

The nutraceutical effect of *Scenedesmus dimorphus* for obesity and nonalcoholic fatty liver disease–linked metabolic syndrome

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ABSTRACT

Obesity is a major cause of nonalcoholic fatty liver disease (NAFLD), which is identified as a characteristic related to metabolic syndrome. This study focuses on the benefits of *Scenedesmus dimorphus* as a nutraceutical to overcome NAFLD-linked metabolic syndrome caused by obesity. The research used 30 mice (*Mus musculus*) divided into six groups: normal control, obesity control (OC), and drug control (Orlistat) and *S. dimorphus* administrated orally for 21 days in obese mice with NAFLD-linked metabolic syndrome with doses of 5 mg/20 g body weight (BW), 10 mg/20 g BW, and 15 mg/20 g BW. The administration of a high-fat diet can cause obese mice to suffer from NAFLD, resulting in an increase in liver enzymes and changes in lipid metabolism. Administration of *S. dimorphus* at 15 mg/20 g BW decreases the activity of liver enzymes and improves liver function. The statistics test showed significant differences ($p < 0.05$ analysis of variance) to decrease the liver enzyme activity of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase compared to OCs. Administration of *S. dimorphus* resulting in metabolism changes showed a significant (at $p < 0.05$) decrease in total cholesterol, triglycerides, and low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol. Microalgae *S. dimorphus* is beneficial as a nutraceutical to manage obesity and reverse NAFLD-linked metabolic syndrome in obese mice.

INTRODUCTION

The prevalence of obesity is currently receiving widespread attention globally, as it is the most common cause of chronic liver disease in almost all countries, especially in Indonesia (WHO, 2017). The prevalence of NAFLD in children and adolescents from the general population study was 7.6% (95% CI: 5.5% to 10%) and 34.2% based on clinical studies in obese children with NAFLD (95% CI: 27.9% to 41.2%). Prevalence is higher in men than in women and increases gradually with greater body mass index (BMI) (Anderson *et al.*, 2015).

Obesity in childhood is associated with a greater likelihood of hepatocellular carcinoma as an adult. NAFLD suffered by children due to obesity can develop into advanced fibrosis (10%–25%); in more severe cases, it can develop into

cirrhosis and high-stage liver disease (Goyal and Schwimmer, 2016). Based on research conducted in the United States, out of 6,000 people tested, 30% suffered from NAFLD and 10% suffered from advanced fibrosis. The risk of death at 5–8 years in NAFLD subjects with advanced fibrosis was significantly higher than in subjects without NAFLD (18% to 35% vs. 2.6% to 5.5%) (Le *et al.*, 2017). Studies conducted in the community in the United States on the trend of NAFLD and the impact on the incidence of metabolic comorbidities, cardiovascular (CV) were higher in NAFLD patients and mortality over a period of 20 years showed that in general, NAFLD in the community increased five-fold, especially in young adults (18–39) years old seven-fold increases (Allen *et al.*, 2018). The research was conducted on populations from two countries, Saudi Arabia and the United Arab Emirates. The study was conducted to predict the progression of NAFLD and nonalcoholic steatohepatitis (NASH) disease based on the prevalence of adults suffering from obesity and diabetes mellitus in both countries. The prevalence of NAFLD increases parallel to the projected increase in obesity and diabetes mellitus and is predicted to double in 2030 (Alswat *et al.*, 2018).

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Obesity is one of the main causes of nonalcoholic fatty liver disease (NAFLD). NAFLD covers almost all liver diseases such as simple steatosis for alcoholic steatohepatitis (NASH) and subsequently becomes noncholestatic cirrhosis and hepatocellular carcinoma. Steatosis is associated with fatty acids (FAs), lipoprotein metabolism, and abnormalities in FA metabolism, which are associated with adipose tissue, liver, and systemic inflammation. This is a key factor involved in the development of insulin resistance, dyslipidemia, and other cardiometabolic risk factors associated with NAFLD (Fabbrini *et al.*, 2010). NAFLD is strongly associated with several metabolic disorders and diseases such as obesity, type 2 diabetes mellitus, and dyslipidemia. NAFLD and dyslipidemia manifested as an increase in serum triglycerides (TG), cholesterol levels, and low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) cholesterol, a major risk factor of heart disease (cardiovascular Diseases) (Zhang and Lu, 2015). The pathological sign of NAFLD is lipid accumulation in hepatocytes, suggesting that there is a close relationship between abnormal lipid metabolism and NAFLD. The majority of patients with NAFLD have metabolic syndrome (Dima *et al.*, 2012), so it is considered a liver manifestation of metabolic syndrome (Ji *et al.*, 2014). The role of obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia in the pathogenesis of NAFLD is an important component of metabolic syndrome. Changes in metabolic profiles due to obesity increase serum lipid levels (dyslipidemia) affect type 2 diabetes mellitus and metabolic syndrome (Norris *et al.*, 2011). Obesity is caused by fat accumulation in the liver through the following mechanism: Increased transport of fat or FAs from the intestine to the liver resulting in FA synthesis increases or decreases lipid oxidation in the mitochondria, both causing an increase in TG synthesis through the esterification process that occurs in the liver accompanied by a disruption of the release of TG from liver cells. The release of TG from liver cells depends on the bond with apoproteins, phospholipids, and cholesterol to form very low-density lipoprotein (VLDL) in the liver (Fabbrini *et al.*, 2010).

There are several drugs that control obesity. Orlistat is a drug commonly used to control body weight (BW) combined with a caloric diet (Kakkar and Dahiya, 2015). There are two main categories of antiobesity drugs: agents that can reduce or limit energy absorption and reduce fat mass by increasing energy expenditure or redistributing adipose tissue (Chatzigeorgiou *et al.*, 2014) and lipase inhibitors of the pancreas that can control weight (Kakkar and Dahiya, 2015). Inhibition of pancreatic lipase decreases free FAs and monoacylglycerol in the intestinal lumen and also decreases triacylglycerol to be absorbed, and orlistat is a drug that inhibits pancreatic lipase (Kang and Park, 2012).

Scenedesmus dimorphus as a nutraceutical because it contains active compounds that have the potential to be drugs and food supplements. *Scenedesmus dimorphus* contains pigments (beta-carotene, astaxanthin, lutein, phycobiliprotein) as antioxidants. The insoluble fiber is the protective effect of heart, hepatoprotective, anti-inflammatory, and antihyperlipidemic (Gammone and D'Orazio, 2015; Hu *et al.*, 2016). Lipid contains *S. dimorphus* (palmitic and oleic) and proteins with 18 types of amino acids (Armaini *et al.*, 2016; Rinaldi *et al.*, 2015). *Scenedesmus dimorphus* as a nutraceutical can repair bone marrow damage due to aplastic anemia. BW, red blood cells, hemoglobin, hematocrit values, and reticulocytes increased in anemia mice (Armaini *et al.*, 2018).

Scenedesmus dimorphus contains beta-glucan, which is an active immunostimulator and has beneficial effects as a free radical scavenger, to reduce blood lipids (Chu *et al.*, 2018). Omega-3, omega-6, poly-unsaturated fatty acid (PUFA), eicosa pentanoic acid (EPA), Docosa hexanoic acid (DHA), and alpha-linolenic acid (ALA) from lipid content, proteins, pigments, vitamins, and minerals reduce the effects of obesity and increase lipid metabolism (Arun *et al.*, 2015; Chen *et al.*, 2017). Nutraceuticals are intended for health and treat degenerative neurons in Alzheimer's disease, Parkinson's disease, heart disease, cancer, and other degenerative diseases, such as diabetes, obesity, and anemia. Omega-3 PUFA is the most widely used nutraceutical because it is useful in preventing and treating coronary heart disease, hypertension, diabetes, arthritis, autoimmune disorders, and cancer (Babcock and Helton, 2000; Howe, 2006; Mohan and Das, 2001; Schmidt *et al.*, 2000). Consumption of omega-6 PUFA can reduce the incidence of deaths from CV disease including ischemic heart disease, nonischemic, myocardial heart disease, and hypertension (Afterburn *et al.*, 2008). Handling obese sufferers can be done a consume dadih (traditional fermented milk products) specifically from Indonesia. Curd made from buffalo milk can improve health because it is rich in probiotics, as an antimicrobial, and can also be used as a diet for weight loss (Syukur *et al.*, 2016). Some herbal products can be used to control hyperlipidemia. Ethyl acetate extract from *Commiphora Mukul* can reduce TG, total cholesterol (TC), and LDL-C and increase HDL-C in the treatment of hyperlipidemia and other related disorders such as atherosclerosis (Shaik and Khan, 2018).

Scenedesmus dimorphus contains omega-3 PUFA, which can inhibit VLDL secretion in the liver to decrease LDL production. This inhibition occurs because omega-3 can inhibit two main enzymes, diacylglycerol acyltransferase (DGAT) and phosphatidic acid phosphohydrolase (PA), which are involved in TG synthesis. Inhibition of liver enzymes causes a decrease in TG production and in the secretion of VLDL and LDL in the liver. Omega-3 can increase the oxidation of β peroxisomes from FAs to reduce the availability of FA for TG synthesis, resulting in a reduction in liver fat (Jacobson, 2008). Based on the description above, this study aims to identify *S. dimorphus* as a useful drug for controlling obesity, which causes metabolic syndrome-linked NAFLD.

MATERIALS AND METHODS

Experimental animals

The research used adult mice (*Mus musculus*) as an experimental animal. Thirty mice were divided into six groups with body weights between 20 and 30 g to obtain weight uniformity. Mice were acclimatized in the laboratory for 14 days and treated with a high-fat diet for 4 weeks to induce obesity (BMI ≥ 30).

Culture of *Scenedesmus dimorphus*

Microalgae *S. dimorphus* was obtained from a collection of the Biochemical Laboratory, Department of Chemistry, Andalas University. This species was isolated from freshwater rivers in the city of Padang, Indonesia. Its morphological and molecular biology characteristics have been identified.

Experimental design

This study used a randomized block design consisting of six groups of five adult mice: group 1 normal control (NC) was

given pellets and drinking water, group 2 obesity control (OC) was given a high-fat diet for four weeks until their BMI reached ≥ 30 (Rohrer index), group 3 drug control (DC) obese rats were given orlistat orally once a day (0.013 mg/g BW), and the remaining three groups of obese rats treated with *S. dimorphus* orally for 1 time a day, respectively consisting of 5 mg/20 g (group 4), 10 mg/20 g (group 5), and 15 mg/20 g body-weight (group 6). Observation of obese mice was carried out 7 and 21 days after treatment.

Biomass production of *Scenedesmus dimorphus*

Cultivation of *S. dimorphus* using bold basal medium (BBM) was carried out in a glass aquarium with a volume capacity of 20 L equipped with an aerator and a lamp with a light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ as light sources, with a ratio of 14:10 hours (light-dark). BBM with a modified source of nitrogen using urea (3 mM) (Rinaldi *et al.*, 2015) was prepared and adding *S. dimorphus* culture into the medium. *Scenedesmus dimorphus* growth was observed every day and optical density was measured using a spectrophotometer at 550 nm. Harvesting was done after two weeks, and biomass was separated by decantation for one day; after the biomass settled, the liquid medium was removed. *Scenedesmus dimorphus* biomass, which still contains liquid, was centrifuged (3,000 rpm) to separate the supernatant, and the biomass was rinsed with distilled water three times until the biomass does not contain a medium. Freeze-dried biomass then crushed to obtain particles of 40–50 mesh size, and the dry biomass was stored at a refrigerator temperature of -20°C .

Blood sampling

Mice blood specimen was taken from the neck vein under deep anesthesia using a blood tube. The blood was then centrifuged ($5,000 \times g$) for 30 minutes to separate the serum and erythrocytes. The blood serum was used for enzyme and lipid profile analysis.

Lipid profile analysis

Lipid profile, with test parameters consisting of TG, TC, LDL cholesterol, and HDL cholesterol, was determined using colorimetric methods with kits (Labtest®).

Liver function analysis

The enzymatic levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase

(ALT) were obtained out using the enzymatic method, also using a kit (Labtest®).

Statistical analysis

Data analysis was performed statistically using analysis of variance (ANOVA) (two factor with replication) in Microsoft Excel 2010. The results of the data are expressed as the average \pm standard deviation with the sample ($n = 5$) at $p < 0.05$ ($\alpha = 0.05$ and power = 0.8). The minimum sample size is calculated based on the value of E, which is the degree of freedom of ANOVA. Any sample size, which keeps E between 10 and 20, should be considered as adequate (Charan and Kantharia, 2013; Ilyas *et al.*, 2017). E can be measured by the following formula:

$$E = \text{Total number of animals} - \text{Total number of groups}$$

Expected attrition or animal death: The final sample size must be adjusted to the expected attrition (Charan and Kantharia, 2013), in this study expecting a 10% reduction, then the sample size calculated by the formula or software should be divided by 0.9 to get the actual sample size.

RESULTS

Analysis of obesity

Induction of high-fat diet (HFD) causes mice to become obese after treatment for four weeks. BW and body length were measured every week to calculate BMI based on the Röhrer index; mice are obese if their BMI value ≥ 30 . Induction of HFD on 25 mice caused obesity with a BMI between 44 and 46.8.

Analysis of lipid metabolism

Obesity in mice can cause NAFLD, characterized by changes in lipid metabolism. Lipid profiles changed because of an increase in cholesterol and TG levels in the OC group after HFD administration compared with the NC group. Based on statistical test results, there are significant differences at $p < 0.05$ with ANOVA (two factor with replication) after administration of *S. dimorphus* compared with NC and OC groups. The Duncan test determined differences between treatments from each group. An increase in total TG also occurred in obese mice with NAFLD compared to normal mice (Table 1). From day 7 to day 21, the DC group was given orlistat, which is usually provided to obese patients who have not been able to reduce cholesterol and TG levels. This is due to the use of orlistat as an inhibitor of pancreatic lipase to BW

Table 1. Measurements of Triglyceride and cholesterol in obese mice suffering NAFLD with the administration of *S. dimorphus*, NC, and OC at 7–21 days of observation.

Treatments	TC (mg/dl)		Total TG (mg/dl)	
	7 Days	21 Days	7 Days	21 Days
NC	87.9 \pm 1.521	90.1 \pm 1.952*	120.7 \pm 3.642	125.2 \pm 2.918*
OC	100.2 \pm 3.299	108.7 \pm 1.863	139.6 \pm 3668	149.1 \pm 4.904
DC	102.9 \pm 2.053	123.1 \pm 1.221	98.8 \pm 3.289	127.3 \pm 4.031
Obesity + <i>S. dimorphus</i> (5 mg/20 mg BW)	131.6 \pm 2.192	126.2 \pm 2.168	134.6 \pm 2.359	87.1 \pm 3.522
Obesity + <i>S. dimorphus</i> (10 mg/20 mg BW)	104.0 \pm 3.742	95.6 \pm 2.509	115.3 \pm 3.606	80.8 \pm 3.426
Obesity + <i>S. dimorphus</i> (15 mg/20 mg BW)	110.3 \pm 5.318	67.7 \pm 4.535*	124.4 \pm 3.641	49.2 \pm 2.395*

Data expressed mean \pm SD ($n = 5$), SD; standard deviation, * $p < 0.05$

control; overcoming obesity requires longer treatment time, and short-term use of the drug has not shown any benefits (Table 1).

Mice suffering from NAFLD experienced changes in their lipid profiles because of an increase in LDL cholesterol and a decrease in HDL cholesterol on day 21 compared with the NC group. Orlistat was able to reduce LDL cholesterol and increase HDL cholesterol levels compared with the OC but not to normal levels (Table 2). The results of the statistical test (ANOVA) at $p < 0.05$ show a significant difference between treatments for the increase in HDL cholesterol. Further testing with Duncan test shows a significant ($p < 0.05$) increase in administering orlistat drugs and *S. dimorphus* on day 21 (Table 2).

Effect of *Scenedesmus dimorphus* on lipid profile changes

The administration of *S. dimorphus* in obese mice with NAFLD can reduce cholesterol and TG levels compared with the OC and NC groups on the 21st day of observation. Based on statistical tests at $p < 0.05$ with ANOVA, very significant inter-treatment differences were seen with the administration of orlistat and *S. dimorphus* with an effective dose of 15 mg/20 g BW, compared with the OC group with NAFLD and the NC group. The administration of *S. dimorphus* at doses of 10 mg/20 g BW and 15 mg/20 g BW caused a decrease in TG levels on day 21, which is very drastic compared with those in the OC group with NAFLD and the DC group. Giving *S. dimorphus* to obese mice is more effective in reducing cholesterol and TG levels compared to giving orlistat (Table 1).

S. dimorphus can reduce LDL cholesterol levels compared to the OC, which is most effective at a dose of 15 mg/20 g BW, which is close to the NC. The administration of orlistat (DC) in obese mice was seen to decrease LDL cholesterol levels, but cholesterol levels were still higher compared to the administration of *S. dimorphus* (Table 2). Based on the results of this study, it can be stated that the administration of *S. dimorphus* is more effective in lowering LDL cholesterol levels than orlistat (Table 2). The results of statistical tests showed that the decrease in LDL cholesterol was not significantly different in each treatment at $p < 0.05$ (two-factor ANOVA with replication). The increase in HDL cholesterol occurred with the administration of *S. dimorphus* at an effective dose

of 15 mg/20 g BW compared to the OC and NC on day 21. HDL cholesterol levels were above the NC but lower than the control for orlistat. Increasing HDL cholesterol levels by giving orlistat is better than through *S. dimorphus*. The statistical test showed that giving *S. dimorphus* and orlistat was significantly different at $p < 0.05$ compared with the NC and obesity groups. Further testing with the Duncan test showed a very significant difference between giving *S. dimorphus* with a dose of 15 mg/20 g BW and administering orlistat on the 21st day of observation (Table 2).

Analysis of liver function

AST, ALT, and ALP enzyme activity is determined to observe decreased liver function. Induction of HFD in mice causes changes in lipid metabolism so that obese mice would have nonalcoholic fatty livers. NAFLD damages liver cells and reduces liver function. This is due to an increase in the release of liver enzymes and affects the increased enzyme activity of AST, ALT, and ALP in obese mice compared with those in the NC group at 7–21 days of observation. The relationship between BMI and AST, ALT and ALP activity looks very real, with increasing BMI, or the more fat the mice lead to an increase in liver enzyme activity (Fig. 1). Giving *S. dimorphus* can reduce mice weight (decrease in BMI value) and improve liver function by decreasing AST, ALT, and ALP enzyme activity (Fig. 1).

Effect of *Scenedesmus dimorphus* on liver function

The administration of *S. dimorphus* in obese mice can reduce the enzyme activity of AST, ALT, and ALP in all dose variations at 21 days of observation (Table 3). Observation on the seventh day showed no decrease in AST and ALT enzyme activity whereas ALP enzyme activity reduced. Statistical analysis with ANOVA from the testing of AST enzyme activity from obese mice showed a significant difference ($p < 0.05$) between each group given *S. dimorphus* compared to the NC and OC groups on observation days 7 to 21 (Table 3). Duncan's test was conducted to observe differences between treatments in all groups. Treatment with orlistat and *S. dimorphus* at a dose of 15 mg/20 g BW showed a very significant difference compared to the NC and OC groups on the 21st day of observation.

Table 2. Measurements of LDL-cholesterol and HDL-cholesterol in obese mice suffering NAFLD with the administration of *S. dimorphus*, NC, and OC at 7–21 days of observation.

Treatments	LDL-Cholesterol (mg/dl)		HDL-Cholesterol(mg/dl)	
	7 Days	21 Days	7 Days	21 Days
NC	8.9 ± 0.63	9.3 ± 0.33	50.2 ± 1.99	50.4 ± 0.96
OC	25.0 ± 3.95	28.6 ± 1.88	48.7 ± 0.87	40.3 ± 0.4
DC	18.5 ± 1.52	15.5 ± 1.49	64.6 ± 1.07	87.1 ± 1.22*
Obesity + <i>S. dimorphus</i> (5 mg/20 mg BW)	14.3 ± 1.88	13.3 ± 0.81	80.4 ± 1.02	83.5 ± 1.68
Obesity + <i>S. dimorphus</i> (10 mg/20 mg BW)	13.1 ± 1.97	13.1 ± 1.15	63.8 ± 0.84	66.3 ± 0.78
Obesity + <i>S. dimorphus</i> (15 mg/20 mg BW)	13.1 ± 1.23	10.2 ± 0.27	87.7 ± 0.89	93.3 ± 1.39*

Data expressed mean ± SD (n = 5), SD; standard deviation, * $p < 0.05$

The statistical analysis of ALT enzyme activity test results from obese mice treated with orlistat and *S. dimorphus* with dose variation compared to NC and OC showed significant differences at $p < 0.05$. Further testing with the Duncan test showed a very significant difference with the treatment of orlistat and *S. dimorphus* at a dose of 15 mg/20 g BW on the 21st day of observation.

DISCUSSION

Metabolic syndrome-related NAFLD

The risk of NAFLD increased in association with obesity. The prevalence of NAFLD in mice increased with increasing BMI (Musso *et al.*, 2009). Steatosis occurred as a characteristic of NAFLD when the absorption of liver FAs from plasma and de novo FA synthesis is greater than FA oxidation levels and the delivery of fat to the liver (as TG in VLDL). Therefore, the amount of excessive intrahepatic TG causes an imbalance between the complex interactions of FA metabolic events in conjunction with

adipose tissue, liver, and systemic inflammation (Fabbrini *et al.*, 2010). The administration of HFD causes fat levels in the body of mice to increase so that dyslipidemia occurs, accompanied by an increase in BMI.

Effect of *Scenedesmus dimorphus* on lipid metabolism

Based on the results, TG and cholesterol levels increased due to obesity (BMI 40–40.8) in experimental mice suffering from NAFLD due to HFD compared with those in NCs. The increase in TG and cholesterol levels in obese mice with NAFLD are caused by changes in lipid metabolism. NAFLD's association with high-risk dyslipidemia and HDL cholesterol levels is low (Chalasani *et al.*, 2018). NAFLD with hepatic steatosis, in which large amounts of free FAs are released to the liver, thereby increasing liver TG synthesis and reducing the ability of the liver to secrete TG, can cause TG accumulation in hepatocytes (Musso *et al.*, 2009; Yamashita *et al.*, 2001). The administration of *S. dimorphus* can affect lipid metabolism because of the omega-3 content of *S. dimorphus*. The lipid content of PUFA from microalgae

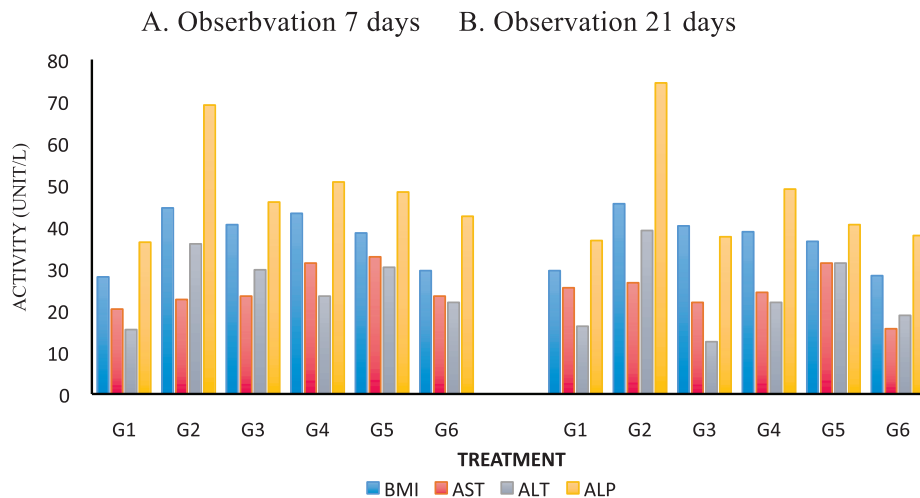


Figure 1. Relationship of BMI with AST, ALT, and ALP enzyme activity (Unit/L) in treatment G1 (NC), G2 (OC), G3 (DC), G4 (Obesity + *Scenedesmus dimorphus* 5 mg/20 g BW), G5 (Obesity + *Scenedesmus dimorphus* 10 mg/20 g BW), and G6 (Obesity + *Scenedesmus dimorphus* 15 mg/20 g BW) on observation (A = 7 days and B = 21 days).

Table 3. Measurements of AST, ALT, and ALP activity on obese mice with treatments *S. dimorphus*

Treatments	AST (Unit/L)		ALT (Unit/L)		ALP (Unit/L)	
	7 days	21 days	7 days	21 days	7 days	21 days
Normal Cont (NC)	20.3 ± 0.41	25.4 ± 0.36	15.4 ± 0.29	16.2 ± 0.53	36.3 ± 0.46	36.7 ± 1.08
Obesity Cont (OC)	22.6 ± 0.34	26.6 ± 0.37	35.9 ± 0.22	39.1 ± 0.49	69.1 ± 0.83	74.4 ± 0.82
DC	23.4 ± 0.35	21.9 ± 0.27*	29.7 ± 0.25	12.5 ± 0.38*	45.9 ± 0.37	37.6 ± 0.83*
Obesity + <i>S. dimorphus</i> (5 mg/20 mg BW)	31.3 ± 0.18	24.3 ± 0.25	23.4 ± 0.31	21.9 ± 0.39	50.7 ± 0.46	49.0 ± 0.83
Obesity + <i>S. dimorphus</i> (10 mg/20 mg BW)	32.8 ± 0.25	31.3 ± 0.31	30.3 ± 0.18	31.3 ± 0.49	48.3 ± 0.57	40.5 ± 0.38
Obesity + <i>S. dimorphus</i> (15 mg/20 mg BW)	23.4 ± 0.31	15.6 ± 0.32*	21.9 ± 0.29	18.8 ± 0.37*	42.5 ± 0.41	37.9 ± 0.29*

Data expressed mean ± SD ($n = 5$), SD; standard deviation, * $p < 0.05$, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, ALP; Alkaline phosphatase

S. dimorphus shows an inhibitory effect on lipid accumulation in the liver. Inhibition of VLDL secretion occurs because omega-3 can inhibit two key enzymes, namely, DGAT and phosphatidic acid phosphohydrolase (PA), which are involved in the biosynthesis of TG in the liver, resulting in decreased TG production (Jacobson, 2008; Zhang and Lu, 2015), causing secretion of VLDL, and LDL in the liver also decreases. Omega-3 can increase peroxisome β -oxidation of FAs resulting in a decrease in the availability of FAs for TG synthesis so that there is a decrease in TG levels, cholesterol, and LDL cholesterol in the liver because of *S. dimorphus*, which can reduce NAFLD due to obesity (Jacobson, 2008; Ryeo-Eun *et al.*, 2016).

The clinical effects of omega-3 FAs in reducing blood cholesterol levels are due to the omega-3 found in *S. dimorphus* influencing the mechanism of production of transport lipoprotein (VLDL and LDL) in the liver secreted into the blood. The high levels of VLDL and LDL can cause cholesterol deposits in the blood because VLDL and LDL are transport proteins that carry TG, cholesterol, and phospholipids from the liver to all tissues (Fabbrini *et al.*, 2010). The administration of *S. dimorphus* with an effective dose of 15 mg/20 g BW can improve lipid metabolism to return to normal, reduce fatty liver in NAFLD cases, and increase HDL cholesterol, which plays a role in transporting cholesterol into the liver which is then broken down into bile acids and expelled through bodily excretion (Zhang and Lu, 2015).

Analysis of liver function

The administration of HFD causes mice to become fat because of the high-fat content and excess fat (triacylglycerol) stored in adipose. Fat accumulation in mice stomach can cause NAFLD; this can be predicted from an increase in liver enzyme secretion. The results of AST, ALT, and ALP tests showed an increase in liver enzyme activity in obese mice with BMI between 44 and 46.8. Liver enzyme activity of AST, ALT, and ALP in obese mice suffering from NAFLD increased compared to NCs on day 21 of observation. Obesity with a BMI ≥ 30 can be used to predict an increase in liver enzyme levels, which are considered as a result of a fatty liver (Ruhl and Everhart, 2003), based on the results of studies that have found an increase in ALT, AST, and gamma-glutamyl transpeptidase levels due to alcohol, hypertension, and fatty liver (Stranges *et al.*, 2005). Studies conducted on adolescents showed that 60% have high ALT levels due to being overweight or obese. In addition, about 1% of obese adolescents show ALT levels more than twice the normal (Strauss *et al.*, 2000). Increasing ALT enzymes in liver toxicity is caused by loss of structural integrity of the liver. Giving orlistat can reduce the enzyme activity of AST, ALT, and ALP on the 21st day of observation with a significant difference at $p < 0.05$ and reaches almost normal conditions. Orlistat can inhibit pancreatic lipases and reduce or limit energy absorption to reduce fat mass by increasing energy expenditure (Chatzigeorgiou *et al.*, 2014; Chen *et al.*, 2017).

Effect of *Scenedesmus dimorphus* on liver function

Giving *S. dimorphus* can reduce the activity of AST, ALT, and ALP at an effective dose of 15 mg/20 g of BW; this is due to high levels of flavonoids and pigments in *S. dimorphus* which can act as antioxidants and have benefits in controlling the liver disease. According to a study conducted by Novilia *et al.* (2018), the content of flavonoids found in red betel leaves can be

hepatoprotective. The high levels of antioxidants in *S. dimorphus* can repair liver cells damaged by the effects of NAFLD due to obesity. Flavonoids are anti-inflammatory and immunomodulatory agents (Fitriyani *et al.*, 2011). Flavonoids can restore permeability and increase capillary vascular resistance (Benayache, 2014). Antioxidants from *S. dimorphus* are expected to reduce liver damage due to the binding of free radicals by antioxidant compounds. Free radicals will interfere with the integrity of the hepatocyte membrane, thus eliminating various enzymes from hepatocytes (Ryeo-Eun *et al.*, 2016). Obesity can interfere with liver function due to NAFLD, which causes damage to liver cells, which then results in increased activity of the enzymes AST, ALT, and ALP (Das *et al.*, 2015).

CONCLUSION

A high-fat diet causes obesity in mice with NAFLD. Obesity causes metabolic syndrome which then causes changes in the lipid profile such as decreased cholesterol, TG, and LDL cholesterol levels and an increase in HDL cholesterol. An effective dose of *S. dimorphus* (15 mg/20 g BW) can reduce fat in hepatocytes from the liver of obese mice with NAFLD so that lipid metabolism becomes normal. NAFLD can cause decreased liver function, and administration of effective doses of *S. dimorphus* can reduce the activity of the enzymes AST, ALT, and ALP and improve liver function. Based on this study, it can be stated that *S. dimorphus* has potential as a nutraceutical because it can control obesity and can be used as a cure for NAFLD-linked metabolic syndrome.

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CONFLICT OF INTEREST

There are no conflicts of interest

ETHICS COMMITTEE

This study has obtained ethical clearance from The Committee of Research ethics of the Faculty of Medicine, Andalas University (registration number: 085/KEP/ FK/2018).

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