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Balinese Green Arabica Coffee Bean (*Coffea arabica*) Extract Did Not Prevent the Increase of Malondialdehyde Level and Progressivity of Non-Alcoholic Fatty Liver Disease in Male Wistar Rats (*Rattus norvegicus*) Induced High Calorie Diet

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Abstract: ***Background: An unhealthy lifestyle accompanied by the consumption of high-calorie foods in large quantities and continuously causes metabolic syndrome. This condition was in line with the increasing incidence of non-alcoholic fatty liver disease (NAFLD). The objective of this study was to determine the effect of Balinese green Arabica coffee bean (Coffea arabica) extract in preventing the increase of malondialdehyde levels and the progression of non-alcoholic fatty liver in male Wistar rats fed a high-calorie diet. Methods: This experimental study was a post-test only control group design method. The research subjects were 30 healthy male Wistar rats which randomly divided into three (3) groups. The control group (K) only given standard food of 20 grams/day, the placebo group (P1) with 2 cc aquades and high-calorie diet with additional of 4 ml of lard oil, 2 egg yolks per day and 60% fructose from corn syrup, the treatment group (P2) with 560 mg/kgBw green Arabica coffee bean extract and high-calorie diet. After 28 days, malondialdehyde level from blood plasma were examined and histopathological observations were made on liver tissue samples using SAF score (Steatosis, Activity, Fibrosis). Results: The mean level of malondialdehyde in group K was 3.52±0.16 nmol/mL and significantly higher compared to groups P1 2.08±0.17 nmol/mL and P2 2,30±0.32 nmol/mL (p=0,00), whilst the mean level of malondialdehyde in group P1 was not significantly difference compared to group P2 (p=0,89). Histopathological analysis with SAF score in group K refer to $S_1A_0F_0$, image of normal liver cells, mild activity of steatosis and the development of NAFLD without fibrosis, compared to groups P1 and P2 refer to $S_0A_2F_0$, slight image of steatosis, moderate activity for the progressivity NAFLD to NASH with the picture of hepatocyte ballooning in almost all liver tissue without fibrosis. Conclusion: These findings concluded that the administration of Balinese green Arabica coffee bean extract did not prevent the increase of malondialdehyde level and the progression of non-alcoholic fatty liver in male wistar rats induced with a high-calorie diet.***

Keywords: balinese coffee bean extract, malondialdehyde, non-alcoholic fatty liver, high-calorie diet

1. Introduction

The main hepatic manifestation of the metabolic syndrome is non-alcoholic fatty liver disease (NAFLD). Changes of lifestyle in developing countries, including Indonesia, by consuming high-calorie diet, also known as the western diet, increase the incidence of NAFLD. A consensus of NAFLD and Non-Alcoholic Steatohepatitis (NASH) reports that Indonesia has the highest incidence of NAFLD in Southeast Asia at 30%, compared to other countries such as Malaysia and Singapore at 17% and 5%.[1–3]

Fat deposition in hepatocytes, in NAFLD cases, can cause an excessive increase in free radicals in the body and lead to oxidative stress that occurs in the liver and subsequently an increase in Reactive Oxygen Species (ROS).[3–5]Generation of ROS, reduced Hepatic Glutathione (GSH) and protein oxidation are conditions associated with mitochondrial dysfunction, representing the initial events for the development of NAFLD. Loss of mitochondrial function leads to secondary inhibition of ß-oxidation of lipids, further increasing steatosis and subsequent NAFLD/NASH progression. The formation of ROS in a lipid-rich environment will further induce lipid peroxidation, lead to the release of malondialdehyde (MDA) with detrimental effects on hepatocytes, increase inflammation, and lead to apoptotic liver cells.[6]This conditions that occur at the molecular level, then lead to an earlier aging process accompanied by metabolic syndrome and the occurrence of various diseases associated with old age.[5]

Until today, there is no approved pharmacological therapy in the management of NAFLD/NASH. International and local NAFLD/NASH management guidelines recommend that lifestyle modification interventions such as diet control, weight loss, physical activity, and behaviour modification are the first and only potential treatment strategies.[7–10]

Green Arabica coffee beans (*Coffea arabica*) are one of the alternative nutraceutical approaches in the pharmaceutical industry. The polyphenol content will act as a natural antioxidant that is able to protect the body against reactive free radicals and overcome oxidative damage caused by the formation of ROS through the induction of antioxidant defensive mechanisms.[10,11]Green Arabica coffee beans (*Coffea arabica*), which are not roasted, contain several

bioactive components, including chlorogenic acids (CGAs), caffeine, tannins, trigonelline, and melanoidins.[12–16]Chlorogenic acid (CGA), an ester of quinic acid and caffeic acid, has antioxidant properties. Animal studies have shown that CGA has anti-diabetic, anti-obesity and anti-lipidaemia properties with beneficial effects on insulin resistance and can lower blood pressure.[13,15,16] In addition, apart from the negative impact of caffein which is also contained in coffee, caffeine can also act as an antioxidant. Several studies have shown the effectiveness of caffeine in preventing the oxidation of low-density lipoprotein (LDL) [17], and together with its metabolites can prevent liver fibrosis and inhibit hepatocarcinogenesis in animal studies.[18]

This study will further study the health benefits of Green Arabica coffee beans (*Coffea arabica*) from the Kintamani region in Bali, against NAFLD, which is thought to prevent the increase in malondialdehyde level and the progression of non-alcoholic fatty liver in rats induced by a high-calorie diet.

2. Methods

This study used a randomized post-test only controlled group design. This research was conducted in the Laboratorium Biomedik Terpadu of Udayana University Bali and Sentra Pathology Laboratory Bali. The extract of Balinese green arabica coffee beans were formulated in Laboratorium Pertanian of Udayana University, Bali, Indonesia.

Experimental Animal

Thirty healthy male Wistar (*Rattus norvegicus*) rats (180-200 g) were taken from animal unit of Medical Faculty, Udayana University. The adaptation period was done in a week with standard food mixture and drinking water were administered *ad libitum*. Animals were maintained in room temperature (25^0C) on a 12:12 h light-dark cycle. The samples then divided randomly into 3 groups (n = 10). The control groups (K) were still treated with standard and normal amount of food (20 g/day), the placebo group (P1) were treated with high calorie diet, consist of standard food with additional of 4 ml of lard oil, 2 egg yolks per day and 60% fructose from corn syrup and 2 cc aquades as placebo, the treatment group (P2) were treated also with high calorie diet and 560 mg/kgbw of Balinese Green Arabica Coffee Bean(*Coffea arabica*) Extract. After 4 weeks of treatment, the rats were anesthetized, sample of blood plasma was taken from medial canthus sinus orbitalis for MDA level measurement and the biopsy of liver was performed to examine the histopathology analysis.

Determination of MDA Level

Malondialdehyde was extracted from blood plasma samples and analyzed using spectrophotometer by thiobarbituric acid method. Add 200 µL ice cold 10% TCA to the 100 µL of each sample and centrifuged at 12,000 rpm for 10 minutes. Transfer 200 µL of each clear supernatant into a new labeled tube. To each of the standards and samples, add 200 µL thiobarbituric acid (TBA) reagent. The solution was incubated in a boiling water bath for 10 min to produce pink color. After cooling at room temperature, samples were read at 535 nm (525 up to 545 nm) using a spectrophotometer.

Examination of Liver Histopathology

Paraffin-embedded liver sections were fixed in 10% formaldehyde and stained with haematoxylin and eosin (H&E). The thick sections of liver were cut from the paraffin block and coated on the glass slide. A single pathologist evaluated the histological characteristics and semi-quantitative evaluation using the steatosis, activity and fibrosis (SAF) score was performed.

Data analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software for Windows version 26. All data were tested for normality using Saphiro-Wilk. The significance test used One-Way Annova (parametric test) for MDA level with normal distribution.

3. Results

Malondialdehyde

Descriptive analysis(Table 1) shows the mean of malondialdehyde in group K was 3.52±0.16 nmol/mL and significantly higher compared to groups P1 2.08±0.17 nmol/mL and P2 2,30±0.32 nmol/mL (p=0,00), whilst the mean level of malondialdehyde in group P1 was not significantly difference compared to group P2 (p=0,89).

Table 1: Descriptive Analysis of Malondialdehyde Level

Groups	n	Mean	Median	Standard Deviation	Minimum	Maximum
Control	10	3.52	3.52	0.16	3.25	3.79
Placebo	10	2.02	2.02	0.17	1.86	2.39
Treatment	10	2.21	2.21	0.32	1.97	2.94

The Saphiro-Wilk was used for normality test. Test results in group K showed that the data were normally distributed (p>0.05). Likewise, the test results in the P1 group were also normally distributed (p>0.05). However, in the P2 group, the data were not normally distributed with (p<0.05). Outlier data was found in the 3rd order, 2.79 nmol/mL and the 4th order, 2.94 nmol/mL, then, the outlier data was removed, and re-evaluate again for the normality test and the P2 group were normally distributed. The results of homogeneity tests show that the data is homogeneous. For comparison test was using parametric test, One-Way Annova.

The results of One-Way Annova test that the value of p = 0.00 (p <0.05). It can be concluded that there was a group that had an average MDA level statistically different from others group. Furthermore, the Bonferonni test was conducted to find out which groups were significantly different. The results shows that the average MDA level in group K compared to P1 were significantly different (p = 0.00), group K compared to P2 was also significantly different (p = 0.00), but not so in the group P1 compared to P2 which was not significantly different (p=0.89).The difference in the mean between groups can be observed in Figure 1.

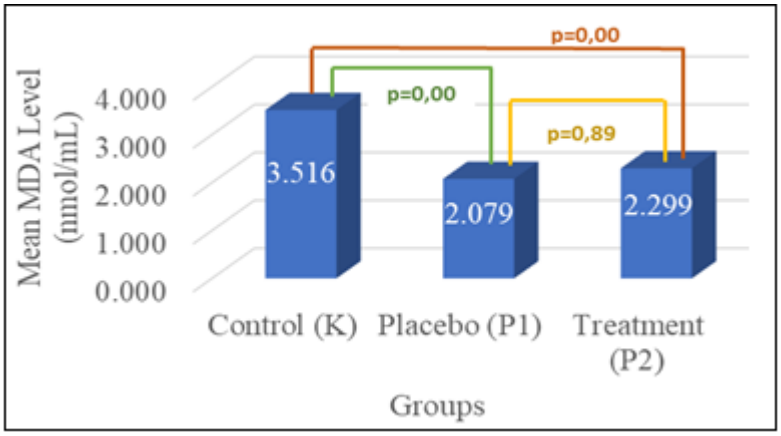
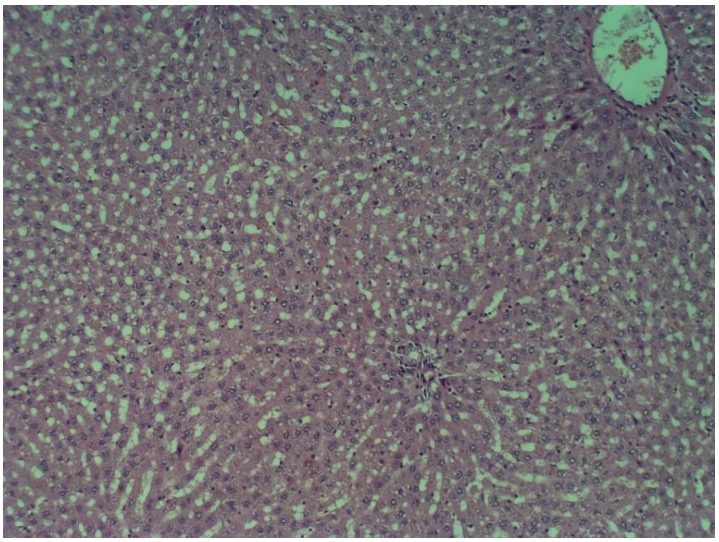


Figure 1: Mean Level Comparation of MDA Level

Histological Findings

Histopathological examination was carried out on rat liver tissue samples taken around 24 hours from the last treatment and stained with hematoxylin and eosin (H&E). The K group demonstrated a normal lobular structure with some steatosis, in which a small, well-defined fat droplet occupies the cytoplasm of the hepatocyte, pushing the nucleus into the periphery (figure 2), compared to the P1 group (figure 3) and P2 group (figure 4), demonstrated hepatocyte degeneration in the form of hepatocellular swelling with few fat droplets.



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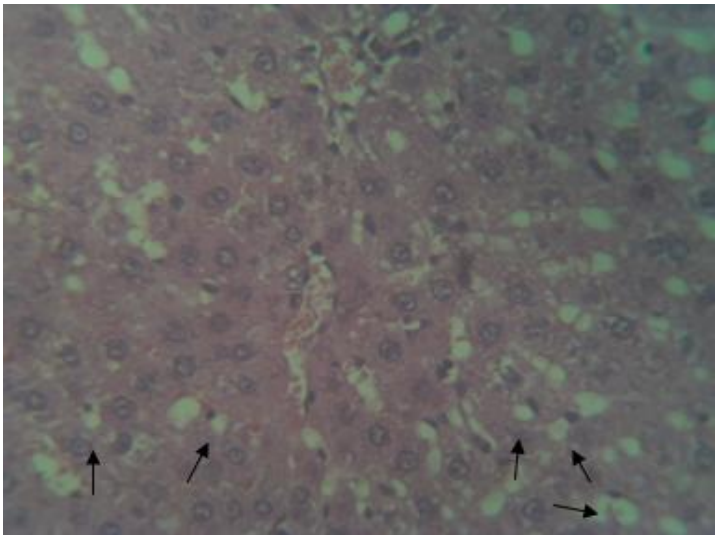
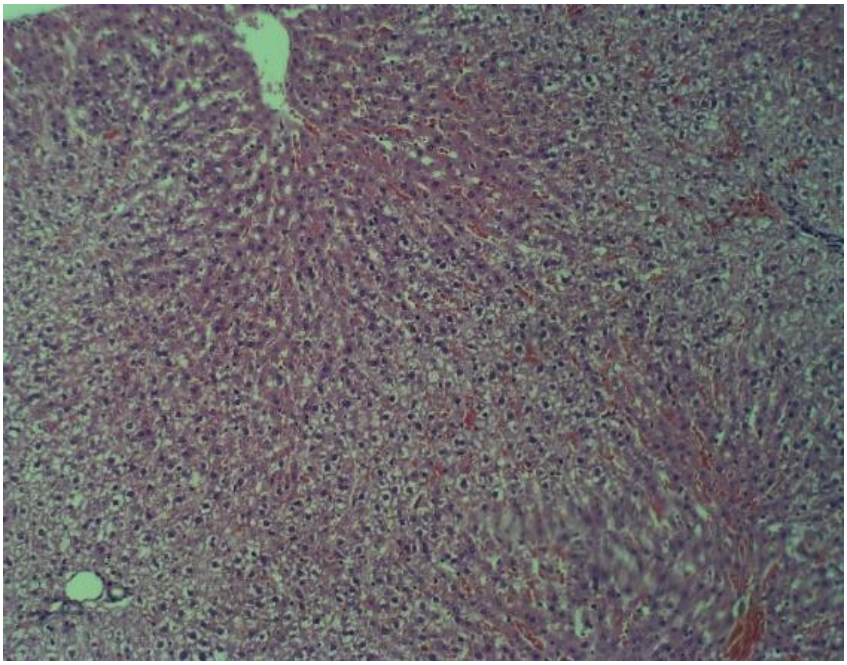


(B)

Figure 2: Histopathology of Control Group (K)
(A) 200x magnification; (B) 400x magnification; bold black arrows indicate steatosis



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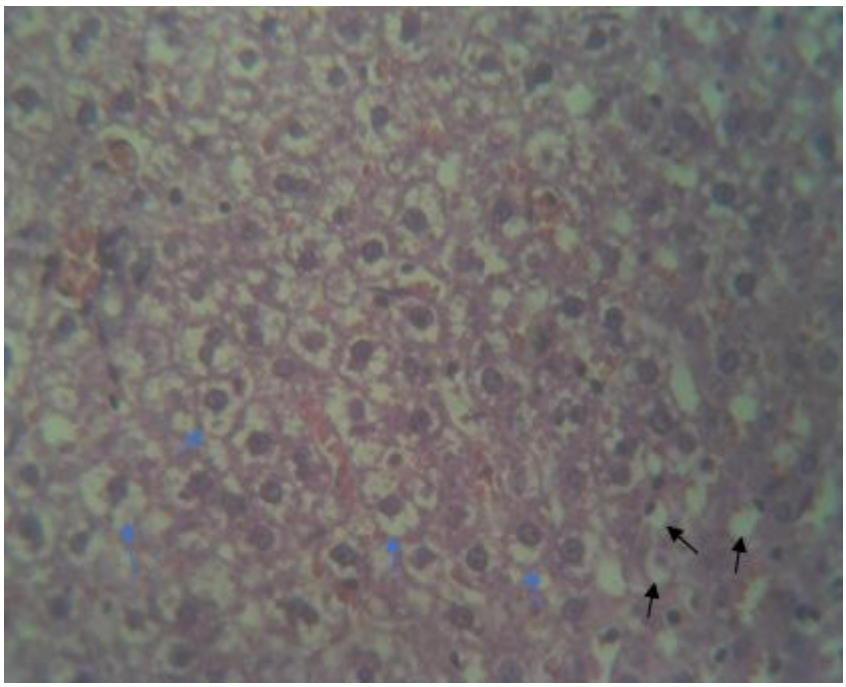
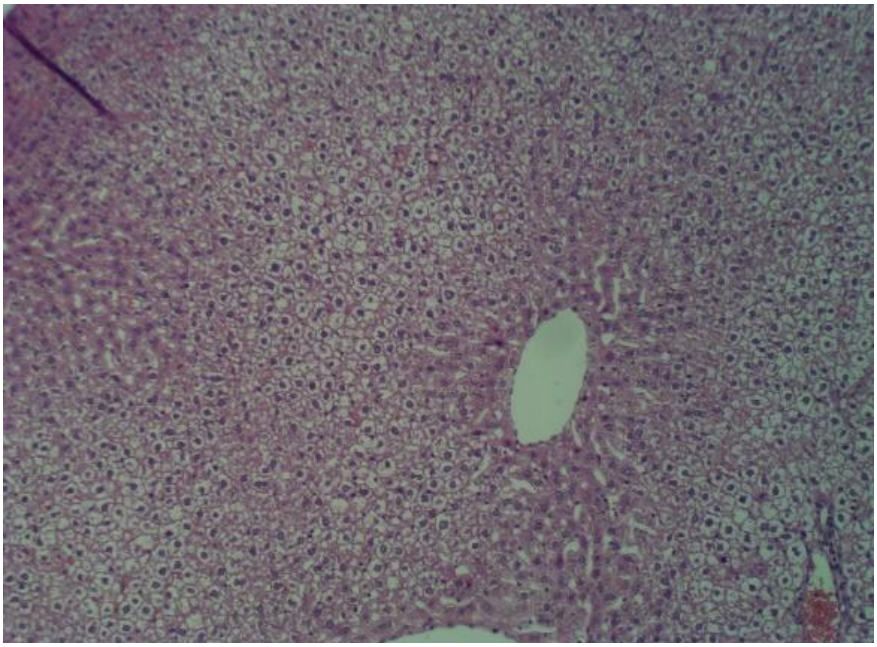


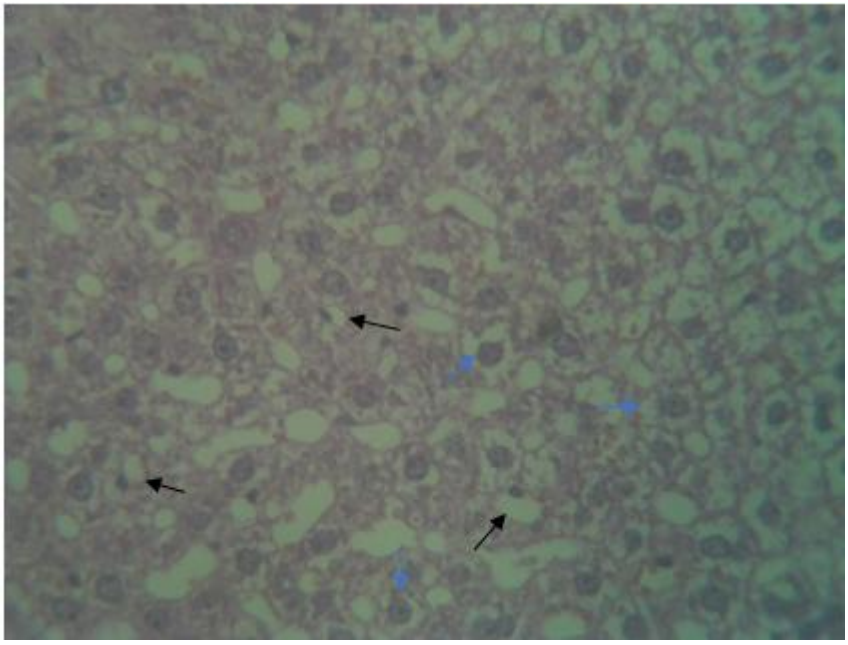
(B)

Figure 3: Histopathology of Placebo Group (P1)

(A) 200x magnification; (B) 400x magnification;Bold black arrows indicate steatosis; Blue bold arrows indicate hepatocyte ballooning



(A)



(B)

Figure 4
Histopathology Treatment Group (P2)
(A) 200x magnification; (B) 400x magnification; Black bold arrows indicate Steatosis; Blue bold arrows indicate hepatocyte balooning

Based on the SAF scoring system (steatosis, activity, fibrosis) [19,20], this study will conduct a semi-quantitative assessment based on the level of NAFLD/NASH activity from the overall view of the tissue samples, for steatosis, hepatocellular swelling and presence or absence of foci of inflammation, also fibrosis.

In accordance with the image of the rat liver tissue samples, the group K (figure 2) show an image of normal hepatocytes with a cuboidal shape and pink eosinophilic cytoplasm, steatosis was found in an estimated number of >5% with medium-sized fat droplets in several areas with no sign of inflammatory and no hepatocyte ballooning, mild activity for the development of NAFLD with no NASH and no fibrosis, hence, the SAF score was $S_1A_0F_0$ (Table 2). The P1 and P2 group demonstrated few of steatosis in an estimated number <5%, enlarged hepatocyte, at least two times of normal cells, but no foci of inflammation in the lobules, moderate activity with development of NASH, no fibrosis, hence, the SAF score was $S_0A_2F_0$ (Table 2)

Table 2: SAF Score of Liver Histopathology Analysis

Groups	Steatosis	Activity of NAFLD/NASH	Fibrosis
Control (K)	S_1	A_0	F_0
Placebo (P1)	S_0	A_2	F_0
Treatment (P2)	S_0	A_2	F_0

Apart from the scores for steatosis and fibrosis, activity scores on the SAF scoring system, to describe the progression from NAFLD to NASH, with observations of hepatocellular swelling and liver lobular inflammation, showed moderate activity in the presence of NASH in groups P1 and P2 (A≥2), compared with group K with mild activity without NASH (A<2).

Supplement Data of Body weight

The analysis of the pre-test and post-test weight measurement data were presented below (table 3). Supplement data for body weight was also an interesting subject to discuss and might had an influence on MDA levels in this study.

Table 2: Analysis of Body Weight Pre- and Post-Test

Variable	Control			Placebo			Treatment		
	Pretest	Posttest	p	Pretest	Posttest	p	Pretest	Posttest	p
Mean±SD	203,5±14,5	242,2±23,5	0,000	207,7±18,8	208,4±22,7	0,768	214,6±19,2	188,7±18,9	0,000
Median	199,8	237,8		199,8	199,3		205,6	184,7	
Min; Max	190,0; 227,6	206,6; 276,8		185,3; 241,7	186,8; 249,3		192,8; 249,6	163,2; 218,7	

Descriptive analysis was performed on supplement data of body weight before and after treatment for 4 weeks. In the control group (K) without treatment, the mean pre-test of body weight was 203.5±14.5 which significantly different with p=0.00 (p<0.05) compared to the mean post-test of body weight was 242, 2±23.5. In the placebo control group (P1), the mean body weight in pre-test was 207.7±18.8 which was not significantly different with p=0.76 (p>0.05) compared to the mean body weight in post-test was 208.4±22.7. In the treatment group (P2), the mean pre-test of body weight was 214.6±19.2 which was significantly different with p=0.00 (p<0.05) compared to the mean post-test of body weight was 188.7 ±18.9.

4. Discussion

Influence Factors to Malondialdehyde Level

Malondialdehyde levels, is a marker of oxidative stress involved in the pathogenesis of NAFLD. The accumulation of fat in hepatocytes causes an increase in the formation of ROS and triggers an increase in the lipid peroxidation process. Animal study of rats fed a high-fat, high-fructose diet (HFFD), after 2 weeks, showed micro-vesicular fat droplets, then, after 4 weeks, liver tissue observations showed additional fat droplet accumulation and hepatocyte degeneration. Level of MDA, as a marker of lipid peroxidation, were also increased in the liver and brain of HFFD-treated rats. The development of non-alcoholic steatosis into steatohepatitis is associated with the formation of ROS in the liver which causes oxidative stress, further damaging unsaturated proteins or lipids in cell membranes.[21]

Another study showed that a diet with high in simple carbohydrates enriched with sucrose (18% simple carbohydrates), resembling the current human diet, over a period (5, 10, 20, and 30 weeks), was able to induce NAFLD-associated obesity, with histologic features. in the form of hepatic steatosis and swelling of hepatocytes, with one of the clinical markers being increased levels of MDA which is a marker of oxidative stress.[22]

In this study, the induction of a high-calorie diet was used in groups P1 and P2 which were given in liquid form mixed with a standard diet through nasogastric administration. Free access to drinking water mixed with fructose, contained in corn syrup, on daily consumption for 4 (four) weeks. Subjects were given a high-calorie diet that contained high simple carbohydrates and fats, similar like the current human diet to induce NAFLD. Histopathological features of hepatocellular ballooning, as explained previously, formation of cell injury that were found in almost all liver tissue samples in groups P1 and P2. In addition, few macro-vesicular of steatosis was also found in liver tissue. However, the results of this study showed that level of MDA in group K were significantly higher than those in P1 and P2, whereas MDA levels were not significantly different between groups P1 and P2.

This is probably due to the samples taken from blood plasma, not samples from liver tissue. Clinical study showed a significantly poor correlation between MDA levels from peripheral and hepatic veins. Measurement of oxidative stress from peripheral venous samples does not describe the state of oxidative stress in the liver.[23]Thus, the measurement of MDA levels from blood plasma samples in this study cannot describe the actual state of oxidative stress in the liver.

In addition, the MDA level measurement tool, thiobarbituric acid (TBA) test was the most common used method with some limitations. Factors of TBA method that might influence the results are the non-specificity of TBA reactivity and malondialdehyde production from reactions other than lipid peroxidation,[24,25]low stability of MDA in biological samples due to its high tendency to react with proteins, amino acids and others, as well as rapid enzymatic degradation.[25] These points that may affect the results of MDA assays using the TBAR method, hence, controversial findings on TBAR related with non-specificity, low stability of standard TBA assay solutions, and lack of data on full validation of TBAR measurements in biological fluids. Those makes the reliability of biomarker measurements oxidative stress with TBAR is questionable.

Based on the results of supplement data analysis of body weight, showed that in the control group without treatment (K), there was a significant difference in weight gain between the pre-test and post-test along with a significant increase in MDA levels compared to the placebo control group (P1) and treatment group (P2). The absence of any handling to experimental animals in group K increasing the amount of food intake then impact to the body weight, however, measurements of food waste were not carried out in this study. The increase in malondialdehyde levels could

be due to the body weight gain causes inflammatory process, yet measurements of pro-inflammatory factors were also not carried out in this study. Subsequently, the formation of steatosis was seen in the picture of liver tissue preparations in the untreated group. An increase in body weight that causes fat accumulation in the body, were further increased the free fatty acid oxidation, ROS and inflammatory factors,[3,4] which then affects the results of malondialdehyde levels.

In the placebo group (P1), there was no significant difference in weight gain between the pre- and post-test. Based on the results of malondialdehyde levels, showed that the MDA levels in the P1 group, were significantly lower than the K group, and were not significantly different from the P2 group. The results of weight measurement in the P1 group may be influenced by the induction pattern. During the treatment period, the P1 and P2 groups, were given a high-calorie diet, which consisted of a high-fat diet accompanied by the addition of egg yolk given through nasogastric tube and fructose that mixed in daily drinking water consumption, caused changes in eat behavior of the rats. Experimental animals in the P1 group did not show a difference in body weight between the pre- and post-test, could be due to satiety cause the induction used in this study, as a result, the amount of food intake was reduced in the P1 group. However, the measurement of food waste in each group of experimental animals was not carried out. High-calorie diet in the P1 group did not cause changes in body weight between pre-test and post-test. The malondialdehyde levels in group P1 significantly lower than group K, but there was no difference in malondialdehyde levels compared to the P2 group that were also given same induction.

The treatment group (P2) result was in line with previous studies, which showed that the administration of Balinese green Arabica coffee bean(*Coffea arabica*) extractcould reduce body weight in obese male wistar rats compared to the placebo group (P1). Caffeine and chlorogenic acid contained in the extract of green Bali arabica coffee (*Coffea arabica*) increase catecholamines and fat oxidation, further increasing body metabolism and reducing food intake.[14] In this study, the treatment group (P2) with a high-calorie diet and the Balinese green Arabica coffee bean(*Coffea arabica*) extract also showed significant weight loss between the pre-test and post-test groups. As explained in the paragraph above, weight loss in the P2 group, could also be influenced by the high-calorie diet that causes a decrease in food intake, but was not measured in this study. As in the P1 group, malondialdehyde levels in the P2 group were also significantly lower than the K group but did not differ from the P1 group. The administration of Balinese green Arabica coffee bean (*Coffea arabica*) extract along with a high-calorie diet in the P2 group, caused significant weight loss between pre-test and post-test, malondialdehyde levels were significantly lower than group K but there was no difference when compared to the P1 group.

High-calorie diet causes weight gain and stimulation of excessive fat deposition in non-adipose tissue, especially hepatocyte. Fat accumulation increased lipolysis in adipocytes and free fatty acid levels, which in turn decreases plasma lipid clearance and increases ß-oxidation. There was also an increase in free radical production that cause biomolecular and cellular damage, as well as the formation of pro-inflammatory genes, which in turn induces the lipid peroxidation process and malondialdehyde levels, also the formation of steatosis in liver cells.[26] This condition was in accordance with the results of malondialdehyde levels and the histopathology of liver tissue at control group compared to placebo and treatment group.

The administration of Balinese green Arabica coffee bean (*Coffea arabica*) extract in the treatment group caused weight loss. The active compounds contained in green coffee bean extract were able to reduce adipogenesis and increase body metabolism.[27] However, in this study, despite weight loss, it did not affect malondialdehyde levels in the P2 group which was not different from the P1 group, however, it was significantly lower than the K group.

High-calorie diet in the treatment group also caused satiety in experimental animals and possibly influenced the amount of food intake as described above, ultimately causing weight loss. Other studies have also shown that coffee consumption before meals could affect food intake,[28] and human studies have shown that consumption of 3 cups of coffee daily for 28 days could reduce plasma ghrelin levels and lead to reduced food intake.[29] Thus, apart from being influenced by the high-calorie diet, seems that the administration of green Bali arabica coffee bean (*Coffea arabica*) extract could also affect the amount of food intake, however, food waste in experimental animal groups were not measured.

Influence Factors to Histopathology of Liver

Excess consumption of carbohydrates (fructose) and fats (fatty acids and cholesterol) plays a key role in the progression of NAFLD through the activation of lipid metabolic pathways that are modulated by a high-fat diet. Excessive carbohydrate and/or fat intake has also been shown to increase blood glucose and free fatty acid concentrations, further contributing to lipid accumulation in the liver. The hepatic fructose metabolite, further, increases the free fatty acid storage in the liver and can lead to much higher degradation of adenosine triphosphate (ATP). Lipogenesis of free fatty acids in the liver then causes impaired glycogen synthesis and insulin resistance in hepatocytes and the release of adipocytokines causes lipotoxicity of hepatocytes, then damages hepatocytes cell.[21] Regarding the histopathological results and the induction pattern used, a study in rats using a hypercholesterolemic diet induction, consisting of 1% cholesterol; 0.5% sodium cholate; 5% butter; 30% sucrose; 10% casein and 53.5% standard diet and the addition of 60% fructose given via nasogastric administration for 4 weeks, increased total and LDL cholesterol, body and liver weight, induced steatosis that further progressed to liver damage.[30]

Another study combined the High Fat Diet (60%) and High fructose (20%) (HFHFr) for 20 weeks, compared to either the High Fat Diet (HF) or the High fructose diet only (HFr). The results obtained were weight gain and intrahepatic fat accumulation more prominent in the HF group compared to the HFr group. In the combined HF and HFr group compared with the HF group only, intrahepatic inflammation and metabolic disturbances were more prominent. Thus, the

addition of fructose to a high-fat diet, not only causes insulin resistance and an increase in blood glucose, but also causes hepatocellular damage as a marker of fatty liver progression.[31]

The induction pattern used in this study was 60% fructose in daily consumption of drinking water, 4 ml/day of lard and 2 ml/day of egg yolk. In accordance with clinical evidence mentioned above, the induction pattern used in this study resulted in lipotoxicity, mitochondrial dysfunction and further degeneration of hepatocytes, not just simple steatosis only. Continuous ROS production causes damage to cell membranes, proteins and DNA, which in turn causes the release of pro-inflammatory cytokines, activation of hepatic stellate cells, fibrogenesis, and direct liver damage.[22]The combination of a high-fat diet and high-fructose diet, referred in this study as a high-calorie diet, resulted in an acceleration of the NAFLD development process towards NASH which was characterized by hepatocellular swelling as a sign of reversible hepatocyte injury. The hallmark of this injury was reduced oxidative phosphorylation with consequent depletion of energy stores in the form of adenosine triphosphate (ATP), and cell swelling caused by changes in ion concentration and influx of water. In addition, various intracellular organelles, such as mitochondria and cytoskeleton, may exhibit changes. This particularly affects cells that are highly susceptible and dependent on fat metabolism, such as hepatocytes.[32]

The concept that excessive fructose consumption may promote the development of biological NAFLD was feasible, given experimental evidence that high-fructose in corn syrup can increase endoplasmic reticulum stress, induce stress-related kinase activation, mitochondrial dysfunction, and increase apoptotic activity in liver cells. Furthermore, an association between dietary fructose intake, gut-derived endotoxemia and NAFLD has been observed in previous studies in humans and animals. Rats fed water enriched with 30% fructose led to accumulation of hepatic triglycerides, altered markers of insulin resistance, portal endotoxemia, increased hepatic lipid peroxidation and TNF-alpha levels. The data suggest that fructose-induced NAFLD or NASH is also associated with intestinal bacterial overgrowth and increased intestinal permeability,[31,33] which further leads to endotoxin-dependent activation of hepatic Kupffer cells, increased lipopolysaccharide and induces ROS production.[31]Habitual fructose consumption can also lead to an unfavorable energy balance in the liver which increases the susceptibility of hepatocytes to injury. The lipogenic and proinflammatory effects of fructose due to its unique metabolism, which involves a transient period of ATP depletion related to rapid phosphorylation in cells.[33]

High-fructose diet characterized by histological features with hepatocellular swelling. In this study, the investigators used 60% fructose into the daily drinking water consumption, which caused the overall liver histopathology in groups P1 and P2 were dominated by hepatocyte ballooning. This finding was a critical pathophysiological condition and likely to occur across the spectrum in NASH. However, being able to accurately measure these cells in hematoxylin and eosin (H&E) a stained liver cell was difficult.

In this study, we used a semi-quantitative analysis by assessing SAF scores; observe steatosis, NAFLD/NASH developmental activity and fibrosis. Analysis of liver tissue sample in group K with SAF score result S1A0F0, showed some steatosis (5%-3%) with mild activity towards the development of NAFLD/NASH and no fibrosis, compared with groups P1 and P2 with SAF scores result S0A2F0, showed a slight steatosis (<5%) with moderate activity towards NALFD/Nash development and no fibrosis. These results indicate that the administration of Balinese green arabica coffee bean (*Coffea arabica*) extract did not prevent the progression of non-alcoholic fatty liver in the group of rats on a high-calorie diet.

The assessment approach mentioned above seems very simple because only certain histopathological patterns were analyzed with semiquantitative measurement, namely the overall picture of steatosis, hepatocellular swelling and lobular inflammation, fibrosis, whereas in NASH presents a more complex histopathological picture. Placement of the threshold at 2 for the NAFLD activity score allows clear recognition in majority cases of NASH with features of hepatocellular swelling with or without steatosis and lobular inflammation, result with the A score was 2. Non-alcoholic steatohepatitis may develop spontaneously or along with therapy throughout all histopathological spectrum, making it difficult to separate the evolutionary processes of hepatocytes, inflammation, steatosis and fibrous lesions.[19]

The SAF score can assess the biopsy results with NAFLD (S≥1; Activity at various stages; Fibrosis at various stages) or with NASH (S1; A≥2; Fibrosis at various stages). In addition, the SAF score can identify a specific group with histopathological features of steatosis and perisinusoidal fibrosis, but no cell injury or lobular inflammation (S≥1; A0; F1) and cases with inflammation and cell injury but no steatosis (S0; A≥). 2; F at various stages)[19]as shown in this research study. Ultimately, there is no precise and perfect NAFLD/NASH scoring system to replace the analytical description of the liver biopsy results. Therefore, in this study, we used the SAF score in the process of analyzing liver tissue samples to determine the developmental activity score of NAFLD/NASH.

Effect of Balinese Green Arabica Coffee Bean(*Coffea arabica*) Extract

In the concept of anti-aging medicine, the process of aging could be prevented. The usage of antioxidants could inhibit damage and aging of cells due to the increased formation of ROS that trigger oxidative stress.[5] It was known with certainty that coffee was a mixture of various chemical compounds. Among the identified constituents, caffeine and chlorogenic acid were the main compounds. The effect of coffee on health, based on clinical evidence, was a controversial topic. Epidemiological data associate coffee consumption with a lower prevalence of chronic liver disease and a reduced risk of elevated liver enzyme levels, advanced liver disease and its complications, and hepatocellular carcinoma. Clinical evidence suggests that coffee consumption protects the liver from damage caused by a high-fat diet. This effect is mediated by reduced hepatic fat accumulation (via increased fatty acid ß-oxidation); systemic and hepatic oxidative stress (via the glutathione

system); liver inflammation (via gene modulation); and the expression, concentration of proteins and cytokines associated with inflammation.[34]

Study of Colombian coffee extract (CE), containing high concentrations of caffeine and diterpenoids, in rat models with metabolic syndrome which were given a diet rich in corn starch compared to groups fed with a high-carbohydrate, high-fat diet with 25% fructose in drinking water for 16 weeks. Groups fed a high-carbohydrate, high-fat diet showed symptoms of the metabolic syndrome leading to cardiovascular remodeling and nonalcoholic fatty liver disease. Colombian coffee extract supplementation improved glucose tolerance, hypertension, cardiovascular remodeling, and nonalcoholic fatty liver disease without altering abdominal obesity and dyslipidemia. This study showed that CE can improved dietary-induced changes in the structure and function of the heart and liver, without altering abdominal fat deposits.[35] The effect of unfiltered coffee on the diet-induced metabolic syndrome with a high-fat diet (35%) and fructose (20%) in drinking water for 14 weeks showed a lower rate of hepatic steatosis compared with controls after long-term coffee consumption. This study also shows the important role of other coffee chemicals and the potential for synergistic work between the compounds contained in coffee.[36]

In this study, the model was fed a high-fat/high-fructose diet, referred to high-calorie diet, which is a western-type diet. This model could induce several signs and symptoms of the metabolic syndrome, in both humans and rodents. The addition of fructose to a high-fat diet has been shown to increase its deleterious effects on hepatic steatosis and lipid metabolism.[36] The induction pattern used in this study caused changes in the shape of the hepatocytes, becomes hepatocyte ballooning which was seen in almost all liver tissue samples in groups P1 and P2, but was not seen in group K. Measurement of these swollen hepatocytes was difficult to do, so semi-quantitative analysis was carried out to assess liver tissue samples with SAF scores as described above.

The general histopathological picture between groups P1 and P2 treated with Balinese green arabica coffee bean (*Coffea arabica*) extract did not show any improvement in liver cells, compared to group K. The pattern of continuous induction of a high-calorie diet, producing ROS and triggering oxidative stress, thus causing lipotoxicity and hepatocellular swelling as progression of NAFLD to NASH. Thus, the administration of Balinese green arabica coffee bean(*Coffea arabica*) extract was not able to provide benefits and help improve the condition of liver cells that were entering the stage of hepatocyte degeneration.

However, the results of this study were in line with recent clinical evidence studying supplementation of the main constituents of coffee, caffeine and/or chlorogenic acid, on liver metabolism, and inflammatory indices in patients with type 2 diabetes and NAFLD. The results obtained showed that the two main ingredients were not superior to placebo in improving fat and other liver outcomes, except for lower total cholesterol levels in the caffeine group and higher insulin levels in the chlorogenic acid plus caffeine group compared to placebo.[37]

The results obtained in this study indicate that the green Balinese Arabica coffee bean (*Coffea arabica*) extract, which contains phytonutrients that could act as antioxidants, has not been proven to prevent the aging process, especially in liver cells. This was due to the induction of high-calorie diet that used, which also affects the research time set in this study. In addition, a high-fat diet plus high-fructose diet has been shown to accelerate the aging process of cells, particularly liver cells. Further research is needed to determine therapeutic doses and comparation of several induction methods to learn more the effect of Balinese green Arabica coffee bean (*Coffea arabica*) extract and determining its effectiveness in NAFLD with progression to NASH.

5. Conclusion

The administration of Balinese green Arabica coffee bean (*Coffea arabica*) extract could not prevent the increase of malondialdehyde level and the progression of non-alcoholic fatty liver in male Wistar rats fed a high-calorie diet.

It is necessary to explore and find the right dosage of Balinese green Arabica coffee bean (*Coffea arabica*) extract to produce a therapeutic effect on the liver. In addition, further research needs to be done for several methods comparation of proper high-calorie diet induction that can be used to study the effect of Balinese green Arabica coffee bean (*Coffea arabica*) extract on metabolic syndrome, especially NAFLD as its livermanifestation.

6. Disclosure

The author reports no conflicts of interest in this work.

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