ORIGINAL ARTICLE

ANTI-INFLAMMATORY EFFECT OF TAMARILLO PEEL EXTRACT ON IL-6 RAT POST CARRAGEENIN INDUCTION

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ABSTRACT

BACKGROUND
Inflammation is a protective response of the body to injury by releasing pro-inflammatory cytokines; one of them is interleukin-6 (IL-6). The peeled fruit of Tamarillo or terung belanda (*Solanum betaceum Cav*.) ethanol extract contains flavonoid compounds that have anti-inflammatory potential. This research was conducted to evaluate the anti-inflammatory effect of terung belanda (*Solanum betaceum Cav*.) peel fruit ethanol extract on carrageenan-induced Wistar strain rat by measuring the IL-6 level.

METHOD
Tamarillo (*Solanum betaceum Cav.*) fruit peel was extracted by maceration methods using 70% ethanol as solvent. The anti-inflammatory was evaluated using 30 rats which were divided into five research groups positive control (diclofenac sodium), negative control (NaCl), and Tamarillo (*Solanum betaceum Cav*.) fruit peel ethanol extract with a dose of 70mg/kg BW, 140mg/kg BW, and 280mg/kg BW. After the treatment, the rats were injected with carrageenan as an inflammatory inducer. Blood samples were taken at 24, 48 and 72 hours to measure the IL-6 level using the ELISA kit. Statistical analysis used one-way ANOVA with a significant difference as 0.05.

RESULTS
The lowest IL-6 levels (13.853 pg/ml) were found in 24 hours at the group of 140 mg/kg BW while in 48 hours at the 280 mg/kg BW group and 72 hours at the 70 mg/kg BW group. In 24 hours, the 140 mg/kg BW group showed lower IL-6 levels than the positive control with a significant difference (p=0.001).

CONCLUSION
Tamarillo (*Solanum betaceum* Cav.) fruit peel ethanol extract is effective as an anti-inflammatory material, especially for 24 hours with a dose of 140 mg/kg BW.

KEYWORDS: Tamarillo, *Solanum betaceum* Cav., Peel Fruit, Ethanol Extract, Carrageenin, IL-6

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ABSTRAK

Efek Antiinflamasi Ekstrak Kulit Tamarillo: Pemeriksaan Kadar IL-6 Tikus Pasca Induksi Karagenin

LATAR BELAKANG
Inflamasi adalah respon protektif tubuh terhadap cedera dengan pelepasan sitokin pro inflamasi salah satunya interleukin-6 (IL-6). Ekstrak etanol kulit buah Tamarillo atau terung belanda (*Solanum betaceum* Cav.) mengandung senyawa flavonoid yang berpotensi sebagai antiinflamasi. Penelitian ini dilakukan untuk mengevaluasi efek antiinflamasi ekstrak etanol kulit buah terung belanda (*Solanum betaceum* Cav.) pada tikus yang diinduksi karagenin dengan mengukur kadar IL-6.

METODE
Kulit buah terung belanda diekstraksi dengan metode maserasi menggunakan pelarut etanol 70%. Uji antiinflamasi menggunakan 30 ekor tikus yang dibagi menjadi lima kelompok penelitian yaitu kontrol positif (natrium diklofenak), kontrol negatif (NaCl), dan ekstrak etanol kulit buah terung belanda dosis 70mg/kgBB, 140mg/kgBB, dan 280mg/kgBB. Pasca pemberian perlakuan, tikus diinjeksikan karagenin sebagai penginduksi inflamasi, dan dilakukan pengambilan sampel darah pada 24, 48 dan 72 jam pasca induksi untuk pengukuran kadar IL-6 dengan metode *enzyme link immunosorbent assay* (ELISA). Uji statistik menggunakan Anova 1 jalan dengan tingkat kemaknaan ditetapkan sebesar 0,05.

HASIL
Kadar Il-6 terendah yaitu 13.,853 pg/ml, ditemukan 24 jam pasca induksi karagenin pada kelompok ekstrak 140 mg/kgBB, 48 jam pada kelompok 280 mg/kgBB, dan 72 jam pada kelompok 70 mg/kgBB. Pada 24 jam, kelompok 140 mg/kgBB menunjukkan kadar IL-6 lebih rendah dibandingkan kontrol positif dengan perbedaan bermakna (p=0.,001)

KESIMPULAN
Ekstrak etanol kulit buah terung belanda efektif sebagai antiinflamasi terutama pada durasi 24 jam dan dengan konsentrasi 140 mg/kgBB.

KATA KUNCI: *Solanum betaceum* Cav., Kulit Buah, Ekstrak Etanol, Karagenin, IL-6

INTRODUCTION

Inflammation is a form of the body's protective response to injury. The cause of inflammation can be infection, injury, microbes or viruses. Immune reactions in the human body will cause inflammation to protect against wounds and microbial or viral infections.[1] Inflammation is characterized by swelling or oedema, pain, redness, heat, and loss of cell function that can cause discomfort. for patients so that there is a need for treatment.[2] In the process of inflammation, there will be an accumulation of leukocytes and an increase in the synthesis and secretion of pro-inflammatory cytokines.[3,4]

In the acute inflammatory stage, pro-inflammatory cytokines are released, one of which is IL-6. (5) IL-6 has a role in regulating inflammation, immune response, and haematopoiesis.[6] IL-6 is often considered a pro-inflammatory cytokine. But this cytokine also has regenerative activity, functions as a pro-inflammatory cytokine, and as an anti-inflammatory secreted by T cells and macrophages to stimulate the body's immune response during infection.[7,8] There are various types of drugs that can be used to treat inflammation, one of which is drugs. steroid and non-steroidal anti-inflammatory drugs, these two classes of drugs have quite severe side effects such as ulcers, suppression of growth, osteoporosis, can aggravate diabetes mellitus, are susceptible to infection, and muscle weakness.[9] Because there are many side effects of these drugs. anti-inflammatory drugs that are commonly used today, more and more anti-inflammatory ingredients derived from plants are being developed.[10]

There are several anti-inflammatory agents derived from natural ingredients that are reported to inhibit inflammation because they contain phytochemicals such as flavonoid compounds, polyphenols, alkaloids, terpenoids, polysaccharides, anthraquinones, lignans, saponins and peptides.[11] Plants containing

flavonoid compounds have the potential to be used as an anti-inflammatory, and one of the plants containing flavonoid compounds is eggplant (Solanum betaceum Cav.).[12] Based on the Central Bureau of Statistics, in 2011, the production of Tamarillo in Indonesia was 519.481 and in 2013 increased by 545.646 tons.[13]

So far, the fruit of Tamarillo that is widely used is the fruit part as a source of antioxidants, which are produced into jam, syrup and Tamarillo juice, leaving the skin part which ends up as waste. However, if viewed from the consumption aspect, the opportunity to increase Tamarillo production is still huge, and this can be the cause of the increase in Tamarillo peel waste, so it is necessary to do an alternative utilization of Tamarillo peel waste. Tamarillo fruit peel contains flavonoid compounds, phenols, anthocyanins, and beta-carotene.[14,15]

Flavonoids are included in the polyphenol group and have a role as an antioxidant that has anti-inflammatory activity.[16] The ethanol extract of Tamarillo skin contains flavonoid compounds from the quercetin group that can function as antioxidants.[17] Antioxidant compounds in flavonoids can play a role in inhibiting inflammation by the mechanism of action. inhibition of cyclooxygenase enzymes so that the formation of prostaglandins is inhibited, and can also inhibit inflammation by scavenging free radicals that cause tissue damage that will trigger arachidonic acid biosynthesis.[18]

Carrageenin itself is a generic name for a group of gelling and thickening polysaccharides obtained from extracts of red seaweed or Chindrus crispus.[1] Carrageenin also has special uses as an irritant compound used in testing anti-inflammatory drugs or finding inflammatory activity and is also used as a compound. Inducer of acute inflammation or as an irritant compound in rats or mice because it can cause damage to the inflamed area. Carrageenin, an acute inflammation-inducing compound or an irritant, is known to stimulate the release of inflammatory mediators such as prostaglandins, leukotrienes, and bradykinins without damaging the tissue at the injection site. Males by giving ethanol extract of Tamarillo skin before injecting carrageenin into their legs. The results showed the anti-inflammatory power of Tamarillo peel extract, especially at a dose of 280 mg/kg BW.[20]

Based on the description above, it is necessary to conduct further research on the anti-inflammatory effect of ethanol extract on the Tamarillo skin by examining the levels of IL-6 in the blood.

METHODS

This type of research is an experimental laboratory in vivo. Therefore, research ethics approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, University of Indonesia (KET-763/UN2.F1/ETIK/PPM00.02/2021).

The research subjects were 30 white male rats (Rattus norvegicus) Wistar strain with a body weight of 180-240 grams and an age of 3-4 months divided into five groups, namely a positive control group, a negative control group, a group with ethanol extract of eggplant skin. Netherlands at a dose of 70 mg/kg BW, the group with ethanol extract of Tamarillo skin at a dose of 140 mg/kg BW, and the group with ethanol extract of Tamarillo skin at a dose of 280 mg/kg BW. The ethanol extract of the Tamarillo skin was obtained by maceration extraction technique with 70% ethanol solvent; then, the extract was carried out by phytochemical tests.

White rats (Rattus norvegicus) Wistar strain totalling 30 were divided into five treatment groups with a total of 6 rats per group. Mice were adapted in cages for one week in a laboratory environment and given the same feed ad libitum; the aim was that during the study, the mice were under the same conditions.

Before the treatment was carried out, the rats fasted for 12-18 hours to eliminate confounding variables from the food. During the study, all rats in each group were stopped from feeding but were still given water.

In the first stage, the experimental animals were treated according to the group. The positive control group was given diclofenac sodium at a dose of 7 mg/kg BW. The negative control group was given saline, the group with ethanol extract of Tamarillo skin at a dose of 70 mg/kg BW, the group with ethanol extract of eggplant skin at a dose of 140 mg/kg BW, and the group with Tamarillo rind ethanol dose of 280 mg/kg BW. The administration was carried out using an oral probe. Oral probe

inserted into the rat's mouth from the side and then affixed to the roof of the rat's mouth, then slowly inserted until the oesophagus and fluid are slowly introduced.

After giving the treatment to the mice in each group, give a pause of 30 minutes to 1 hour. Furthermore, the mice were anaesthetized with a combination of 0.05 ml of xylazine and 1 ml of ketamine. The anaesthetized rats were measured by measuring the thickness of the buccal mucosa using a calliper. The rats were injected with 0.1 ml of 1% carrageenin dissolved in 0.1 ml of saline submucosa on the buccal mucosa of rats and repeated measurements of the thickness of the buccal mucosa three times, namely, on 24 (first day), 48 (second day) and 72 (third day) hours after rats were induced by carrageenin. Blood samples from the heart of rats for examination of IL-6 levels were carried out at 24 (first day), 48 (second day) and 72 (third day) hours after rats were induced by carrageenin. Blood samples were taken as much as 3 mL of blood from the hearts of rats by inserting a 3 mL syringe directly into the heart. The blood sample was put into a tube and then centrifuged at 3,000 rpm for 10 minutes to separate the serum from the sediment. The serum obtained was then put into a tube and stored at -80°C before testing using the Rat IL-6 ELISA kit. After that, the rats were euthanized by injection using a combination of 1.5 ml of xylazine and 3 ml of ketamine. The data obtained were statistically tested using ANOVA 1 way with a significance level set at 0.05.

RESULTS

Based on the results of the phytochemical tests, the ethanol extract of the Tamarillo peel was proven positive for containing flavonoids, phenolics, tannins and alkaloids.

The rats that had been treated and anaesthetized were then measured for the thickness of the buccal mucosa using callipers. Then the rats were injected with 0.1 ml of 1% carrageenin dissolved in 0.1 ml of saline submucosa on the buccal mucosa of the rats. Again the thickness of the buccal mucosa was measured. Three times, namely on 24 (first day), 48 (second day) and 72 (third day) hours after rats were induced with carrageenin.

Table 1. The results of measuring the thickness of the buccal mucosa of rats before, 24 hours, 48 hours, and 72 hours after carrageenin injection.

Time	Study Group	Before Karagenin Injection (mm)	After Karagenin Injection (mm)
24 hours	Positive Control	0.85	1.77
	Positive Control	0.82	1.94
	Negative Control	1.19	1.64
	Negative Control	0.94	1.74
	Extract 70mg/ kgWB	1.45	1.88
	Extract 70mg/ kgWB	1.24	1.88
	Extract 140mg/ kgWB	1.28	1.72
	Extract 140mg/ kgWB	1.16	1.62
	Extract 280mg/ kgWB	1.35	1.86
	Extract 280mg/ kgWB	1.12	1.69
48 hours	Positive Control	1.2	1.98
	Positive Control	1.25	3.05
	Negative Control	1.02	1.68
	Negative Control	1.1	2
	Extract 70mg/ kgWB	1.37	1.7
	Extract 70mg/ kgWB	1.27	3.34
	Extract 140mg/ kgWB	1.23	1.73
	Extract 140mg/ kgWB	1.25	1.69
	Extract 280mg/ kgWB	1.26	1.93
	Extract 280mg/ kgWB	1.11	1.6
72 hours	Positive Control	1.65	1.56
	Positive Control	1.11	1.42
	Negative Control	1.05	1.8
	Negative Control	1.08	1.33
	Extract 70mg/ kgWB	1.34	1.42
	Extract 70mg/ kgWB	1.19	2.11
	Extract 140mg/ kgWB	1.26	1.59
	Extract 140mg/ kgWB	1.26	1.43
	Extract 280mg/ kgWB	1.06	1.81
	Extract 280mg/ kgWB	1	1.64

Table 2. The results of the examination of IL-6 levels from the heart blood of rats at 24 hours, 48 hours, and 72 hours after carrageenin injection.

Time	Sample	Level (pg/ml)
24 hours	Positive Control	23.941
	Positive Control	15.294
	Negative Control	27.088
	Negative Control	29.088
	Extract 70mg/kgWB	24.324
	Extract 70mg/kgWB	18.912
	Extract 140mg/kgWB	14
	Extract 140mg/kgWB	13.853
	Extract 280mg/kgWB	22.676
	Extract 280mg/kgWB	33.588
48 hours	Positive Control	15.765
	Positive Control	11
	Negative Control	26.029
	Negative Control	13.059
	Extract 70mg/kgWB	19.353
	Extract 70mg/kgWB	18.765
	Extract 140mg/kgWB	23.412
	Extract 140mg/kgWB	23.088
	Extract 280mg/kgWB	23.529
	Extract 280mg/kgWB	15.529
72 hours	Positive Control	26.765
	Positive Control	16.147
	Negative Control	12.735
	Negative Control	4.324
	Extract 70mg/kgWB	18.882
	Extract 70mg/kgWB	20.971
	Extract 140mg/kgWB	29.324
	Extract 140mg/kgWB	20.882
	Extract 280mg/kgWB	29.5
	Extract 280mg/kgWB	28.059

The average value of IL-6 levels in all study groups is shown in the diagram below (Figure 1) From the results of the measurement of the buccal mucosa of rats thickness, it was seen that there was thickening of the buccal mucosa in all groups, as indicated by the increasing number of measurement results.

Furthermore, blood samples were taken from the hearts of rats. Blood samples were taken three times, namely, on 24 (first day), 48 (second day) and 72 (third day) hours after rats were induced with carrageenin.

From the results of the statistical analysis test, the results of the normality test using the Shapiro-Wilk method showed that the data had

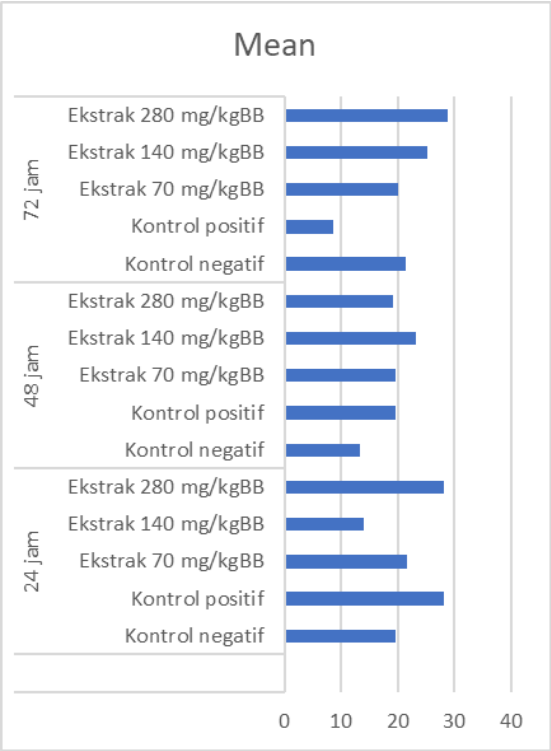

Figure 1. The average value of the measurement results of IL-6 levels.

a normal distribution with a value (p> 0.05), so further tests could be carried out using the parametric statistical method, namely One Way ANOVA which showed a significant difference between the group with a value (p=<0.001). It is necessary to do a post hoc test. Based on the results of the post hoc test to find out which groups have differences, it can be seen that all samples in all groups have a significant difference, namely (p = <0.001).

DISCUSSION

The results of the phytochemical tests showed that the ethanol extract of the rind of the Tamarillo (Solanum betaceum Cav.) was positive for flavonoids, phenolics, tannins and alkaloids. This is in accordance with another study, which stated that the ethanol extract of the Tamarillo skin contains flavonoid compounds and has anti-inflammatory properties.[20]

In the health sector, flavonoids have been used as anti-inflammatory, antioxidant, antibacterial, and antidiabetic.[21] The antioxidant compounds in flavonoids can play a role in inhibiting inflammation by inhibiting the cyclooxygenase enzyme mechanism so that the formation of prostaglandins is inhibited and by

scavenging free radicals that cause tissue damage will trigger the biosynthesis of arachidonic acid. (18)

In this study, there were five research groups, namely the positive control group, the negative control group, and the group with the ethanol extract of the Tamarillo skin at a dose of 70 mg/kg BW, 140 mg/kg BW, and 280 mg/kg BW. From the results of the ELISA test with the Rat IL-6 ELISA kit, the following results were obtained:

At 24 hours post-carrageenin induction, among the group given the extract, the 140 mg/kg BW dose group had the lowest levels of IL-6 at 13,853 pg/ml, and this level was still lower than the positive and negative control groups. The levels of Il-6 in this group were also lower than those obtained by the other groups at both 48 hours and 72 hours of observation. In previous studies, it was stated that the most effective dose to reduce oedema was a dose of 280 mg/kg BW (20), while in this study, the most effective dose to reduce oedema and reduce blood levels of Il-6 was a dose of 140 mg/kg BW. This indicates that the anti-inflammatory power of the ethanol extract of the Tamarillo peel plays a role in reducing the levels of pro-inflammatory cytokines in the blood, which in this case is Il-6.

The group of ethanol extract of Tamarillo skin at a dose of 140 mg/kg BW at 24 hours post-carrageenin induction was the most effective in reducing IL-6 levels (13,853pg/ml). The anti-inflammatory ability of this group surpassed the positive control group. Based on the data obtained in the study, it turns out that the anti-inflammatory ability of the extract used is not only influenced by the dose but also by the time it takes for the active ingredients contained in the extract to function. In this study, the most effective time was 24 hours after carrageenin induction. The results of the study will be compared more accurately if the research also prepares groups of research subjects who are not induced by inflammatory substances.

CONCLUSIONS

Ethanol extract of Tamarillo skin (Solanum betaceum Cav.) with a dose of 140 mg/kg BW within 24 hours post-carrageenin induction is the most effective natural ingredient in reducing IL-6 cytokine levels. The results of this study can be developed and used as alternative herbal ingredients to treat inflammation.

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AUTHORS' CONTRIBUTION

Study conception and design: JS. Data collection: SAS. Analysis and interpretation of result: JS and SAS. Draft manuscript preparation: JS and SAS. Review the result and approved the final version of the manuscript: JS and SAS.

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The fund for the research is covered by the researcher.

CONFLICT OF INTEREST

Competing interests: No relevant disclosures

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