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The Effect of Chayote (*Sechium Edule*) Extract Cream Can Reduce Matrix Metalloproteinase-2 (MMP-2) Levels and Increase Collagen Density in The Wound Healing Process of Male Wistar Rats (*Rattus Novergicus*) With Diabetes Mellitus

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ABSTRACT

Introduction: The main complication in Diabetes Mellitus patients is delayed wound healing resulting in chronic wounds. Chayote is known as a vegetable, which has anti-inflammatory properties. The tannin and saponin compounds in chayote produce antibacterial agents. This study aims to prove the role of chayote extract cream on the wound healing process of diabetic rats through MMP-2 markers and collagen density. ***Methods:*** This study was an experimental post-test only control group design using male rats. Samples were induced hyperglycaemia with 46 mg of nicotinamide and 10 mg of streptozotocin one week before the experiment. The sample was divided into 2 groups: the placebo group (P0) and the treatment group (P1) with a total of 11 samples per group. The treatment group received cream of chayote extract 60% for 1 week. On the 8th day, both groups were taken by punch biopsy to assess collagen density, then examined for MMP-2 levels in the blood. ***Results:*** The average MMP-2 level in the control group was 4.97 ± 0.36 ng/ml, while the average MMP-2 in the treatment group was 4.59 ± 0.42 ng/ml. The average collagen level in the control group was 37.08 ± 12.92 %/ body surface, while the mean collagen in the treatment group was 54.21 ± 15.76 %/ body surface. ***Conclusion:*** Administration of ethanol extract of chayote (*Sechium edule*) significantly reduced matrix metalloproteinase-2 (MMP-2) levels and increased collagen density in the wound healing process of rats.

Keywords: chayote; MMP-2; collagen; diabetes mellitus

INTRODUCTION

Diabetes Mellitus (DM) and its complications are the leading cause of death in most countries. The main complication in DM patients is delayed wound healing resulting in chronic wounds. These complications can include damage to small and large blood vessels and nerves that cause loss of vision and kidney function, heart attacks, strokes, and amputations of the lower limbs, as well as causing disability and mortality. [1,2]

The wound healing process in diabetes is primarily characterized by chronic inflammatory conditions, impaired angiogenic processes, decreased endothelial cells, and extracellular matrix imbalance. Higher Matrix metalloproteinase (MMP) levels have been reported in diabetic wounds because high glucose levels can directly induce MMP production and reduction of TIMPs thereby contributing to impaired healing processes.

Matrix metalloproteinases are involved in various stages of wound healing, such as cell migration through ECM degradation, leukocyte invasion, processing of several cytokines and growth factors involved in the healing process.[3] Matrix metalloproteinase-2 co-localizes with keratinocytes, fibroblasts and macrophages in the dermis, sweat glands, hair follicles, and sinus passages. Decreased expression of MMP-2 can restore collagen types I and III which results in accelerated wound healing.[4]

Medicinal plants have a lot of benefit because of their antioxidant ingredient such as tannins, alkaloids, flavonoids, and saponins they contain.[5] Chayote is known as a vegetable, which has anti-inflammatory properties. The tannin and saponin compounds in chayote produce antibacterial agents. Saponins and tannins are components of complex organic substances known as secondary metabolites that produce antimicrobial and antibacterial activity.[5]

This study aims to prove the role of chayote extract cream on the wound healing process of diabetic rats through MMP-2 markers and collagen density.

METHOD

This study was an experimental post-test only control group design using male rats, aged 10-15 weeks, with a body weight of 200-250 grams.

Prior to the study, the samples were induced hyperglycemia with 46 mg of nicotinamide and 10 mg of streptozotocin one week before the experiment. The sample was divided into 2 groups: the placebo group (P0) and the treatment group (P1) with a total of 11 samples per group. The treatment group received cream of chayote extract 60% for 1 week. On the 8th day, both groups were taken by punch biopsy to assess collagen density, then examined for MMP-2 levels in the blood. Data is recorded and analyzed. This study has cleared the ethical clearance with number B/37/UN14.2.9/PT.01.04/2023.

RESULTS

The average MMP-2 level in the control group was 4.97 ± 0.36 ng/ml, while the average MMP-2 in the treatment group was 4.59 ± 0.42 ng/ml. The average collagen level in the control group was 37.08 ± 12.92 %/ body surface, while the mean collagen in the treatment group was 54.21 ± 15.76 %/ body surface. MMP-2 and collagen data were tested for normality using the Shapiro-Wilk test. The results showed that the MMP-2 and collagen data were normally distributed (p>0.05). The results of the analysis are presented in Table 1. Treatment effect analysis was tested based on the average MMP-2 between the control and treatment groups. The results of the significance analysis with the independent t-test are presented in Figure 1.

TABLE 1: Descriptive Analysis of MMP-2 and Collagen Variables.

Variables	Group	n	Mean	SD	Min	Max
MMP-2 (ng/ml)	Control	11	4,97	0,36	4,49	5,68
	Treatment	11	4,59	0,42	3,96	5,20
Collagen density (%/body surface)	Control	11	37,08	12,92	13,20	59,10
	Treatment	11	54,21	15,76	29,40	71,30

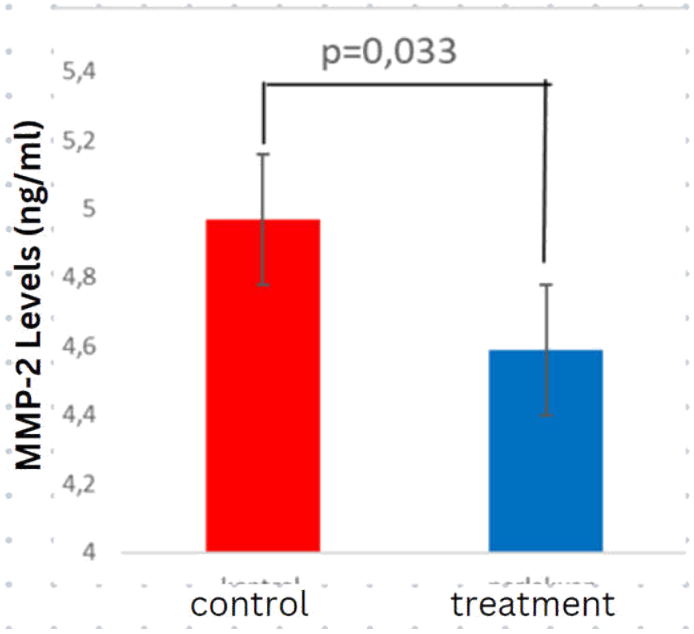

FIGURE 1: Comparison of MMP-2 levels between control and treatment groups.

Analysis of significance using the t-independent test showed that the amount of collagen was higher in the treatment group than the control group with p = 0.011.

The results of the significance analysis with the independent t-test are presented in Figure 2.

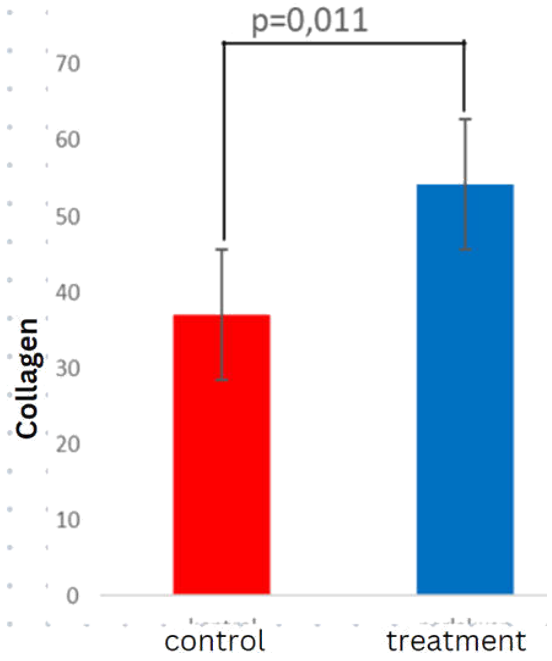

FIGURE 2: Comparison of Collagen between control and treatment groups.

On histological examination, the results showed an increase in the percentage of collagen with picosirius red staining and 400x magnification.

The results showed that the control group showed that the collagen fibers looked thin, irregular and had lots of empty gaps, while in the treatment group, the collagen fibers looked denser, regular and thicker Figure 3.

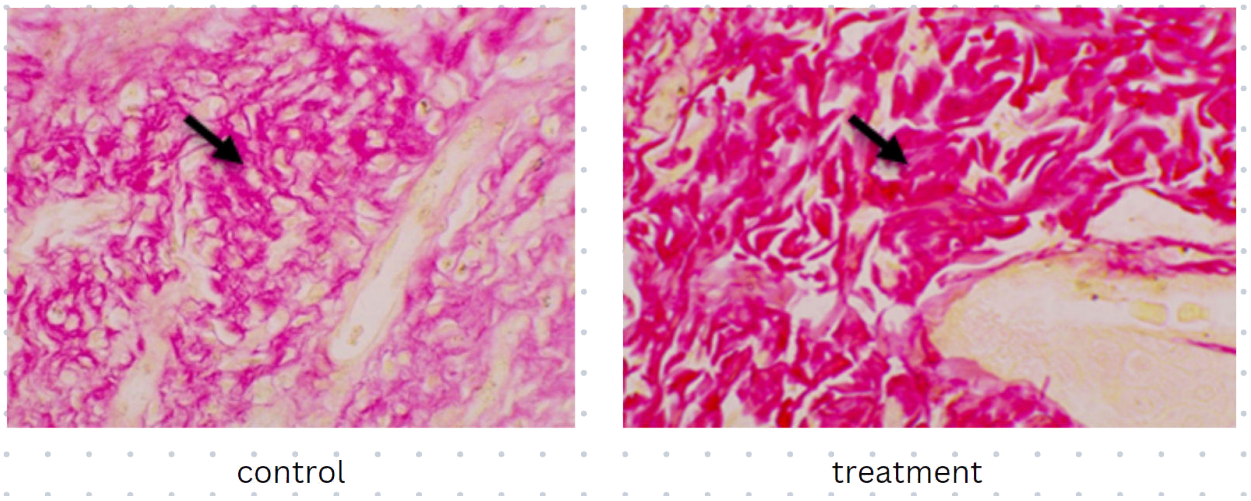

FIGURE 3: Histology of collagen density with Picrosirius red staining seen with the 400x magnification Opti lab camera shows that the collagen fibers in the treatment group are thicker, red, and wider.

DISCUSSION

The study was divided into 2 groups: the control group which was given a placebo-based cream, and the treatment group which was given 60% chayote extract cream, each of which was given a spread of 2x1 a day for 7 days. The average result of MMP-2 in the control group was 4.97 ± 0.36 ng/ml, while in the treatment group it was found to be an average of 4.59 ± 0.42 ng/ml, this indicates that there was an inhibition of MMP-2 levels in the control group. against the treatment group.

This is presumably because the active substance of pumpin contains flavonoid compounds which function as antioxidants and anti-inflammatories.

Flavonoids are able to inhibit the increase in MMP-2 levels thereby increasing the rate and amount of collagen synthesized by fibroblasts which is needed for wound healing. This shortens the inflammatory and proliferative phases so that wounds can heal more quickly. [6,7]

In the process of wound healing, flavonoids act as anti-inflammatories through inhibition of cyclooxygenase and lipooxygenase, causing a limitation in the number of inflammatory cells that migrate to injured tissue, resulting in a shorter inflammatory phase.[8] (Dewantari et al., 2015). The role of flavonoids as antioxidants through inhibition of TNF-α can reduce MMP-2 which results in a decrease in ROS in wounds thereby accelerating wound healing.[9]

In addition, the active ingredient naringenin in chayote acts intracellularly by reducing the expression of the miR-17-3p microRNA which functions to inhibit the expression of the SOD, GPx and CAT genes, which causes a decrease in OxS.[10]

The tannin content in chayote acts as an antioxidant, proangiogenesis, and can increase oxygen supply and nourish injured skin. Tannins are also known as strong antioxidants, even tannins have the ability to chelate metal ions, slow down oxidation, and inhibit lipid peroxidation through inhibition of cyclooxygenase activity.[11]

Tannins have cellular mechanism activities, namely cleaning free radicals and reactive oxygen, increasing wound healing, and increasing capillary blood vessel formation and fibroblast activation.[12] Tannin compounds attack microorganisms by damaging the bacterial cell membrane and shrinking the bacterial cell walls in the wound so that it will interfere with the permeability of the bacterial cells which causes bacterial cell growth to be delayed and even die.[13]

During wound healing, MMPs are secreted by different cell types such as keratinocytes, fibroblasts, and inflammatory cells at different stages and locations. In this process, various transcription factors regulate the expression of proteases and cytokines.[10]

The contribution of MMPs to inflammation is characterized by the activation of cytokine-mediated proinflammatory pathways and also the degradation products of MMPs induce infiltration of immune cells. TGF-β has been shown to upregulate MMP-2. Matrix metalloproteinase-2 activates a signaling pathway that induces ROS formation. The increase in ROS production induced by MMP-2 is dependent on EGFR activation. MMP-2-induced HB-EGF-mediated EGFR is inhibited by EGFR kinase inhibitors.[14]

The average yield of collagen in the control group was 37.08 ± 12.92 %/body surface, while in the treatment group the average was 54.21 ± 15.76 %/body surface. This shows that there was an increase in collagen in the treatment group compared to the control group. This is consistent with the results of histopathological examination using Picrosirus red staining where there was an increase in collagen density in the treatment group which was marked by redder, thicker, and wider colored collagen fibers compared to the control group.

The increase in collagen in the wound healing process is due to the content of active substances in chayote, flavonoids which function to increase collagen synthesis, besides that flavonoids also work to activate T cells, differentiate and proliferate into TH1, TH2, and TH3. TH3 cells will produce Transforming Growth Factor-β (TFG-β) which can stimulate fibroblast proliferation.[15]

In this study, drug administration was used topically. To obtain a more precise topical penetration of the drug, it is necessary to carry out further research related to these topical preparations. Are cream, gel or ointment dosage forms more effective for the wound healing process, especially in DM conditions. The use of herbal-based alternative therapies is one of the therapies of choice in the wound healing process, it is hoped that it can be used at the first stage in the family. Direct application to humans needs to be re-tested whether there are significant results like in experimental animals.

CONCLUSION

Administration of ethanol extract of chayote (*Sechium edule*) significantly reduced matrix metalloproteinase-2 (MMP-2) levels and increased collagen density in the wound healing process of rats.

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