 120 mg/kg BW in 0.05 M dissolved in citrate buffer (pH 4.5).\textsuperscript{23} After three days, blood glucose level were measured and only animal with glycaemia higher than 250 mg/dL were considered diabetic.

Fibroblast cell isolation and culture

Rats were sacrificed with an overdose of ketamine and xylazine. Fibroblast cells were isolated according to Seluanov *et al.*,\textsuperscript{24} after modification. Underarm skin area of animal were shaved and dissected to collect skin samples. Skin was cut approximately 6 cm\textsuperscript{2} using sterile technique and cultured using explant method. The cells were cultured under standard conditions, with Dulbecco’s modified Eagle’s medium (DMEM low glucose) containing 10% fetal bovine serum (FBS), 1% penstrep, and 0.5% fungizone (GIBCO\textsuperscript{®}, Grandisland, NY, USA), then incubated at 37°C and 5% CO\textsubscript{2}. The medium was changed thrice a week and cells were trypsinned at 80% confluence. Passages 4-7 of cell were used for all experiments. The sample then divided into six groups i.e. KN as normal fibroblast cell + low glucose medium (5 mM); K(+) as fibroblast of diabetic rat + high glucose medium (25 mM) + Algisite-M\textsuperscript{®} dressing; K(-) as fibroblast of diabetic rat + high glucose medium (25 mM); AV500 as fibroblast of diabetic rat + high glucose medium (25 mM) + *A. vera* 500 µg/mL; AV250 as fibroblast of diabetic rat + high glucose medium (25 mM) + *A. vera* 250 µg/mL; AV125 as fibroblast of diabetic rat + high glucose medium (25 mM) + *A. vera* 125 µg/mL.

Preparation of solution

About 5 mg of the ethanolic extract of *A. vera* was diluted in 100 µL dimethyl sulphoxide (DMSO; SIGMA\textsuperscript{®}; St Louis, MO, USA) to obtain a stock solution (50.000 µg/mL) and then dissolved inhight glucose medium (DMEM 25 mM glucose, FBS 10%, penstrep 1%, fungizone 0.5%) and created in various concentration of *A. vera* (500, 250, 125 µg/mL). Algisite-M\textsuperscript{®} dressing extract solution was created.
by using 2 cm² of Algsite-M® dressing (Smith & Nephew®) and incubated in 8 mL of high glucose medium at 37°C for 24 h. Positive control (Algsite-M®) was used for each assay.

**Cell proliferation examination**

Cell proliferation examination was performed by counting the number of cells in hemocytometer slide Neubauer. Cells were seeded in microplate-96 wells with 10⁴ cells/mL per well in volume 100 µL, and incubated in high glucose medium (DMEM 25 mM glucose) for 24 hours at 37°C and 5% CO₂. Furthermore, cells were incubated with A. vera extract concentration 500, 250, 125 µg/mL and Algisite-M® extract in high glucose medium. The number of cells was then counted in 24, 48 and 72 h after incubation.

**Cell migration examination**

Cell migration test carried out using in vitro scratch assay method. The number of cells to examine cell migration was 5x10⁴ cells per sample in microplate-24 well. Cells were incubated in high glucose medium (DMEM 25 mM glucose) at 37°C and 5% CO₂ for 24 h. Furthermore, cells were incubated with A. vera extract concentration 500, 250, 125 µg/mL and Algisite-M® dressing in high glucose medium and incubated at 37°C and 5% CO₂ for 48 h. Total RNA isolated by using RNA ISO Plus or GENEzol™ (GZR100) with recommended procedures. RNA were quantified by using spectrophotometer. PCR cDNA mix were carried out by mixed 5x buffer (Toyobo, Cat. No. TRT-101), random primer (TaKaRa, Cat. No. 3801), deoxyribonucleotide triphosphate (dNTP) (TaKaRa, Cat. No. 4030), and Rever TraAce (Toyobo, Cat. No. TRT-101). cDNA product, PCR water, and PCR mixture were mixed and carried out PCR running with conditions: denaturation at 30°C (10 min), annealing at 42°C (60 min), and extension at 99°C (5 min). Gene amplification was performed by using polymerase chain reaction (PCR) method with specific primer. GoTaq® Green Master Mix (Promega, USA) in 12.5 µL were used to amplifications and using total reaction volume total 25 µL. Furthermore, 10 µL amplification products were visualized by using electrophoresis.

| Primer | Urutan |
|--------|--------|
| VEGF-A | F: 5'-ACCTCCACCATGCCAAGT-3'  
R: 5'-TTGGTCTGACATTCACTCTG-3' |
| JNK-1  | F: 5'-GCCAGTCAGGCGAGAGATTT-3'  
R: 5'-GGACGCATCTATCACCAGCA-3' |
| GAPDH  | F: 5'-ATGACATCAAGAAGGTGGTG-3'  
R: 5'-CATACCAGAAATGAGCTTG-3' |
TABLE 2. PCR conditions used to examine VEGF-A, JNK-1 and GAPDH gene expressions

| PCR condition | Pre Denaturation | Denaturation | Annealing | Extension | Final Extension | Cycle | Sample |
|---------------|------------------|--------------|-----------|-----------|-----------------|-------|--------|
| VEGF-A        | 94°C2 min        | 94°C10 sec   | 65°C30 sec| 72°C1 min | 72°C10 min      | 35x   | 4µL    |
| JNK-1         | 94°C2 min        | 94°C10 sec   | 60°C30 sec| 72°C1 min | 72°C10 min      | 35x   | 4µL    |
| GAPDH         | 94°C2 min        | 94°C10 sec   | 60°C30 sec| 72°C1 min | 72°C10 min      | 35x   | 4µL    |

Statistical analysis

Statistical analysis was carried out with the SPSS program for windows. Data were expressed as the mean ± standard deviation (SD). The differences among the mean values from four independent experiments were analyzed with one-way ANOVA followed by post hoc LSD test analysis. A p value <0.05 was considered significant.

RESULTS

Phytochemical screening of A. vera

Phytochemical analysis of the A. vera extract by TLC showed the presence of compounds on length of wave 366 nm with rate of flow (Rf) values of 0.32; 0.45; 0.64; 0.84; and 0.90, respectively. HPLC examination of A. vera extract resulted in the level of mannose compound < 32 µg/g (FIGURE 1).

FIGURE 1. The TLC and HPLC chromatogram of A. vera extract. (A) Examination by UV 254 nm; (B) Examination by UV 366. nm. (C) Examination by UV 366 nm after Sitroborat spraying. (D) Graphic of HPLC examination (the arrow shows mannose level).
Cell proliferation

Cell proliferation is presented in FIGURE 2. The cell proliferation of the negative control group after 24, 48, 72 h incubations were significantly lower than that normal fibroblast cell group (p<0.05). The cell proliferation of the AV500 and AV250 groups after 24 and 48 h incubations were higher than that the negative control group, but they were not significantly different (p>0.05). However, after 72 h incubation, significantly different between AV500, AV250, and AV125 groups compared to that the negative control group was observed (p<0.05).

![Cell proliferation graph](image)

FIGURE 2. Cell proliferation (mean ± SD 10⁴ cells/mL) after 24, 48 and 72 h incubations. One way ANOVA examination in 24 h (p = 0.001), 48 h (p = 0.000) and 72 h (p = 0.000). Post hoc LSD: a (p < 0.05) compared to positive control; b (p < 0.05) compared to negative control; c (p < 0.05) compared to normal fibroblast cell control.

Cell migration

The cell migration of the normal fibroblast cell group after 24 h incubation was highest compared to all of the groups (p<0.05). The cell migration of the AV500, AV250 and AV125 groups after 24 h incubation was significantly higher than that the negative control group (p<0.05). However, after 48 and 72 h incubation, no significantly different in all groups were observed (p>0.05). It was indicated that scratch wound has closed at the hour 48 after treatments. The cell migration of all groups are represented in FIGURE 4.
FIGURE 3. Image of cell migration in hour 0, 24, 48, and 72 after treatments. The yellow line was the margin of the scratch. Magnification 40x. The scale of measurement was 100 µm. The migration rate was quantified by the Image-J software and data were expressed as mean ± SD.
FIGURE 4. Cell migration (mean ± SD%) in 24, 48, 72 h after incubations. One way ANOVA examination in 24 h (p = 0.000), 48 and 72 h (p > 0.05). Posthoc LSD: a (p < 0.05) compared to positive control; b (p < 0.05) compared to negative control; c (p < 0.05) compared to normal fibroblast cell control.

VEGF-A expression

The normal fibroblast cell control group showed highest VEGF-A/GAPDH expression compared to all of the groups (p<0.05). The AV500 group had higher VEGF-A/GAPDH expression compared to that positive control, negative control, AV250, and AV125 groups (p<0.05).

FIGURE 5. (A) Resultsof electrophoresis VEGF-A and GAPDH geneafter 48 hours incubation. (B) Chart bar of mean± SD VEGF-A/GAPDH in hour 48. One way ANOVA test (p = 0.000). Posthoc LSD: a (p < 0.05) compared to positive control; b (p < 0.05) compared to negative control; c (p < 0.05) compared to normal fibroblast cell control.
JNK-1 expression

The normal fibroblast cell control groups showed highest JNK-1/GAPDH expression compared to all of the groups (p<0.05). The AV500 group had higher JNK-1/GAPDH expression compared to that negative control, AV250, and AV125 group (p<0.05).

![Image of electrophoresis results for JNK-1 and GAPDH genes](image)

FIGURE 6. (A) Result of electrophoresis for JNK-1 and GAPDH gene after 48 hours incubation. (B) Chart bar of mean±SD JNK-1/GAPDH in hour 48. One way ANOVA test (p = 0.000). Posthoc LSD: a (p<0.05) compared to positive control; b (p<0.05) compared to negative control; c (p<0.05) compared to normal fibroblast cell control.

DISCUSSION

In this study, hyperglycemia condition caused cell proliferation and cell migration disturbances, also decreased VEGF-A and JNK-1 gene expression on fibroblast of diabetic rat. It was indicated by significantly fewer cell proliferation of the negative control group compared to that control normal group after 24, 48 and 72 h incubations (p<0.05). In addition, cell migration of negative control group was significantly lower compared to that control normal group (p=0.000) and the scratch wounds of control normal group was almost completely closed compared to the other groups. The VEGF-A and JNK-1 gene expression of negative control group after 24 h incubation was significantly lower compared to that normal control group (p=0.000).

The result of this study was supported by previous studies which confirmed that hyperglycemia condition caused the cell proliferation and cell migration disturbance. Moreover, hyperglycemia condition can diminish VEGF production and JNK phosphorylation lead to disturbance of cell proliferation and cell migration on wound healing process.

The impairment of cell proliferation on hyperglycemia can caused by several factors such as increase of L-lactate production, inflammatory mediators and advanced glycation end-product (AGE) which stimulate apoptosis through activation of ROS, pro-apoptotic transcription factor FOXO1 and caspase 3. The increase of ROS production affects the protein structure and function, as well as inhibition of cell migration directly by over-activation of the small Rho GTPase Rac1 and altered polarity and morphology of cells. Diminished VEGF production on hyperglycemia occurs by inhibition of JAK-2 and
STAT3 (Signal transducer and activator of transcription-3). Hyperglycemia obstructs JNK phosphorylation which is the downstream of VEGF, as a result there are disturbance on cell proliferation and cell migration.11-13,26

Ethanolic A. vera extract can stimulate proliferation and migration of fibroblast cell of diabetic rat in this study. The cell proliferation after 24 and 48 h incubations with A. vera extract at concentration of 500 and 250 µg/mL were higher than that the negative control group, but they were not significantly different (p>0.05). However, after 72 h incubation, significantly different between A. vera extract at concentration of 500, 250 and 125 µg/mL compared to that the negative control group was observed (p < 0.05). The cell migration after 24 h incubation with A. vera extract at concentration of 500, 250 and 125 µg/mL were significantly higher than that the negative control group (p <0.05). The result of this study was also support by the previous studies which confirmed that A. vera can increase cell proliferation and cell migration.17,27 The increase of cell proliferation and cell migration occurred in concentration dependent manner. The scratches wounds almost completely closed in each group after 48 h incubation. The increase of the cell migration activity is likely occurred before 24 h incubation, so after 24 h the migration has been achieved>50% in each group.

The VEGF-A and JNK-1 expressions after incubation with A. vera extract at concentration of 500 µg/mL were significantly higher compared to that the negative control group (p<0.05). It was indicated that A. vera can stimulate VEGF production and the other growth factors which has a role in wound healing process as previously reported.20,28 The incubation of A. vera extract at concentration of 250 and 125 µg/mL on the VEGF-A and JNK-1 expressions were not significantly different compared to the negative control. This may be related to the small concentrations that do not affect the increased expression of VEGF-A and JNK-1.

The glycoproteins and polysaccharides components in A. vera can trigger cell growth and significantly increase cell proliferation and cell migration by stimulating TGF-β1 and bFGF production, also affecting Gap junctional intercellular communication (GJIC) which is the important factors in growth of tissue and organs.26,29 Aloesin compound of A. vera stimulates cell growth through induce cell cycle protein regulators such as cyclin E, cyclin dependen kinase-2 (CDK2), and cell division cycle-25A (CDC25A) which synergistically up-regulate the cyclin E/CDK2 kinase activity.30 β-Sitosterol compound of A. vera increase VEGF expression on brain ischemic reperfusion animals.27

The phytochemical analysis of the A. vera extract by TLC showed the presence of compounds on length of wave 366 nm with Rf value of 0.32; 0.45; 0.64; 0.84; and 0.90. It was indicated that A. vera extract has various compounds that taken along by mobile phase with various distance based on polarity of compounds. Citroborate spraying was used to examine of flavonoid existence in the A. vera extract. The major polysaccharide compound of A. vera is acemannan (mannose-6-phosphat) which is composed of one or more polymers of various chain length with molecular weights 30-40 kDa and consists of repeating units of glucose and mannose.31 In this research, mannose compound was analyzed by using HPLC and had mannose content <32 µg/gr.

Previous studies reported that A. vera accelerates diabetic wound healing4,16 by increase the glycosaminoglycans (GAGs), and plasma insulin level, also diminish blood glucose level.32 Acemannan accelerates oral and diabetic wound healing of animal
Firdaus I, et al., Aloe vera stimulate cell... modelsby stimulates KGF-1, VEGF production, collagen synthesis, and also increase collagen cross link activity for wound contraction.20,28 The active accemanan should bind to mannose receptor on the cell surface by recognize polysaccharide chains terminating with mannose, fucose or N-acetylglusamine and stimulate growth factor production and cell proliferationby induce the intracellular signaling pathway.20,33 Acemanan promotes skin wound healing and accelerates cell proliferation through stimulate synthesis of protein translationcyclin D1 which is mediated by AKT/mTOR signaling pathway.34 Antioxidant activity of A. vera like flavonoid significantly decreases ROS, lipid peroxidase and glycosylated hemoglobin level which are the cause of cell proliferation and cell migration impairment, also diminished of VEGF on diabetic wound.7,10,35 This antioxidant activity also has a role in radical scavenging by increase in activity ofsuperoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes.7,10,35

CONCLUSION

Ethanolic extract of A. vera increases cell proliferation, cell migration, VEGF-Aand JNK-1expression onfibroblast cell cultured of diabetic rat skin.

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REFERENCES

1. Alberti KG, Zimmet PZ. Definition, diagnosisandclassificationofdiabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med. 1998; 15(7):539-53. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S
2. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer- a review on pathophysiology, classification and microbial etiology. Diabetes Metab Syndr Clin Res Rev 2015; 9(3):192-9. https://doi.org/10.1016/j.dsx.2015.04.007
3. Blakytny R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 2006; 23(6):594-608. https://doi.org/10.1111/j.1464-5491.2006.01773.x
4. Chithra P, Sajithlal GB, Chandrakasan G. Influence of Aloe vera on the healing of dermal wounds in diabetic rats. J Ethnopharmacol. 1998; 59(3):195-201. https://doi.org/10.1016/S0378-8741(97)00124-4
5. Lien Y-H, Tseng MM, Stern R. Glucose and glucose analogs modulate collagen metabolism. Exp Mol Pathol 1992;57(3):215-21. https://doi.org/10.1016/0014-4800(92)90012-Z
6. Folkman J. Angiogenesis inhibitors generated by tumors. Mol Med 1995; 1(2):120. https://doi.org/10.1007/BF03401559
7. Lamers ML, Almeida MES, Vicente-Manzanares M, Horwitz AF, Santos MF. High glucose-mediated oxidative stress impairs cell migration. Blagosklonny MV. PLoS One. 2011; 6(8):e22865.
8. Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol. 2003; 162(1):303-12. https://doi.org/10.1016/S0002-9440(10)63821-7

9. Ferrara N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol-Cell Physiol. 2001; 280(6):C1358-66. https://doi.org/10.1152/ajpcell.2001.280.6.C1358

10. Johnson KE, Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. Adv Wound Care 2014; 3(10):647-61. https://doi.org/10.1089/wound.2013.0517

11. Hehenberger K, Heilborn JD, Brismar K, Hansson A. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of L-lactate. Wound Repair Regen 1998; 6(2):135-41. https://doi.org/10.1046/j.1524-475X.1998.60207.x

12. Desta T, Li J, Chino T, Graves DT. Altered fibroblast proliferation and apoptosis in diabetic gingival wounds. J Dent Res 2010; 89(6):609-14. https://doi.org/10.1177/0022034510362960

13. Xuan YH, Huang BB, Tian HS, Chi LS, Duan YM, Wang X, et al. High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation. PLoS One 2014; 9(9):e108182. https://doi.org/10.1371/journal.pone.0108182

14. Rajeswari R, Umadevi M, Rahale CS, Selvavenkadesh S, Kumar KS, Bhowmik D. Aloe vera: the miracle plant its medicinal and traditional uses in India. J Pharmacogn Phytochem 2012; 1(4): 118-24.

15. Dat AD, Poon F, Pham KB, Doust J. Aloe vera for treating acute and chronic wounds. In: The Cochrane Collaboration, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2012. https://doi.org/10.1002/14651858.CD008762.pub2

16. Takzare N, Hosseini M, Hasanzadeh G, Mortazavi H, Takzare A, Habibi P. Influence of Aloe vera gel on dermal wound healing process in rat. Toxicol Mech Methods 2009; 19(1):73-7. https://doi.org/10.1080/15376510802442444

17. Yao H, Chen Y, Li S, Huang L, Chen W, Lin X. Promotion proliferation effect of a polysaccharide from Aloe barbadensis Miller on human fibroblasts in vitro. Int J Biol Macromol 2009; 45(2):152-6. https://doi.org/10.1016/j.ijbiomac.2009.04.013

18. Curto EM, Labella A, Chandler HL. Aloe vera: an in vitro study of effects on corneal wound closure and collagenase activity. Vet Ophthalmol. 2014; 17(6):403-410. https://doi.org/10.1111/vop.12163

19. Moon E-J, Lee YM, Lee O-H, Lee M-J, Lee S-K, Chung M-H, et al. A novel angiogenic factor derived from Aloe vera gel: β-sitosterol, a plant sterol. Angiogenesis 1999; 3(2):117-23. https://doi.org/10.1023/A:1009058232389

20. Jettanacheawchankit S, Sasithanasate S, Sangvanich P, Banlunara W, Thunyakitpisal P. Acemannan stimulates gingival fibroblast proliferation; expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and type I collagen; and wound healing. J Pharmacol Sci 2009;
21. Atiba A, Ueno H, Uzuka Y. The effect of Aloe vera oral administration on cutaneous wound healing in type 2 diabetic rats. J Vet Med Sci. 2011; 73(5):583-9. https://doi.org/10.1292/jvms.10-0438

22. Moniruzzaman M, Rokeya B, Ahmed S, Bhowmik A, Khalil M, Gan S. In vitro antioxidant effects of Aloe barbadensis Miller extracts and the potential role of these extracts as antidiabetic and antilipidemic agents on streptozotocin-induced type 2 diabetic model rats. Molecules 2012; 17(12):12851-67. https://doi.org/10.3390/molecules17112851

23. Szkudelski T. Streptozotocin – nicotinamide - induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med 2012; 237(5):481-90. https://doi.org/10.1258/ebm.2012.011372

24. Seluanov A, Vaidya A, Gorbunova V. Establishing primary adult fibroblast cultures from rodents. JOVE 2010; (44): pii:2033. https://doi.org/10.3791/2033

25. Liu Z, Lei M, Jiang Y, Hao H, Chu L, Xu J, et al. High glucose attenuates VEGF expression in rat multipotent adult progenitor cells in association with inhibition of JAK2/STAT3 signalling. J Cell Mol Med 2009; 13(9b):3427-36. https://doi.org/10.1111/j.1582-4934.2008.00502.x

26. Huang C. MAP kinases and cell migration. J Cell Sci 2004; 117(20):4619-28. https://doi.org/10.1242/jcs.01481

27. Choi S, Kim K-W, Choi J-S, Han S-T, Park Y-I, Lee S-K, et al. Angiogenic activity of β-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian gerbil. Planta Med 2002; 68(4):330-5. https://doi.org/10.1055/s-2002-26750

28. Atiba A, Nishimura M, Kakinuma S, Hiraoka T, Goryo M, Shimada Y, et al. Aloe vera oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor-β and fibroblast growth factor production. Am J Surg 2011; 201(6):809-18. https://doi.org/10.1016/j.amjsurg.2010.06.017

29. Abdullah KM, Abdullah A, Johnson ML, Bilski JJ, Petry K, Redmer DA, et al. Effects of Aloe vera on gap junctional intercellular communication and proliferation of human diabetic and nondiabetic skin fibroblasts. J Altern Complement Med 2003; 9(5):711-8. https://doi.org/10.1089/107555303322524553

30. Lee KY, Park JH, Chung MH, Park YI, Kim KW, Lee YJ, et al. Aloesin up-regulates cyclin E/CDK2 kinase activity via inducing the protein levels of cyclin E, CDK2, and CDC25A in SK-HEP-1 cells. IUBMB Life. 1997; 41(2):285-292. https://doi.org/10.1080/15216549700201291

31. Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera. J Environ Sci Health Part C 2006; 24(1):103-154. https://doi.org/10.1080/10590500600614303

32. Daburkar M, Rathore A, Tangadpaliwar S, Bhutada P, Lohar V. 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; 6(3):205-12. https://doi.org/10.4103/0975-7406.135248

33. Davis RH, Donato JJ, Hartman GM, Haas RC. Anti inflammatory and wound healing activity of a growth substance in Aloe vera. J Am Podiatr Med Assoc 1994;84(2):77-82. https://doi.org/10.7547/87507315-84-2-77
34. Xing W, Guo W, Zou C-H, Fu T-T, Li X-Y, Zhu M, et al. Acemannan accelerates cell proliferation and skin wound healing through AKT/mTOR signaling pathway. J Dermatol Sci 2015; 79(2):101-9. https://doi.org/10.1016/j.jdermsci.2015.03.016

35. Ozsoy N, Candoken E, Akev N. Implications for degenerative disorders: antioxidative activity, total phenols, flavonoids, ascorbic acid, β-carotene and α-tocopherol in Aloe vera. Oxid Med Cell Longev 2009; 2(2):99-106. https://doi.org/10.4161/oxim.2.2.8493