

Cytotoxicity and Anti-Inflammatory Activity of Tamarillo (*Solanum betaceum Cav.*) Peel Extract in Lipopolysaccharide Stimulated RAW 264.7 Cells

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Abstrak: Obat anti inflamasi sering diresepkan dalam bidang kedokteran gigi. Umumnya obat-obat ini memiliki efek samping beragam, dari yang ringan hingga parah seperti perdarahan lambung. Oleh karena itu, bahan anti inflamasi yang alami dan lebih aman diperlukan sebagai alternatif. *Solanum betaceum Cav.*, dikenal sebagai terong Belanda, merupakan buah eksotik yang dapat juga dipergunakan sebagai bahan obat. Limbah buah seperti kulit dan biji biasanya tidak dikonsumsi. Beberapa penelitian menunjukkan bahwa limbah dari berbagai jenis buah mengandung senyawa bioaktif yang bermanfaat. Penelitian ini bertujuan untuk mengetahui sitotoksistas dan kemampuan anti-inflamasi dari ekstrak kulit terong Belanda terhadap makrofag lini RAW 264.7 yang diinduksi oleh lipopolisakarida (LPS). Sitotoksistas diuji untuk menentukan konsentrasi yang aman dengan menggunakan metode MTS. Aktivitas anti-inflamasi dinilai dengan membandingkan kadar PGE-2, TNF- α , dan IL-1 β pada sel RAW 264.7 yang distimulasi LPS antara kelompok yang diberikan ekstrak dan yang tidak diberikan ekstrak menggunakan metode ELISA. Hasil penelitian mendapatkan bahwa ekstrak kulit terong Belanda dapat menekan produksi PGE-2, TNF- α , dan IL-1 β pada sel RAW 264.7 yang diinduksi dengan LPS. Simpulan penelitian ini ialah ekstrak kulit *Solanum betaceum Cav* memiliki kemampuan antiinflamasi melalui penekanan produksi PGE-2, TNF- α , dan IL-1 β pada sel RAW 264.7 yang diinduksi LPS.
Kata kunci: ekstrak kulit *Solanum betaceum Cav.* (terong Belanda), sitotoksistas, antiinflamasi

Abstract: Anti-inflammatory agents are commonly prescribed in dentistry. Most of them have side effects varying from mild to severe such as gastric bleeding. Therefore, a safer and natural anti-inflammatory agent is needed as an alternative. *Solanum betaceum Cav.*, known as tamarillo, is one of exotic fruits used in traditional medicines. The fruit wastes such as peels and seeds, are usually unconsumed. However, many investigators have reported that wastes from numerous fruits had potent bioactive compounds. This study was aimed to assess the cytotoxicity and anti-inflammatory activities of tamarillo peel extract (TPE) in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophage cell line. The cytotoxicity of TPE was performed to determine the non-toxic concentration by using MTS method. The inflammatory markers measured in this study were PGE-2, TNF- α , and IL-1 β . Their concentrations were measured by using ELISA based assay. The anti-inflammatory activity was determined by comparing the reduction of the inflammatory mediators between the LPS stimulated RAW 264.7 cells treated with TPE and the non-treated group. This study revealed that TPE could reduce the production of PGE-2, TNF- α , and IL-1 β in LPS stimulated RAW 264.7 cells. In conclusion, tamarillo peel extract possess an anti-inflammatory effect by reducing the production of PGE-2, TNF- α , and IL-1 β in LPS stimulated RAW 264.7 cells.

Keywords: *Solanum betaceum Cav.* (tamarillo) peel extract, cytotoxicity, anti-inflammatory

INTRODUCTION

The utilization of medicinal plants for disease management has played a central role

since ancient times.^{1,2} Dentistry also has started to utilize medicinal plants to relieve oral problems.³ Thus, the demand for medi-

cial plants is also increasing nowadays.^{1,4} This might be due to the inexpensive price of herbal medicines compared to Western medicines.¹ Moreover, the problems due to adverse drug reactions of some Western medicines are very prominent.⁴ On the other hand, medicinal plants are considered to be safe and effective.⁵ As the demand of medicinal plants is increasing, pharmacologists become more aware to study the safety, efficacy, and toxicity of the medicinal plant.⁶ One of its efficacy is the anti-inflammatory potential of natural compounds.⁷ Medicinal plants possess numerous of active phytochemical compounds with various biological activities. Scientists are highly interested in studying plants in order to isolate novel herbal drugs to substitute synthetic drugs.⁸ Therefore, the initial step to evaluate the cytotoxicity of plant extracts is important to promote successful development of pharmaceutical preparation of minimal to no toxic effect herbal drugs to the host.⁹

Inflammation is a vital defense mechanism of human body against harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation, removes harmful stimuli, and initiates healing.¹⁰ In the past, inflammation was known as part of healing process. But nowadays, inflammation is seen as an unwanted response which is harmful to the host.¹¹ During inflammatory responses, cellular and molecular processes and their interactions minimize the injury or infection. However, uncontrolled inflammation may turn to chronic state, contributing to various chronic inflammatory diseases. Several pro-inflammatory cytokines are involved in inflammatory process, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6).¹⁰ IL-1 β is released by monocytes, macrophages, fibroblasts, and endothelial cells during inflammation, invasion, infection and cell injury.¹² IL-1 β up-regulates other pro-nociceptive mediators, thus facilitates synaptic activity and pain transmission, and contribute to the development of chronic pain. TNF- α plays a well-known role in some pain models and acts on several different signaling pathways through two cell surface receptors.¹³ On the other

hand, prostaglandin (PG) production, especially abundantly detecting PGE-2, can also exacerbate inflammatory response. PGE-2 induces acute inflammatory reaction by activating mast cells via EP3 receptor. PGE-2 also induces chronic inflammatory reaction by differentiation of Th-1 (T helper-1), proliferation of Th-12, and production of IL-22 by Th-22 via EP2 and EP4 pathways.¹⁴

The most commonly prescribed medications for pain and inflammation, the non-steroidal anti-inflammatory drugs (NSAIDs), are highly effective, but are also known for multiple adverse effects such as gastrointestinal bleeding, cardiovascular effects, and nephrotoxicity.¹⁵ Due to the side effects of NSAIDs, high interests are found toward herbal remedies to reduce pain and inflammation.¹⁶ Many of these herbal materials possess anti-inflammatory effect, can stop bleeding, and act in similar pathway as NSAIDs, which are necessary in dental treatment.^{3,16} The natural compound that is commonly identified in natural herbs is flavonoid. Flavonoids belong to an important class of natural compounds which possesses the anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic properties. Flavonoids are widely found in fruits, vegetables, and certain beverages.¹⁷ An example of the fruits is tamarillo (*Solanum betaceum Cav.*), an exotic nutritious fruit which is easily found, processed, and consumed. Both tamarillo peel and flesh contains several phytochemicals including flavonoids, anthocyanins, and carotenoids.^{18,19} Therefore, the aim of this study was to evaluate the cytotoxic and anti-inflammatory effects of various concentrations of tamarillo peel extracts (TPE) in LPS stimulated RAW 264.7 cell line. Based on the literatures given, we hypothesized that TPE in various concentrations were able to reduce the production of PGE-2, IL-1 β , and TNF- α as pro-inflammatory cytokines.

METHODS

This was an experimental study by posttest only with control group design that compared the cytotoxicity of various concentrations of tamarillo peel extracts (TPE) and

its effect toward the concentrations of PGE-2, IL-1 β , and TNF- α . Figure 1 A and B showed the fresh tamarillo fruits as well as dried tamarillo fruits.



Figure 1. A) Fresh tamarillo fruits; B) Dried tamarillo fruits

Tamarillo peel extracts (TPE) was obtained by using maceration technique with 70% ethanol.

The macrophage cell line RAW 264.7 (ATCC TIB-71) culture was done on Dulbecco's modified eagle's medium (DMEM, Gibco 11995065) added with 10% of fetal bovine serum (FBS, Biowest S181H), and 1% of antibiotic and antimycotic (ABAM, Gibco 1772653). The culture was incubated at 37°C with 5% of CO₂ until the 70-80% of the cells were confluent. The culture was then washed and harvested.²⁰⁻²²

Cytotoxicity test was measured by MTS assay based on the conversion of yellow tetrazolium salt to form a purple formazan product. MTS (Abcam, ab197010) was used. Cells were seeded in a 96-well plate (5 x 10³ cell/well) then were incubated for 24 hours at 37°C with 5% CO₂. After 24 hours, each well was supplemented with 180 μ l new culture medium along with 20 μ l of the TPE in various concentration (12.5 μ g/ml, 25 μ g/ml, 50 μ g/ml, and 100 μ g/ml). The cells were then incubated for another 24 hours at 37°C with 5% CO₂. After 24 hours, 20 μ l of MTS were added on each well then be incubated for 3 hours at 37°C with 5% CO₂. The absorbance was measured by using spectrophotometry at 490 nm.²⁰⁻²²

The pro-inflammatory activation of RAW 264.7 cell process was, as follows:

cells were plated in a 6-well plate (1000 cells/plate) then were incubated for 24 hours at 37°C with 5% CO₂. After 24 hours, the culture medium was discharged, the plate was added with 1600 μ l of new culture medium along with 200 μ l TPE (12.5 and 50 μ g/ml) then was incubated for 1-2 hours at 37°C with 5% CO₂. Further, 200 μ l of 1 μ g/ml LPS (Sigma Aldrich, L2880) was added into each well and the plate was incubated for 24 hours at 37°C with 5% CO₂. The conditioned medium was collected in a small tube and centrifuged at 2000xg for 10 minutes. The supernatant was collected and stored at -80°C for quantification of PGE-2, IL-1 β , and TNF- α concentration.²⁰⁻²²

In quantification of PGE-2, IL-1 β , and TNF- α concentrations, the process was as follow: PGE-2 concentration was measured using ELISA PGE-2 mouse kit from Elabscience (E-EL-M0052). An amount of 100 μ l of standard solution was added twice into the wells, then the wells were sealed and incubated for 90 minutes at 37°C. The solution was discharged, then 100 μ l of biotinylated detection Ab solution was added to each well, sealed, and incubated for 1 hour at 37°C. After one hour, the solution was washed away using wash buffer repeated for three times. 100 μ l of HRP conjugate was then added to each plate and covered an incubated for 30 minutes at 37°C. The plate was then washed for five times and 90 μ l of reagent was added to each well followed by incubation for 15-30 minutes at 37°C. After incubation, 50 μ l of stop solution was added to each well then optical density was measured at 450 nm using spectrophotometer. The similar procedure was repeated for TNF- α and IL-1 β using ELISA TNF- α mouse kit (Elabscience, E-EL-M0049) and ELISA IL-1 β mouse kit (Elabscience, E-EL-M0037).²⁰⁻²² All experiments done in this study were performed in triplicate for each group.

Statistical analysis was conducted by using SPSS software. All data were presented as mean \pm deviation standard. Any significant differences between the groups presented in this study were determined by ANOVA followed by Tukey HSD post hoc test.

RESULTS

The cytotoxic effects of TPE were evaluated against raw 263.7 cells using MTS assay. The concentrations tested were 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml. The cytotoxic test showed that only 72.77% of cells survived after stimulated with 100µg/ml of TPE. Whereas, the stimulation of 12.5 µg/ml, 25 µg/ml, and 50 µg/ml showed high cell viabilities which were 108.96%, 104.44%, and 99.26% respectively. Based on ANOVA Tukey post hoc test, the viability of RAW 264.7 cells induced by 100 µg/ml of TPE was statistically lower compared to the viabilities of RAW 264.7 cells induced by 12.5 µg/ml, 25 µg/ml, and 50 µg/ml of TPE. Whereas, the viabilities of RAW 264.7 cells induced by 12.5 µg/ml, 25 µg/ml and 50 µg/ml TPE were not

statistically significant (Figure 2).

DISCUSSION

Viability cell assays are used to measure cell proliferation, testing for its cytotoxic effect.²³ This viability test is important in pharmacology prior to clinical use of any drug or chemical compound.²²⁻²⁴ In this study, the viability cell assay was carried out by using MTS assay. Non toxicity was recorded by 80% or more viable cells.²⁴ Based on this study, the concentrations of TPE ranged between 12.5-50 µg/ml were non-toxic, and cells' viabilities were more than 80%. These groups were considered to be safe for living cells. However, group of 100 µg/ml of TPE showed lower viability which was less than 80% (72.77%), therefore, it was considered as low toxicity.²⁴

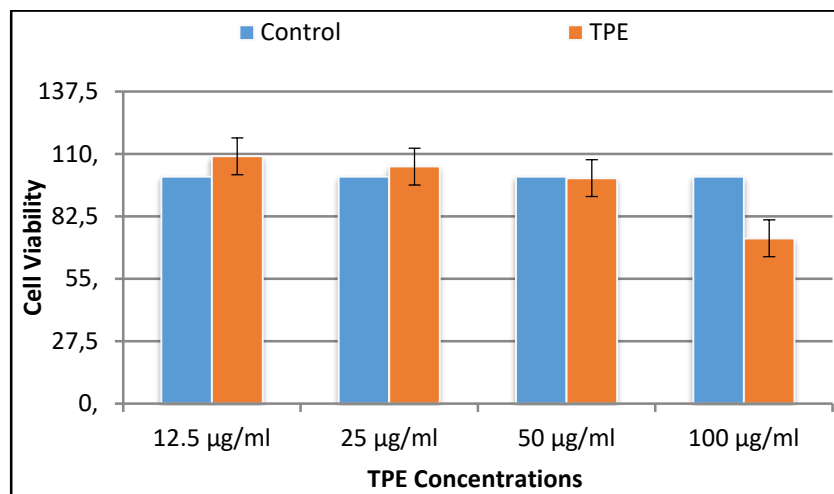


Figure 2. Viability of RAW 264.7 cells induced by TPE of various concentrations

Table 1. PGE-2, IL-1 β , and TNF- α concentration on RAW 264.7 cell line after induction with TPE

Samples	PGE-2 Concentration (pg/mL) \pm SD	IL1- β Concentration (pg/mL) \pm SD	TNF- α Concentration (pg/mL) \pm SD
(+) Control	866,44 \pm 39,02 ^e	437,06 \pm 25,72 ^d	582,63 \pm 25,23 ^c
(-) Control	404,51 \pm 10,31 ^a	139,04 \pm 7,91 ^a	242,17 \pm 18,95 ^a
12,5 µg/ml TPE	747,63 \pm 25,32 ^d	347,55 \pm 23,67 ^c	438,81 \pm 33,53 ^b
75 µg/ml TPE	501,12 \pm 32,56 ^b	176,15 \pm 15,84 ^a	276,48 \pm 43,82 ^a

Note: All data were presented as mean \pm SD. Different lowercase letters in the same column were statistically significant at $p < 0.05$ (Tukey HSD post hoc test).

The anti-inflammatory potential of TPE extracts in this study was observed through the inhibitory activity against inflammatory markers such as PGE-2, IL-1 β , and TNF- α on LPS induced macrophage cell line (RAW 264.7). PGE-2 was the most abundantly produced prostaglandins in human body. During inflammation process, PGE-2 involves in almost all inflammatory signs, such as redness, swelling, and pain.²⁵ The reduction of PGE-2 production will reduce the inflammation and enhance healing process.²⁶ IL1- β acts in homeostatic functions. However, IL1- β overproduction occurs in pathophysiological changes. IL1- β can be released by immune cells and non-immune cells and plays a role in inflammation and pain by Caspase-1 via the inflammasomes. On the other hand, IL1- β can lead to the release and/or activation of nociceptive molecules such as prostaglandins, IL-6, substance P, and MMP9.¹⁴ TNF- α is a pro-inflammatory cytokine that is simultaneously produced with interleukins which modulates pro-inflammatory signals by coordinating vascular and cellular alterations in the immune system.²⁷

This study showed that application of 12.5 $\mu\text{g/ml}$ and 75 $\mu\text{g/ml}$ of TPE significantly decreased the production of PGE-2, IL1- β , and TNF- α compared to the LPS stimulated group. In addition, the reductions of IL1- β and TNF- α production after induction with 75 $\mu\text{g/ml}$ of TPE were similar to the non-stimulated group. Based on this study, 12.5 $\mu\text{g/ml}$ and 75 $\mu\text{g/ml}$ of TPE may be a potential anti-inflammatory agent which did not possess toxic effect toward RAW 264.7 macrophage cell line. These results were consistent with the previous research that supplementation of *Cyphomandra betacea* (tamarillo) extracts successfully decreased the production of inflammatory biomarkers.²⁸ According to Kadir et al,²⁸ *Cyphomandra betacea* (tamarillo) supplement given to obese induced rats were able to reduce the concentration of TNF- α and IL-6 in blood.

This anti-inflammatory potential of TPE is caused by the content of phytochemical compounds such as flavonoids, tannins, saponin, and alkaloids.²⁹ The flavonoids found in TPE are flavons, flavonols, and

isoflavons.¹⁹ Many researches have showed that bioactive compounds found in plants have pharmacologic effect towards diseases including inflammatory related diseases.³⁰ Flavonoids are able to inhibit several enzymes including cyclooxygenase,³¹ phospholipase A2 and lipoxygenase, thus reduce the concentrations of prostanoids and leukotrienes.³² Flavonoids was believed to be able to inhibit neutrophil degranulation.³³

Other bioactive compounds found in TPE is flavons. Flavons act as an anti-inflammatory agent through modulation of pro-inflammatory gene expression such as cyclooxygenase-2 (COX-2), nitric oxide synthase (NOS), and several cytokines.³² Cyclooxygenases and lipoxygenases play some important roles during inflammatory process. These enzymes are involved in arachidonic acid formation which is the initial process of inflammation process. This activity releases cytokines, thus, the inhibition of these enzymes will reduce the production of inflammatory metabolites.³³

Tannins possess an anti-inflammatory activity through inhibition of inflammatory mediator expression, such as several types of cytokines, inducible NOS, and COX-2.³⁴ Tannin inhibits the expression of COX-2 through NF- κB and MAPK pathways, thus the pro-inflammatory gene transcriptions such as TNF- α , IL-6, dan IL-1 β are also reduced. Inhibition of COX-2 expression also reduces prostaglandin production.³⁵

CONCLUSION

TPE in lower concentration (12.5 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, and 50 $\mu\text{g/ml}$) shows no toxic effect towards macrophage cell lines and has anti-inflammatory potential proven by its ability to reduce the production of pro-inflammatory mediators including PGE-2, IL-1 β , and TNF- α on LPS induced macrophage cell lines.

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Conflict of Interest

The authors declare no conflict of interests in this study.

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