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The Immunomodulatory Activities of Alkaloid (Vf-1) Isolated from Stem Bark of Tampa Badak (*Voacanga Foetida* (Bl) Rolfe) on Raw 264.7 Cells

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ABSTRACT

Voacanga foetida (Bl) Rolfe (Apocynaceae) locally called Tampa Badak is a widely distributed plant in Indonesia. Indonesian people use this plant as a traditional medicine to treat infections, pain, diarrhea, itching, and swelling. VF-1 is an alkaloid compound that has been previously isolated from the dichloromethane fraction of the stem bark of the Tampa Badak. Based on the melting point value and interpretation of the ^{1}H-NMR spectrum, it was described that the VF-1 alkaloid compound was similar to voacangine. This study was conducted to determine the immunomodulatory activity of VF-1 compounds from the stem bark of the Tampa Badak. Cell viability and immunomodulatory activity of the compounds were carried on cultured RAW 264.7 cells (macrophages). The effect of VF-1 compound on cell viability was carried out using the MTT method to determine the safety or toxicity of the compound against macrophage cells. The immunomodulatory activity of VF-1 was tested on lipopolysaccharide (LPS)-induced RAW 264.7 cells using the sandwich ELISA method by measuring the production of proinflammatory cytokines IL-6 in cell supernatants. The results showed that RAW 264.7 cells treated with VF-1 at concentrations of 1, 10, and 100 g/mL appeared safe and non-toxic and showing cell viability values >80%. The percentage inhibition of IL-6 production at each concentration of VF-1 was 45.85; 58.20; 23.25% respectively. Based on the results obtained, it was concluded that the compound has the potential as an immunosuppressant in RAW 264.7 macrophage cells induced by LPS.

Keywords: *Voacanga foetida, immunomodulatory, IL-6, macrophages, LPS*

1. INTRODUCTION

The immune system plays an important role in our health. It is known that suppression of immune system function can lead to an increased incidence and severity of diseases such as infectious diseases [1]. Based on WHO data in 2014 stated that diseases caused by microbial infections, such as viruses, bacteria, protozoa, worms, and fungi are the second cause of death in the world after cardiovascular which indicates that the presence of immunity is very important as a body defense. If there is a reduction in the number and function of immune cells, infectious diseases by microorganisms will easily arise [2]. Activation of the immune system is needed to help the body against antigenic substances. Immune system activation can be stimulated using immunomodulators Immunomodulators are substances or agents that can help repair impaired immune system function [3]. Immunomodulatory mechanisms include restoring impaired immune function (immunorestoration), improving immune

system function (immunostimulation) and suppressing immune responses (immunosuppression) [4].

The innate immune response is the first line of nonspecific defense against pathogens and is carried out by macrophages, granulocytes, monocytes and natural killer (NK) cells. Among them, macrophages play an important role and are involved in many functions such as phagocytosis, surveillance, chemotaxis, and destruction of target organisms [5]. Macrophages can be activated to release several inflammatory mediators and cytokines. Cytokines are glycoproteins derived from helper T cells, natural killer (NK) cells and macrophages, which play an important role in the body's response to infection [6]. During infection, bacterial products such as LPS activate macrophages and other cells to produce and release various proinflammatory cytokines such as IL-1, IL-6 and TNF-α [7].

IL-6 is a cytokine with various biological activities. IL-6 is also an indicator of inflammation in the body. IL-6 is an endogenous biochemical that is active during B cell maturation and inflammatory processes. IL-6 can act as a pyrogen and can cause fever during infectious, non-infectious and autoimmune diseases. IL-6 is produced by macrophages and monocytes in the early stages of infectious inflammation immediately after stimulation of Toll-Like Receptors (TLR)-like receptors with separate Pathogen Related Molecular Patterns (PAMP). When non-infectious inflammation occurs such as traumatic injury or burns, Damage Related Molecular Patterns (DAMPs) from the site of damage activate TLRs to produce IL-6 [8]. IL-6 functions both in non-specific and specific immunity. In non-specific immunity, IL-6 stimulates hepatocytes to produce APP and together with CSF stimulates progenitors in the bone marrow to produce neutrophils. In specific immunity, IL-6 stimulates the growth and differentiation of B cells into antibody-producing mast cells [7]. LPS is a component of the cell wall of Gram-negative bacteria, promoting the release of various pro-inflammatory cytokines [7].

The modulation of the immune response to treat diseases has long been attracting attention. Numerous recent studies have made advances in the research on ethnomedicinal plants as immunomodulatory agents. The possible uses of immunomodulators in clinical medicine include the reconstruction of immune deficit, such as AIDS treatment, and the suppression of normal immune response or exaggerated immune response in autoimmune diseases. Medicinal plants and their active components are important sources of immunomodulators [3]. *Voacanga foetida* (Bl) Rolfe is one of the plants that have been used by the people of Indonesia as a medicine, including as a medicine for wounds, pain, itching and swelling (inflammation), leprosy, diarrhea, seizures and madness as well as curing gonorrhea [9], for the treatment of psychosis and infections [10], as a remedy for skin diseases, aches, headaches and stomachaches [11]. *Voacanga foetida* (Bl) Rolfe has been shown to possess several pharmacological activities such as antimicrobial [12], analgesic [9], antipyretic [13], anti-inflammatory [14], anti-cancer [15]. *Voacanga foetida* (Bl) Rolfe is known to contain a lot of alkaloid compounds, the alkaloid content of *V. foetida* is 5-10% in the root bark, 4-5% in the stem bark, 0.3-0.45% in the leaves and 1.5% in the seeds [13]. Four types of alkaloids were identified from the *V. foetida* species, namely voacangine, voacristin, coronaridine, and lombine [12]. Based on the melting point and ^{1}H NMR spectra data in previous research showed that the alkaloid (VF-1) was similar to the voacangine alkaloids [16]. Voacangine has been shown to possess several pharmacological activities such as anti-angiogenic activity [17] and effectively inhibits the growth of oral cancer cells by inducing apoptosis [18]. Several alkaloid compounds that have been show immunomodulatory agents are berberine, chelerythrine, gelselegine, pseudocoptisine, leonurine, piperine, sinomenine, koumine, lycorine, sophocarpine, rhynchophylline, tetrandrine, matrine [19], punarnavine, papaverine [20] .

In the present study, the immunomodulatory activity of alkaloid compounds (VF-1) from *Voacanga foetida (*Bl.) Rolfe) were studied using 264.7 cells on the production of proinflammatory cytokine IL-6 . This plant is thought to have potential as an immunomodulator because of its alkaloid chemical compound.

2. METHODS

2.1. Chemicals and Reagent

The mouse macrophage RAW 264.7 cell line (ATCC® TIB-71™), VF-1 alkaloid compound isolated from Voacanga foetida (Bl.) Rolfe which has been obtained from previous research, Dulbecco's Modified Eagle's Medium (DMEM) (Gibco®), Fetal Bovine Serum (FBS) (Gibco®), Phosphate Buffered Saline (PBS) (Thermo Scientific®), Dimethyl Sulfoxide (DMSO) (Vivantis®) Pen-strep (Gibco®), trypsin-EDTA (Gibco®), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Sigma®), Lipopolysaccharide (LPS) (Sigma®), ELISA kits for IL-6 (Bioassay Technology Laboratory®),

2.2. Cell Culture

A murine macrophage cell line RAW 264.7 was cultured in DMEM supplemented with 1% penicillin–streptomycin anda 10% fetal bovine serum (FBS). The cells were cultured at 37°C in a 5% CO2 atmosphere incubator. The cells were subcultured when at 70-80% confluence.

2.3. Cell Viability Assay

The Microtetrazolium (MTT) assay was used to determine the effect of samples on RAW264.7 cell viability. The cells were plated in 96-well cell culture plates at a density of 9×10^3 cells/well in complete DMEM medium and were treated with

various concentrations of samples at the final concentrations of 1, 10, and 100 µg/mL were incubated for 24 hours at 37°C and 5% CO2. Then, 20 µL of 5 mg/mL MTT solution was added to each well. After 4 h of incubation at 37°C, the media and MTT were aspirated, and 100 µL of DMSO was added to dissolve the yellow MTT tetrazolium salt produced by metabolism to acquire purple MTT formazan salt. The amount of MTT formazan salt produced is proportional to the amount of viable cells, and the cell viability rate is determined by measuring the absorbance at 570 nm using a microplate reader.

2.4. Level Measurement of IL-6

The capability of samples to stimulate proimflamatory cytokine production in the RAW 264.7 cell was evaluated using ELISA kits. The cells were plated in 24-well cell culture plates at a density of 1×10^5 cells/well were incubated in complete DMEM medium for 24 hours at 37°C in 5% CO_2. The cells were treated with various concentrations of samples and incubated for 2 hours and then stimulated with LPS (1 µg/mL) for 24 hours at 37 °C in 5% CO2. The medium was removed before treatment with LPS, and cells were washed with PBS and replenished with complete medium. Cells treated with 1 µg/mL LPS alone were used as the control. After incubation for 24 hours, all the supernatant was collected in conical tube for testing. IL-6 in the macrophage supernatants were assayed using ELISA kits according to the manufacturer's instructions.

Add 50µL standard with various concentrations to standar wells and add 40µL sample to sample wells and than add 10 µL anti-IL-6 antibody to sample wells, than add 50 µL streptavidin-HRP to sample wells and standard wells. Mix well. Cover the plate weith sealer, than incubate 60 minutes at 37ºC. After that, remove the sealer and wash the plate 5 times with at least 0,35 wash buffer for 30 seconds to 1 minute for each wash. Blot the plate onto paper towels or other absorbent material. Add 50µL substrate solution A to each well and than add 50µL substrate solution B to each well. Incubate plate covered with new sealer for 10 minutes at 37ºC in the dark. Add 50µL stop solution to each well, the blue color will change into yellow immediately. Determine absorbance of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding stop solution.

2.5. Statistical Analysis

The values are presented as mean ± standard division (SD). The data are statistically analyzed through one-way analysis of variance (ANOVA) and Duncan post hoc test, with $P \leq 0.05$ considered as statistically significan. All statistical analyses were carried out using SPSS for Windows, Version 26.0.

3. RESULTS AND DISCUSSION

The sample used in this study was an alkaloid compound (VF-1) isolated from the dichloromethane fraction of the stem bark of tampa badak (*Voacanga foetida* (Bl) Rolfe) which was available at the Cell Culture Laboratory, Faculty of Pharmacy, Andalas University, Padang. The separation and purification process of this compound used column chromatography and thin layer chromatography (TLC). On organoleptic examination, this compound is in the form of white crystals. In a previous study conducted by Putri (2020) it was found that the VF-1 alkaloid melted in a temperature range of 130 ºC – 132 ºC, in which the voacangine alkaloid that had been isolated from *V. foetida* according to Hadi (2002) had a melting point of 131ºC. In addition, the ^{1}H-NMR spectra data for the VF-1 alkaloid compound showed typical proton signals for the indole alkaloid compound which has a structure with a heterocyclic nitrogen atom in its carbon ring which is similar to the ^{1}H-NMR spectrum of the voacangine compound. Based on the melting point data and ^{1}H-NMR spectra data, the VF-1 alkaloid compounds are similar to the voacangine alkaloids [16]. In relation to the immune system, alkaloid compounds can act as immunomodulators through various mechanisms [19]. This research focuses more on the nonspecific immune system (innate immunity). The non-specific immune system is the body's first line of defense against various microorganisms and can respond directly to antigens. The non-specific immune system is a response to an antigen that can arise even though the body has not previously been exposed to an antigen and can induce a subsequent immune response, namely a specific immune response [21].

RAW 264.7 cell is a monocyte-macrophage cell line that is widely used in research on the immune system because it is similar to macrophages produced by bone marrow. RAW 264.7 cells were derived from *Mus musculus* or mice induced by Abelson murine leukemia virus. Macrophages are the main cells that play a role in the non-specific immune system and play an important role in defense against pathogens. RAW 264.7 cells were used in this study because they have a fast growth rate or division with a replication time of 12-24 hours [22] and have the ability to divide indefinitely (immortally) through the transformation process [23].

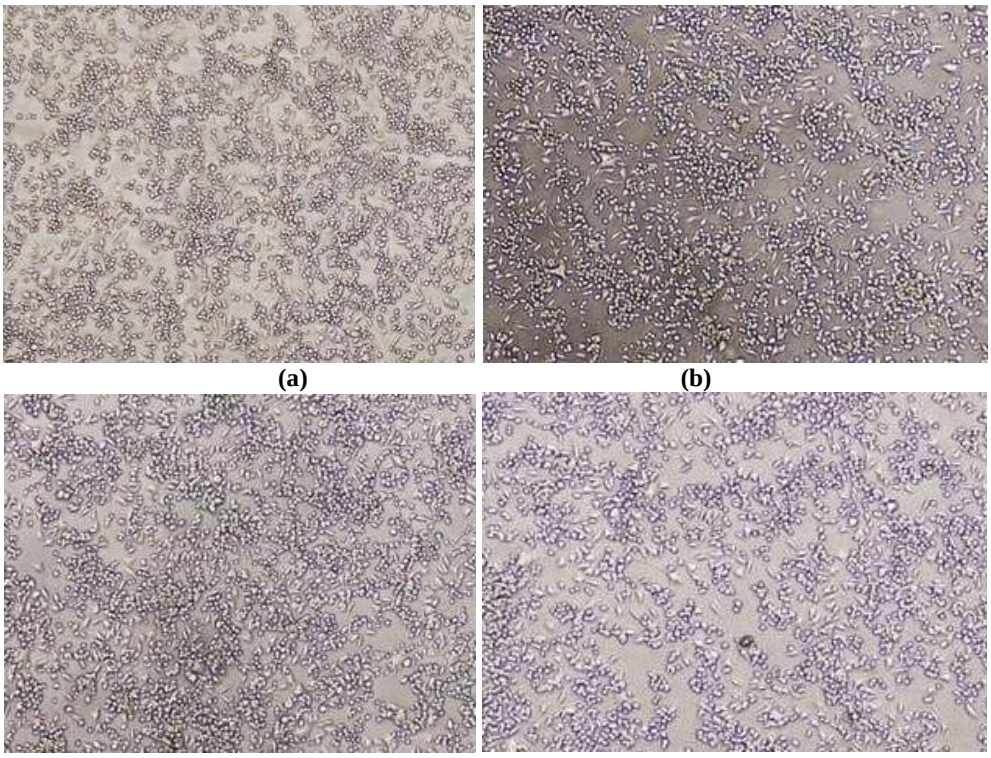

Figure 1 Normal RAW 264.7 cell (a); RAW 264.7 cells treated with VF-1 1µg/mL(b); RAW 264.7 cells treated with VF-1 10µg/mL(c); RAW 264.7 cells treated with VF-1 100µg/mL(d), All images are magnified at 40X in inverted microscope

The Microtetrazolium (MTT) assay is used to measure cellular metabolic activity as an indicator of cell viability The MTT assay was used to determine the effect of samples on RAW 264.7 cell viability. This test is important to ensure that samples used is safe or not toxic to RAW 264.7 cells so the immunomodulator activity test can be continued and to avoid false positives in immunomodulatory testing. The MTT method is a sensitive, quantitative, and reliable colorimetric method for measuring cell viability [24]. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain succinate tetrazolium reductase enzymes which reduce the MTT to formazan. MTT produces a low absorbance value in the absence of viable cells. The intensity of the purple color formed is proportional to the number of viable cells. Dead cells cannot be stained by MTT salts so they not turn purple color as in viable cells [25]. Dead cells lose the ability to reduce tetrazolium salts to colored formazan products [26].

Table 1 RAW 264.7 cell viability test results

Concentration (µg/mL)	Absorbance			RAW 264.7 Cell Viability (%)			Average RAW 264.7 cell viability ± SD
	1	2	3	1	2	3	
VF-1 (1)	1,502	1,508	1,546	108,26	108,70	111,50	109,49 ± 1,76
VF-1 (10)	1,430	1,436	1,441	102,95	103,39	103,76	103,37 ± 0,41
VF-1 (100)	1,425	1,427	1,437	95,58	96,53	97,20	96,44 ± 0,82

The results demonstrated that sample at concentration 1; 10; and 100 µg/mL had no toxic effect on viability of the cells with viability percentage above 80%. The percentage value of viability, from the smallest sample concentration was 109.49 ± 1.76; 103.37 ± 0.41; and 96.44 ± 0.82%, respectively (Table 1). Based on the relation between the concentration of VF-1 compounds with the percentage of cell viability of RAW 264.7, statistical analysis was carried out with one way ANOVA test using the IBM SPSS Statistics version 26, obtained a significant value with $p < 0.05$. This shows that

cells treated with VF-1 at concentrations of 1, 10, and 100 µg/mL significantly affected the percentage of cell viability of RAW 264.7. To find out whether there was a significant difference between each treatment group on the percentage of viability of RAW 264.7 cells, the data analysis was continued with Duncan's post hoc test which gave the result that there were significant differences between each treatment group variation on the percentage of viability of RAW 264.7 cells. Figure 1(a) shows normal RAW 264.7 cells (control group), Figures 1 (b) and (c) show the VF-1 group with concentrations of 1 µg/mL and 10 µg/mL respectively, which shows an increase in the number of RAW 264.7 cells compared to control. In contrast to Figure 1 (d), VF-1 with a concentration of 100 µg/mL which shows the presence of dead cells, but has not been categorized as toxic to cells.

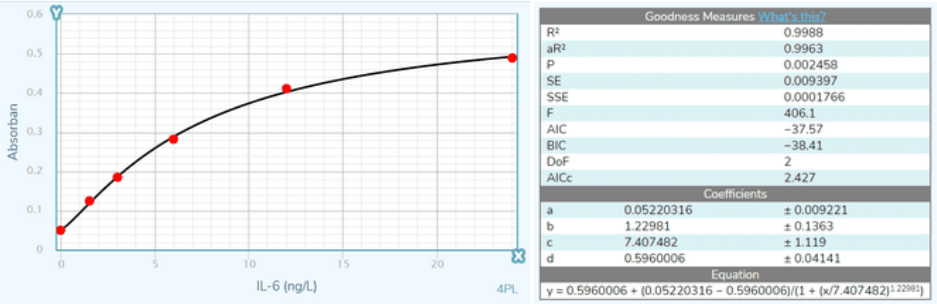

Figure 2 IL-6 standard curve measured on a Microplate Reader (λ=450 nm)

The effect of alkaloid (VF-1) on the production of IL-6 proinflammatory cytokines in RAW 264.7 macrophages was evaluated and measured using sandwich ELISA. The principle of the sandwich ELISA method is the occurrence of a complex bond between the antigen and 2 antibodies, the first antibody (capture antibody) is coated into the well on the microplate and the second antibody is conjugated with an enzyme (detection antibody). The addition of the substrate will cause a color change which indicates the presence of antigen in the sample. Absorption measurements were carried out to determine the amount of antigen in the sample [27]. Streptavidin-HRP consists of streptavidin protein, as a secondary detection reagent which is conjugated with HRP enzymes which will form enzyme-antibody conjugates with detection antibodies. Antigen that is not bound to antibody will be washed from the microplate with wash buffer. The washing process aims to remove components that are not specifically bound to antibodies. Then substrate solutions A and B were added to each well. The substrate solution A contains H_2O_2, while the substrate solution B contains Tetra Methyl Benzidine (TMB). Substrate solution is a solution that reacts with Streptavidin-HRP which converts H_2O_2 into H_2O. The reduced amount of H_2O_2 in solution causes TMB to be oxidized continuously which causes the concentration of TMB to increase and precipitate in solution. This precipitate consists of the TMB molecule which is a blue diazene (N_2H_2) derivative. The amount of TMB oxidized was stopped by giving stop solution (H_2SO_4) to each well. The addition of H_2SO_4 causes a decrease in the pH of the solution so that the color changes from blue to yellow and the color change is measured by a microplate reader with a wavelength of 450 nm [28]. The level of IL-6 in the sample is determined by comparing the absorbance value of the sample obtained with the standard solution curve. The standard dilution concentration for IL-6 is 24 ng/L, 12 ng/L, 6 ng/L, 3 ng/L, 1,5 ng/L, and 0 ng/L. The absorbance results obtained from each standard solution were made into a standard curve using a four-parameter logistic curve (4PL) curve using My Curve Fit software (https://mycurvefit.com/). Figure 2 shows the results of the standard curve obtained. On the standard IL-6 curve, the R square value is 0.9988. It means that the value of IL-6 levels in the standard solution simultaneously affects the absorbance value of 99.88%. RAW 264.7 cells unstimulated with LPS were used as negative controls, because no treatment on the cells was thought to produce the lowest levels of the IL-6 proinflammatory cytokines. While, the positive control was LPS-stimulated RAW 264.7 cells. Positive control was estimated to produce the highest levels of IL-6.

Table 2 IL-6 production in RAW 264.7 cells

Concentration (µg/mL)	Absorbance			IL-6 levels (ng/L)			Average levels of IL-6 ± SD
	1	2	3	1	2	3	
Untreated	0,108	0,114	0,116	1,27	1,40	1,44	1,37 ± 0,09
LPS	0,211	0,226	0,245	3,60	4,00	4,55	4,05 ± 0,47
VF1 (1)	0.161	0,153	0,155	2,40	2,22	2,27	2,30 ± 0,09
VF1 (10)	0,130	0,127	0,128	1,73	1,67	1,69	1,69 ± 0,03
VF1 (100)	0,185	0,196	0,193	2,96	3,22	3,15	3,11 ± 0,14

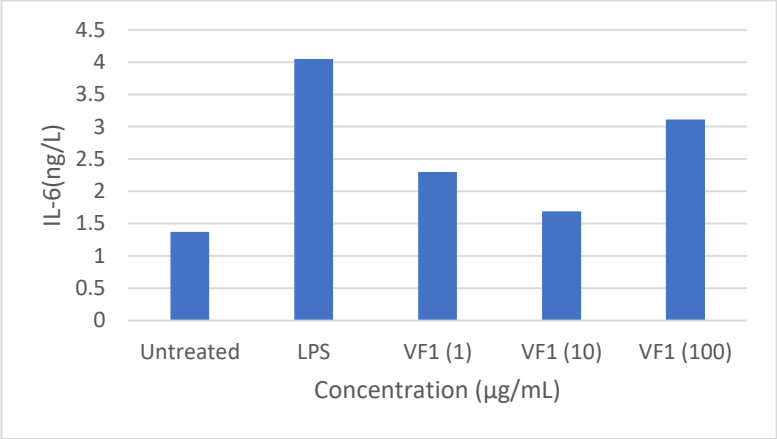

Figure 3 Effects of VF-1 alkaloid compound on IL-6 production in RAW 264.7 cells**.**

Table 2 shows the ELISA test results of IL-6. The results of this study showed that LPS was shown to significantly increase levels of the proinflammatory cytokine IL-6 compared to normal RAW 264.7 cells ($p<0.05$). IL-6 levels in LPS-induced RAW 264.7 cells were 4.05 ± 0.47 ng/L compared to normal RAW 263.7 cells which produced IL-6 levels of 1.37 ± 0.09 ng/L. The increase in levels of pro-inflammatory cytokines is due to Lipopolysaccharide (LPS) is one of the most potent antigens to induce an immune response [29]. LPS, also known as endotoxin, is a major component of the outer membrane of the cell wall of Gram-negative bacteria. LPS toxicity is related to the presence of the lipid A component of LPS [30]. LPS is a PAMP which is a pathogen that can be recognized by receptors on the surface of macrophages. Macrophages can bind LPS through the TLR (toll-like receptor). Signals from TLR will activate non-specific immune responses, stimulate the production of transcription factors that result in the production of a number of cytokines that play a role in the immune response including IL-6 [7]. As an inflammatory stimulus, LPS acts by interacting with TLR4 and causes stimulation of the MAPK inflammatory pathway. After MAPK activation by LPS, phosphorylation of I-κB via I-κB kinase occurs, leading to cleavage of the I-κB complex followed by activation of NF-κB. Then NF-κB translocates to the nucleus and activates the transcription of various genes including IL-6 as well as other proinflammatory mediators [31]. The levels of IL-6 produced by LPS-induced RAW 264.7 cells after treated with alkaloid (VF-1) at concentrations 1, 10 and 100 µg/mL were significantly lower than the positive control ($p<0.05$), which was 2.30. ± 0.09 ng/L at a concentration of 1 µg/mL, at a concentration of 10 µg/mL the IL-6 level was 1.69 ± 0.03 ng/L and at a concentration of 100 µg/mL the IL-6 level was 3 ,11 ± 0.14 ng/L. Figure 3 shows a graph between the effects of VF1 alkaloids on IL-6 production in RAW 264.7 cells. The largest percentage inhibition of IL-6 production was at concentration of 10 µg/mL compared to concentrations of 1 µg/mL and 100 µg/mL, which was 58,20% while at a concentration of 1 g/mL the percentage of inhibition of IL-6 production was 45,86% and at a concentration of 100 µg/mL the percentage inhibition of IL-6 production produced was 23,25% (Table 3).

Table 3 Percentage inhibition of IL-6 production result

Concentration (µg/mL)	IL-6 levels (ng/L)	IL-6 production inhibition (%)
Untreated	1,37	-
LPS	4,05	0
VF-1 (1)	2,19	45,86
VF-1 (10)	1,69	58,20
VF-1 (100)	3,11	23,25

Based on the relation between the concentration of VF-1 compounds and IL-6 levels produced by RAW 264.7 cells, statistical analysis was carried out with one way ANOVA test using the IBM SPSS Statistics version 26 program, overall a significant value was obtained ($p<0.05$). This indicates that RAW 264.7 cells treated with VF-1 alkaloid at concentrations of 1, 10, and 100 g/mL significantly affected IL-6 levels in LPS-induced RAW 264 cells. Duncan's test is a further test of ANOVA which was conducted to compare whether between each treatment of each group there was a significant difference in the levels of IL-6 produced in LPS-induced RAW 2647 cells. The results of Duncan's test on IL-6 levels showed that there was no significant difference between the test compound at a concentration of 10 µg/mL and the normal RAW 264.7 cell group (untreated). This indicates that this group is very effective in reducing IL-6 levels to resemble the non-inflammatory state (not induced by LPS).

It is known that previously no research has been conducted on the immunomodulatory activity of isolates from *Voacanga foetida* (Bl.) Rolfe). This plant is thought to have potential as an immunomodulator because of its alkaoid chemical content. Based on this research, it is known that RAW 264.7 cells treated with alkaloid (VF-1) isolated from the stem bark of *Voacanga foetida* at concentrations of 1, 10, and 100 g/mL can inhibit the production of the pro-inflammatory cytokine IL-6 on LPS-induced RAW 264.7 cells so that it has the potential to be a potential immunosuppressant agent.

Several alkaloid compounds are known to be a source of immunosuppressant agents, many alkaloid compounds have been shown to suppress the production of pro-inflammatory cytokines [19]. Based on research by Yun *et al.* (2009) concluded that the pseudocoptisin alkaloid isolated from *Corydalis turtschaninovi* was a potential immunosuppressant which was able to suppress the production of the proinflammatory cytokines IL-6 in RAW 264.7 cells by inhibiting NF-κB [32]. It can be assumed that the suppression of the production of the proinflammatory cytokine IL-6 in alkaloids (VF-1) also occurs through inhibition of NF-κB activity on RAW 264.7 cells. Nuclear factor-κB (NF-κB) is a transcription factor that plays an important role in regulating genes involved in the innate immune system and inflammatory response. Activation of NF-κB will induce the expression of inflammatory genes and increase the production of proinflammatory cytokines [33].

The data in this study showed that the higher concentration of samples on LPS-induced RAW 264.7 cells, was not a factor in decreasing levels of IL-6. In this study, the percentage of inhibition of the proinflammatory cytokine IL-6 was highest at a concentration of 10 µg/mL, while at a higher dose of 100 µg/mL the percentage was lower. The inconsistency between the inhibition of cytokine production and the concentration of the test compound also occurred in the research of Wang *et al.* in 2020 where in the results of his research showed that the alkaloid isolated from *Dendrobium aphyllum* (DAA) at concentrations of 0.03 mg/mL and 0.07 mg/mL resulted in the greatest decrease in the proinflammatory cytokine IL-6 while at higher concentrations of 0, 09 mg/mL resulted in a lower decrease in the proinflammatory cytokine IL-6. This is due to the activation of NF-κB at higher doses which stimulates the production of proinflammatory cytokines so that the effectiveness of the alkaloid (VF-1) decreases IL-6 levels at a concentration of 100 µg/mL to be less effective [34].

Voacanga foetida is also one of the plants that have been used by Indonesian people traditionally, such as as a treatment for swelling or inflammation conditions [12]. The VF-1 alkaloid compound in this study may explain the therapeutic efficacy of using this plant to treat inflammation. In this study, it was found that the effective concentration of VF-1 in suppressing the highest number of proinflammatory cytokine IL-6 produced by RAW 264.7 cells at a concentration of 10 µg/mL. The results of this study indicate that these compounds have immunosuppressant activity on RAW 264.7 macrophage cells induced by LPS. This can make this compound a strategy for the management of various immune system disorders and inflammatory diseases so that it can serve as a basis for the development of potential immunomodulatory drugs such as for the treatment of patients with auto-immune disorders. Auto-immune disease is a condition in which the immune system sees healthy body cells as foreign organisms, so the antibodies released by the immune system attack these healthy cells. Some examples of autoimmune diseases such as rheumatoid arthritis (RA), Systemic Lupus Erythematosus (SLE), and Psoriasis. Patients with autoimmune diseases are more susceptible to infection, so use this immunosuppressant agent with caution in people with infection.

4. CONCLUSION

The data presented in this study demonstrate that alkaloid (VF-1) isolated from the stem bark of the tampa badak (*Voacanga foetida* (Bl) Rolfe) at concentrations of 1, 10, and 100 µg/mL was safe or non-toxic to RAW 264.7 cells which gives cell viability value >80%, and could significantly reduce IL-6 levels. The highest percentage inhibition of IL-6 production was indicated by the concentration 10 µg/mL of 58.20%. Based on these results, it can be concluded that the compound has the potential as an immunosuppressant agent in RAW 264.7 macrophage cells induced by LPS. This can make this compound a strategy for the management of various immune system disorders and inflammatory diseases so that it can serve as a basis for the development of potential immunomodulatory drugs such as for the treatment of patients with auto-immune disorders.

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