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Original Research Article

# Effects of Ethanol Extract of Sungkai (*Peronema canescens* Jack.) on Fertility of Female Wistar Mice (*Mus musculus* L.)

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ABSTRACT

Sungkai leaf (*Peronema canescens* Jack.) is a natural source of flavonoids. Flavonoids have been implicated in increasing fertility in humans. This study aims to determine the effect of oral ethanol extract of Sungkai leaf on the fertility of female mice. Twenty-four female mice randomly divided into four groups were used in this study. The test (II-IV) groups were administered orally ethanol leaf extract of *Peronema canescens* at doses of 200 mg/kg bw, 400 mg/kg bw, and 800 mg/kg bw, respectively, for 14 days. The negative control (I) group received 0.5% Na-CMC only. On the 15th day, the mice were mated with male mice in one cage until pregnancy was established. On the 10th day of gestation, the mice were sacrificed by cervical dislocation, and both ovaries were excised. The result shows increased primary ovarian follicles, corpus luteum, and ovulation rate. The optimal increase of the ovaries occurred at the dose of 200 mg/kg bw. There was however a decrease in the above parameters at extract doses of 400 and 800 200 mg/kg bw. The study concludes that the leaf extract of *Peronema canescens* has the potential to treat infertility.

***Keywords***: Fertility, Follicle, Corpus Luteum, Ovulation Rate, *Peronema canescens* Jack.

## Introduction

Infertility is a medical condition in which a sexually active couple fails to conceive a pregnancy within one year of unprotected intercourse.[1] In Indonesia, the prevalence of infertility among childbearing-age couples is estimated to be between 10-15%, which means that around 4 to 6 million couples may require medical assistance to conceive.[2] According to the Central Statistics Agency 2011, there are approximately 39.8 million women of childbearing age in Indonesia, and around 10-15% are infertile. Hence, there is a significant need for treatment options to address infertility. Plants-derived remedies are treatments that have proven efficacious in managing female infertility. Sungkai (*Peronema canescens*) is a plant commonly found in Southeast Asia. It has been used in traditional medicine to treat various ailments, including female reproductive system disorders such as infertility.[3]
The Indonesians see several herbal plants as natural fertility enhancers. One such herb is pegagan dew which contains isoflavone flavonoids, saponins, and alkaloids that promote mammal estrogen production. Pegagan dew leaf extract effectively increases the number of ovarian follicles at low doses. According to studies, gandarusa leaf, which contains flavonoid compounds in the form of isoflavones, can increase the endogenous hormone estradiol and the number of secondary follicles.[4,5] These plants contain secondary metabolites, almost the same as the sungkai plant.

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The results of the phytochemical test on Sungkai revealed that the plant contains various secondary metabolites, including alkaloids, steroids, terpenoids, tannins, and saponins.[6] Another research shows that the ethanol extract of sungkai leaf also contains phenolics, anthraquinones, and flavonoids. Flavonoids are polyphenols found in many plants.
Based on their chemical structure, flavonoids are classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, and proanthocyanidins.[7] Flavonoids are active ingredients that affect estrogen and testosterone hormones.[8] Flavonoids are also called phytoestrogens because they are found in plants (phyto) and their ability to react like the hormone estrogen in the human body.[9]
This study aims to investigate the potential of the sungkai plant as a natural fertility enhancer. Flavonoids in the sungkai plant are known to affect estrogen and testosterone secretion and have been classified based on their chemical structure. The study seeks to explore the effectiveness of the sungkai plant extract in increasing the number of ovarian follicles and endogenous hormone levels in mammals, with the ultimate goal of providing more natural options for treating infertility-related problems.

## Materials and Methods

*Extract preparation*
Fresh leaves (3 kg) of Sungkai leaf were collected and dried under the shade. The sample was obtained in January 2021 with voucher number 102/K-ID/ANDA/II/2021. The leaves were ground, and 300 g of the powdered plant material was extracted exhaustively by macerating in ethanol (70%) (2.5 L x 3) for three days with intermittent stirring. The macerate in each extraction was filtered with filter paper, and the filtrates were combined and concentrated using a rotary evaporator at 35-45°C, yielding 80 grams of a concentrated extract. The extract was further exposed to a water bath at 40°C to ensure the complete removal of extracting solvent. Finally, the phytochemical content of the extract was examined by a colourimetric method to identify the presence of flavonoids[10]. A positive result would indicate the presence of flavonoids in the extract.

*Ethical Approval*
Ethical approval was obtained from the ethical committee of the Medical Faculty of Andalas University under reference number 842/UN.162/KEP-FK/2022.

*Preparation of experimental animals*
Female Wistar strain mice, aged 2-3 months with an average weight of 20-35 grams, were acclimatized for seven days before treatment. The test animals were divided into four groups (I-IV): Group I, the negative control group, received a 0.5% Na-CMC suspension at a dose of 0.5 mL, and test groups II, II, and IV received oral doses of sungkai extract at 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively, for 14 days. On day 15, the test and negative control groups' female mice were mated with male mice (at a 4:1 mating ratio) in one cage, usually at night. The next day, vaginal swabs were examined to determine fertilization, and the presence of sperm was considered day 0 of pregnancy in female mice.[11]

*Observation of Ovarian Follicle Number, Corpus Luteum, and Ovulation Rate.*
The reproductive organs of mice were examined on the 10th day of pregnancy. The mice were sacrificed using cervical dislocation, and surgical procedures were performed to remove all reproductive organs. The successfully removed organs were then placed into an ointment pot containing 0.9% NaCl and were drained using filter paper. The drained ovaries were stored in an ointment pot containing 10% NBF[12] for subsequent preparation and observation. The ovarian preparations were observed for the number of primary follicles, secondary follicles, de Graafian follicles, and corpus luteum, using an Olympus BX 51 microscope with 400x magnification. Tissue processing and staining for histological assay were carried out to investigate the observations further. The number of ovulation rates was calculated to indicate the number of ovaries that had undergone ovulation. The ovulation rate was determined by calculating the percentage of the number of follicles compared to the number of corpus luteum.

$$\text{Ovulation rate} = \frac{\text{corpus luteum}}{\text{follicle}} \text{x}100\%$$

*Statistical analysis*
The data were statistically analyzed using the one-way Analysis of Variance (ANOVA) and the Duncan test. A statistical significance level of $p < 0.05$ was considered significant.[13]

## Results and Discussion

The secondary metabolites test of sungkai leaf showed positive results for alkaloids, flavonoids, phenolics, steroids, and saponins. Observations on ovarian follicles included the average number of primary, secondary, de Graafian, and corpus luteum[14]. The average number of primary follicles, secondary follicles, de Graaf follicles,s and right and left ovarian corpus luteum in female mice after being treated with sungkai leaf extract are presented in Tables 1 and 2. *Peronema canescens* leaf extract affected the number of primary follicles, secondary follicles, de graff follicles, and corpus luteum significantly ($p$<0.05).
The findings revealed no meaningful distinctions in the average primary, secondary, and de Graff follicle count between the negative control and the group treated with 800 mg/kg bw of the extract. Nonetheless, the results for the average count of primary and secondary follicles and de Graaf follicles on both the right and left ovaries exhibited a rise in the number at the 800 mg/kg bw dose compared to the negative control group.
The increase in follicles is believed to be caused by the administration of *Peronema canescens* leaf extract, which contains flavonoids and estrogenic compounds that can act like estrogen in the body. These phytoestrogens constituents of the plant may bind to endogenous estrogen receptors in the ovaries, causing an increase in the average number of follicles.[15,16] The hormone estrogen plays a role in increasing the number of Follicle-stimulating hormone (FSH) receptors on granulosa cells; thus improving ovarian follicles development.[17] *Peronema canescens* leaf extract in low doses can increase the number of ovarian follicles, while at high doses, it can reduce the number of follicles.[4] The estrogenic properties of flavonoids affect estrogen production in the ovarian follicles.
Estrogen in moderate amounts can increase the number of follicles, while in chronic or high doses, it can affect the neuroendocrine system to be disrupted. High levels of estrogen can prevent the production of FSH so that follicle development is inhibited.[18]
The right and left ovaries showed no significant difference between the negative control group at 800 mg/kg bw. The average number of right and left ovarian corpus luteum increased at 200 mg/kg bw compared to the negative control. There was an increase in the corpus luteum and the number of follicles in the treatment group. The corpus luteum is the largest producer of the hormone progesterone and the placenta. Increasing the corpus luteum in early pregnancy is necessary to increase progesterone production in maintaining pregnancy. Progesterone has an essential role in maintaining pregnancy.[19]

**Table 1:** The average number of primary follicles, secondary follicles, de graaf follicles, and corpus luteum in the left ovary of female mice after administration of *Peronema cannescens* leaf extract for 14 days.

| Group | Average Number of Primary Follicles ± SD | Average Secondary Follicle Number ± SD | Average De Graaf Follicle Count ± SD | Average Number of Corpus Luteum ± SD |
|---|---|---|---|---|
| Negative Control (I) | 8.667 ± 1.966 | 8.500 ± 1.643 | 8.667 ± 1.211 | 7.500 ± 1.788 |
| 200 mg/kgbw (II) | 13.167 ± 2.401 | 10.666 ± 2.066 | 7.833 ± 1.941 | 13.000 ± 3.559 |
| 400 mg/kgbw (III) | 11.333 ± 3.327 | 10.833 ± 1.472 | 7.333 ± 2.503 | 10.333 ± 2.422 |
| 800 mg/kgbw (IV) | 8.667 ± 1.966 | 9.333 ± 2.066 | 4.000 ± 1.673 | 5.667 ± 3.2710 |

**Table 2:** The average number of primary follicles, secondary follicles, de graaf follicles, and corpus luteum in the right ovary of female mice after giving *Peronema cannescens* leaf extract for 14 days

| Group | Average Number of Primary Follicles ± SD | Average Secondary Follicle Number ± SD | Average De Graff Follicle Count ± SD | Average Number of Corpus Luteum ± SD |
|---|---|---|---|---|
| Negative Control (I) | 8.000 ± 2.000 | 8.167 ± 1.835 | 9.000 ± 1.549 | 8.833 ± 2.714 |
| 200 mg/kgbw (II) | 12.500 ± 2.345 | 11.000 ± 1.414 | 8.500 ± 2.588 | 12.833 ± 2.804 |
| 400 mg/kgbw (III) | 9.833 ± 4.355 | 10.167 ± 2.714 | 7.333 ± 2.503 | 12.667 ± 3.076 |
| 800 mg/kgbw (IV) | 7.333 ± 3.386 | 9.000 ± 2.098 | 4.000 ± 1.673 | 8.333 ± 2.926 |

Progesterone concentration in the mother's serum influences the fetus's life in the uterus.[20] If the hormone progesterone is insufficient, it will cause continuous uterine contractions, leading to embryo implantation failure and abortion.[21] The hormone estrogen plays a role in maintaining the corpus luteum to keep secreting progesterone. The endometrium will proliferate under the influence of progesterone by increasing the effectiveness of the glands and their secretions into the uterus. This uterine fluid will affect the development of the blastula to become a fetus. This fluid will become a nutrient for the ovum from maturation until implanted in the uterus.[22]

Figure 1 shows the histological structure of the primary, secondary, De Graafian and corpus luteum follicles after administering Sunkai extract for the treatment duration. The increase in ovulation rate is very effective in improving the fertility rate of experimental animals. As such, the ovulation rate can be affected by increased gonadotrophin-dependent follicles or increased follicle rate in Table 3.

**Table 3:** Shows the percentage value of the ovulation rate at a dose of 200 mg/kg BW

| Ovaries | Negative Control | Dosage 200 mg/kgbw | Dosage 400 mg/kgbw | Dosage 800 mg/kgbw |
|---|---|---|---|---|
| Left | 32.14% | 41.26% | 36.68% | 37.87% |
| Right | 38.97% | 43.50% | 40.78% | 28.81% |

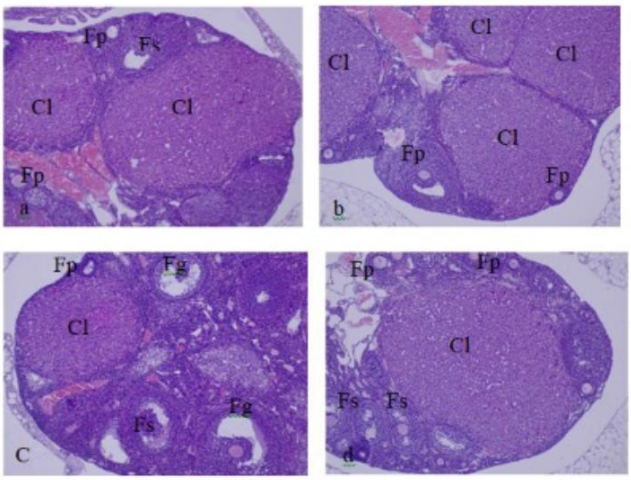


Figure 1. Histological results of experimental animal ovary tissue showed Primary Follicle (Fp), Secondary Follicle (Fs), De Graaf Follicle (Fg), and Corpus Luteum (Cl), using 400x magnification. (a): Negative control, (b): 200 mg/kgbw, (c): 400 mg/kgbw, (d): 800 mg/kgbw.

## Conclusion

Different doses of ethanol leaf extract of *Peronema cannescens* have been shown to increase the number of ovarian follicles, corpus luteum, and ovulation rates of both ovaries of female Wistar mice (*Mus musculus* L). This increase was optimal at an oral dose of 200 mg/kg bw. The study concludes that the leaf extract of *Peronema canescens* is a potential natural product that could be used in treating female infertility.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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