

Turmeric Rhizome's Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats

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Turmeric rhizome extract has been shown to have antifertility effects as antiestrogenic and is reversible. This study aims to rate turmeric rhizome extract (*Curcuma longa* L.) antiestrogenic potential towards epithelium cell and endometrium layer thickness reduction on female rats. Twenty-eight female rats aged around 6-8 weeks old and weighing around 200-250 g were divided into four groups using a completely randomized design. The control group received only aquadest. Treatment groups 1, 2, and 3 received 250, 500, and 1.000 mg/kg BW turmeric rhizome extract, respectively, for five days. At the end of the examination, there was a significant decrease in the number of endometrial epithelial cells in the turmeric group ($p=0,000$), in line with the increase in the dose given. This research also shows the presence of antiestrogenic potential effects associated with an endometrium layer thickness ($p=0.013$), and there was a decrease in endometrium thickness associated between the control group and treatment group ($p<0,05$). Conclusions: Turmeric rhizome extract has an antiestrogenic potential and can reduce the total of epithelium cells and endometrium layer thickness on female rats.

Keywords: Antiestrogenic Potential, Endometrium Epithelium Cells,
Endometrium Layer, Turmeric Rhizome Extract.

Interaction between blastocyst, implantation, and endometrium stages during initiation and nidation is a complex matter. When blastocysts carry out adhesion and invasion, the endometrium will be able to respond. Structurally and molecularly, the initiation of trophoblast apposition and adhesion in the endometrium of primate species is not very well known. In rodents, exceptions occur after fertilization, the trophoctoderm does not undergo adhesion, and the microfilic portion will assist the application of blastocysts. During adhesion and implantation,

the gap between the trophoctoderm cell membrane and the surface of the endometrium epithelium will decrease¹.

It added that the situation was caused by extensive endometrium destruction. It results in amenorrhea and habitual abortion, which is believed to occur due to inadequate endometrium to support implantation of the fertilization results so that failure of implantation of embryos in the endometrium will cause abortion^{2,3}.

Turmeric rhizome extract has been shown to have antifertility effects as antiestrogenic and

is reversible. Giving turmeric rhizome extract with cumin (*Carum carvi*) reduces levels of the hormone Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) significantly through the inhibition of positive feedback to the pituitary, thereby inhibiting the formation of estrogen¹.

In addition, turmeric rhizome extract affects the female reproductive organs, evidenced by the administration of turmeric ethanol extract using multi-level doses orally showing the higher dose given the thinner the layer and the less number of uterine endometrium glands formed⁴. On the other hand, it was explained that turmeric extract could reduce the number of endometrium luminal epithelium cells in LH-induced mice, and post-coitus given turmeric extract showed no implantation or fetal sites⁵.

METHODS

Plant Materials

Turmeric rhizomes were collected from Bandar Lampung city area, Lampung, Indonesia. The collected turmeric rhizomes (*Curcuma longa* L.) were labeled and transferred to the laboratory, washed with tap water, air dried, and stored until further investigations.

Preparation of the Plant Extract

The obtained turmeric rhizomes are then cleaned and then processed to dry using a 70°C oven for 15 minutes. After drying, the plants are milled using a blender to produce a powder obtained 283.9 grams. Turmeric rhizome's which was crushed, was put into a 2.000 ml glass beaker then macerated using 96% ethanol solvent for 3 x 24 hours to obtain macerate. The filtrate obtained is concentrated or thickened using a rotary evaporator at a temperature of 50°C for 1 hour.

Animal Treatment

Rats conducted acclimatization for one week under laboratory conditions in cages prepared. Appropriate 28 female white rats Sprague Dawlye's strain age around 6-8 weeks old weighed around 200-250 g that splits into four groups and can be maintained 100 cm x 50 cm, placed in a research room, or placed in a research room rat cage. The rat cage contained a bowl filled with food, rice husks, and a place to drink. Rats are fed with small chicken feed and drink water daily. Husks are replaced every two days due to moisture

due to spillage of food or drink rats to prevent bacteria or fungi growth.

Turmeric Rhizome's Extract Treatment

Rats received an oral dose of freshly prepared turmeric rhizome extract. The Control group only received aquadest. The treatment group 1, 2, and 3 received 250, 500, and 1.000 mg/kg BW turmeric rhizome extract, respectively, for five days.

Sample Collection

After five days, all rats were sacrificed using ketamine (80-100 mg/kg BW). The endometrium was taken and fixed in 10% phosphate-buffered formalin. After being fixed for 48 hours, the endometrium was then made histopathological preparations with Mayer Hematoxylin staining, carried out following the protocol prepared by the Department of Pathology, Faculty of Medicine, University of Lampung. The number of epithelial endometrium cells was observed using a light microscope with 100x magnification in 10 visual fields. The thickness of the endometrium layer was observed using the Olympus Stream Software.

Statistical Analysis

The number of epithelial endometrium cells and the thickness of the endometrium layer data were tested using One Way Anova followed by the Least Significant Difference (LSD) test. All tests were performed at a 95% confidence level.

Ethical Clearance

This study was approved by the Health Research Ethics Committee with EC number: 3813/UN26.18/PP/05.02.00/2019.

RESULTS

Turmeric rhizome extract decreases the number of endometrium epithelium cells.

The results showed that the group of female rats given turmeric rhizome extract had a significantly lower number of endometrial epithelial cells when compared to the control group. A decrease in the number of endometrial epithelium occurs with an increase in the dose of the extract given (Tabel 1).

Turmeric rhizome's extract decreases the endometrium layer thickness

Similar to the number of endometrium epithelium cells, the endometrial layer thickness in the turmeric rhizome's extract group was

significantly thinner when compared to the control group. The highest dose of turmeric rhizome extract had the thinnest endometrial layer thickness (Tabel 1).

Histological observations on preparations with 1000x magnification showed a microscopic picture of endometrium epithelium cells of white rats and their cell nuclei. Endometrium epithelium cell counts are only performed on epithelium cells that have a normal appearance. Normal endometrium epithelium cells in female white

rats (*Rattus norvegicus*) are ciliated columnar epithelium cells with oval-shaped nuclei arranged in the mucosal lining of the white rat's uterus. The abnormal picture of endometrium epithelium cells in mice includes necrosis or lysis of endometrium epithelium cells⁶.

DISCUSSION

This study showed a decrease in the average number of epithelium cells in each

Table 1. The average number of endometrium epithelium cells and endometrium layer thickness of white rats

Group	Number of endometrium epithelium cells		Endometrium layer thickness	
	Mean±SD (cells)	<i>P</i> value	Mean±SD (µm)	<i>P</i> value
C	291,00 ± 43,9 ^a	0,000*	764,74 ± 80,19 ^a	0,013*
T1	234,86 ± 55,7 ^a		615,06 ± 119,50 ^b	
T2	174,29 ± 24,93 ^b		646,17 ± 139,29 ^b	
T3	167,00 ± 55,5 ^b		566,18 ± 68,74 ^c	

*indicates a significant difference based on the One Way Anova test at á 5%. The mean followed by different letters indicates a significant difference based on the LSD test at 5%.

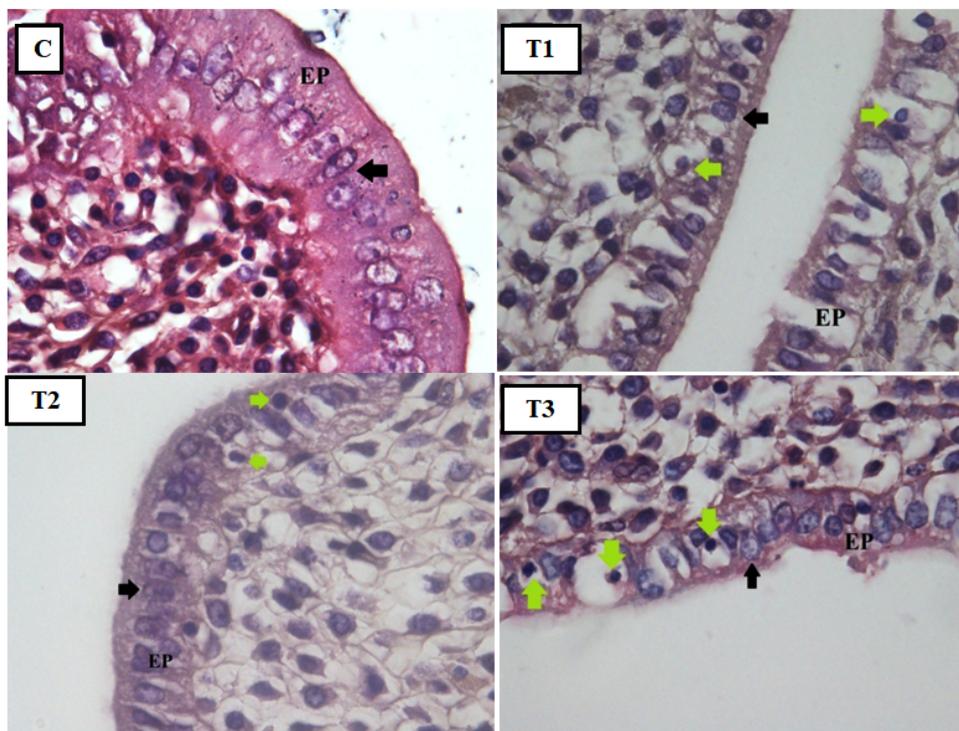


Fig. 1. Endometrium epithelium cells with H.E. staining (magnification 1000x). Epithelial cells (EP); Nucleus (black arrow); Apoptosis (green arrow); Control Group (C); Treatment 1 (T1); Treatment 2 (T2); Treatment 3 (T3)

treatment group, along with the increasing dose of turmeric rhizome extract given. The lowest mean number occurred in the T3 group, given the highest dose of turmeric rhizome extract at 1.000 mg/kg with mean epithelium cells 167 ± 55.5 cells. The results obtained in this study support the results of research⁵, which states that the administration of 500 mg/kg BW turmeric extract after coitus for five days showed the absence of implantation and fetal sites in rat endometrium.

The decrease in the number of normal endometrium epithelium cells occurs due to the antifertility mechanism of turmeric rhizome extract to the cascade of female reproductive cycles. Meanwhile, the reproductive cycle is an endometrium maturation to become a blastocyst implantation medium. It is related to maturation occurring when the endometrium is exposed to estrogen and progesterone⁷.

The antifertility mechanism of a turmeric extract inhibits positive feedback from the hormone estrogen. Physiologically, the hormone estrogen is formed when the ovarian follicles are stimulated by the hormones FSH and LH produced by the anterior pituitary. FSH and LH help the development of ovarian follicles, in line with this internal theca cells under the influence of LH produce androgens. On the other hand, granulosa cells under the influence of FSH will produce the aromatase enzyme, which helps convert androgens to the hormone estrogen. Estrogen is a sex hormone responsible for cellular proliferation and tissue growth in the reproductive system. Estrogen has a significant role in the endometrium proliferation phase⁸.

The formation of estrogen will constantly give a signal in the form of positive feedback resulting in a surge in LH that triggers ovulation. If positive feedback is inhibited, ovulation does not occur so that the corpus luteum, which produces the hormone progesterone, is not formed. In addition, FSH suppression occurs, which results in inadequate development of ovarian follicles so that de Graaf follicles are not formed⁴.

Estrogen will work if it binds to receptors in endometrium epithelium cells, the receptors ER α , ER β , and G-protein-coupled estrogen receptor 1 (GPER1)⁹. Like estrogen, progesterone works when it binds to progesterone receptors (PR), namely PR-A and PR-B¹⁰. The receptors are

scattered on the surface of the endometrium¹¹. This regulation is regulated explicitly by epithelium cells and endometrium stroma under the influence of estrogen¹². For these reasons, if the number of endometrium epithelium cells is reduced, the number indicates that fertility is also declining.

Together with the blastocyst, the endometrium expresses adhesion molecules important for the implantation process, namely integrin $\beta 3$, fibronectin, trophoblasts, and blystin. The endometrium widely produces integrin $\beta 3$ during the menstrual cycle and pregnancy, which functions for adhesion, migration, invasion, and regulation of cellular signals. The expression integrin $\beta 3$ illustrates the value of endometrium receptivity¹³. It is known that if the number of endometrium epithelium cells is reduced, it can be assumed that the production of adhesion molecules is also reduced, which results in decreased endometrium receptivity.

Curcuminoid is one of the many turmeric contents consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin¹⁴. Curcumin is an ingredient that has the main therapeutic effect of turmeric¹⁵. Curcumin inhibits the formation of luteal steroid hormones (steroidogenesis) by inhibiting the accumulation of cyclic adenosine 3', 5' monophosphate (cAMP). It makes an obstacle in extracellular signal-regulated kinase (ERK) phosphorylation in the steroidogenesis pathway so that it inhibits the conversion of cholesterol into pregnenolone¹⁶.

It is known that curcuminoids can bind to the active site of enzymes that play a role in steroidogenesis, such as P450 side-chain cleavage (P450_{scc}), CYP17A1, CYP19A1 (aromatase), and CYP21A2 so that enzymes are inhibited. The P450_{scc} enzyme is an enzyme in charge of converting cholesterol into pregnenolone in the mitochondria. CYP17A1 functions to convert pregnenolone to 17OH-pregnenolone, whereas CYP19A1 or aromatase is an enzyme that converts androstenedione to estrogen. Thus, if ERK phosphorylation and steroidogenesis enzymes are inhibited, steroid metabolism will decrease, which causes the production of sex steroid hormones will decrease, thereby inhibiting fertility¹⁷.

Prostaglandin E₂ (PGE₂) is the key to the de Graaf follicle ovulation. Follicles produce the cyclooxygenase COX-1 and COX-2 enzymes in the

event of an LH surge that triggers the expression of PLA2G4A in granulosa cells. PLA2G4A is a phospholipase that cleaves arachidonic acid from the phospholipid membrane at the time of pre-ovulation. PGE₂ helps in the process of cumulus expansion, oocyte maturation, ruptured ovarian follicles, and oocyte release in the fallopian tubes¹⁸. The antifertility effect of curcumin in turmeric acts on the COX enzyme. Curcumin works to inhibit COX-2 downregulation. Therefore, the process of converting arachidonic acid to prostaglandins is inhibited¹⁹. After uterine examination in the treatment group of white rats that were given turmeric extract (*Curcuma longa* L.) for five days at a dose level of 250 mg/kg BW, 500 mg/kg BW, 1.000 mg/kg BW, it was found that there were differences in the average thickness of the endometrium layer of the strain rat *Sprague Dawley* in each group. The highest average endometrium thickness was found in the control group, the only group given feed and aquadest. The lowest mean endometrium thickness was obtained in Treatment group 3. Namely, the group was given turmeric extract (*Curcuma longa* L.) at a dose of 1.000 mg/kg BW. The control group showed an average endometrium thickness of $764.74 \pm 80.19 \mu\text{m}$. Treatment Group 1 (T1), namely the administration of turmeric extract (*Curcuma longa* L.) at a dose of 250 mg/kg BW, showed an average thickness of the endometrium layer of $615.06 \pm 119.50 \mu\text{m}$. Treatment Group 2 (T2), namely the administration of turmeric extract (*Curcuma longa* L.) at a dose of 500 mg/kg BW, showed an average thickness of the endometrium layer of $646.17 \pm 139.29 \mu\text{m}$. Treatment Group 3 (T3), namely the administration of turmeric extract at a dose of 1000 mg/kg BW showed the average thickness of the endometrium layer $566.18 \pm 68.74 \mu\text{m}$.

In this study, the results obtained were lower endometrium thickness in the treatment group compared with the control group. Curcumin has antifertility and antioovulation effects in the presence of antiestrogenic activity that inhibits the pituitary hypothalamus, causing estrogen receptor obstruction or decreased estrogen synthesis due to reduced cholesterol metabolism or both. Curcumin can reduce estrogen, namely estradiol 17- β , which plays a role in tissue proliferation, making up the endometrium layer¹. The addition of turmeric extract (*Curcuma longa* L.) in the treatment

group caused a decrease in the thickness of the endometrium layer. It illustrates the antifertility effect of turmeric extract (*Curcuma longa* L.).

In the *One-way Anova* test, the value of $P= 0.013$ ($P<0.05$) means that there is an antifertility effect of turmeric extract (*Curcuma longa* L.) on the thickness of the endometrium layer of Sprague Dawley rats. The antifertility effect is caused by turmeric (*Curcuma longa* L.) extract, which is weak in estrogen, causing changes in the usual biochemical environment of reproduction⁵. Turmeric extract (*Curcuma longa* L.) has antioovulation, antiimplantation, antiestrogenic effects that inhibit pregnancy. Antiestrogenic activity causes hypothalamic-pituitary inhibitors, which can inhibit ovulation and implantation²⁰. Adhesion molecules such as integrin $\alpha 3$ play a role in the process of implantation. If there is interference or damage to the endometrium can inhibit the expression of integrins $\alpha 3$, which can affect the window/implantation window or implantation window¹³.

The Post hoc LSD test showed that administration of turmeric extract (*Curcuma longa* L.) could significantly reduce the thickness of the endometrium layer between the control group and treatment group 1 (T1) with the value of $P= 0.014$ ($P> 0.05$), Treatment 2 (T2) with a value of $P = 0.047$ ($P> 0.05$). Treatment 3 (T3) with a value of $P = 0.002$ ($P<0.05$).

CONCLUSION

Turmeric rhizome extract has an antiestrogenic potential, reducing the female rat's total epithelium cells and endometrium layer thickness. It showed that turmeric has a potential effect as an antifertility agent. It can be made as an evaluation for embryo implantation to the endometrium mechanism onto the following research.

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Conflict of interest

The authors declare no conflict of interest.

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