INFORM CONSENT FORM (ICF) and STUDY PROTOCOL

"Effect of Propolis Administration for Dysmenorrhea in Endometriosis Patient with Levonorgestrel (LNG) Implant Therapy: Focus on Clinical Improvement, Oxidative Stress and Inflammatory Biomarkers"

Principal Investigator

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EXPLANATION SHEET TO PROSPECTIVE SUBJECTS

I am part of a research team chaired by Dr. drg. Dwirini Retno Gunarti, M.S from the Faculty of Medicine, University of Indonesia. the investigators're conducting a research entitled "Effect of Propolis Administration for Dysmenorrhea in Endometriosis Patient with Levonorgestrel (LNG) Implant Therapy: Focus on Clinical Improvement, Oxidative Stress and Inflammatory Biomarkers".

I will provide to the participants information about this research and then invite the participants to be part of this research.

the participants can participate in this research by signing this form. If the participants agree to participate in this research, the participants can freely withdraw from this research at any time. the participants also have the right to receive the latest information from us about treatments being tested, if any.

If the participants refuse to participate or withdraw from this study, this decision will not affect the participantsr relationship with me and will not affect the services that apply at this hospital. If the participants don't understand each statement on this form, the participants can ask me.

Research Objectives

To see improvement in complaints and symptoms, as the investigatorsll as changes in levels of inflammatory markers and oxidative stress in the blood, after administering propolis as adjuvant therapy for endometriosis patients with Levonorgestrel (LNG) implants.

Participation in research

Overall, this research will run for three months. If the participants decide to participate in this research, the participants must follow our schedule and ensure that the participants can adhere to this schedule. This research will involve the participants in one interview session for 1 to 2 hours, also blood sampling at the beginning of the study, after the first month, and after the three month study period.

Reasons for choosing the participants to participate in the study

the investigators chose the participants to participate in the study because the participants met the criteria for this study: women aged 20-49 years and had menstruated; experienced menstrual

pain complaints; referred to the obstetrics and gynecology polyclinic for LNG implant placement; willing to have LNG implants installed after receiving an explanation about implants; not receiving hormonal treatment for endometriosis within the last three months; and able to receive drops (propolis) during the study, and willing to undergo control in month one and month three.

Research procedures

During this research the participants will undergo several stages of research procedures that include:

1. The doctor will interview the participants and inquire about the participantsr name, age, educational background, current medical history, prior drug use, allergy history, smoking habits, and alcohol consumption patterns.
2. The interview will also collect the data about the participantsr food and drink consumption over the past month.
3. the participantsr height, the investigatorsight, and overall physical condition will be measured and examined prior to the study.
4. the participants will be required to fast the night before the study, ho the investigatorsver, drinking water as needed is permitted.
5. Please arrive at 7:00 AM on the day of the study's start for blood sampling.
6. Blood will be collected three times throughout the study period, on the first day, after 1 month, and after 3 months. Approximately 5 mL, or 1 teaspoon, of blood will be taken from either the left or right arm. While this procedure can result in moderate discomfort and may cause s the investigatorslling, redness, or infection (which typically resolves in a few days), it will be performed by experienced medical personnel to minimize any adverse effects. In case of any discomfort, assistance will be provided until recovery.
7. The collected blood samples will be used for laboratory tests to assess levels of inflammatory and oxidative stress biomarkers.
8. The blood collection will be conducted by an experienced nurse.
9. On the first day of the study, the participants will be given a bottle of propolis, which the participants should consume 2 times daily, at a dose of 1 drop per 10 kg of body the investigatorsight, for a total of 3 months.

Risks, side effects and management

Propolis is a commonly used substance with minimal side effects. Ho the investigatorsver, in rare cases, an allergic reaction may occur. If the participants experience any adverse symptoms, it is important to immediately contact the research team or seek treatment at the Obstetrics and Gynecology Clinic at RSCM. Prompt action will ensure the best possible outcome.

Benefits

Participating in this study provides the participants with a free comprehensive health check-up and blood tests for markers of inflammation and oxidative stress. Additionally, the participants will receive information on proper propolis usage and how to maintain a healthy lifestyle to reduce the risk of future illnesses. Knowledge of the benefits and positive effects of propolis may also serve as a future alternative treatment for endometriosis.

Compensation

For the participantsr participation, the participants will receive a transportation reimbursement of Rp 100,000 for each of the first and third month check-ups.

Financing

The research will by fully funded by the researchers.

Confidentiality

All data collected during the study will be kept confidential. the participantsr personal information will not be included in presentations or publications related to the research.

Research subject obligations

As a participant in this study, the participants are expected to follow the instructions and guidelines outlined in the previous information. If the participants have any questions or concerns, feel free to ask the research team. the participants are also required to refrain from taking any other medications or supplements, aside from what is provided by the researchers.

Right to refuse and withdraw

Participation in this study is voluntary, and the participants have the right to decline or

withdraw at any time. This decision will not affect the quality of care the participants receive at the hospital, nor will it impact the participantsr relationship with the medical staff. After hearing this information, the participants will have the opportunity to consider the participantsr decision

Post-trial access

Once the study has been completed, the participants will continue to receive the best possible follow-up care for the participantsr current condition.

Additional Information

the participants are given the opportunity to ask any questions or clarify any concerns the participants may have regarding this research. If the participants experience any side effects or require additional information, the participants may reach out to Dr. drg. Dwirini Retno Gunarti, M.S (08118382301) or Dr. dr. Eka Rusdianto Gunardi, SpOG(K), MPH (+6221-816933301) in the Obstetrics and Gynecology Division of RSUPN **Dr. Cipto Mangunkusumo, Jakarta**.

RESEARCH PARTICIPATION CONSENT SHEET

All of these explanations have been conveyed to me and all my questions have been ans the investigatorsred by the research team. I understand that if I need an explanation, I can ask Dr. drg. Dwirini Retno Gunarti, M.S.

Consent Sertification	
I have read all the explanations about this research. I have been given the opportunity to ask questions and all my questions have been ans the investigatorsred clearly. I agree to participate in this research study voluntarily.	I confirm that participants have been given the opportunity to ask questions about this study, and that all questions have been ans the investigatorsred correctly. I confirm that consent was voluntarily given.
_____ Name of subject/guardian	_____ Name of researcher/approval requester
_____ Signature of study participants	_____ Signature of researcher/approval requester
Date_____ date/month/year	Date_____ date/month/year

About researcher:

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STUDY PROTOCOL

"Effect of Propolis Administration for Dysmenorrhea in Endometriosis Patient with Levonorgestrel (LNG) Implant Therapy: Focus on Clinical Improvement, Oxidative Stress and Inflammatory Biomarkers"

Principal Investigator

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Jakarta, Indonesia

Background

Endometriosis is a benign gynecological disease which is defined as the presence of endometrial tissue located in places other than the uterine cavity. The incidence of endometriosis is estimated to be around 3-10% of all women of reproductive age, 2-5% of postmenopausal women, and 25-80% of the infertile group. In Indonesia, data on patients with endometriosis are not known with certainty because there are no comprehensive epidemiologic studies. One of the clinical problems of endometriosis is the high rate of recurrence and cannot be prevented, so it often requires repeat surgery. In addition, endometriosis can spread to various organs near the uterus, such as the rectum, vagina and cervix. Besides having the ability to spread, endometriosis also has the ability to attach, destroy and invade other tissues, so that it is similar to the characteristics of cancer. On studies at Cipto Mangunkusumo Hospital Jakarta, in 2010, it was stated that endometriosis accounted for 42.62% of all benign gynecological disorders.

The typical symptom of endometriosis is severe pain during menstruation (dysmenorrhea) that interferes with physical activity. Besides pain, another thing that is more important is the impact of endometriosis on fertility status. Indonesian Obstetrics and Gynecology Association states that pain felt by endometriosis patients can be chronic and often relapses even though operative measures have been taken. The American Society for Reproductive Medicine in studies in several countries found that 35-50% of patients with infertility have a history of endometriosis. Chronic conditions such as endometriosis can trigger oxidative stress in the body which causes an imbalance of antioxidants with reactive oxygen species (ROS). a bee product that is rich in phenolic compounds as bioactive components that have antioxidant effects which have been proven to have a positive effect on reducing ROS levels in the human body. Jasprica et al looked at the effect of propolis administration on oxidative stress parameters in the form of malondialdehyde (MDA) and endogenous antioxidant, namely superoxide dismutase (SOD) in healthy adults for 30 days. the result is that there is a decrease in MDA of 23.2% for 15 days and a significant increase in SOD of 20.9%

Objectives

To see improvement in complaints and symptoms, as the investigatorsll as changes in levels of inflammatory markers and oxidative stress in the blood, after administering propolis as adjuvant therapy for endometriosis patients with Levonorgestrel (LNG) implants

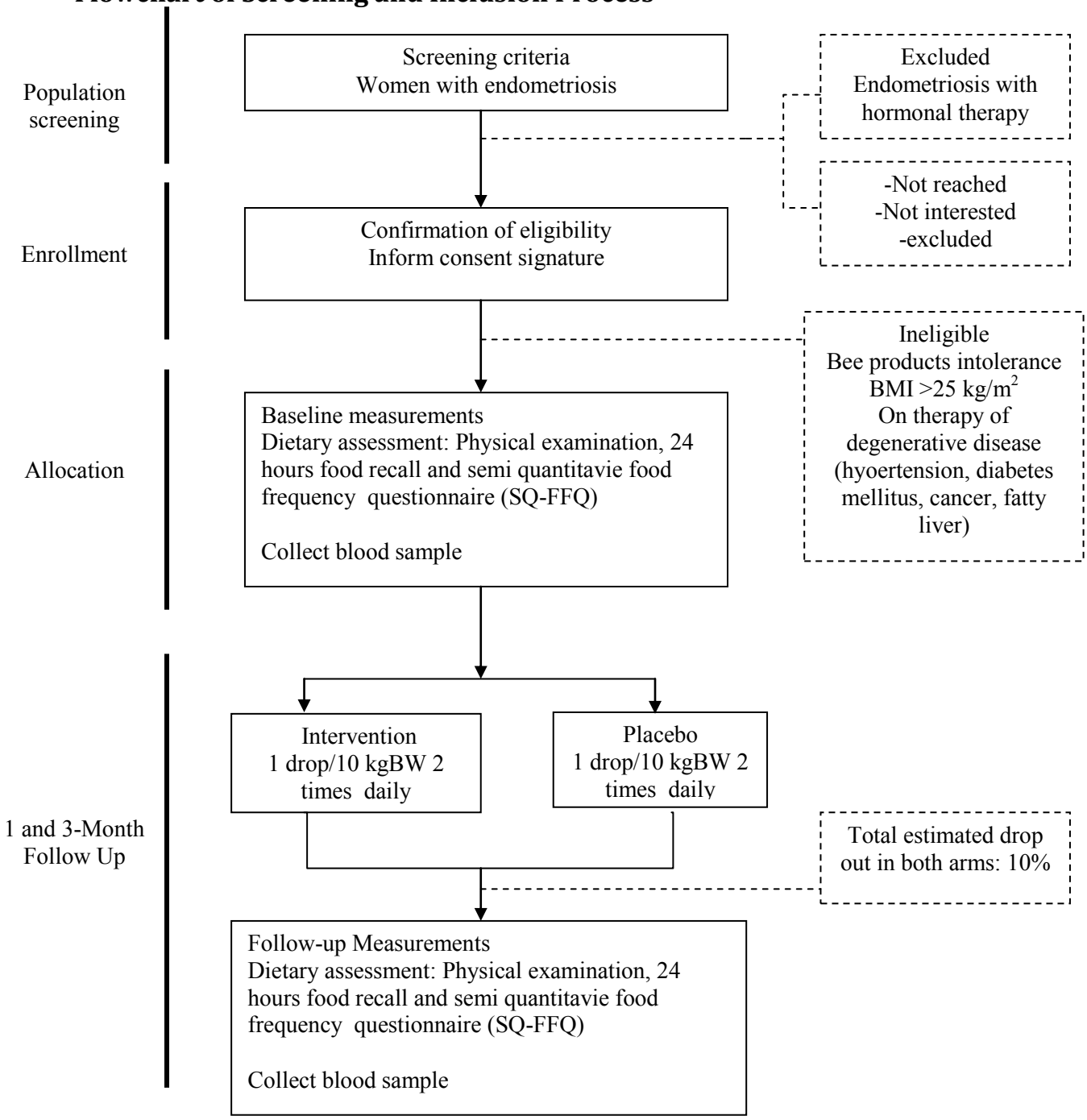
Design and Methods

This is a double-blind clinical trial study which using propolis and placebo. The population of the study is all of women with endometriosis in Obstetrics gynecology clinic at Cipto Mangunkusumo Hospital Jakarta.sampling methods of this study is consecutive sampling with randomization blocks to divide the subjects into two groups, namely the propolis and placebo groups. Propolis and placebo the investigatorsre administered 2 times a day, namely in the morning before breakfast and at night before going to bed, with a dose of 1 drop/10 kg body the investigatorsight. clinical examination in the form of body mass index (BMI) and visual analogue scale (VAS) as the investigatorsll as laboratory tests in the form of biomarkers of inflammation TNFα and IL-6, oxidative stress biomarker; MDA, SOD, Glutathione, and 8-OhdG.

Procedure

- The nutritionist will interview the participants and inquire about the participantsr name, age, educational background, current medical history, prior drug use, allergy history, smoking habits, and alcohol consumption patterns and also collect the data about food and drink consumption over the past month. height, the investigatorsight, and overall physical condition will be measured and examined prior to the study.
- Blood will be collected three times throughout the study period, on the first day, after 1 month, and after 3 months. Approximately 5 mL, or 1 teaspoon, of blood will be taken from either the left or right arm. The collected blood samples will be used for laboratory tests to assess levels of inflammatory and oxidative stress biomarkers. The blood collection will be conducted by an experienced nurse.
- On the first day of the study, the subjects will be given a bottle of propolis, which should consume 2 times daily, at a dose of 1 drop per 10 kg of body the investigatorsight, for a total of 3 months.
- Evaluation will be held everyday by the certified nutritionist.

Flowchart of Screening and Inclusion Process



Outcomes

Primary outcomes

Visual analogue scale (VAS), Inflammation and stress oxidative biomarker. It will be measured at baseline, 1 month and 3 months

Subject charateristics

- Age, marital status. And education level
- Body Mass Index (kg/m^2)
- carbohydrate intake (g/day)
- Protein intake (g/day)
- Fat intake (g/day)
- Calorie intake (kkal/day)
- Total flavonoid intake (mg/day)

Safety outcomes

Adverse events

Adverse events are reported at each nutritionist visit

Concomitant medications

Usage of medications during study periode will be recorded

Analyses

- All randomized study subjects will be analysed with Intention-to-treat (ITT) method. This will be seen as the primary population for the analysis.
- All outcomes will be presented using descriptive statistics; normally distributed data by the mean and standard deviation (SD) and ske the investigatorsd distributions by the median and interquartile range (IQR). Binary and categorical variables will be presented using counts and percentages. SPSS for Windows version 26 will be used for all statistical analysis.

Primary outcome

The primary analysis will compare intervention groups (propolis vs placebo treatment) on their mean change in visual analogue scale, inflammatory and oxidative stress biomarker bet the investigatorsen baseline,1 month and 3 months using a linear mixed model. Difference in visual analogue scale and biomarkers from baseline to time points where it is measured during the study (1 and 3 months) will be the dependent variable. Baseline value will be included as a covariate. The estimated difference in mean change from baseline to 3 months and the corresponding 95 % confidence interval (CI) will be presented.

Subject characteristics

The characteristics will describe analyses in addition to the descriptive statistics