EFFECT OF PSIDIUM GUAJAVA LYNN
ON MMP-9 AND TIMP-1 RATIO
OF PATIENTS WITH DIABETIC ULCER

Andina Setyawati

BADAN PENELITIAN DAN PENGEMBANGAN KESEHATAN
JAKARTA
dan
PROGRAM STUDI ILMU KEPERAWATAN FAKULTAS KEDOKTERAN
UNIVERSITAS HASANUDDIN
2015
Effect of Psidium Guajava Lynn on MMP-9 and TIMP-1 Ratio of Patients With Diabetic Ulcer

Andina Setyawati¹, Nuurhidayat Jafar², Andi Wahyuni³

¹ Lecture of Nursing Science Program, Medical Surgical Department, Hasanuddin University
² Lecture of Nursing Science Program, Community Department, Hasanuddin University
³ Andi Wahyuni, Lecture of Public Health Science, Hasanuddin University

Email : salsabila02@gmail.com

Abstract

Diabetic ulcer is a main health problem, which was found in 15% of 200 million Diabetes Mellitus patients worldwide and will increase 2.5% annually. Ulcer can become infection’s port d’entry, disrupt peripher blood flow progressively and can cause delayed healing. Hence, it needs innovation on each phase of wound healing, including herbal medicine such as Psidium Guajava Lynn. This study aims to determine the effect of Psidium Guajava Lynn on MMP-9 and TIMP-1 ratio of patients with Diabetic ulcer. This was a quasy experiment study with pre and post-test design with controlled group. Subjects were accidentally selected in this study. MMP-9 and TIMP-1 was evaluated on first visit and baseline laboratories were taken by ELISA. Each subject in the group of intervention took psidium guajava lynn to wash the ulcer each three days. After 10 times, repeat laboratory examinations were taken. No adverse were reported during the trial. The data were analyzed using paired T-test with significance level of $\alpha \leq 0.05$. This study found a significant on decreasing ratio on intervention group and increasing ratio on control group ($p=0.000$). This study found significant different of psidium guajava lynn on changing the ratio of MMP-9 and TIMP-1 ($p=0.035$). This study concludes that psidium guajava lynn could decrease MMP-9 and TIMP-1 ratio for diabetic ulcer patients.

Key words: Psidium Guajava Lynn, MMP-9 and TIMP-1 ratio, diabetic ulcer
BACKGROUND
Diabetic ulcer has become a financial problem in the health care system with expenses approximately 4500 USD per patient besides the psychosocial issues which must be borne by the patient as a result of a decrease in quality of life. Ulcers can be infection’s port d’entry and can cause progressive tissue damage and also delayed healing. Therefore, innovation needs to be done to wound healing time in every phase to prevent amputation (Wu et al., 2007).

Diabetic ulcer’s basic healing include adequate perfusion, debridement, infection control and epitelization management (Wu et al., 2007). Epitelization management can be done by proteolitic control. Proteolitic control helpful to cell migration, angiogenesis and tissue remodeling during wound healing phase (Li et al., 2013). Proteinase plays an important role on cell migration and tissue remodeling, through various biological process. One of the proteinase is matrix metalloproteinases (MMPs) which is a family of zinc-containing endoproteinase (Budiarta, 2007). MMPs especially MMP-9 or gellatinase-B naturally present in all healing phases and influence the wound healing by degrading extracellular matrix (Kilpadi et al., 2006). Tissue Inhibitor Metallo proteinases (TIMP) especially TIMP-1 produced by mRNA at the tissue beneath the wound in response to wound contraction and serves to inhibit MMP-9’s activation with its ability to bind to zinc-binding site in active MMP-9’s catalytic domain (Budiarta, 2007). A high ratio of MMP-9 and TIMP-1 describes pathologic condition of chronic wound, so it can be predictor on diabetic ulcer wound healing (Kilpadi et al., 2006).

It has now expanded various modalities of wound care which is currently recommended, but not an option because it is a commercial treatment (Liu et al., 2009).

According to Patil et al (2008), psidium guajava lynn is known containing flavonoid which has effect of decreasing MMP-9 and TIMP-1 ratio by binding zinc thus inhibiting MMP-9 activation and result on angiogenesis supression. Laboratory test results of quality testing and food safety in 2014, water boiled filter of guajava Lynn contain 90,04% water, 8,95% quercetin, 0,84% essential oil, 0,08% fatty oils, 0,05% psidoklat acid, and 1,78% tannin. The toxicity test by Traul et al (2008), water boiled filter of Psidium guajava lynn essentially non-toxic in some animals, not irritant to eyes or skin tissue, and do not have capacity to inflict hypersititvity. The use of Psidium guajava lynn as a topical on human chronic wounds grading up 10,98% was confirmed on some clinical researches.

METHODOLOGY
This was experimental study, with pre test and post test data collections. The populations was all patients with diabetic ulcer registered or ever registered in enterostoma nursing centre (ETN centre), public health care and wound care clinic in Makassar area. Sample was taken by accidental sampling. Sampling allocation was used to divide samples on experiment and control group. Number of experiment sample was 15 and control samples were 14. The inclusion criteria were: 1) granulation phase of wound, 2) 1 or 2 Wagner criteria of wound grading, 3) long-suffering of diabetic less than 10 years. The exclusion criteria were: 1) had been treated with psidium guajava lynn, 2) had undergone major surgery. Dropped out criteria were: 1)patients withdrew from this study, 2) patients
who undergo surgery during this study, and 3) patients with infection and discomfort during this study.

Wound care on experiment group is done by soaking the wound with Psidium Guajava Lynn and Normal Salin combination (PGL-NS) and closing the wound with gauze’s moistened by PGL-NS as primary dressing. While wound care on control group is done in standard way, that is soaking the wound with NS and closing the wound with gauze’s moistened by NS as primary dressing. Secondary dressing in both group using a dry gauze. Wound care is done every 3 days for 10 times.

Blood serum was taken twice, in order to examine MMP-9 and TIMP-1 proteinases, a day before first wound care and a day after last wound care, by the method of enzyme linked immunoassay (ELISA). Blood glucose, nutritional intake and wound scoring were evaluated in every wound care visit.

**RESULT**

The majority of subjects were elderly (>60 years old), that was 10 on experiment group (66.7%) and 8 on control group (57.1%). The majority of subjects were female, that was 9 on experiment group (60%) and 8 on control group (57.1%). All respondents (100%) having wound grade of 2 on both groups. Most of the subjects had high blood (> 200 mgs/dl), that was 8 on experiment group (53.3%) and 10 on control group (71.4%). After 10 times of wound care, almost all subjects experiencing regeneration, that was 15 on experiment group (100%) and 10 on control group (92.9%). Most of experiment subject did not use insulin, that was 7 (46.7%), while half of control subjects used insulin, that was 7 (50%).

Most subjects of both groups having a nutritional intake of less than American Diabetes Association (ADA) recommendation by the year 2010 specifically targeted to chronic wound healing (<20kcal/kg), that was 10 subjects on experiment group (66.7%) and also 10 on control group (71.4%).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Paired T-Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 and TIMP-1 Ratio</td>
<td>Group</td>
</tr>
<tr>
<td>Experiment</td>
<td>Pre</td>
</tr>
<tr>
<td>Control</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td>Post</td>
</tr>
</tbody>
</table>

Table 1 shows that MMP-9 and TIMP-1 ratio in experiment group was decrease at average value decline of 4.81, with 2.53 standar deviation. Paired T-Test shows that there was a significance decrease of MMP-9 and TIMP-1 ratio after wound care in experiment group (p=0.000), while there was a significance increase in control group (p=0.000).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Independent T-Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Different of MMP-9 dan TIMP-1 Ratio</td>
<td>Group</td>
</tr>
<tr>
<td>Experiment</td>
<td>4.81</td>
</tr>
<tr>
<td>Control</td>
<td>-3.09</td>
</tr>
</tbody>
</table>

Table 2 shows that MMP-9 dan TIMP-1 ratio mean in experiment group was 4.81 positive, while negative 3.09 in control group. Mean different both group was 1.72 with 0.77 error standard. Independent T-Test result with $\alpha=0.05$.
concluded that there was significant different of MMP-9 and TIMP-1 ratio mean after intervention on experiment and control group (p=0.000). The final conclusion is there was effect of PGL on MMP-9 and TIMP ratio of diabetic ulcer patients.

**DISCUSSION**

Matrix Metalloproteinases (MMPs) especially MMP-9 has an important contribution to wound healing process, because of its function to degrade extracellular component. MMP-9 will tend to increase and TIMP-1 as specific MMP-9 inhibitor will tend to decrease on chronic diabetic ulcer. MMP-9 and TIMP-1 ratio above 0.39 will disturb microbiology balance on each phase of wound healing (Muller et al, 2007). The theory proved in this research, that mean of MMP-9 and TIMP-1 ratio before intervention increased in both group, that was 9.08 on experiment group and 4.96 on control group. On good wound healing, MMP-9 secreted by inflammation cell diminishes rapidly as much as 82% on the first 4 weeks, while on delayed wound healing, MMP-9 diminishes less than 82% (Muller et al, 2007). Muller’s study shows that there was no significant different of MMP-9 on good healing and delayed healing in the first week, but MMP-9 increased in the fourth week on delayed healing. This study used PGL-NS combination as fluid washer and as primay dressing. This intervention was done 10 times, each 3 days during 28 days. Specified time in this study referred to infection prevention and control (IPC) of Indonesia that the standard of wound care is every 3 days in order to prevent infection or infection development. Each wound care visits, blood glucose and wound scoring were measured and nutrition intake was documented. Blood glucose was measured with automatic set of measuring blood sugar *Easy Touch* brands. Wound scoring was measured with *Bates Jensen* method by evaluating 12 items of wound development consisting size, depth, edges, and undermining of the wound, also necrotic tissue type and amount, exudate type and amount, skin colour surrounding wound, peripheral tissue edema and induration, the amount of granulation tissue and epithelization. Nutrition intake measured by dietary daily record documented by subject and researcher guide as follow. Dropped out subjects were not included in the final analysis.

This study shows there was significant different of MMP-9 and TIMP-ratio before and after treatment on both group (p=0.000). However, the experiment group shows positive effect and control group shows negative effect. This study shows that MMP-9 and TIMP-1 ratio is not in accordance with Bates-Jensen wound scoring. There were 100% subject of experiment group experiencing wound regeneration and also almost 100% of control group. According to study of Chuan-Yang et al (2009) about wound healing dynamics of 21 diabetic rats and 21 non diabetic rats, there was conclude that wound healing on non diabetic rat faster than those diabetic rats 6 weeks after injury, microbiology measured by RT-PCR to identify mRNA of MMP-9 and TIMP-1 and also ELISA to identify MMP-9’s and TIMP-1’s protein through skin tissue biopsy. Its result shows that mRNA MMP-9’s protein and mRNA is higher in diabetic rats (p<0.05), and mRNA TIMP-1’s protein and mRNA was lower in diabetic rats. MMPs expressed temporary in skin tissue, but expressed permanently in blood, with various quantity in diabetic patients based on exogenous factor signals, cytokine,
growth factor, cellular matrix interaction, histology changes and cell function. Therefore, diabetic patients are more vulnerable injured and experiencing delayed wound healing. Study of Davis et al (2006) shows that mRNA level of MMP-9 in diabetic pastients are higher than non diabetic, even before tissue injury, while mRNA level of TIMP-1 in diabetic patients are lower. The decreasing of TIMP-1 in diabetic ulcer will cause disorganized connective tissue and the distance between the collagen is widening. Adequacy inflammation contribute to wound healing process, so that the cytokine accumulation, that was TNF-α and IL-1β, which would stimulate macrofag and fibroblast to secrete MMP-9 and inhibit TIMP-1 production. The unbalance of MMP-9 and TIMP-1 ratio will cause excessive extracellular matrix degradation and ends on delayed healing after 3 days injured, so that they need therapy considering component ability to to decrease or push MMP-9 and TIMP-1 ratio. It is also recommended by Rosch et al (2014), on their study that the increasing of MMP-9 and decreasing of TIMP-1 will make excessive proteolitic environment, so it will inhibit diabetic wound healing. According to their study, They wrote that therapy which aims to decrease MMP-9 and increase TIMP-1 will really help the healing. Study using these PGL-NS combination, there was a significant decrease of MMP-9 and TIMP-1 ratio in experiment group, otherwise there was a significant increase of MMP-9 and TIMP-1 in control group. Based on the theory underlying the treatment, PGL could be expected to inhibit MMP-9 production through its flavonoid component, quercetin, by binding pro-MMP-9’s zinc. PGL consists of essential oil of quercetin component, that was 18.81% caryoppylene, 11.80% copaene, 10.27% Azulene and 7.36% Eucalyptol. Quercetin is also called avicularin, 3-L-4-4-arabino-furanosid, beside being anti-inflammatory, also well in antibacterial (Begum et al, 2012). This theory proved in this study that there was significant decrease of MMP-9 and TIMP-1 ratio in experiment group. Muruganandan et al (2010) proved that either young or old PGL have an effect as anti-inflammatory with 58.27% inhibition on topical therapy. Ticzon (2011) proved that young and fresh PGL have an effect as antispasmodic on oral therapy. Water boiled PGL has also gone through study of Suzuki et al (2010) in atopic dermatitis subjects, this water consists of antialergy activity 4-8 weeks after treatment. PGL has a role in imperfecting wound healing proliferative phase, and MMPs have many roles inside, especially MMP-9 which degrades denaturized collagen, called gelatin. MMP-9 trashes the collagen and other partially denaturized extracellular matrix during injury. This was very important because collagen should specifically interact to form imperfect fibril. Partially degraded collagen would not perfectly interact with new collagen molecule forming during proliferation phase, so that the tissue wall formed fragile (Wadood et al, 2015). MMP-9 and TIMP-1 disregulation in diabetic patients will develop to cardiovascular disease if not treated, and also will cause delayed healing. Study of Wadood et al (2015) in 24 diabetic patients without dyslipidemia experienced diabetic less than 5 years, 30 diabetic patients with dyslipidemia experienced diabetic more than 10 years and 26 healthy persons, proved that significant increase of MMP-9 is one of risk factors of cardiovascular
complication in diabetic patients with
dyslipidemi experienced diabetic less
than 5 years and more than 10 years
compared to healthy persons.
One subject of experiment group who is
experiencing sinister pedis wound found
being hypergranulated. Regarding to
subject’s statement that she is too much
walking so the dressing opened and the
wound scarred. This was stated by Vuolo
(2013) that factor triggering
hypergranulation is recurrent trauma and
infection, allergy reaction, foreign object
irritation or occlusive dressing. Physical
examination of this subject shows there
was no pus, tenderness, bed smelling and
other infection manifestations, and there
was found no foreign subject in ulcers,
but there was scarred wound bleeding.
This subject was observed that she wears
strict shock daily. This occlusive
dressing can cause hypergranulasi trough
2 mechanisms, exudate accumulation
beneath the dressing which can cause
tissue edema and cytotoxic effect of
occlusion (Rheinecker, 2013).
Hypergranulation also found in control
group, regarding the observation that the
wound was already open before caring
and closed by rigid towel. These can be
one of the risk factor of the appearance
of new wound. These subject also
observed of red exudate on the sixth
treatment.
This study concluded that soaking the
wound and dressing the wound with
PGL-NS combination could decrease
MMP-9 and TIMP-1 ratio. But, there
was many factors influence its healing,
especially subject’s characteristic, that
should be communicate to be controlled.
Mendes dan Neves (2012) write that sever ulcers need multidiscipline
approach, because the treatment should
be aligned among medication, nutrition
and physiotherapy so that result in
optimal wound healing.

CONCLUSION
Increasing ratio of MMP-9 and TIMP-1
as predictor factor of worsening injury
was decreased by soaking and dressing
wound with PGL-NS combination.
Further research is needed to create
innovation of various PGL preparation
as topical therapy and primary
dressing.

REFERENSI
1. Begum, S., Hasan.I., & Siddiqui,
B.S.(December, 2012). Two new
triterpenoids from the fresh leaves of
Psium Guajava. Planta Med,
68(12) : 1149-52.
2. Chuan-Yang., Zhu, Ping., Yan, Li.,
et al. (2009). Dynamic changes of
matrix metalloproteinase-9 and tissue
inhibitor metalloproteinase-1
during wound healing in diabetic
rats. JAMA, 293:217-228
3. Davis. (2006). Moleculare balance of
capillary tube formation versus
regression in wound repair : role of
matrix metalloproteinaises and their
inhibitor. J Investig Dermatol Symp
Proc, 11 : 44-56.
4. Kilpadi, D.V., Stechmiller, J.K.,
Composition of wound fluid from
pressure ulcers treated with negative
pressure wound therapy using
V.A.C. therapy in home health or
extended care patients: a pilot study.
Wounds, 18(5) : 119-126.
http://www.medscape.com/viewarticl
e/533859_4
5. Li, Z., Guo,S., Yao, F, et al. (July-
August 2013). Increased ratio of
serum matrix metalloproteinase-9
against TIMP-1 predicts poor wound
healing in diabetic foot ulcers. J
Diabetes Complications, 27(4) : 380-
2. DOI : 23357650
6. Li, Z., Guo,S., Yao, F, et al. (July-
August 2013). Patterns of matrix
metalloproteinase and TIMP


http://www.care.diabetesjournals.org