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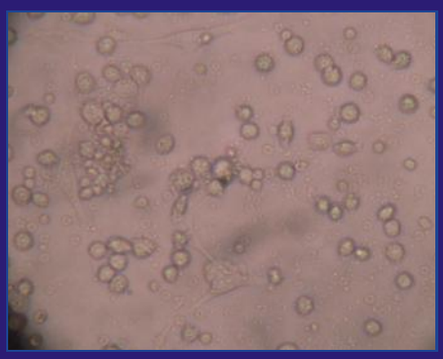

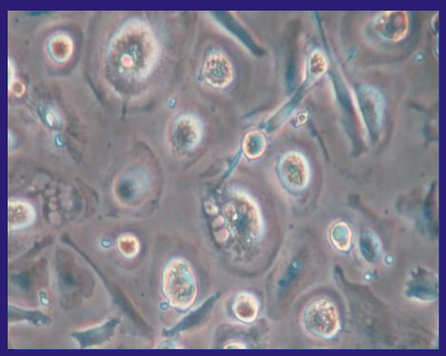

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Research Report

THE IMMUNOSTIMULAN POTENTIAL OF TENGGULUN (*PROTIUM JAVANICUM*) LEAVES TOWARDS T CELL CD4+ AND IFNγ SECRETION ON PBMC CHICKEN

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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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ secretion on the macrofag cells culture and limfosit cells.

***Keywords**: Protium javanicum, imunostimulan, Newcastle Disease Virus, CD4⁺, IFNγ*

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 IFNγ 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 IFNγ menggunakan metode ELISA dari ektrak daun Tenggulun daun dosis 10μg yang diinokulasi setelah terinfeksi virus ND memiliki potensi immonostimulant dalam meningkatkan sekresi IFNγ yaitu 120,91 ± 6,44 yang secara signifikan berbeda dengan K-, K +, dan P1 namun tidak berbeda nyata dengan P0. Kandungan terpenoid dapat meningkatkan IFNγ sekresi pada kultur sel macrofag dan sel limfosit.

***Kata Kunci**: Protium javanicum, imunostimulan, Newcastle Disease Virus, CD4⁺, IFNγ*

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-α is secreted mainly by leucocytes, while IFN-β is produced by fibroblasts. Type II interferon, now referred to as IFN-γ, 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 IFNγ 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

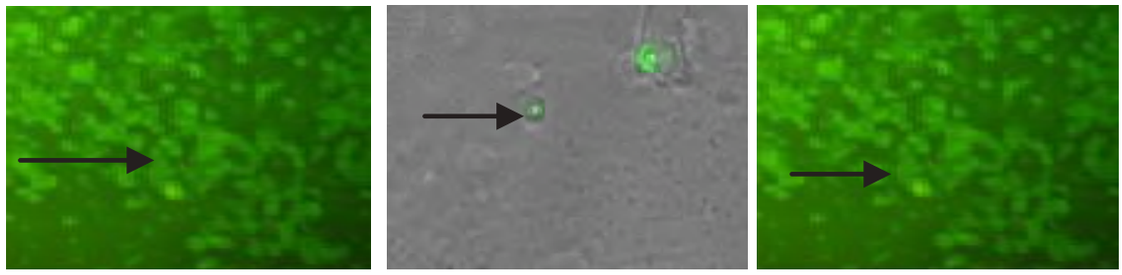

Figure 1. The expression results in $CD4^+$ in PBMC cells per treatment. It is observed using a fluorescent microscope magnification of 400x. It positively glows green and is pointed by black arrow (⟶).

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^-)	$5,60^a \pm 1,51$	$74,76^a \pm 10,99$
2	PBMC + ND + 5 µg Meniran (K^+)	$25,60^b \pm 5,17$	$93,51^b \pm 18,06$
3	PBMC + ND + DMSO (P0)	$12,40^c \pm 5,36$	$110,47^{bc} \pm 1,62$
4	PBMC + ND + DMSO + Tenggulun 5 µg (P1)	$55,80^d \pm 1,64$	$100,25^b \pm 0,93$
5	PBMC + ND + DMSO + Tenggulun 10 µg (P2)	$68,80^e \pm 0,83$	$120,91^c \pm 6,44$

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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Case Report

SEZARY SYNDROME MIMICKING GENERALIZED PSORIASIS VULGARIS

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ABSTRACT

Sezary syndrome is the leukemic variant of cutaneous T cell lymphoma. This disease is characterized by some reddish patches or plaques all over the skin which extends to the whole body into erythroderma, lymphadenopathy. It is also indicated by the presence of atypical lymphocytes called Sezary cells. This case report is aimed to know clinical manifestation, examination and management of Sezary syndrome which clinically resembles generalized psoriasis. A 60 years old man came with scaly reddish brown plaques almost all over his body. It was accompanied by lymphadenopathy on the supraclavicular lymph node right and left as well as intense itchy. Other clinical features were alopecia, palmoplantar hyperkeratosis, onychodysthropy, facies leonine without anesthesia on the lesion and enlargement of peripheral nerve. From a laboratory test, an increase in the number of leukocytes and, Sezary cells were found in peripheral blood smear examination; while the histopathology showed focal athrophy and acanthosis of the epidermis and dense infiltration of lymphocytes in the dermo-epidermal junction and superficial dermis. Patient received 3 x 5 mg (1 cycle) of methotrexate (MTX) with 0,1% cream mometasone furoate and 3x1 tablet of CTM for adjunctive therapy. Methotrexate was discontinued because there was a disturbance in liver function and deterioration of patient's condition. After 25 days of treatment, the patient got sepsis and then passed away. Early onset of Sezary syndrome in this case is difficult to know because the clinical manifestation is similar with psoriasis vulgaris. Supporting examination such as laboratory test, blood smears and histopathology examination could help the diagnosis. The presence of lymphadenopathy, and atypical lymphocytes in the peripheral blood and the extensive skin involvement reflect the poor prognosis. The most common cause of death was sepsis.

Keywords: *Sezary syndrome, cutaneous T cell lymphoma, psoriasis vulgaris, erythroderma, Sezary cells*

ABSTRAK

Sindrom Sezary merupakan salah satu jenis limfoma pada sel T kutaneus. Penyakit ini ditandai dengan bercak atau plak kemerahan pada kulit yang meluas ke seluruh tubuh menjadi eritroderma, limfadenopati dan terdapatnya limfosit atipikal yang disebut dengan sel Sezary. Tujuan dari laporan kasus ini adalah untuk mengetahui manifestasi klinis, pemeriksaan dan penatalaksanaan Sindroma Sezary yang secara klinis menyerupai generalized psoriasis. Seorang pria berusia 60 tahun datang dengan plak coklat kemerahan dengan skuama tebal pada hampir seluruh tubuh disertai limfadenopati kelenjar getah bening supraklavikular kanan dan kiri yang disertai rasa gatal pada lesi. Gambaran klinis lainnya adalah alopesia, hiperkeratosis palmoplantar, onikodistrofi, facies leonina tanpa anestesi pada lesi dan tanpa pembesaran saraf tepi. Pada pemeriksaan laboratorium terdapat peningkatan jumlah leukosit, pada pemeriksaan hapusan darah tepi ditemukan sel Sezary dan gambaran histopatologi menunjukkan atrofi dan akantosis fokal pada epidermis dan infiltrasi padat limfosit pada dermo-epidermal junction dan dermis superfisial. Pasien mendapatkan pengobatan methotrexate (MTX) 3 x 5 mg (1 siklus) dengan terapi tambahan krim mometason furoate 0,1% dan CTM 3 x 1 tablet. Pemberian methotrexate tidak dilanjutkan karena terjadi gangguan pada fungsi hepar pasien dan terjadi pemburukan kondisi pasien. Setelah 25 hari perawatan, pasien mengalami sepsis dan kemudian pasien meninggal dunia. Onset awal dari sindrom Sezary pada kasus ini sulit untuk diketahui karena gambaran klinisnya yang mirip dengan psoriasis vulgaris. Pemeriksaan penunjang berupa pemeriksaan

laboratorium, hapusan darah tepi dan pemeriksaan histopatologi membantu menegakkan diagnosis. Adanya limfadenopati dan ditemukannya sel limfosit atipikal pada darah tepi dan keterlibatan kulit yang luas menggambarkan prognosis yang buruk pada kasus ini. Penyebab kematian tersering adalah sepsis.

Kata kunci: *sindrom Sezary, limfoma sel T kutaneus, psoriasis vulgaris, eritroderma, sel Sezary*

INTRODUCTION

Lymphoma is the name for various types of cancer which emerge in the lymphocytes (immune cells). There are three types of lymphocytes: B-lymphocyte (B-cell), T-cell and the Natural Killer lymphocyte (NK-cell). In general the B-cell lymphoma is more prevalent, while T-cell lymphoma is specifically common for the skin.[1] The Non-Hodgkin Cutaneous T-cell Lymphoma is manifested on the skin. It has a variety of skin manifestations like skin patch or plaque, tumor, or erythrodermic lesions.[1,2] The proportion of T-cell lymphoma was accounted for approximately 4% of the whole Non-Hodgkin lymphoma.[1] Among many types of Cutaneous-T cell lymphoma, Mycosis Fungoides is the slow growth clinical type while the Sezary Syndrome (SS) is an aggressive, leukemic cutaneous T-cell lymphoma variant which manifests as erythroderma. Sezary Syndrome is the most aggressive type of lymphoma due to its influence to skin and blood.[1,2]

The incidence of cutaneous lymphoma has been increasing since the last decades and mostly occurred among black people at the age of 50–60 years old.[1,3.] Changes in the amount of tumor suppressor and gen that control apoptosis were found in Cutaneous T cell lymphoma. However, how these changes affect the T cell activities are still not clear.[3] Genetic disorder of NAV3 expression (the tumor suppressor) was found in 50-85% patient. Mutations of p53, p15, p16, junB and PTEN gen were observed in advance cases.

The clinical manifestation of MF and SS may be similar with some mild dermatoses like dermatitis, psoriasis vulgaris, pityriasis lichenoides chronic, and pityriasis lichenoides et varioliformis acuta.[2,4] There are trias in clinical symptom of SS which consist of erythroderma, lymphadenopathy and the typical Sezary cell.[3,5] Itchy skin lesions and thick squamae especially over the palmar and plantar areas, often occurred along with alopecia. Histopathologically, the SS shows non-specific inflammatory dermatosis, but in immuno-histochemistry staining some atypical CD4 T-cells are found with the increase ratio of CD4 / CD8 recorded at more than 10.[3,6,7] The prognosis of SS is not suitable with low remission and slow response to the few available treatment modalities.[3,8,9]

CASE

A 60 years old man was hospitalized in the Dermatology ward of Dr. Soetomo Hospital, complaining about itchy and thick patches all over the body in the last 3 days before admission. The erythematous plaques started in the back for a year previously and spread to the other parts of the body. He was treated at the Health Center and got some tablets and injections without any improvement; then he was referred to the hospital. One month prior to the admission to the Dermatology ward, the patient was hospitalized in the Internal Department for granulomatous lymphadenitis and treated with 3 series of Methotrexate (MTX) course. Some improvements were observed and the patient was discharged from the hospital.

In clinical examination the patient looked weak though all vital signs were normal, except for the body temperature (39^0C). The enlargements of both supraclavicular lymph nodes were palpable. Thick brown plaques with some erythematous skin areas were found all over the body, while hyperkeratosis of the skin was observed with some fissures on his palms and soles. Erythematous nodules were found all over the face and alopecia of the scalp was also present. However, there was no skin anesthesia, madarosis, or thickening of the ear lobes. There was no peripheral nerve enlargements on palpation either (Figure 1-4).

Laboratory results of peripheral blood revealed a leukocytosis ($17.9x10^3$/µl) with the increasing neutrophil. Hemoglobin of 11.2 g/dl and low albumin level (2.4 g/l) were also observed. The liver and renal function tests were in normal limits. Peripheral blood smear examination showed a typical lymphoid cell, and the Sezary cell was found (Figure 5).

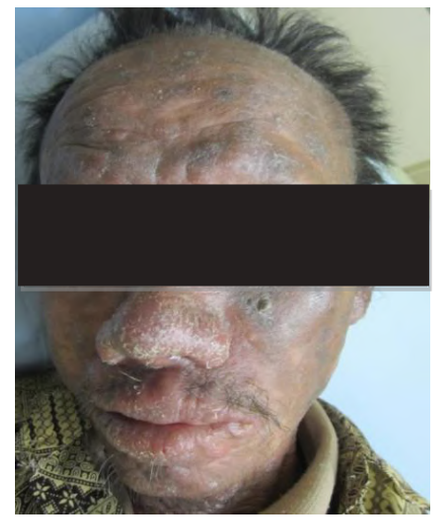

Figure 1. Erythematous nodules on face and madarosis.

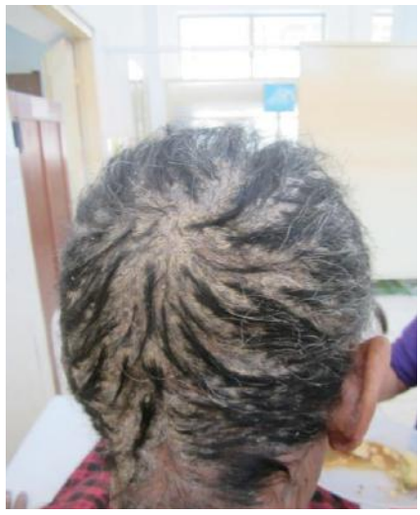

Figure 2. Alopecia

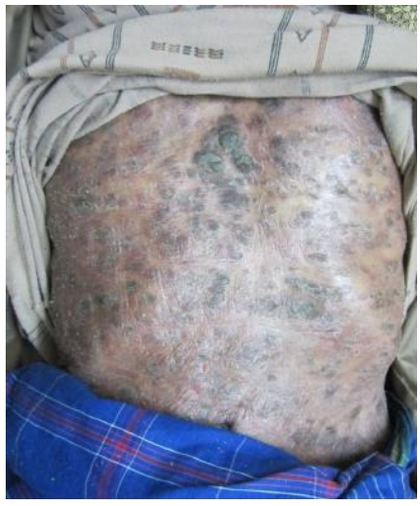

Figure 3. Psoriasiform plaques and infiltrated plaques with hyperkeratosis and scaling

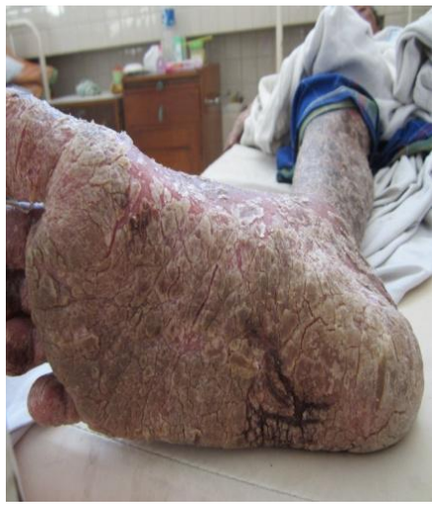

Figure 4. Plaques with superficial erosion and fissuring on the soles

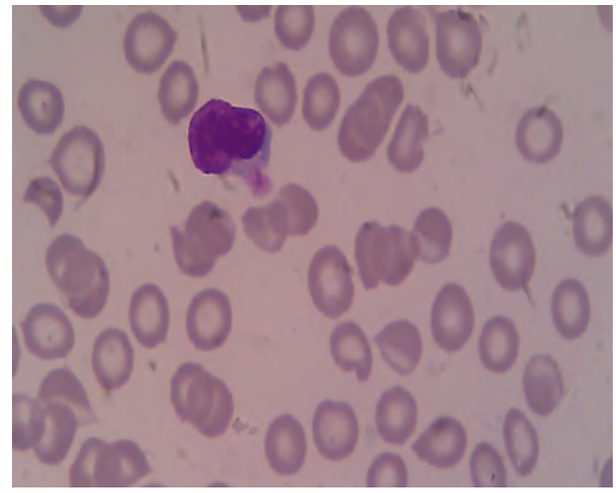

Figure 5. Sezary cell

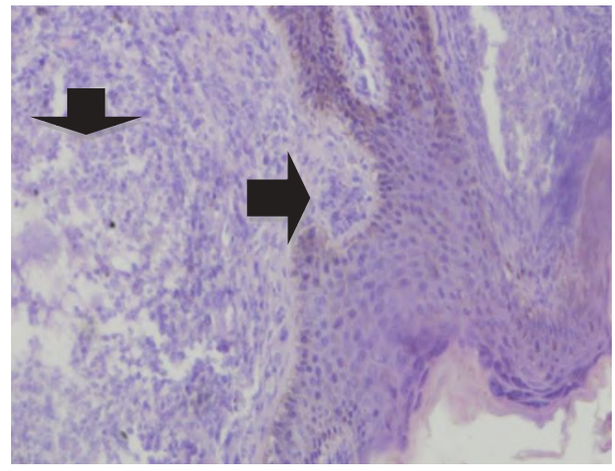

Figure 6. Focal acanthosis and dense lymphocytes (400x ampl)

In histopathological examination of the skin plaque biopsy, a hyperkeratotic corneum layer and epidermic atrophy with inflammatory process in the dermis were identified. It was indicated by the dense lymphocyte infiltrating at the dermis-epidermis junction and some focal acanthosis. There was no Acid Fast Bacilli (AFB) found in the Fite Faraco staining (Figure 6).

The diagnosis of Sezary Syndrome was established, then the treatment using Methotrexate (MTX) 3x5 mg every 12 hours (one cycle) combined with Ciprofloxacin 2x500 mg daily and Chlorpheniramine Maleat (CTM) 3x4 mg daily as the antipruritic drug was started. Physiologic saline with 10% Dextrose and 20% albumin was administered through infusion. Emollients was also applied all over his body as a topical therapy.

After one cycle of MTX therapy (3 times 5 mg, every 12 hour), the general condition of the patient got worse. Blood culture showed a positive *Staphylococcus aureus* growth, the liver function test was abnormal and the patient passed away after 25 days being hospitalized due to sepsis.

DISCUSSION

Sezary Syndrome (SS) can mimic several mild dermatoses like dermatitis, psoriasis vulgaris, pityriasis lichenoides chronica, pityriasis lichenoides et varioliformis acuta, and actinic reticuloid.[2,4,10] These mild dermatoses can progress to erythrodermic skin lesions due to the extension of the plaques; thus it is spread all over the body. The SS patients were mostly 50-70 years old. The trias of SS are pruritic erythroderma, lymph nodes enlargement and Sezary cells in the blood circulation.[7,11,12]

Sometimes it is difficult to differentiate SS from erythroderma which is caused by other agents, especially in a case of severe skin desquamation. However, there is an exception in some a case where the infiltration is more dominant than the desquamation.[13]

This patient fulfilled those three criteria of SS with severe skin manifestations and many other systemic symptoms. The first differential diagnosis is psoriasis vulgaris due to the thick scales; however, based on the history of early lesions sites, it does not occur in the traumatic area like elbow, knee and shoulder. Clinically, the typical scales of psoriasis were not present and the Auspitz' sign was also negative. The second differential diagnosis is leprosy reaction, such as ENL reaction, but this can be excluded if there is by no cardinal signs of leprosy identified.

Both differential diagnosis could be excluded by histopathological examination because psoriasis vulgaris and leprosy have some specific histopathological patterns. The results of the histopathological examination for the thick scale revealed focal atrophy and acanthosis of the epidermis, as well as dense infiltration of lymphocytes in the dermo-epidermal junction and superficial dermis. The result of peripheral blood smear showed the presence of Sezary cells which ensures a definite diagnosis of Sezary Syndrome on this patient.

The staging system of Mycosis Fungoides is usually applicable for SS and the clinical manifestation of this case was classified as Grade 3. The malignancy related to this condition was identified prior to or after the diagnosis of MF and SS.[14]

Only a few data or reports on SS cases are available. Likewise, there are limited amount of treatment available in the clinical setting due to rarity of the case. Some topical treatments could be given for SS cases, such as nitrogen mustard, corticosteroids or bexarotene. The Ultra Violet treatment has been used to control the plaques; while the systemic treatment is used when topical therapy shows no progress.[3,8,9] Methotrexate (MTX) and other antimitotic drugs are the other available choices to manage SS.[14]

In this case, MTX was only applied for one cycle and had to be discontinued due to the clinically worsening condition of the patient and the disturbance of his liver function. The low immune level of the patient was the main factor in the fatal sepsis caused by *Staphylococcus aureus* infection. This kind of situation was also reported by Mirvish et al who worked on SS cases.[15]

In general, the prognosis of SS is not suitable with low remission and slow response to therapy, especially for the pruritus of the skin which influences the patient's quality of life. A guide of SS management has been proposed by US Cutaneous Lymphoma Consortium (USCLC) and modified by The National Comprehensive Cancer Network (NCCN).[11]

CONCLUSION

Sezary Syndrome (SS) is rare and relatively difficult to diagnose due to the similarities of the clinical manifestation with any skin diseases. The presence of lymphadenopathy, and atypical lymphocytes in the peripheral blood and the extensive skin involvement reflect the poor prognosis.

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Research Report

BETLE LEAF ESSENTIAL OIL FOR HEMOPHILIAC PATIENTS AND ITS ANTIBACTERIAL EFFECTS ON *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Betle leaf (Piper betle L.) is a medicinal plant. It contains essential oil and shows various biological activities, such as antibacterial, anticoagulant, etc. It is further reported to have low anticoagulant activities; thus, it is highly potential as a candidate for coagulant drug. Coagulant is used to prevent bleeding for patients with blood clotting disorders like hemophilia. In Indonesia, 1,236 people were reported with hemophilia. The standard parameters of anticoagulant activity are the freezing period and the compound concentrations. The purpose of this study was to determine the effect of betle leaf's essential oil on blood coagulation in patients with factor VIII and IX of blood plasma disorders. The isolation of essential oil is conducted through steam distillation method with two kinds of solvents, namely distilled water and n-hexane. The obtained n-hexane extract is then separated from the liquid-liquid extraction and rotary evaporator. Essential oil is diluted with citrate plasma solution. The test results of blood clots increase as the concentration of essential oils increase. The results are recorded as such: essential oils ½ times dilution of 99.67 seconds; ¼ times dilution of 127 seconds; 1/8 times dilution of 179 seconds; and 1/16 times dilution of 242.67 seconds. The test above proves that the piper betle extract possesses a coagulant activity. The ethanol extract contained in the piper betle could stimulate clotting in the blood cells. It is caused by the increase of blood plasma concentration which further escalate the plasma fluid into the blood cells. Based on this study, the activity of Mycobacterium tuberculosis can be obstructed by betle leaf in ½ times dilution. The extract significantly reduces acid which accelerates bacteria development.

***Keywords**: Betle Leaf, Liquid-Liquid Extraction, Blood Clotting, Coagulant, Anti-Mycobacterium tuberculosis*

ABSTRAK

Daun Sirih (Piper betle L.) merupakan tanaman obat. Daun sirih terdapat kandungan minyak atsiri dan menunjukkan berbagai aktivitas biologi, diantaranya adalah antibakteri, antikoagulan, dan lain sebagainya. Di Indonesia, jumlah penderita hemofilia dilaporkan 1.236 orang. Koagulan digunakan untuk mencegah pendarahan pada penderitan kelainan pembekuan darah seperti hemofilia. Daun sirih dilaporkan memiliki aktivitas antikoagulan rendah, sehingga sangat potensial untuk kandidat obat koagulan. Parameter standar untuk aktifitas antikoagulan adalah waktu pembekuan dan konsentrasi senyawa. Tujuan dari penelitian ini adalah mengetahui pengaruh minyak atsiri daun sirih terhadap pembekuan darah pada penderita kelainan faktor VIII dan IX plasma darah. Isolasi minyak atsiri dilakukan dengan metode destilasi uap menggunakan dua macam pelarut yaitu aquades dan n-heksana. Ekstrak n-heksana yang diperoleh dipisahkan dengan ekstraksi cair-cair dan rotary evaporator. Minyak atsiri didilusi dengan larutan plasma sitrat. Hasil uji pembekuan darah minyak atsiri meningkat seiring konsentrasi minyak atsiri yaitu pengenceran 1/2 kali 99.67 detik; pengenceran 1/4 kali 127 detik; pengenceran 1/8 kali 179 detik; dan pengenceran 1/16 kali 242.67 detik. Pengujian di atas menunjukkan bahwa ekstrak piper betle memiliki aktivitas koagulan. Ekstrak etanol yang terkandung dalam piper betle dapat menyebabkan pembekuan dalam sel-sel darah. Hal ini disebabkan konsentrasi plasma darah naik, yang meningkatkan cairan plasma ke dalam sel darah. Berdasarkan penelitian ini, aktivitas Mycobacterium tuberculosis dapat dihambat oleh ekstrak daun sirih pada pengenceran 1/2 kali. Ekstrak secara signifikan mengurangi sifat asam yang dapat mempercepat perkembangan bakteri.

***Kata kunci** : Daun Sirih, Ekstraksi Cair-Cair, Pembekuan Darah, Koagulan, Anti-Mycobacterium tuberculos*

INTRODUCTION

Indonesia has a tropical climate suitable to grow various medicinal plants, one of them is Betle leaf (Piper betle L.).[1] Indonesian people who live in rural areas particularly use betle leaf to cure various diseases. A part of the leaf is mainly used for some health treatments, such as nosebleed (epistaxis). The leaf is rolled up and put into one's nostrils.[2] Moreover, betle leaves can also be used as a mouthwash. A dried betle leaf can also be used as a traditional medicine, such as cough medicine, drugs, or eye wounds. Betle is a chemical plant which consists of saponins, flavonoids, polyphenols, and essential oil.[3] There is an increase usage of natural materials through a large scale of fabrication. The use of traditional medicine is considered having fewer side effects compared with the chemical drugs and more affordable.[4] Modern drug is widely believed to cause spasm of the bile duct sphincter and impede bile flow; whereas the effect on renal development receives less attention.[5]

Meanwhile, hemophilia is a hereditary disorder which is heavily associated with a deficiency or an abnormality of biological factor VIII and factor IX in blood plasma.[6] This genetic disorder affects many people. In Indonesia, the number of hemophiliac was reported at 1,236 people.

The contents of the essential oil in a betle plant are chavicol, eugenol, cineol, and carvacrol. Essential oils functions as antibacterial, antioxidant, antifungal, anti-ulcerogenic, anti-amoebic, anti-inflammatory, anti-filarial, anti-microbial, anti-fertility, anti-hyperglycemic, anti-dermatophytid, anti-naceptive, and radioprotective properties.[1] Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis,* is a highly infectious disease. Its morbidity and mortality continue being a cause of concern. There has been a substantial increase in these last decades in the investigation of medicinal plants to find out their biological efficacies for the treatment of various disorder. In the field of anti-TB agents, several studies on potential medicinal plants have been reported from various parts of the world. P*iper nigrum* extract, a combination of acetone and ethanol extracts of 50 µg/ml each, was effectively tested against anti-*Mycobacterium tuberculosis*.[7] The antibacterial activity from the plant is caused by secondary metabolic compounds with phenolic compounds. This study chooses betle plant leaves as the research object, as it is often used as nosebleed cure.[8] This study uses *Piper betle L.* species from Jajar village, Kediri District, East Java, Indonesia.

On the description above, the researchers look for a new solution by leveraging the existing knowledge which increases the potential betle leaf's essential oil extracts (Piper betle L.) on hemophiliac patients. The purpose of this study is to determine the effects of betle leaf's essential oil on hemophiliac patients in vitro using clotting time method and study of anti-*Mycobacterium tuberculosis* activity.

MATERIAL AND METHOD

Betle Leaf's Essential Oil Extraction

Betle leaf's essential oil is isolated using steam distillation technique. Prior to the first steam distillation, the betle leaves are cut into small pieces to facilitate the distillation and insulate the essential oil inside the betle leaf. Solvents are used for the distillation. Time required to isolate the essential oil is about 2 hours until the solution in the distillation equipment condenser becomes colorless. The color indicates that the essential oil has been all isolated.

Isolation is separated between water phase and organic phase using liquid-liquid extraction with an organic n-hexane solvent. The extraction is performed 5 times to perfectly separate the essential oil in the water. The essential oil will be mixed with the n-hexane solvent.

The last phase of separation is conducted using a rotary evaporator. The essential oil in n-hexane is separated using the principle of boiling point. The heating process is carried out at approximately 60 to 70°C. N-hexane's boiling point is recorded at 63°C in which it is still in the form of gas; while the essential oil remains in liquid form due to the extremely high boiling point of the volatile oil. The heating process produces two products, namely n-hexane and pure essential oil of betle leaf. The essential oil obtained in this isolation is 4.5 mL with a percentage of 0.9%. This is because the properties of essential oil is volatile, thus, it reduces their products. Volatile chemical compounds have a high vapor pressure at ordinary room temperature.[6]

Extract Dilutions

Extract dilutions is conducted using PZ solution (saline), because this solution is deemed to have the same osmotic pressure with the fluid contained in human body. Dilution is done by extracting the essential oil of betle leaf as much as 1 cc, added with 1 cc solution of PZ, then ½ times dilution of the extract concentrated essential oil is obtained. A quarter times, 1/8 times, and 1/16 times dilutions are also conducted to determine the most effective dilution to speed up blood clotting.[6]

Separation of Plasma from Red Blood

Twenty-five cc blood from normal individual is mixed with 3.8% sodium citrate in 9:1 ratio; then, the mixture is put in a tube of blood plasma and made sure it is perfectly blended. The mixture is centrifuged for about 30 minutes at 1500 rpm. Tubes are excluded from clinical centrifuges. At the top of the tube, there is clear yellowish liquid; while at the bottom, red sediment can be seen. The clear liquid, which is called citrate plasma, is then extracted and stored in a refrigerator.

Control Solution

Citrate plasma of 0.8 ml for each blood group is mixed with 0.2 cc PZ solution, then they are shaken and left for some time to mix. Next, 0.2 cc and 0.2 cc plus $CaCl_2$ are taken for control solution until the first fiber is formed. The fiber is in the form of white threads called fibrin.

Blood Coagulation Experiment using the Essential Oil of Betle Leaf

The 0.8 cc citrate plasma solution is added to 0.2 cc betle leaf's essential oil extract. After making sure it is blended well, 0.2 cc from the mixed solution is added to 0.2 cc $CaCl_2$. The researchers then observe and record the freezing time. The same procedures are performed to ½ times, ¼ times, 1/8 times, and 1/16 times dilutions. Citrate plasma uses all blood types. The experiments are performed at 37°C.

Anti-*Mycobacterium tuberculosis* test

M*ycobacterium tuberculosis* refers to the strain H37Rv. The preparation for medium 7H10 was dissolved with aquadest, then autoclaved in 121°C for 10 minutes. Medium 7H10 is added with essential oil and incubated for 4-3 weeks at 37°C in a CO_2 incubator. The tested essential oil concentrations consist of ½ times dilution, ¼ times dilution, 1/8 times dilution, and 1/16 times dilution.

RESULT AND DISCUSSION

Blood plasma is the most important [object] in this research, because it consists of plasma proteins which have a big impact on blood clotting. The blood plasma was separated by a mixture of blood centrifuged between normal and 3.8% sodium citrate. This study uses the normal blood group B. Therefore, sodium citrate anticoagulant of 3.8%, which slows down clotting process, is added to make the normal blood to have the same nature with the blood with clotting factor disorders. Although both citrate and heparin are used as anticoagulants during apheresis, citrate is preferred for most exchange procedures because of its safety and effectiveness.[9] The 9:1 (blood to sodium citrate 3.8%) ratio is used for the anticoagulants as an ideal comparison, because the anticoagulation in a greater portion of blood clotting takes longer processing time.[10] The plasma from the reaction above is called citrate plasma (Figure

The control solution is used as a comparison to the blood clotting process using betle leaf's essential oil. A control solution is considered as successfully made if the color is brownish yellow (Figure 2).

Blood clotting test is conducted by mixing the essential oil dilution with plasma citrate in four different reaction tubes. A solution of $CaCl_2$ is then added into the mixture. The fourth solution is formed in yellow-brown color with different intensities of concentration. The color intensity for the solution concentration of essential oil with ½ times dilution is higher or more concentrated than the essential oil

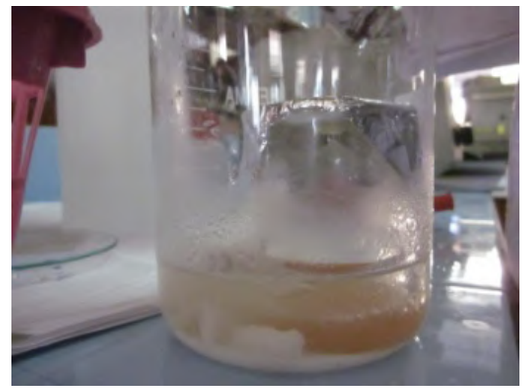


Figure 1. Citrate plasma

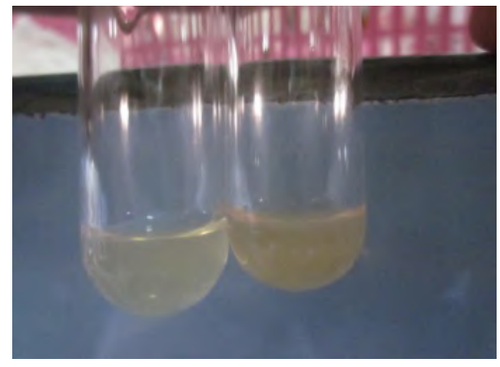


Figure 2. Control solution

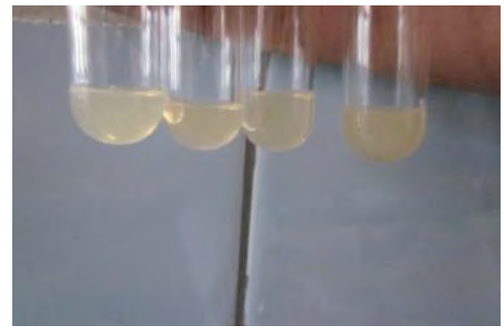


Figure 3. Dilution of essential oil with citrate plasma

with 1/16 times dilution. This solution results in the same color with the earlier control solution (Figure 3-4).

Blood clotting mechanism cannot be shown directly in this study, as this study is only conducted in vitro; however, there are some visual data obtained. $CaCl_2$ solution acts as the activation for prothrombin. The study is conducted at 37°C, matching the temperature of human body. The result of blood clotting test is showed in Table 1.

ANOVA test is then performed to analyze the results. A significant difference between four conditions of essential oil is obtained (F-count is recorded at 232.69, greater than the F-table at 4:07).

Figure 4. Freezing blood test with essential oil

Table 1. The results of blood clotting in seconds

Concentration of Essential Oil	Time (second)
1/2 times	99.67
1/4 times	127
1/8 times	179
1/16 times	242.67

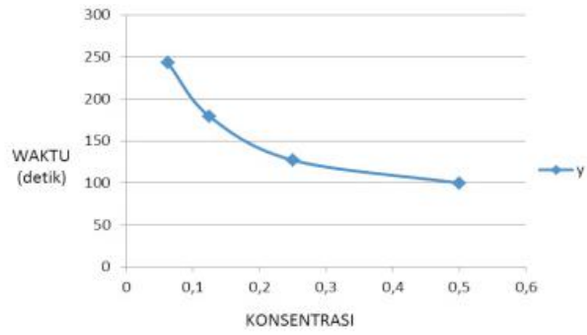


Figure 5. Curve blood clotting in betle leaf's essential oil

According to Table 1, the frozen blood from each donor was shown in time difference. Normal blood clotting occurs from 3 to 18 minutes.[11] Blood clots occurred because plasma protein prothrombin is changed into thrombin. Thrombin is an enzyme which catalyzes the forming of fibrinogen. It is a soluble protein which changes into fibrin. In a few second, fibrin polymerized a mesh which is composed by some fibrin threads. The thread runs to every direction and forms a net which catches blood element and forms a clot.[12]

Based on blood clotting curve of the betle leaf's essential oil, it can be concluded that the higher the concentration is, the faster the blood clotting process takes place.[13] The test results of blood clots increase as the concentration of essential oil increases. The results are as follows: essential oils ½ times dilution for 99.67 seconds; ¼ times dilution for 127 seconds; 1/8 times dilution for 179 seconds; and 1/16 times dilution for 242.67 seconds. This research successfully demonstrates that the essential oil of betle leaf can be used as blood clotting. The betle leaf is known to have antibacterial activity. Staphylococcus aureus' activity was inhibited by betle leaf in 200 mg/ml concentration. The extract is found to significantly reduce acid production of the bacteria.[14] The test above indicates that the piper betle extract possesses the coagulant activity. Blood clotting is a complex procedure which involved numerous factors in the plasma and tissues. Both intrinsic and extrinsic pathways play vital roles. Inhibitors of the blood coagulation affect some factors in blood (Figure 5).[15]

The chemical components of betle leaf's essential oil are monoterpenes, sesquiterpenes, alcohols, esters, aldehydes, and phenols.[16] According to Tangkery in 2013, ethanol causes clotting in the blood cells to stick to each other; however, the red blood cells, or erythrocytes, no longer have any forms, because the cell's wall has been destroyed. It is caused by the increase of blood plasma concentration which further escalates plasma fluid into the blood cells.[11]

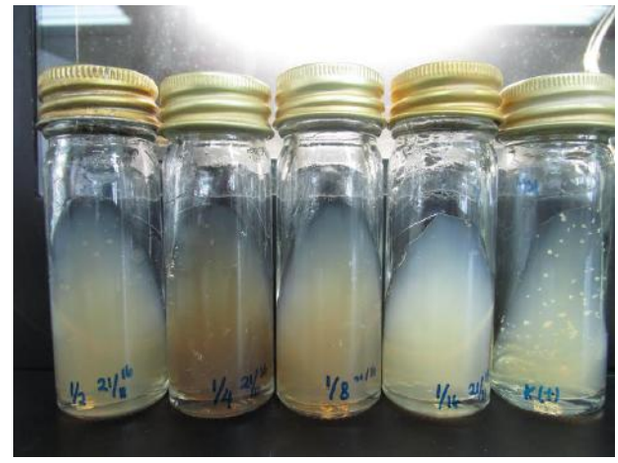
Anti-*Mycobacterium Tuberculosis* Test

Amidst the emerging drug resistance in infectious diseases field, the use of medicinal plants provides an alternative therapy. Unfortunately, there are limited report on the anti-*Mycobacterium tuberculosis* in Indonesian medicinal plants. Essential oil from *Piper betle L.* was shown to have anti-*Mycobacterium tuberculosis* activity (Figure 6).

The essential oil with 1/2 times dilution concentration demonstrates an inhibitory activity against *Mycobacterium tuberculosis*. It proves its effectiveness for the inhibited activity of *Mycobacterium tuberculosis*. Some drops of fungal are shown in the ½ times dilution bottle, however, there are no bacteria showed.

M*ycobacterium tuberculosis* and fungal are shown in ¼ times dilution, 1/8 times dilution and 1/16 times dilution. Non-active essential oil and activity of *Mycobacterium tuberculosis* are demonstrated in the lower concentration of the essential oil.

Meanwhile, piperine is an active compound in *Piper belte L.* extract. In the literature, piperine of 1.0 and 10 µg/ml showed an up-regulation of IFN-γ and IL-2 production in *Mycobacterium tuberculosis*. An effective immune-stimulant can complement the host cellular immune response by specifically inducing the type 1 (Th-1) response.[17] In



1/16 times 1/8 times ¼ times ½ times

Figure 6. Culture of *Mycobacterium tuberculosis* containing essential oil from *Piper betle L.*

this regard, the key cytokine in mice and humans seems to be gamma interferon (INF-γ) which activates bactericidal effector mechanisms in the mycobacterial host cell, the macrophage.[18] Piperine (1 mg/kg) in mice which are infected with *Mycobacterium tuberculosis* activates the differentiation of the T cells into Th-1 sub-population ($CD4^+/CD8^+$ subsets).[17] Protective immunity against *Mycobacterium tuberculosis* requires the generation of cell-mediated immunity. The secretion of Th-1 cytokines by antigen-specific T cells plays an important role in protective granuloma formation and stimulates the antimicrobial activity of the infected macrophages.[19]

CONCLUSION

The dilution of the betle leaf's essential oil extraction at ½ dilution have the most rapid blood clotting effectiveness for hemophilia treatment in vitro. It is caused by the capability of ethanol compound in the betle leaf's extract to yield clotting in blood cells. The blood clot further increases blood plasma concentration and plasma fluid in blood cells. Plasma fluid is an important component for blood clot factor, because it contains prothrombin and fibrinogen. Betle leaf extract provides anti-infection activity, mainly against *Mycobacterium tuberculosis*.

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Case Report

BACTERIAL COLONY GROWTH IN THE VENTILATOR CIRCUIT OF THE INTENSIVE OBSERVATION UNIT AT RSUD DR. SOETOMO SURABAYA

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ABSTRACT

Ventilator-associated pneumonia (VAP) remains a problem with the highest cos, morbidity and mortalityt in the Intensive Care Unit (ICU). The correlation between mechanical ventilation and pneumonia is considered as common sense, yet scientific evidence to support this statement is still needed. This research aims to analyze the bacterial colony grows in mechanical ventilation circuit and those grew in the patient's sputum culture. We performed an observational study. Samples for bacterial culture were taken from ventilator circuit and patient sputum on Day-0, Day-3 and Day-7.. Sputum samplings are collected using double catheter tracheal aspiration technique; Results are then analyzed with Chi-square test. While the similarity of bacteria species in ventilator circuit to patient's sputum is analyzed with Binomial test. Two samples are dropped out immediately due to the rate of bacterial growth on Day-0. Bacterial colony growth in ventilator circuit shows a significant difference on Day-3 and Day-7 at 50% and 92% respectively (p = 0.05). A comparison for the bacterial similarity of the ventilator circuit and patient's sputum shows that the bacterial growth on Day-3 is 7 out of 14 (50%) and 3 with more than 10^5 CFU/ml colony; while on Day-7, there are 13 out of 14 positive bacterial growth, both in the circuit and the patient's sputum. Among them, 5 out of 14 (35%) of the bacterial colony which grow in the circuit have the same species as those grow in patient's sputum. The recent study shows that there is bacteria colony growth in the ventilator circuit after Day-3 and a significant increase on Day-7. Almost half of the colony illustrates similar species from both ventilator circuit and patient's sputum. This suggests that the bacterial growth on Day-7 in the ventilator circuit might be related to those growth in patient's sputum.

Keywords: *Ventilator Circuit, VAP, Bacterial Colony, Bacteria species*

ABSTRAK

Ventilator-associated pneumonia (VAP) masih menjadi problematik perawatan pasien di ICU dan menghabiskan biaya yang besar. VAP menyebabkan morbiditas dan mortalitas yang tinggi. VAP spesifik untuk infeksi nosokomial yang terjadi pada pasien yang mendapat ventilasi mekanik. Hubungan antara sirkuit ventilasi mekanik dengan terjadinya infeksi paru sudah dianggap sebagai suatu fakta, walaupun tanpa bukti ilmiah. Penelitian ini bertujuan untuk menganalisa pertumbuhan koloni bakteri pada sirkuit ventilator yang digunakan pada pasien di ruang observasi intensif RSUD Dr. Soetomo Surabaya. Penelitian ini menggunakan analisis observasional. Kultur bakteri diambil dengan menggunakan swab pada bagian inspirasi dari sirkuit ventilator pada 16 pasien yang dirawat dengan ventilasi mekanik di ruang observasi intensif RSUD Dr. Soetomo Surabaya. Sirkuit ventilator dilakukan swab pada hari ke-0, ke-3 dan ke-7, kemudian dilakukan perhitungan . pengambilan sampling sputum menggunakan tehnik double catheter tracheal aspiration. Hasil kemudian dianalisa menggunakan uji Chi square dan kesamaan spesies bakteri pada sirkuit dengan sputum pasien dianalisa dengan uji binomial. 2 sampel drop out karena terdapat pertumbuhan koloni bakteri pada hari ke-0. Terdapat perbedaan yang signifikan pertumbuhan koloni bakteri pada sirkuit ventilator hari ke-3 dan ke-7, 50% dan 92% dengan nilai p=0,05. Terdapat pertumbuhan koloni bakteri pada sirkuit ventilator 7 dari 14 sampel (50%) pada hari ke-3 dan 3 dengan jumlah koloni > 10^5 CFU/ml, sedangkan pada hari ke-7 terdapat pertumbuhan koloni 13 dari 14 sampel baik pada sirkuit ventilator maupun pada sputum pasien. Dan 5 (35%) diantaranya memiliki kesamaan spesies bakteri yang tumbuh pada sirkuit ventilator maupun pada sputum pasien. Penelitian ini menunjukkan ada nya pertumbuhan koloni bakteri setelah hari ke-3, dan terjadi peningkatan jumlah koloni yang signifikan pada

hari ke-7. 50% dari pertumbuhan koloni ini menunjukkan kesamaan spesien bakteri antara sirkuit ventilator dan sputum pasien. Hal ini menunjukkan ada kemungkinan pertumbuhan koloni bakteri pada harike-7 berkorelasi dengan kultur sputum pasien.

Kata kunci : *Sirkuit ventilator, VAP, Koloni Bakteri, Spesies bakteri*

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection which occurs at the Intensive Care Unit (ICU) and plays an important role in increasing the number of mortality and morbidity. VAP specifically occurs in patients who are treated with mechanical ventilation. VAP which occurs in the first 48-72 hours after intubation is categorized as an early onset of VAP. It might be caused by aspiration as a complication during endotracheal tube insertion. VAP which occurs for more than 72 hours is identified as the late onset of VAP. It is directly correlated with mechanical ventilatory support.[1–4]

VAP as the most commonly occurs as nosocomial infection with 9%-40% incidence rate might prolong hospitalization (2-3x longer), prolongs the length of stay in ICU for 5-7 days, increases health cost, and increases mortality rate to 15-45%. VAP's cost is estimated to increase about $40,000 per patient and around $1.2 billion annually. Previous researches showed that pneumonia infection rate obtained in the hospital (nosocomial) was 15% of all hospital nosocomial infection and 24-27% of it occured at the ICU.[1,2]

Various researches about risk factors related to VAP (such as age, sex, trauma, COPD) and the use of mechanical ventilation have been done several times. Understanding the pathogenesis and epidemiology of VAP is important to formulate a preventive strategy to fight this infection. The most common source for VAP endemic is oropharynx colonization which is caused by endogenous flora or even pathogen flora from ICU environment; especially from the hand of the healthcare workers, contaminated mechanical ventilation, and the air and the water at the ICU. The digestive tract is also a potential source of secondary colonization and gram-negative of nosocomial bacteria storage. Microorganism aspiration from the oropharynx, gaster, and tracheal secretion around balloon of endotracheal tube towards lower respiratory tract, which are supposed to be sterile in normal condition, are the most common endemic source of VAP. Epidemically, VAP is mostly caused by contaminated ventilation, bronchoscopy, inhalation drugs, water (eg: Legionella sp.), and air (eg: Aspergillus sp.). A strategy to combat microbes from the oropharynx and digestive tract is by using chlorhexidine as an oral treatment, antimicrobial prophylaxis for inhalation drugs, or sucralfate as prophylaxis stress ulcer. Moreover, aspiration prevention through patient positioning and continuous suction in the subglotic area are also proven to decrease VAP risks. Disinfection of mechanical ventilation circuit and bronchoscopy, legionella-free hospital water, and infection control from the medical aerosol are important for VAP prevention. Routine inspection towards VAP as an early detection is important.[1,3–5]

The correlation between mechanical ventilation circuit and pulmonary infection is stated as a fact, however, there is no scientific explanation about it. Ting-Chang Hsieh did a cohort of observational study towards 96 patients who were divided into 2 groups; Group 1 underwent circuit replacement every 3 days, while Group 2 underwent circuit replacement every 7 days. Though there was no statistically significant difference, an increasing number of VAP incidence was observed. There was 13% in Group 1 and around 16% in Group 2; besides, there was an increase in mortality rate at 22% in Group 1 and 36% in Group 2. Other researches showed that the main cause of pneumonia in mechanical ventilation users was the colonization of the gastrointestinal tract, followed by aspiration around balloon of the endotracheal tube, and bacteria-transmitted health products.[5,6]

Although there is no research about VAP number in Indonesia, based on the worldwide database, it is stated that VAP incidence is high. The correlation between the mechanical ventilation and the occurrence of VAP is stated as a fact, because the mechanical ventilation circuit is proven to be contaminated by pathogen bacteria, especially from the patient's secretion.

At the Intensive Observation Unit of RSUD Dr. Soetomo, there is no procedure about the continual replacement of ventilation mechanic circuit, thus, it might become a primary cause of VAP infection there. This study is conducted to understand the bacterial colony growth in mechanical ventilation circuit at the Intensive Observation Unit of RSUD Dr. Soetomo and expected to standardize the procedure of mechanical ventilation circuit replacement at the Intensive Observation Unit of RSUD Dr. Soetomo.

METHOD

This study employs observational analysis design in time series (3x measurement) with all patients at the Intensive Observation Unit of RSUD Dr. Soetomo as the research subject. The inclusion criteria of this research are the patients who are using mechanical ventilation and exhibit no pneumonia sign and symptom previously based on CPIS criteria. The research consents are obtained from the family members to comply with the Medical Research Ethical regulation from RSUD Dr.Soetomo. While the exclusion criteria are patients with underlying diseases (COPD, ARDS, neuromuscular disease) and those who refuse to participate in this experiment. The drop out criteria are the patients with less than 7 days of extubation, died

in less than 7 days, and develop bacterial growth within 48 hours.

The methods exercised in this research aim to understand the growth of bacteria using bacterial colony measurement. Measuring bacterial colony can be done using pour plate method. Colony Counter is also used to facilitate the accurate calculation of the colony. The sample is taken in ventilator circuit on Day-0 before the circuit is connected to the patient, then it is re-done on Day-3 and Day-7 after the ventilator application. After bacterial colony is being calculated, it will be scored. Besides calculating the bacterial growth in different days, the bacterial culture is performed to study the bacterial species in patient's sputum. All subjects are treated with usual standardized VAP prevention. T-test analysis is also conducted to understand the difference between various colony growth on different days (Day-0, Day-3, and Day-7) for the patients who undergo mechanical ventilation treatments.

RESULT AND DISCUSSION

From 16 samples, 2 samples are dropped out due to bacterial growth on Day-0. The characteristic analysis and data distribution illustrate that male respondents are recorded in 42.9% and female in 57.1%.

Various colony growth is obtained from the sample. The data is categorized into 3 groups, namely a sterile group, a group with the colony number less than 105 CFU/ml, and a group with colony number more than 105 CFU/ml. Table 5.2 explains about the observed bacterial growth on Day-3 in which the number of sterile group is 7 (50%), while the less than 10^5 CFU/ml bacteria colony has 7 samples (50%). On Day-7, the number of sterile group is 1 sample (7.1%); the less than 10^5 CFU/ml bacterial colony has 10 samples (71.5%); and the more than 10^5 CFU/ml bacterial colony has 3 samples (21.4%).

Table 2 and Figure 1 illustrate the bacteria species growing in ventilator circuit on Day-3 are derived from 5 samples of Acinetobacter spp. (35.7%); 1 sample of Pseudomonas aeruginosa (7.1%); 1 sample of Klebsiella pneumonia (7.1%); and 7 samples with no bacteria growth (50%). On the 7th day, 7 samples (50%) of Acinetobacter spp.; 3 samples (21.4%) of Klebsiella pneumonia; 1 sample of Pseudomonas aeruginosa (7.1%); 2 samples of enterobacter aerogenes (14.3%); and 1 sample with no bacteria colony growth.

Table 1. Bacterial colony growth of the ventilator circuit on day 3 and day 7

Counted Bacterila Colony in the Ventilator Circuit	Observation Time	
	Day 3 (%)	Day 7 (%)
Steril	7 (50,0)	1 (7,1)
< 10^5 CFU/ml	7 (50,0)	10(71,5)
≥ 10^5 CFU/ml	-	3(21,4)

Table 2. Bacterial species in the ventilator circuit and sputum culture of the patient on day 3 and day 7

Bacterial Species	Ventilator Circuit		Sputum Culture	
	Day 3 (%)	Day 7 (%)	Day 3 (%)	Day 7 (%)
Steril	7(50,0)	1(7,1)	8(57,1)	1(7,1)
Acinobacter spp	5(35,7)	7(50,0)	5(35,7)	11(78,6)
Klebsiella pneumonia	1(7,1)	3(21,4)	-	-
Pseudomonas aeruginosa	1(7,1)	1(7,1)	1(7,1)	2(14,3)
Enterobacter aerogenes	-	2(14,3)	-	-

Furthermore, Table 2 and Figure 2 also displays the bacterial culture results of patient's sputum on Day-3, namely 5 samples of Acinetobacter sp (35.7%); 1 sample of Pseudomonas aeruginosa culture (7.1%); and 8 samples with no bacteria growth (57.1%). On the 7th day of evaluation, 11 samples of Acinetobacter sp. (78.6%); 2 samples with Pseudomonas aeruginosa culture (14.3%); and 1 sample with no bacterial growth (7.1%) are obtained.

Table 3 demonstrates data recapitulation about bacterial colony growth in ventilator circuit on Day-3 and Day-7. According to the data, Chi-square statistic test is done to understand the significant difference in bacterial colony growth in different days.

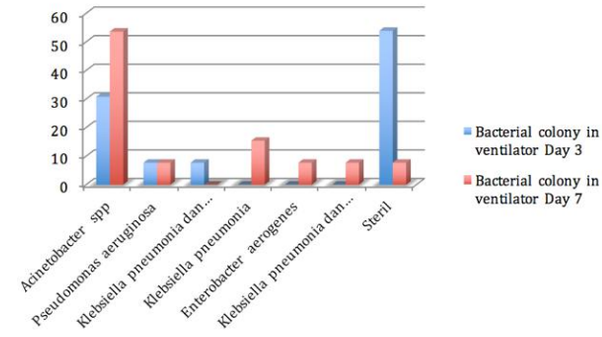

Figure 1. Bacterial species in the ventilator circuit on day 3 and day 7

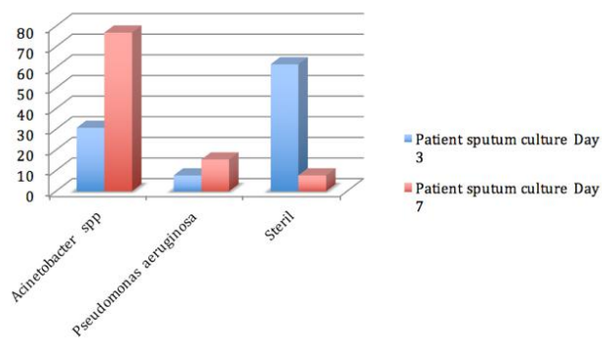

Figure 2. Patient Culture on Day 3 and Day 7

Table 3. Comparison test bacterial colony growth in the ventilator circuit on day 3 and day 7

Day 3	Day 7		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	6 (42,9)	1 (7,1)	0,429	0,049
Negative Result (%)	3 (21,4)	4 (28,6)		

Table 4. Binomial test for similarity bacterial species between ventilator circuit and patient's sputum culture on day 3

Ventilator Circuit	Sputum Culture		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	4 (28,6)	1 (7,1)	0,689	0,010
Negative Result (%)	1 (7,1)	8 (57,1)		

Table 5. Binomial test for similarity bacterial species between ventilator circuit and patient's sputum culture on day 7

Ventilator Circuit	Sputum Culture		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	4 (28,6)	1 (7,1)	0,689	0,010
Negative Result (%)	1 (7,1)	8 (57,1)		

Table 6. Correlations between bacterial colony growth in the circuit ventilator with CPIS >6

CPIS 3 Day 3	CPIS 7 Day 7		CPIS 3 Day 3 and CPIS 7 Day 7
	Negative	Positive	
Negative	4	7	13
Positive	0	2	0.016

The statistic shows that there is a significant difference between bacterial growth in Day-3 and Day-7 (p = 0.049).

Table 4 shows that the observation on Day-3 found 7 samples with no bacterial colony in ventilator circuit or in patient's sputum (50%); 3 samples with similar bacterial species (21.4%); and 4 samples with no similar bacteria species in both ventilator circuit and patient's sputum (28.6%). Based on the statistical analysis using binomial test, there is a significant difference between both groups on Day-3 (p = 0.01).

Table 5 shows the observation on Day-7, it proves that there are 7 samples containing similar bacterial species in the ventilator circuit and patient's sputum (50%) and 7 samples without similar bacterial species in both locations. After being statistically tested using binomial test, the bacterial species in ventilator circuit and the patient's sputum on Day-7 show no significant difference (p = 0.515).

The correlation analysis of bacterial colony in ventilator circuit (CPIS> 6) is also tested in this research. Table 5.6 shows that during the Day-3 observation, there are 2 patients with CPIS more than 6; while during the Day-7 observation, there are 12 patients with CPIS more than 6. Using binomial test, it is proven that there is no significant difference in the amount of bacterial colony in ventilator circuit (CPIS> 6) on patients who undergo mechanical ventilation treatment.

Based on this research's comparative study, it is found that there is a significant difference between bacterial colony growth on Day-3 and Day-7 (p = 0.049). Bacterial colony growth in ventilator might be caused by many factors, such as humidifier which produces microorganism-carrying water drops, thus, the water inside the humidifier might be contaminated as well. Therefore, the water refill in humidifier should be done aseptically and use sterile fluid. The condenser in ventilator circuit might also contaminate the ventilator circuit, if the drainage is conducted thoroughly. Around 1980, there was a high risk of infection related to the contaminated nebulizer reservoir. A drug used by nebulizer might contaminate the ventilator circuit and penetrate into patient's respiratory tract. The contamination suffered by some health workers may also play a role in ventilator circuit contamination; therefore, all treatments (such as sterilization, ventilator circuit formation, and ventilator circuit maintenance) must be performed aseptically.[1,6–8]

The increase of bacterial colony significantly on Day-3 to Day-7 in this research still cannot predict the main role of ventilator circuit in VAP contamination, because of the VAP's complex pathogenesis and various factors possibly cause VAP. However, the bacterial colony growth in ventilator circuit still cannot be ignored, because the colony growth in ventilator circuit might penetrate the lung tissue through inhalation and further cause pneumonia. Based Long and Fink's researches, the suspension of maintenance and ventilator circuit replacement from once a day to twice a day might decrease VAP occurrence. While CDC and The Healthcare Injection Control Practices Advisory Committee have issued some guidelines for preventing health care-associated pneumonia, ventilator circuit replacement is recommended to be conducted only if the circuit is dirty or malfunctioned. Other research shows that ventilator circuit replacement over 48 hours is safe and might not increase VAP prevalence .

Table 7. Result of ventilator circuit and sputum culture

	Day 0			Day 3			Day 7		
	Bacterial colony growth in the circuit ventilator	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**	**Bacterial colony growth in the circuit ventilator**	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**	**Bacterial colony growth in the circuit ventilator**	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**
Patient 1	Steril	-	Steril	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp
Patient 2	Steril	-	Steril	45 CFU/ ml	Acinetobacter sp	Acinetobacter sp	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 3	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Pseudomonas aeruginosa
Patient 4	Steril	-	Steril	75 CFU/ ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp
Patient 5	Steril	-	Steril	100 CFU/ ml	Acinetobacter sp	Pseudomonas aeruginosa	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 6	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 7	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp
Patient 8	Steril	-	Steril	Steril	-	Steril	25 CFU/ ml	Enterobacter aerogenes	Steril
Patient 9	Steril	-	Steril	Steril		Steril	Steril	-	Acinetobacter sp
Patient 10	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp	12.2 × 10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp
Patient 11	Steril	-	Steril	Steril	-	Steril	100 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 12	Steril	-	Steril	100 CFU/ ml	Pseudomonas aeruginosa	Steril	12.5 × 10^5 CFU/ ml	Pseudomonas aeruginosa	Acinetobacter sp
Patient 13	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Pseudomonas aeruginosa
Patient 14	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Enterobacter aerogenes	Acinetobacter sp

CFU : Colony Form Unit

This research is expected to inform the readers about the treatment and ventilator circuit replacement at the ICU. The similarity test, which is conducted to analyze bacteria growth in ventilator circuit and patient's sputum on Day-3, indicates a significant difference (p = 0.010). It might be caused by the complex pathogenesis of VAP. Therefore, the bacteria growth might be caused by many factors. The bacteria in patient's sputum might come from the digestive tract colonization of the pathogenic microorganism and increase the chance of contaminated secrete aspiration to lower respiratory tract; further, it might also colonize the oropharynx mucosal surface.[1,3,4] Optic fiber bronchoscopy, tracheal mucous suction, and respirator might induce pathogen bacteria contamination into the lower expiratory tract. The bacterial colony in ventilator circuit might be coming from the healthcare worker's hand contamination, water, air, or even patient's own secrete. Bacteria test similarity shows that the bacteria growth in ventilator circuit and patient's sputum culture on Day-7 has no significant difference (p = 0.515). From the Day-7 of observation, there is no proof whether the bacterial colonization of the patient's respiratory is coming from the ventilator circuit or not. The bacterial colony growth in patient's sputum reaches 50% of the total sample. It might be caused by the microorganism mapping at the ICU itself. Meanwhile, the bacteria commonly found in the ventilator circuit, humidifier, and respirometer are *Acinetobacter calcoaceticus, Burkholderia cereus,* and *Pseudomonas aeruginosa*.[1]

In this research, there is no conclusion can be drawn on whether bacterial colonization in patient's sputum is related to

the bacterial colonization in the ventilator circuit. Those factors may affect this research's results. This research also analyzes the correlation of the bacterial colony in ventilator circuit with CPIS criteria more than 6 on the patient with mechanical ventilation help and diagnosed as pneumonia. This research shows no significant correlation between bacterial colony size in ventilator circuit with CPIS > 6 with the help of mechanical ventilation, stated by p=0.016.

On the third day of observation, there are 2 patients with CPIS>16; while on the 7th day of observation, there are 13 patients with CPIS> 6 out of 14 patients in this research's sample. The early onset of VAP might be observed in less than 4 days and the late onset of VAP can be observed over 4 days after the patient is treated with mechanical ventilation. Pathogen which causes pneumonia on the early onset of VAP and late onset of VAP is usually different. The early onset of VAP mostly has a good prognosis. Moreover, the early onset of VAP might be caused by *Haemophilus influenzae* and *Streptococcus pneumonia*; while the late onset of VAP might be caused by high-risk pathogens, such as *Pseudomonas aeruginosa, Acinetobacter spp.,* and *Stenotrophomonas rnaltophilia*. As stated by Kollef, these high-risk pathogens record a high mortality rate (65%) compared to the late onset of VAP which is caused by other pathogen microorganisms (31%). Besides the contaminated ventilator circuit, there are various risk factors causing VAP, such as surgery. Post-surgery patient is exposed to higher rate of VAP occurrence. Based on American Society of Anesthesiologist, the risk factors of pneumonia post surgery include smoking, long inpatient treatment, longer surgery operation, thorax, and upper abdomen surgery.[8–10]

The irrational antibiotic usage also plays a role in the occurrence of nosocomial pneumonia and the infection of antibiotic resistant microbes. Stress ulcer prophylaxis also influences bacterial colonization in the digestive tract. The prophylaxis stress ulcer which is unable to change gastric pH have a lower bacterial colonization rate and in turn, may lower the pneumonia nosocomial rate as well. Endotracheal tube, tracheostomy, and reintubation may also lower patient's immune system and further causes local trauma and inflammation. It can increase the probability of pathogen bacterial aspiration from the oropharynx area, specifically around the cuff. Oropharyngeal secretion's continuous suction, elevated head position, the use of nasogastric tube, and enteral feeding play a part in VAP occurences.[9,10]

CONCLUSION

The recent study shows that there are bacterial colony growth in the ventilator circuit after Day-3 and significant increase in the number of colony on Day-7. Almost half of the colony displays similar species in both ventilator circuit and patient's sputum. This suggests that the growth of bactery on Day-7 in the ventilator circuit might be related to the growth in patient's sputum.

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Literature Review

THE USAGE COMPARISON OF CEFTRIAXONE AND CHLORAMPHENICOL FOR TYPHOID FEVER TREATMENT: AN EVIDENCE BASED CASE REPORT

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ABSTRACT

Typhoid fever is a disease caused by the gram-negative bacterium Salmonella typhi. Since its introduction in 1949, chloramphenicol has become the first-line treatment of typhoid fever for decades. Until now, chloramphenicol is still the first line treatment of typhoid fever in rural areas in Indonesia, due to its low cost. However, in addition to the problem of bacterial resistance, chloramphenicol is known to cause some side effect such as bone marrow suppression. Currently, many other antibiotics are used as the regimens for the treatment of typhoid fever, one of which is ceftriaxone. However, there are evidences on reemergence of chloramphenicol sensitivity in typhoid fever treatment. This report is created to answer the clinical question on whether ceftriaxone is more effective compared to chloramphenicol as the first-line treatment of typhoid fever. A structured search was performed on PubMed, EBSCO, and ScienceDirect and after a screening process and appraisal using the criteria from Center of Evidence Based Medicine at Oxford University, only one article was selected. The article shows higher efficacy of ceftriaxone in term of defervescence rate (P = 0.0001). No other study that compares the efficacy of ceftriaxone and chloramphenicol for typhoid fever treatment during the last ten years could be found during article searching. In conclusion, ceftriaxone shows better efficacy in the treatment of typhoid fever compared to chloramphenicol but with the rise of microbial sensitivity to chloramphenicol in recent years, more studies on this topic are needed to support this conclusion.

Keywords: *Typhoid fever, enteric fever, Ceftriaxone, Chloramphenicol, Effectiveness*

ABSTRAK

Demam tifoid merupakan penyakit disebabkan oleh bakteri gram negatif Salmonella typhi. Sejak diperkenalkan pada tahun 1949, chloramphenicol selama puluhan tahun menjadi lini pertama pengobatan demam tifoid. Hingga saat ini chloramphenicol masih merupakan lini pertama untuk pengobatan demam tifoid di daerah-daerah di Indonesia terutama karena biayanya yang murah. Namun, selain masalah resistensi kuman, chloramphenicol diketahui menimbulkan efek samping berupa supresi sumsum tulang, sehingga saat ini banyak digunakan antibiotik lain sebagai rejimen pengobatan demam tifoid seperti ceftriaxone. Laporan ini dibuat untuk menjawab pertanyaan klinis apakah ceftriaxone lebih efektif dibandingan chloramphenicol sebagai lini pertama untuk pengobatan demam tifoid. Pencarian artikel terstruktur dilakukan pada PUBMED, EBSCO, dan ScienceDirect. Setelah proses penyaringan dan appraisal menggunakan kriteria Center of Evidence Based Medicine dari Universitas Oxford, didapatkan satu artikel terpilih. Artikel tersebut menunjukkan efektivitas ceftriaxone dalam menurunkan demam yang lebih baik dengan P = 0,0001. Tidak ditemukan penelitian lain mengenai perbandingan efektivitas ceftriaxone dengan chloramphenicol dalam menangani demam tifoid pada pencarian artikel. Kesimpulan yang ditarik adalah ceftriaxone menunjukkan efektivitas yang lebih baik dalam tatalaksana demam tifoid dibandingkan dengan chloramphenicol, namun dengan meningkatnya sensitivitas bakteri terhadap chloramphenicol dalam tahun-tahun terakhir, penelitian mengenai topik ini masih sangat diperlukan.

Kata kunci: *Demam tifoid, Demam tipus, Ceftriaxone, Chloramphenicol, Efektivitas*

INTRODUCTION

Typhoid fever is a disease which is caused by gram negative bacterium *salmonella typhi*. It is categorized as an endemic disease in Indonesia. In 2006, there are 500 cases of typhoid fever reported out of 100,000 people, with 0.65% death rate.[1]

Since it was introduced in 1949, chloramphenicol has been used as the first-line treatment for typhoid fever. It is still preferred in many areas in Indonesia due to its relatively affordable price. In many other countries, the use of chloramphenicol has been less and less because many bacteria strains have already resisted it.[2,3] However, a six years' study conducted by Moehario LH et al showed that 90% of bacteria were still susceptible to this drug.[4] Other studies in India also showed a reemergence of chloramphenicol sensitivity in typhoid fever treatment.[5–9]

The recommended dose of chloramphenicol is 2000 mg per day, divided to 4 dose orally or intravenous for at least 7 days. However, aside from bacteria resistance, chloramphenicol is known to induce bone marrow suppression. With that in mind, other antibiotics are often used as a therapy regiment for typhoid, one of which is a 3rd generation cephalosporin ceftriaxone.[2] Aside from avoiding the said side effect, the length of treatment using ceftriaxone is shorter than chloramphenicol and can improve a patient's adherence to the treatment. The recommended dosage for ceftriaxone is 3-4 grams in 100 cc of 40% dextrose solution per day for 3 to 5 days.[4]

CASE

A 18 years old female patient arrived with a chief complaint of fever for 1 week prior to the admission. The fever was accompanied with watery stool up to 3 times a day. Serological widal examination showed a positive result, thus, the patient was treated with intravenous ceftriaxone antibiotic 3 grams per day.

CLINICAL QUESTION

Is ceftriaxone more effective than chloramphenicol as the first-line treatment for typhoid fever?

MATERIAL AND METHOD

The method of this study is a systematic review on some articles relevant to the topic. A structured search was performed on three databases, namely PUBMED Clinical Queries, EBSCO Medline, and ScienceDirect,

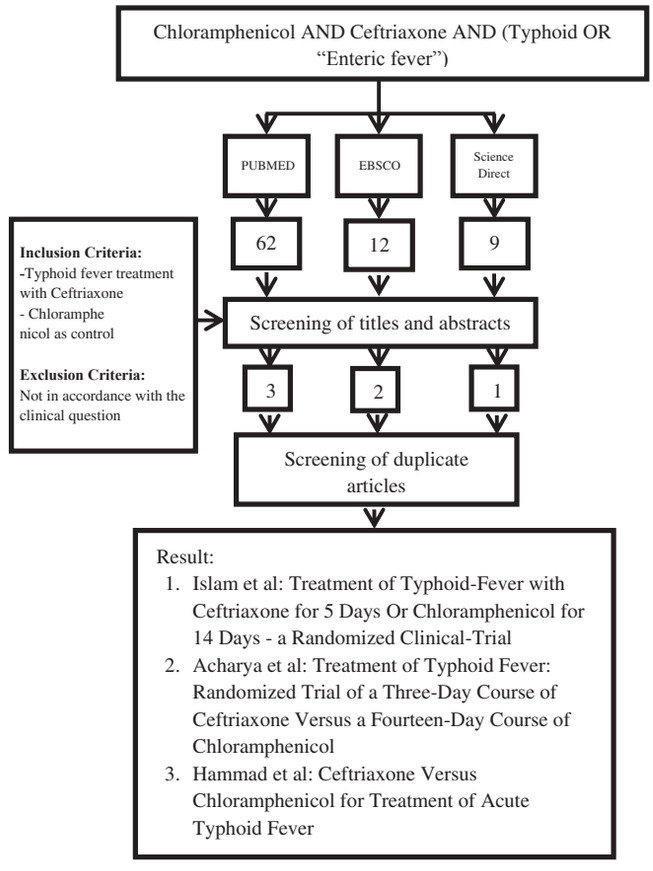

Figure 1. Article searching method and result

Table 1. Keywords and filters for article searching

	Keywords	Filter
PUBMED Clinical Queries	chloramphenicol AND ceftriaxone AND (typhoid OR "enteric fever")	Therapy; Broad Human species, English language, Full text available
EBSCO Medline	chloramphenicol [AB Abstract] AND ceftriaxone [AB Abstract] AND AND (typhoid OR "enteric fever") [AB Abstract]	Human, English, Full text available
Science Direct	chloramphenicol AND ceftriaxone AND (typhoid OR "enteric fever")	Journal

Table 2. *The critical appraisal* of articles validity

	Islam *et al* Antimicrobial Agents and Chemotherapy (1993)	**Acharya *et al* American Journal of Tropical Medicine and Hygiene (1995)**	**Hammad *et al* Life Science Journal (2011)**
Was the assignment of patients to treatments randomized?	Yes	Yes	Yes
Were all patients who entered the trial accounted for at its conclusion?	Yes	Yes	Yes
Were patients and clinicians kept "blind" to which treatment was being received?	Yes	Yes	No
Aside from the experimental treatment, were the groups treated equally?	Yes	Yes	Yes
Were the groups similar at the start of the trial?	Yes	Yes	Yes

using chloramphenicol AND ceftriaxone AND (typhoid OR "enteric fever") as the keywords (Table 1). From those keywords, we found as many as 62 articles from PUBMED, 12 articles from EBSCO, and 9 articles from Science Direct.

The title and abstract of those articles were then screened (as seen on Figure 1) with inclusion criteria being: (1) a trial on typhoid fever treatment with Ceftriaxone and (2) chloramphenicol as control.

The articles found were as follows: (1) Treatment of Typhoid-Fever with Ceftriaxone for 5 Days Or Chloramphenicol for 14 Days - a Randomized Clinical-Trial, by Islam et al; (2) Treatment of Typhoid Fever: Randomized Trial of a Three-Day Course of Ceftriaxone Versus a Fourteen-Day Course of Chloramphenicol, by Acharya et al; and (3) Ceftriaxone Versus Chloramphenicol for Treatment of Acute Typhoid Fever, by Hammad et al.[10]

These articles were appraised using the criteria from Center of Evidence Based Medicine Oxford University (Table 2). Articles by Islam et al and Acharya et al were published more than 20 years ago and therefore are not included in this review.

RESULT AND DISCUSSION

Hammad et al did a study on 2007 to re-asses the effectiveness of chloramphenicol as typhoid treatment in response to the increase of multidrug resistance to the first-line antimicrobials in Egypt for the last 30 years.[10]

Fifty-two patients of acute typhoid fever with positive blood culture for Salmonella typhi were divided into 2 groups. Twenty-seven patients were randomly allocated to be treated with chloramphenicol (50 mg/kg bw/day orally or intravenously) which is given 6 times hourly until defervescence for further 5 days.[10]

Twenty five patients were randomly allocated to be treated with ceftriaxone parenterally (80 mg/kg/day for children and 2 gm/day for adults) the treatment is given once a day for 7 days.[10]

Clinical cure occurred on all patients. The mean time (mean±SD) of defervescence for ceftriaxone and chloramphenicol was 3.3±1.2 and 5.8±1.2 days respectively (P = 0.0001, 95% CI = 1.8-3.2). Ceftriaxone treatment showed a shorter time of defervescence compared to chloramphenicol.[10]

We found only one article on PUBMED Clinical Queries, EBSCO Medline, and ScienceDirect using Center of Evidence Based Medicine Oxford University criteria.

A study by Hammad et al showed that ceftriaxone has more efficacy than chloramphenicol in treating typhoid fever. Ceftriaxone treatment had a shorter time of defervescence (3.3±1.2 days) compared to chloramphenicol (5.8±1.2 days).

This study also showed an increased risk of bone marrow suppression in using chloramphenicol as a

treatment. It was showed by the decreased of hematocrit mean value compared to the ceftriaxone group.

Unfortunately, no other clinical trial that compares the efficacy of ceftriaxone treatment and chloramphenicol treatment in the last 10 years was found during article searching.

Although ceftriaxone showed better efficacy and less side effect, chloramphenicol treatment can still be considered effective in treating typhoid. All patients experienced clinical cure after being treated with either ceftriaxone or chloramphenicol. This can be considered an improvement from years ago when chloramphenicol was rendered ineffective as a treatment because of widespread microbial resistance.[7]

CONCLUSION

In conclusion, the use of chloramphenicol is still effective for the treatment of typhoid fever. However, ceftriaxone showed greater effectiveness in typhoid fever treatment as shown by shorter time of defervescence compared to chloramphenicol. The use of ceftriaxone also poses less risk on bone marrow suppression compared to cephalosporin. Another advantage of using ceftriaxone as a treatment is the shorter length of treatment which can improve a patient's adherence to the treatment.

Only one clinical trial was found from article searching and with the rise of microbial sensitivity to chloramphenicol in recent years[5–9], more studies on this topic are needed to support this conclusion.

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Notes to authors

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