

THE EFFECT OF DIFFERENT FREE FATTY ACID FRACTIONS FROM HYDROLYZED VIRGIN COCONUT OIL ON CHANGES OF LIPID PROFILE AND LIVER MORPHOLOGY INDUCED BY HIGH FAT DIET: AN *IN VIVO* STUDY

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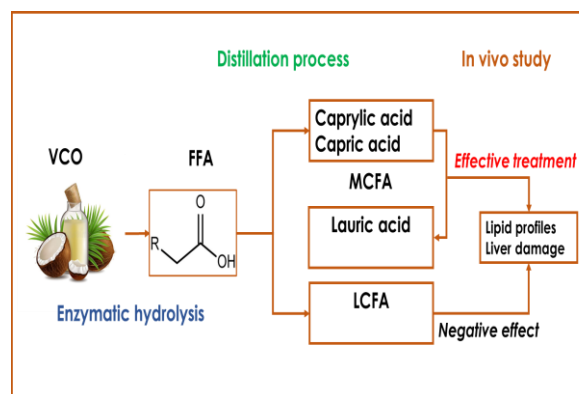
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Graphical abstract



Abstract

This study investigated effect of virgin coconut oil (VCO)-derived fatty acids on the alteration of lipid profiles and liver tissues of high fat diet (HFD) fed mice. The initial fatty acids mixture was successfully obtained via hydrolysis process using *Candida rugosa* lipase (CRL). Enrichment of medium chain fatty acids (MCFA) from the initial fatty acid mixture using distillation process was developed to achieve 3 fractions of fatty acids mixture including Fraction I (MCFA mainly from C8 to C10), Fraction II of lauric acid (C12), and Fraction III (long chain fatty acids from C14 to C20). Among these agents, VCO only diminished ALT level but the initial FFA mixture decreased both ALT and AST levels compared with HFD-induced mice. Moreover, Fraction I and Fraction II showed an obvious difference in decreasing ALT, AST, total cholesterol, and LDL cholesterol levels as well as increasing HDL cholesterol level compared to other agents. It was observed a microvesicular steatosis and mild inflammation in liver section of mice fed with HFD whereas VCO, initial fatty acids, and Fraction II showed the beneficial effect on liver damage.

Keywords: Medium chain fatty acids, virgin coconut oil, liver, hepatic steatosis, inflammation, high fat diet

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1.0 INTRODUCTION

Fat in daily meals is indispensable due to its important nutritive energy source for activities of people. However, fat generally used for a daily diet is long chain fatty acids (LCFA), which are mainly responsible for adipose tissue constituents and several metabolic

diseases owing to their tendency to convert into fat in the liver and adipose tissue. These LCFA have been easily found in vegetable oil or fat sources. Nowadays, medium chain fatty acids (MCFA) have been drawing the attention of many researchers due to their biological benefits. MCFA are either transported directly to the liver via the portal venous system or

reformed into new triglycerides, which enter the lymphatic system. However, they tend to enter the portal vein to go to the liver, in which they are metabolized rapidly by β -oxidation, increasing diet-induced thermogenesis [1]. A previous study which was related to absorption and metabolism pathway of fatty acids in rat intestine showed that 72% of lauric acid (C12), 58% of myristic acid (C14), 41% of palmitic acid (C16:0) and 28% of stearic acid (C18:0) were evaded from lymphatic pathway [2]. This obviously suggested that MCFA were directly transported to the liver rather than entering the lymphatic pathway as LCFA [3]. As the result, MCFA do not contribute to weight gain as they are mostly converted into energy and do not convert into fat in adipose tissues as LCFA; therefore, it could be possibly used for preventing and treating obesity. Moreover, a replacement of LCFA-rich diet by MCFA-rich diet has been shown to reduce the levels of hepatic steatosis and hepatitis [1].

Virgin coconut oil (VCO) is well recognized as a rich source of MCFA including Caprylic acid (C8), Capric acid (C10), and Lauric acid (C12) which is accounted for 45-53% of the total fatty acid content in VCO. VCO is rapidly metabolized as its MCFA composition is easily absorbed and transported [3]. It was previously demonstrated that the treatment of VCO decreased significantly blood total cholesterol, LDL levels, along with the increase of HDL in the hypercholesterolemic rats [4]. Mice fed with VCO combined diet were found to be the most effective approach to increase HDL cholesterol while decrease LDL cholesterol compared to copra oil, olive oil, and sunflower oil [5]. Most recent studies have usually been conducted to investigate the beneficial effects of medium chain triglycerides (MCT) on lipid profiles and liver tissues. The biological benefits of fatty acids in free form have yet to be fully addressed. The aim of this study was to extract free fatty acids (FFA) in VCO and evaluate their biological activities in lipid profiles and their preventive effect on hepatic steatosis and hepatitis induced by high cholesterol diet in mice. Besides, to emphasize the role of specific fatty acid composition, these initial fatty acids were fractionated to enrich the content of specific fatty acids and were compared to VCO, the initial fatty acid mixture upon their biological efficacy.

2.0 METHODOLOGY

2.1 Materials

Virgin coconut oil was supplied from Luong Quoi Co. Ltd, Ben Tre, Vietnam. Lipase from *Candida rugosa* sp (CRL) (type VII, ≥ 700 unit/mg solid) was supplied from Sigma-Aldrich Co. Ltd, St. Louis, Missouri, USA. Potassium dihydrogen phosphate (KH_2PO_4), and sodium chloride (NaCl) were from Ajax Finechem (Auburn, NSW, Australia). Potassium chloride (KCl), disodium hydrogen phosphate anhydrous (Na_2HPO_4) were from Elago Enterprises Pty. Ltd. (Sydney,

Australia). Ethanol, acetone, iso-octane, diethyl ether, silymarin, and n-hexane were purchased from Sigma-Aldrich Co. Ltd, St. Louis, Missouri, USA.

Male Swiss albino mice (aged at two months, with body weight about 25-30g) were provided from Pasteur Institute of Ho Chi Minh City, Vietnam. The commercial standard chow (3,840 kcal/kg, Anifood) was purchased from the Institute of Vaccine and Medical Biologicals, Nha Trang city, Vietnam. Ground nut oil was purchased from Quy Nguyen company, Viet Nam and cholesterol powder was provided by Merck Millipore, USA. Calcium carbonate was supplied from Novats Pharma Ltd., Pakistan. H&E Staining Kit (Hematoxylin and Eosin) was purchased from Abcam plc., Cambridge, UK.

2.2 Obtaining Free Fatty Acids from Virgin Coconut Oil

The hydrolysis process of VCO was adopted from Nguyen *et al.* (2017) with optimized process parameters [6]. Briefly, VCO was dissolved in iso-octane (1:1 of w/w) to enhance its solubility in phosphate buffer saline (PBS) [7]. The as-prepared VCO mixture was emulsified in PBS (pH 7) at the ratio of 1:5 (w/w) by using high speed homogenizer (IKA T25 digital ULTRA-TURRAX, Germany) at 10000 rpm in 15 min. 1.5% (w/w VCO) of CRL was then dissolved in the emulsified mixture by using an overhead stirrer (OS20, USA) at 350 rpm in 5 min. The hydrolysis reaction was conducted in an orbital shaker incubator (LM-2575RD, Yihder Technology Co., Taiwan) at the speed of 150 rpm and temperature of 40°C for 16 hours [6]. An aliquot of ethanol (99.5%) was added to the hydrolyzed mixture to stop the reaction.

The extraction process of FFA was then conducted by using a separatory funnel, following the method of Shimada *et al.* [8]. The released FFA in the hydrolyzed mixture was firstly neutralized by using the excess of 0.5 N KOH. The addition of n-hexane at a ratio of 1:1 (v/v) in the mixture was to dissolve unlipolyzed VCO (tri-, di-, or monoglyceride), resulting in the upper phase in the separatory funnel while the lower phase was the mixture of neutralized FFA. The neutralized FFA was then acidified with 4 N HCl solution for the conversion of fatty acids to free form. The mixture was again mixed with n-hexane solution to obtain the FFA, followed by a purification process of FFA using a rotary evaporator at 60°C (IKA RV digital V, Germany).

2.3 Experimental Set-up of Vacuum Distillation Process

The experimental set-up of vacuum distillation system was custom-made by connecting Trivac oil sealed vacuum pump (15 mm Hg, Leybold, USA) with laboratory distillation apparatus. Briefly, a two-neck round bottom flask (250 ml) was connected to the condenser via distillation head; a thermometer (200°C) was inserted into the other neck of the flask and was sealed by a hole rubber stopper. The other part of the condenser was covered by receiving flask via

distillation receiver, which promoted a vacuum inlet to connect to the vacuum pump. The extracted FFA in the round bottom flask was heated using a heating mantle (Joan Lab Equipment Co., Ltd, Zhejiang, China). The temperature at the pressure of 15 mm Hg of the experimental set-up was preliminarily calculated to obtain three assigned fractions of FFA. FFA from C8:0-C10:0 (fraction I), C12:0 (fraction II) were achieved by adjusting the temperature of 160-165°C and 175-180°C, respectively. The remaining FFA at the bottom flask (fraction III) were determined as LCFA from C14:0 to C20:0 [9].

2.4 Analysis of Fatty Acid Composition

Characterization of FFA composition in each achieved FFA mixture was determined by using Gas Chromatography with Flame-Ionization Detection (GC-FID) analysis. The FFA was initially converted to fatty acid methyl esters (FAME) by adding 950 ml of n-hexane 50 mg of FFA solution followed by 50 ml of 30 ml/100 ml sodium methoxide in methanol. The mixture was vortexed for 5 s and was kept settling for 5 min. An aliquot (2 µl) at the top layer of the mixture was injected into a GC-FID device (SHIZMADZU 2010 Plus, Japan) by split injection mode at the ratio of 1:25. The column DBFFAP (0.25 mm internal diameter, 30 m length, and 0.25 µm film thickness) was operated at a pressure of 12 psi. Helium as the carrier gas was used; FID and injector temperature were both maintained at 250°C over the process. The initial column oven temperature was 100°C and the temperature was programmed to 230°C at 8°C/min and maintained consistently until the analysis was completed [10].

2.5 Animal Experiments

Mice were acclimatized in the animal cage under standard husbandry conditions with a 12 h light-dark cycle (8:00-20:00) for 1 week before the experiment. The experimental procedure was in strict compliance with the Declaration of Helsinki (1964). Briefly, mice were divided into 8 groups (5 mice/group) which had different treatments by using the oral gavage method [4].

Group 1 (Control): Mice were fed with commercial standard pellet for 28 days

Group 2: Mice were fed with high fat diet (powdered standard commercial pellet + 8% (w/w) of groundnut oil + 2% (w/w) of cholesterol + 0.1% (w/w) Calcium) (HFD) for 28 days without treatment [11]

Group 3: Mice were fed with HFD and treated by Silymarin with a dose of 50 mg/kg bodyweight for 28 days

Group 4: Mice were fed with HFD and treated by VCO with a dose of 2 ml/kg bodyweight for 28 days

Group 5: Mice were fed with HFD and treated by FFAs with a dose of 2 ml/kg bodyweight for 28 days

Group 6: Mice were fed with HFD and treated by fraction I with a dose of 2 ml/kg bodyweight for 28 days

Group 7: Mice were fed with HFD and treated by fraction II with a dose of 2 ml/kg bodyweight for 28 days

Group 8: Mice were fed with HFD and treated by fraction III with a dose of 2 ml/kg bodyweight for 28 days

2.6 Measurement of Bodyweight, Liver Weight, and Relative Liver Weight

At the beginning (day 1) and the end of the experiment (day 28), all experimental animals were fasted overnight to reduce the difference in feeding. The body weight was directly measured using an electronic balance. At the end of the experiment, mice were euthanized by carbon dioxide inhalation. Blood was collected via a cardiac puncture into the heparinized tube for biochemical analysis. The liver was collected, washed with ice-cold saline, and recorded its weight. The relative weight of livers was calculated by the following formula:

$$\text{Relative liver weight} = \frac{\text{liver weight}}{\text{body weight}} \times 100 \text{ (g/100g)}$$

Subsequently, livers were immediately fixed in 10% formalin for histological studies.

2.7 Determination of Lipid Profile and Hepatic Enzymes

Blood was collected into heparinized tubes; the plasma was then separated by centrifugation. Lipid profile including triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol), triglyceride (TG), as well as plasma levels of hepatic enzymes such as alanine transaminase (ALT), and aspartate transaminase (AST) were determined using commercial diagnostic kits (Diagnosticum Zrt, Hungary) according to manufacture instructions.

2.8 Histological Analysis

Livers preserved in 10% of formalin were prepared for histological studies with Hematoxylin and Eosin staining. The specimen was dehydrated in different grades of alcohol and was cleared in xylol, followed by paraffin embedding, sectioned at 4-6 µm thick and stained with Hematoxylin and Eosin. The liver sections then were examined under a microscope for estimation of the extent of hepatic damage.

2.9 Statistical analysis

Statistical analysis was performed using R software (R Foundation, Vienna, Austria). The data were presented as mean ± standard deviation. Differences between means of different groups were analyzed using analysis of variance (Nested ANOVA) before using Duncan

test, the criterion of statistical significance was set as $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Composition of Fatty Acids in Developed Fraction

Figure 1 presents the composition of free fatty acids in each achieved Fraction after the distillation process, compared to the initial FFA mixture. The initial FFA mixture was successfully achieved via hydrolysis of VCO using CRL which was consistent with our previous studies [6,12]. As shown in Figure 1, the primary fatty acid components in the initial FFA mixture were found to be MCFA in which lauric acid (C12:0) was predominant, about 47.23%. This result was compatible

with reported studies [3,13,14]. In this study, three fractions of desirable fatty acids were well developed via the experimental set-up of the vacuum distillation process. In fraction I, MCFA including caprylic acid (C8:0) and capric acid (C10:0) took up 64.76% compared to those of 13.74% in the initial FFA mixture. Fraction II was the result of enriched lauric acid (C12:0), around 76.49% compared to 47.23% in the initial FFA mixture. And Fraction III was representative for LCFA (C14:0 to C20:0) which were proportional to approximately 88%. This method could promote a promising approach for the use of selected medium chain fatty acid components in virgin coconut oil. The obtained fatty acid fraction could be applied to further studies in the evaluation of its beneficial activities.

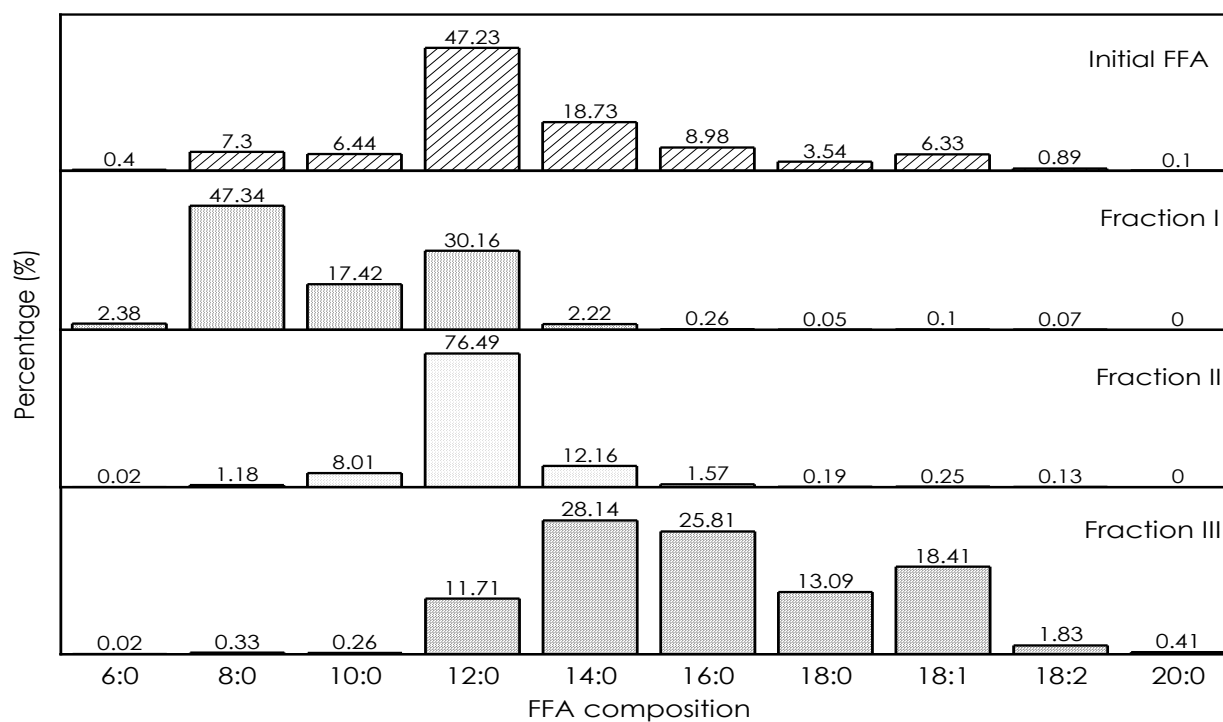


Figure 1 The percentage of each type of fatty acids (C8:0 to C20:0) in the mixture of initial FFA, Fraction I, Fraction II, and Fraction III

3.2 The Change of Bodyweight and Relative Liver Weight in Experimental Mice

Table 1 shows the change of body weight of mice under different treatments. At the beginning of the experiment, all mice showed an average value of initial body weight (~32 grams). The body weight of mice with HFD appeared to dramatically increase to 37.78 ± 1.31 grams as compared to control mice (33.86 ± 1.03 g, respectively, $p < 0.05$). In this study, VCO and fraction III treatment in 4 weeks did not alter the increase of body weight when mice were fed with HFD (38.76 ± 1.02 and 37.78 ± 1.31 g, respectively, $p > 0.05$). Coconut oil was reportedly

found to reduce the HFD-increased body weight of mice but required an extended time of treatment (12 weeks) [15]. The main components of fraction III were LCFA which entered the lymphatic pathway and contributed to fat accumulation, elucidating insignificant body weight-lowering effect [3]. However, the initial FFA, fraction I, and fraction II treated mice had a positive effect in reducing body weight. This could be explained by medium chain fatty acid components of these fractions. MCFA were previously reported to be absorbed via portal system and was transported directly to the liver, preventing fat accumulation in adipose tissues [1]. In this study, it was clearly demonstrated that MCFA in free form

could effectively promote better body weight-lowering effect than medium chain triglyceride in VCO. As seen in Table 1, among these MCFA, lauric acids (C12:0) in fraction II were considerably the most effective in preventing weight gain of mice (30.86 ± 1.89 g) compared to caprylic acid (C8:0) and capric acid (C10:0) in fraction I (34.32 ± 1.07 g). This result could possibly contribute to the applicability of lauric acid in free form in preventing obesity and several lifestyle-related diseases in further studies. In terms of relative liver weight, Fraction I and Fraction III treated groups were higher than the control group (5.31 ± 0.22 , 5.55 ± 0.19 , and 5.43 ± 0.38 versus 4.09 ± 0.30 g/100g, $p < 0.05$), which implied some alteration of liver morphology in these groups, such as hepatitis or hepatic steatosis. Liver weight of treated mice with initial FFA, VCO, and fraction II were fairly consistent with control group (~ 4 g/100g B.W). Long chain triglycerides, medium chain triglycerides and long-medium chain triglycerides treated rats in the study of Shinohara *et al.* (2005) did not significantly differ in liver weight [16].

Table 1 Bodyweight and relative liver weight of experimental mice exposed to different treatments

	Initial B.W. (g)	Final B.W. (g)	Relative L.W. (g/100g)
Control	31.12 ± 0.74^a	33.86 ± 1.03^b	4.09 ± 0.30^a
HFD	31.25 ± 1.01^a	37.78 ± 1.31^{de}	5.31 ± 0.22^b
Silymarin	31.41 ± 1.07^a	35.20 ± 1.14^{bc}	4.14 ± 0.28^a
VCO	31.65 ± 0.75^a	38.76 ± 1.02^e	4.27 ± 0.28^a
Initial FFA	31.30 ± 0.95^a	35.05 ± 1.22^{bc}	4.32 ± 0.25^a
Fraction I	31.25 ± 1.05^a	34.32 ± 1.07^b	5.55 ± 0.19^b
Fraction II	32.24 ± 1.13^a	30.86 ± 1.89^a	4.48 ± 0.52^a
Fraction III	32.23 ± 1.29^a	36.69 ± 1.34^{cd}	5.43 ± 0.38^b

Values with different letters (a, b, c, d, e) within the column are significantly different ($p < 0.05$). B.W., bodyweight; L.W., liver weight

3.3 The Change of Lipid Profile and Hepatic Enzymes In Experimental Mice

The hepatic enzyme level of mice after different types of treatment is illustrated in Figure 2. Administration with HFD caused a significant increase in AST (Figure 2A), ALT (Figure 2B) compared to control mice. The alteration of HFD on hepatic enzymes and cholesterol was in agreement with past reports [17, 18]. Silymarin was used as a standard drug for treating liver disease, which noticeably decreased ALT (Figure 2B) and AST (Figure 2A) levels as compared to the control group [19]. VCO could only decrease ALT (Figure 2B) in this study which was incompatible with the previous study of Harini *et al.* (2009) [4]. This could be ascribed to the higher fat diet using in this study, leading to insignificant ALT-lowering effect.

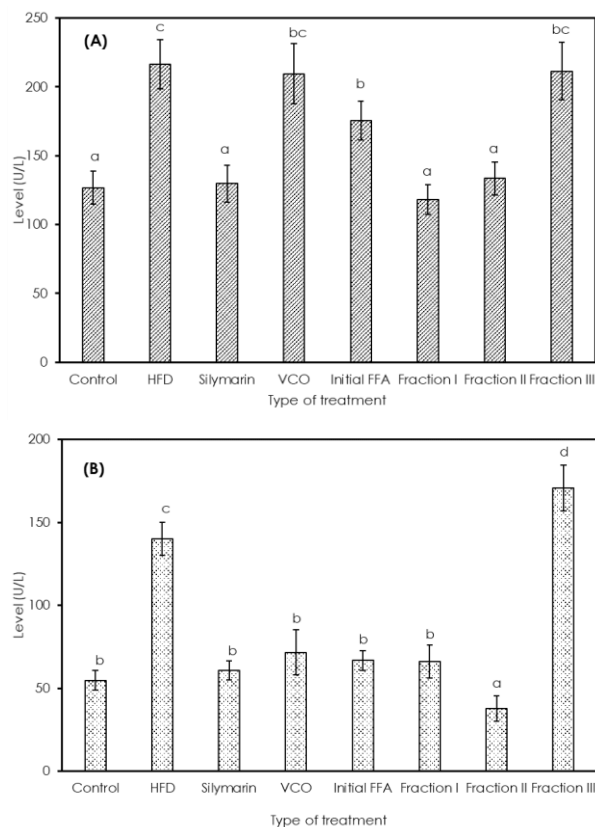


Figure 2 The changes of hepatic enzymes A) Aspartate transaminase (AST) and B) Alanine transaminase (ALT) in experimental mice. Superscripts (a, b, c) indicate significant difference in each type of treatment

LCFA in Fraction III did not exert the beneficial effect on ALT and AST level but they contributed to increase ALT (Figure 2B) level, which implied serious liver damage. Wang *et al.* (2016) showed that LCFA could induce the apoptosis and necrosis of hepatocytes, inhibited the hepatic antioxidant enzymes, and triggered oxidative stress and inflammation [20]. Therefore, administration of LCFA in Fraction III resulted in the increase of ALT (Figure 2B) and AST (Figure 2A), two hepatic enzymes, which were activated by liver injury and liver diseases. The initial FFA, fraction I, and fraction II treatment caused a significant reduction in ALT (Figure 2B) and AST (Figure 2A) level of treated mice. Moreover, treated mice by fraction I and II were found to return AST (Figure 2A) value to normal level compared to the control group (~ 125 U/L). ALT (Figure 2B) level of fraction II- treated mice (~ 40 U/L) was significantly lower than control mice (50 U/L) after treatment. Hence, supplement with Fraction I and Fraction II could be a viable option in decreasing ALT, AST levels.

Table 2 presents serum biochemical parameters including plasma triglyceride, total cholesterol, and LDL cholesterol level of mice treated by different therapies. Silymarin, in this study, only had the effect in reducing the total cholesterol level from 4.57 ± 0.16 to 4.13 ± 0.24 mmol/L and LDL value from 1.29 ± 0.22 to

0.89±0.20 mmol/L. Although the initial FFA reduced noticeably AST and ALT levels, the total cholesterol of initial FFA was the same as HFD, therefore it still maintained HDL cholesterol and LDL cholesterol level when compared to HFD treated rats. Note that VCO could reduce plasma triglyceride, which was consistent with the previous study [21], whereas the FFA did not alter plasma triglyceride level. Besides,

Fraction III decreased LDL cholesterol significantly, leading to a fall of total cholesterol compared to HFD mice. This could be possibly explained that the unsaturated fatty acids, such as oleic acid (C18:1) in Fraction III (18.41%) might promote hypocholesterolemic effect [22].

Table 2 Plasma lipid profile of experimental mice exposed to different treatments

	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Control	3.38±0.19 ^e	0.62±0.08 ^{cd}	2.34±0.24 ^{bc}	0.75±0.09 ^c
HFD	4.57±0.16 ^{bc}	0.84±0.14 ^{ab}	2.09±0.23 ^{cd}	1.29±0.22 ^a
Silymarin	4.13±0.24 ^d	0.77±0.13 ^{abc}	2.22±0.20 ^{bcd}	0.89±0.20 ^c
VCO	4.19±0.13 ^{cd}	0.61±0.06 ^d	1.96±0.14 ^{de}	1.12±0.22 ^{ab}
Initial FFA	5.88±0.31 ^a	0.70±0.14 ^{bcd}	2.96±0.16 ^a	1.20±0.06 ^{ab}
Fraction I	4.05±0.41 ^d	0.57±0.09 ^d	2.02±0.21 ^{de}	1.20±0.21 ^{ab}
Fraction II	4.80±0.16 ^b	0.82±0.08 ^{ab}	2.42±0.21 ^b	0.98±0.11 ^{bc}
Fraction III	3.33±0.39 ^e	0.86±0.6 ^a	1.73±0.20 ^e	0.44±0.17 ^d

^{a,b,c,d,e} Values with different letters within the column are significantly different ($p < 0.05$). LDL-cholesterol, low density lipoprotein cholesterol and HDL-cholesterol, high density lipoprotein cholesterol.

In this study, the treatment with Fraction I reduced significantly blood lipid parameters (total cholesterol and triglyceride) as compared to HFD mice. Blood ALT and AST level of Fraction II – treated mice were found to be noticeably reduced compared to HFD mice. Although the result showed that the total cholesterol level of Fraction II treated mice was equivalent to HFD mice, it increased the HDL cholesterol and decreased LDL cholesterol level. Therefore, it could be a good point of FFA at Fraction II. In a previous study, MCFAs could inhibit hepatic apoptosis, oxidative stress, and inflammatory activity [20]. Moreover, Liu *et al.* (2017) [23] also showed that MCFAs could reduce serum cholesterol by regulating the metabolism of bile acid in mice. Taken together, administration with MCFA-enriched fractions could ameliorate elevation of hepatic enzymes and reversed the alteration of serum cholesterol. Among of these agents, FFA at Fraction I and Fraction II were the most active agents because they showed obviously the decrease of ALT and AST, as well as LDL cholesterol. Therefore, enhancing the content of MCFA in Fraction I and II could probably increase the efficiency in decreasing blood lipid parameters.

3.4 Histological Changes of Livers of Experimental Mice

Histological changes of livers are presented via photomicrograph of liver tissues in Figure 3. The data from histological studies were strongly supportive to findings from biochemical analysis. The HFD diet caused mild hepatic inflammation and micro-vesicular fatty liver, which was demonstrated with lipid accumulation in hepatic cytoplasm and

infiltration of immune cells in the liver section of HFD treated mice as compared to the normal structure of the liver section of control mice (Figure 3B and 3A, respectively). Inflammatory activation in HFD treated mice was accounted for the increase of plasma AST and ALT levels, as well as relative liver weight (Figure 2 and Table 2).

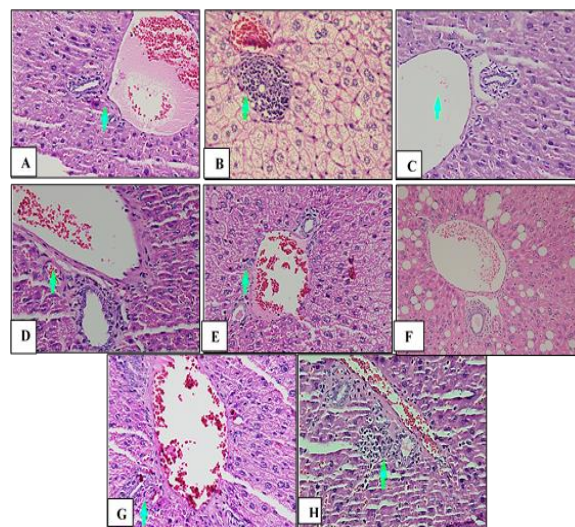


Figure 3 Photomicrograph of liver tissues from mice treated by different agents. Control group (A), HFD diet group (B), Silymarin treated group (C), VCO treated group (D), initial FFA treated group (E), Fraction I treated group (F), Fraction II treated group (G), and Fraction III treated group (H)

The treatment with silymarin reversed the inflammation and recovered the normal structure of the hepatic portal and parenchymal regions (Figure 3C). Therefore, AST and ALT values of silymarin-treated mice were decreased into normal values of control mice. Moreover, VCO and initial FFA-treated groups did not show the inflammation of liver tissue (Figure 3D and 3E). Fraction III also caused mild inflammation for the liver tissue (Figure 3H). It could be explained that Fraction III comprised of the high percentage of LCFA, which contributed to apoptosis of hepatocytes and activated inflammatory markers; therefore, administration of Fraction III could induce the inflammatory response in the liver along with the increase of relative liver weight, ALT and AST levels (Figure 2 and Table 2). This explanation was equivalent to the study of Wang *et al.* (2016) [20], LCFA with HFD increased apoptosis, oxidative stress, and chronic inflammatory responses in the hepatic cells with steatosis, which made the inflammation in mice liver become more serious.

Fraction I treated liver showed the normal structure of portal area with no inflammation, which was consistent with biochemical analysis (Figure 3F and Figure 2). Although the biochemical analysis showed that Fraction I could decrease the plasma ALT, AST, and total cholesterol levels, treatment with Fraction I caused macrovesicular steatosis with many lipid droplets in the liver section and an increased of relative liver weight (Figure 3F, Table 2). Therefore, the use of Fraction I or Fraction III in liver diseases should be limited. Note that, liver tissue of mice using Fraction II showed the normal structure of portal and surrounding parenchymal regions with no inflammation (Figure 3G). It suggested that Fraction II not only decreased ALT, AST but also exerted as an anti-inflammatory agent. This result was similar to the finding from the study of Wang *et al.* [20], in which MCFA could reduce the inflammatory response in liver cells.

4.0 CONCLUSION

Giving the treatment for mice with HFD using different agents showed different outcomes. Among these agents, Fraction I and Fraction II were considerably the most active agents affecting blood lipid parameters compared to control mice and HFD mice. In the HE staining, VCO, initial FFA, Fraction II showed the normal status of rat liver tissue. Therefore, FFA at Fraction II was the most beneficial agent to reinforce the treatment of high fat diet induced metabolic disorders in term of the decrease of body weight, lowering plasma cholesterol effect and inhibition of hepatic inflammation.

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