RESEARCH ARTICLE

Ganoderma lucidum Polysaccharide Peptide Reduce Inflammation and Oxidative Stress in Patient with Atrial Fibrillation

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Abstract

BACKGROUND: Atrial fibrillation (AF) could be triggered by inflammation and oxidative stress. *Ganoderma lucidum* has an active substance in the form of β-glucan that can reduce inflammatory process and oxidative stress in rats. The objective of this study was to evaluate the effect of *Ganoderma lucidum* polysaccharide peptide (GLPP) in paroxysmal AF subjects with parameters of anti-inflammatory antioxidant, electrocardiography and health-related quality of life (HRQoL).

METHODS: A randomized closed-label clinical trial with pre- and post-test design was conducted. After AF subjects selection, the subjects were randomized, interviewed and veni-punctured to isolate blood plasma. AF Subjects were then treated with placebo or GLPP for 90 days. Post-test blood plasma was collected on the following day after the 90th day. Then anti-inflammatory and antioxidant parameters were measured. After that, echocardiographic and HRQoL assessments were performed.

RESULTS: A total of 38 subjects, 11 males and 27 females, completed the study with no significant changes in diets, physical activities, or medications. Comparing to control, the 90-days GLPP-treated subject characteristics were significant difference in systolic blood pressure, heart rate, malondialdehyde, high-sensitivity C-reactive protein, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, primary (P)-wave dispersion, P-max, physical functioning, limitation to physical health, energy/fatigue, pain, and physical limitation.

CONCLUSION: GLPP has several potential effects in AF subjects, including anti-inflammatory, antioxidant, and atrial remodelling, so that HRQoL of AF subjects could be improved. Hence, GLPP could suggested as a potential supplementing agent for AF management.

KEYWORDS: atrial fibrillation, *Ganoderma lucidum*, inflammation, antioxidant, atrial remodelling, quality of life

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Introduction

Atrial fibrillation (AF) is the most popular and the most discussed sustained arrhythmia by the researcher and the physician in the last two decades.(1) In Indonesia, AF prevalence ranges 1-2 %.(2) The actual number is believed to be higher than that. Two major factors were needed to develop AF, trigger and substrate. A trigger is needed as an initiator, while to keep the arrhythmia sustained or maintained, substrate is needed.(3) Connection between inflammation and AF has been long being studied.(4,5) However, the role of inflammation as the cause or the response of AF is remained unclear.

Targeting inflammation is a part of upstream AF therapy. The majority of upstream AF therapies are targeting

the process of inflammation and atrial remodelling.(6) Several agents have been widely used, including Statin, Angiotensin II Converting Enzyme Inhibitors (ACE-I), Angiotensin II Receptor Blocker (ARB) and Colchicine. In the most recent guidelines, the use of ACE-I and ARB is recommended in patient with heart failure and reduced systolic function. The use of these agents as sole treatment of AF without any other indication is not recommended.(7)

Ganoderma lucidum, a type of mushroom, has been investigated due to its anti-inflammatory and antioxidant. β-Glucan was reported as the active component of *Ganoderma lucidum* polysacharide peptide (GLPP).(5) *Ganoderma lucidum* could improve atherosclerotic rat after 12 weeks of treatment. In human, *Ganoderma lucidum* has also been proven to reduce inflammatory biomarkers in patient with high-risk coronary artery disease.(8) Since GLPP could be beneficial for AF patients, current study was conducted to know whether GLPP could serve as an future upstream AF therapy. To our knowledge, similar study has not been conducted. We aimed to evaluate the effect of GLPP in paroxysmal AF subjects with parameters of anti-inflammatory antioxidant, electrocardiography and health-related quality of life (HRQoL).

Methods

Clinical Trial Design and Subject Selection

A randomized closed-label clinical trial with pre- and post-test design was conducted at Cardiology Outpatient Clinic of Saiful Anwar General Hospital, Malang, Indonesia, assisted by Lavalette Hospital Malang, Indonesian Heart Association and Geriatric Association in Malang, Indonesia. Subjects aged >18 years old and diagnosed with Paroxysmal AF were selected. All subjects were recorded in electrocardiogram (ECG). Paroxysmal AF Subjects with hyperthyroidism, palpitations, light headedness, chest pain, and dyspnea were included. Meanwhile, subjects with myocardial infarction, autoimmune disease, heart failure, unstable hepatic or renal function, pneumonia, diarrhea and other medical problems with the life expectancy of <2 years old, were excluded.

All subjects have signed informed consents. The protocol of this study was approved by the Ethical Committee of Universitas Brawijaya, Malang, Indonesia (Approval No. #400/79/K.3/302/2018) and Institutional Review Board of Tianjin Medical University General Hospital, Heping, China (Approval No. #20101207A).

Data Collection, Intervention and Sample Collection

Each subject was individually interviewed with a structured questionnaire to obtain related data, including age, gender, body mass index (BMI), blood pressure, heart rate (HR), history of smoking, medical history, physical exercise and psychological status. Selected subjects were randomly assigned to control group (CG) or intervention group (IG). Randomization was performed using a computer generated random number algorithm.

Subjects of IG consumed 250 mg GLPP (PT Sahabat Lingkungan Hidup, Surabaya, Indonesia) 3 times a day, while CG consumed placebo for 90 days. GLPP was confirmed to contain β-Glucan by using the β-Glucan United States Pharmacopeia Reference Standard (Catalog No. 1048288, Lot No. FOK129, Merck, Kenilworth, NJ, USA). A 250 mg freeze-dried GLPP contained 180 mg β-Glucan.

Peripheral blood was collected from each subject on the day before and the day after the last of placebo (CG) or GLPP (IG) consumption for 90 days. Plasma was then isolated and used for various laboratory measurements.

Assessment of HRQoL

Short Form Survey (SF)-36 questionnaire was used for accessing HRQoL in various cardiac conditions.(9) The Assessment in the HRQoL was performed to measure the physical functioning, limitation to physical health, limitation to emotional problem, energy/fatigue, emotional well-being and social functioning.

Assessment of Primary (P)-wave Parameters and

A standard 12-lead surface ECG (25-mm/s, 1-mV/cm, and 100-Hz) was used for evaluating P-wave duration and P-wave dispersion. An image analysis software system (Image Tool 3.0) was used to perform quantitative assessments. P-wave dispersion was calculated as the difference between maximum and minimum P-wave duration. Both P-wave measurements were corrected for heart rate using Bazett's formula.(10)

Two cardiologist with >5 years of experience evaluated the left atrial volume index (LAVI). Inter-observer variabilities were less than 10%. Modified biplane area length method was used according to guideline of American Society of Echocardiography with 4-chamber and 2-chamber view for evaluating left atrial volume that was corrected for body surface area.(11)

Nitric Oxide (NO) Assay

NO StressXpress Colorimetric Detection Kit (Eagle Biosciences, Nashua, NH, USA) was used for quantitative

determination of nitrate (NO_3) and nitrite (NO_2) in plasma. NO content was derived from the sum of NO_3 and NO_2. The colored product is read at 550 nm. The limit of detection was determined as 0.94 µM in the NO_2 and 3.0 µM in the Total NO.

Malondialdehyde (MDA) Assay

To measure MDA of collected plasma, QuantiChrom Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit (DTBA-100) (BioAssay Systems, Hayward, CA, USA) was used. This kit was based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink colored product. The color intensity at 535 nm was used to determine the MDA activity. The kit's detection range was 1-30 µM.

High-sensitivity C-reactive Protein (hs-CRP) Assay

Sandwich enzyme linked immunosorbent assay (ELISA) was used to detect hs-CRP in plasma using hs-CRP ELISA kit (Diagnostics Biochem Canada, London, Ontario, Canada). The color development was measured with a microplate reader capable of measuring absorbance at 450 nm. The sensitivity of the kit was 10 ng/mL.

Interleukin (IL)-1β, IL-6, and TNF-α Assays

To measure IL-1β, IL-6 and TNF-α of collected plasma, legend max human IL-1β, human IL-6 and human TNF-α ELISA Kits (BioLegend, San Diego, CA, USA) were used. These kits were based on sandwich ELISA with mouse monoclonal anti-human IL-1β, rat monoclonal anti-human IL-6 or mouse monoclonal anti-human TNF-α antibody pre-coated plates. The detection antibody was a biotinylated goat polyclonal anti-human IL-1β, rat monoclonal anti-human IL-6 or mouse monoclonal anti-human TNF-α antibody. Minimum detectable concentration of IL-1β was 0.5 pg/mL, IL-6 was 1.6 pg/mL, or TNF-α was 3.5 pg/mL.

Statistical Analysis

All data were made in mean±standard deviation (SD). Paired t-test was used to perform statistical analysis in parametric data. For not homogenous data, Mann-Whitney was used to test significant difference. All analyses were performed using SPSS version 17 (SPSS Inc, Chicago, IL, USA). The *p*-value<0.05 was considered statistically significant.

Results

A total of 38 subjects, 11 males and 27 females, completed the study with no significant changes in diets, physical activities, or medications. The mean of Morisky scale for the treatment compliance was 6.8±1.2. All subjects had Morisky scale scores of ≥6. There were 18 subjects of CG and 20 subjects of IG. Baseline subject characteristics of CG and IG were not significantly different (Table 1).

Compared with the baseline (pre-test), the 90-days (post-test) GLPP-treated subject characteristics (IG) were significant lower in SBP, DBP, HR, LDL, IL-1β, IL-6, hs-CRP, and TNF-α. Meanwhile, no significant changes were observed in the CG. To evaluate the effect of GLPP, the change in each parameter (post-test scores were subtracted with pre-test scores) of the CG was compared with that of the IG (Table 2). Significant differences were detected between the 2 groups for SBP, HR, MDA, hs-CRP, TNF-α, IL-1β, IL-6, P-wave dispersion, P-max, physical functioning, limitation to physical health, energy/fatigue, pain, and physical limitation (Table 2). There was no difference in rehospitalization between CG and IG (Hazard Ratios 1.12; 95% CI 0.84 to 1.8.92; *p*=0.81).

Discussion

In this study we found that GLPP significantly reduced the IL-1, IL-6, hs-CRP and TNF-α inflammatory markers in the subjects with paroxysmal AF. In current study, GLPP has been confirmed to contain β-Glucan. Some studies suggested that β-glucan can inhibit inflammatory process due to blockage of nuclear factor (NF)-κB activation. (12) By reducing the inflammation, further process of atrial remodelling such as apoptosis, extra cellular matrix deposition and fibroblast recruitment can be stopped. In current study, NO bioavailability was increased in IG, although not statistically significant. Meanwhile in IG, SBP was significantly decreased. NO could play a role in facilitating sodium excretion, so that an increase in NO generation will lead to decrease blood pressure. Ganoderma lucidum has been reported to prevent oxidative stress via suppressing Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase subunit.(10)

Increased MDA levels enhance the production of free radical and reduce the antioxidant activity.(7) Another study has shown that β-glucan can lower MDA level in the liver, kidney, heart, lung, diaphragm and brain tissues on sepsis model rats.(5,13) In diabetic rats, β-glucan could lower the MDA after 21 days of treatment.(10) MDA lowering agents could be beneficial and considered as therapeutic agents for cardiovascular disease, because they can attenuate the oxidative injuries in the body.(10) In current result, we

Table 1. Baseline subject characteristics (pre-test).

Variable	CG (n=18)	IG (n=20)	*p*- value
Age (years old), Mean±SD	62.50±10.50	63.61±8.50	0.65
Gender (ratio of male/female)	33.33%	25.00%	0.89
History of smoking	22.22%	25.00%	0.93
Diabetes mellitus	22.22%	30.00%	0.82
Hypertension	55.56%	55.00%	0.96
History of CVA	5.56%	10.00%	0.28
CAD	33.33%	25.00%	0.46
ACE inhibitor/ARB	94.44%	80.00%	0.16
CCB	22.22%	25.00%	0.86
b-Blocker	94.44%	90.00%	0.88
Statin	77.78%	75.00%	0.98
Digoxin	5.56%	10.00%	0.64
Warfarin	88.89%	80.00%	0.68
BMI (kg/m^2), Mean±SD	26.40±4.00	26.30±5.40	0.62
SBP (mmHg), Mean±SD	132.20±16.40	138.80±18.70	0.68
DBP (mmHg), Mean±SD	87.97±10.20	86.50±14.00	0.63
HR (bpm), Mean±SD	75.83±12.25	80.40±14.70	0.18
P-wave Dispersion (ms)	40.70±23.60	40.18±24.28	0.88
P-max (ms)	118.30±9.00	119.30±14.80	0.84
LAVI (mL/m^2)	24.48 ± 1.76	26.45 ± 2.85	0.52
NO (mM)	36.50±14.34	33.70±12.61	0.35
MDA (ng/mL)	1.66±0.43	1.53±0.56	0.28
hs-CRP (ng/mL)	2.83±1.13	2.99±0.86	0.49
IL-1b (pg/mL)	13.74±1.26	14.82±1.88	0.14
IL-6 (pg/mL)	14.46±1.71	15.60±2.17	0.11
TNF-a (pg/mL)	13.28±2.40	14.79±1.98	0.14
PT (s)	13.46±2.41	12.20±3.17	0.61
APTT (s)	24.26±3.61	23.20±4.47	0.62
INR	2.26±1.21	2.04±1.17	0.81
Physical Functioning	88.11±17.90	90.22±9.79	0.82
Limitation to Physical Health	85.70±34.50	86.50±20.50	0.64
Limitation to Emotional Problem	80.95±35.40	80.50±27.72	0.71
Energy/Fatigue	75.30±19.69	78.78±15.90	0.68
Emotional Well-being	78.90±16.15	82.77±15.50	0.17
Social Functioning	83.60±28.50	88.75±15.00	0.18
Pain	83.19±28.50	86.08±24.10	0.52
General Health	79.55±16.70	77.50±14.30	0.83
Physical Limitation	76.30±19.90	79.30±18.40	0.18

ACE: angiotensin-converting enzyme, APTT: activated partial thromboplastin time, ARB: angiotensin receptor blocker, BMI: body mass index, CAD: coronary artery disease, CCB: calcium-channel blocker, CVA: cerebrovascular accident, DBP: diastolic blood pressure, HR: heart rate, hs-CRP: high-sensitivity C-reactive Protein, IL: interleukin, INR: international normalized ratio, LAVI: left atrial volume index, MDA: malondialdehyde, NO: nitric oxide, P-max: P-wave maximum, PT: prothrombin time, SBP: systolic blood pressure, SD: standard deviation, TNF: tumor necrosis factor.

Table 2. Changes of subject characteristics. Post-test scores were subtracted with pre-test scores.

Variable	CG (n=18)	IG (n=20)	*p*- value
D BMI (kg/m^2) (Mean±SD)	-0.04±1.00	-0.01±0.78	0.76
D SBP (mmHg) (Mean±SD)	-10.50±15.03	-22.50±17.60	0.002
D DBP (mmHg) (Mean±SD)	-3.30±11.30	-7.74±10.90	0.109
D HR (bpm) (Mean±SD)	-2.02±5.50	-4.97±10.50	0.15
D P-wave dispersion (Mean±SD)	-1.18±24.28	-4.70±23.60	0.046
D P-max (ms)	-1.30±10.80	-10.80±40	0.004
D LAVI (mL/m^2)	-1.08±3.46	-2.38±2.04	0.15
D NO (mM)	2.26±2.40	4.54±10.50	0.125
D MDA (ng/mL)	0.04±0.24	-0.29±0.52	0.002
D hs-CRP (ng/mL)	-0.39±0.69	-1.62±0.90	0.038
D IL-1b (pg/mL)	-0.87±1.10	-2.59±1.75	0.013
D IL-6 (pg/mL)	-0.80±1.20	-2.63±1.69	0.001
D TNF-a (pg/mL)	-1.08±1.10	-3.27±1.80	0.006
D PT (s)	1.32±1.26	1.22±1.42	0.64
D APTT (s)	2.20±1.47	2.26±2.61	0.82
D INR	0.04±1.17	0.16±1.21	0.68
D Physical Functioning	-3.98±2.73	3.02±2.73	0
D Limitation to Physical Health	0.22±13.90	4.78±11.09	0.03
D Limitation to Emotional problem	1.71±13.40	2.75±3.76	0.18
D Energy/Fatigue	-2.71±13.40	4.38±13.46	0.044
D Emotional Well Being	2.41±3.92	3.81±17.15	0.48
D Social Functioning	0.20±4.80	2.28±7.70	0.86
D Pain	0.60±7.66	4.61±13.09	0.026
D General Health	-2.54±4.60	2.95±7.58	0.16
D Physical Limitation	-2.66±7.80	4.46±10.54	0.008

Δ: subtraction from post- to pre-test scores, ACE: angiotensin-converting enzyme, APTT: activated partial thromboplastin time, ARB: angiotensin receptor blocker, BMI: body mass index, CAD: coronary artery disease, CCB: calcium-channel blocker, CVA: cerebrovascular accident, DBP: diastolic blood pressure, HR: heart rate, hs-CRP: high-sensitivity C-reactive protein, IL: interleukin, INR: international normalized ratio, LAVI: left atrial volume index, MDA: malondialdehyde, NO: nitric oxide, P-max: P-wave maximum, PT: prothrombin time, SBP: systolic blood pressure, TNF: tumor necrosis factor.

found that GLPP could reduce MDA levels of subjects with paroxysmal AF after 90 days treatment.

P-wave dispersion and P-max had valuable prognostic measurement to predict the new onset of AF, reoccurrence of AF after rhythm conversion with direct current cardioversion and AF ablation.(14,15) Increased P-wave dispersion and P-max indicated the disorganization of intra- and inter-atrial electrical impulse propagation, that found in AF. P-wave dispersion and P-max could are also non-invasive markers for atrial remodelling. In current study, the P-wave dispersion and P-max of IG were improved, suggesting the possibility of atrial remodelling. The atrial remodelling might lead to the improvement of hs-CRP levels of this study. The hs-CRP level has been reported to be associated with reverse atrial remodelling.(11)

Decrease cardiac output, shorter diastolic filling time and sensation of irregular heart beat can caused several symptoms in AF patients. Such as dyspnoea, fatigue, syncope, palpitation and chest discomfort.(16) Which in turn, those symptoms can cause alteration in patient's HRQoL. Patients with AF were reported to have worse HRQoL compared to healthy control, and even to other cardiac conditions such as heart failure and myocardial infarction.(17) In current study, we found that GLPP improved HRQoL of AF subjects. GLPP-treated subjects had better physical functioning, lower limitation to physical activity, less fatigue and pain.

Although this study showed positive effect of GLPP, there are several limitations, including small sample size and indirect method in evaluating the AF. Therefore, further study with larger number of subjects and continuous ambulatory ECG monitoring are required.

Conclusion

GLPP has several potential effects in AF subjects, including anti-inflammatory, antioxidant, and atrial remodelling, so that HRQoL of AF subjects could be improved. However, further study is required. Hence, GLPP could suggested as a potential supplementing agent for AF management.

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