

anatomical pathologist blindly evaluated the histopathological features of the tissue using a light microscope. The histologic features were grouped based on the re-epithelialization, ulceration, neovascularization, presence of inflammatory cells, and granulation maturation. Ultimately, the total score of the wound healing process were calculated based on the scoring system depicted in Table 1 below. Obtained data were tested using the SPSS version 19.0 computer program.

Discussion

This study generally shows that the application of topical Ageratum leaf extract in acute excisional wounds of diabetic models can accelerate the epithelialization process. This epithelialization process is influenced by an

increase in the number of fibroblasts. The same result was shown in collagen formation which revealed an increase in collagen thickness on day 3 (inflammatory phase), which served as the main structure of the new extracellular matrix of wound tissue and decreased on the 10th day (proliferation phase). From these results, it can be seen that Ageratum leaf extract can increase the collagen thickness in the inflammatory phase but reduce the thickness of collagen during the proliferation phase.

Table 2 shows that all control rats were categorized into the re-epithelialization 0 group while in the treatment group only 1 (12.5%) was classified in the re-epithelialization 0 group and 7 (87.5%) rats were categorized into the re-epithelialization 1 group. In the control group, 6 (75%) rats were categorized into ulceration 0 group, and 2 (25%) rats were categorized into ulceration 1 group while in the treatment

Table 1 Histopathologic score to assess wound healing (cited from “Effects of Systemic Erythropoietin on Ischemic Wound Healing in Rats”¹⁶)

Scoring criteria	Score			
	0	1	2	3
Re-epithelialisation	None	Partial	Complete, but immature or thin	Complete and mature
Neovascularisation	None	Up to 5 vessels/HMF	6-10 vessels/HMF	>10 vessels/HMF
Amount of granulation tissue	None	Scant	Moderate	Abundant
Maturation of granulation tissue	Immature	Mild maturation	Moderate maturation	Fully matured
Inflammatory cells	None	Scant	Moderate	Abundant
Ulcer	Wide and deep ulcers, abscesses	Wide ulcers	None or very small	None

Table 2 Comparison between Re-epithelization and Ulceration in Control and Treatment Groups

Variable	Group		P value
	Control n=8	Treatment n=8	
Reepithelization			0.001**
0	8 (100.0%)	1 (12.5%)	
1	0 (0.0%)	7 (87.5%)	
Ulcers			0.041**
0	6 (75.0%)	1 (12.5%)	
1	2 (25.0%)	7 (87.5%)	

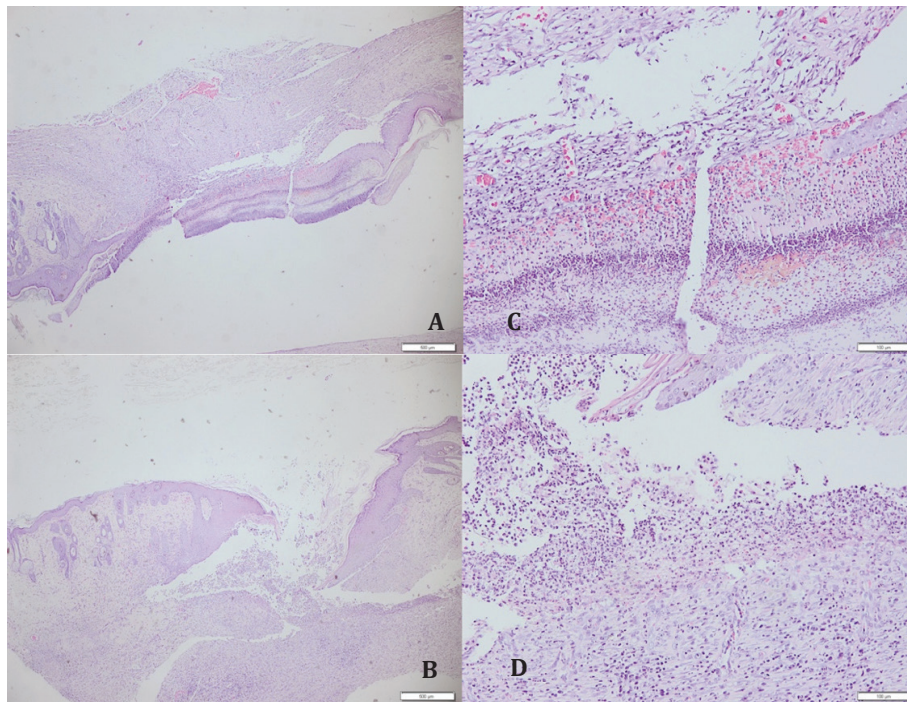


Figure 1 Histologic Appearance of Wound from Control Group in 20x (A) and 100x Magnification, and from Treatment Group in 20x (C) and 100x (D) Magnification

group, only 1 (12,5%) rat was categorized into ulceration 0 group and 7 (87.5%) rats were categorized into ulceration 1 group. Table 2 showed that there were significant differences ($p < 0.05$) in the re-epithelialization and ulceration between the two groups. Table 3 shows that in the control group, 2 (25%) rats were categorized

into neovascularization 0 group and 6 (75%) rats were categorized into neovascularization 2 group. Meanwhile, in the treatment group, there was 1 (12.5%) rat in the neovascularization 0 group, 1 (12.5%) rat in the neovascularization 1 group, 2 (25%) rats in the neovascularization 2 group, and 4 (50%) rats in the neovascularization

Table 3 Comparison between Neovascularization and Presence of Inflammatory Cells in Control and Treatment Groups

Variable	Group		p value
	Control n=8	Treatment n=8	
Neovascularization			0.270
0	2 (25.0%)	1 (12.5%)	
1	0 (0.0%)	1 (12.5%)	
2	6 (75.0%)	2 (25.0%)	
3	0 (0.0%)	4 (50.0%)	
Inflammatory cells			0.001**
0	1 (12.5%)	0 (0.0%)	
1	1 (12.5%)	0 (0.0%)	
2	6 (75.0%)	0 (0.0%)	
3	0(0.0%)	8(100.0%)	

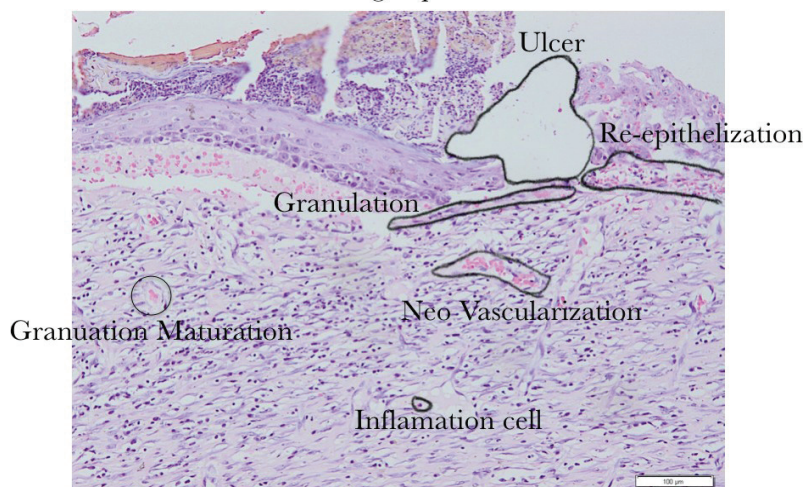


Figure 2 Histologic Appearance of Wound from Control Group in 100x Magnification

3 group. In the control group, 1 (12.5%) rat was categorized into inflammatory cell 0 group, 1 (12.5%) rat was categorized into inflammatory cell 1 group, and 6 (75%) rats were categorized into inflammatory cell 2 group. In the treatment group, all rats were categorized into the inflammatory cell 3 group. As shown in Table 3,

it can be concluded that there were no significant differences in neovascularization between the two groups whereas significant differences in inflammatory cells produced were seen between the two groups.

Previous studies only investigated the component of Ageratum leaves but did not apply

Table 4 Comparison between Granulation Network and Granulation Maturation in Control and Treatment Groups

Variable	Group		Value p
	Control n=8	Treatment n=8	
Granulation tissue			0.001**
0	2 (25.0%)	0 (0.0%)	
1	6 (75.0%)	0 (0.0%)	
2	0 (0.0%)	6 (75.0%)	
3	0 (0.0%)	2 (25.0%)	
Mature granulation			0.001**
0	3 (37.5%)	0 (0.0%)	
1	5 (62.5%)	0 (0.0%)	
2	0 (0.0%)	6 (75.0%)	
3	0 (0.0%)	2 (25.0%)	

Table 5 Comparison between Total Scores of Control and Treatment Groups

Variable	Group		Value P
	Control N=8	Treatment N=8	
Total score			0.000**
Mean±Std	4.75±1.832	11.37±1.407	
Median	5.000	11.500	
Range (min-max)	1.00-7.00	9.00-13.00	

the leaves directly to see the effect on wound healing in vivo, while this study examined the application of Ageratum leaves on acute open wound healing.¹⁴ Rahman et al. did an experimental study in 2012 to explore the wound healing properties of Ageratum on acute open wound healing in non-diabetic rats while this study tested the application of Ageratum leaves in diabetic rats.¹⁵ Table 5 shows a statistically significant difference in the total variable score between the control and treatment groups. The analysis showed an increase in the overall score of the treatment group.

Ageratum leaf extract carries high antioxidant activities because it contains a high amount of polyphenols. The polyphenol contained in Ageratum leaves is ellagic acid. Ellagic acid has been observed to have a fibroblast synthesis stimulating property. Fibroblasts are the principal cells during the proliferation phase of wound healing that play a major role in providing extracellular matrix as a framework for keratinocyte migration. Dense fibroblasts assist the formation of denser and more compact extracellular matrices so that the epithelialization process by keratinocytes is triggered. The thicker collagen layer observed in the treatment group was presumably caused by the increase in fibroblasts and led to faster re-epithelialization when compared to the control group. In this study, no analytical statistic was performed to compare the size of the epithelialization of the wound between the control and the treatment groups.

In a previous study conducted by Oladejo and Imosemi, it was found that Ageratum conyzoides contained phytochemicals which could shorten the healing time of acute open wounds. The wound healing process in the treatment group went aptly in this study because Ageratum leaf extract was shown to accelerated epithelialization process. Other study conducted by Prasad also showed that Ageratum conyzoides application to open wound in rat is able to increase tissue regeneration. Rahan et al. found that Ageratum conyzoides extract contains anti-inflammatory properties by inhibiting the synthesis and release of inflammatory mediators such as prostaglandin, histamine, and serotonin. The substances that are suspected to be responsible for this anti-inflammatory effect are quercetin, kaempferol, glycosides, tannins, and phenols.⁷⁻⁹ These findings were not shown in the current study where the inflammatory cells were found to be statistically more abundant in the control group when compared to the treatment

group.

This experiment shows that Ageratum leaf extract might accelerate the healing of acute open wounds in diabetes patients. Topical administration of Ageratum leaf extract stimulates the healing process of excisional wounds in type II diabetes mellitus models by increasing the thickness of collagen in the inflammatory phase and decreases collagen thickness in the proliferation phase during the healing of excisional wounds and accelerates epithelialization in type II diabetes mellitus models.

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