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Evaluation of lipid profile and liver function after administration of *Scenedesmus dimorphus* in obese mice

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Abstract

Obesity is a major public health problem in developing countries and is a significant risk factor for metabolic disorders. Microalgae *Scenedesmus dimorphus* (*S. dimorphus)* contains bioactive compounds such as pigment function as antioxidants, and omega-3 and omega-6 PUFAs have the potential as nutraceuticals. The study aimed to evaluate the lipid profile and liver function after the administration of *S. dimorphus* in obese mice. The research design uses mice which are divided into 6 groups; Group 1 (G1) normal control, G2 control Obesity, G3 treatment with Orlistat, G4 treatment *S. dimorphus* (0.25mg/g BW), G5 treatment *S. dimorphus* (0.5 mg/g BW) and G6 treatment *S. dimorphus* (0.75 mg/g BW) each group consisted of 5 mice and 21 days of observation time. The parameters observed were the lipid profile and liver function of mice. Based on the results of the study, the effective dose for treating obesity is a dose of *S. dimorphus* 0.75 mg/g (BW) can reduce cholesterol, triglycerides, and LDL levels, respectively 67.7 mg/dl, 49.2 mg/dl, 10 , 2 mg/dl, and increased HDL, 68.32 mg/dl compared to control of obesity (G2), respectively 108.7 mg/dl, 139.1 mg/dl, 20.6 mg/dl and HDL 60, 28 mg/dl, this dose is also effective for improving the function of blood pressure by reducing AST and ALT 15.6 U/L and 18.8 U/L, respectively, compared to the obesity group (G2), which is 26.6 U/L, and 29,7 U/L. Based on the results of the study it can be concluded that *S. dimorphus* is useful for anti-obesity for mice (*Mus musculus*).

Keywords: *Scenedesmus dimorphus; obesity; lipid profile; liver function*

Introduction

Obesity is a metabolic disorder characterized by excessive accumulation of body fat, which is caused by excess nutrient intake, lack of physical activity, lifestyle, and genetic factors that influence the endocrine system and nerves that can increase the energy reserves of adipose[1]. Excess fat (triacylglycerol) is stored in adipose, which can function as endocrine cells that secrete biologically active mediators. This mediator is known as adipokine including leptin, adiponectin, and resistin. Adipokine can change insulin sensitivity, glucose, and lipid metabolism in muscles, liver tissue, and adipose[2]. In obese people, there is an increased risk of developing type 2 diabetes, dyslipidemia, hypertension, cardiovascular (CVS), non-alcoholic fatty liver disease (NFLD), and certain types of cancer[3]. Obesity is caused by 2 factors, namely, exogenous factors and endogenous factors.

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The main cause of exogenous factors is nutritional (90%), due to excessive appetite, especially in foods high in calories and high fat, excess energy is stored as fat (triacylglycerol). Endogenous factors are caused by genetic disorders due to hormonal abnormalities (10%)[4,5]. Obesity is called the metabolic syndrome which can cause several metabolic-related diseases: insulin resistance, increased plasma insulin levels, total lipid profile changes namely increased cholesterol levels and cholesterol fraction, triglycerides, low-density lipoprotein (LDL) and high-density levels decrease lipoproteins (HDL)[6]. The main risks associated with obesity/metabolic syndrome are related to cardiovascular implications due to their association with other risk factors, such as hypertension, insulin resistance, NAFLD, and dyslipidemia[7,8]. Giving a high-fat diet causes mice to be obese, then there is an increase in liver enzymes and changes in lipid metabolism so mice suffer from NAFLD[9]. Obesity is a major cause of non-alcoholic fatty liver disease which has been identified as a characteristic associated with metabolic syndrome. This includes an increase in free fatty acids along with a decrease in Beta-oxidation, causing an increased susceptibility to liver fibrosis[10,11].

Obesity can increase the lipolysis of adipose tissue and the release of fatty acids (FA) into the plasma. Increased levels of intrahepatic triglycerides (TG) and intra-myocellular triglyceride (TG) due to obesity. Intracellular fatty acids sent from plasma or derived from intracellular lipolysis triglycerides can be transported to mitochondria for oxidation, esterified to TG, and partially metabolized into several lipid intermediates (acyl-CoA, ceramide, lysophosphatidate) and fatty acid phosphatidic acid (LPA), also diacylglycerol (DAG)[12]. Fat accumulation correlated with biomass index (BMI) and liver function related to the enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alanine phosphatase (ALP) enzymes. The role of independent central adiposity in BMI in predicting elevated liver enzyme levels, possibly as a result of fatty liver[13]. The increase in ALT, AST, and gamma-glutamyl transpeptidase (GGT) in the obese patient group, along with the increase in BMI rates and beyond normal limits[14].

S. dimorphus contains bioactive compounds that have the potential as drugs and supplements because they contain pigments as antioxidants, and insoluble fiber provides heart protection, hepatoprotective, anti-inflammatory, and antihyperlipidemic effects[15,16]. The content of folic acid and pigments that function as antioxidants from *S. dimorphus* can treat aplastic anemia in rats, by increasing blood hematological parameters and repairing bone marrow damage[17]. *S. dimorphus* contains beta-glucan, which is an active immunostimulator and has beneficial effects as free radical scavengers, to reduce blood lipids[18], and can also prevent skin aging[19]. *S. dimorphus* as *a* nutraceutical can 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 PUFAs as nutraceuticals are used to prevent and treat coronary heart disease, hypertension, diabetes, arthritis, autoimmune disorders, and cancer[20–23]. Consumption of omega-6 PUFAs can reduce mortality due to cardiovascular diseases, including ischemic heart disease, nonischemic heart disease, myocardial heart disease, and hypertension[24].

This research uses the microalgae *S. dimorphus* collection of the Biochemistry laboratory, at Andalas University. The approach method was carried out in vivo using mice as animal models induced with a High Fat Diet (HFD) to make obese mice (*Mus musculus*) treated with *S. dimorphus* microalgae. The study aimed to evaluate the lipid profile and liver function after the administration of *S. dimorphus* in obese mice.

Experimental

Materials

Male mice (*Mus musculus* L) aged six weeks weighing between 20-30 g, 30 mice consisted of

6 groups of 5 mice each. Microalgae *Scenedesmus dimorphus,* High-fat diet to make mice obese (Pellet HFD32 commercial from Clea Japan Inc), basal food for normal mice, kits (Labtest®) to test Profile lipids (triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol) and liver enzymes (AST, ALT and ALP activity), Bold Basal Medium (BBM).

Instruments

Spectrophotometer UV-VIS (Thermo Scientific Genesyis 20), Autoclave (E-Scientific), aerator (Amara), oven (Memmert), centrifuge (Nesco 80-2), micro tube vacutainer EDTA anti-coagulant, micropipette (MRO), Freeze dryer (Telstar – LyoQuest Laboratory Freeze Dryers).

Method

Culture Microalgae Scenedesmus dimorphus

Scenedesmus dimorphus is a collection from the Biochemistry Laboratory. Department of Chemistry, Andalas University. Cultivation of *Scenedesmus dimorphus* in Bold Basal Medium (BBM) was modified with a nitrogen source using urea with a harvest time of 2 weeks[25]. Biomass *S. dimorphus* is dried using a freeze-dryer.

Experimental Animal Design

Adult mice are divided into 6 groups, each group consisting of 5 mice; Group G1 normal control (pellet + drinking water), G2 Obesity Control (High-fat diet), G3 Obesity treatment with Orlistat, G4 Obesity treatment S. dimorphus (0.25 mg/g BW), G5 Obesity treatment S. *dimorphus* (0.50 mg/g BW), G6 obesity treatment S. *dimorphus* (0.75 mg/g BW). Mice before treatment with S. *dimorphus* and Orlistat, mice were made obese by giving a high-fat diet for 4 weeks. Obesity indicators based on BMI values > 30 indicate that the mice are obese. Observations on days 7 and 21 on obese mice after S. *dimorphus* treatment compared to orlistat aimed to find out how long the treatment given was so that the mice were normal and healthy.

The method to prepare the oral formulation

Preparation of the oral formulation of S. *dimorphus* was carried out by making a suspension of S. *dimorphus* in water with a concentration of 25 mg/mL, prepared from 0.25 g of dry biomass of S. *dimorphus* dissolved in 10 ml of distilled water. Administration of S. *dimorphus* was orally treated to mice daily. This research, used mice with a body weight of 25 g, for a dose of 0.25 mg/g BW, 6.25 mg/25g BW was required. The volume suspension needed 0.25 ml for doses of 0.25 mg/g BW. For doses of 0.50, and 0.75 mg/g BW, the volume of the S. *dimorphus* suspension given to mice was 0.5 and 0.75 ml, respectively.

Drug formulation

Administration of orlistat 20 mg/kg (0.02 mg/g BW) orally treatment daily on mice obese after the administration of a high-fat diet for 4 weeks.

High Fat Diet Commercial Formulation

HFD32 (Clea Japan Inc) is a super-high-fat diet with a content of crude fat of 32% and a fat origin calorie rate of 60% from gross energy (Fat kcal%).

Measurement of mice's body weight and length

Measurement of body weight (g) and length (naso – anal in cm) for all treatment groups to determine BMI from normal and obese mice calculated by Rõhrer index,

$$\text{Rõhrer index} = \frac{\text{Body weight (g)}}{\text{(naso – anal) length (cm)}} \times 10^3$$

Rõhrer index value >3 (obese)

Collect the blood of mice

Blood of mice was taken from the neck vein and then stored in a microtube vacutainer EDTA anti-coagulant, and centrifuged (5000 X g) for 30 minutes, then separating the serum and erythrocytes, Serum is used for the analysis of lipid profiles and tests of AST, ALT and ALP activity.

Lipid Profile Analysis

Determination of lipid profile using mice blood serum to calculate triglycerides used Triglycerides Assay Kit Quantification (ab.65336). Total cholesterol, LDL-cholesterol, and HDL-cholesterol measurement used

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Cholesterol Assay Kit - HDL and LDL/VLDL (ab.65390), detection methods are colorimetric (spectrophotometry at λ= 570 nm) to observe the lipid profile with the kit (Labtest®) from ABCAM-USA.

Procedure: Prepare 200 µL solution test, 0.25 µg/µL Cholesterol Working Standard by diluting 25 µL of the provided Cholesterol Standard (2 µg/µL solution) with 175 µL of Cholesterol Assay Buffer. Mix 100 µL sample with 100 µL 2X Precipitation Buffer in microcentrifuge tubes, then Incubate for 10 minutes at room temperature. Centrifuge sample for 10 minutes at RT at 2000 xg (5,000 rpm on a bench microcentrifuge). Transfer the supernatant into a new tube. This is the HDL fraction.

Centrifuge the precipitate once more for 10 minutes at room temperature at 2000 xg to remove any HDL left in the sample. Remove the trace amount of supernatant carefully. Resuspend precipitate in 200 µL PBS. This is the LDL/VLDL fraction (Procedure according to Kit instructions).

Analysis of Liver Function

Liver function analysis using mouse blood serum to observe levels of enzymes alkaline phosphatase (ALP) used Assay Kit (ab83369), aspartate aminotransferase (AST) used Assay Kit (Ab105135), and alanine aminotransferase (ALT) was performed by enzymatic method, determined used ALT Assay Kit (ab.241035) (Labtest®) from ABCAM-USA.

Results and Discussion

Based on the results of measurements of length and body weight calculated BMI based on the Rõhrer index in normal mice (G1) and obese mice (G2 - G6) obtained a Rõhrer index of more than 30 indicated mice obesity (table 1). The group of mice obese by giving a High Fat Diet was the group to be treated with orlistat drugs and *S. dimorphus*.

The administration of high-fat diets in mice can cause obesity due to increased lipid metabolism, levels of fat storage in adipose tissue, and fatty liver[26]. The high-fat diet given to mice becomes obese after four weeks of treatment calculated based on the value of the Rohrer index[9]. Mice before treatment with *S. dimorphus* and Orlistat, mice were made obese by giving a high-fat diet for 4 weeks. Obesity indicators based on BMI values > 30 indicate that the mice are obese. After four weeks of administration of HFD, the obese mice were with *S. dimorphus* in various doses. Observations were made at 7 and 21 days after administration of *S. dimorphus* in obese mice and compared with the control drug Orlistat was commercially available for obese patients.

Table 1. Body weight, length of the body, and Body Mass Index of mice after obesity treatment with High Fad Diet (HFD) at observation 0 - 21 days

Treatments	Body Weight Mean (g) Days		Length of body Mean (cm) Days		BMI Mean ((Rõhrer index) Days	
	0	21	0	21	0	21
G1 (Normal Control)	23.1	26.1	9.16	9.20	30.05	33.51
G2 (Obesity Control)	33.9	37.7	9.16	9.30	44.10	46.87
G3 (Orlistat)	37.3	37.7	9.00	9.20	51.16	48.41
G4 (*S. dimorphus* 0.25 mg/g BW)	36.1	36.0	9.16	9.20	46.97	46.23
G5 (*S. dimorphus* 0.50 mg/g BW)	39.2	39.5	9.33	9.45	48.26	46.81
G6 (*S. dimorphus* 0.75 mg/g BW)	36.9	36.2	9.50	9.52	43.03	41.95

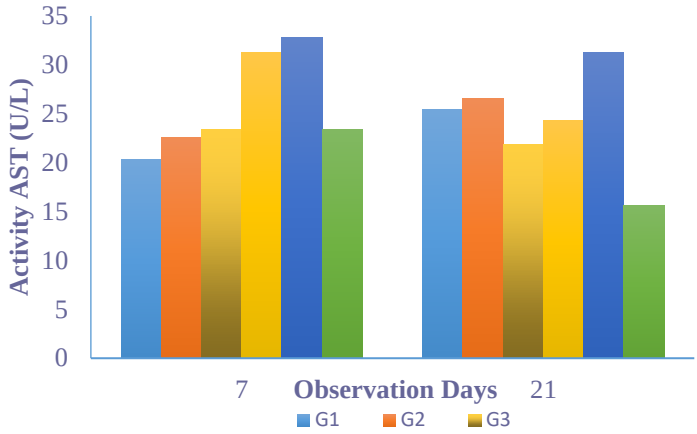

Figure 1. AST enzyme activity in treatment variation between group G1-G6 and observations were done on days 7 to 21, after mice obesity

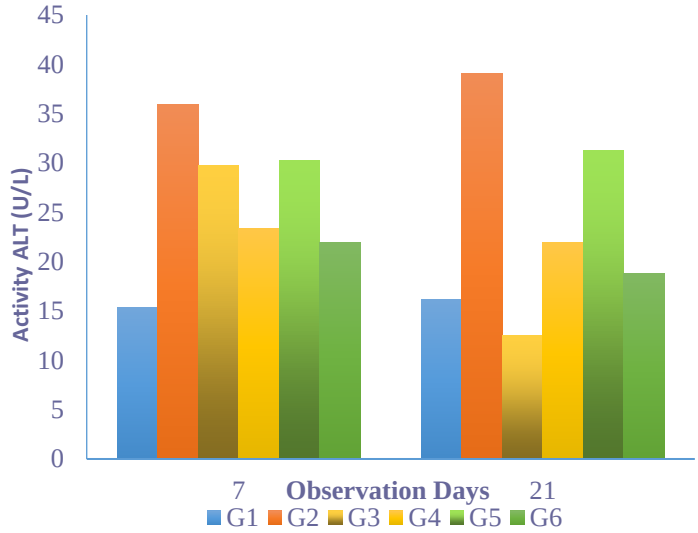

Figure 2. ALT enzyme activity in various treatments between group G1-G6 and observations were done on days 7 to 21, after mice obesity

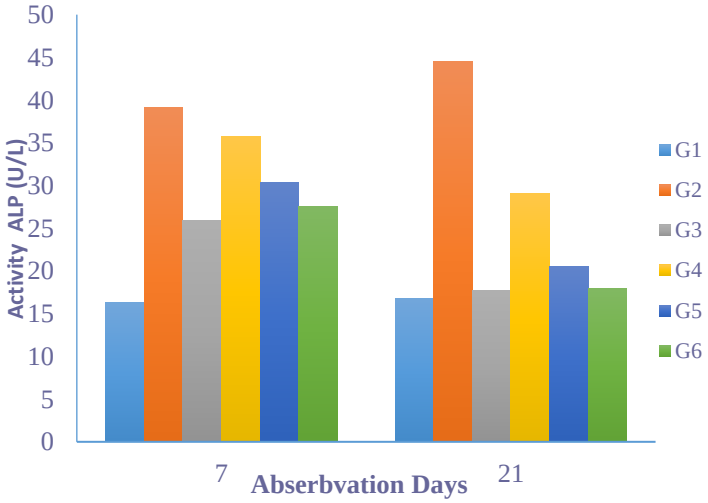

Figure 3. ALP enzyme activity in treatment between group G1- G6 and observations were done on days 7 to 21 after mice obesity

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Based on the results of statistical analysis using ANOVA at $p<0.05$, it gave a significant difference in BMI between orlistat (G3) and *S. dimorphus* (G4, G5, and G6) treatments against obesity control (G2).

Liver Function

Obese mice have decreased liver function seen to the increase in liver enzyme activity (AST, ALT, and ALP) due to an increase in fat levels and changes in lipid metabolism which can lead to a fatty liver[26]. Giving *S. dimorphus* to obese mice can reduce the activity of AST, ALT, and ALP enzymes by 24.3, 21.9, and 29.0 U/l, respectively, at a dose of 0.25 mg/g BW in G4 treatment. Administration of a dose of 0.5 mg/g (G5) can cause a decrease in AST, ALT, and ALP enzyme activity 31.3, 31.3, and 20.5 U/l respectively, on the 21st day. The decrease was very significant in the administration of S. dimorphus whit a dose of 0.75 mg/g BW on day 21 was are 15.6, 18.8, and 17.9 U/l (G6) respectively (Figure 1, 2, and 3), compared with obese mice (treatment G2). On the 7th day, there was no decrease in the activity of the AST and ALT enzymes, whereas, in the ALP activity, there has been a decrease in enzyme activity (Figure 3).

Obesity can cause an increase in liver enzyme levels (ALT, AST, and GGT)[9]. Giving *S. dimorphus* a dose of 0.75 mg/g body weight in obese mice can reduce liver enzyme activity, so it can improve liver function to become normal. Based on the research of Arun et al. 2015, the administration of a mixture of microalgae *Scenedesmus* and *Schroederiella* can reduce obesity-linked metabolic syndrome[28]. Treatment with *S. dimorphus* in obese mice with NAFLD can improve liver function, reduces the activity of AST, ALT, and ALP enzymes, and control obesity by reducing body weight[9]. Giving orlistat can reduce the enzyme activity of AST, ALT, and ALP on the 21st day of observation in almost normal conditions. Orlistat can inhibit pancreatic lipases and reduce or limit energy absorption to reduce fat mass by increasing energy expenditure[26].

Lipid Profile

The administration of High-fat diets can cause mice obesity. Obese mice experience changes in lipid metabolism that result in lipid profile changes marked by increased triglyceride levels, total cholesterol, LDL, and decreased HDL, which can harm the body[23],[26]. The total cholesterol value in the group given microalgae *S. dimorphus* decreased cholesterol values at all doses among the three doses *S. dimorphus* 0.75 mg/g BW is a better dose in reducing cholesterol values compared to an obese group and under normals control (Figure 4A).

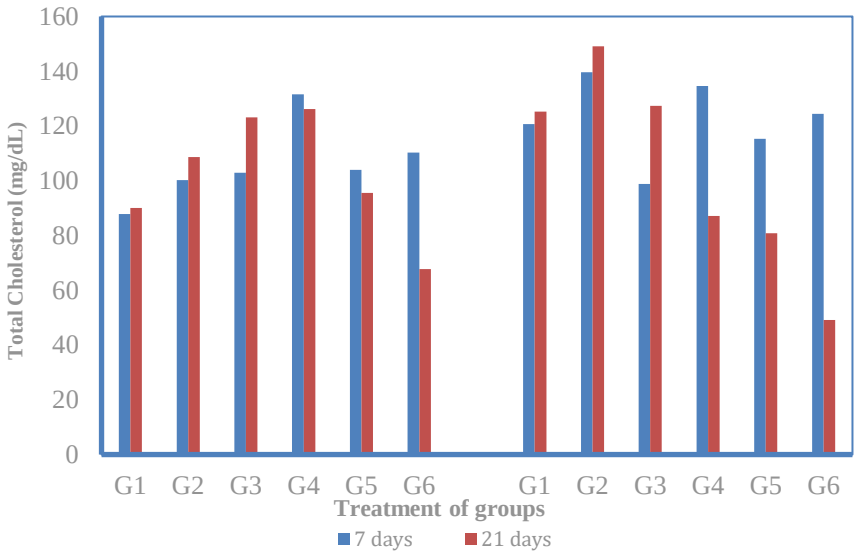

Figure 4. Total cholesterol (A) and Total Triglyceride (B) with variations in treatment between groups G1- G6 were done observations on days 7 to 21 after mice obesity

The group that wares given orlistat experienced an increase in cholesterol values at this condition may be orlistat not work optimally at 21 days of treatment because the use of orlistat tended to have a long consumption time.

The administration of *S. dimorphus* can reduce total cholesterol levels because the omega-3 and lipid content of PUFA found in *S. dimorphus* shows a reals inhibitory effect on lipid accumulation in the liver. The reduction of VLDL secretion by omega-3 occurs due to the inhibition of two key enzymes in the biosynthesis of triglycerides in the liver namely diacylglycerol acyltransferase (DGAT) and phosphatidic acid phosphohydrolase (PAP) are inhibitors of VLDL[27]. Inhibition leads to decreased triglyceride production so the secretion of VLDL and LDL in the liver is also inhibited[20],[22]. Omega-3 can increase peroxisome oxidation of FA to reduce the availability of FA for TG synthesis, resulting in a reduction in liver fat[27]. Microalgae S. dimorphus has dietary fiber, and PUFAs including EPA and DHA to control obesity and its side effects such as diabetes, hypertension, and cardiovascular[21],[24].

The administration of S. dimorphus in obese mice can reduce cholesterol and triglyceride levels compared to the control obesity (G2) and normal (G1) at the 21st-day observation. Cholesterol levels decreased in the administration of *S. dimorphus* at doses of 0.5 mg/g and 0.75 mg/g BW on day 21. The value of triglycerides in the group given *S. dimorphus* decreased in all variations of doses. *S. dimorphus* 0.75 mg/g BW is a better dose than the other two doses in reducing the value of triglycerides. In the group given orlistat, the triglyceride value increased, this was because during the 21 days the orlistat treatment had not worked optimally. In obese mice, there is a change in lipid profile due to an increase in LDL cholesterol and a decrease in HDL cholesterol on 21th-days. The LDL value was decreased in the group given microalgae *S. dimorphus* at two doses of 0.25 and 0.5 mg / g BW (Figure 5), the effective dose of S. dimorphus to reduce LDL value is 0.75 mg / g BW. The administration of S. dimorphus is better for reducing LDL cholesterol levels than orlistat.

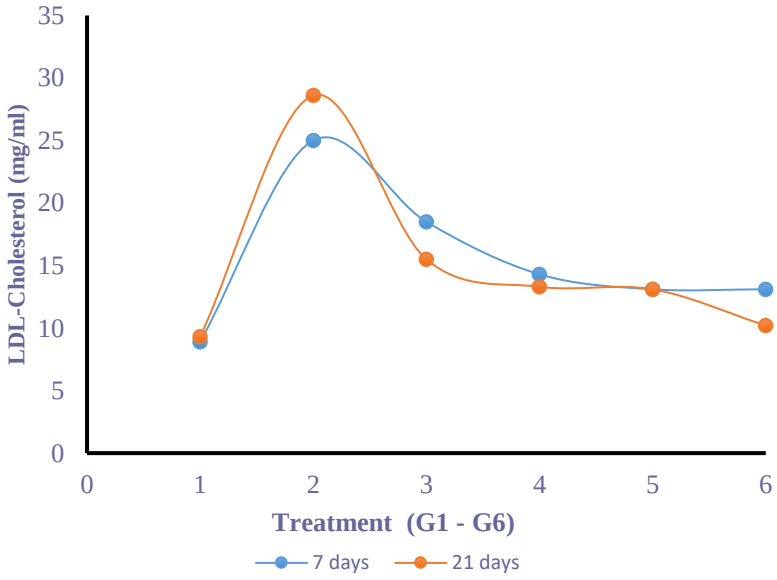

Figure 5. LDL-Cholesterol Levels with variations in treatment between groups G1-G6 and observations were done on days 7 to 21 after mice obesity.

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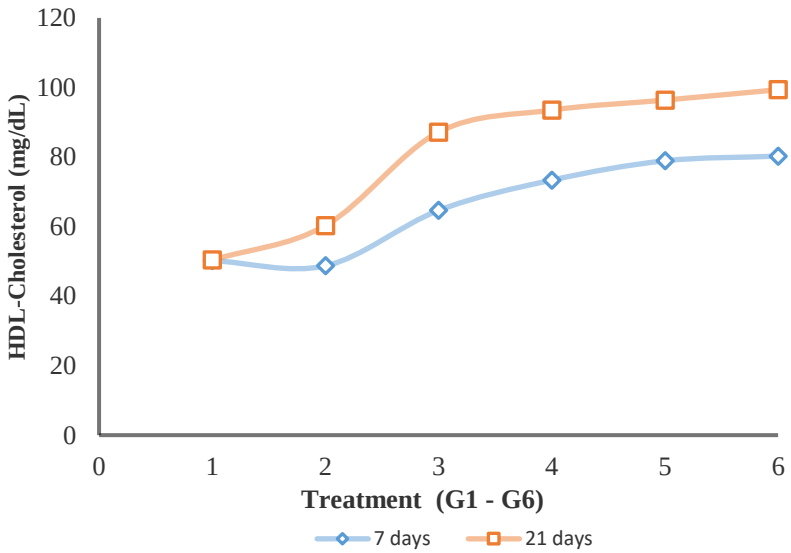

Figure 6. HDL-Cholesterol Levels with variations in treatment between groups G1- G6 and observations were done on days 7 to 21 after mice obesity

In obese mice, a decrease in HDL cholesterol levels is caused by changes in lipid metabolism[22]. Omega-3, omega-6, polyunsaturated fatty acids (PUFA), eicosa pentanoic acid (EPA), docosa hexanoic acid (DHA), and alpha-linolenic acid (ALA) from the content of lipids, proteins, pigments, vitamins, and minerals. Minerals reduce the effects of obesity and improve lipid metabolism[26],[28]. The administration of *S. dimorphus* causes the increase of HDL values in each dose variation on 21th-days (Figure 6). *S. dimorphus* with doses of 0.75 mg/g BW effectively increased HDL values. The orlistat can increase HDL in obese mice at the 21st-day observation (Figure 6).

Omega-3 fatty acids contained in *S. dimorphus* can increase HDL which is a transport lipoprotein in the liver to secrete into the blood. HDL has the role of transporting cholesterol into the liver and then breaking it down into bile acids and removed by excretion of the body. Increasing HDL will reduce cholesterol levels in the blood[29]. Given *S. dimorphus* with an effective dose of 0.75 mg/g BW can improve lipid metabolism back to normal and increase HDL cholesterol which plays a role in transporting cholesterol into the liver which is then broken down into bile acids and excreted through the body's excretions[30].

Conclusions

The Administration of a high-fat diet (HFD) causes obesity in mice, leading to decreased liver function and changes in lipid profile. Treatment with *S. dimorphus* can improve liver function, and the lipid profile becomes normal. *S. dimorphus* is beneficial as an anti-obesity for mice models (*Mus musculus).*

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