The Effects of β-glucan Extract from Oyster Mushroom
(*Pleurotus ostreatus*) on Expression of Serum
Malondialdehyde in *Sprague dawley* Rats Induced by HFHF Diet

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Abstract. The obesity prevalence in the world continues to increase yearly, which further cause clinical problems related to metabolic syndrome and lipid peroxidation. This study aims to determine the effect of β-glucan extract from oyster mushrooms on lipid peroxidation markers, namely serum MDA levels in rats. Therefore, *Sprague dawley* rats were divided into four groups, namely the KN group, which was fed with AIN-93M standard diet, the KP group was given the AIN-93M modified HFHF diet, the P1 group was fed with AIN-93M modified HFHF + β-glucan diet 125 mg/kgBW, and the P2 group was given the AIN-93M modified HFHF + β-glucan diet 375 mg/kgBW. The β-glucan detection test in oyster mushroom extract used an FTIR spectrophotometer, while the content analysis used the Mega-Calc™ from Megazyme, and also, the MDA levels were determined through the TBARS method. Furthermore, based on FTIR spectrum results, it was proven that oyster mushroom extract contained β-glucan. The provision of HFHF diet for 14 weeks caused the rats to be pre-obese, resulting in lipid peroxidation due to the free radicals induction. The average Lee index rats at the end of treatment were 294.00 ± 6.40 (KN), 292.78 ± 6.37 (KP), 291.85 ± 9.60 (P1), and 286.88 ± 10.60 (P2), with a p value of 0.687. Meanwhile, the average serum MDA level (ng/mL) obtained were 507.833 ± 35.95 (KN), 504.184 ± 29.17 (KP), 540.397 ± 29.80 (P1), and 553.996 ± 86.78 (P2), with a p value of 0.001. The values of serum MDA levels that were statistically significant were KN vs P2, KP vs P1, KP vs P2, and P1 vs P2. These results showed that the dose and duration of β-glucan administered were not sufficient to prevent the lipid peroxidation process. Keywords: β-glucan, High-Fat-High-Fructose, oyster mushroom, *Pleurotus ostreatus*, serum malondialdehyde
1. Introduction

Obesity is the excess fat accumulation due to the imbalance in energy intake and usage. In 2016, more than 1.9 billion adults in the world aged 18 years and over were obese, therefore, obesity prevalence in the world tripled between 1975 and 2016 [1]. In Indonesia, the population aged more than 18 years experienced an increase in obesity prevalence from 11.7% in 2010 to 15.4% in 2013 [2].

This increasing incidence has become a problem in the health sector, which has caused several clinical metabolic syndromes, such as diabetes, cardiac ischemia, and cancer [2, 3]. This pathological condition is related to an imbalance of lipid levels in the body, resulting in oxidative stress. Therefore, making oxidative damage characterized by the Reactive Oxygen Species (ROS) and triggers lipid peroxidation [4].

Lipid peroxidation involves free radicals that interact with unsaturated fatty acids in cell membranes and plasma lipoproteins. These radicals do not have paired electrons therefore, they are very reactive and interact very quickly with others to achieve stability. Their increase in the body cause damage to nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins. For example, an increase in free radicals, result to a rise in lipid peroxidation, causing damage to membrane function, due to decrease in cell permeability. This condition causes cell membrane to become rigid and result to various diseases [5, 6].

Free radicals in the body is determined by measuring the product of lipid peroxidation, namely malondialdehyde (MDA), which is estimated by reaction with thiobarbituric acid. The results shows a fluorescent red color indicating that the substance is reactive [5]. Overall, a decrease in oxidative stress is characterized by reduction in MDA levels [7].

Oyster mushroom (Pleurotus ostreatus) is a type of wood fungus that has high nutritional value, and often consumed since it is easily affordable. Its contents include β-glucan [8], a glucose polymer which binds to the β-glycoside ring on β-1,3-glucan and β-1,6-glucan. Meanwhile, the fungus cell wall contains β-glucan as much as 80-90%. Several studies have shown that β-glucan is used as anti-tumor, antioxidant, anti-cholesterol, anti-aging, and also increase the body's immune system [9, 10].

Based on the research conducted by Alp et al. (2012), which proved that β-glucan provision inhibited lipid peroxidation in the diabetic rats’ brain [6]. Furthermore, it significantly reduce LDL cholesterol (Low Density Lipoprotein), IL-6 (Interleukin-6), and AGE products (Advanced Glycation End) in obese conditions [11]. The presence of oyster mushrooms increases the β-glucan activity in reducing serum MDA levels, however, the research related to this occurrence is still limited. Therefore, this study aims to determine the effect of β-glucan extract from oyster mushrooms (Pleurotus ostreatus) on serum MDA levels in rats, induced by AIN-93M diet, a modification of High-Fat-High-Fructose (HFHF).

2. Materials and Methods

2.1 Preparation and Plant Materials Extraction

Oyster mushrooms were obtained from Sidorejo Village, Jabung District, Malang Regency, East Java, Indonesia. The detection test was carried out at the Taxonomy, Plant Structure and Development Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya. The results showed that this plant species was Pleurotus ostreatus (Identification Number: 0249/UN10.F09.42/03/2019).

The oyster mushroom extract solution was incubated with ethanol 96% (1:1) and stored in refrigerator at 4 °C for 24 hours. Then separated from the solvent using a rotary evaporator.

2.2 Research Design

This research was conducted experimentally with post-test using control group design. The materials used included, Sprague dawley rats as the experimental animals, divided into 4 groups, namely KN (standard diet AIN-93M), KP (AIN-93M modified HFHF diet), P1 (AIN-93M modified HFHF + β-glucan 125 mg/kgBW), and P2 (AIN-93M modified HFHF + β-glucan 375 mg/kgBW). This research was approved by the Health Research Ethics Commission of Faculty of Medicine, Universitas
Brawijaya with an ethical acceptance letter number of 280/EC/KEPK/10/2019. Prior to the commencement of this study, the rats were acclimatized by being given a normal diet for 14 days. Furthermore, the treatment stage was carried out for 14 weeks.

2.3 Identification of β-glucan Functional Groups
The β-glucan content in oyster mushrooms was tested using a FTIR (Fourier-transform Infrared Spectroscopy) spectrophotometer. The fine KBr powder was weighed as much as 100 mg and the sample extract powder was evaluated to 10 mg. Furthermore, both powders were mixed into the mortar agate, crushed until smooth and well mixed, then placed into the pellet mold set. The established KBr pellets were placed in a tablet holder and the functional groups were identified using an FTIR spectrophotometer [12].

2.4 Measurement of Total Glucan (α-glucan + β-glucan) plus D-Glucose in Oligosaccharides, Sucrose and free D-Glucose
The β-glucan levels test in oyster mushrooms used Mega-Calc™ method from Megazyme. While the β-glucan content calculation was carried out as follows, total glucan minus α-glucan.

2.5 AIN-93M Standard Feed Making
The ingredients content of standard feed included, cornstarch, sucrose, maltodextrin, gelatine, fish meal, egg white flour, casein, fiber, AIN-93 M vitamine, AIN-93 M mineral mix, L-cysteine, coline bitartrate, and soybean oil [13].

2.6 Feed Making of AIN-93M Modified High-Fat-High-Fructose (HFHF)
The ingredients composition of AIN-93M standard feed was modified with the addition of fat to achieve a high fat content according to the previous studies [14, 15]. This process was carried out by mixing all the necessary dry ingredients to form pellets, while fructose was administered as a test animal drink made of 30% fructose solution [16].

2.7 Lee Index Measurement
Each rat was weighed, and its body length was measured from nasal to anus. The calculation of Lee index value used a formula, namely \{Body weight (g)\^{1/3}/Naso-anal length (cm)\} x 10\(^3\). Rats were declared obese when the Lee index value was > 300 [17].

2.8 Lipid Peroxidation Activity Measurement
This study used a malondialdehyde (MDA) marker to determine lipid peroxidation activity. The MDA levels were measured using the TBARS (thiobarbituric acid reactive substances) method. The rat blood sample was taken as much as 4 mL, centrifuged for 10 mins at a speed of 100 rpm at 4°C, then the separated plasma was taken and placed into a microtube. Furthermore, 400 µL plasma was added with 400 µL sterile thiobarbituric acid, plus 1 mL distilled water, and 200 µL HCl 1 N into an Eppendorf tube. The solution was homogenized, centrifuged at 500 rpm for 10 mins, and incubated in a water bath at 95 °C for 15 min. Furthermore, the solution was cooled in a refrigerator at 8°C for 15 mins, centrifuged again at 10,000 rpm for 10 mins, and the absorbance was measured at a maximum wavelength of 512 nm, which was included in linear regression equation to obtain the MDA levels [18].

2.9 Data Analysis
All Lee index values and MDA levels were reported as average ± standard deviation (SD) and, examined using one-way analysis of variance (ANOVA), since the data were normally distributed. While the MDA level used the Kruskal-Wallis analysis with the Mann Whitney post-hoc test, since the data were not normally distributed. Both analyses in the treatment groups were considered statistically significant when the p value was < 0.05.
3. Result and Discussions

3.1 The Identification Results of β-glucan Functional Groups by FTIR

The characterization was carried out using FTIR test to determine the β-glucan functional group as follows, in figure 1, the infrared spectrum of the compounds was detected by the presence of absorption peaks at wave numbers of 3750-3000 cm\(^{-1}\) (-OH or alcohol groups), 3000-2700 cm\(^{-1}\) (-CH groups), and 1260-1050 cm\(^{-1}\) (-C-O-C groups) [19]. The results of β-glucan functional group analysis using FTIR were shown in Table 1.

![Figure 1. Structure of 1,3/1,6 β-glucan [19]](image)

### Table 1. Analysis of β-1,3 Glucans Functional Groups Using FTIR

| Wavenumbers (cm\(^{-1}\)) | Wave Numbers (cm\(^{-1}\)) | Functional Groups          |
|---------------------------|-----------------------------|-----------------------------|
| Standard from Sigma Oyster Mushroom Extract | 3363.62 | 3403.92 | 3750-3000 | Alcohol and hydroxyl (OH) |
| 2891.10 | 2925.61 | 3000-2700 | CH alkane |
| 1157.21 | 1078.90 | 1260-1050 | -C-O-C (ether) |

![Figure 2. FTIR Spectra Standard of β-glucan from Sigma](image)
Table 1, Figures 2 and 3 showed that the oyster mushroom extracts containing β-glucan had a very similar infrared spectrum compared to β-glucan standard from Sigma. The presence of ether and alcohol groups were used to indicate a glycosidic bond, since it was a linkage between sugar hydroxyl and alcohol. The fingerprint area (1500–700 cm⁻¹) was used to confirm the presence of an organic compound by comparing the test structure with the standard. The β-glycoside bond was detected by IR spectra at wave numbers of 1024 and 867 cm⁻¹, while β-1,3-D-glucan bonds was indicated by the absorption band at 895 cm⁻¹. In the standard FTIR spectra of β-glucan from Sigma and oyster mushroom extract, there was an absorption band of β-1,3 glucan bonds, respectively indicated by the presence of absorption bands at wave numbers of 894.91 and 853.24 cm⁻¹ [19]. Based on the FTIR spectra data, the oyster mushroom extract showed the presence of β-glucan, however, did not indicate β-1,6 glucan branch chain. This result was consistent with the study of Baeva et al. (2019), which also did not show the presence of β-1,6 glucan in β-glucan of oyster mushroom extract using water extraction technique [20].

3.2 Analysis Results of β-glucan Levels with Megazyme
The β-glucan levels were measured by the Megazyme method as follows, the gel extracted from β-glucan of oyster mushrooms were analyzed using Mega-Calc™ Yeast & Mushroom Beta-Glucan (K-YBGL) Determination from Megazyme. The result showed that β-glucan levels in oyster mushroom gel was 2.05% w/w.

3.3 Lee Index Results
Lee et al. (2011) described that in experimental animals, such as rat, the calculation of obesity index used several methods, namely the Rohrer, Lee index, and TM index [17]. The measurement results of Lee index was shown in Table 2. The rats that fed on HFHF diet (KP, P1, and P2) were not obese, since the Lee index value for this condition was > 300 [17]. However, the rats were already advancing
towards obesity or were pre-obese, since the purpose of this study was to prevent their metabolic syndrome. Therefore, obesity is one of the triggers for metabolic syndrome that further led to pathological processes, such as oxidative stress, characterized by the increase in MDA levels [3, 17]. This condition was certainly life threatening, since it caused major illness, such as type II diabetes and cardiovascular disease [3].

Table 2. One-Way ANOVA Analysis Results of Lee Index Values

| Group | Average Body Weight (g) ± S.D | Average Body Length (cm) ± S.D | Average Lee Index ± S.D | p       |
|-------|-------------------------------|-------------------------------|------------------------|---------|
| KN    | 431.25 ± 35.66                | 25.69 ± 0.80                  | 294.00 ± 6.40          | 0.687   |
| KP    | 417.00 ± 17.47                | 25.45 ± 0.55                  | 292.78 ± 6.37          |         |
| P1    | 412.75 ± 34.31                | 25.35 ± 0.78                  | 291.85 ± 9.60          |         |
| P2    | 400.50 ± 89.16                | 25.44 ± 1.24                  | 286.88 ± 10.60         |         |

*It was statistically significant when the p value < 0.05.

In this study, fructose was used in the composition of HFHF diet, since it was widely used in daily food as a substitute for sweetener, such as glucose or sucrose. The HFHF diet induced obesity due to hyperleptinaemia and endothelial dysfunction. Furthermore, a diet high in refined carbohydrates, such as fructose, glucose, or sucrose increased the risk of dyslipidemia and cardiovascular disease. In conditions of further pathogenesis, the HFHF diet caused inflammation and oxidative stress. Meanwhile, the series of oxidative stress contributed to several pathological conditions, namely the aging process, metabolic syndrome, and diabetes [22].

Mushrooms are natural source for nutraceuticals and functional food, due to their bioactive components, which are beneficial to human health [23]. Their β-glucan compositions were active substances with the potential to improve health, for cardiovascular protection, and anti-obesity activity [19, 20].

The research results showed that the mixture of HFHF diet with β-glucan at doses of 125 and 375 mg/kgBW produced lower Lee index than rats that were only given the diet without β-glucan, although the values were not statistically significant. Furthermore, the Lee index value of the rat feed with β-glucan 375 mg/kgBW was also lower than those given β-glucan 125 mg/kgBW. This was in line with Zhu et al. (2015) and Bulam et al. (2018) study, which stated that β-glucans acted as antiobesity [19, 20]. The β-glucan as a constituent of dietary fiber was difficult to digest, due to lack of hydrolase in humans and rats. The glucans provided less energy as a substitute for nutrients in the diet and increased satiety. The soluble dietary fiber increased stool volume and water, and intestinal contents' viscosity, which makes it easy for undigested food to be transported into the large intestine [25].

The mushrooms from Pleurotus ostreatus species were known to be edible, with the potential to produce lovastatin, a pharmaceutical agent for lowering cholesterol, and an inhibitor of HMG-Co-A reductase. This HMG-Co-A reductase is an enzyme in cholesterol metabolism that catalyzes the reduction of HMG-Co-A to mevalonate. The addition of Pleurotus ostreatus to the hyperlipidemic diet prevented the accumulation of cholesterol and triacyl-glycerols and reduced the atherogenic effect in patients with coronary disease [26].

3.4 The Role of β-glucan in Reducing Lipid Peroxidation

A lot of evidence had shown the existence of oxidative stress process as an imbalance between oxidants and antioxidants, therefore, allowing many biochemical changes to occur. It played an important role in pathological development of human diseases on a broad spectrum. The oxygen free radicals were very reactive and attack almost every cell component causing damage to the surrounding tissue. The heaviest impact of oxidative stress was in lipid peroxidation in the pathogenesis of various
diseases, namely atherosclerosis, diabetes, cancer, and aging. Lipid peroxidation was a chain reaction that started with hydrogen abstraction or the addition of oxygen radicals causing oxidative damage of polyunsaturated fatty acids [3]. The increase in lipid peroxidation reflected a decrease in enzymatic and nonenzymatic antioxidants in the body's defense system [6]. The serum MDA level test was the most widely used method in clinical practice, due to its sensitivity and simplicity [3]. Several studies had shown that MDA levels were markers for oxidative stress in obesity [3, 23].

Table 3: Kruskal-Wallis Analysis Results of Serum MDA Levels

| Group | Average MDA Levels (ng/mL) ± SD | p     |
|-------|---------------------------------|-------|
| KN    | 507.833 ± 35.95                 | <0.001*|
| KP    | 504.184 ± 29.17                 |       |
| P1    | 540.397 ± 29.80                 |       |
| P2    | 553.996 ± 86.78                 |       |

Kruskal-Wallis and Mann-Whitney Post-hoc Test within a column showed that the values with different superscript were significantly different when \( p < 0.05 \), and statistically significant when the \( p \) value < 0.05.

The presence of \( \beta \)-glucan reduced oxidative stress through its antioxidant capacity [28]. The \( \beta \)-glucan is an antioxidant with a scavenger ability between \( \alpha \)-tocopherol and incorporated in the lipid bilayer, water-soluble antioxidants, and mannitol. It also reduced oxidants and increased antioxidants in the brain and nerve tissue of diabetic rats [6]. However, the relationship between serum MDA levels and \( \beta \)-glucan in pre-obese rat has not been studied.

The purpose of this study was to determine the effect of \( \beta \)-glucan on serum MDA levels in pre-obese rats. The results showed that the HFHF modified AIN-93M diet had caused pre-obesity, which triggered lipid peroxidation, indicated by an increase in serum MDA levels. However, this study showed that \( \beta \)-glucan in 125 and 375 mg/kg BW dosage did not reduce serum MDA levels compared to the control group (Table 2). It was observed that the \( \beta \)-glucan dose administered for 14 weeks were inadequate to prevent lipid peroxidation in serum. Furthermore, the \( \beta \)-glucan had hydroxyl radical scavenging activity, which was affected by the decrease in molecular weight. Therefore, the size of molecular weight was an important factor for the antioxidant activity of \( \beta \)-glucans [29]. The \( \beta \)-glucan content identification in the oyster mushroom extract solution only found \( \beta \)-1,3 glucan branch chains and did not find any \( \beta \)-1,6 glucan branch chains. This affected the inability of \( \beta \)-glucan in this study to reduce free radicals from serum MDA levels, which also requires further research.

4. Conclusion
The administration of HFHF diet for 14 weeks caused pre-obesity in the experimental rats, and not obese, while the provision of \( \beta \)-glucan at 125 and 375 mg/kg BW doses with the HFHF modified AIN-93M diet for 14 weeks was not sufficient to prevent lipid peroxidation as observed from serum MDA level markers. Therefore, further research is needed to comprehensively determine the cause of increase in serum MDA levels.

5. Research Limitations
There were no plasma lipid data, therefore, the occurrence of dyslipidemia which was the main requirement for lipid peroxidation was not determined.

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