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Original Article

Potential of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth) in Regulating TNF-α, VEGF and Histopathological Changes in Wounds in Mice with Diabetic Ulcers

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ABSTRACT

Background: Diabetes Mellitus (DM) is a chronic and progressive metabolic disorder characterized by an increase in blood glucose levels (hyperglycemia) because the pancreas does not produce enough insulin or the body is unable to use the insulin produced effectively. One of the complaints that occur in DM patients is the appearance of wounds that are difficult to heal or are called diabetic ulcers. Diabetic ulcers and gangrene cases in Indonesia are the most common cases encountered in hospitals. The general aim of this study was to analyze the effect of ethanol extract of suruhan leaves (*Peperomia pellucida* L. Kunth) on levels of TNF-α, VEGF and wound histopathology in white Wistar rats (*Rattus norvegicus*) as a model of diabetic ulcers. **Materials and Methods:** The research design with a posttest-only controlled group design is a laboratory experiment on male white Wistar rats, a model of diabetes mellitus with ulcers. **Results:** Phytochemical screening results revealed the presence of flavonoids, alkaloids, saponins, phenols and tannins in rosella extract. In this study, a significant difference ($p<0.05$) was found between all research groups but not find a significant difference ($p>0.05$) between all research groups on VEGF levels. **Conclusion:** The results of the study showed that ethanol extract at a dose of 40 mg/kg BW was better in terms of reducing inflammation levels and collagen formation in diabetic rat ulcer wounds. As well as improving the histopathology of diabetic ulcer wounds. This shows that the ethanol extract at a dose of 40 mg/kg BB has the potential to heal wounds.

Keywords: Diabetic ulcers, Ethanol Extract Effect Suruhan Leaves, White rat, Wistar strain.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder that is chronic and develops progressively, which is characterized by increased blood glucose levels (hyperglycemia) resulting from a lack of insulin production by the pancreas or the body's inability to use insulin effectively.[1] The number of diabetes mellitus sufferers continues to increase every year, making it a significant health problem.[1,2]

One of the problems often experienced by Diabetes Mellitus (DM) sufferers is the appearance of wounds that are difficult to heal, known as diabetic ulcers.[3] The incidence of diabetic ulcers in Indonesia reaches 12%, with a risk of diabetic ulcers of 55.4%. Cases of diabetic ulcers and gangrene are problems that are often encountered in Indonesian hospitals. The death rate from ulcers and gangrene ranges from 17-23%, while the amputation rate ranges from 15-30%.[4] Diabetic ulcers are a chronic and serious complication that occurs in 5 to 10% of the population of DM sufferers.[3,4]

Wound healing is a complex and dynamic process, occurring through several phases, namely the inflammation, proliferation and remodeling phases. Wound healing in DM sufferers takes longer than in non-diabetic sufferers because the inflammatory phase is prolonged. Wound care is carried out to reduce the occurrence of infections and amputations, improve function and quality of life and reduce health care costs.[5,6]

Prolonged wound healing in diabetes sufferers also occurs due to a relationship between cell function disorders, inflammatory

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imbalances, proteases, cytokines and growth factors. In diabetic foot ulcers, there is an increase in fibroblast apoptosis and a decrease in fibroblast cell proliferation and prolongation of the inflammatory reaction, as evidenced by the presence of large numbers of neutrophil granulocytes in the wound. Granulocyte neutrophils secrete proinflammatory cytokines, especially Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β). These 2 cytokines stimulate the synthesis of Matrix Metalloproteinases (MMPs), causing degradation of matrix proteins and growth factors so that wound healing becomes interrupted and uncoordinated.[6,7]

One of the parameters for determining diabetic wound healing is microscopic observation of skin tissue, namely the expression of Vascular Endothelial Growth Factor (VEGF). VEGF is a growth factor that has an important role in neovascularization in wound healing. VEGF acts as a potent mitogen of endothelial cells that induces migration and sprouting of endothelial cells for the formation of new blood vessels through regulating several integrin receptors on endothelial cells. The role of VEGF in wound healing is as a stimulator of angiogenesis. Angiogenesis in the wound healing process includes several stages, namely vasodilation, basement membrane degradation, migration, proliferation and maturation of endothelial cells. The growth of capillary blood vessels functions to distribute nutrients and various healing mediators. The wound healing process will be weakened if there is inhibition of angiogenesis.[8,9]

Treatment of diabetic ulcer wounds requires several types of therapy, namely therapy to lower blood glucose levels, therapy for wounds such as dressings and repeated debridement of necrotic tissue and requires antibiotic therapy for infections from diabetic ulcers.[10] Treatment of diabetic ulcers requires various types of chemical drugs of different types and complex wound care to treat diabetic ulcers. Meanwhile, it is widely known that quite a few synthetic medicines cause undesirable side effects and because of this, people end up tending to use traditional medicines both from within the country and abroad, because basically traditional medicines give negative side effects smaller compared to synthetic drugs and the use of traditional medicine can be another alternative that can provide healing besides modern medicine. One of the plants is thought to be able to be used to replace synthetic drugs, such as *Peperomia pellucida* L. Kunth leaves, which belong to the Piperaceae family.[11]

Suruhan plants (*Peperomia pellucida* L. Kunth) is one of the medicinal plants commonly used in Indonesia. This plant has many benefits and properties for body health, including being used empirically to treat fever, headaches and stomach aches.[12,13] This plant can be used as an anti-bacterial, anti-inflammatory and analgesic. Several research results that have been carried out show that the suruhan plant has the potential to be anti-inflammatory, antipyretic, antimicrobial and anti-cancer and has an analgesic effect.[14,15]

The herb plant has antihyperglycemic activity. However, so far there is no information regarding the suruhan plant with varying doses as an anti-inflammatory and wound healing process, therefore, it is necessary to carry out research regarding the use of suruhan leaves as an anti-diabetic agent.[16] Based on the above background, the researchers wanted to know the potential of ethanol extract of suruhan leaves (*Peperomia pellucida* L. Kunth) on the levels of TNF-α, VEGF and wound histopathology in male white Wistar rats (*Rattus norvegicus*) as a model of diabetic ulcers induced by Streptozotocin (STZ). Based on the above background, the researchers wanted to know the potential of ethanol extract of suruhan leaves (*Peperomia pellucida* L. Kunth) on the levels of TNF-α, VEGF and wound histopathology in male white Wistar rats (*Rattus norvegicus*) as a model of diabetic ulcers induced by Streptozotocin (STZ).

MATERIALS AND METHODS

The experimental design utilized a posttest only controlled group design conducted in a laboratory setting using male white Wistar rats with diabetes mellitus and diabetic ulcer. This design enables researchers to assess the impact of treatment (intervention) on experimental groups by comparing them to control groups. However, in this design, researchers did not quantify the extent of change since the assessments were conducted at the conclusion of the treatment period.

Place and Time of Research

Research Sites

The study was conducted at the Laboratory of the Faculty of Medicine, Methodist University of Indonesia, to produce ethanol extract from suruhan leaves (*Peperomia pellucida* L. Kunth) and to provide a facility for surgical procedures on experimental animals.

Research Time

This research was conducted in June-November 2023, around ±6 months.

Research Sample

Male white Wistar rats were utilized as the experimental subjects in this study. The decision to use rats as experimental animals was made due to their genetic resemblance to humans and their adaptability to laboratory conditions. The allocation of samples into groups was performed based on specific inclusion and exclusion criteria.

Inclusion criteria

For the experimental rats were as follows: aged between 2.5 to 3 months, with a body weight ranging from 150 to 220 g, male gender, in a healthy condition (active and non-disabled) and with

blood sugar levels exceeding 200 mg/dL (indicating Type II DM). Additionally, the rats were surgically incised.

Exclusion criteria

For this study include male white rats that do not exhibit active movement and rats that died during the research period.

Each treatment group contained a minimum of 5 male mice. The researchers chose to use 6 male rats per group to anticipate the death of the experimental animals with a total of 5 treatment groups so that the total number of research samples was 30, which were divided into:

a. The normal group was not given any treatment, only given normal food and drink (*ad libitum*) in their cage.

b. Negative control, only induced by STZ 40 mg/kgbb for 5 consecutive days.[17-19]

c. Positive Control, treatment group induced by STZ 40 mg/kg BW with metformin 50 mg/kg BW/day (human dose with a body weight of 70 kg in mice with a body weight of 200 g is 0.018. The dose of metformin used for adults is 500 mg, so the dose for a 200 g rat is 9 mg. For a 100 g rat it is 4.5 mg).[20]

d. Treatment Group I, treatment group induced by STZ 40 mg/kgBW with Ethanol Extract of Suruhan Leaves (EESL) 40 mg/kgBW, p.o.[21]

e. Treatment Group II, treatment group induced by STZ 40 mg/kgBW with ethanol extract of suruhan leaves 80 mg/kgBW, p.o.[21]

RESULTS

This study constitutes a laboratory-based experimental investigation employing a post-test only controlled group research design. It was conducted at the Phytopharmaceutical Laboratory, Veterinary Laboratory and Integrated Laboratory of the Faculty of Medicine, Universitas Methodist Indonesia.

Analisis Determinasi Suruhan Leaves (*Peperomia pellucida* L. Kunth)

Determination of sample materials in this study using (Table 1).

Based on the results of identification and determination carried out by the Indonesian Biology Generation Foundation, the sample sent was Ketumpang water (*Peperomia pellucida* (L.) Kunth var. pellucida).

Classification

Regnum : Plantae

Divided : Magnoliophyta

Class : Magnoliopsida

Nation : Piperales

Family : Piperaceae

Genus : Peperomia

Type : *Peperomia pellucida* (L.) Kunth

Analysis Screening Phytokimia Extrak Ethanol Suruhan Leaves (*Peperomia pellucida* L. Kunth) Phytochemical Screening Results of Suruhan Leaves (*Peperomia pellucida* L. Kunth) (Table 2).

Bivariate Analysis

Analysis of the Relationship between Differences in Activity of Serum TNF-α Levels Between Groups of Male Wistar White Rats

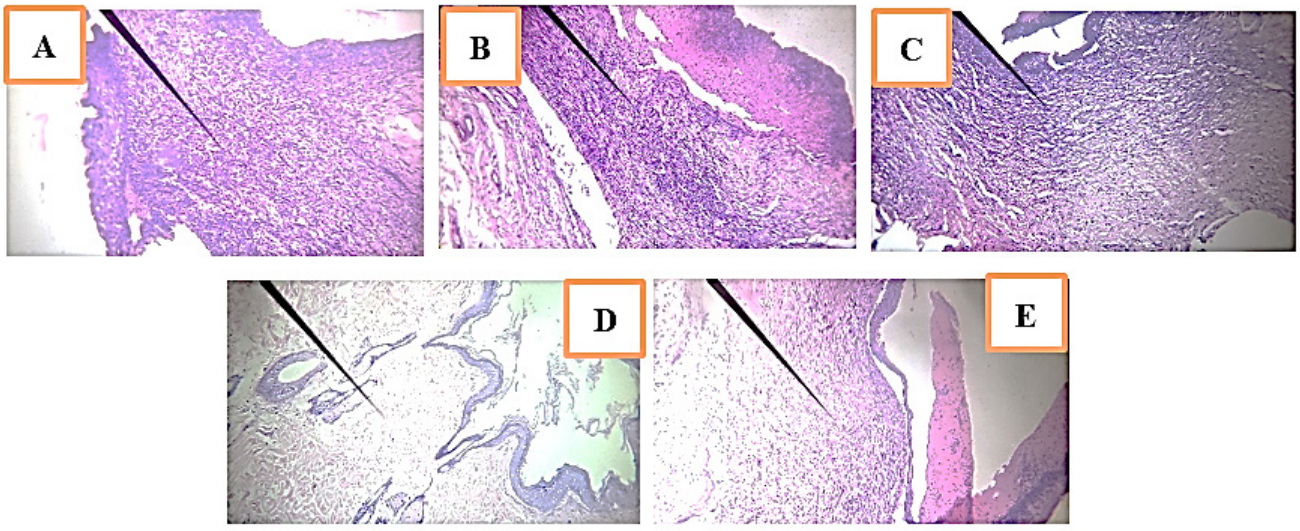


Figure 1: Histopathological picture of extensive collagen density wounds. (A) Normal group collagen density; (B) Negative group collagen density; (C) Collagen density positive group; (D) Group EESL40 collagen density; (E) Group EESL80 collagen density.

(*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth).

Results of analysis of the relationship between differences in serum TNF-α levels between groups of male white rats of the Wistar strain model of diabetic ulcer after administration of ethanol extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth) (Table 3).

In this study, 30 samples were divided into 5 groups. The Normal group had samples with TNF-α levels of Mean=141.54 and SD=20.25, the Negative group had Mean=213.17 and SD=32.06, the Positive group Mean=172.24 and SD=14.24, group I Mean=163.39 and SD=14.47 and group II Mean=176.39 and SD=28.16. The results of this analysis showed a significant relationship between groups of serum TNF-α levels ($p<0.001$).

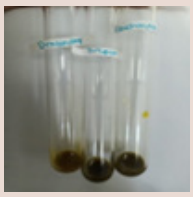
Analysis of the Relationship between Differences in the Activity of Serum VEGF Levels Between Groups of Male Wistar White Rats (*Rattus norvegicus*) in the Diabetic Ulcer Model after Giving Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth).

Results of analysis of the relationship between differences in serum VEGF levels between groups of male white rats of the Wistar strain model of diabetic ulcer after administration of ethanol extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth) (Table 4).

Table 1: Determination of Suruhan Leaves.

Name	Kind	Tribe
Daun Pegagan	*Peperomia pellucida* (L.) Kunth	Piperaceae

Table 2: Phytochemical Screening of Suruhan Leaves Ethanol Extract.

Test	Picture	Result
Flavonoid		(+)
Alkaloids (bouchardad mayer dragen drof)		(+)
Saponins		(+)
Tannin		(+)
Phenol		(+)

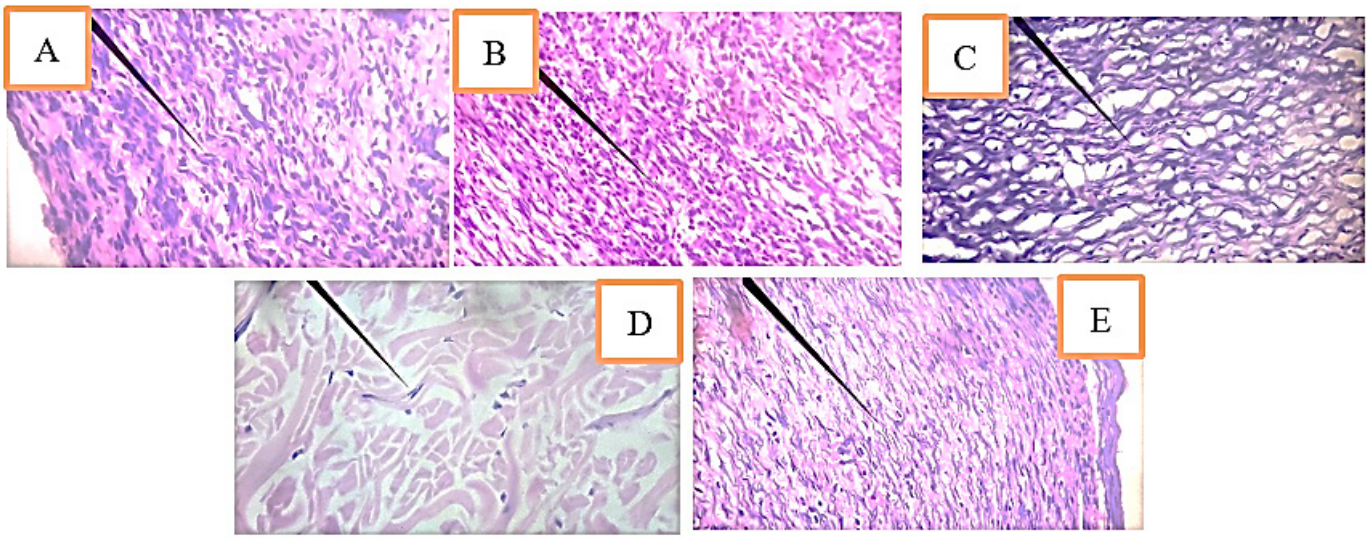


Figure 2: Extensive histopathological picture of fibroblast cell injury. (A) Normal group fibroblast cells; (B) Negative group fibroblast cells; (C) Positive group fibroblast cells; (D) Group EESL40 fibroblast cells; (E) Group EESL80 fibroblast cells.

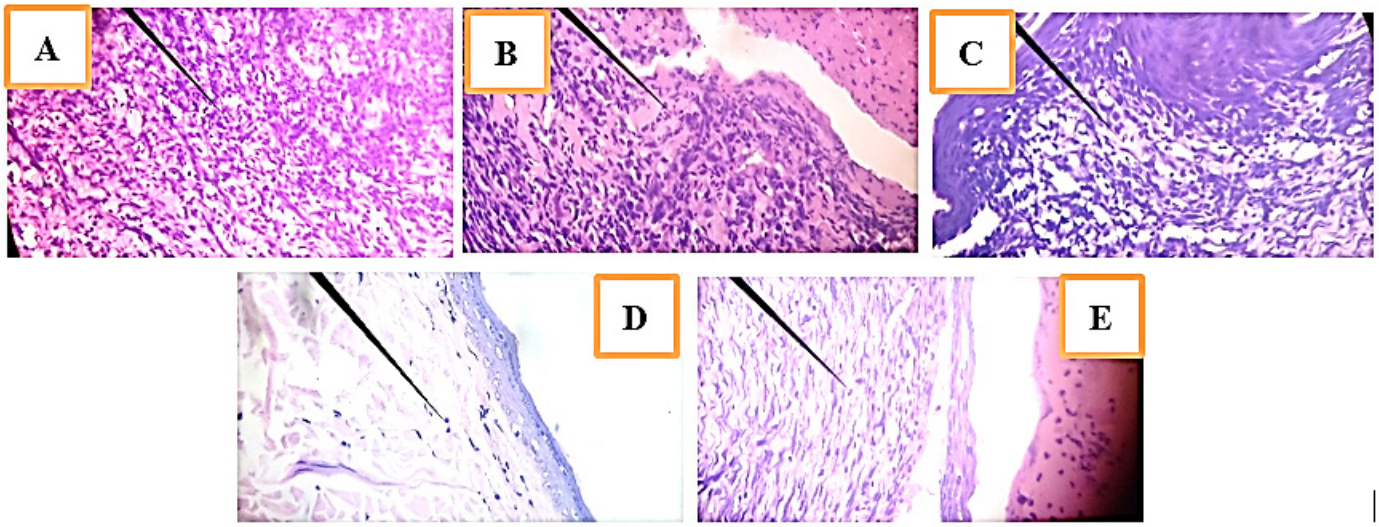


Figure 3: Histopathological picture of extensive inflammatory cell lesions. (A) Inflammatory Cells normal group; (B) Negative group inflammatory cells; (C) Inflammatory cells positive group; (D) Group EESL40 inflammatory cells; (E) Group EESL80 inflammatory cells.

In this study, 30 samples were divided into 5 groups. The Normal group had samples with VEGF levels of Mean=297.69 and SD=44.95, the Negative group had Mean=302.73 and SD=63.19, the Positive group Mean=300.21 and SD=56.54, group I Mean=275.45 and SD=45.40 and group II Mean=307.68 and SD=12.47. The results of this analysis showed no significant relationship between groups of serum VEGF levels (p>0.05).

Histopathological Analysis of Wounds of Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Seruhan Leaves (*Peperomia pellucida* L. Kunth).

Results of histopathological analysis of wounds from male white rats with a model of diabetic ulcers after administration of ethanol extract of suruhan leaves (Table 5).

The results of wound histopathology in this study from 30 samples showed that the highest average collagen density was in group EESL40 compared to the other groups. The highest numbers of fibroblast cell formations were in group EESL80 compared to other groups. Meanwhile, the lowest inflammation score was in group EESL40 compared to the other groups.

The histopathological description of the extent of the wound for collagen density, fibroblast cells and inflammation is shown in Figures 1-3.

Table 3: Results of Relationship Analysis of Differences Between Groups in Activity of Serum TNF-α Levels in Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth).

Group	Mean±SD	*p*
Normal Group	141.54±20.25	
Negative Group	213.17±32.06	
Positive Group	172.24±14.24	<0.001*
Group I	163.39±14.47	
Group II	176.39±28.16	

*Anova Test (Significant<0.05).

DISCUSSION

Diabetic foot ulcers are one of the most common complications in patients with diabetes mellitus who are not well controlled. This is usually caused by poor glycemic control, underlying neuropathy, peripheral vascular disease, or poor foot care. This disease is also one of the common causes of foot osteomyelitis and lower extremity amputation. These ulcers usually occur in areas of the foot that experience repeated trauma and pressure sensations.[22]

Wound healing is a complex and dynamic process of restoring cellular structure and tissue layers.[23] Healing of diabetic ulcers can be impaired due to four factors, namely persistent hyperglycemia, proinflammatory environment, peripheral arterial disease and peripheral neuropathy, as well as impaired neovascularization.[24] Wound healing requires control of infection, repair of inflammation, regeneration of the connective tissue matrix, angiogenesis or vasculogenesis, wound contraction and reepithelialization.[25,26]

Treatment is carried out in treating cases of diabetic ulcers in several ways. First, surgery is performed to remove pus and reduce tissue necrosis. Second, use antibiotics to eradicate bacteria such as *Staphylococcus* or *Streptococcus*. Third, how to care for wounds properly and correctly to help speed up the wound healing process.[27,28] Of the three treatments, the use of antibiotics has weaknesses, this is due to the possibility of bacterial resistance to antibiotics which will have an impact on morbidity and mortality rates as well as socio-economic conditions.[29,30]

Table 4: Results of Relationship Analysis of Differences Between Groups in Activity of Serum VEGF Levels in Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* L. Kunth).

VEGF	Mean±SD	*p*
Normal Group	297.69±44.95	
Negative Group	302.73±63.19	
Positive Group	300.21±56.54	0.801*
Group I	275.45±45.40	
Group II	307.68±12.47	

*Anova Test (Significant<0.05).

Table 5: Results of Histopathological Analysis of Wounds of Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Seruhan Leaves (*Peperomia pellucida* L. Kunth).

Group	Collagen Density (x̄)	Fibroblast Cells (x̄)	Inflammation (x̄)
Normal Group	0.33	117.00	3
Negative Group	0.33	89.67	3
Positive Group	0.67	89.67	3
Group I	4.00	3.67	0
Group II	1.67	141	1.33

Phytochemical Screening

Results of Ethanol Extract of Suruhan Leaves

This research carried out a phytochemical screening examination of the ethanol extract of suruhan leaves, secondary metabolites were obtained such as: flavonoids, alkaloids, saponins, tannins and phenols (Table 2). This means that suruhan leaves have potential as an antioxidant and anti-inflammatory. The part of the wort plant most often used in medicine is the leaves. This is the same as research by Ashibna SF and Nurkholidah (2023), showing that the ethanol extract of suruhan leaves contains flavonoid, alkaloid, phenolic and terpenoid compounds.[31] Based on the research results of Ibrahim (2020), phytochemical tests of suruhan leaves (*Peperomia pellucida* L. Kunth) show that suruhan leaves contain alkaloids, flavonoids, tannins and saponins.[32]

Results of Analysis of Differences Between and Within Activity Groups in Serum TNF-α Levels of Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Seruhan Leaves (*Peperomia pellucida* L. Kunth)

The pathological condition (Negative Group) shows a significant increase in TNF-α level (213.17±32.06) compared to normal conditions (141.54±20.25), with higher data variability indicating disease condition instability. Treatment administration in the Positive Group successfully reduced TNF-α level (172.24±14.24) with lower variability, demonstrating consistent treatment effectiveness. Group I shows the most promising results with the lowest TNF-α levels among treatment groups (163.39±14.47) and controlled variability, approaching normal conditions. Group II shows more moderate effects (176.39±28.16) with higher variability, indicating less consistent responses. The results of this study showed a significant relationship between groups (p<0.001) (Table 3). These results indicate that the ethanol extract of suruhan leaves can reduce blood levels of TNF-α in the blood of male white Wistar rats with a model of diabetic ulcers. The flavonoid content in suruhan leaves has anti-inflammatory potential; this can be seen from the reduction in blood levels of TNF-α in this experiment. Flavonoids can regulate the activity of enzyme expression involved in carbohydrate metabolism, in other words the inflammatory process can be inhibited and inhibit the pro-inflammatory activity of TNF-α, resulting in improvements in pancreatic β cells through cell regeneration and improvement of pancreatic β cell organs.[33] TNF-α is a cytokine known to induce insulin resistance.[34]

The tannin compounds contained in the extract also play a role in wound healing by neutralizing inflammatory proteins and inhibiting hypersecretion of mucosal fluid. Another secondary metabolite, namely saponin, also plays a role in wound healing by increasing tissue epithelialization and stimulating the formation of collagen fibers which play an important role in the wound closure process.[35] Alkaloid compounds in the wound healing process are able to initiate fibroblasts towards the ulcer wound area so that the presence of an increasing number of fibroblasts can speed up wound healing.[36]

Results of Analysis of Differences between Activity Groups in Serum VEGF Levels of Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Seruhan Leaves (*Peperomia pellucida* L. Kunth).

The Negative Group (302.73±63.19) showed slightly elevated VEGF levels compared to the Normal Group (297.69±44.95), with higher variability as indicated by the larger SD, suggesting disease-state induced fluctuations. The Positive Group (300.21±56.54) maintained levels similar to both normal and negative groups, indicating minimal intervention effect on VEGF expression. Group I (275.45±45.40) demonstrated the lowest VEGF levels among all groups, suggesting a potential regulatory effect, though not statistically significant. Group II (307.68±12.47) showed the highest mean value but with the lowest variability (SD), indicating consistent but slightly elevated VEGF levels. These results indicate that there is no difference in the ethanol extract of syringe leaves between treatment groups. However, it can be seen in the Table 4. Although no significant difference was found, the EESL80 group was able to increase VEGF levels compared to the other groups.

One of the parameters for determining diabetic wound healing is microscopic observation of skin tissue, namely the expression of Vascular Endothelial Growth Factor (VEGF). VEGF acts as a potent mitogen of endothelial cells that induces migration and sprouting of endothelial cells for the formation of new blood vessels through regulating several integrin receptors on endothelial cells. The role of VEGF in wound healing is as a stimulator of angiogenesis. Angiogenesis in the wound healing process includes several stages, namely vasodilation, basement membrane degradation, migration, proliferation and maturation of endothelial cells. The growth of capillary blood vessels functions to distribute nutrients and various healing mediators. The wound healing process will be weakened if there is inhibition of angiogenesis.[37]

The flavonoids contained in the ethanol extract of suruhan leaves act as anti-inflammatory and antioxidants. The role of these two compounds influences the inflammatory phase of diabetic wound healing in the form of increasing anti-inflammatory mediators so that it will influence the inflammatory phase by reducing free radicals (ROS) and helping to kill bacteria. Another effect is that macrophages produced during the inflammatory phase and proliferation phase will also increase, especially M2 which secrete VEGF. VEGF induces angiogenesis which plays a role in diabetic wound healing.[38] This plant accelerates the production of cell and tissue growth factors, induces fibroblast proliferation and increases wound oxygenation, thereby accelerating capillary circulation and the wound healing process due to the antioxidant and antimicrobial effects of phytochemicals such as flavonoids and glycosides.[39]

Results of Histopathological Analysis of Wounds of Male Wistar White Rats (*Rattus norvegicus*) Diabetic Ulcer Model after Administration of Ethanol Extract of Seruhan Leaves (*Peperomia pellucida* L. Kunth).

The results of histopathological research on wounds in diabetic ulcer model mice showed that group EESL40 (Ethanol Extract of Suruhan Leaves 40 mg/Kgbb) was better in the wound healing process, this was seen in the formation of collagen and a decrease in inflammatory cells (Table 5). In terms of fibroblast cell formation, group II was better than the other treatment groups (Table 5).

The flavonoid compounds contained in surhan leaf extract are thought to have anti-inflammatory properties. Flavonoids inhibit inflammatory processes in two ways. Inhibits capillary permeability and inhibits arachidonic acid thereby reducing prostaglandin production. Apart from flavonoids, the compounds found in Suruhan leaves are tannins, saponins and steroids. Tannin has preservative and antibacterial properties. The saponin content can stimulate collagen formation which plays a role in the wound healing process, besides the steroid content can reduce wound pain as an anti-inflammatory.[40] Wound healing involves a biological wound healing process in which cells are regenerated and tissue damage is repaired.[41] This is a normal reaction because it is a reaction to injury or damage to skin tissue. Wound healing is characterized by closing the wound surface, accelerating epithelialization time, collagen contraction and connective tissue density. The wound healing process has several stages, namely the inflammatory, proliferation and remodeling phases.[42,43]

Alkaloid compounds in the wound healing process can initiate fibroblasts towards the ulcerated wound area, so that increasing the number of fibroblasts will encourage wound healing. In addition, flavonoid compounds also have antioxidant, antibacterial and anti-inflammatory effects on diabetic wounds.[36] The ability of flavonoids as antioxidants is to inhibit lipid peroxidation and protect tissue from oxidative stress thereby increasing wound contraction. The tannin compounds contained in the extract also play a role in wound healing by neutralizing inflammatory proteins and inhibiting hypersecretion of mucosal fluid.[44] Another secondary metabolite, namely saponin, also plays a role in wound healing by increasing tissue epithelialization and stimulating the formation of collagen fibers which play an important role in the wound closure process.[45]

CONCLUSION

The results of the phytochemical screening of the ethanol extract of Suruhan leaves are flavonoids, alkaloids, saponins, tannins and phenols. The ethanol extract of suruhan leaves at a dose of 40 mg/kgBB is better in terms of reducing pro-inflammatory cytokine levels, repairing wound tissue through increasing serum levels of VEGF and forming collagen in the healing process of diabetic ulcers.

ACKNOWLEDGEMENT

The researcher would like to thank all participants who have helped us in this research.

ABBREVIATIONS

DM: Diabetes Mellitus; **TNF-α:** Tumor Necrosis Factor alpha; **IL-1β:** Interleukin-1β; **MMPs:** Matrix metalloproteinases; **STZ:** Streptozotocin; **VEGF:** Vascular Endothelial Growth Factor; **ROS:** Reactive oxygen species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

All experimental procedures in this study were carried out in accordance with the Institutional Animal Care guidelines of the Animal House of the Faculty of Medicine, Methodist University of Indonesia and approved by the Ethics Committee of the Faculty of Medicine, Methodist University of Indonesia (code of ethics: No. 05/KEPK-FKUMI/EC/2024).

SUMMARY

This study explored the healing potential of *Peperomia pellucida* L. Kunth leaf extract on diabetic ulcers, a common complication in diabetes patients. Using male Wistar rats as models, researchers investigated how the extract affects key wound healing factors. The extract, rich in compounds like flavonoids and alkaloids, showed promising results. It significantly reduced levels of TNF-α, an inflammatory marker, suggesting anti-inflammatory properties. While the extract didn't significantly alter VEGF levels, which are important for blood vessel formation, it did improve overall wound healing as observed through histopathological analysis. Notably, a dose of 40 mg/kgBW emerged as the most effective, demonstrating superior collagen formation and reduced inflammation in wound tissues. These findings indicate that *Peperomia pellucida* L. Kunth extract could be a valuable natural remedy for enhancing diabetic wound healing, potentially offering a new avenue in the management of diabetic ulcers. This research not only contributes to our understanding of natural product applications in wound care but also opens doors for further studies and possible clinical applications in treating diabetic wounds.

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