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Original Research Article

Effects of *Physalis angulata* L. Fruit Extract on Endothelial Cell Migration and VEGF Concentration during Wound Healing under Hyperglycemic Conditions: An *in vitro* Study

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ABSTRACT

Diabetes mellitus cases and complications, including difficult-to-heal wounds, are increasing worldwide. Diabetic wounds frequently exhibit impaired angiogenesis due to hyperglycemia, which disrupts many factors that promote angiogenesis, including the release of vascular endothelial growth factor (VEGF). *Physalis angulata* L, an herb in the *Solanaceae* family, is used as a herbal medicine for many ailments, such as treating wounds. The fruit of *P. angulata* is rich in lupeol, a compound that can enhance wound healing by upregulating the expression of certain growth factors. This study aimed to determine the effect of *P. angulata* fruit extract on endothelial cell migration and VEGF concentration in wound healing in hyperglycemic endothelial cell cultures. Human umbilical vein endothelial cells (HUVECs) were exposed to high glucose (22 mM/dL) for 6 and 24 h with or without 0.025%, 0.05%, and 0.1% concentrations of *P. angulata* fruit extract. Cell migration was examined in HUVECs by scratching and measuring the distance. VEGF concentration was measured using enzyme-linked immunosorbent assay methods. *P. angulata* fruit extract at a concentration of 0.025% for 24 h significantly stimulated cell migration and VEGF concentration in HUVECs exposed to hyperglycemia. *P. angulata* fruit extract is a promising herb for treating diabetic wounds.

Keywords: *Physalis angulata L.* Wound healing, Cell migration, Vascular endothelial growth factor, Human umbilical vein endothelial cells.

Introduction

Diabetes mellitus (DM) is a chronic disease that arises when the pancreas fails to generate sufficient insulin or the body does not efficiently use insulin. The most common effect caused by uncontrolled diabetes is hyperglycemia or increased blood sugar.[1] Hyperglycemia in patients with DM disrupts cellular responses and the healing process, leading to pathological wound healing and chronic nonhealing wounds.[2] Patients with DM have an imbalance in the expression of growth factors and cytokines, which are significant variables affecting wound healing.[3] In hyperglycemic conditions, human umbilical vein endothelial cells (HUVECs) show a decrease in vascular endothelial growth factor (VEGF) and maximal inhibition of endothelial cell proliferation, ultimately promoting endothelial cell apoptosis.[4] Numerous pharmaceuticals and stem cells stimulate diabetic wound healing via VEGF.[5,6]VEGF is a growth factor that regulates the wound healing process. This growth factor is expressed and secreted by endothelial cells to improve blood vessel homeostasis, which acts as the primary regulator of angiogenesis.[7,8]

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Endothelial cell migration is a crucial process in angiogenesis, encompassing three mechanisms: chemotaxis, haptotaxis, and mechanotaxis. Growth factors, particularly VEGF, commonly induce endothelial cell chemotaxis. VEGF-A and the chemokine receptor CXCR4 are critical for influencing Notch-dependent signaling pathways that control the selection and activity of sprouting cells.[9] Recently, treatment for chronic wounds has not advanced substantially. Other currently approved therapeutic implications include growth factor and cell therapies, but high costs and undesirable side effects still limit these therapies. Therefore, new therapeutic agents for treating chronic diabetic wounds should be investigated.[10,11] The leaf of *Physalis angulata L.* is promising as a natural ingredient for wound healing because of its ability to enhance angiogenesis, collagenation, and reepithelialization, which facilitates fibrous tissue production at the base of the wound.[12] Using phytochemical techniques, the chemical content between leaf and fruit was almost the same, including lupeol, ursolic acid, and *β*-sitosterol.[13]*β*-sitosterol stimulates VEGF signaling pathways in rat models of diabetic ulcer wounds.[14] This study determined the effect of *P. angulata fruit extract* on endothelial cell migration and VEGF concentration during wound healing in hyperglycemic endothelial cell cultures.

Materials and Methods

Isolation and HUVEC culture

The umbilical cord was obtained following a cesarean section (CS) operation, adhering to specific conditions for inclusion (normal mother, Hemoglobin < 11 g/dl), healthy baby (weight >2.5 kg, Apgar score 7–9), and exclusion (such as mothers with hypertension, diabetes, cardiovascular disease, preeclampsia, and hyperlipidemia), and was cultured within 12 h post-CS procedure. The umbilical vein was incubated (37°C, 8 min) in collagenase type 1 (Worthington) to isolate endothelial cells and then plated in 48-well plates covering 0.2% gelatin (Sigma G1393) with culture medium containing M199 (Sigma),

100 µg/mL penicillin, streptomycin (Gibco), and 10% fetal bovine serum (Gibco). Cells were incubated at 37°C with 5% CO_2. There were five groups: HUVECs with normal glucose (5 mM/dL, K−), high glucose (22 mM/dL, K+), and three groups of high-glucose cells that were exposed to extract concentrations of 0.025%, 0.05%, and 0.1% (P1, P2, and P3, respectively) for 6 or 24 h. The study participants received comprehensive information and provided signed informed consent before taking part.. This study was conducted under ethical guidelines and was approved by the Ethics Committee, Faculty of Medicine, Brawijaya University in Malang (No. 223/EC/KEPK/10/2022).

Extraction of Physalis angulata L. fruit

The *P. angulata* fruit was identified in Materia Medica, Batu, Indonesia. The ripe fruit (yellowish green) was carefully washed and dried after removing the calyx covering. It was then blended to a smooth consistency, strained, and freeze-dried to yield a concentrated extract. This extract was diluted with distilled water to achieve the required concentrations of 0.025%, 0.05%, and 0.1%.

Cell migration assay

After the cells were monolayer, they were scratched manually using a white tip to represent the wound. Photomicrographs of the initial scratch were taken at 100× magnification using a phase contrast microscope. Then, photomicrographs were taken at 6 and 24 h after treatment. The calculation formula used to determine the percentage of cell migration is as follows:

$$\% \text{ Migration of cells} = 100 - [(x/y) \times 100\%]$$

Where x is the wound area at a given time (6 and 24 h) and y is its initial area.

ELISA for VEGF

An enzyme-linked immunosorbent assay (ELISA) (Human-VEGF-ELISA kit, Bioassay Technology, China) was used to measure VEGF in the cell culture medium. Microplates (96 wells) were filled with 40 µL of sample solution and 10 µL of anti-VEGF antibody. All wells were filled with 50 µL streptavidin-horseradish peroxidase, covered with adhesive paper, and shaken at 37°C for 60 min. The washing process was then repeated five times using a washing buffer. Subsequently, 50 µL of substrate A solution and 50 µL of substrate B solution were added to the well plates and shaken in dark conditions before incubation at 37°C for 10 min. Next, 50 µL of stop solution was added to each well. Absorption was measured using an ELISA reader to determine the amount of VEGF in the medium at a wavelength of 450 nm.

Statistical analysis

The results are presented as mean ± SD. One-way ANOVA was used to examine multiple comparisons, with Tukey's *posthoc* test. The Pearson correlation test was used to determine the correlation between two variables. The statistical tests were performed using SPSS software version 23, with a significance level (p) <0.05.

Results and Discussion

Endothelial cell migration is one of the crucial signs indicating angiogenesis. The results of this study, % HUVEC migration from the group exposed to 22 mM/dL for 6 and 24 h (K+), were the lowest among other groups. Tukey's *post hoc* test indicated that % cell migration of the group administered with *P. angulata* fruit extract at a concentration of 0.025% for 24 h had a significant difference compared with other groups that included normal glucose (K−), K+, and cells exposed to glucose 22 mM/dL with extract concentrations of 0.05% and 0.1% (P2 and P3) for 24 h. However, there was no significant difference between all groups in the treatment for 6 h (Figures 1 and 2).

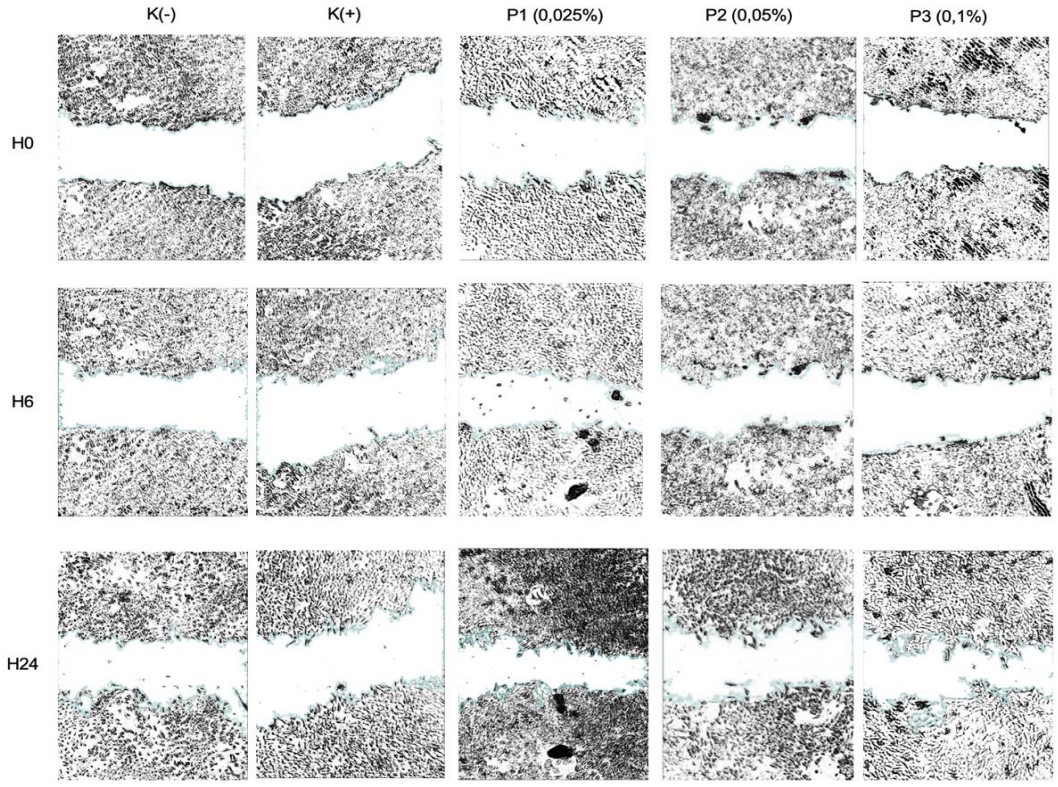

Figure 1: Image of HUVECs migration in the control and treatment groups from a scratch assay at 0 (H0), 6 (H6), and 24 h (H24)

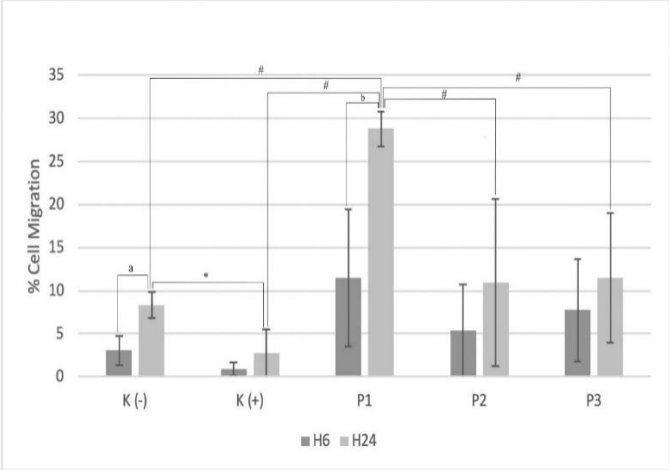

Figure 2: Mean HUVECs migration in the control and treatment groups. #p < 0.05 compared between groups (Tukey's *post hoc* test)

The results showed that high glucose conditions inhibit the migration of endothelial cells and ultimately hinder the healing process. In endothelial cells, migration was virtually nonexistent because of elevated glucose levels.[15] The glucose concentration could significantly affect the stiffness of HUVECs, which subsequently results in remarkable alterations in cell migration and proliferation capabilities.[16]*P. angulata* fruit extract contains *lupeol, β-sitosterol, and ursolic acid*.[13]Lupeol can heal wounds in hyperglycemic environments by reducing the inflammatory process and increasing the number of markers involved in angiogenesis.[17] The migratory activities of endothelial cells were significantly and time-dependently enhanced by ursolic acid purified from apple peel.[18] In rats with diabetic ulcers, *β*-sitosterol promotes wound angiogenesis and the release of anti-inflammatory cytokines, thereby accelerating the healing process.[14] VEGF is a pivotal proangiogenic growth factor in angiogenesis, stimulating the development of new blood vessels by encouraging migration, proliferation, differentiation, and viability of endothelial cells.[8,19] In a wound, VEGF production is increased by several types of cells, including endothelial cells.[20] This study showed that the group exposed to high glucose for 6 and 24 h (K+) had the lowest VEGF levels. The observation at 24 h showed a significant difference, especially between the control group with normal glucose and the high glucose treatment group given the extract of *P. angulata at 0.025%* and 0.1%, respectively (Figure 3).

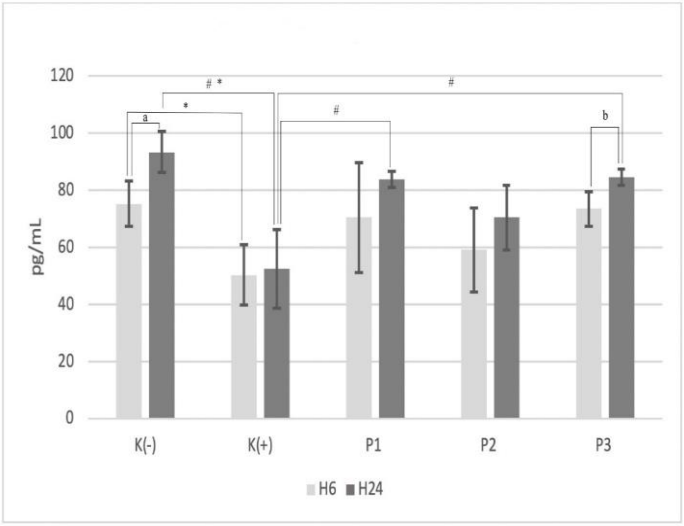

Figure 3: Mean VEGF concentration (pg/mL) in the control and treatment groups. #p < 0.05 compared between groups (Tukey's *post hoc* test)

In this study, the lower VEGF concentration in hyperglycemic conditions was in line with conditions in patients with DM, which showed a reduction in growth factors such as platelet-derived growth factor, keratinocyte growth factor, transforming growth factor-beta (TGF-β1), and VEGF.[2] The administration of *P. angulata* fruit extract maintained VEGF concentration in endothelial cells in a high-glucose environment. This result was supported by research that found that lupeol increases the immunostaining of Ki-67 and gene expression and the immunolabeling of epidermal growth factor and VEGF, thereby promoting new blood vessel formation.[17] The presence of *β*-sitosterol in aloe vera improves the ability of diabetic mice to heal full-thickness excisional wounds more quickly by stimulating angiogenesis, VEGF, and reepithelialization.[21]

Pearson's correlation analysis, used to determine whether both parameters correlate, showed no significant correlation. This phenomenon indicated that other than the VEGF pathways in the extract, it increased the migration of endothelial cells under high glucose conditions. Previous research has shown that lupeol can increase the intensity of growth factors fibroblast growth factor-2 (FGF-2), TGF-*β*1, and collagen III.[22] In contrast, angiogenic growth factors like VEGF and FGF-2, as well as their receptors, were increased by ursolic acid.[23] *P. angulata* has been shown to have antioxidant properties in an assessment using DPPH and ABTS+ radicals.[24] Additionally, *G.arabica*, which possesses free radical scavenging properties, has enhanced various stages of wound healing, including fibroplasia, collagen production, and wound contraction.[25] This suggests that the antioxidant properties of the *P. angulata* fruit extract may also play a role in angiogenesis.

Conclusion

P. angulata fruit extract significantly increased VEGF concentration and HUVEC migration when exposed to glucose at 22 mM/dL for 24 h. Further research is recommended to explore the effects of *P. angulata* fruit on other growth factors that promote endothelial cell migration.

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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