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VEGF Expression in the Wound Healing Process of Hyperglycemic Wistar Rats After Administration of Roselle Flower (Hibiscus sabdariffa) Extract: An In Vivo Experimental Laboratory Study

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ABSTRACT

Background: Wound healing is a complex process significantly impaired by hyperglycemia, primarily due to disruption of angiogenesis mediated by Vascular Endothelial Growth Factor (VEGF). Roselle (Hibiscus sabdariffa) flower extract, rich in antioxidants and anti-inflammatory compounds, holds potential as a phytotherapy agent to support wound recovery. This study aimed to analyze the effect of Roselle extract administration on VEGF expression during incision wound healing in Wistar rats under acute hyperglycemic conditions.

Methods: An experimental study was conducted using Wistar rats induced with acute hyperglycemia. Rats were divided into control groups (hyperglycemic without extract), normoglycemic groups with extract, and hyperglycemic groups with Roselle extract (K3). An incision wound was created, and the extract was administered. VEGF expression in the wound tissue was measured on days 3 and 7 post-incision using immunohistochemistry (IHC).

Results: Roselle extract administration significantly increased VEGF expression on day 7 post-incision, coinciding with the proliferative phase. The hyperglycemic group treated with Roselle extract (K3) showed a statistically higher mean VEGF expression compared to the hyperglycemic control group (K1). This finding indicates that Roselle extract effectively mitigates the angiogenic impairment caused by acute hyperglycemia. The bioactive compounds in Roselle are thought to stimulate the HIF-1α – VEGF pathway and reduce oxidative stress, creating a conducive environment for tissue regeneration.

Conclusion: Roselle (Hibiscus sabdariffa) flower extract is effective in enhancing VEGF expression during the incision wound healing process in Wistar rats, especially under acute hyperglycemic conditions. Roselle has significant potential to be developed as a herbal-based adjuvant therapy to accelerate wound healing, particularly in patients with metabolic disorders.

KEYWORDS: Roselle, Hibiscus sabdariffa, VEGF, Angiogenesis, Wound Healing, Hyperglycemia.

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INTRODUCTION

Wound healing is a precise biological process, but it is severely compromised by hyperglycemia, a condition prevalent in diabetic patients. High blood glucose levels impair various healing mechanisms, notably by inhibiting immune cell function, reducing collagen synthesis, increasing oxidative stress, and promoting chronic inflammation (Murray et al., 2009).

Crucially, hyperglycemia disrupts angiogenesis—the formation of new blood vessels—which is essential for the proliferative phase. This process is heavily dependent on Vascular Endothelial Growth Factor (VEGF). In hyperglycemic states, VEGF expression is often reduced, leading to inadequate oxygen and nutrient supply and delayed wound closure (Chen et al., 2020).

Roselle flower (Hibiscus sabdariffa) is a potential natural therapy, rich in bioactive compounds like flavonoids and anthocyanins with known antioxidant and anti-inflammatory properties. Traditional use and preliminary studies suggest Roselle can reduce oxidative stress, mitigate inflammation (Kusumastuti et al., 2013), and potentially enhance VEGF expression and angiogenesis, thereby accelerating tissue regeneration, even under hyperglycemic conditions (Chen et al., 2020).

Given Roselle's promise in counteracting the metabolic hindrances to healing, stronger evidence is needed regarding its molecular action on VEGF and the angiogenic pathway. Therefore, this study aims to evaluate the effect of Roselle flower extract on VEGF expression and the wound healing process in Wistar rats under conditions of acute hyperglycemia. This research seeks to contribute to the development of plant-based therapies for complicated wounds in metabolically compromised patients.

MATERIALS AND METHODS

This study employed an analytical laboratory experimental design utilizing a Randomized Control Group Post-Test Only Design to investigate the effect of Hibiscus sabdariffa extract on wound healing in male Wistar rats. The rats (250-300 g, 1-2 months old) were divided into three groups: K1 (hyperglycemic control, no extract), K2 (normoglycemic with extract), and K3 (hyperglycemic with extract). Hyperglycemia was acutely induced via intraperitoneal Alloxan (150 mg/kg BW), confirmed by blood glucose levels $>200 \text{ mg/dL}$. A standardized 1.0 cm incision wound was created on the dorsal skin under Ketamine anesthesia. The Roselle extract was prepared as a 2% topical gel following maceration in 70% ethanol, and was applied once daily to the K2 and K3 groups. Biopsies of the wound tissue were collected on days 1, 3, and 7 post-incision, fixed in 10% Formalin, and processed for histopathological examination. VEGF expression, the primary dependent variable, was assessed using Immunohistochemistry (IHC) with a monoclonal anti-VEGF antibody, DAB as the chromogen, and Hematoxylin-Eosin counterstaining. Quantitative data on VEGF expression were analyzed using descriptive statistics (mean and standard deviation). The Shapiro-Wilk and Levene's tests were performed to check for normality and homogeneity, respectively. Statistical hypotheses were tested using One-Way ANOVA followed by the Post Hoc Tukey test for normally distributed and homogeneous data, or the Kruskal-Wallis Test followed by the Mann-Whitney U test for non-parametric data.

RESULTS

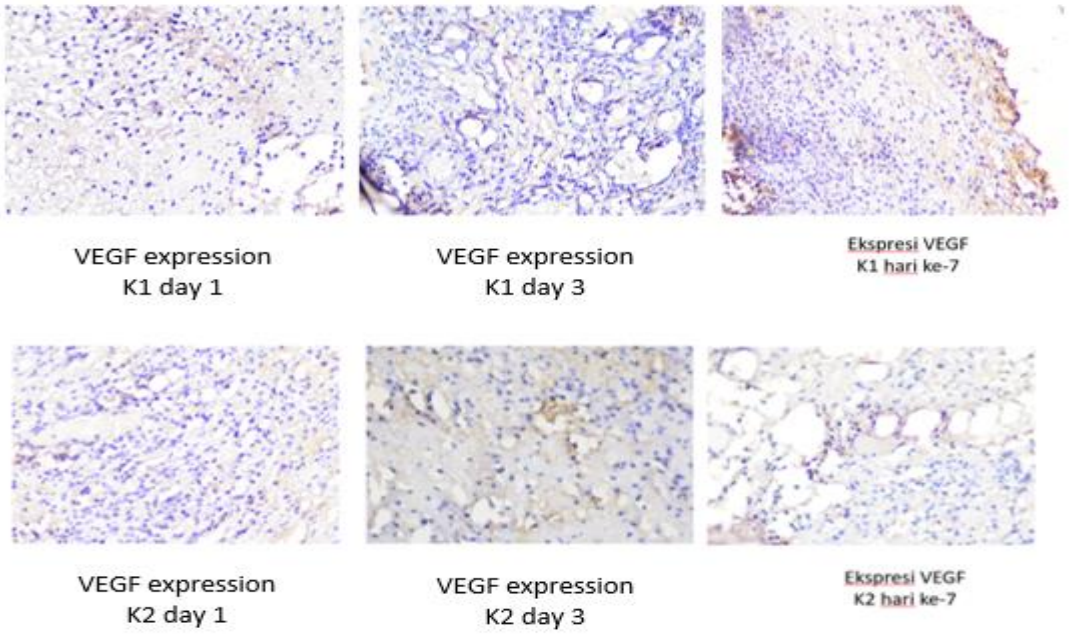
This study evaluated the effect of Hibiscus sabdariffa (roselle) extract on VEGF expression during the healing of incisional wounds in hyperglycemic Wistar rats. VEGF expression was examined using immunohistochemistry on days 1, 3, and 7 after incision. The expression levels were compared between hyperglycemic and normoglycemic rats, with and without roselle extract administration.

Table 1 presents the mean VEGF expression in all experimental groups. Rats treated with roselle extract, both normoglycemic (K2) and hyperglycemic (K3), showed higher VEGF expression compared to untreated hyperglycemic rats (K1). VEGF expression gradually increased over time in all groups, with a notably steeper increase observed in the roselle-treated groups.

Table 1. Mean VEGF Expression in Each Experimental Group

Group	Condition	Day 1	Day 3	Day 7
K1	Hyperglycemic without roselle extract	2.25	2.75	3.50
K2	Normoglycemic with roselle extract	4.50	6.00	7.00
K3	Hyperglycemic with roselle extract	4.00	5.50	6.50

Immunohistochemical staining revealed brownish-golden coloration in the cytoplasm of fibroblast and endothelial cells, indicating VEGF expression. In the untreated hyperglycemic group (K1), very minimal staining was observed on day 1, followed by a restricted increase up to day 7, suggesting delayed angiogenesis. In contrast, both roselle-treated groups (K2 and K3) showed more intense and widespread staining beginning on day 3 and becoming prominent by day 7, reflecting enhanced angiogenic activity. The Shapiro–Wilk test demonstrated that some groups had normally distributed data ($p > 0.05$), whereas others did not ($p < 0.05$). Considering this distribution pattern, the data analysis was continued using non-parametric tests, specifically Kruskal–Wallis followed by Mann–Whitney post hoc comparisons.



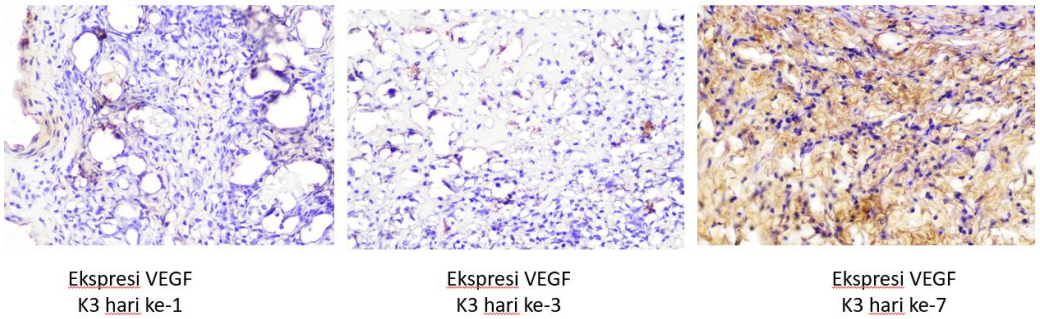


Figure 1. The results of VEGF immunohistochemistry staining on Wistar rat wound tissue, which was divided into 3 groups and observed on days 1, 3, and 7. VEGF expression is indicated by a golden-brown color in the cytoplasm of fibroblast or endothelial cells."

The Kruskal–Wallis test revealed statistically significant differences in VEGF expression among groups on days 1, 3, and 7 ($p < 0.05$). Subsequent Mann–Whitney testing showed that VEGF expression in hyperglycemic rats without extract (K1, day 1) was significantly lower than in all roselle-treated groups (K2 and K3) across days 1, 3, and 7 ($p < 0.05$). Significant differences were also found between K1 (days 3 and 7) and both K2 and K3 (days 3 and 7), indicating suppressed VEGF levels in untreated hyperglycemic animals. Within roselle-treated groups, VEGF expression significantly increased over time, particularly:

- K2 day 1 vs. K2 day 7 ($p = 0.028$)
- K3 day 1 vs. K3 day 3 ($p = 0.036$)
- K3 day 1 vs. K3 day 7 ($p = 0.028$)
-

RESULTS

This study demonstrates that Hibiscus sabdariffa (roselle) extract significantly increases the expression of Vascular Endothelial Growth Factor (VEGF) during the healing of incisional wounds in Wistar rats. The highest increase was observed on days 3 and 7, corresponding to the proliferative phase of wound healing. The roselle-treated groups, in both normoglycemic and acute hyperglycemic conditions, showed higher mean VEGF expression compared with controls, supporting the hypothesis that the bioactive compounds of roselle enhance angiogenesis.

The most notable finding was observed in the hyperglycemic rats treated with roselle (K3), which exhibited significantly higher VEGF expression compared with untreated hyperglycemic rats (K1). Hyperglycemia is known to impair angiogenesis and delay tissue repair; however, our findings indicate that roselle extract can counteract this metabolic impairment. Similar results were reported by Rambe et al. (2022), where roselle gel accelerated diabetic wound closure by enhancing angiogenesis and reducing local inflammation. Mohammed et al. (2021) also demonstrated that roselle stimulates not only VEGF but also TGF-β expression, supporting granulation tissue formation.

VEGF is a key regulator of angiogenesis released by keratinocytes, macrophages, and endothelial cells during tissue repair. It promotes endothelial cell proliferation, migration, and vascular permeability, making it essential in the transition from the inflammatory phase to the proliferative phase (Ferrara, 2009; Bao et al., 2009). Reduced VEGF expression is directly associated with delayed wound healing (Johnson & Wilgus, 2014).

The bioactive components of roselle—particularly anthocyanins, flavonoids, ascorbic acid, and organic acids—regulate VEGF expression through multiple molecular pathways. Flavonoids stimulate the PI3K/Akt and HIF-1α signaling pathways, enhancing VEGF synthesis. Cho et al. (2023) demonstrated that roselle extract upregulates HIF-1α in endothelial cells, increasing VEGF secretion. In addition, roselle reduces inflammatory cytokines (TNF-α, IL-6) (Alzubairi et al., 2022) and decreases excess reactive oxygen species (ROS), providing a microenvironment conducive to angiogenesis. These mechanisms explain the restoration of VEGF expression observed in acute hyperglycemia in this study.

The biological effects of roselle vary according to the metabolic condition. In normoglycemia, wound healing progresses physiologically and roselle acts primarily as an antioxidant and anti-inflammatory agent. In acute hyperglycemia, as used in this study (approximately one week duration), oxidative stress and inflammation increase without chronic structural vascular damage. Roselle rapidly neutralizes ROS via Nrf2 activation and suppresses inflammatory pathways such as NF-κB, enabling an early transition to the proliferative phase. This is consistent with Ajay et al. (2007) and Peng et al. (2011), who reported significant anti-inflammatory and hypoglycemic effects of roselle.

In contrast, chronic hyperglycemia produces persistent inflammation, protein glycation, endothelial dysfunction, and impaired fibroblast activity. In such conditions, roselle remains beneficial but requires longer administration and may need combination therapy with other pro-angiogenic agents, such as topical VEGF or stem cells (Li et al., 2020). Therefore, the strong VEGF response observed in the hyperglycemic rats in this study is associated with an acute rather than chronic hyperglycemic state.

Based on the present findings and previous literature, roselle may accelerate wound healing in acute hyperglycemia through the following mechanisms:

1. Activation of Nrf2 Pathway, enhancing antioxidant defenses and reducing ROS (Ajay et al., 2007).
2. Upregulation of HIF-1α–VEGF Signaling, promoting angiogenesis (Bao et al., 2009; Yang et al., 2021).
3. Suppression of Inflammatory Cytokines (TNF-α, IL-6, MMP-9), facilitating earlier transition to the proliferative phase (Peng et al., 2011).
4. Stimulation of Fibroblast Proliferation and ECM Synthesis, improving tissue structure and wound strength (Chen et al., 2005; Li et al., 2020).
5. Hypoglycemic Effect, resulting in a more favorable metabolic environment for healing (Ajay et al., 2007).

These mechanisms collectively explain the significant improvement in VEGF expression and angiogenesis observed in the roselle-treated hyperglycemic group.

LIMITATIONS

This study focused solely on VEGF as an angiogenic indicator and did not evaluate other vital growth factors such as FGF-2, TGF-β, or PDGF. The assessment relied on semi-quantitative IHC scoring without performing vascular density quantification or advanced histomorphometric analysis. Furthermore, the hyperglycemic model represented acute rather than chronic diabetes, limiting generalization to long-term diabetic conditions. Although rats provide valuable biological insights, extrapolation to human wound physiology remains limited.

CLINICAL IMPLICATIONS

The findings indicate that roselle extract has strong potential as an adjuvant therapy for acute hyperglycemic wounds, including postoperative or traumatic wounds in diabetic patients. Roselle may be formulated into topical gels, creams, or oral supplements within standardized phytopharmaceutical products. Further clinical trials are essential to determine optimal dosage, duration, safety profiles, and potential synergy with other pro-angiogenic therapies.

CONCLUSIONS

The present study demonstrates that Hibiscus sabdariffa (Roselle) extract significantly increases VEGF expression during incisional wound healing in Wistar rats. Enhanced VEGF activity was observed in both normoglycemic and acute hyperglycemic states, with peak expression on day 7, aligning with the proliferative phase. Roselle-treated hyperglycemic rats exhibited notably higher VEGF expression than untreated controls, indicating its ability to counteract angiogenic impairment caused by metabolic stress. These findings highlight Roselle extract as a promising pro-angiogenic phytotherapeutic agent for improving wound healing outcomes.

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