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Research Report

# THE IMMUNOSTIMULAN POTENTIAL OF TENGGULUN (*PROTIUM JAVANICUM*) LEAVES TOWARDS T CELL CD4+ AND IFN$\gamma$ SECRETION ON PBMC CHICKEN

**Andi Jayawardhana[1a], Heni Puspitasari [2], Dewa Ketut Meles[3], Setiawan Koesdarto[4]**
[1] Nursery Department Faculty of Health Sciences Merdeka University
[2] Toxoplasma Study Group Institute of Tropical Disease Universitas Airlangga
[3] Basic Medicine Veteriner Department Faculty of Veterinary Medicine Universitas Airlangga
[4] Parasitology Veteriner Department Faculty of Veterinary Medicine Universitas Airlangga
[a] Corresponding author: andi.jayawardhana@gmail.com

***ABSTRACT***

*One of the plants with immunostimulant activity is Tenggulun leaves which contain of flavonoid, like terpenoid. The aim of this research is to find the potential of Tenggulun's leaves extract to have the immunostimulant activities. The potential of immunostimulant activity is identified by the increasing the amount of T-cell CD4$^+$ expression and IFN$\gamma$ secretion. The research method is conducted through cultured chicken PBMC which is infected by ND virus; it is then treated with Tenggulun's leaves extract with immunostimulant. The result of immunocitochemistry examination CD4$^+$ secretion on PBMC cultures shows how tenggulun is significantly different from the control in the secretion CD4$^+$. The 10µg of tenggulun extract can modulate the T cell CD4$^+$ secretion 68.8±0.83. It is significantly different from K (control) ($p<0,05$) and treatment group K+, P0, and P1. The examination of IFN$\gamma$ level using ELISA from tenggulun leaves extract of 10µg doses were inoculated after being infected by ND virus contained immonostimulant potential in increasing the secretion of IFN$\gamma$ 120.91±6.44. It is significantly different from K-, K+, and P1, yet not significantly different from P0. The content of terpenoid can increase IFN$\gamma$ secretion on the macrofag cells culture and limfosit cells.*

***Keywords**: Protium javanicum, imunostimulan, Newcastle Disease Virus, CD4$^+$, IFN$\gamma$*

***ABSTRAK***

*Salah satu tanaman yang memiliki aktivitas imunostimulan adalah daun Tenggulun yang mengandung flavanoid seperti terpenoid. Tujuan dari penelitian ini adalah untuk menemukan potensi ekstrak daun Tenggulun memiliki aktivitas imunostimulan. Potensi aktivitas imunostimulan dengan meningkatkan jumlah ekspresi sel T CD4 + dan sekresi IFN$\gamma$ sekresi. Metode penelitian dengan memberikan kultur PBMC ayam berbudaya dengan virus ND dan ekstrak daun Tenggulun sebagai imunostimulan. Hasil pemeriksaan immunocitochemistry sekresi CD4 + pada kultur PBMC yang diberikan ektrak daun Tenggulun berbeda nyata dengan kontrol. Ekstrak 10µg dari Tenggulun mampu memodulasi sel T CD4 + sekresi 68,8 ± 0,83 yang secara signifikan berbeda dari K (kontrol) ($p <0,05$) dan kelompok perlakuan K +, P0, dan P1. Pemeriksaan sekresi IFN$\gamma$ menggunakan metode ELISA dari ektrak daun Tenggulun daun dosis 10µg yang diinokulasi setelah terinfeksi virus ND memiliki potensi immonostimulant dalam meningkatkan sekresi IFN$\gamma$ yaitu 120,91 ± 6,44 yang secara signifikan berbeda dengan K-, K +, dan P1 namun tidak berbeda nyata dengan P0. Kandungan terpenoid dapat meningkatkan IFN$\gamma$ sekresi pada kultur sel macrofag dan sel limfosit.*

***Kata Kunci**: Protium javanicum, imunostimulan, Newcastle Disease Virus, CD4$^+$, IFN$\gamma$*

## INTRODUCTION

Plants are the sources of herbal ingredients with various benefits, including for medicines against various diseases. Disease prevention by increasing the body's immune system maintenance is highly important, because high level of immunity enables us to face the attack of foreign substances, like pathogenic microorganisms. One way to maintain immune system is by administering immunomodulatory compound which can improve the immune function of the human body.[1]

Immunostimulatory is a compound capable of boosting the immune system and reactivate immune system in various ways, such as by increasing the number and activity of T cells, NK cells and macrophages and by releasing interferon and interleukin.[2] The use of immunostimulatory therapy is rather difficult as it is mostly imported and expensive.

The incidence of immunosuppression is a problem mostly found a chicken farm. The cause is divided into two factors, namely non-infectious factors and infectious factors. The cases of immunosuppression in a farm are caused by one of those factors or combination of both factors. The combination of both factors may cause more severe immunosuppression. The research on the prevention of immunosuppression factor in farms and its application through vaccination and biosecurity to improve livestocks' livelihood are conducted as a farming industry strategy nowadays. Biosecurity aims to prevent disease and consists of isolation, traffic control, and sanitation.[3] A research on the increase of cytokine secretion can be an alternative to cure various diseases through the immune system. One of the strategies to increase the expression of cytokines is to use herbal ingredients.

Tenggulun (*Protium javanicum*) is used because it has certain bioactive compounds which influence the movement of the immune system or *immunomodulator*. The in-vitro and in-vivo research on medicinal plants have shown its potential to modulate cytokine secretion of various kinds, one of which is interferon gamma. Divided into two major types, type I IFNs are induced and act effectively in responses against viruses: IFN-$\alpha$ is secreted mainly by leucocytes, while IFN-$\beta$ is produced by fibroblasts. Type II interferon, now referred to as IFN-$\gamma$, is synthesized mostly by T lymphocytes and NK cells after this cell is activated by immune and inflammatory stimuli, rather than by viral infection.[4] The materials from the plants can modulate the performance of cytokines and spark a variety of materials from the original plants. They can also have the function of a spur of the various components of the immune system non-specific (phagocytes, NK cells) and specific immune system (the proliferation of T cells and B cells which produce antibodies) to improve the healing for various infectious diseases.[5]

Tenggulun (*Protium javanicum*) can be used as anti-inflammatory drugs. The plant is known from the experimental pharmacological research which explains that steroids and terfenoid contained in Tengggulun can act as anti-bacteria, such as *Escherichia coli* and *Staphylococcus Aures*.[6] The carrageenan-induced peritonitis is known as acute inflammatory model in which fluid extravasation and leukocyte migration are involved in the inflammatory response. The anti-inflamatory activities of rat's edema are induced by karagenan.[7] The inflammatory process consists of diverse physiological and pathological activities.[8] The anti-inflammatory activity and increased capacity can provide Protuim hepthaphyllum in male mice.[8] Successful group issolation of terfenoid terpinene which is contained in the volatile constituent of *Protium heptaphyllum*.[9] Terfenoid terpinene group can boost the immune response in-vivo and in-vitro.[8]

## MATERIAL AND METHOD

The study group is divided into five groups, namely control group (K); Meniran comparator control ($K^+$); ND (*Newcastle Disease*) infection control (P0); treatment one (P1); and treatment two (P2). While the division of the treatment groups follows the control groups: PBMC (*Peripheral Blood Mononuclear cell*) cultures are inoculated by 5µl of 0.01% (K) DMSO (*Dimethile Sulfoxide*); PBMC cultures are exposed to Newcastle Disease virus which is inoculated by meniran extract of 5g ($K^+$); P0: PBMC cultures are exposed to Newcastle Disease virus then inoculated by 0.01% DMSO; P1: infection control group, PBMC cultures are infected by Newcastle Disease virus which is inoculated by 5µl of 0.01% DMSO and Tenggulun leaf extract of 5g; P2: PBMC cultures are infected by Newcastle Disease virus which is inoculated by 5µl of 0.01% DMSO and Tenggulun leaf extract (*Protium javanicum*) of 10 mg. The production of PBMC cultures is conducted by taking peripheral blood aseptically from the brachial vein using a syringe of 15 ml of 3 broiler chickens aged 35 days. Tenggulun dried leaf powder is then extracted using methanol.

Viral infections are performed with the suspension taken from *Newcastle Disease* virus titer of 1.1 x 105 EID50 as 50µl / ml per *wells*. It is subsequently inoculated cautiously in PBMCs cultured cells with a sterile pipette. The examination of T-cell expression is accomplished using immunocytochemistry techniques, while the IFN$\gamma$ examination is conducted using *ELISA*.

## RESULT AND DISCUSSION

### Cell expression T $CD4^+$

The immunocytochemistry assay results from the observation using fluorescent microscopy T cell expression of each control and treatment group are shown in Figure 1.

The potential of methanol extract from Tenggulun leaves through the activation of specific immune system can be observed by T cell expression. The average and

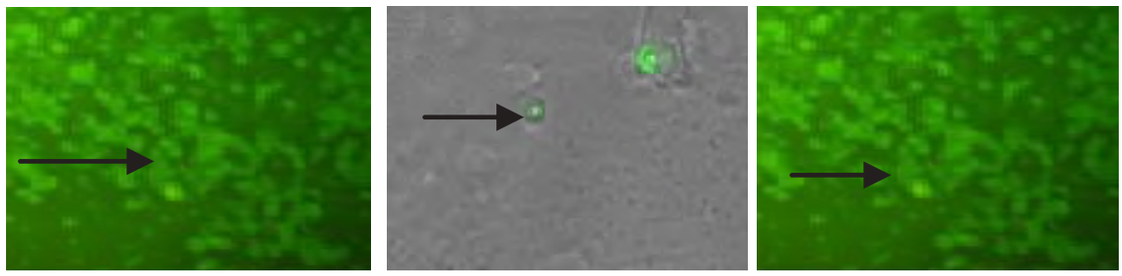

**Figure 1.** The expression results in CD4+ in PBMC cells per treatment. It is observed using a fluorescent microscope magnification of 400x. It positively glows green and is pointed by black arrow (⟶).

standard deviation of Tenggulun leaves' extracts are shown in percentage using immunocytochemistry method (Table 1).

The expression at the second day after adding the methanol extract for Tenggulun leaves are analyzed using ANOVA and Duncan test ($p \leq 0.05$). The results indicate that P2 groups record higher expression than the control group (K), P0, and P1. Different P2 groups also express significantly higher than the control group (K), P0, and P1. The statistic results state that the secretion of CD4+ group which is not given immunostimulant and not inoculated by ND virus is lower than the treatment group (K+, P0, P1, P2). It is also stated that a viral infection of ND and extract meniran and Tenggulun leaves can increase the secretion of CD4+. The ND virus infection which can increase the specific adaptive immunity are mediated by T lymphocytes.[10] The infection itself is an important factor in the development and protection of chickens against viruses. CMI will be stimulated by CD4+ lymphocytes and MHC II. The ND virus infection in-vitro induced by Nitric Oxide on heterophile chicken.[11] PBMC also showed that IFNγ that IFNß in mRNA can be detected in macrophages. The discovery of drugs derived from plants which modulate the function of CD4+ and CD8+ are important for the potential therapeutic of compounds.[12]

The average treatments group (K+, P0, P1, P2) are expressing CD4+ higher than the control groups due to the influence of ND viruses and their immunostimulant. The Cell Mediated Immunity (CMI) response to poultry may be obtained by ND vaccination. Th-1 cells and the expression of IL-18 can be detected at the third day of post-vaccination inactivated ND. IL-18 cells can induce the expression of cytokines IL-4 and IFN-γ plays an important role in the immune response of Th-1.[13] Active immunization or active vaccination aims to give a specific antigen to animals to form a protective immunity against the disease.[14]

**The secretion levels of interferon gamma (IFNγ)**

The examination results of the potential immunostimulatory Tenggulun leaf's extract (*Protium javanicum*) against IFNγ secretion by ELISA can be seen in Table 5.1. The extract (*Protium javanicum*) can increase the secretion of IFNγ. It is indicated by the lower

**Table 1.** The average and standard deviation of Tenggulun leaves' extracts (*Protium javanicum*) on the expression and secretion of IFNγ

| No. | Treatment | CD4+ expression in % | IFNγ levels in pg/ml |
|---|---|---|---|
| 1 | PBMC + DMSO (K-) | 5,60[a] ± 1,51 | 74,76[a] ± 10,99 |
| 2 | PBMC + ND + 5 µg Meniran (K+) | 25,60[b] ± 5,17 | 93,51[b] ± 18,06 |
| 3 | PBMC + ND + DMSO (P0) | 12,40[c] ± 5,36 | 110,47 [bc] ± 1,62 |
| 4 | PBMC + ND + DMSO + Tenggulun 5 µg (P1) | 55,80[d] ± 1,64 | 100,25[b] ± 0,93 |
| 5 | PBMC + ND + DMSO + Tenggulun 10 µg (P2) | 68,80[e] ± 0,83 | 120,91[c] ± 6,44 |

Description: different superscripts convey significantly different meaning

secretion of IFNγ for negative control group (K) than for the positive control group (K+); the treatment groups (P0, P1 and P2) also indicates significantly different secretions ($p$ <0.05) in which P2 group records the highest value of IFNγ secretion. The data analysis results for the secretion of IFNγ showed that the control group + (K+), P0, P1 and P2 has a value secretion higher than the group control- (K), because ND virus inoculation can increase the secretion of IFNγ.[11,15] Among the highest valued treatment group for the secretion of IFNγ, the treatment group 2 (P2) shows that the administration of 10µg dose Tenggulun leaf extract is the most effective to increase the secretion of IFNγ. The increased secretion of IFNγ may be caused by Tenggulun leaves' terpenoids as the antibacterial and anti-inflammatory compounds. Terpenoids is a substance which modulates the immune system by signaling pathway Nuclear Transcription Factor kappa B (NF-κb).[8] The modulated pathways of NF-κB lines include cytokines, limphotoxin, TNFα, and IFNγ-IFNß. Terpenoids flavonoids can increase IFNγ secretion in cultured macrophages and lymphocytes.[3,16]

The treatment of K+ (inoculated ND and extract meniran) with P1 (inoculated with ND virus and Tenggulun leaf extracts of 5µg) are not significantly different in

secreting IFNγ, because the competition between the plant extracts with the viral antigen induces similar APC cells (dendritic cells); thus, the extract's effect of 5µg dose is not enough to modulate the secretion of IFNγ post virus infection.[12,17]

## CONCLUSION

Based on this study we can conclude that the leaf extract of 10µg Tenggulun has higher immunomodulatory potential than the 5µg dose in increasing the expression and secretion of CD4 + IFNγ in PBMC cultures which are infected by ND virus.

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