

ORIGINAL ARTICLES

The Effect of *Virgin coconut oil (VCO)* on *Staphylococcus aureus* Infection in Mice (*Mus musculus*) Observed from different Organ Histopathology

¹Ahmad fauzi, ²Iwan Setiawan, ²Fitri ariyanti

¹Faculty of Veterinary Medicine, University of Brawijaya. Haryono st 169 Malang 65145, Indonesia

²Faculty of Veterinary Medicine, Gadjah Mada University. Fauna 2 st Yogyakarta 55281, Indonesia

ABSTRACT

The aim of this research was to identify the role of *Virgin coconut oil (VCO)* in treatment of infections caused by *Staphylococcus aureus* which were examined by histopathologic pictures of the heart, lungs and skin. This study used 25 female mice of Swiss strain, 4 weeks old with average weight of 23 grams. The mice were randomly divided into five groups, each group was consisted of 5 mice. Group I (K 1) as a control, mice were given *phosfat buffered saline (PBS)* 0.05 ml per oral (po). Group II (K2) mice were infected by *Staphylococcus aureus* 1×10^2 bacteria/ml via intraperitoneal (IP) at the 1st day and were given 0.05 ml PBS. Group III (K3) mice were infected by *Staphylococcus aureus* IP at the 1st day, then in the 3rd day mice were given VCO 0.05 ml per oral (po) until 1 week. Group IV (K4) mice were infected by *Staphylococcus aureus* topically at the 1st day. Group V (K5) mice were infected by *Staphylococcus aureus* topical at the 1st day and then in the later day the mice were given VCO by topical application until 1 week. In the 4th week, mice which were infected by *Staphylococcus aureus* via intraperitoneal (Group II and III) were necropsied for histopathology of liver and lungs observation. While, skin of mice which were infected by *Staphylococcus aureus* Group IV and V) were necropsied at the 3rd, 6th and 9th day to examine the histopathologic of skin. The results showed that *Staphylococcus aureus* infection caused infiltration of inflammatory cells (polymorphonuclear cells) in the liver and necrosis of hepatocytes, granulomatous inflammation in lung, narrowing the pulmonary septa alveoli and skin necrosis. Mice which were infected by *Staphylococcus aureus* then treated with VCO have ability to repair their liver tissue, lung and skin from damage caused by *Staphylococcus aureus* infection.

Key words: histopathology, *Staphylococcus aureus*, *virgin coconut oil (VCO)*,

Introduction

Staphylococcus aureus has been known to spread widely in the world and caused many anomalies of the skin and the mucous membranes of animals and human beings (Todar, 2002). All tissues can possibly be infected by *Staphylococcus aureus* and cause disease with typical signs of inflammation, necrosis and abscess formation (Anonymous, 1994). According Joklik *et al.* (1992) *Staphylococcus aureus* is also a major pathogen in humans that cause many diseases associated with toxic shock syndrome (TSS) as a result of food poisoning. In addition, *Staphylococcus aureus* is responsible for 80% of suppurative disease, the skin surface as their natural habitat. *Staphylococcus aureus* clinical manifestations in humans including impetigo, scalded skin syndrome, pneumonia, osteomyelitis, pioarthritis, endocarditis, metastatic, food poisoning, toxic shock syndrome (TSS), meningitis and sepsis (Joklik *et al.*, 1992;. Emmerson, 1994; Ena *et al.*, 1994; Todar, 2002; Salasia *et al.*, 2005).

Staphylococcus aureus infection caused many losses, one of which is the cause of subclinical mastitis (inflammation of the udder) which commonly occurs in dairy cattle in the world. Mastitis caused substantial losses due to the decrease of milk production which reached 70%, residues of antibiotics in milk, health and activity, culling, milk dairy cows loses, and mortality in cattle (Salasia *et al.*, 2005). Intra-mamaria infection can cause significant economic losses due mainly to the decrease of milk production between 10-25% of total milk production (Han *et al.*, 2001).

Virgin coconut oil (VCO) has been known to have functions as an antiviral, antibacterial and antiprotozoa, lowers blood sugar levels and LDL cholesterol and increase HDL cholesterol, cure hepatitis, help thyroid function, prevent premature and as an ingredient in cosmetics natural aging. Many benefits of VCO was due to its content of lauric acid (monolaurin) and acid capric which improve metabolism and immunity. Monolaurin can damage membranes and kill bacteria (Karjono *et al.*, 2010).

Based on the results of chemical analysis by gas chromatography, it was known that VCO contains lauric acid 45-50%, miristic acid 12-16%, caprylic acid 12.8%, capric acid 8.10%, palmitic acid <5%, oleic acid 2%,

stearic acid 1.5%, and in lesser extend of caproic acid (Marina *et al*, 2009). High levels of lauric acid which is the chain of saturated fatty acids (middle-chained fatty acid-MCFA) in VCO has functions as a natural antimicrobial which is able to kill various bacteria, viruses and parasites (Anonymous, 2006).

Materials And Methods

Materials:

Virgin coconut oil (VCO) were purchased from local markets in Yogyakarta, Indonesia, mice were supplied by General Laboratory of Pharmacy Faculty, Gadjah Mada University, phospat buffer saline (PBS), *Staphylococcus aureus* strain BY7 (Clinical Pathology, Gadjah Mada University), todd hewith broth (THB) (Pronadisa, Spain), manitol salt agar (MSA), blood agar plate (BAP), agar base (Oxoid, German), aquades, formalin 10 %, alcohol 70 % and picric acid.

Preparation of *Staphylococcus aureus*:

Staphylococcus were grown on BAP containing sheep blood defibrination and incubated at 37°C for 24 hours. The bacteria were planted in THB, incubated at 37°C for 18-24 hours, mixed using vortex and centrifuged at 3000 rpm for 10 minute. The supernatant was discarded and the pellet was added with 10 ml PBS, mixed using vortex and centrifuged at 3000 rpm for 10 minutes, the supernatant was discarded again. The pellet was then added with 2 ml of PBS and then read with 10% Spectrophotometric absorption in λ 620 nm, the result was 10^9 bacterial cells (Salasia, 1994).

Experiment:

This study used 25 female mice aged 3-4 weeks with a body weight of $\pm$ 23 gram. The mice were fed on pellets food, provided drinking water *ad libitum*. The mice were randomly divided into five groups. Each group was treated with different treatment. Group I (K 1) as a control treatment, the mice were given *phosfat buffered saline* (PBS) 0.05 ml per oral (po). Group II (K2), the mice were infected by *Staphylococcus aureus* 1×10^2 bacteria/ml via intraperitoneal (IP) on the 1st day and given 0.05 ml PBS. Group III (K3), the mice were infected by *Staphylococcus aureus* IP on the 1st day and given VCO 0.05 ml per oral (po) from the 3rd day until 1 week. Group IV (K4), the mice were infected by *Staphylococcus aureus* topical on the 1st day. Group V (K5), the mice were infected by *Staphylococcus aureus* topical on the 1st day and then given VCO topical application until 1 week. In the 4th week, mice which were infected by *Staphylococcus aureus* via intraperitoneal (Group II and III) were necropsied for histopathology of liver and lungs observation. While, skin of mice which were infected by *Staphylococcus aureus* (Group IV and V) were necropsied on the 3rd, 6th and 9th day to examine the histopathological of skin.

Data analysis:

Histopathological images and microscopic observation of liver, lungs and skin were descriptive analyzed by comparing those of treated groups with those of control group.

Results and Discussion

Data of microscopic observation of the liver, lungs, and skin tissues of control group and the treated groups were presented on Table 1 and 2. Microscopic image of the tissues on control group and treated group were presented on the Figure 1-12. Microscopic image of liver on control group (Figure 1) showed liver cells which was constituted with radier and the nucleus which contained average polihedral and sinusoids as on normal liver. The figure was in conformity with microscopic image of the normal liver as reported by Dellmann and Brown (1992). The liver of mice which were infected by *Staphylococcus aureus* via intraperitoneal route have visible necrosis of hepatocytes cells, the infiltration of inflammatory cells, and polymorphonuclear cells (Figure 2). *Staphylococcus aureus* infection can cause inflammation of liver (Shulman, 1994). Two bacterial wall components, peptidoglycan and lipoteichoic acid from *Staphylococcus aureus*, work together to cause systemic inflammation and multiple systems failure associated with Gram-positive organisms (Kimpe *et al*, 1995). Peptidoglycan of *Staphylococcus aureus* causes organ injury/dysfunction, organ inflammation, and systemic inflammation in the rat, involving nuclear factor- κ B and possibly activator protein 1 (Wang, *et al.*, 2004)

Table 1: Microscopic observation of liver and lung of control and treated mice after 4 weeks.

Treatment	No Mice	Microscopic observation	
		Liver	Lung
Group I Control	I	Liver cells arranged radier, polihedral shaped, core in the middle, normal sinusoids (Figure 1)	lung cells, alveoli, and normal alveolar septa, there is alveolar macrophages (Figure 4)
	II	Normal	Normal
	III	Normal	Normal
	IV	Normal	Normal
	V	Normal	Normal
Group II Infected by <i>Staphylococcus aureus</i> Intraperitoneal (IP)	I	hepatocyte cell necrosis, inflammatory cell infiltration, Polimorfonuclear (PMN) cells (Figure 2)	Thickening of inter alveolar septa, inflammatory granulomatous, inflammatory cell infiltration (Figure 5)
	II	hepatocyte cell necrosis, inflammatory cell infiltration	Thickening of inter alveolar septa, inflammatory cell infiltration
	III	hepatocyte cell necrosis	Inflammatory granulomatous, inflammatory cell infiltration
	IV	Normal	Normal
	V	hepatocyte cell necrosis	Thickening of inter alveolar septa, inflammatory cell infiltration
Group III <i>Staphylococcus aureus</i> + Treatment by VCO	I	Radier hepatocyte, no necrosis, normal sinusoids (Figure 3)	Normal septa interalveoli, there is no infiltration of inflammatory cells (Figure 6)
	II	Radier hepatocyte	Septa interalveoli and alveolar is normal
	III	Radier hepatocyte, normal sinusoids	Thickening of inter alveolar septa, inflammatory cell infiltration
	IV	normal sinusoids structure	inflammatory cell infiltration
	V	normal sinusoids structure	Septa interalveoli and alveolar is normal

VCO which was given to mice could improve the capability of the mice to repair tissue including liver. The liver showed structures with normal sinusoids and its cells contain radier more towards the central vein (Figure 3). In this study VCO could be able to induce the body's defense system, improving the ability of mice immunity due to the increase of levels of lauric acid (monolaurin) and capric acid from VCO which enhance immunity and improve metabolism and also monolaurin could damage the cell membranes of bacteria and kill them (Karjono *et al*, 2010).

Microscopic picture of lung tissue of control mice showed lung with normal composition of cells, alveoli and alveolar septa and there is alveolar macrophages in interalveolar septa (Figure 4). Histopathological of mice lung tissue which were infected by *Staphylococcus aureus* showed inflammatory cell infiltration and granulomatous and thickening inter-alveolar septa (Figure 5). Pelczar and Chan (1988) reported that *Staphylococcus aureus* infection can cause pneumonia. According to Banks (1993) in the lungs there is a special mechanism of defense against pathological agents that are not specific: the mucosa siliaris movement, macrophages alveolar, phagocytosis, the passive transport of lymphocytes. Infectious disease in the lungs can occur aerogenous, hematogenous, and directly to the lungs. *Staphylococcus aureus* infection in lung is by hematogenous.

Histopathology of lung tissue of mice were infected *Staphylococcus aureus* and given VCO showing an improvement in the cells of the mice with a remarkable expansion of interalveoli partitions and non-visible infiltration of inflammatory cells (Figure 6). Capacity of VCO to improve lung tissue conditions is probably due to high content of lauric acid in VCO. Lauric acid in the body becomes monolaurat, compound of monoglycerides which are as antimicrobial (Purnomo, 2006). VCO contains also very high antioxidant, such as alpha tocopherol and beta carotene (Setiaji and Prayugo, 2006). Alpha tocopherol is the main form of vitamin E, such as intracellular antioxidant tocopherol has powerful protection of lymphocytes and monocytes from the interference of radicals DNA and is able to improve the hypersensitivity reaction of the immune system, a response to combat cancer, parasites and chronic infections. The beta carotene helps prevent damage to the tissues and DNA, as a stimulator of the enzymatic destruction of carcinogens, the increase of white blood cells effects, and stimulates the body's ability to convert toxic substances into harmless compounds (Alamsyah, 2005).

The microscopic observation of mice skin of control group and treated group are presented in Table 2. Microscopic image of skin in control group showed the distinguished epidermal layer and dermis, there was a section dermis sebaceous glands and hair follicles. Preview histopathology of mice skin were infected *Staphylococcus aureus* topical, on the 3rd day showed that around the incision visible inflammatory cell infiltration, the presence of bacterial colonies, large necrotic areas, and there were plenty of fibrinogen (Figure 7). *Staphylococcus aureus* infected mice and then given VCO topical, indicated to the healing on the 3rd day which was characterized by the appearance of the epitelization, although there were fibrinogen and cells polymorphonuclear (Figure 8).

Table 2: Microscopic image of the abdominal skin of mice on 3rd, 6th and 9th days infection

Treatment	No Mice	Microscopic observation		
		3 rd day	6 th day	9 th day
Group IV Infected by <i>Staphylococcus aureus</i> topically	I	inflammatory cell infiltration, bacterial colonies, contained fibrin, tissue necrosis	there are colonies of bacteria, vacuole former abscess, tissue necrosis	inflammatory cell infiltration, epithelialization
	II	tissue necrosis	there are colonies of bacteria and necrosis	Necrosis, epithelialization
	III	tissue necrosis	there are colonies of bacteria and necrosis	Necrosis, epithelialization
Group V <i>Staphylococcus aureus</i> + Treatment by VCO	I	epithelialization, visible fibrin and polymorphonuclear cell	inflammatory cell infiltration, hair follicles formed, epithelialization	healing epithelial, hair follicles and dermis is formed
	II	Epithelialization	epithelialization	dermis layer formed
	III	Epithelialization	epithelialization	formed hair follicles

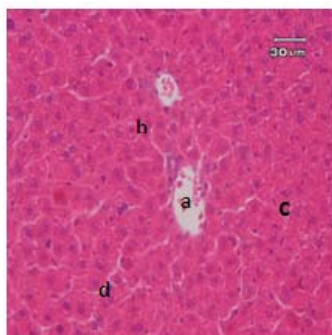
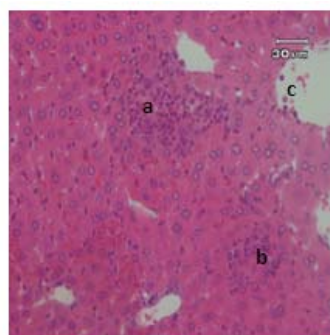
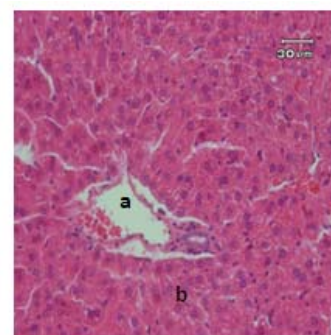
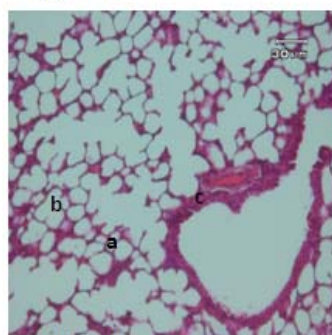
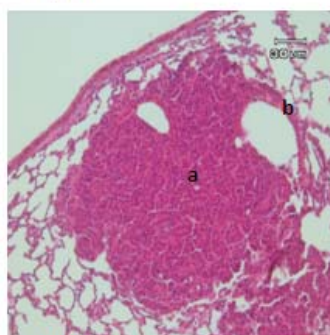
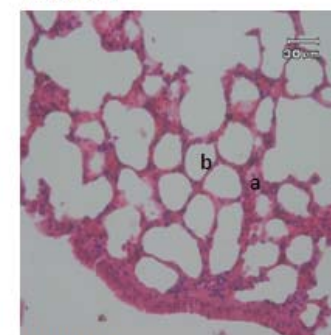
**Figure 1****Figure 2****Figure 3****Figure 4****Figure 5****Figure 6**

Fig. 1: Fotomikrograf liver of mice normal-control group, (a) central vein, (b) liver cells (hepatocytes), (c) Kupffer cells, (d) sinusoid (H & E staining, 400x).

Fig. 2: Fotomikrograf liver of mice infected by *Staphylococcus aureus*, (a) necrosis of hepatocytes, (b) polymorphonuclear (PMN) and mononuclear cell infiltration, (c) central venous (H & E staining, 400x).

Fig. 3: Fotomikrograf liver of mice infected by *Staphylococcus aureus* and given VCO dose 7800 mg / kg bw / day, (a) central vein, (b) Structure of liver cells radier (H & E staining, 400x).

Fig. 4: Fotomikrograf lung of mice normal, (a) interalveoli septa, (b) alveoli, (c) respiratory bronchioles (H & E staining, 400x).

Fig. 5: Fotomikrograf lung of mice infected by *Staphylococcus aureus*, (a) granulomatous inflammation, (b) thickening of septa interalveoli (H & E staining, 400x).

Fig. 6: Fotomikrograf lung of mice infected by *Staphylococcus aureus* and given VCO dose 7800 mg / kg bw / day, (a) interalveoli septa consists of a thin connective tissue, (b) normal alveoli (H & E staining, 400x).

Histopathology skin of mice infected by *Staphylococcus aureus* topical on 6th day after infection showed colonies of bacteria, necrosis and abscess (Figure 9). However, on 9th day after infection, the skin showed infiltration of inflammatory cells and begin to occur epithelialization (Figure 11). Microscopic image of mice skin were infected by *Staphylococcus aureus* topical and then given VCO topical application, on 6th day after infection still showed visible infiltration of inflammatory cells in small quantities, the epithelialization and hair

follicles begin to form (Figure 10). However, on the 9th day after infection, the mice showed epithelial skin recovery, the formation of the hair follicle and formed layer dermis (Figure 12).

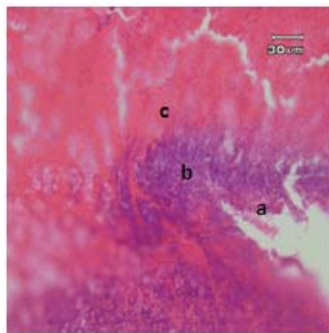


Figure 7

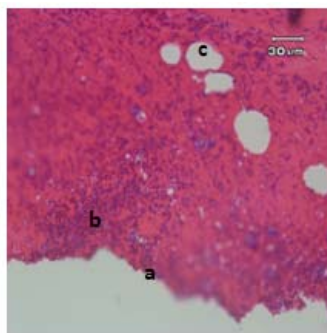


Figure 8

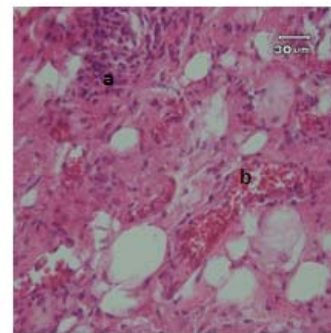


Figure 9

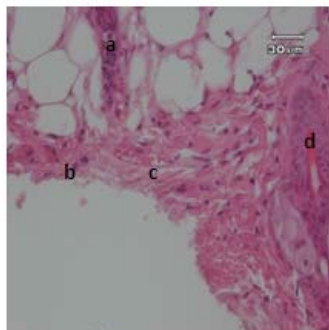


Figure 10

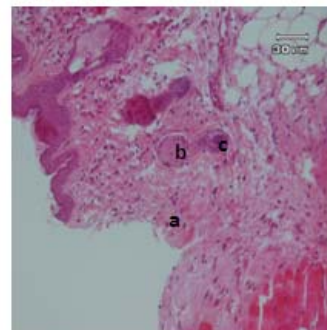


Figure 11

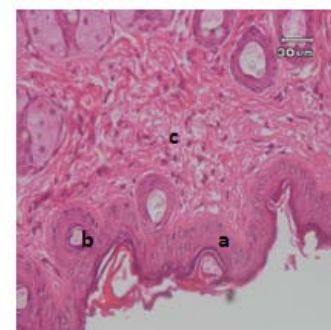


Figure 12

- Fig. 7:** Fotomikrograf skin of mice on 3rd days post infection *Staphylococcus aureus*, (a) bacterial colonies, (b) necrosis, (c) fibrin (H & E staining, 400x).
- Fig. 8:** Fotomikrograf skin of mice on 6th days post infection of *Staphylococcus aureus*, (a) bacterial colonies, (b) necrosis, (c) the former abscess vacuoles (H & E staining, 400x)
- Fig. 9:** Fotomikrograf skin of mice on 9th days post infection of *Staphylococcus aureus*, (a) infiltration of inflammatory cells (b) epitelization (H & E staining, 400x).
- Fig. 10:** Fotomikrograf skin of mice on 3rd days post infection of *Staphylococcus aureus* and given VCO topically, (a) the infiltration of inflammatory cells, (b) epitelialization, (c) fibrin, (d) hair follicles (H & E staining, 400x).
- Fig. 11:** Fotomikrograf skin of mice on 6th days post infection of *Staphylococcus aureus* and given VCO topically, (a) epitelization, (b) hair follicles, (c) infiltration of inflammatory cells (H & E staining, 400x).
- Fig. 12:** Fotomikrograf skin of mice on the 9th days post infection of *Staphylococcus aureus* and given VCO topically, (a) skin epitelium, (b) hair follicles, (c) layer of dermis (H & E staining, 400x).

In this study, *Staphylococcus aureus* infection can make damage to various organs including liver, lung and skin. VCO can improve the immune system and body's defense system which immediately reacts to any foreign objects that enter the body including bacteria and other microorganisms (Mc Caleb, 2002). The possible mechanism of the improve of ability of immunity on the VCO was as reported by (Karjono *et al*, 2010).

The increasing ability of phagocytosis by polymorphonuclear cells stimulated by VCO can be an information that can boost immunity. In addition, the possibility of vitamin E function as antioxidants which help in overcoming various damaged tissue by *Staphylococcus aureus* infection. VCO contains quite high antioxidants such as alpha tocopherol and beta-carotene. Alpha tocopherol contains biologically active components which are generally accepted as the activity of vitamin E in maintaining the immune system (Setiaji and Prayugo, 2006). Beta carotene helps to prevent tissue and DNA from damage, as a stimulator of enzyme destructing carcinogens, enhance the effect of white blood cells, and stimulate the body's ability to convert toxic substances into harmless compounds (Alamsyah, 2005).

Conclusion:

Staphylococcus aureus infection in mice causes tissue damage as characterized by inflammatory polymorphonuclear cell infiltration and necrosis of hepatocytes, granulomatous inflammation in the lungs,

narrowing of the pulmonary alveolar septa and infiltration of inflammatory cells occurs, abscesses and skin necrosis. *Staphylococcus aureus* infected mice which was given VCO 0.05ml were able to restore the damaged tissues caused by infection to be normal.

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