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The effect of oyster mushroom (*Pleurotus ostreatus*) beta-glucan extract on brain -derived neurotrophic factor and neuron in high-fat-high-fructose diet-induced male Sprague Dawley rats

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Abstract

Obesity is a condition where there is an accumulation of excess body fat, which can increase oxidative stress either in the blood or in tissues, such as brain tissue. This situation can trigger atrophy in neurons and reduce brain-derived neurotrophic factor (BDNF) levels, which can increase the risk of impaired cognitive function. This study aimed to ascertain the effect of oyster mushroom (*Pleurotus ostreatus*) β-glucan extract on the number of neurons and levels of BDNF in rats. The study design was a true experimental with a randomized control group post-test only with white male Sprague Dawley rats as the subjects. A total of twenty-four rats were divided into four groups, namely the normal diet group fed AIN-93M standard diet (KN), the high-fat-high-fructose (HFHF) diet group (KP), and the HFHF diet group with the addition of β-glucan extract dose of 125 mg/kg body weight (P1), and dose of 375 mg/kg body weight (P2). The BDNF was measured by ELISA, and the number of neurons was by histological staining approach. Although there was no significant difference in the number of neurons ($p = 0.692$), the data revealed a significant difference in BDNF levels ($p = 0.005$). There was no relationship between the number of neurons and BDNF levels ($p = 0.874$) and between the β-glucan dose and the number of neurons ($p = 0.796$). However, there was a relationship between β-glucan dose and BDNF levels ($p = 0.001$; $r = 0.695$). This study concludes that the dose of β-glucan from oyster mushroom extract positively affects BDNF levels but not the number of neurons. There was no relationship between the number of neurons with BDNF levels and β-glucan dose. It was presumable because the staining technique was less specific, so the possibility of other cells counted as neurons may occur.

1. Introduction

Obesity is a state of excess body fat accumulation. Increased body mass index (BMI) can elevate the risk of cancer, type 2 diabetes, and cardiovascular diseases (World Health Organization (WHO), 2020). In 2016, 11% (men) and 15% (women) had a BMI of 30 kg/m^2, or nearly half a billion people in the world over the age of 18 were obese (WHO, 2020). In Indonesia, the obese population shows an increase every year. The 2018 national survey known as Indonesia Baseline Health Research showed that the population aged >18 years who were obese (BMI 27 kg/m^2) in 2007 was 10.5% and increased to 21.8% in 2018 (The Ministry of Health of Indonesia, 2018). The obesity prevalence in Indonesia is estimated to grow, with an increase of 3.9% per year to reach 22 million cases of obesity (BMI 30 kg/m^2) in 2030 (World Obesity Federation, 2022).

Obesity is often associated with the incidence of non -degenerative diseases, but another problem that can also arise from obesity is neurodegenerative, which is characterized by impaired cognitive function. Meo *et al*. (2019) reported that school adolescents aged 12-15 with a higher body mass index (BMI) experienced a significant decline in cognitive function compared to healthy adolescents (Meo *et al*., 2019). The decline in

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cognitive function may be due to atrophy in the hippocampus, along with an increase in a person's BMI (Buie *et al.*, 2019). A study in high-fat-high-fructose (HFHF) diet-induced obesity model rats showed an inflammatory response in the hippocampus characterized by Toll-like Receptor 4 (TLR4) and Nuclear Factor Kappa B (NF-κB) activation and proinflammatory cytokine production. In addition, neuronal cell apoptosis was relatively high in the HFHF diet group than in the normal group (Yu *et al*., 2019).

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor functioning in the neurogenesis process and has a role in learning and memory (Krabbe *et al*., 2007). BDNF levels decreased due to increased food intake, hyperphagia, and obesity (Zhang *et al*., 2013; Janićijević *et al.*, 2020). Several studies have proven that BDNF plays a role in energy homeostasis and weight management by regulating satiety. Decreased levels of BDNF can increase appetite through serotonergic mechanisms in the hypothalamus and inhibition of stimulation of glutamate or gamma-aminobutyric acid-ergic (GABAergic) receptors resulting in changes in neuronal activity that cause obesity (Pandit *et al*., 2020; Bumb *et al*., 2021).

β-glucan has long been studied for its benefits against obesity. The results show that β-glucan can improve hypertriglyceridemia, hypercholesterolemia, hypertension, hyperglycemia, and insulin resistance (Zheng *et al*., 2013; Bashir and Choi, 2017). Improvement of the above conditions will ultimately help reduce oxidative stress. Oxidative stress can trigger neuronal cell death in the hippocampus by activating microglia, releasing cytochrome c and apoptosis-inducing factor (Apaf-1), and causing neurotoxic effects by disrupting the brain barrier (Shah *et al.*, 2009; Kusumastuty *et al.*, 2020; Mullins *et al*., 2020).

Currently, research on the benefits of β-glucan on the number of neurons and BDNF levels in the obese condition is limited, particularly in Indonesia. Therefore, the purpose of this study was to investigate the impact of oyster mushroom (*Pleurotus ostreatus*) β-glucan extract on the number of hippocampal neurons and BDNF levels in HFHF diet-induced rats.

2. Materials and methods

2.1 Research design

The study design was true experimental with a randomized control group post-test only. The ethical permission was granted by the Health Research Ethics Commission, Faculty of Medicine, Universitas Brawijaya (No. 136/EC/KEPK/07/2020). The subjects used in this study were male Sprague Dawley rats. Rats were divided into four groups, namely the negative control group (KN) fed AIN-93M standard diet, the positive control group (KP) fed HFHF diet, the intervention 1 group (P1), and the intervention 2 group (P2). The intervention groups were given HFHF diet with the addition of β-glucan doses of 125 and 375 mg/kg body weight (mg/kg BW).

2.2 Research instruments

The tools used are rat cages, cage drinking water bottles, analytical scales, oral probes, syringes, blenders, pans, beakers, measuring cups, and rotary evaporators. The materials used were oyster mushrooms, male Sprague Dawley rats, 96% ethanol, distilled water, ketamine, 10% formalin or 10% buffer, BDNF ELISA kit (Catalog No.: E-EL-R1235 96T), husks, diet for rats, drinking water, fructose solution, gloves, and mask.

2.3 Subject preparation

A total of 24 rats aged between 40-60 days were acclimatized for 14 days, then randomized and divided into four groups. The treatment period lasted 14 weeks.

2.4 Preparation of beta-glucan

Extraction of β-glucan was carried out based on the method described by Ariyanti (2016) and consists of 3 main stages, namely, (1) making a mushroom solution; (2) mixing the oyster mushroom solution with 96% ethanol and incubating for 24 hrs; and (3) separating the mushroom extract with ethanol solvent using a rotary evaporator. The β-glucan was identified in 2 stages, using FTIR (Fourier-transform Infrared Spectroscopy) spectrophotometry to identify the functional group of β-glucan as well as calculating total glucan levels and then measuring total glucan (α-glucan + β-glucan) levels using the Mega-Calc^{TM} from Megazyme. The β-glucan content was calculated as total glucan minus α-glucan (Yunita *et al*., 2020).

2.5 Normal diet and high-fat-high-fructose diet making

A normal diet was made using a mixture of ingredients such as cornstarch, sucrose, maltodextrin, gelatine, white egg flour, fish meal, agar flour, casein, mineral and vitamin mix AIN, L-cystine, and choline bitartrate, then added with soybean oil and water. The HFHF diet replaced soybean oil with lard and animal fat. In addition, a 30% fructose solution was given with 0.02% red food coloring.

2.6 Calculation of hippocampal neurons and brain-derived neurotrophic factor levels

After 14 weeks of treatment, the rats were injected with 0.005 mL/g body weight of ketamine and then

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sacrificed for their brain organs (Winarto, 2009). After that, histological preparations of the rat brain were made, which were stained with hematoxylin-eosin (HE) staining. The observation of hippocampal neurons was carried out by observing the results of the scan preparations using double-blinding. Hippocampal neurons were counted with OlyVIA application at 400× magnification of 10 fields of view per preparation (Arjadi *et al*., 2014). Meanwhile, plasma BDNF levels were calculated using the ELISA technique.

2.7 Data analysis

SPSS 16.0 was used to analyze the data. First, normality and homogeneity tests were carried out using the Shapiro-Wilk and Levene tests. Furthermore, different tests were carried out on body weight and the number of hippocampal neurons using the One-Way ANOVA test. In contrast, the Lee index and BDNF levels used the Kruskal Wallis test. Spearman Rank correlation test was used to test whether there was a relationship between β-glucan dose, the number of hippocampal neurons, and BDNF levels.

3. Results and discussion

3.1 Body weight and Lee's index

Rat body weight was observed and measured once per week for 14 weeks of treatment to monitor changes in body weight. The average weight gain of rats can be seen in Table 1. Each group experienced an increase in body weight by 209.67 g (KN), 192.33 g (KP), 184.50 g (P1), and 193.33 g (P2). However, statistical analysis revealed no significant differences in initial body weight, final body weight, and weight gain between the groups. The highest Lee index value was in the P1 group (296.10), and the lowest was in the P2 group (289.47). However, no rats were obese based on the Lee index value because the value was still <300.

Rat body weight increased in all groups, although there was no significant difference. In this study, rats were given a high-fat and high-fructose diet, which contained a high caloric density that could lead to weight gain (Inbar *et al*., 2020). The increased body weight in rats due to the high-calorie intake resulted in large calorie stores in the body and an increase in visceral fat, body fat, and adiposity index (Matias *et al*., 2018). The Lee index is one of the indicators used to assess obesity in rats. If a rat has a Lee index >300, then the rat is said to be obese (Nesti, 2015). In this study, rats fed the HFHF diet (KP, P1, and P2) were not said to be obese because the Lee index was still <300. However, with Lee's index close to 300, it can be said that the rat was pre-obese.

3.2 Food and fructose intake

Food and fructose intake were determined by measuring the total food in grams and fructose in ml given to the rats and subtracting the remaining food and fructose every 24 hrs. The average increase in food intake can be seen in Table 2. There were significant differences in food and fructose intake between groups, as well as carbohydrate and fat intake. However, the groups had no significant difference in energy and protein intake.

3.3 High-fat-high-fructose, beta-glucan and neuron

The number of hippocampal neurons was calculated from the total number of cells in 10 visual fields taken from five areas in the hippocampus, namely CA2-3, CA1, dentate gyrus (granular cell layer and hilum or polymorphic cell layer), and subiculum. The average number of neurons in the hippocampus can be seen in Table 3. The highest number of neurons was found in the P1 group (408.21), then the KP group (384.00), the KN group (364.00), and finally, the P2 group (360.92). The statistical analysis showed that there was no significant difference in the number of hippocampal neurons between the groups ($p = 0.692$; $p>0.05$). However, the number of neurons in the P1 group (which was given β-glucan at a dose of 125 mg/kg BW) appeared to be higher than the positive control group (KP).

Administration of the HFHF diet in mice can lead to obesity, insulin resistance, hyperinsulinemia, and dyslipidemia, similar to the pathogenesis of humans

Table 1. Average body weight of rats and Lee's index.

Parameter	KN (N = 6)	KP (N = 6)	P1 (N = 6)	P2 (N = 6)	*P*-Value
Initial body weight (g)	214.67±21.91	224.83±15.56	225.67±27.10	222.50±20.38	0.809[a]
Final body weight (g)	424.33±39.25	417.17±20.82	410.17±37.62	415.83±51.51	0.938[a]
Weight gain (g)	209.67±39.78	192.33±25.78	184.50±25.73	193.33±35.10	0.594[a]
Lee's index	291.63±3.26	294.04±7.47	296.10±9.45	289.47±6.67	0.555[b]

Values are presented as mean±SD. KN: Negative Control Group (KN), KP: Positive Control Group (HFHF), P1: Intervention Group 1 (HFHF + β-glucan dose 125 mg/kg BW), P2: Intervention Group 2 (HFHF + β-glucan dose 375 mg/kg BW). Weight gain was calculated by subtracting the final body weight from the initial body weight.
[a]Parametric test (One Way ANOVA), [b]Non-parametric test (Kruskal Wallis Test).

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Table 2. Average food intake of rats.

Parameter	KN (N = 6)	KP (N = 6)	P1 (N = 6)	P2 (N = 6)	*P*-Value
Food (g)	22.53±1.37	11.90±0.75	11.07±1.07	11.52±1.42	0.003[b]
Fructose (g)	0	25.71±2.72	22.86±3.82	19.19±3.18	0.000[b]
Energy (kcal)	92.40±5.55	95.83±5.12	87.86±8.14	85.92±10.14	0.129[a]
Carbohydrate (g; %)	12.37±0.74; 53.54%	11.82±0.84; 49.34%	10.68±1.26; 48.58%	9.77±1.27; 45.49%	0.002[a]
Protein (g; %)	3.18±0.20; 13.78%	3.11±0.20; 12.98%	2.88±0.30; 13.15%	3.02±0.38; 14.07%	0.297[a]
Fat (g; %)	0.62±0.04; 6.01%	2.67±0.16; 25.11%	2.48±0.23; 25.40%	2.59±0.33; 27.08%	0.002[b]

Values are presented as mean±SD. KN: Negative Control Group (KN), KP: Positive Control Group (HFHF), P1: Intervention Group 1 (HFHF + β-glucan dose 125 mg/kg BW), P2: Intervention Group 2 (HFHF + β-glucan dose 375 mg/kg BW).
[a]Parametric test (One Way ANOVA), [b]Non-parametric test (Kruskal Wallis Test).

Table 3. Average BDNF levels and number of neurons.

Parameter	KN (N = 6)	KP (N = 6)	P1 (N = 6)	P2 (N = 6)	*P*-Value
BDNF Levels (pg/mL)	452.83±218.85	81.58±63.24	116.17±63.12	394.92±221.66	0.005[b]
Number of Neurons	364.00±65.37	384.00±70.53	408.21±97.81	360.92±66.76	0.692[a]

Values are presented as mean±SD. KN: Negative Control Group (KN), KP: Positive Control Group (HFHF), P1: Intervention Group 1 (HFHF + β-glucan dose 125 mg/kg BW), P2: Intervention Group 2 (HFHF + β-glucan dose 375 mg/kg BW).
[a]Parametric test (One Way ANOVA), [b]Non-parametric test (Kruskal Wallis Test).

(Zhuhua *et al*., 2015). This condition, through a complex mechanism, can indirectly affect neurons in the hippocampus. In conditions of insulin resistance, tumor necrosis factor-α (TNF-α) levels are elevated, which can trigger the synthesis of free fatty acids and interleukin-6 (IL-6), which is correlated with an increase in triglyceride levels (Fernández-Sánchez *et al*., 2011). Increased levels of triglycerides can trigger increased levels of protein carbonyl and 4-hydroxynonenal (HNE), which are pro-oxidants so that oxidative stress will increase. Increased oxidative stress can also be caused by low levels of high-density lipoprotein (HDL), which will trigger an increase in amyloid precursor protein (APP) followed by the formation of amyloid (Aβ) and the presence of excessively oxidized fat (Fernández-Sánchez *et al*., 2011; Kusumastuty *et al*., 2020). Oxidative stress can trigger the death of neurons in the rat hippocampus.

β-glucan is a dietary fiber that can generally be found as a constituent of most cell walls in oats and mushrooms (El Khoury *et al*., 2012). Some of the benefits of β-glucan include reducing the adverse effects of a high-fat diet (HFD), such as weight gain, fat mass development, excess fasting glucose levels, glucose intolerance, fat accumulation in the liver, and hypercholesterolemia. In addition, hypercholesterolemic rats showed a decrease in triglyceride levels in the blood (Kusmiati and Dhewantara, 2016).

The microbiota-gut-brain relationship is believed to be the primary regulator of neural function. One of the gut microbiotas, namely *Bacteriodetes*, will decrease in number due to the influence of the HFHF diet and will increase lipopolysaccharide (LPS) serum which can affect microglia activation and increase proinflammatory cytokine activation in the hippocampus. Administration of β-glucan will increase *Bacteriodetes* and microbiota diversity which will help reduce microglia activation and proinflammatory cytokine formation in the hippocampus (Shi *et al*., 2020). β-glucan from mushrooms and oats can increase IL-10 expression, decrease TNF-α and IL-6 expression in the colon, increase BDNF levels in the prefrontal cortex, and reduce the number of microglia in the prefrontal cortex and hippocampus (Hu *et al*., 2022). However, different results were obtained in this study where there was no significant difference in the number of neurons, and there was no relationship between neurons with the dose of β-glucan and also BDNF levels (Table 4). This was probably because the HE staining technique cannot specifically differentiate neuronal cells from microglia, where under conditions of increased oxidative stress, the number of microglia cells will rise (Rimbun and Kalanjati, 2012; Shi *et al*., 2020).

Another possibility is the fat percentage in the diet and the treatment or intervention period. In a study by Lozano *et al*. (2016), the administration of HFD and HFHF diets with a fat content of 21.4% for two months was able to have an effect in the form of increased oxidative stress in plasma and tissues. However, a significant increase in TNFα up to 4-5-fold could only be seen after eight months of diet (Lozano *et al*., 2016). In this study, the percentage of fat in the diet that was given

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Table 4. The correlation test between BDNF levels, number of neurons, and dose of β-glucan.

Parameter		Group	*P*-Value	*r*
BDNF Levels (pg/mL)	Number of Neurons	KN, KP, P1, P2	0.874[b]	0.034
Dose of β-glucan	BDNF Levels (pg/mL)	KP, P1, P2	0.001[b]	0.695
Dose of β-glucan	Number of Neurons	KP, P1, P2	0.796[b]	-0.066

KN: Negative Control Group (KN), KP: Positive Control Group (HFHF), P1: Intervention Group 1 (HFHF + β-glucan dose 125 mg/kg BW), P2: Intervention Group 2 (HFHF + β-glucan dose 375 mg/kg BW).
[b]Non-parametric test (Kruskal Wallis Test).

was only about 30%, with the duration of the diet for 14 weeks. Therefore, it is possible that the existing oxidative stress conditions can only activate the microglia activation response but have not reached the limit that can have a negative histological effect. In addition, the age of the experimental animals also affects the response to oxidative stress conditions. Where the older the experimental animal, the more likely it is to release proinflammatory cytokines throughout the amygdala and hippocampus and increase microglial immunoreactivity (Spencer *et al*., 2019; Milanova *et al*., 2021).

3.4 High-fat high-fructose, beta-glucan, and brain-derived neurotrophic factor

The highest levels of BDNF were found in the KN group (452.83), the P2 group (394.92), the P1 group (116.17), and finally, the KP group (81.58). Statistical analysis showed that BDNF levels between groups significantly differed ($p = 0.005$; $p<0.05$). BDNF is part of a group of neurotrophic factors that greatly influence neuron function and development (neurogenesis) and cognitive function. Animal research has shown that BDNF can also be found in the periphery besides being produced by the brain. In the brain, BDNF is widely expressed in high plasticity areas, such as the hippocampus, cortex, and basal areas (Krabbe *et al*., 2007; Tapia-Arancibia *et al.*, 2008; Mudjihartini, 2021). BDNF circulating in the periphery does not only originate from the brain because BDNF can be produced by peripheral tissues (Gravesteijn *et al.*, 2021; Mudjihartini, 2021). Peripheral BDNF production appears to be similar between animals and humans. At the periphery, most of the BDNF is stored in platelets while the remainder circulates in plasma. There are indications that peripheral and central BDNF concentrations are positively related, so blood BDNF concentrations can be considered as an indication of BDNF concentrations in the Central Nervous System (CNS), although this finding is still open to further investigation (Gravesteijn *et al*., 2021).

Studies have shown that BDNF functions in energy homeostasis and weight management by controlling satiety (Pandit *et al*., 2020). BDNF levels were found to decrease in conditions of increased food intake, hyperphagia, and obesity (Zhang *et al*., 2013; Janićijević *et al*., 2020). Several studies in rats have shown that feeding a diet high in saturated fat and sugar has a negative effect on BDNF levels in the hippocampus (Virtuoso *et al*., 2022). Research on mice fed a HFD showed that BDNF levels significantly decreased after four weeks (Ramalho *et al*., 2018). Decreased levels of BDNF can increase appetite through serotonergic mechanisms in the hypothalamus and inhibition of stimulation of glutamate or GABAergic receptors resulting in changes in neuronal activity that cause obesity (Pandit *et al*., 2020; Bumb *et al*., 2021). In line with this study, where the levels of BDNF in the KP group had lower BDNF levels than in the KN group and intervention groups (P1 and P2).

Dietary fiber, including β-glucan, has the potential to impact the microbiota ecosystem and thus improve brain function via the microbiota-gut-brain axis. The study conducted by Hu *et al*. (2022) showed that β-glucan supplementation was good from mushroom, curdlan, and oats bran because it can increase BDNF in the Prefrontal Cortex (PFC). This outcome aligns with this study's results. This study also showed a positive relationship between β-glucan dose and BDNF levels ($p = 0.001$; $r = 0.695$). The highest BDNF level was found in P2 with a dose of β-glucan as much as 375 mg/kg BW. However, several studies have stated that plasma concentrations can be considered an indication of the concentration of BDNF in the CNS (Gravesteijn *et al*., 2021). It seems that β-glucan can provide protection to neurons in the pre -obesity phase.

3.5 Neurons and brain-derived neurotrophic factor

The statistical test results in Table 4 showed no significant relationship between BDNF levels and the number of neurons in all groups ($p = 0.874$; $p>0.05$). Statistical analysis of the HFHF diet groups (KP, P1, and P2) revealed that there was no significant relationship between the administration of β-glucan dose and the number of hippocampal neurons ($p = 0.796$; $p>0.05$). However, different results were shown by the relationship between β-glucan dose and BDNF levels, with a significant relationship between these two variables. The direction of the correlation is positive, which means that the higher the β-glucan dose, the

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higher the BDNF levels and the strength of the correlation is strong (r = 0.695).

In this study, the sample used to analyze BDNF levels was plasma. Most immunoassays used to measure BDNF concentrations cannot differentiate between precursor BDNF (proBDNF) and mature BDNF (mBDNF) (Lim *et al.*, 2015; Gravesteijn *et al*., 2021; Mudjihartini, 2021). On the other hand, studies also mention that the plasma concentration of BDNF can be considered an indication of BDNF concentration in the CNS (Gravesteijn *et al*., 2021). The results of this study revealed an increase in BDNF levels following the administration of the β-glucan dose. This was an interesting finding because β-glucan has been shown to provide qualitative protection to neurons by increasing BDNF.

4. Conclusion

This study concluded that the β-glucan dose from oyster mushroom extract positively correlated with BDNF levels in rats induced by a high-fat and high-fructose diet. Higher doses of β-glucan were proportional to an increase in BDNF levels. Increasing BDNF levels helps to regulate appetite through a complex mechanism, which is beneficial for reducing obesity. The β-glucan dose from oyster mushroom extract was not associated with the number of neurons. It is presumable because the staining technique was less specific, so the possibility of other cells counted as neurons may occur. Although there are still shortcomings, the results of this study showed that β-glucan can provide qualitative protection to neurons by increasing BDNF.

Conflict of interest

The authors declare no conflict of interest.

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