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Combination of Spirulina Supplementation and Moderate Physical Activity Reduced Weight, Abdominal Fat and Malondialdehyde in Obese Rats

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Abstract: ***Background**: Obesity is a multifactorial disease that appears as an accumulation of excess fat tissue due to an imbalance in calorie intake. The phycocyanin pigment in spirulina is a powerful antioxidant that helps weight loss by reducing Malondialdehyde (MDA), a free radical degradation that increases obesity cases and complicates the management. This study aimed toseethe effect of spirulina supplementationwith moderate physical activityto reduce body weight, abdominal fat, and MDA levels in obese male Wistar rats. **Method**: The randomizedposttest only control group design was conducted on 36 obese male Wistar rats, aged 3-4 months. The total sample was divided evenly into a control group and a study group. The study group received a standard diet and 10 mg/ml oral spirulina supplementation twice a day while the control group was given a standard dietwithNaCl 0.9%, both accompanied by moderate physical activity for 28 days. MDA level examination, weighing, and abdominal fat weight measurements were carried out after the intervention was done. **Results**: The results showed that the mean body weight and weight of abdominal fat were significantly lower in the study group compared to the control group, 229.8±7.18 gr versus 235.5±8.33 gr, p=0.036 and 4.55±0.86 gr versus 5.43±0.72 gr, p=0.002. The MDA level median was also lower in the study group than the control group, 0.73(0.62-0.81) µm versus 1.79(1.64-2.82) µm, p<0.001. **Conclusion**: Spirulina supplementation with moderate physical activity reduced body weight, abdominal fat weight, and MDA levels in obese male Wistar rats.*

Keywords: Lipid metabolism,Malondialdehyde,Obesity, Spirulina

1. Introduction

Unhealthy lifestyles such as high carbohydrates and fats diet and a sedentary lifestyle where daily physical activity is minimal will cause excess body fat, especially abdominal fat deposits. The accumulation of abdominal visceral fat is one of the causes of increased morbidity and mortality.[1]Obesity happened because of an imbalance in the amount of energy consumed than the amount of energy used. Genetic factors, diet, physical activity, and lifestyle are risk factors that play a significant role in obesity.[2–4]Hormonal issues also responsible as the risk factor, sex hormones such as estrogen and testosterone affect the distribution of body fat and adipose tissue differentiation. In addition, estrogen and testosterone receptors also regulate several aspects of glucose and lipid metabolism. This metabolic signal disturbance can cause a metabolic syndrome which is characterized by abdominal obesity and impaired lipid and glucose profiles.[5,6]

In particular, obesity accelerates the aging process by shortening telomere length, compromises the immune system, and quickens the premature onset of age-related conditions.[7]It is known that overweight and obese people have accumulated fat in the body. The excess fat releases bioactive substances that trigger inflammation in the body, which will lead to the formation of excessive reactive oxygen species (ROS). This process causes oxidative stress, which will trigger the premature aging process.[8–10]

In 2016, the World Health Organization (WHO) estimated that more than 1.9 billion adults in the world over 18 years of age are overweight, 13% of whom are obese.[11] Increased obesity will increase risk factors for non-communicable diseases such as heart disease, diabetes, osteoarthritis, cancer.[2,12,13]Obese sufferers experience intracellular fat accumulation resulting in lipotoxicity in cells, which will trigger free radicals. Free radicals adversely affect human body cells and cause damage. The reaction of free radicals with lipids in the cell membrane produces lipid peroxidases. Lipid peroxidase compounds consist of malondialdehyde (MDA), Hydroxinonenal (HNE), and isoprostane. Free radical reactions with proteins produce cross-links resulting in protein dysfunction, and DNA and nucleotides' reactions may cause gene mutations. The protein dysfunction, damage cell,and gene mutations resulting n cell death and accumulation of this damage cause the aging process. An increase in MDA characterizes free radicals because MDA is one of the lipid peroxidase reaction results. The MDA profile in serum serves as a marker of cellular damage due to free radicals.[8,9,14]

Spirulina platensis, contain vitamins, minerals, and carotene known to function as exogenous antioxidants, including carotene, xanthophyll, chlorophyll, and phycocyanin pigments.[15] The combination of the content of rich blue-green microalgae is known to break the radical chain reaction so that it can inhibit ROS and oxidative stress in obese people as a hepatoprotector and help reduce blood sugar levels.[16–18] Zhao et al. mentioned the anti-obesity potential of spirulina in mice in his research and recommended that protein hydrolyzate from *Spirulina platensis* supplements has anti-obesity effects. [19] The same results were also shown by Moradi et al., who stated the positive effects of spirulina supplementation on research subjects suffering from obesity.[20]

Spirulina supplementation contained high protein and antioxidants to bind free radicals that may reduce the MDA levels, thus treat obesity and slowing down the aging process. This study aims to observe the ability of spirulina supplementation and moderate physical activity combination in reducing body weight, the thickness of abdominal fat, and MDA levels in obese male Wistar rats.

2. Methods

An experimental study with a randomized posttest only control group design was conducted on male Wistar rats (*Rattus norvegicus*) with obesity. The study samples were withdrawn randomly from healthy male Wistar rats with an approximate weight between 140 to 160 grams initially before they were induced to be obese. A total sample of 36 rats had been used in the study following the Federer sample formula calculation before the study started.

All experimental animals were adapted to the environment for seven days and coded by their group. After four weeks of administered a high carbohydrate and fat diet, the subjects match obese rats' criteria with an average bodyweight of 200 grams. The 36 subjects were divided evenly into two groups (n=18) randomly, and the diet of the two groups was changed to standard food.

The control group (P0) was given moderate physical exercise for four weeks, astandard diet, and NaCl 0.9%, then weighed the rest of the food every day. The study group (P1) was given moderate physical exercise, a standard diet, and spirulina according to the dose per gram of body weight dissolved in 1 cc of aquadest for four weeks 30 minutes before meals, once every other day, then weighed the rest of the food every day. Physical exercise is being done by swimming in a bucket with a 35 cm diameter, a depth of 20 cm for 45 minutes (75% of the maximum time).

The spirulina supplement's dosage was converted to rats weighing 200 grams, resulting in a 10 mg supplement every day. After four weeks, the rat's blood was taken with a capillary pipette in the orbital sinus (2 ml) to measure the MDA level. Wistar rats were terminated with ketamine 10% and xylazine 2% (0.2 ml) intracardially, then weighed using the *Camry* scales. The rat surgical procedure was performed after termination, and the abdominal fat is separated. Abdominal fat is taken from the subcutaneous and intraperitoneal fat, then measured by a sartorius scale. The institutional ethical committee from Udayana University approved all the study procedures with ethical clearance number #1373/UN14.2.2.VII.14/LT/2020

3. Results

The descriptive results in table 1 show that the mean final body weight, MDA levels, and fat weight were lower in the study group compared to the control group. Besides, median and minimum and maximum values in the study group also showed a consistent lower result than the control group.

Table 1: Descriptive Analysis of Weight, MDA Level, and Abdominal Fat Weight in The Study and Control Group

Variable	Group	Freq.	Mean±S.Dev	Median	Min	Max
Body Weight	Control	18	235,5 ± 8,33	234,5	225	253
	Study	18	229,8 ± 7,18	230	218	241
MDA Level	Control	18	1,84 ± 0,25	1,79	1,64	2,82
	Study	18	0,73 ± 0,06	0,73	0,62	0,81
Abdominal Fat Weight	Control	18	5,43 ± 0,72	5,5	4,2	6,7
	Study	18	4,55 ± 0,86	4,3	3,5	6,5

Comparability tests on the variables of body weight (grams), MDA levels (µm), and fat weight (grams) were carried out to compare the mean of each variable in the control and study groups. The result shows that all variables in the study group are significantly lower than the control group. The significance analysis was tested using the Independent Sample T-Test on weight (grams) variables and fat weight (grams). The Mann Whitney U test was used to compare differences in MDA levels (µm). The results of the comparability test between groups are presented in Table 2.

Table 2: Comparability Test of Body Weight, MDA Level, and Abdominal Fat Weight Between Study and Control Group

Variable	Groups		p
	Control (Freq. 18)	Study (Freq.18)	
Body Weight	235,5 ± 8,33	229,8 ± 7,18	0,036
Abdominal Fat Weight	5,43 ± 0,72	4,55 ± 0,86	0,002
MDA Levels	1,79(1,64-2,82)	0,73(0,62-0,81)	<0,001

4. Discussion

The study group's examination results that were given oral spirulina extract as much as 10 mg/ml twice a day had an average body weight of 229.8 grams, MDA levels of 0.73 µm, and fat weight of 4.55. These results indicate that even with Spirulina administration and moderate physical activity for four weeks, the mean body weight, MDA levels, and fat weight still exceed normal limits. Therefore, apart from giving spirulina as a dietary supplement, it can be concluded that modification of a low-fat and carbohydrate diet and a better combination of physical activity needs to pay more attention to lowering the body mass index.

The descriptive analysis shows that the mean body weight (grams) in the study group (229.8 ± 7.18) showed a lower mean difference of 2.41% than the control group (235.5 ± 8.33). The comparability test between the two groups showed significant results with p-value = 0.036. These results indicate that giving oral spirulina 10 mg/ml twice a day for four weeks resulted in a significant reduction in obese male Wistar rats' body weight compared to the control group. These results are supported by research conducted by Abd El-Rahman, where giving supplementation of a combination of spirulina and cinnamon for four weeks accelerated weight loss of Wistar rats given a high-fat diet. [21]

Since obesity is considered a low-grade inflammatory state, anti-inflammatory nutritional interventions are recommended to manage the pro-inflammatory status in obese people. Omega-3 fatty acids and polyphenols are a major component of the anti-inflammatory diet and are also a major spirulina component. Also, phycocyanin and ß-carotene are the most effective anti-inflammatory and antioxidant components of spirulina. This is used as the basis for research conducted by Abd El Rahman (2018) and Yousefi et al. (2018) in explaining the mechanism of weight loss in research subjects.[15,21]

Descriptive analysis of the mean fat weight (grams) in the study group (4.55 ± 0.86) showed a lower mean difference of 16.24% than the control group (5.43 ± 0.72). The comparability test between the two groups showed significant results with p-value = 0.002. These results indicate that giving oral spirulina 10 mg/ml twice a day for four weeks resulted in a significant reduction in obese male Wistar rats' fat weight compared to the control group. These results are supported by Chen et al., where giving spirulina supplementation increases fat metabolism, reducing total fat levels in Wistar rats' body and ultimately losing weight.[16] This study's results are also supported by a review by Gómez-Zorita et al., which states that spirulina can significantly inhibit body fat accumulation.[22] This is possible because Spirulina sp is a producer of various active ingredients essential for health, including polyunsaturated fatty acids, namely linoleic acid (LA) and a-linolenic acid (GLA). LA and GLA are useful for the treatment of hypercholesterolemia and lipid control. Spirulina's mechanism is supported by its properties that stimulate the production of antibodies and cytokines to stimulate the body's ability to repair and control lipids, which can increase weight gain and subcutaneous fat accumulation.[23,24]

The median MDA levels (μm) in the study group (0.73 (0.62-0.81) μm) showed a lower median difference of 60.4% than the control group (1.79 (1.64-2, 82) μm). The comparability test between the two groups showed significant results with a value of p = 0.000. These results indicate that giving oral spirulina 10 mg/ml twice a day for four weeks resulted in a significant reduction in MDA levels of obese male Wistar rats compared to the control group. These results are supported by Nasirian et al. 's research, where the provision of 20 mg/ml spirulina supplementation for 35 days significantly reduced MDA levels in Wistar rats with DM. Although the dose of administration was higher than this study, Wistar rats in the Nasirian study experienced DM, which could complicate spirulina supplementation in reducing lipid peroxidase such as MDA.[25,26]

Spirulina contains several active ingredients, especially phycocyanin and β carotene, which have potent antioxidant and anti-inflammatory activity. Phycocyanin can scavenge free radicals, including alkoxyl, hydroxyl, and peroxyl radicals, reducing nitrite production and suppressing inducible nitric oxide synthase (iNOS) expression, and inhibit lipid peroxidation.[20,27,28] Research conducted by Gargouri found that the high calcium and low sodium content of spirulina positively affected blood pressure. Spirulina's activity can inhibit lipid peroxidation and is a strong anti-inflammatory, allows changes to the abdominal area abdominal fat accompanied by moderate physical activity, and reduces MDA levels that increase due to lipid peroxidation.[29]

5. Conclusions

The expected effect of giving spirulina extract in this study is to help reduce body weight, MDA levels, and fat weight in obese male Wistar rats compared to controls. The results showed a strong lipid peroxidation and anti-inflammatory effect of oral spirulina, which was shown to have a significant reduction in body weight, MDA levels, and fat weight in the control group's treatment. However, this study has several drawbacks, including Wistar rats' weight, which still revolves around the obesity classification. This encourages further research regarding the effect of spirulina on the weight loss process by choosing the right dose, a combination of administration with other supplements, and considering the duration of intervention and optimal physical activity. This study is expected to add to the research evidence on the potential of spirulina on weight loss, lipid peroxidation, and fat content in obese male Wistar rats.

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