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Research Article

Effect of *Arthrospira maxima* and *Chlorella vulgaris* to Lipid Profile and Visceral Fat Index Alteration in Streptozotocin-Induced Hyperglycemia Rats

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ABSTRACT
Arthrospira maxima and *Chlorella vulgaris* contain protein, carbohydrates, antioxidants, omega-3 fatty acids, and many micronutrients. Those compounds have potency of antidiabetic and hypolipidemic activity. This study aimed to evaluate the effect of A. *maxima* and C. *vulgaris* powder administration on alteration of body weight, lipid profile, glucose levels, and visceral fat index of hyperglycemia rats. Twenty male rats were divided into 5 groups i.e. negative control (NC), hyperglycemia control (HC), metformin (M), A. *maxima* (AR), and C. *vulgaris* (CH). Body weight and visceral fat index were measured and calculated by semianalytic and analytical scales. Serum glucose levels were measured by Easy Touch GCU (Glucose, Cholesterol, Uric acid). Lipid profile levels were measured using the photometric enzymatic method. The results showed no differences in body weight between groups, except in AR group was found significantly decreased in body weight on the 20th day. Glucose serum, total cholesterol, HDL and triglyceride levels in microalgae treatment groups were not significantly different be compare to control group. LDL levels of D30 significantly different from D0, but neither between groups. The visceral fat index of a control group was higher compared to that of a microalgae group and significantly different. In conclusion, the administration of microalgae A. *maxima* and C. *vulgaris* for 30 days are effective to reduce visceral fat index but not effective to maintain body weight, glucose level, as well as not effective to improve lipid profile.

Keywords: *Arthrospira maxima, Chlorella vulgaris*, lipid profile, glucose serum level, visceral fat index

INTRODUCTION
In recent years, the prevalence of diabetes mellitus (DM) continues to increase and become a major health problem in all countries (Aizzat et al., 2010). Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, around 90% total incidence of diabetes mellitus (Ekoe, 2019). T2DM develops through a combination of genes, and environmental factors arise as a manifestation of the hyperglycemia phenotype (Hupfeld & Olefsky, 2016). Hyperglycemia is one of the main symptoms of DM. Pharmaceutical factory products which most widely used to overcome the state of hyperglycemia are metformin (Hossain & Pervin, 2018).

Nowadays, the demand of microalgae is increasing in the pharmaceutical field (Udayan et al., 2017). Microalgae that are often used are *Arthrospira* and *Chlorella,* which contain important nutrients including carbohydrate, lipid, protein, nucleic acid, minerals (especially iron), γ-linolenic acid, and antioxidants (Udayan et al., 2017; Yousefi et al., 2019).

Several studies have assessed the potential cytotoxic effects of *Arthrospira* in vitro. There were no adverse effects in rats that were fed A. *maxima* up to 30% in food for three months (Bigagli et al., 2017). A meta-analysis conducted by Serban et al. (2016), showed a significant effect of supplementation with *Arthrospira* in reducing plasma concentrations of total cholesterol, LDL, triglycerides, and increasing HDL levels.

Chlorella's role is to prevent dyslipidemia in high-fat feed rats. The treatment for 8 weeks showed a decrease in total cholesterol, LDL, and triglyceride

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levels (Jong-Yuh & Mei-Fen, 2005). *Chlorella* consumption is useful in preventing the development of type 2 diabetes mellitus. The research conducted by Aizzat et al. (2010), showed that the administration of *Chlorella vulgaris* during 4 weeks in diabetic rats has no hypoglycemic effect but has a protective function against STZ-induced rats by reducing oxidative DNA damage and lipid peroxidation.

Measurement of serum cholesterol levels has a major function in the diagnosis and treatment of several diseases such as cardiovascular disease, hypothyroidism, and diabetes (Narwal et al. 2019). In some studies, there is a relationship between lipid profile levels and type 2 diabetes (Pantoja-Torres et al., 2019). Therefore, this study aimed to evaluate the effect of A. *maxima* and C. *vulgaris* administration for 30 days to serum lipid profile, visceral fat index, glucose serum level, and body weight of hyperglycemia rats induced by single low-dose of streptozotocin.

MATERIALS AND METHODS

Microalgae *A. maxima* and *C. vulgaris* powder were obtained from Blue-Green Algae Biotechnology. Male Wistar rats at 15 weeks old, 200-300 gram body weight from Integrated Laboratory Research and Testing (Laboratorium Penelitian dan Pengujian Terpadu, LPPT) UGM. This research was approved by UGM Animal Ethics Commission with certificate number 00167/04/LPPT/1/2018.

Experimental Design

The animal models were maintained in middle photoperiode (12L-12D cycles) and room temperature. The rats routine feed and drinking with Reverse Osmosis (RO) water ad libitum. The twenty male rats were randomly divided into five groups with 4 replicants. Every group of animals were taken care of in a communal cage and acclimated for a week. A group of rats for normal control (NC). Four other groups were induced hyperglycemia using single low-dose of STZ was 30 mg/kg b.w. in 0.1 M citrate buffer pH 4.5 intraperitoneally. After that, without any other treatments for hyperglycemia control (HC) group. Metformin (M) group treated with metformin 10 mg/kg b.w. as medical treatment. AR group treated with *A. maxima* 2,500 mg/kg b.w. CH group treated with *Chlorella vulgaris* 2,500 mg/kg b.w. Blood glucose levels were measured three days and seven days after induction. This level of glucose later was marked as day 0 (0^{th}). Metformin and microalgae were given orally once a day at 3.00 p.m. - 4.00 p.m. The treatment lasts for 30 days.

Body weight and Visceral Fat Index

Body weight was measured every 10 days. At the end of the experiment, the rats were anesthetized briefly before sacrificed. The adipose tissues were collected and measured its weight to obtained visceral fat index. Visceral fat index was calculated using the formula below:

$$\frac{weight\ of\ visceral\ fat}{body\ weight - weight\ of\ visceral\ fat} \times 100\% \quad (1)$$

Biochemical Analysis

Serum glucose level and serum lipid profile were measured at the start, 15^{th}, and 30^{th} days. Blood samples were collected from the supra-orbital sinus of rats after 8 hours fast. Serum glucose level measured using Easy Touch GCU (Glucose, Cholesterol, Uric acid). Lipid profile consists of total cholesterol, HDL-C, LDL-C, and triglycerides. Total cholesterol was measured using enzymatic photometric CHOD-PAP method. While HDL-C, LDL-C, and TG were measured using enzymatic photometric GPO method.

Statistical Analysis

Body weight, blood glucose level, and lipid profile data were analyzed using One way ANOVA, followed by Duncan test at a significance level of 5%

RESULTS AND DISCUSSION

The results of this research are body weight, serum glucose level, lipid profile, and visceral index shown at the figures and tables below. The age of rats used was 15 weeks with high carbohydrate diet in the previous research period (Hartantyo et al., 2018). Hartantyo et al. (2018) showed that high carbohydrate diet caused metabolic disorders such as obesity and hyperglycemia. In obese animals, insulin resistance can increase with age (King & Austin, 2017). Microalgae used in the study were *A. maxima* and *C. vulgaris*. Some nutrients in A. *maxima* and C. *vulgaris* are omega 3, omega 6, linoleic acid, EPA, DHA, tannins, saponins, Zn, and Fe (Widiyanto et al., 2018). There were no adverse effects or negative effects during the treatment. All of the test animals were kept alive during the treatment.

Body Weight

Body weight of the test animal measurements were used as a parameter for growth. The body weight range of rats was 200-300 grams to minimize mortality (Mu'allimah, 2017). The body weight rats of all groups continued to increase until the 30^{th} day. Body weight gain was linear with age (Stevani, 2017). Fluctuations showed in body weight gain or decrease during microalgae and metformin intervention

Table 1. Serum glucose level of hyperglycemia Wistar male rats (*Rattus norvegicus* Berkenhout,1769) treated with A. *maxima* and C. *vulgaris* on days (-7), 0, 15, and 30 of treatments

Group	Serum Glucose Level (mg/dL)				Δ (D30-D0) (%)
	D(-7)	D0	D15	D30	
NC	81.25 ± 12.69^{a,x}	**87.00 ± 4.08^{a,x}**	105.50 ± 10.60^{a,y}	95.75 ± 8.77^{a,xy}	10.06 ± 10.88
HC	**249.50 ± 99.95^{c,x}**	295.75 ± 55.09^{b,x}	183.75 ± 104.98^{a,x}	185.75 ± 75.26^{a,x}	-37.19 ± 17.03
M	237.25 ± 105.75^{bc,x}	292.00 ± 134.32^{b,x}	192.75± 82.10^{a,x}	223.25 ± 127.06^{a,x}	-23.54 ± 39.92
AR	189.50 ± 14.39^{bc,x}	210.25 ± 79.19^{ab,x}	175.25 ± 62.95^{a,x}	187.25 ± 110.62^{a,x}	-10.94 ± 107.28
CH	139.25 ± 33.46^{ab,x}	277.25 ± 185.48^{b,x}	142.25 ± 62.89^{a,x}	152.75 ± 68.16^{a,x}	-44.91 ± 33.88

Note : ^{a,b,c} = differences between days, ^{x,y,z} = differences between groups, (-) = decreasing of levels, bold : significant differences between groups or days, NC : negative control, HC : hyperglycemia control, M : metformin, AR : A. *maxima*, and CH : C. *vulgaris*

(Figure 1.) However, there were no significant differences between the treatment groups at Δ1 and Δ3. Whereas at Δ2, the *Arthrospira maxima* group had a significant decrease in weight. Δ : the difference between two data; Δ1: body weight difference between D0 and D10; Δ2: body weight difference between D10 and H20; Δ3: body weight difference between D20 and D30.

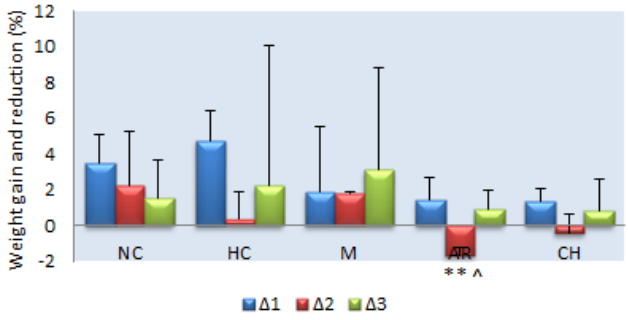

Figure 1. Body weight of hyperglycemia Wistar male rats (*Rattus norvegicus* Berkenhout, 1769) treated with A. *maxima* and C. *vulgaris* for 30 days; Δ1: BW difference between D0 and D10; Δ2: BW difference between D10 and H20; Δ3: BW difference between D20 and D30; ** = significantly different between groups, ^ = significantly different between deltas. NC: negative control, HC: hyperglycemia control, M: metformin, AR: A. *maxima*, and CH: C. *vulgaris*

The smallest or lowest weight gain was at Δ2. This is related to high serum glucose levels in D10 to D20 (day 10 and day 20 of treatment) and rats undergoing early diabetes. So that the increase of body weight was only slightly due to the limitations of cells utilizing glucose to be converted into energy. At the end of the treatment, blood glucose levels had improved so that the body weight began to increase again even though the increase in body weight was lower than at the beginning of the treatment. Body weight reductions of 1.70 ± 0.48% and 0.45 ± 1.11% in the AR and CH groups, respectively, can be caused by the saponin in both microalgae. Although it can not be certain that saponin is the single cause. According to Stevani (2017), saponins can suppress and maintain body weight in the normal range by inhibiting the activity of the pancreatic lipase enzyme thereby preventing the accumulation of fat in the body.

Serum Glucose Levels

Streptozotocin (STZ) has been widely used to induce hyperglycemia in experimental animals by inhibit insulin secretion because it is analogous to glucose (Radenkovic et al., 2015). Pancreatic beta cells have a high concentration of glucose transporter 2 (GLUT2). STZ has the same structure with glucose, so it will absorbted by pancreatic beta cells (Papich, 2016).

In this study, induction of hyperglycemia was used single low-dose 30 mg/kg b.w. of STZ. Some previous studies used higher STZ dose variations to induce type 2 diabetes mellitus (Ghadge et al., 2016; Rouhi et al., 2017). Meanwhile, according to Radenkovic et al (2015), the most widely dose used to induce diabetes is 35-80 mg/kg b.w. intraperitoneally and 35 mg/kg b.w. intravenously. The recommended dose is 60 mg/kg b.w. intraperitoneally. Based on these recommendations, it can be assumed that the effect of a single low-dose STZ induction will only temporarily increase glucose levels.

The decreased of glucose levels in the HC group was happen because this is still the initial stage

of induction to diabetes, so that insulin resistance and impaired glucose tolerance can still return to normal glucose tolerance (Hupfeld & Olefsky, 2016). Whereas in the M and AR groups respectively decreased by 23.54 ± 39.92% and 10.94 ± 107.28% (Table 1.). The cause of decreased serum glucose levels in the microalgae group is explained as follows. According to Jong-Yuh & Mei-Fen (2005), *Chlorella* can reduce glucose levels by reducing the level of DNA damage and lipid peroxidation. Whereas according to Vo et al. (2015), the water-soluble *Arthrospira* fraction is effective in reducing serum glucose levels in a fasting state.

Lipid Profile

Lipid profiles are the main data for this research**.** Acording to Table 2. showed the normal levels for each of the Wistar rat lipid profile parameters (*Rattus norvegicus* Berkenhout,1769) for males (Stevani, 2017) and females (Karima & Mulyati, 2019). Normal levels showed as two normal line in the result's figures, that is upper and lower normal limits.

Table 2. Normal ranges of lipid profile parameters in male and female Wistar rats (*Rattus norvegicus* Berkenhout,1769)

Lipid Profile Parameters	Normal range of lipid profile levels (mg/dL)	
	Karima & Mulyati, 2019	Stevani, 2017
Total cholesterol levels	75.86 – 82.00	53.70 – 73.60
HDL-C levels	59.08 – 64.60	14.90 – 30.90
LDL-C levels	53.72 – 57.92	18.70 – 36.90
Triglycerides levels	46.10 – 65.52	48.00 – 167.00

Total Cholesterol Levels

Cholesterol is part of lipids, with the main structure of steroids. Cholesterol is the basic structure for the synthesis of steroid hormones such as estradiol, progesterone, testosterone, and cortisol. Cholesterol levels in the blood are closely related to the dynamics of steroid hormone levels. It is also related to the individual reproduction phase and stress conditions. Cholesterol levels in the blood of test rats with the treatment of *A. maxima* and *C.vulgaris* are presented in the Table 3.

Based on Table 3., total cholesterol level of the microalgae group was higher than the HC and M groups, and almost the same as the NC group at the end of the treatment (D30). Total cholesterol levels of the HC group increased in D0. This is linear to statement of Rouhi et al. (2017) that the state of diabetes can increase total cholesterol levels. An increase of total cholesterol levels in hyperglycemia individuals due to the state of insulin resistance can reduce the level of cholesterol absorption (Andrade et al., 2019). Even the CH groups in D15 and D30 were slightly higher than normal levels of total cholesterol (Stevani, 2017). NC group levels also exceed normal levels at D0 and D30. However, when viewed from D15, the total cholesterol level in the CH group was lower at D30. The increase and decrease are not significantly difference.

Cholesterol levels of negative control and microalgae group higher than the hyperglycemia control group caused by high cholesterol synthesis and absorption (Andrade et al., 2019), as well as the accumulation of cholesterol metabolism in the body (Stevani, 2017). An increase in cholesterol levels also caused by an increase in LDL levels. This is related to the function of LDL as a cholesterol transporter from the liver to the peripheral cells. Therefore, factors that influence total LDL concentrations will affect total cholesterol. So, an increase in LDL levels is proportional to an increase in total cholesterol levels (Arifah, 2006).

HDL-C levels

At D30, all groups except AR group showed an increase in HDL levels. NC and CH groups had HDL levels exceeding the normal range. The group with the highest HDL levels was *Chlorella g*roup, followed by the negative control, *Arthrospira*, hyperglycemia control, and metformin group (Table 4.).

According to Tabel 4. when compared between the two microalgae groups, the *Chlorella vulgaris* group (CH group) has higher HDL-C levels than AR group. The omega 3 content may be higher in *Chlorella* than *Arthrospira.* The results are consistent with the statement of Harris & Jacobson (2009), that omega 3 has a minor effect on HDL however, omega 3 can increase HDL levels, although not significantly. Mechanisms that possible to increase HDL levels are HDL receives cholesterol from peripheral cells and takes it to the liver for bile production (Wickramasinghe & Weaver, 2018). HDL has two mechanisms in transporting cholesterol esters to the liver i.e. direct and indirect reverse transport. Reverse transportation is indirectly mediated by Cholesterol Esters Transfer Protein (CETP) (Jim, 2013). According to Purnomo (2014), omega-3 can reduce CETP activity so that the transfer of ester cholesterol from HDL to VLDL, IDL, and LDL also decreases. In addition, according to Riggs and Rohatgi (2019), Apo A1 is the main protein constituent of HDL particles, mediating reverse cholesterol transport. Then decrease the concentration of VLDL and apo B due to the fall of

CETP which is influenced by omega-3. This result has implications for the secretion of apo B and VLDL which slows down into circulation or faster VLDL catabolism in the liver (Ryu et al., 2014). So that HDL levels increase.

LDL-C Levels

LDL lipoprotein is commonly used as an indicator of health-related to the heart and blood vessels. LDL is a part of lipid in blood circulation that carries a lot of cholesterol and triglyceride components. The two components have a large proportion, consequently forming molecules that are also large. In blood circulation, this is often seen as a barrier to the rate of circulation. The measurements of LDL-C levels of this research at D0, D15, and D30 were showed in Table 5.

Based on Table 5., all groups have a significant difference increase in LDL-C levels when compared to D0. According to previous research, A. *maxima* and C. *vulgaris* have the effect of lowering LDL cholesterol levels (Jong-Yuh & Mei-Fen, 2005; Torres-Duran *et al.,* 2007; Karima & Mulyati, 2019). The results in this study are not in accordance with the study. However, based on a meta-analysis in patients with type 2 diabetes who were given an omega-3 diet, they have a significant difference increase of LDL cholesterol levels (Chaves et al., 2019).

LDL cholesterol increase caused by two things: an increase in the number of LDL particles or an increase in LDL particle size, with each particle carrying more cholesterol. Larger LDL particles are less atherogenic than smaller particles, the denser characteristics found in the hypertriglyceridemia state (Harris & Jacobson, 2009). This is in accordance with Purnomo (2014), which states that in the process of catabolism of VLDL to LDL, there are two types of LDL produced, large VLDL will produce small, dense, and atherogenic LDL. Whereas a small VLDL will produce large and non-atherogenic LDL. In Metformin groups, the difference in increasing LDL-C is the highest compared with the other groups. Metformin has an effect on reducing blood glucose levels. Through certain mechanisms, then blood glucose is converted to triglycerides so that levels gradually increase with time. In group Microalgae, there was a slight increase compared to control.

Triglyceride Levels

Triglycerides are part of a lipid with a structured form of glycerol and fatty acids. In general, high triglyceride levels also indicate an unhealthy circulation system. Associated with LDL-C levels, the possibility of triglycerides is moving dynamically on free TG with TG on LDL-cholesterol (LDL-C). The results of measurements of triglyceride levels at D0, D15, and D30 are shown in Table 6.

Triglyceride levels in the microalgae group were lower than the hyperglycemia group. Whereas in the metformin group the triglyceride levels were higher but also decreased in D30. This is caused by the ability of metformin to improve glucose levels. Increasing the utilization of peripheral glucose has the potential to reduce the production of fatty acids and triglycerides (Srinivasan et al., 2018). C. *vulgaris* can lower triglyceride levels better than A. *maxima* because the ratio of omega-6 / omega-3 to C. *vulgaris* is lower than A. *maxima* (Gonzalez-Periz et al., 2009; Zanwar et al., 2018)

Previous studies have revealed that *Arthrospira* and *Chlorella* can reduce triglyceride levels (Jong-Yuh & Mei-Fen, 2005; Ou et al., 2012; Karima & Mulyati, 2019). The decrease of triglyceride levels may be influenced by omega-3 content in both microalgae (Ghadge et al., 2016). *Chlorella* is a good source of dietary fiber that affects lymphatic cholesterol and triglyceride absorption by increasing intestinal viscosity, changing the composition of bile acid pools or producing fermented products in the intestine (Ryu et al., 2014).

A potential mechanism by which omega-3 fatty acids (FA) in both microalgae were affecting hepatic triglyceride (TG) metabolism. Feeding omega-3 FA in mice has been shown can inhibit lipogenesis and diacylglycerol acyltransferase (DGAT) activity, phosphatidic acid phosphohydrolase (PA), and hormone-sensitive lipase; and to stimulate oxidation, phospholipid synthesis, and degradation of apolipoprotein (apo) B. The result is a very low level of TG lipoprotein (VLDL) secretion. Nonesterified fatty acid serum (NEFA) also provides FA for TG synthesis (Harris & Jacobson, 2009).

Visceral Fat Index

The visceral fat index shows the amount of fat found in the abdominal cavity. The fat appears to envelop or cover the internal organs. These fats can indicate the presence of excess glucose in the blood that is in a chronic state, the glucose is then converted to glycogen and fat. The visceral fat index is often associated with metabolic syndrome, impaired lipid and glucose metabolism, and cardiovascular disease (Hameed & AbdulQahar, 2019). It is a good predictor of visceral adiposity associated with T2DM. Serves as an indicator of fat distribution and an indicator of adipose tissue function that can change due to insulin resistance (Babiker et al., 2018).

The visceral fat index of hyperglycemia group

Table 3. Total cholesterol level of hyperglycemia male Wistar rats (Rattus norvegicus Berkenhout, 1769) with A. maxima and C. vulgaris treatment on days 0, 15, and 30 of treatments.

Groups	Total Cholesterol Levels (mg/dL)			Δ (D30-D0) (%)
	D0	D15	D30	
NC	77.50 ± 12.34^{a,x}	68.68 ± 6.05^{a,x}	74.20 ± 9.50^{a,x}	-4.26 ± 5.62
HC	59.68 ± 10.17^{a,x}	69.43 ± 8.24^{a,x}	67.60 ± 11.09^{a,x}	13.27±12.97
M	58.98 ± 11.08^{a,x}	61.90 ± 14.05^{a,x}	67.03 ±16.40^{a,x}	13.65 ± 0.09
AR	67.90 ± 4.24^{a,x}	68.78 ±11.66^{a,x}	73.48 ± 21.08^{a,x}	8.22 ± 31.52
CH	70.35 ± 16.89^{a,x}	75.65 ± 16.72^{a,x}	74.78 ± 12.70^{a,x}	6.30 ± 21.49

Note : ^{a,b,c} = differences between days, ^{x,y,z} = differences between groups, (-) = decreasing of levels, bold : significant differences between groups or days, NC : negative control, HC : hyperglycemia control, M : metformin, AR : A. *maxima*, and CH : C. *vulgaris*

Table 4. HDL cholesterol levels of hyperglycemia male Wistar rats (Rattus norvegicus Berkenhout, 1769) with A. maxima and C. vulgaris treatment during 30 days.

Groups	HDL-C Levels (mg/dL)			Δ (D30-D0) (%)
	D0	D15	D30	
NC	34.18 ± 6.76^{ab,x}	36.83 ± 4.21^{a,x}	34.20 ± 4.54^{a,x}	0.06 ± 8.34
HC	29.30 ± 6.73^{a,x}	33.18 ± 3.22^{a,x}	30.18 ± 3.54^{a,x}	3.00 ± 22.28
M	26.60 ± 3.98^{a,x}	29.88 ± 4.38^{a,x}	30.05 ± 4.89^{a,x}	12.97 ± 18.43
AR	39.35 ± 5.56^{c,x}	32.93 ± 4.66^{a,x}	33.83 ± 6.54^{a,x}	-14.03 ± 28.31
CH	35.78 ± 7.20^{ab,x}	35.48 ±5.54^{a,x}	40.50 ± 13.15^{a,x}	13.19 ± 42.22

Note : ^{a,b,c} = differences between days, ^{x,y,z} = differences between groups, (-) = decreasing of levels, bold : significant differences between groups or days, NC : negative control, HC : hyperglycemia control, M : metformin, AR : A. *maxima*, and CH : C. *vulgaris*

Table 5. LDL-cholesterol levels of hyperglycemia male Wistar rats (*Rattus norvegicus* Berkenhout,1769) treated with A. *maxima* and C. *vulgaris* during 30 days

Groups	LDL-C Levels (mg/dL)			Δ (D30-D0) (%)
	D0	D15	D30	
NC	**14.65 ± 2.45^{a,x}**	22.80 ± 2.32^{a,y}	23.78 ± 3.47^{a,y}	62.32 ± 19.82
HC	**13.13 ± 3.83^{a,x}**	23.68 ± 4.91^{a,y}	21.55 ± 2.59^{a,y}	64.13 ± 58.42
M	**10.95 ± 3.00^{a,x}**	20.10 ± 0.16^{a,y}	23.58 ± 2.93^{a,y}	**115.34 ± 63.80**
AR	12.63 ± 2.21^{a,x}	21.05 ± 5.14^{a,x}	20.70 ± 9.87^{a,x}	63.90 ± 96.39
CH	**13.43 ± 5.14^{a,x}**	22.60 ± 5.30^{a,y}	22.65 ± 4.15^{a,y}	68.65 ± 78.60

Note : ^{a,b,c} = differences between days, ^{x,y,z} = differences between groups, bold : significant differences between groups or days, NC : negative control, HC : hyperglycemia control, M : metformin, AR : A. *maxima*, and CH : C. *vulgaris*

showed the lowest compared to other groups especially normal control or nomale group. Visceral fat index of all groups was lower than normal controls. All hyperglycemia treatment groups became less fatty. This may be due to the body cells experiencing glucose deficiency so that there is no conversion to glycogen or fat. The results of treatment with *A maxima* and *C. vulgaris* are better than hyperglycemia conditions. Furthermore, between the two kinds of microalgae, *A maxima* is better than *C vulgaris* (Figure 2.).

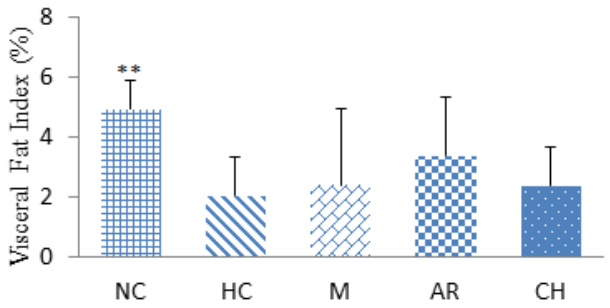

Figure 2. Visceral fat index of hyperglycemia male Wistar rats (*Rattus norvegicus* Berkenhout, 1769) treated with *A. maxima* and *C. vulgaris* after 30 days of treatment; sign (**) = significantly different between treatment groups. NC : negative control, HC : hyperglycemia control, M : metformin, AR : A. *maxima*, and CH : C. *vulgaris*

According to Eckel (2018), the main sites for the regulation of insulin and glucagon are liver, fat tissue, and muscle. The liver functions in the process of glycogenesis, glycogenolysis, gluconeogenesis, and ketogenesis. While fat cells function for lipolysis (Eckel, 2018). Because there is a disruption in energy production, alternative energy sources are used, namely the adipose tissue. So the adipose tissue mass in the HC group is low.

Increasing the utilization of peripheral glucose has the potential to reduce the production of fatty acids and triglycerides (Srinivasan et al., 2018). The results of this study indicate that the omega-3 content reduces triglyceride levels. When triglyceride levels go down, triglyceride stores in adipose tissue will also decrease. Therefore, the visceral fat index in the microalgae group was lower than the NC group but was already higher than the HC visceral fat index due to induction hyperglycemia using metformin and microalgae.

CONCLUSION

In conclusion, the administration of microalgae A. *maxima* and C. *vulgaris* were effective to reduce the visceral fat index but not effective to maintain body weight, glucose serum levels, as well as not effective to improve lipid profile.

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