β-1,3/1,6-D-glucan of Mycelia Extract Posses Renal Protection Potential and Reduces Nitric Oxide in Obese Subjects

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ABSTRACT

BACKGROUND: Obesity has been widely reported to be associated with loss of kidney function. The efficacy of β-1,3/1,6-D-glucan as a traditional medicine for the improvement of inflammation and vascular status in obesity has known. However, there have been no further studies that prove the effect of β-1,3/1,6-D-glucan in inhibiting kidney injury as an impact of chronic inflammation exposure on obesity. This study aimed to investigate the impact of β-1,3/1,6-D-glucan from mycelia extract supplementation on renal function improvement based on serum nitric oxide (NO), ureum, and creatinine levels.

METHODS: This was a randomized control trial study involving 69 obese subjects treated with or without β-1,3/1,6-D-glucan supplementation. The serum NO, ureum, and creatinine levels of the subjects were measured at baseline and post-treatment using enzyme-linked immunosorbent assay (ELISA) and then statistically analyzed with paired T-test.

RESULTS: Although slightly decrease, no significant difference was found between the ureum and creatinine level at the baseline and and post-treatment (p=0.806, p=0.306, respectively) after β-1,3/1,6-D-glucan supplementation. Serum NO levels significantly decrease after treatment of β-1,3/1,6-D-glucan (p<0.001).

CONCLUSION: Current study concludes that β-1,3/1,6-D-glucan from mycelia extract does not significantly lower urea and creatinine level, however, significantly able to reduce the serum NO concentration in obese subjects. Therefore, β-1,3/1,6-D-glucan from mycelia extract might have the renal protection potential in obesity.

KEYWORDS: β-1,3/1,6-D-glucan, Ganoderma lucidum, renal function improvement, obesity
previous study as visceral fat accumulation biomarkers. (5,6) Obesity can affect renal function due to indirect etiologies, such as arterial hypertension progression and DM, or by the adipose tissue accumulation in the kidneys. (7) Lipid accumulation in renal tissue induces structural disruption of mesangial cells, podocytes, and proximal tubules, culminating in impaired glomerular function due to increased glomerular permeability-induced proteinuria.(1)

Although the mechanism is not clear, fat cells that produce pro-inflammatory cytokines likely stimulate inducible NO synthase (iNOS) to inhibit proliferation and potential to mediate DNA damage through the reactive nitrogen species (RNS) generation.(8) When stimulated by inflammatory cytokines, iNOS produces up to 1,000 fold more NO than endothelial NOS (eNOS), iNOS-derived NO increased vascular reactive oxygen species (ROS) lead to endothelial dysfunction and vascular remodeling.(9)

Obesity can be effectively tackled with lifestyle modifications. Unfortunately, the effects of lifestyle modification are rarely successfully sustained for the long term, pharmaceutical intervention is required but there is no single drug for the treatment of obesity until now.(10) *Ganoderma lucidum* is a Chinese traditional medicine from mushrooms that have been widely used to repair systemic diseases complications for a long time ago.(11) Recently, several studies have shown that *Ganoderma lucidum* is good enough to tolerate and improve blood pressure dysregulation, triglyceride, blood glucose, and lipid profile. (12-14)

The results of our previous study have shown that the structural characterization of our β-1,3/1,6-D-glucan from *Ganoderma lucidum* including molecular weight, complex branching structure, and triple helix solution proves to be following the β-1,3/1,6-D-glucan characterization that has a high potency as a strong immunomodulator.(12) Supplementation of β-1,3/1,6-D-Glucan with a dose of 540 mg daily for 90 days as adjuvant therapy significantly lowered inflammation serum markers including interleukin (IL)-6, TNF-α, C-reactive protein (CRP), malondialdehyde (MDA), and circulating endothelial cells (CEC) on stable angina pectoris patients. Other finding also suggest that polysaccharide peptide (PSP) of *Ganoderma lucidum* increases endothelial progenitor cell (EPC) and reduces CEC concentration, H_{2}O_{2}, triglyceride , total cholesterol and insulin resistance.(15,16)

It is well known that the bioactive content β-1,3/1,6-D-glucan acts as anti-inflammatory, anti-tumor, antioxidant, immunomodulating and radioprotective, but its effect on kidney function has not been explored. This study aimed to evaluate the efficacy and safety of β-1,3/1,6-D-glucan from mycelia extract from *Ganoderma lucidum*, to counteract inflammation in renal microenvironment related to obesity as one of the modified cardiovascular risk factors.

### Methods

#### Study Design and Subjects Recruitment

This was a randomized control trial study with pre-test and post-test design to determine the effect of β-1,3/1,6-D-glucan from mycelia extract of *Ganoderma lucidum* on obese subjects. The study was conducted at General Hospital of Universitas Brawijaya, Malang, from October 1, 2021 to January 30, 2022. This clinical trial had been registered with the US National Clinical Trial (No.: NCT05079529).

Total 69 eligible obese patients with body mass index (BMI) of 25 or higher were recruited. The subjects who has currently established CVD or another severe disease, pregnant or lactation women, severe cancer, alcoholic or drug user, and died during the follow-up period were excluded (Figure 1). After the subject exclusion, a final of 68 subjects were enrolled in this study.

At baseline, each subject was taken for medical examination, venous puncture procedure for a biological test, and survey interview. During the monitoring period, the investigator regularly evaluated subjects' adherence to consumption of placebo or β-1,3/1,6-D-glucan. Written voluntary consent was obtained from each participant before the data collection.

#### Interventions

Sixty-eight subjects were divided into two groups with randomization using a blocking schema via an interactive web-response system. The treatment group was given β-1,3/1,6-D-glucan from mycelia extract provided by “Environmental Workmate” Surabaya, Indonesia. The extract was loaded into capsule which contained 180 mg of β-1,3/1,6-D-glucan from mycelia extract, and given to the treatment group three times per day for 3 months. Meanwhile, the other group was given placebo for 3 months Regular monitoring of adverse event was conducted by the cardiologist. Before and after the treatment, clinical examination and blood serum was taken for the measurement.

#### Clinical Examinations

All subjects were physically assessed to capture anthropometrics of weight and height examined for
calculating body mass index (BMI). Height was measured using a Stadiometer (SECA, Hamburg, Germany) in a standing position, while weight was measured using digital scale (Tanita, Tokyo, Japan). All measurements were performed twice and averaged for reliability and accuracy. Blood pressure measurements were done using a calibrated sphygmomanometer.

Serum Collection and Parameters Measurement
Blood parameters including fasting plasma glucose (FPG), lipid profile, urea, creatinine, HbA1c, and high-sensitivity C-reactive protein (hsCRP), and NO were examined at the beginning and at the end of treatment, subjects were asked to fast for 12 h before collection. At least, 5 mL of venous blood samples were collected under aseptic precautions, subsequently centrifugation to separate between serum and cellular debris for further investigations.

NO concentration was measured in fasting serum using Griess assay (Invitrogen #EMSN0, Bender MedSystems GmbH, Vienna, Austria) after converting nitrate to nitrite by nitrate reductase enzyme according the manufacturer procedure, with range 0 to 100 µM. Whole blood was allowed to clot for 30 minutes at room temperature before the serum was centrifuged at 1500 rpm for 10 minutes. The separated serum was transferred to a 3 mL tube and the samples were stored at -20°C or below until measurement.

TNF-α serum levels were measured using human TNF-α enzyme-linked immunosorbent assay (ELISA) test kits (No. Cat. 181421, Abcam, Cambridge, UK). Blood samples were centrifuged at 3,000 rpm for 5 to 10 min in 10 cc tubes, and then the serum was collected and stored at -20°C until the measurement. With a similar method, the MDA level as a determinant of oxidative stress, was examined using MDA Assay Kit (No. Cat: MAK085, Sigma-Aldrich, St. Louis, MO, USA).

Lipid profile, FPG, HbA1c and hsCRP were measured by examining the fasting plasma using Lipid, FPG, HbA1c and hsCRP kits from Roche Diagnostics (Indianapolis, IN, USA) in Hitachi 902 autoanalyzer (Boehringer Mannheim, Ingelheim, Germany).

Renal Function Determination
Renal function was defined based on urea and creatinine concentration measured before and after treatment with ELISA. Serum urea and creatinine assay were performed by standard clinical chemistry procedures based on the Jaffe compensated traceable to an isotope dilution mass spectrometry method (Olympus AU 5400 analyzer, Beckman-Coulter, Brea, CA, USA).

Statistical Analysis
All the obtained data were analyzed with SPSS 25.0 (IBM Corporation, Armonk, NY, USA). After the normality test, all numeric data were presented as mean±SD. T-test analysis was used to identify the statistical difference between β-1,3/1,6-D-glucan from the mycelia extract group and placebo groups for urea, creatinine, and NO levels. The comparison within baseline measurement of serum markers and after 3-month treatment was analyzed using the Paired T-test. The $p<0.05$ was considered statistically significant.

Results

Baseline Characteristics
The average age of subjects was 58.27±2.5 years old in the treatment group and 59.50±2.1 years old in the placebo group ($p=0.721$). The results of the data analysis in Table 1 showed that there was no significant difference between the characteristics of the treatment group and the characteristics of the placebo group at baseline ($p≥0.05$). This indicates that there was participant homogeneity at the start of the clinical trial. It was known that all participants did not show an increased in urea and creatinine levels, suggesting that the negative impact of obesity on changes in kidney function was not found.
Serum Urea Level
The treatment group showed a higher urea level at baseline (34.30±17.4 mg/dL) than the placebo group (27.27±6.0 mg/dL). After given β-1,3/1,6-D-glucan from mycelia extract treatment for 90 days, urea level was slightly decreased (32.95±15.5 mg/dL) but not statistically significant (p=0.806). Meanwhile, subjects in placebo group had a significant increase in urea level compared to the baseline urea level (p=0.015) (Figure 2A).

Serum Creatinine Level
As well as urea level, the treatment group showed higher creatinine level at baseline (1.03±0.5 mg/dL) than the placebo (0.8±0.5 mg/dL). After given β-1,3/1,6-D-glucan treatment for three months, creatinine level also slightly decreased (0.94±0.4 mg/dL) but not statistically significant (p=0.304). Furthermore, a statistically significant increase in the serum creatinine level was found in the subjects in placebo group, as compared to the baseline creatinine levels (1.1±0.5 mg/dL; p=0.006) (Figure 2B).

The declining renal function was reflected by the increase of creatinine concentration, likewise elevated serum NO, therefore, representing the declining renal function.

Serum NO Level
At baseline measurement, the concentration of NO in the treatment group was 38.41±6.9 μM, which was slightly

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Table 1. Baseline characteristic of participant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β-1,3/1,6-D-glucan from Mycelia Extract (n=34)</th>
<th>Placebo (n=34)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.27±2.5</td>
<td>59.5±2.1</td>
<td>0.721</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130.18±2.7</td>
<td>133.27±2.7</td>
<td>0.421</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.64±1.5</td>
<td>83.64±1.7</td>
<td>0.370</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.54±13.76</td>
<td>74.87±5.2</td>
<td>0.975</td>
</tr>
<tr>
<td>Abdominal girth (cm)</td>
<td>95.55±11.15</td>
<td>92.87±2.8</td>
<td>0.690</td>
</tr>
<tr>
<td>BMI</td>
<td>31.02±6.2</td>
<td>32.01±7.1</td>
<td>0.506</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>130.38±3.7</td>
<td>126.42±3.6</td>
<td>0.856</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.52±3.2</td>
<td>52.71±8.2</td>
<td>0.593</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>118.58±4.8</td>
<td>112.45±4.1</td>
<td>0.834</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>198.60±4.9</td>
<td>201.48±7.1</td>
<td>0.688</td>
</tr>
<tr>
<td>hs-CRP (mg/mL)</td>
<td>971.24±120.2</td>
<td>908.42±101.8</td>
<td>0.993</td>
</tr>
<tr>
<td>HbA1c (mmol/L)</td>
<td>16.13±2.6</td>
<td>31.81±9.2</td>
<td>0.386</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>97.06±5.6</td>
<td>93.77±3.7</td>
<td>0.468</td>
</tr>
</tbody>
</table>

BMI: body mass index, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, hs-CRP: high sensitivity C-reactive protein, HbA1c: haemoglobin A1c, FBG: fasting blood glucose.

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Figure 2. Analysis of serum ureum, creatinine, and NO levels in treatment and placebo group. Black bar: β-1,3/1,6-D-glucan from mycelia extract group; White bar: placebo group.
higher than the placebo group (32.56±9.1 μM). The result indicated that significant decrease of serum NO levels from the baseline to 3 months after the treatment were found in the treatment and placebo group (p=0.001, p=0.005, respectively) (Table 2, Figure 2C).

Inflammatory Markers and Oxidative Stress Levels
The results of this study showed that the administration of β-1,3/1,6-D-glucan from mycelia extract significantly reduced TNF-α levels (p=0.001) (Table 2). Besides the anti-inflammatory effect and the immunomodulator that we have already found, β-1,3/1,6-D-glucan from mycelia extract was also able to significantly reduce MDA levels in the subjects’ serum (p=0.047). Although it was not specific, it was suggested that the NO measured in this study might be synthesized by iNOS, but the improvement in the determinants of inflammation and oxidative stress in this study were strongly suspected to be related to the decrease in iNOS activity.

Table 2. Comparison of serum markers level according the treatment groups and time of measurement.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum Level</th>
<th>p-value1</th>
<th>p-value2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 Month</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>β-1,3/1,6-D-glucan from mycelia extract</td>
<td>34.30±7.4</td>
<td>32.95±5.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>27.27±5.9</td>
<td>37.69±2.5</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.658</td>
<td>0.356</td>
</tr>
<tr>
<td>Creatinine</td>
<td>β-1,3/1,6-D-glucan from mycelia extract</td>
<td>1.03±0.5</td>
<td>0.94±0.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.80±0.2</td>
<td>1.10±0.6</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.751</td>
<td>0.743</td>
</tr>
<tr>
<td>Nitric Oxide</td>
<td>β-1,3/1,6-D-glucan from mycelia extract</td>
<td>38.41±6.9</td>
<td>32.08±5.6</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>32.56±9.1</td>
<td>26.93±8.1</td>
</tr>
<tr>
<td></td>
<td>p-value1</td>
<td>0.136</td>
<td>0.171</td>
</tr>
<tr>
<td>TNF-α</td>
<td>β-1,3/1,6-D-glucan from mycelia extract</td>
<td>243.12±31.5</td>
<td>102.45±3.7</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>217.38±7.1</td>
<td>100.19±2.6</td>
</tr>
<tr>
<td></td>
<td>p-value1</td>
<td>0.428</td>
<td>0.557</td>
</tr>
<tr>
<td>MDA</td>
<td>β-1,3/1,6-D-glucan from mycelia extract</td>
<td>47.58±5.1</td>
<td>34.01±2.8</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>40.58±4.2</td>
<td>32.18±3.2</td>
</tr>
<tr>
<td></td>
<td>p-value1</td>
<td>0.293</td>
<td>0.675</td>
</tr>
</tbody>
</table>

p-value1 for comparison between β-1,3/1,6-D-glucan from mycelia extract and placebo (T-test); p-value2 for intra-group comparisons between baseline and after 3 months post-treatment (Paired T-test).

Discussion
Elevation in NO levels has been reported correlated with chronic kidney disease (CKD), serum NO had increased steeply after creatinine level of 8 mg/dL or higher represent poor renal function.(16-18) Based on this statement, further investigation is intended to demonstrate whether β-1,3/1,6-D-glucan has efficacy for lowering serum NO. In our present study, urea and creatinine levels slightly decreased after β-1,3/1,6-D-glucan from mycelia extract treatment, although was not statistically significant. On the other hand, supplementation of β-1,3/1,6-D-glucan from mycelia extract induced a significant decrease in NO level after 3 months. In obesity, perivascular adipose tissue undergoes hypoxia, inflammation, and oxidative stress culminating in the production of adipokines and pro-inflammatory cytokines as major inducers of endothelial dysfunction and vascular stiffness.(19-22) It is well known that BMI >30
Conclusion

In the current study, supplementation of 180 mg β-1,3/1,6-D-glucan from mycelia extract for 3 months does not statistically significant to lower urea and creatinine levels, but significantly decreases serum NO level. Therefore, it is suggesting that β-1,3/1,6-D-glucan from mycelia extract might has nephroprotective effect for renal function improvement in obesity.

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Authors Contribution

DS and AR were involved in planning and supervising the work;YW, AR, AFR, CK, and PAK performed the measurements; DS and WN processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures; IDR, MRF, BBP, YFK, SLD, and WN performed the data calculations and statistical analysis; DS and WN aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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