



THE ROLE OF MORINGA LEAF EXTRACT (*Moringa oleifera*) ON INTERLEUKIN-10 LEVELS IN CHRONIC INFLAMMATION OF THE DERMIS OF WHITE WISTAR RATS (*Rattus norvegicus*)

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Abstract

Traumatic injury can cause chronic inflammation that lasts several months to years. Interleukin-10 plays an important role in the repair of tissue fibrosis and accelerates wound healing. The active compounds of secondary metabolites in Moringa leaves include flavonoids, saponins, alkaloids, and tannins that can accelerate wound closure and act as anti-inflammatory effect to help vasoconstriction in blood vessels so as to minimize signs of inflammation. The aim of this study was to analyze the role of Moringa leaf extract on Interleukin-10 levels in chronic dermis inflammation. This study used a true experimental research with a post-test only control group design. This experimental procedure using 20 male Wistar rats, aging 2-3 months, weighing 150-200 grams and divided into four groups, a negative control group and experiment group with treatment of Moringa leaf extract at concentration of 5%, 10% and 15%. The extract was administered for 3 days after the creation of chronic inflammation in the dermis. Blood serum were taken after 3 days administration of Moringa leaf extract. Analysis using Kruskal Wallis with 5% of error rate. The results showed $p=0.040$ ($p<0.005$), which means interleukin-10 levels in experiment group with treatment of Moringa leaf extract were significantly increased. The leaf extract of Moringa oleifera had a role in increasing Interleukin-10 levels in chronic dermis inflammation of male white Wistar rats (*Rattus norvegicus*) with the best concentration of 15%.

Keywords: Chronic Dermis inflammation, Moringa oleifera Leaf Extract, Interleukin-10

Abstrak

Cedera traumatis dapat menyebabkan peradangan kronis yang berlangsung berbulan-bulan hingga bertahun-tahun. Interleukin-10 berperan penting dalam perbaikan fibrosis jaringan dan mempercepat penyembuhan luka. Komponen aktif dari metabolit sekunder pada daun kelor termasuk flavonoid, saponin, alkaloid dan tanin yang dapat mempercepat penutupan luka dan berperan sebagai efek anti inflamasi untuk membantu vasokonstriksi pada pembuluh darah untuk meminimalisir inflamasi. Penelitian ini dilakukan untuk menganalisis peran ekstrak daun kelor (*Moringa oleifera*) terhadap kadar interleukin-10 pada radang kronis dermis. Penelitian ini merupakan penelitian true experimental dengan rancangan penelitian post-test only control group design. Sampel penelitian ini adalah 20 ekor tikus putih Wistar (*Rattus norvegicus*), jantan, berusia 2-3 bulan, berat badan 150-200 gram dan dibagi menjadi empat kelompok, kelompok kontrol negatif dan kelompok perlakuan dengan pemberian ekstrak daun kelor konsentrasi 5%, 10% dan 15%. Ekstrak diberikan selama tiga hari setelah pembuatan radang kronis dermis. Sampel serum diambil setelah tiga hari pemberian ekstrak daun kelor. Analisis data menggunakan uji Kruskal-Wallis dengan eror 5%. Hasilnya menunjukkan $p=0,040$ ($p<0,005$), artinya kadar interleukin-10 pada kelompok perlakuan dengan pemberian ekstrak meningkat secara signifikan. Ekstrak daun kelor berperan meningkatkan kadar interleukin-10 pada radang kronis dermis tikus putih Wistar jantan (*Rattus norvegicus*) dengan konsentrasi terbaik adalah 15%.

Kata Kunci: Radang Kronis Dermis, Ekstrak Daun Kelor (*Moringa oleifera*), Interleukin-10



1. PENDAHULUAN

Trauma or injury is still one of the leading reasons for death among populations worldwide. Inflammation is a reaction to infection or injury and involves many mediators (Netea et al., 2017). Acute inflammation can become a chronic condition when cells that respond to the inflammatory environment are unable to eliminate pathogens, as a result, prolonged hypoxia in traumatic injury causes a prolonged inflammatory phase and disruption of the wound healing process that leads to chronic inflammation (Guo and DiPietro, 2010).

Chronic inflammation is referred to as long-term inflammation that lasts from months to years. The American Wound Association, MedMarket Diligence, in 2009 reported that chronic wound inflammation affects 6.5 million wound-related patients. Management of chronic inflammation from wounds National Health Service in the UK confirmed in 2005 the cost of treating patients with chronic inflammation was estimated at US\$3.4-4.6 billion per year representing about 3% of the total estimated expenditure on health over the same period (Järbrink et al. al., 2016). One of the therapies used to reduce chronic inflammation or anti-inflammatory drugs is corticosteroids, but long-term use of corticosteroids can cause impaired wound healing caused by inhibition of collagen synthesis (Crown and Lightman, 2005).

Interleukin-10 is known as an immunosuppressive and anti-inflammatory cytokine that plays an important role in preventing further tissue damage in chronic inflammation by suppressing inflammatory pathways and playing a role in repairing tissue fibrosis and accelerating wound healing (Sziksz et al., 2015; Fard et al., 2015).

Moringa leaf extract (*Moringa oleifera*) contains secondary metabolites that contribute to the wound healing process. The active components of secondary metabolites in Moringa leaves include flavonoids,

saponins, alkaloids and tannins that can accelerate wound closure and act as an anti-inflammatory effect to help vasoconstrict blood vessels to minimize inflammation. Quercetin is the largest flavonoid belonging to the flavonol class which has an antioxidant effect that can prevent the increase of free radicals and also functions as an anti-inflammatory which works by blocking Ik-B kinase and preventing NF- activation and also accelerating epithelialization (Luetragoon et al. , 2020). Anti-bacterial from Moringa leaves has a mechanism of action by damaging bacterial cell membranes, Moringa leaves can be used to inhibit gastric and gastrointestinal ulcers. Moringa leaf extract contains low molecular weight protein which has anti-bacterial and anti-fungal activity (Widowati et al., 2014). Moringa leaves also have active components such as isothiocyanates (ITC) which function as anti-inflammatory, namely Moringa isothiocyanate-1 (MIC-1) (Sailaja et al., 2021).

This study was conducted to analyze the role of Moringa leaf extract (*Moringa oleifera*) on interleukin-10 levels in chronic inflammation of the dermis of male Wistar white rats (*Rattus norvegicus*).

2. METHOD

This research was conducted in August-September 2021 at the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga. This research is a true experimental study with a post-test only control group design. The samples of this study were 20 white Wistar rats (*Rattus norvegicus*), male, 2-3 months old, weighing 150-200 grams which were obtained from the Biochemistry Laboratory, Faculty of Medicine, Airlangga University. Researchers made chronic inflammation of the dermis for 3 days by giving 10% H₂O₂ in rat wounds. This study had 4 groups, namely a negative control group (KN) and a treatment group with a concentration of 5% (P1), 10% (P2) and 15% (P3) Moringa leaf extract, each group consisted of 5 rats. The gel and extract



of Moringa leaves were made by maceration process at the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya. Interleukin-10 levels were checked by ELISA method at the Clinical Pathology Laboratory, Dr. Hospital. Soetomo Surabaya. The data obtained were analyzed using IBM SPSS Statistic version 23. The normality test of the distribution of the research data used the Shapiro-Wilk test and the data analysis test used Kruskal Wallis with 95% confidence level and 5% error. This research proposal has been reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Airlangga University, Surabaya with ethical clearance 403/HRECC.FODM/VII/2021.

Acclimatization was carried out for one week at the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga before the experiment was carried out. Then the rats were randomly divided into 4 groups (KN, P1, P2, and P3) each containing 5 mice. Then the rats were anesthetized for making cuts on the rats' backs and given 10% H₂O₂ 2 times a day for 2-3 minutes for 3 days to create chronic inflammation. After 3 days the rats were chronically inflamed, on day 4,5,6, Moringa leaf extract gel was added topically to each rat treatment group. Then on day 7 all rats were sacrificed for their blood serum. Blood was taken from the heart and stored in a chemical vacutainer tube (yellow or red cap) then centrifuged to obtain the serum for examination of interleukin-10 levels using the ELISA method at the Clinical Pathology Laboratory, Dr. Hospital. Soetomo Surabaya.

3. RESULT

Tabel 1. Deskripsi data kadar interleukin-10

Group	N	IL-10 (pg/ml)	P Value
		Mean ± Standard Deviation	
KN	5	26,600 ± 6,7402	0,040
P1	5	17,540 ± 1,4328	
P2	5	28,980 ± 9,8004	
P3	5	36,480 ± 17,2250	

Based on Table 1. it is known that the highest mean level of interleukin-10 in the P3 group is 36.480 pg/ml, inversely proportional to P1 which has interleukin-10 levels with a low average compared to the KN, P2, and P3 groups, namely 17.540 pg/ml. . In the Kruskal-Wallis test, it was found to be significant below 0.05 (p<0.05), namely p=0.040. The differences in each group can be observed in the graph below:

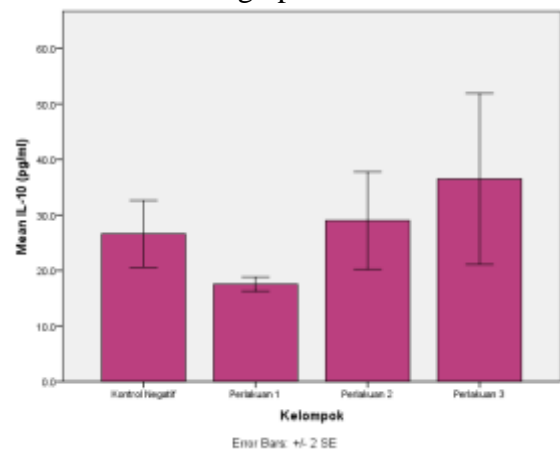


Figure 1. Graph of Interleukin-10 levels in all groups

Tabel 2. Mann-Whitney test results for Interleukin-10 levels

Group		Significance
KN	P1	0,117
	P2	0,602
	P3	0,917
P1	KN	0,117
	P2	0,009*
	P3	0,009*
P2	KN	0,602
	P1	0,009*
	P3	0,530
P3	KN	0,917
	P1	0,009*
	P2	0,530

*Significant value

Based on the table above (P<0.05, it shows a significant difference); found a significant difference between groups P1 and P2 and groups P1 and P3. While not



significant in the KN and P1 groups, KN and P2 groups, KN and P3 groups, and P2 and P3 groups. The significant difference showed that the levels of interleukin-10 were significantly different between treatments. The insignificant difference showed that the levels of interleukin-10 were not significantly different between treatments.

4. DISCUSSION

Moringa leaf extract (*Moringa oleifera*) in this study played a role in increasing interleukin-10 in chronic inflammation of the dermis with the best administration of Moringa leaf extract was 15%. The purpose of giving 10% H₂O₂ to rat wounds is to make the wound area hypoxic. Prolonged hypoxia can disrupt the wound healing process and lead to chronic inflammation (Guo and DiPietro, 2010).

In this study, the level of interleukin-10 in the KN group was higher than that of P1 (5% Moringa leaf extract) because the 5% concentration of Moringa leaf extract had not been able to maximally increase interleukin-10 levels.

Moringa leaves are one of the best natural sources of antioxidants for the treatment of inflammatory diseases. The flavonoids in Moringa leaves have anti-inflammatory properties by reducing levels of pro-inflammatory cytokines such as PGE₂, TNF- α , IL-6, IFN- γ and COX and increasing anti-inflammatory cytokines in particular. interleukin-10 (Carvalho et al., 2021).

Cell necrosis caused by hypoxia releases intracellular contents known as DAMPs. DAMP binds to TLR4 on macrophages and then activates the transcription factor NF- κ B to induce pro-inflammatory cytokines, including IL-1 β , IL-6, IFN- γ , and TNF- α (Julier et al., 2017). Moringa leaf extract functions as an anti-inflammatory which works by blocking I κ B kinase so that I κ B degradation does not occur (p50 and p65) which can prevent NF-activation so that there is no increase in pro-inflammatory levels (Luetrogon et al., 2020)

Research conducted by Masyhur et al. (2011) showed that quercetin (a group of flavonoids) can inhibit the activation of kinase enzymes that can cause phosphorylation reactions of NF- (Masyhur et al., 2011).

Research conducted by Tan et al. (2015) showed that treatment with 80% hydroethanol extract of *Moringa oleifera* flower had a significant effect in inhibiting NO production and expression of inflammatory mediators (NF- κ B, iNOS and COX-2) and pro-inflammatory cytokines (TNF- α , IL-1 β), IL-6 and PGE₂) by blocking the degradation of I κ B- α and defending NF- κ B in the cytoplasm from further activation and increasing the expression of the anti-inflammatory cytokine interleukin-10 (Tan et al., 2015). This is in accordance with the research of Fard et al. (2015) that the bioactive hydroethanolic extract of *Moringa oleifera* leaves induces a significant amount of interleukin-10 as an anti-inflammatory cytokine in a dose-dependent manner that is compatible with the bioactive anti-inflammatory properties of *Moringa oleifera* extract (Fard et al., 2015).

5. CONCLUSIONS AND SUGGESTIONS

Moringa leaf extract plays a role in increasing interleukin-10 levels in chronic inflammation of the dermis of male Wistar white rats (*Rattus norvegicus*) with the best concentration of 15%. Suggestions for further researchers are to add a confirmation test of chronic inflammation in wounds, increase the number of samples and increase the concentration of Moringa leaf extract.

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