INTRODUCTION

Endometriosis is a benign gynecological disease whose pathogenesis mechanism is not fully understood because it involves various theories and underlying pathophysiology. Endometriosis in its development is progressive, angiogenesis, invasive and metastatic, and resistant to apoptosis. This chronic disease depends on the hormone estrogen, which is characterized by the presence of endometrial glands and stroma that are not in place.

Endometriosis treatment is widely carried out by hormonal and surgical methods aiming to relieve symptoms, suppress progression, and increase fertility. However, hormonal treatment is generally expensive and disrupts the cycle of follicular growth in the ovaries, so the treatment of endometriosis with infertility should not be hormonal. This study was conducted to find new alternative therapies for endometriosis, so it would be unethical to do it directly on humans. It is increasingly being used, which contributes to anti-inflammatory and anti-angiogenic therapy. Therefore, finding the right anti-inflammatory, anti-angiogenic, anti-oxidant, and apoptotic regulators is an important issue for treating endometriosis implants in mice model of endometriosis.

The effect of Chromolaena odorata on endometriosis implants in mice model of endometriosis

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ABSTRACT

Introduction: Endometriosis is a benign gynecological disease whose pathogenesis mechanism is not fully understood with treatment modality with hormonal and surgical methods aiming to relieve symptoms, suppress progression, and increase fertility. This study aims to observe the differences in the administration of Chromolaena odorata leaf extract on the area of endometriosis implants in the mice model of endometriosis.

Materials and Methods: This study was an experimental study with a Randomized Separated Pre and Post Test Design research conducted with the control group. Thirty mice (Mus musculus) were injected with 0.2 ml/mice of cyclosporine A. Then endometrial tissue was injected into the peritoneal cavity, followed by 0.1 ml Ethinyl estradiol/mice injections on the 1st and 5th days. Mice were reared for 14 days until the endometriosis model was formed. Mice models of endometriosis were randomly divided into five groups: endometriosis mice that were terminated on day 14 (pretest), then the other groups are endometriosis mice given Aquadest as placebo (P0), endometriosis mice treated with Chromolaena odorata leaf extract at a dose of 400 mg per kilograms of body weigh (mg/kgBW) (P1), 800 mg/kgBW (P2) and 1200 mg/kgBW (P3) for 14 days. On the twenty-eighth day, mice were dissected to examine and assess the extent of endometriosis implantation macroscopically in hyperemic areas by taking samples in the most hyperemic areas of the peritoneal wall. Research data were analyzed using SPSS Version 22.

Results: From this study, the average value of endometriosis implant area in the group given Chromolaena odorata leaf extract was 400 mg/kgBW (P1 = 15.60 mm²), 800 mg/kgBW (P2=14.23 mm²) and 1200 mg/kgBW (P3=0.00 mm²) was lower than the pretest group (P=104.72 mm²) and the placebo group (P0 = 153.89 mm²), showing a significant difference (p<0.001). There was a tendency that the area of endometriosis implants was also lower in the group receiving a higher dose of Chromolaena odorata leaf extract 1200 mg/kgBW (P3=0.00 mm²) than the group receiving a dose of 400 mg/kgBW (P1=15.60 mm²) and 800 mg/kgBW (P2=14.23 mm²).

Conclusions: It can be concluded that the administration of Chromolaena odorata leaf extract can reduce the area of endometriosis implants in mice models of endometriosis. Higher doses result in the lower endometrial implant area.

Keywords: endometriosis, Chromolaena odorata, implant area.

endometriosis.*

Chromolaena odorata is one of the flowering shrubs from the Asteraceae family. In several chemical analyses, it has potential in medicine containing active phytochemicals, namely essential oils, flavonoids glycones, quercetagetin, kaempferol, acacetin, naringenin, chalcones, quercetin, lutolin, and sinensetin, terpenes, terpenoids, saponins, tannins, alkaloids, phytoprostanes and phenolic acids.** This plant has biological activity as an anti-inflammatory, antioxidant, and cytotoxicity. Also, it has an inhibitory effect on the viability and proliferative properties of cancer cells as well as a cytotoxic effect and enhances apoptosis.***

This study aims to show the effect of Chromolaena odorata with different doses on decreasing the area of endometriosis implants in mice models of endometriosis.

**MATERIALS AND METHODS**

**Experimental Design**

This experimental study used a Randomized, Separated Pre and Post Test Design. Thirty mice (Mus musculus) weighing 20-30 grams and 3-4 months old were obtained from the Laboratory of Reproductive Physiology Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Chromolaena odorata leaf extract, Laboratory of Organic Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh.

**Endometrial Tissue Sampling**

Endometrial cells were taken from benign uterine tumor material. The endometrial tissue needed to make preparations for the mice was stored in phosphate-buffered saline (PBS).

**Experimental Implementation**

After, the mice underwent an adaptation process for one week in the cage for adjustment by getting the same food. The mice were injected with cyclosporine A at 10 mg/kgBW intramuscularly at 0.2 ml. Cyclosporine injection aims to suppress the immune system of mice so that the growth of endometriosis implants in the peritoneal cavity of the mice is not disturbed. The endometrial tissue is then injected into the peritoneal cavity. This endometrial tissue was stored in phosphate-buffered saline (PBS). Before being injected into the mice, it was washed twice with a centrifuge of 2,500 rpm. The supernatant was discarded, and PBS, penicillin 200 IU/ml, and streptomycin 200 g/m were added. The wet endometrial tissue was taken with a 3 ml syringe. Each mouse will receive an intraperitoneal injection of 0.1 ml using a 1 ml syringe with an 18G needle so that endometrial tissue can enter. Furthermore, injection of ethynyl estradiol with a dose of 54 IU intramuscularly on days 1 and 5, each mouse will get an injection of 0.1 ml.

The mice were reared for 14 days to wait for the formation of endometrial tissue in the peritoneum. After that, the mice model of endometriosis was randomly divided into five groups consisting of endometriosis mice which were dissected on day 14 (pretest) to prove the formation of the mice model of endometriosis and assess the extent of the endometriosis implant in the peritoneal wall macroscopically. Then mice models of endometriosis were treated with Aquadest as placebo (P0), and endometriosis mice were treated with Chromolaena odorata leaf extract at a dose of 400 mg/kgBW (P1), 800 mg/kgBW (P2) and 1200 mg/kgBW (P3) for 14 days. On the twenty-eighth day, the mice were operated on to assess the extent of the endometrial implants macroscopically in the hyperemic area of the peritoneal wall.

**Sampling**

The mice model of endometriosis was euthanized, and the abdominal wall was dissected, then the peritoneal tissue was collected and photographed on millimeter block paper and documented with photographs to assess the extent of endometriosis implants.

**Endometriosis Implant Area Calculation**

The size of the endometrial implant was assessed macroscopically in the hyperemic area. Measurement of implant area and/or length of hypervascularization is carried out in mm², which is calculated by tracing the method using the Image Raster application on a computer.

**RESULTS**

Data on the area of endometriosis implants based on treatment with Chromolaena odorata leaf extract in 30 mice are shown in Table 1.

The analysis showed that the lowest implant area of the mice model of endometriosis was in the P3 group. The lowest median area of endometriosis implants was in the P3 group with a value of 0.00 mm² and the highest in the K-group with a value of 135.02 mm² (Table 1). Analysis between treatment groups showed a significant difference in the endometriosis implants in the mice model of endometriosis (p<0.001), so it could be concluded that there were differences in the area of endometriosis implants based on the administration of Chromolaena odorata leaf extract. In Table 2 shows, the results of the analysis of the area of endometriosis implants are shown between treatment groups giving Chromolaena odorata leaf extract to a mice model of endometriosis.

Table 2, showed that the area of endometriosis implants in the posttest and K-groups significantly differed from that of the group that received Chromolaena odorata leaf extract. Meanwhile, the group that received Chromolaena odorata leaf extract did not have a significantly different area of endometriosis implants. It can be concluded that the administration of the extract can produce differences in the area of endometriosis implants in a mice model of endometriosis.

Figure 1 shows that the area of endometriosis implants in the group given Chromolaena odorata leaf extract was lower than in the pretest and K-groups. There were a tendency for the endometriosis implant area to be lower in the group that received a higher dose of Chromolaena odorata leaf extract. It can be concluded that the administration of Chromolaena odorata leaf extract can affect the decrease in the area of endometriosis implants in the mice model of endometriosis. The gross tissue of endometriosis can be seen in figure 2.

**DISCUSSION**

Endometriosis is the finding of endometrial tissue, glands, and stroma outside the...
uteroine cavity. Ectopic endometrial tissue is generally located in the pelvic cavity, with the most frequent sites in the ovaries, Douglas cavity, sacrouterine and broad ligaments, fallopian tubes, sigmoid colon, and appendix. Endometriosis as a chronic disease has three main types: peritoneal type, deep infiltrating, and ovarian endometrioma, where endometriosis lesions will experience a growth cycle and bleeding following the menstrual cycle. The decrease in the endometrial implants and serum levels of estradiol (E2) and tumor necrosis factor (TNF) alpha compared to the control group. The results showed that quercetin treatment significantly increased antioxidant activity. The beneficial effects of natural active ingredients affecting major pathophysiological pathways of endometriosis demonstrated in preclinical models require evidence in clinical trials. Phenolic acids as polyphenols in the management of endometriosis have beneficial effects of bioactive compounds in preventing chronic non-communicable diseases, including cardiovascular disease and cancer. Several studies have evaluated the association between the administration of polyphenols, such as flavonoids and phytoestrogens, and women's cancer risk. Regular

Table 1. Analysis of Endometriosis Implant Areas in Mice Model of Endometriosis Based on the Provision of Chromolaena odorata Leaf Extract (n=30).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretest</th>
<th>K-</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>14.23</td>
<td>15.60</td>
<td>14.23</td>
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<tr>
<td>Median</td>
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<td>13.52</td>
<td>12.00</td>
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<td>0.00</td>
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<tr>
<td>Standard deviation</td>
<td>20.65</td>
<td>19.22</td>
<td>22.06</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Min</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Max</td>
<td>30.00</td>
<td>46.32</td>
<td>43.88</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>p*</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>p**</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 2. Analysis of Inter-Group Areas of Endometriosis Implants in Mice Model of Endometriosis Based on Provision of Chromolaena odorata Leaf Extract (n=30).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretest</th>
<th>K-</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Median</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Standard deviation</td>
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<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Min</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Max</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
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<tr>
<td>p*</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>p**</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
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</tr>
</tbody>
</table>

*data normality test; ** Kruskar Wallis test; Pretest: endometriosis (+); K-: Endometriosis (+), Aquaedest; P1: Endometriosis (+), Chromolaena odorata extract 400 mg/KgBW; P2: Endometriosis (+), Chromolaena odorata extract 800 mg/KgBW; P3: Endometriosis (+), Chromolaena odorata extract 1200 mg/KgBW
The consumption of flavonoid-rich foods is associated with a reduced risk of breast and ovarian cancer. In a meta-analysis, it has been found that a higher dietary intake of phytoestrogens (isoflavones) reduces the risk of breast cancer mortality and recurrence. Endometriosis and breast cancer are estrogen-dependent conditions and share similar cellular processes such as proliferation, invasion, neo-angiogenesis, metastasis, and reproductive-associated risk factors. Significant similarities between endometriosis and cancer prompt research into the effects of polyphenols on endometriosis.

Increasing the dose of *Chromolaena odorata* can suppress the extent of endometriosis implants in the peritoneum mice model of endometriosis. The implant area of the group that was given *Chromolaena odorata* leaf extract at a dose of 1200 mg/kg BW was the best compared to the group with a dose of 800 mg/kg BW and 400 mg/kg BW. In this study, the leaf extract of *Chromolaena odorata* with a dose of 1200 mg/kg BW was the best in reducing the area of endometriosis implants in the peritoneum. This shows that the efficacy of *Chromolaena odorata* is highly dose-dependent, where the anti-inflammatory, antioxidant, immunomodulatory, and regulating apoptosis synergistic effects increases its efficacy to reduce the area of endometriosis implants in the mice model of endometriosis.

**CONCLUSIONS**

Endometriosis lesions given *Chromolaena odorata* extract can cause a decrease in the area of endometriosis implants. The decrease in implant area is dose-dependent, whereas a higher dose results in a lower endometrial implant area.

**ETHICAL ISSUES**

All methods were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Syiah Kuala–Zainoel Abidin Hospital, Banda Aceh with ethical clearance reference number: No. 083/EA/FK-RSUDZA/2022

**CONFLICT OF INTEREST**

The author declares that there is no conflict of interest.

**FINANCIAL SUPPORT**

None.

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**AUTHOR CONTRIBUTION**

All author had contributed to manuscript writing and agreed for the final version of manuscript for publication.

**REFERENCES**


