UNIVERSITAS INDONESIA

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STUDY PROTOCOL

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The Role of Beta-1,3/1,6-D-Glucan from Mycelium Extract of Indonesian *Ganoderma Lucidum* on Ulcerative Colitis: A Double-Blind Randomized Controlled Trial

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Title

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Background

Inflammatory bowel disease (IBD) is an autoimmune disorder there is marked by chronic inflammation in gastrointestinal system.[1] IBD is classified to two types, ulcerative colitis (UC) and Crohn's disease (CD). These are based on difference pathology and clinical manifestation. The common symptom of IBD is chronic diarrhoea with or without blood, abdominal cramp and weight loss.[2,3] IBD has high incidence and prevalence in Europe and America. Incidence of UC and CD in Europe are 24.3 and 29.3 by 100,000 respectively.[4] On the last few decades, there was an increasing incidence and prevalence IBD on Asia and other developing countries.[5] In Indonesia, incidence of UC and CD are 0.55 and 0.33 by 100.0000 respectively.[6] Trends in epidemiology of IBD was related to westernized lifestyle, urbanization, and hygiene as a part of socioeconomic development in developing countries.[7]

Nowadays, IBD treatment focus to prevent complication, maintain remission phase and improve quality of life. Drugs that are widely used for improving symptoms of IBD are anti-inflammatory drugs (5-aminosalicylic acid), immunomodulator (azathioprine, mercaptopurine, methotrexate), and biologic agent. These drugs work by increasing Th2-mediated response that cause a decrease in inflammation mediated by Th1.[8] Long term use of these drugs will cause different side effects. Side effects from immunomodulator are myelosuppresion, hepatitis and increase risk of malignancy. Use of anti TNF-α will cause lupus like syndrome and malignancy.[9] Therefore, we need alternative treatment of IBD that effective but minimal side effects.

Ganoderma lucidum is a species from *Ganodermataceae* family that has Beta-1,3/1,6-D-Glucan as one of active compound from isolated mycelium extract *Ganoderma lucidum*. This compound has biologic effect as immunomodulator and has been used to prevent and treat diseases such as gastric ulcer, arthritis, atherosclerosis, asthma, diabetes, cancer and other diseases related to immune disorders.[10,11] A study on patient with CD showed that *Ganoderma lucidum* decrease TNF-α, IFN-γ, IL-17A from inflammation of intestinal mucosa.[9] On the other hand, there is no study about the role of Beta-1,3/1,6-D-Glucan *Ganoderma lucidum* in UC.

Objective

General objective

To know the role of Beta-1,3/1,6-D-Glucan from mycelium extract of Ganoderma lucidum administration in ulcerative colitis

Specific objectives

1. To know the quality of life from patient with ulcerative colitis after Beta-1,3/1,6-D-Glucan mycelium extract of Ganoderma lucidum administration
2. To know the level of inflammatory marker from patient with ulcerative colitis after Beta-1,3/1,6-D-Glucan mycelium extract of Ganoderma lucidum administration
3. To know the Mayo score from patient with ulcerative colitis after Beta-1,3/1,6-D-Glucan mycelium extract of Ganoderma lucidum administration

Methods

Study Design

This research is a double blind, randomized clinical trial method. Analysis will be conducted pre and post administration: control (placebo) and intervention (polysaccharide peptide Ganoderma lucidum) 3 times daily for 90 days.

Location and Time

This research will be conducted on Indonesia from August 2019 to December 2020

Population and Subject

Population

The population of this research is patients diagnosed with ulcerative colitis in Indonesia

Subjects Criteria

- Inclusion criteria
  1. Subjects aged ≥ 18 years old
  2. Ulcerative colitis patients who are treated by 5-ASA 3x500 mg
  3. Agreed to participate

- Exclusion criteria
  1. Allergic with *Ganoderma lucidum*

2. Ulcerative colitis patients who are treated by corticosteroid, immunosuppressive agent, biologic agent
3. Could not be randomised and participate in this study by clinical judgement

Drop Out Criteria

Drop out subjects are subjects who have signed the informed consent but decide to withdraw before research is completed

Estimated Sample Size

Minimum sample size is determined using the following equation for proportion equation.

$$N1 = N2 = \frac{(Z_\alpha\sqrt{2PQ} + Z_\beta\sqrt{P_1Q_1 + P_2Q_2})^2}{(P_1 - P_2)^2}$$

Description :

$Z\alpha = 1.96$ $Z\beta = 0.84$

$P_1\text{-}P_2 = 0.2$ $P_1 = 0.554$[12]

N1 = N2 = 92.25, thus with predicted drop out 10% the minimal sample for each group is 102 subjects

Randomization

Randomization is generated by computer and subjects will be given code and divided into two groups (control and intervention). Researcher and subjects are blinded to treatment.

Variables

Independent Variables

1. Intervention: administration of capsule contain Beta-1,3/1,6-D-Glucan as active form of polysaccharide peptide from mycelium extract *Ganoderma lucidum* 3 times daily
2. Control (placebo): administration of empty capsule 3 times daily

Dependent Variables

1. Quality of life; assessed by Indonesian validated SF-36 questionnaire
2. Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), Tumor Necrosis Factor Alpha (TNF-α) and Interleukin 6 (IL-6) from blood samples

3. Fecal calprotectin from stool examination
4. Mayo Score from colonoscopy
5. Biopsy

Procedure

1. Eligibility assessment

   The subjects will be assessed for eligibility using inclusion and exclusion criteria.
2. Subjects sign the informed consent form
3. Randomization
4. Pre-administration

   Before administration of polysaccharide peptide or placebo based on randomization, subjects will be analysed for his/her quality of life by SF 36 questionnaire, ESR, CRP, TNF-α, IL-6, fecal calprotectin, colonoscopy (Mayo Score) and biopsy.
5. Administration (90 days)

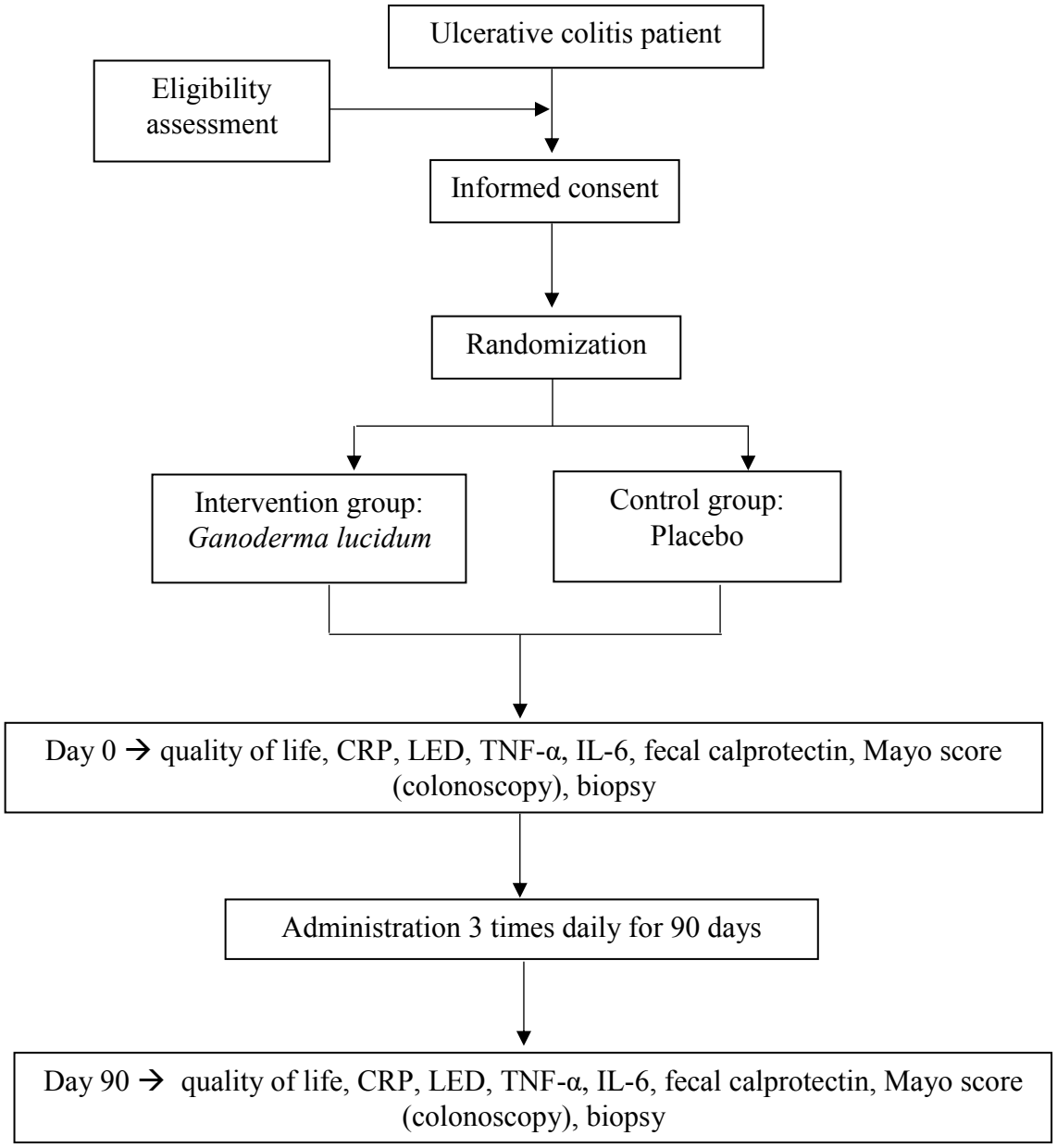
   Subjects will be given *Ganoderma lucidum* and placebo that will be taken three times daily for 90 days. The drugs are given monthly to the subjects.
6. Compliance monitoring

   Compliance monitoring of administration is conducted by the investigators coordinating with his/her family and checked monthly when the patients came to take the capsule for the next month
7. Post administration

   After 90 days the subjects will be analysed for his/her quality of life by SF 36 questionnaire, ESR, CRP, TNF-α, IL-6, fecal calprotectin, colonoscopy (Mayo Score) and biopsy.
8. Data analysis
   a. Normality analysis using Kolmogorov-Smirnov (Normally distributed if $p>0.05$)
   b. Bivariate analysis by comparing numeric data between two groups: if data is normality distributed using T-test, if not using Mann-Whitney test (significant if $p<0.05$)
   c. Bivariate analysis by comparing categorical data between two groups: if data is normality distributed using T-test, if not using Mann-Whitney test (significant if $p<0.05$)

d. Bivariate analysis by comparing numeric data between preadministration and postadministration: if data is normality distributed using paired T-test, if not using Wilcoxon test (significant if $p<0.05$)
e. Bivariate analysis by comparing categorical data between preadministration and postadministration: if data is normality distributed using Mc Nemar test, if not using Wilcoxon test (significant if $p<0.05$)

Study flow chart



Ethical committee approval

Approval from the Ethical Committee at the Faculty of Medicine Universitas Indonesia must be obtained before starting the trial.

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