

Increase of Fibroblast Cells in Wound Healing of Diabetes Mellitus Male Rats (*Rattus Norvegicus*) Using Aloin

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ABSTRACT

The healing process wound on the skin diabetic experience slowdown because dysfunction fibroblasts. Aloins boost expression fibroblasts that can accelerating the process of wound healing in patients with Diabetes Mellitus (DM). Objective study that is for now effect enhancement cell fibroblasts in wound healing wound I t mouse male DM with aloe giving. Method study use principle true experimental with approach post-test serial only control group design. Treatment form wound cut on mice. Group study using 4 groups with a negative control in the form mouse healthy, positive control form DM mouse. group action 1 in the form of DM rats were given 1.25 mg/ kg BW aloin and treatment 2 was in the form of DM rats given 2.5 mg/ kg BW aloin. Expression fibroblasts calculated on day 7 and day 14. Analysis bivariate using the Friedman test continued with the Wilcoxon Post- Hoc test. Research results that are there is enhancement fibroblasts significant in the group treatment given with aloin a dose of 1.25 mg/ kg and a dose of 2.5 mg/ kg. The conclusion is that Aloin accelerates wound healing wound incision in DM rats with mechanism enhancement expression fibroblasts with minimum dose of 1.25 mg/ KgBB. Research suggestions that is examination of re-epithelialization and density Dermal collagen in wound healing wound I t DM rats given aloin in the study next.

Keywords: Fibroblasts, Wound Healing, Aloin, Diabetes Mellitus

INTRODUCTION

Skin diabetic experience disturbance function dermal fibroblasts and keratinocytes result accumulation of Reactive Oxygen Species (ROS). ROS buildup results activating oxidative stress cells pro-inflammatory cytokines persistent, activation enzyme matrix metalloproteinase and decrease matrix extracellular. slowdown migration fibroblasts resulted slowing of angiogenesis, angiogenesis and synthesis collagen. Decreased fiber deposits collagen cause delay in the process of wound healing resulting in wound chronic or bad wound. (Labib, et.al., 2022 ; Y.Zheng,et.al., 2018)

Stated that happen slowdown migration and decline function fibroblasts in diabetic skin wound compared to healthy skin (Khamaisi, 2016). Abnormal fibroblast function underlies poor wound healing in patients with diabetes; however, the mechanisms that impair wound healing are poorly defined. Here, we evaluated fibroblasts from individuals who had type 1 diabetes (T1D) for 50 years or more (Medalists, n = 26) and from age-matched controls (n = 7).

Compared with those from controls, Medalist fibroblasts demonstrated a reduced migration response to insulin, lower VEGF expression, and less phosphorylated AKT (p-AKT), but not p-ERK, activation. Medalist fibroblasts were also functionally less effective at wound closure in nude mice. Activation of the isoform of protein kinase C (PKC) was increased in postmortem fibroblasts from Medalists, fibroblasts from living T1D subjects, biopsies of active wounds of living T1D subjects, and granulation tissues from mice with streptozotocin-induced diabetes. Diabetes-induced PKCD mRNA expression was related to a 2-fold increase in the mRNA half-life. Pharmacologic inhibition and siRNA-mediated knockdown of PKC or expression of a dominant-negative isoform restored insulin signaling of p-AKT and VEGF expression in vitro and improved wound healing in vivo. Additionally, increasing PKC expression in control fibroblasts produced the same abnormalities as those seen in Medalist fibroblasts. Our results indicate that persistent PKC elevation in fibroblasts from diabetic patients inhibits insulin signaling and function to impair wound healing and suggest PKC inhibition as a potential therapy to improve wound healing in diabetic patients} Ibuki et al, 2018 stated that woman Japan diabetic experience obesity and aging early. Aging observed early is enhancement Advanced Glycation Ends (AGEs), decrease hydration of the stratum corneum, increase transepidermal waterloss and decline density collagen. Aging early that occurs in diabetes becomes factor risk For slowdown wound healing (A.Ibuki et.al, 2018)

Aloins is compound active class anthraquinones found in Aloe vera. Aloins are anti-inflammatory, antibacterial and antioxidant (R.Bista, et.al, 2018 ; Vani, 2022) . Renuga et al, 2019 stated that aloin compound is compound functioning antioxidants as soothing gel that is maintain moisture and skin barrier, as well speed up healing wound (G.Renuga, 2019) . Liu et al, 2015 stated that aloin as protection fibroblasts from damage consequence warmup with mechanism suppress ROS and activation glutathione as well as enzyme Super Oxide Dismutase (SOD).

Based on research that has done, researcher want to see aloin effect on enhancement fibroblasts in the wound healing process of diabetes. Objective study is known amount expression fibroblasts the 7th day and the 14th day with aloin administration, as well know effectiveness giving aloin to enhancement amount expression fibroblasts in the wound healing process.

METHOD

Study characteristic true experimental laboratory with principal post-test serial only control group design. Population study is mouse male (*Rattus norvegicus*) diabetes mellitus (DM) with gift Streptozocin (STZ), 3 months old with body weight 150–200 grams. Study This use technique taking sample Simple Random Sampling.

Study consists top 4 groups, i.e. control negative (K (-)), control positive (K (+)), Treatment 1 (P1) and Treatment 2 (P2). K (-) uses mouse Healthy without STZ induction and therapy wound with 0.9% NaCl. K (+) uses DM mice with

administration of STZ and therapy wound with 0.9% NaCl. P1 use DM mice with administration of STZ and therapy wound with aloin dose of 1.25 mg/ kg BW. P2 uses DM mice with administration of STZ and therapy wound with aloin dose of 2.5 mg/ kg BW.

Aloin got from extraction Aloe vera ethanol. A total of 80 kg of wet Aloe vera extracted and yielded aloin content of 0.113%. Analysis of aloin using the HPLC technique. After aloin extraction, research next with adaptation mouse for 1 week, which was continued with STZ induction with dose of 55 mg/kg body weight. Measurement rate glucose blood performed on the 4th and 11th post injection. Mouse stated DM if rate glucose blood > 150 mg/dL.

Making wound done with do incision of 20mm x 10mm x 4mm. incision done after mouse bathed and fur shaved clean. Giving aloin extract and 0.9% NaCl was carried out very a day. Taking preparations done on the day to 7 and day to 14 for count expression fibroblasts.

Fibroblasts counted with see 3 airy views with a side-to-side area preparation. Then done counting average fibroblasts. Analysis expression fibroblasts done with normality and homogeneity tests for now data distribution. Normality test using the proven Shapiro-Wilk distributed data abnormal. Homogeneity test use Test of Homogeneity of Variances which is proven by the data is not homogeneous. Furthermore, Friedman's non- parametric test was carried out which was continued with the Wilcoxon Post- Hoc test. hypothesis study that is Aloin administration increases expression cell fibroblasts in wound healing I t mouse male (*Rattus norvegicus*) diabetes mellitus.

RESULTS AND DISCUSSION

Recovery process wound covers closing surface wound, closure vessels torn blood, regeneration from cells peripheral as well as replacement network muscles by fibers collagen. Healing process grouped wounds in four phase that is phase homeostasis, phase inflammation phase proliferation, and phase remodeling. If happen wound , so functions from skin No can walklike Which should (SA Gunawan, et.al, 2019) . Fibroblast's role important phase proliferation. fibroblasts move active going to damaged tissue, persistence collagen and produce networkgranulation For return unite network wounds (Annisa, 2019). Fibroblasts is cellmain in mechanism healing wound. Fibroblasts migrate to wound area and proliferate in a manner active. Amount cell fibroblasts start experience improvement on day 3 and continues increased on day 5 to reach peak day to 7 as well as become that cell dominant. On the 14th day of recovery wound Already enter phase remodeling. Remodeling process form network with size, shape, And strength Which optimum (Haraharap, 2024 ; N.Primadina, et.al, 2019).

Expression 7th Day Fibroblasts

Counting expression fibroblasts, the 7th day showed expression highest in group K (-), i.e. mouse healthy. Group DM rats experienced slowdown expression fibroblasts. Hyperglycemia cause dysfunction fibroblasts, that is

disturbance formation myofibroblasts, damage matrix extracellular as well as hypoxia network. Damage continues to fibroblast apoptosis that causes delay healing cuts and wounds become chronic (R.Wan, 2021 ; LM Morton and Tj Philips, 2016). Wang et al, 2019 stated that on healing diabetic sores exist dysregulation Transforming Growth Factor- β (TGF- β) which results weak contractile fibroblasts, slowdown change fibroblasts to myofibroblasts and delayed re-epithelialization (Wang, et.al, 2019) . Expression fibroblasts the 7th day is shown in table 1.

Table 1. Expressions fibroblasts on day 7

Group	N	Mean \pm Std.Dev	Min Value	Max Value
K -	6	25.17 \pm 5.307	20	33
K +	6	6.83 \pm 2.137	4	9
P1	6	9.50 \pm 1.871	10	13
P2	6	14.33 \pm 4.320	4	21

Giving aloin to the group treatment show expression more fibroblasts Lots compared to with administration of 0.9% NaCl even with dose of 2.5 mg/ kg BW mark expression fibroblasts reach expression fibroblasts in normal mice. aloin can increase angiogenesis and proliferation fibroblasts with induce expression factor epidermal growth, so speed up healing wound. Besides that , aloin also makes fiber collagen skin more regular after healing fibroblasts so that reduce formation used wounds , aloin also inhibits response inflammation (Vera, et.al, 2021 ; W.Li, et.al, 2022)

Expression Fibroblasts Day 14

On the 14th day, normal skin already enter phase marked remodeling with decline expression fibroblasts. on the skin diabetic Still happen enhancement expression fibroblasts. this seen in the K (+) and treatment groups, where expression fibroblasts in the group that was given more aloin tall than group treatment given 0.9% NaCl. expression results fibroblast shown in table 2.

Table 2. Expressions Fibroblasts on Day 14

Group	N	Mean \pm Std.Dev	Min Value	Max Value
K-	6	8.67 \pm 2.338	5	12
K+	6	12.00 \pm 1.549	10	14
P1	6	17.00 \pm 3.578	12	22
P2	6	20.17 \pm 3.189	15	24

Expression fibroblasts highest found in the group giving aloin with dose of 2.5 mg/ kg B. However, secondly aloin dose showed more expression tall compared to with group DM rats given 0.9% NaCl. Based on study this, seen that aloin improves expression fibroblasts on healing diabetic wound.

Process of re-epithelialization with migration and proliferation fibroblasts have role important in process healing normal. On diabetic, happen decline growth factor so on the day to 7 cells fibroblasts Still little and will Keep going

increase until peak day 14. Fibroblasts differentiate become myofibroblast as well as seen formation signaling collagen he started stages healing (Hidayat, et.al, 2018)

Process healing wound experience disturbance on patient diabetes. Disturbance healing related wounds with diabetes caused by multiple complex that is decline growth factor, production cytokines, dysfunction endothelium, disorder cell inflammation as well as decrease migration and proliferation fibroblasts (Liu, et.al., 2015)

Enhancement Expression Fibroblasts with Aloins gift

Healing wound I t with administration of aloin shows enhancement fibroblasts with administration of two doses of aloin, where enhancement expression fibroblasts biggest got with gift dose of 1.25 mg/ KgBB. Improvement results expression fibroblasts shown in table 3.

Table 3. Analysis Bivariate Expression Fibroblasts

Group	N	Normality Test Shapiro-wilk	Test Homogeneity	Friedman's test	Wilcoxon test
K- H7	6	0.270	0.012	0.000	
K+ H7	6	0.331			
P1 H7	6	0.80			
P2 H7	6	0.434			
K- H14	6	0.896			
K+ H14	6	0.456			
P1 H14	6	0.974			0.027
P2 H14	6	0.653			0.043

In the bivariate test obtained P value <0.05 so can stated that hypothesis study accepted. Analysis results statistics state that Aloin administration increases expression fibroblasts in wound healing I t DM mouse. then the Wilcoxon test states that second aloin concentration increases expression fibroblasts in a manner significant. Furthermore, if compared to meaning between second dose, then obtained dose of 1.25 mg/ KgBB more meaning for enhancement expression fibroblasts wound healing.

Research Donkor et al, 2020 stated that aloin with the formulated dose of 2.5 mg/ kgBB in preparations ointment show inhibition growth infection of the skin, accelerate healing wound , and pressing growth microorganisms pathogen skin (Donkor, et.al, 2020) . Different with Study others who use dose more low , Seyed et al, 2017 stated that aloin concentration of 5% or concentration dose most effective low For use acceleration wound healing topically compared concentration of 10% (Vijayaraghavan, et.al 2017)

On research this, no seen sign dermal toxicity. Dermis toxicity is characterized with degeneration collagen, cells fibroblasts atypical or cell apoptosis, and degeneration matrix. Based on description dermal histology in the study, a dose of 2.5 mg/ KgBB no dose topical aloin administration.

Study others, Li et al, 2017 stated that giving aloin to speed up healing

very effective wound, through mechanism acceleration migration fibroblasts, increase proliferation fibroblasts and endothelial cells, promote re-epithelialization, and acceleration formation regular and tight collagen. Based on study This can estimated, the dose of aloin is 2.5 mg/ KgBB effective For acceleration healing wound , so that on the 14th day , skin DM mice have enter stage remodeling , so has happen decline expression fibroblasts (LJ Li, et.al, 2017). Phase remodeling can see If inspection description histology includes re-epithelialization as well as density collagen. Inspection This No conducted on research this, which becomes weakness study as well as should examined in research next.

CONCLUSION

Research conclusion This is Aloin administration increases expression cell fibroblasts in wound healing wound I t mouse male (*Rattus norvegicus*) diabetes mellitus with minimum dose of 1.25 mg/ KgBB. Study furthermore should see re-epithelialization and density collagen wound cut on giving aloin to find out rate increase in fibroblasts as well acceleration phase wound remodeling I t mouse male DM.

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