Characterization of virgin coconut oil fermented using starter culture prepared with probiotic bacterial strain

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ABSTRACT

Fermented Coconut Oil (FCO) stands out as a unique form of pure coconut oil, processed through fermentation with a starter culture. Primarily composed of medium-chain saturated fatty acids, it inherits the biological properties of coconut oil, extensively studied for its remarkable antibacterial potential. The high concentration of medium-chain fatty acids, particularly lauric acid and its monoglyceride derivative, monolaurin. endows FCO with potent antibacterial properties. This enables FCO to combat a range of pathogenic microorganisms effectively. This study investigated the antibacterial activity of FCO against cultured pathogens, including Listeria monocitogenes, Bacillus cereus and Salmonella typhimurium. The results revealed the ability of FCO to inhibit the growth of these pathogens. Regarding in vivo testing with mice, parameters such as weight gain, blood sugar, cholesterol. and triglyceride levels were assessed in blood serum. Histopathological examination of the spleen, liver, kidneys, and intestines did not reveal any adverse changes. However, mice infected with Escherichia coli and simultaneously given FCO showed focal necrosis in the liver due to E. coli infection. Observation of kidney tissue showed glomerular swelling and renal tubular epithelial necrosis in some groups, but did not appear in the control group. In particular, the group infected with E. coli and given FCO showed glomerular swelling and renal tubular epithelial cell necrosis. This shows the potential of FCO in reducing bacterial infections and highlights its impact on kidney health by preventing inflammation and necrosis in the kidneys.

Keywords:
Fermented Coconut Oil
Starter Culture
Antibacterial
Pathology
Histopathology

1. INTRODUCTION

The popularity of coconut oil has indeed surged in recent years. driven by various health claims and endorsements from celebrities. Influencers, and even medical professionals, Coconut, often referred to as the "tree of life" has been a significant part of the diet in tropical and subtropical regions for centuries. Some studies suggest that the high levels of lauric acid in coconut oil may contribute to raising high-density lipoprotein (HDL)

and lowering low-density lipoprotein (LDL). However, the scientific consensus on this matter is still evolving (BBC News. 2018; Medical News Today. 2018; New Straits Times. 2018).

According to the universally accepted Lipid-Heart Theory, high saturated fats cause hypercholesterolemia and coronary heart disease (Dayrit & Calleja, 2003). Because coconut oil is rich in saturated medium-chain fatty acids, it is believed to be cholesterogenic. Animal studies that showed these harmful effects were flawed because of their use of hydrogenated coconut oil. Hydrogenation of coconut oil is a process that prevents peroxidation or rancidity (Rethinam & Muhartoyo, 2003). This process saturates the small amount of the essential fatty acid linoleic acid that makes hydrogenated coconut oil cholesterogenic.

Without linoleic acid supplementation, the animals suffered from essential fatty acid deficiency (Dayrit & Calleja, 2003). Coconut oil, in fact, raises the HDL and lower the LDL:HDL ratio (Rethinam & Muhartoyo, 2003). Compared to long chain triglyceride fats, these medium-chain triglycerides are easier to digest, absorb and oxidize. It is absorbed and is carried to the liver, where it undergoes rapid oxidation to release energy (Rethinam & Muhartoyo, 2003). This property allows them to deposit less into adipose tissue and not cause obesity. They also decrease protein catabolism in hypercatabolic states, raise thyroid function, and do not form esters with cholesterol. When supplied with sufficient polyunsaturated fatty acids to avoid essential fatty acid deficiency, medium-chain saturated fats cannot raise cholesterol levels (Dayrit & Calleja, 2003).

One of the amazing qualities of coconut oil is its antibacterial properties. Monolaurin, an ingredient in coconut oil, has long been recognized for its bug-fighting properties. It is found in breast milk, perhaps in part to help protect the developing baby from infection (Clarke & May, 2007). It appears that coconut milk can protect against several different kinds of bacteria and fungi of clinical impact and can further benefit the skin by treating and preventing skin infections (Carpo et al., 2007; Clarke & May, 2007).

According to Abbas et al. (2017), this virgin coconut oil, which is a potent nondrug or natural yeast fighter, contains three medium-chain fatty acids, i.e. lauric (50–53%), caprylic, and capric. All of which have antibacterial and antifungal effect against lipid coated bacteria such as staphylococcus species and fungi (Candida spp). Lauric acid a twelve carbon chain acids, is one of the medium-chain fatty acids gotten from some plants oil particularly coconut oil and others related oil such as palm kernel oil which has been known as one of the most active ingredient and more predominant in the total saturated fat present (Fife, 2000). It is found in many vegetables, fats particularly in coconut oil and palm kernel oil (Chuah et al., 2014), and has been known as one of the most active ingredient and composed over 52% of the total 92% saturated fats present in the coconut oil and is claimed to play a significant role in the healing miracle that is revealed in coconut oil (Fife, 2003). Medium-chain free fatty

acids which lauric acid fall under have been found to have a broad spectrum of microbicidal activity though the mechanisms by which the lipids kill bacteria are not known, but electron microscope studies indicate that they disrupt cell membranes (Ogbolu, 2007).

Free fatty acids of various chain lengths (C8-C18) have antibacterial activity against a range of Gram-positive bacteria, but not against several Gram-negative bacteria (Georgel et al., 2005; Skrivanova et al., 2005; Drake et al., 2008). This resulted from the medium-chain triglycerides (MCTs) present in coconut oil, which antibacterial influence because it can disintegrate bacterial cell walls; MCTs are also presenting the ability to treat severe bacterial infections that are antibiotic resistant (Fife, 2000). Despite the vast impact of coconut plants and its health importance to humanity hitherto, most people still lack the basic knowledge in these plants and relatively few studies has been done to ascertain its health impact. In this study, antibacterial activity of coconut oil and its derivative as lauric acid were investigated.

The primary purpose of the present study was to determine the effect of fermented coconut oil on lipid profile (serum cholesterol), low-density lipoprotein, high-density lipoprotein, triglycerides and blood sugar levels using animal as an experimental model. In addition, we determined the antibacterial activity of extracted FCO through agar disc diffusion assay. agar well diffusion and broth dilution assay.

2. MATERIALS AND METHODS

Preparation of Fermented Coconut Oil

The coconut kernels were ground in a grinding machine into viscous slurry. Thereafter, squeezed through cheesecloth to get coconut milk and then were put into glass jars. The glass jars containing the squeezed coconut milk were left for a few minutes to allow the cream coconut milk and skim coconut milk to separate into a layer of curd which appears at the top of the jars. The cream milk was inoculated with probiotic starter culture derived from *Lactobacillus plantarum* and incubated for overnight to allow the coconut curd and oil to separate into a layer of oil which appears at the beneath of the curd. Thereafter, the curd was scooped out and discarded, leaving the fermented coconut oil in the jars. The obtained FCO was decanted into a bottle with a plastic screw cap and stored at room temperature for the present study (Sulistyo, 2009).

Animal Model Experiment

Male Sprague-Dawley rats weighing about 100–200 g, obtained from Central Veterinary Research. were used. The animals were housed in colony plastic cages at an ambient temperature of 25°C–27°C under a 12-hour light/dark cycle and free admittance to standard rat diet and tap water. The rats were allowed to adapt to the laboratory

environment for 1 week before starting the experiment. All the experimental procedures were performed according to the ethical guidelines for the use and care of laboratory animals.

Blood Specimen Collection

The method of collecting blood specimens is related to the volume of blood required for research purposes. The blood volume in mice was around 6-8% of body weight, thus a mouse weighing 200-300 grams has a total blood volume of around 12-24 ml. One blood specimen taken were not be more than 10% of the total blood volume of the experimental animal (Handajani et al., 2016).

Measurement of Blood Glucose, Cholesterol, Triglyceride

Blood glucose level was measured using One Touch Ultra test strips. Blood was obtained from the rats at the tip of the rat's tail. The blood was dropped on the test strips already inserted in One Touch Ultra Easy Glucometer. The glucose levels of the animal were displayed on the glucometer in about 5 sec. Blood glucose level was measured at the beginning of the experiment and after 4 weeks. Plasma samples were analyzed using methods according to the procedures contained in the KIT lipoprotein manual. Blood samples in EDTA solution were centrifuged for 10 minutes at a speed of 3000 rpm.

Furthermore, blood plasma fluid was taken for further examination. including checking total cholesterol. 100 µl of blood plasma was mixed with 1000 µl of cholesterol reagent, left for 10 min, then the absorbance value was measured using a spectrophotometer at a wavelength of 500 nm. To examine high density lipoprotein (HDL). 200 µl of blood plasma is added to distilled water and HDL reagent (100 µl: 400 µl), then left for 10 min, and centrifuged for 10 min at a speed of 3000 rpm. 100 µl of the supernatant was taken. mixed with 1000 µg of cholesterol reagent and left for 15 min, then the absorbance value was read using a spectrophotometer at 500 nm. Triglyceride was measured by mixing 100 µl of plasma with 1000 µl of triglyceride reagent and left for 10 min, then the absorbance was read using a spectrophotometer at 500 nm. (Runge et al., 2014).

Observation of Organ Specimens

Organ specimens were taken from experimental animals and underwent several checks according to the research variables. From the chest of experimental animals, thymus, heart and lungs were taken. From the abdomen, digestive system organs can be taken, including intestine, cecum, liver, spleen and also pancreas. Other organs in the abdomen that were taken the kidneys and gallbladder (Parkinson et al., 2011). For histopathological examination, organs can be stored in 10% formalin and all organs must be

immersed in the preservation solution. Organs taken can be subjected to histopathological examination to determine the damage that has occurred to them.

Antibacterial Activity Test

Antibacterial susceptibility test was carried out in each of the plate using agar disc diffusion method as described by Bauer-Kirby in Hudzicki (2009). This involves an inoculation of an agar plate with the test organisms. A disc of Whatman filter paper was impregnated with an appropriate concentration of lauric acid and was placed on a plate of susceptibility testing to be uniformly inoculated with the test organisms and equally spaced on the inoculated plate. The antibacterial agent was diffused from the disc into the medium and the growth of the test organism was inhibited at a distance from the disc that is related to the susceptibility of the organisms. Strains susceptible to the antibacterial were inhibited at a distance from the disc, whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc (Cheesbrough, 2006). Following incubation, the agar plate was examined for zones of inhibition surrounding the discs. Zone of inhibition indicates antibacterial activity against the tested bacterial strains (*Listeria monocytogenes* and *Salmonella typhimurium*). Absence of zone of inhibition indicates that the lauric acid was ineffective against the test organisms or the organisms were resistant to the acid.

3. RESULTS AND DISCUSSION

Production of Fermented Coconut Oil

The FCO is obtained from fresh and mature kernel of the coconut (*Cocos nucifera* L.) by mechanical or natural means with or without the application of heat, unless processed by fermentation using a probiotic starter culture, which does not lead to alteration of the nature of the oil. The FCO has not undergone chemical refining, bleaching and deodorizing. It can be consumed in its natural state without the need for further processing. FCO consists mainly of medium-chain triglycerides, which are resistant to peroxidation. The fatty acids in FCO are distinct from animal fats, which contain mainly of long chain saturated fatty acids. The FCO is colorless, free of sediment with a natural fresh coconut scent. It is free from rancid odor or taste (Figure 1). The FCO extraction process was carried out through a fermentation process involving probiotic bacterial cells and enzymes that were able to break down emulsions of fat, protein and water. However, its activity was influenced by several environmental conditions. including substrate viscosity, stimulated enzyme, pH. temperature and incubation time. Our FCO was produced using a starter culture of *Lactobacillus plantarun,* which functioned as an enzyme producer and furthermore was purified using filter paper without heating or adding synthetic chemical solvents. Hence, it meets the quality according to APCC specification (Table 1 and Table 2).

(A) (B)

Figure 1. Coconut Milk Fermented by Probiotic Starter Culture after Incubation at Room Temperature (A); and the Obtained FCO after Filtration using Whatman Filter Paper (B).

Table 1. Essential Composition of FCO According to APCC Standard (2009)

Essential Composition & Quality Factors	APCC Standar	FCO
Identity Characteristic		
Relative density	0.915 - 0.920	0.92
Reactive Index at 40ú C	1.4480 - 1.4492	1.45
Moister % wt. Max	0.1 - 0.5	0.12
Isoluble impurities % by mass max	0.005	0.04
Saponification Value	250 - 260 min	252.00
Iodine Value	4.1 - 11.0	4.90
Unsaponifiable matter % by mass Max	0.2 - 0.5	0.32
Specific gravity at 30 deg./ 30 deg.C	0.915 - 0.920	0.92
Acid Value max	0.5	0.42
Polenske Value min.	13	14.00
GLC Ranges of FA Composition (%)		
C 6 : 0 (Caproic)	0.4 - 0.6	0.51
C 8 : 0 (Caprylic)	5.0 - 10.0	6.01
C 10 : 0 (Capric)	4.5 - 8.0	7.5
C 12 : 0 (Lauric)	**43.0 - 53.0**	**48.02**
C 14 : 0 (Myristic)	16.0 - 21.0	17.02
C 16 : 0 (Palmitic)	7.5 - 10.0	7.21
C 18 : 0 (Stearic)	2.0 - 4.0	3.11
C 18 : 1 (Oleic)	5.0 - 10.0	5.41
C 18 : 2 (Linoleic)	1.0 - 2.5	1.3
C 18 : 3 - C 24 : 1 (Linolenic, etc)	> 0.5	0.00
Quality Characteristic		
Colour	Water Clean	Clean
Free Fatty Acid	≤ 0.5 %	0.31
Peroxide Value	° 3 meg/Kg oil	2.00
Total Plate Count	< 10 cfu	3.00
Odour and Taste	Free from foreign and rancid odour and taste	Free
Contaminants		
Matter volatile at 105° C	0.20%	0.17
Iron : (Fe)	5 mg/Kg	3.00
Copper	0.4 mg/Kg	0.19
Lead	0.1 mg/Kg	0.05
Arsenic	0.1 mg/Kg	0.00

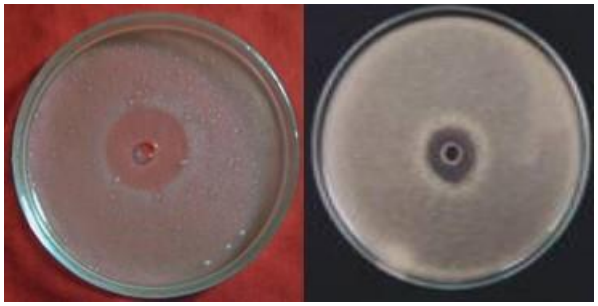
Table 2. Gas Liquid Chromatography (GLC) Ranges of Fatty Acid Component.

Fatty Acid	Composition	(%)	FCO
Caproic acid	C 6:0	0.10 – 0.95	0.51
Caprylic acid	C 8:0	4 – 10	6.01
Capric acid	C 10:0	4 – 8	7.5
Lauric acid	C 12:0	45 – 56	48.02
Myristic acid	C 14:0	16 – 21	17.02
Palmitic acid	C 16:0	7.5 – 10.2	7.21
Stearic acid	C 18:0	2 – 4	3.11
Oleic acid	C 18:1	4.5 - 10	5.41
Linoleic acid	C 18:2	0.7 – 2.5	1.3

Test for Antibacterial Activity

Effectiveness of synthesized monoasil glycerol of coconut oil as antibacterial has been tested *in vitro* at SEAFAST Center IPB (Figure. 2). The provided information describes a synergistic effect observed in the antibacterial properties of FCO resulting from the enzymatic fermentation process using a starter culture derived from L. plantarum bacteria. Enzymatic fermentation of FCO is initiated using starter culture that was produced by L. plantarum bacteria. The fermentation process involves lauric acid, which is present in FCO due to the L. plantarum cultures produce organic acids during the logarithmic phase in the growth medium. These organic acids are referred to as antibacterial metabolites. FCO contains fatty acids that do not dissociate due to ester groups, thus the organic acids from the L. plantarum cultures can lower the pH of the cytoplasm. The antibacterial properties of FCO are tested on an agar medium using the diffusion method. The test includes cultures of Listeria monocytogenes. Bacillus cereus. Escherichia coli. and Salmonella.

The combined presence of fatty acids and organic acids enhances the antibacterial activity of FCO. Monoglycerides from FCO and metabolites from the starter culture work synergistically. The test results indicate that FCO. when mixed with the starter culture extract of *L. plantarum*, inhibits the growth of L. monocytogenes, B. cereus and S. typhimurium. The synergy mechanism was attributed to the combined action of monoglycerides and metabolites. which diffuse into the medium containing the tested bacterial strains. It appears that the combination of FCO and the organic acid-producing starter culture creates a potent antibacterial mixture. inhibiting the growth of various tested bacteria. The synergy between the fatty acids and organic acids, derived from both FCO and the bacterial culture, contributes to the enhanced antibacterial properties observed in the testing (Figure 1).



A B

Figure 2. Antibacterial Activity of Monoasil Glycerol of FCO Against Pathogenic Microbial Growth of *L. Monocytogenes* (A) and *S. Typhimurium* (B)
Source: *photograph courtesy by Dr. Asriani*

Measurement of Blood Glucose, Cholesterol and Triglyceride

The parameters observed and measured in this study were body weight, blood sugar, cholesterol, HDL, LDL, triglycerides, pathology, and histopathological changes. In vivo tests using DDY mice as an animal model were carried out to determine the effectiveness of FCO in controlling blood chemistry. The parameters measured included the weight gain of the mice and the levels of blood sugar, cholesterol, LDL, HDL, and triglycerides in the mice's serum. Pathological and histopathological examinations of the liver, kidney, spleen, and intestine of mice were carried out to determine the effects of FCO on these organs.

A pre-clinical trial using 160 mice aged 2-3 months with an average weight of 20 grams was conducted for a 28-day observation period. The mice were divided into 8 groups, each consisting of 20 mice, according to the treatment protocol. Group (I): control mice; Group (II): mice administered with a dose of 50 µl FCO; Group (III): mice administered with a dose of 500 µl FCO; Group (IV): mice administered with a dose of 500 µl sunflower oil; Group (V): mice administered with a dose of 50 µl FCO prior to infection with *E. coli*; Group (VI): mice infected with *E. coli* prior to administration of a dose of 50 µl FCO; Group (VII): mice administered with a dose of 500 µl FCO prior to infection with *E. coli*; Group (VIII): mice infected with *E. coli* prior to administration of a dose of 500 µl FCO.

Figure 3 shows that up to day 14, the body weight of mice in groups V and VII declined. However, in group VII, which was provided with a higher dose of FCO (500 µl), weight loss could be kept compared to group V, which was provided with a lower FCO dose (50 µl) (Sulistyo et al., 2006). Figure 3 also shows that the body weight of mice in groups II, III, and IV increased following the treatment. This increase was attributed to administering doses ranging from 50 µl to 500 µl of FCO and doses of 500 µl of sunflower oil, which could increase the body weight of mice. Oral administration of a 500 µl dose of sunflower oil resulted in a higher increase in body weight compared to administering a 500 µl dose of FCO. It shows that sunflower oil is a type of hydrogenated oil that proliferates body weight.

Mice in groups V and VII showed higher weight loss compared to the control group. This was due to the mice being infected with *E. coli* prior to the administration of FCO, which exacerbated the infection. Providing FCO after the infection did not lead to an increase in body weight, as FCO had to work extra hard to improve metabolism and protect the body's cells from damage. Mice in groups VI and VIII, which were infected with *E. coli* and simultaneously given FCO, were able to gain body weight. This was because FCO, which entered the body instantly, could be used to help the body defend against the occurrence of infection. Therefore, oral administration of a 500 µl dose of FCO to group VIII resulted in a higher weight gain compared to group VII, which was given a 50 µl dose of FCO.

These results demonstrate that administering FCO prior to infection with *E. coli* resulted in a higher difference in weight gain compared to administering FCO after infection with *E. coli* occurred. Consequently, oral administration of FCO prior to infection enabled the body to better overcome infections, resulting in higher weight gain (Sulistyo et al., 2009).

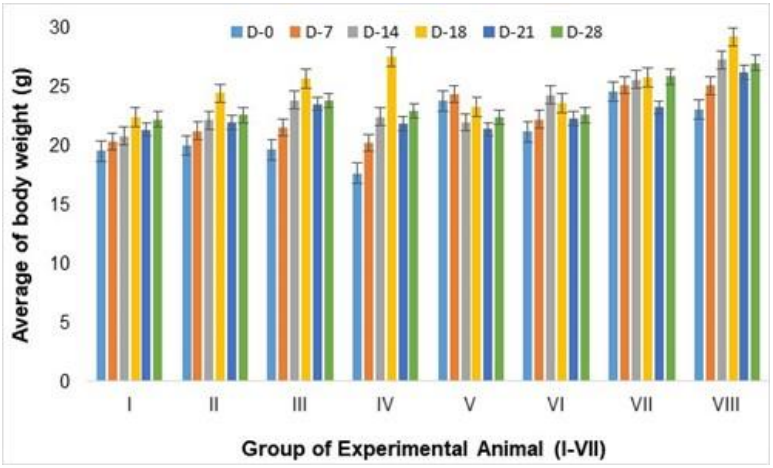


Figure 3. Observations on Body Weight Changes of Mice Before and After Treatment with FCO and *E. Coli* Infection

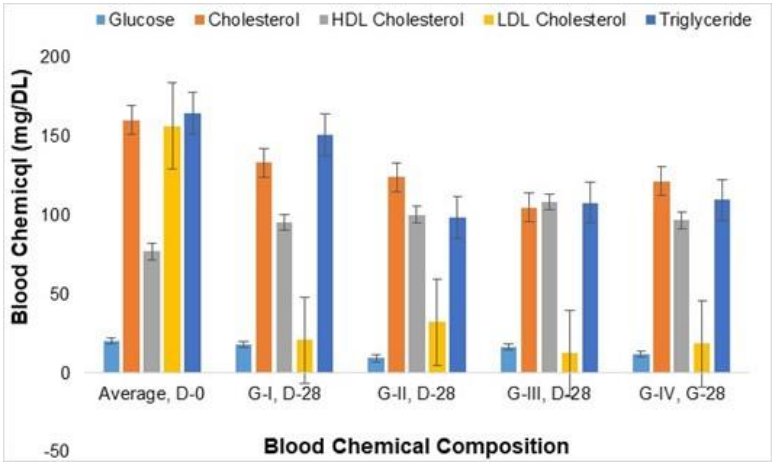


Figure 4. Observation on Blood Serum of Mice on Days-0 and Day-28

Analysis of blood sugar levels in mice in groups II and III, which were administered doses of 50 µl and 500 µl of FCO, respectively, showed a decrease of 5.33 mg/dL and 0.33 mg/dL, respectively, on day 14, and a decrease of 8.5 mg/dL and 1.2 mg/dL on day 28, respectively. This suggests that the decrease in blood sugar levels required more time. Serum taken on day 28, following administration of FCO, showed lower blood sugar levels compared to serum taken on day 14 (Sulistyo et al., 2009) (Figure 3).

Analysis of blood sugar levels in mice in groups II and III, which were administered doses of 50 µl and 500 µl of FCO showed a decrease of 5.33 mg/dL and 0.33 mg/dL, respectively, on day 14, and a decrease of 8.5 mg/dL and 1.2 mg/dL on day 28. This suggests that the decrease in blood sugar levels required more time. Serum taken on day 28, following administration of FCO, showed lower blood sugar levels compared to serum taken on day 14 (Sulistyo et al, 2009).

Analysis of blood cholesterol levels in mice in group II, which were administered a dose of 50 µl of FCO, showed a decrease of 9 mg/dL on day 28. Meanwhile, cholesterol levels in mice of group III, administered with a dose of 500 µl of FCO, decreased to 3.6 mg/dL on day 14 and 28.33 mg/dL on day 28, respectively. Mice in group IV, administered with a dose of 500 µl of sunflower oil, showed a decrease in blood cholesterol starting from day 14 (3.3 mg/dL) to day 28 (11.7 mg/dL). HDL cholesterol levels in mice of groups II and III, administered with doses of 50 µl and 500 µl of FCO, and 500 µl of sunflower oil, respectively, showed an increase on day 14 (4.67 mg/dL, 12.67 mg/dL, and 1.0 mg/dL, respectively), but decreased on day 28 (10.2 mg/dL, 11.5 mg/dL, and 1.5 mg/dL, respectively) (Figure 4). Although the administration of FCO and sunflower oil increased HDL levels, administration of a 500 µl dose of FCO was more effective compared to a 50 µl dose of FCO, as well as compared to a 500 µl dose of sunflower oil (Sulistyo et al, 2011; 2009).

Analysis of LDL cholesterol in mice from group II, administered with 50 µl doses of FCO, showed a decrease on day 14 (5.6 mg/dL), followed by an increase on day 28 (11.5 mg/dL). Mice in group III, administered with 500 µl doses of FCO, exhibited a decrease in LDL cholesterol levels to 8.2 mg/dL. When compared to group IV, administered with sunflower oil (2.2 mg/dL), the mice in the FCO 500 µl group showed lower LDL cholesterol levels. Oral administration of 50 µl doses of FCO resulted in a reduction in triglyceride levels from day 14 (18.6 mg/dL) to day 28 (52.5 mg/dL). Administration of 50 µl doses of FCO was more effective in lowering triglycerides compared to both 500 µl doses of FCO and 500 µl doses of sunflower oil. These results indicate that lower doses of FCO (50 µl) required a longer time to produce optimal results, while higher doses (500 µl) required less time to achieve effective results and were more efficient than the use of sunflower oil.

Figure 5 displays microscopic observations of tissue samples from mice spleens. Observations of liver tissue in all groups were conducted on days 14, 17, 23, and 28. Histopathological examination of spleen and intestine tissues showed no changes from day 13 to day 28, whereas samples of liver and kidney tissues revealed changes occurring. Histopathological examination of the intestinal tissue of mice showed no changes. Similarly, group II, administered with doses of 50 µl of FCO, and group III, administered with doses of 500 µl of FCO, exhibited no changes in their liver tissue samples compared to mice administered with sunflower oil. Microscopic observation revealed that the livers of mice infected with E. coli and simultaneously administered with FCO exhibited focal necrosis due to the pathogenic infection of *E. coli*. Oral administration of 500 µl doses of FCO resulted in fatty liver occurrence, while administration of 50 µl doses of FCO indicated the absence of fatty liver. Therefore, the use of FCO is suggested at appropriate doses (Figure 5).

The presence of fat vacuoles in the livers of mice administered with sunflower oil indicates the occurrence of fatty liver due to the administration of high-fat oil. A different result was observed in the liver samples of mice administered with FCO at the same dose, as FCO, containing saturated fatty acids, does not cause fat deposits in the liver organ. In contrast, sunflower oil, with a high percentage of unsaturated fatty acids, is converted into triglycerides in the blood, leading to fatty liver formation (Figure 5).

Histopathological changes in the kidneys were observed from day 14 to day 28, such as swelling of the glomerulus and necrosis of renal tubule epithelial cells, in some mice across different treatment groups (Sulistyo et al., 2006). However, histopathological changes in the kidneys were not found in the control group. Administration of a 50 µl dose of FCO did not cause histopathological changes in the kidneys of mice from day 13 until the end of the study on day 28, compared to mice administered with a 500 µl dose of FCO, which exhibited changes such as swelling of glomeruli and necrosis of renal tubule epithelial cells (Table 3).

Oral administration of a 500 µl dose of FCO resulted in increased kidney workload, affecting kidney function, characterized by swelling of the renal glomerulus. Mice infected with *E. coli* and simultaneously administered with FCO exhibited swelling of the glomerulus and necrosis of kidney tubule epithelial cells. *E. coli* infection may lead to sepsis, allowing bacteria to penetrate the kidneys, resulting in nephritis characterized by inflammation and necrosis of glomerular epithelial cells and renal tubules (Sulistyo et al, 2006).

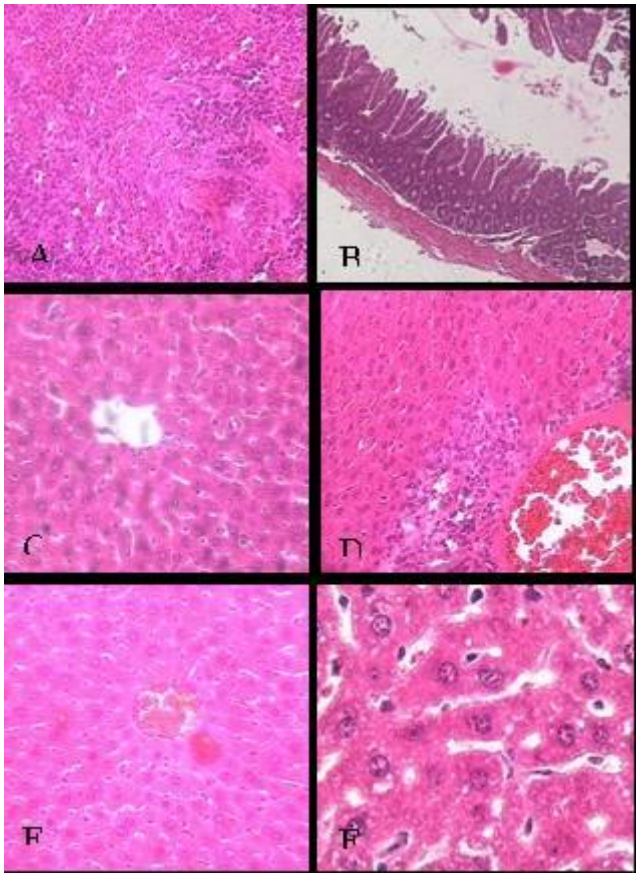


Figure 5. Microscopic Observation of Spleen Preparations from Mice (A), Intestinal Preparations (B), Liver Preparations from the Control Group (C), Liver Preparations from Mice Infected with E. Coli Followed by Oral Administration of FCO (D), Liver Preparations from Mice Treated with a Single Dose of FCO (E), and Liver Preparations from Mice Treated with Sunflower Oil (F), All at 400x Magnification

Table 3. Histopathology Observation on Samples of Mice Kidney

Group	Day-14	Day-18	Day-21	Day-28
I	NC	NC	NC	NC
II	NC	NC	NC	NC
III	NC	NC	NC	Glomerular swelling and necrosis of epithel tubule
IV	NC	NC	Glomerular swelling and necrosis of epithel tubule	Glomerular swelling and necrosis of epithel tubule
V	NC	NC	NC	NC
VI	NC	NC	Glomerular swelling and necrosis of epithel tubule	NC
VII	NC	Glomerular swelling and necrosis of epithel tubule	Glomerular swelling and necrosis of epithel tubule	Glomerular swelling and necrosis of epithel tubule
VIII	NC	Glomerular swelling and necrosis of epithel tubule	Glomerular swelling and necrosis of epithel tubule	Glomerular swelling and necrosis of epithel tubule

*) NC;

4. CONCLUSION

The antibacterial activity of FCO has been tested against pathogenic cultures, especially L. monocytogenes and S. typhimurium. The results demonstrate FCO's ability to inhibit the growth of these pathogens. Histopathological examination of the spleen, liver,

kidneys, and intestines did not reveal any adverse changes. However, mice infected with E. coli followed by the administration of FCO showed focal necrosis in the liver due to the E. coli infection and glomerular swelling and renal tubular epithelial necrosis in several experimental animal treatment groups. Test animals infected with E. coli at the same time as FCO was administered showed the potential of FCO in reducing E. coli infections by preventing inflammation and necrosis in the kidneys.

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