Anti-Anemic and Analgesic Activity of Sauropus Androgynous L Merr on Female Mice Model

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ABSTRACT

Leaves of *Sauropus androgynus* (L) Merr (LSA) empirically have been used as a maternal breast milk (milk), anti-anemic and analgesic (dysmenorrhea). This study aims to determine the pharmacological activity of LSA extract as anti-anemic and analgesic. This research was conducted with 2 methods in vivo that are anti-anemic and analgesic. Anti-anemic was performed using 24 female mice randomly grouped into 6 groups: group 1 (as normal control received drug carrier), group 2 (as positive control received drug carrier), group 3 (received Sangobion supplement 1.95 mL / kg), and group 4-6 (received LSA extract dose of 50, 100, and 200 mg/kg). All groups except the normal group received $NaNO_2$ (sodium nitrite) as an induction of anemic state and test drugs for 7 days. Blood collection was performed on day 8 to measure hematocrit volume. Analgesic activity was performed by the writhing method, where the pain was induced by 1% glacial acetic acid 0.5 mL intraperitoneally. Aspirin as a standard drug was given at a dose of 39 mg/kg. The parameters measured were the amount of stretching in each treatment group every 5 minutes for 1 hour of observation. The results showed that LSA at dose of 50, 100, and 200 mg/kg can increase the number of red blood cells and decrease the writhing number. The results of this study can be concluded that LSA dose of 200 mg/kg has the effect as anti-anemic and analgesic simultaneously.

Key Words: Sauropus Androgynus L Merr, Anti-Anemic, Analgesic, Aspirin, Glacial Acetic Acid, Sodium Nitrite.

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INTRODUCTION

Menstruation is part of a woman's life journey which is experienced since menarche and ended when menopause. In one menstrual cycle, there are 4 phases, namely the follicular phase, the ovulatory phase, the luteal phase and the menstrual phase. In menstrual phase, dysmenorrhea can occur. The duration of dysmenorrhea ranges from five days (range 3-6 days) which occurs repeatedly during every menstrual cycle within 22-35 days. The pain experienced during the bleeding phase in one menstrual cycle is called dysmenorrhea [1].

Primary dysmenorrhea is a menstrual pain without abnormalities of reproductive organs. Primary dysmenorrhea commonly occurs in young women with pain complaints such as cramps in the lower middle of the uterus. Almost all women have experienced menstrual pain (dysmenorrhea) with various levels, ranging from aches in the hips from the inner side to pain that is very severe. The usual pain under the abdomen occurs on the first and second day of menstruation. Pain will be reduced after a lot of blood out [2].

The prevalence of menstrual pain in the world is enormous. On average, more than 50% of women in every country experience menstrual pain, in America the percentage is about 60% and in Sweden about 72%. While in Indonesia the figure is estimated to be 55% of women of productive age. The incidence of menstrual pain ranges from 45-95% among women. Although generally harmless, it is felt disturbing by women who experience it. The degree of pain and levels of disorders are not the same for every woman [3].

The phase of bleeding in the menstrual cycle can lead to anemia. Anemia occurs in most of the world's population, with the main cause being iron deficiency. Anemia is characterized by symptoms of chronic fatigue, disturbance

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of intelligence and disrupts health. Proper anemia treatment can improve the quality of life, improve the symptoms of anemia, and reduce the need for blood transfusions [4]. The status of iron deficiency in patients with chronic heart function disorders affects their exercise capacity [5].

Anemia is a condition in which the hemoglobin level in the blood is less than normal. Normal hemoglobin levels are different for each age group and sex. Anemia is a state of the body characterized by an insufficient number of erythrocytes or hemoglobin levels for O_2 and CO_2 exchange functions between tissues and blood [6]. In anemia condition, the number of hematocrits also increases which indicates the decrease of red blood cell count [7].

Leaves of Sauropus androgynous (LSA) have many benefits in everyday life. LSA is efficacious for protecting cell structure, improving the effectiveness of vitamin C, preventing bone loss and as a natural antibiotic [8].

LSA has been used as an enhancer of breast milk production in Indonesia empirically. To date, many studies have proven their efficacy as enhancers of breast milk production [9]. LSA plays a role in improving nutrients and food absorption [10].

Based on the above description, this study aimed to determine the effect of LSA as an anti-anemic and analgesic. Tests were performed on the animal model of anemia induced by sodium nitrite and chemically pain-induced animal models of mice using 1% glacial acetic acid intraperitoneally.

MATERIALS AND METHODS

The study was conducted using 2 in vivo methods in Swiss Webster's female mice. Anti-anemic activity testing was performed on anemia-induced mice using sodium nitrite with 7 days of testing drugs. Testing of analgesic activity was performed by chemically painless induction method using 1% glacial acetic acid intraperitoneally. The number of stretches of each group was observed every 5 minutes for 1 hour.

Data collection

LSA was obtained from the community located in Subang, West Java, Indonesia. A determination was done at Herbarium Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia (no 011 / HB / 01/2017).

The standard drugs used were Aspirin (generic name) and Sangobion (Merck brand) obtained from local pharmacies in Bandung, Indonesia. Aspirin was given intraperitoneally at a dose of 39 mg/kg. Sangobion was a drug containing the active substances Fe, Mn, Co, folic acid, Vitamin B12 and vitamin C which is indicated to overcome anemia. Sangobion was given orally at a dose of 1.95 ml/kg.

Animal preparation

The test animals used in this study were Swiss Webster's female mice with age ± 2 months, and the average body weight of 20-30 grams. Mice were adapted in cages for 10 days with a light-dark cycle of 12 hours, free access to food and drinking water. The procedures in this study have been approved by the local ethics committee (No 769/UN6.C.10/PN/2017).

Preparation Material and extraction

The selected LSA was fresh with undamaged leaves. The leaves were washed with running water and dried in a temperature oven of 40ºC. The dried leaves obtained was powdered using a blender.

The LSA powder was extracted by maceration method using 96% ethanol for 3 days. The filtrate was then filtered and evaporated using a rotary evaporator at a temperature of 40-50ºC until a viscous extract was obtained.

Test of anti-anemic activity

A total of 24 female mice were grouped randomly into 6 groups: group 1 (as a normal control received drug carrier), group 2 (as positive control received drug carrier), group 3 (received Sangobion® supplement 1.95 mL / kg orally) and group 4 - 6 (received the test extract at doses of 50, 100, and 200 mg / kg orally). All groups except the normal group received $NaNO_2$ (sodium nitrite) as an induction of anemia and test drugs for 7 days. On the 8th day, blood samples were taken to measure hematocrit volume. The difference in hematocrit volume in each treatment group showed the effect of the test drug as anti-anemic.

Test of analgesic activity

Analgesic activity was performed by the method of stretching, where the pain was induced by glacial acetic acid 1% 0.5 mL intraperitoneally. A total of 20 female mice were grouped randomly into 5 groups consisting of group 1 (receiving drug carriers), group 2 (receiving aspirin dose 39 mg/kg orally), group 3-5 receiving the test extract at doses of 50, 100, and 200 mg/kg orally. All groups were induced by 1% glacial acetic acid intra-peritoneally at 30 minutes after administration of the test drug. The parameters measured were the number of writhing in each treatment group every 5 minutes for 1 hour of observation. A decrease in the amount of writhing indicates an analgesic effect.

Data analysis

The data obtained were analyzed statistically at 95% confidence level. The effect of the test drug was marked by a significant difference in p-value <0.05 compared to the control group.

RESULTS AND DISCUSSIONS

Phytochemical Screening

The results of phytochemical screening of LSA dry matter showed that LSA contains secondary metabolites of alkaloids, flavonoids, tannins, saponins, and steroids.

Analgesic activity

The results showed that glacial acetic acid administration showed a pain response in group 2 indicated by the more significant amount of writhing compared to the group of animals receiving the test drug (Table 1). Animal groups receiving LSA extract at doses of 50, 100, and 200 mg/kg showed less amount of writhing compared to the control group ($p < 0.05$). The group receiving a dose of 200 mg/kg showed an analgesic effect comparable to aspirin ($p > 0.05$).

Table 1. The accumulated amount of writhing for each treatment group during 1 hour of observation

Group	Treatment and Dose	Number of writhing ± SD
1	Received a drug carrier	150.8 ±22.4
2	received aspirin dose of 39 mg / kg orally	49.0 ± 5.4*
3	Received LSA extract at a dose of 50 mg / kg orally	100.5 ± 9.9 *
4	Rreceived LSA extract at a dose of 100 mg / kg orally	94.3 ± 13.6*
5	Received LSA extract at a dose of 200 mg / kg orally	48.3 ± 9.8*

* LSA = leaves of Sauropus androgynous. Showed a significantly different effect compared to group 1 ($p<0.05$)

Primary dysmenorrhea is known to cause mood disorders in women and even decreases the quality of life due to the pain of each menstrual cycle. Dysmenorrhea can lead to decreased mood, sleep quality, physical activity. Although, the prevalence of dysmenorrhea is very high in women, lack of attention in an effort to overcome it. Interventions to treat dysmenorrhea pain may reduce the risk of chronic pain in women [11].

LSA extract provides a beneficial nutrient, it also has analgesic effects that are comparable to aspirin. Aspirin is one of the non-steroidal anti-inflammatory drugs that are the first choice to overcome dysmenorrhea. However, long-term aspirin use can cause peptic ulcers.

This study is in line with previous research that LSA extract has analgesic and antinflammatory activity comparable to papaverine [12]. Leaf LSA has been reported to contain papaverine which serves as spasmolysis with the mechanisms' action that increases blood flow in the body, and relaxes the muscle of blood vessels [13].

Anti-anemic activity

Anti-anemic testing is done by calculating the amount of plasma volume. Blood plasma is a component of yellow blood fluid which is the medium of blood cells. Blood plasma consists of 90% water and 10% in the form of protein solution, glucose, a coagulation factor, mineral ion, hormone, and carbon dioxide. Blood plasma is obtained by adding anticoagulants in fresh blood, then centrifuging until the blood cells fall to the bottom of the tube, and the blood plasma is above the layer.

Sodium nitrite solution causes anemia by altering and oxidizing Fe2 + (Ferro) ions in hemoglobin (Hb) into Fe 3+ (Ferri) ions resulting in methemoglobin. In this case, mice experience the lack of oxygen needed for their own body. Sangobion as a standard drug plays a role in increasing the formation of red blood cells and platelets in the blood and maintains the normal levels.

Induction of anemia was performed by administering a solution of 3.75 mg / 1 mL sodium nitrite at a dose of 0.3 mL orally. Furthermore, test drugs are given for 7 days. The results showed that sodium nitrite can increase plasma volume due to decreased red blood cell component, which indicates anemia (Table 2).

Table 2. Effect of LSA on plasma volume after induction of sodium nitrite and test drugs for 7 days for each treatment group

Group	Treatment and Dose	Plasma Volume ± SD (µL)
1	Received a drug carrier	13 ± 0.7
2	Received NaNO2 and drug carrier	25 ± 0.1
3	Received NaNO2 and Sangobion at a dose of 1.95 mL/kg	7 ± 0.4*
4	Received NaNO2 and LSA extract at a dose of 50 mg / kg	23 ± 0.1
5	Received NaNO2 and LSA extract at a dose of 100 mg/kg	20 ±0. 8
6	Received NaNO2 and LSA extract at a dose of 200 mg/kg	11±0.7*

* LSA = leaves of Sauropus androgynous.Showed a significantly different effect compared to group 2 ($p<0.05$)

Dysmenorrhea is often accompanied by severe menstrual bleeding. It increases the risk of anemia. So, women with dysmenorrhea need an analgesic drug and also they need to get nutritional supplements to increase the formation of red blood cells [14].

Our results showed that LSA extract has an anti-anemia effect characterized by increased red blood cell volume and decreased hematocrit volume (Table 2). LSA extract doses of 200 mg/kg showed the best anti-anemic effect. This study is in line with previous studies that LSA chlorophyll has an antioxidant effect that can prevent the process of oxidation of hemoglobin into methemoglobinemia which is a sign of damaged blood cells or lysis. This leads to decreased iron in the body. Our results, are in line with previous reported studies that chlorophyll from LSA has

anti-anemic activity through mechanisms as antioxidants [15].

Anemia is prevalent throughout the world population, and most cases of anemia are caused by lack of iron [16]. It has been reported that LSA extract contains higher amounts of iron in young leaves than in older leaves [17]. In our study, the LSA used were young and fresh leaves so that the anti-anemic effects were caused by iron content and secondary metabolites in leaves.

Based on our results, the group receiving LSA extract showed analgesic activity comparable to those receiving aspirin. In addition, the group receiving LSA extract showed a significantly different increase in red blood cell count compared to the control group.

CONCLUSION

This finding is very important, as LSA can be an alternative therapy for dysmenorrhea. It can reduce drug use and avoid long-term side effects of NSAIDs. Because most women experience dysmenorrhea accompanied by severe menstruation, it is often necessary to use NSAIDs every month during menstruation and increase the risk of anemia. Based on our results, it can be concluded that Sauropus androgynus leaf extract (L) Merr has analgesic and anti-anemic activity at a dose of 200 mg / kg as an effective dose. The results of this study reinforce the benefits of LSA in women who experience menstrual dysmenorrhea to overcome the pain due to dysmenorrhea and the risk of anemia due to heavy menstruation. The biological effects of LSA occur because of the secondary metabolite content in the extracts including alkaloids, flavonoids, tannins, saponins, and steroids

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