HISTORY OF MANUSCRIPT PUBLICATION

(Biomedical and Pharmacology Journal)

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

Hendri Busman[1]

Sutyarso[1]

Salman Farisi[1]

Fukrapti[2]

Aulia Rika Fahrumnisa[2]

[1]Department of Biology, Faculty Mathematics and Natural Science, University of Lampung, Indonesia
[2]Faculty of Medicine, University of Lampung, Indonesia

DAFTAR ISI

I. SUBMISSION

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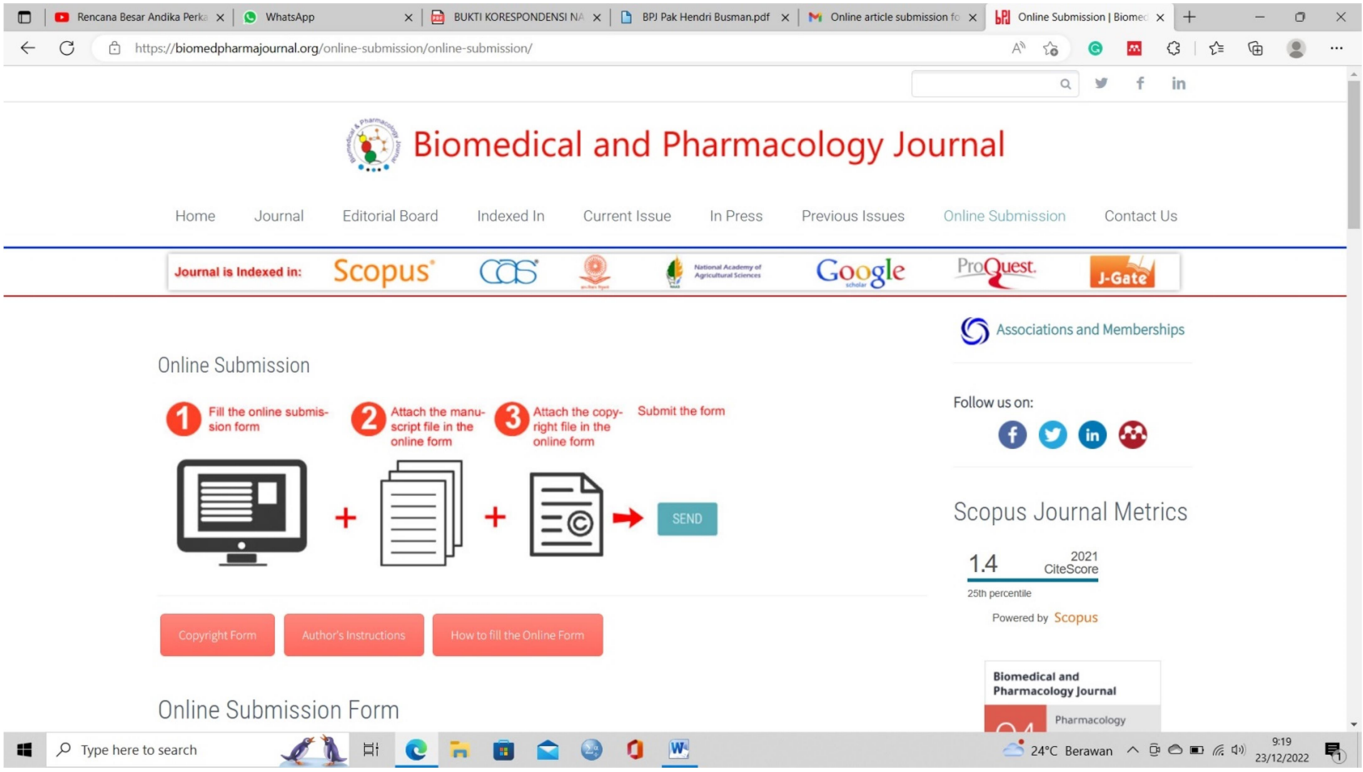

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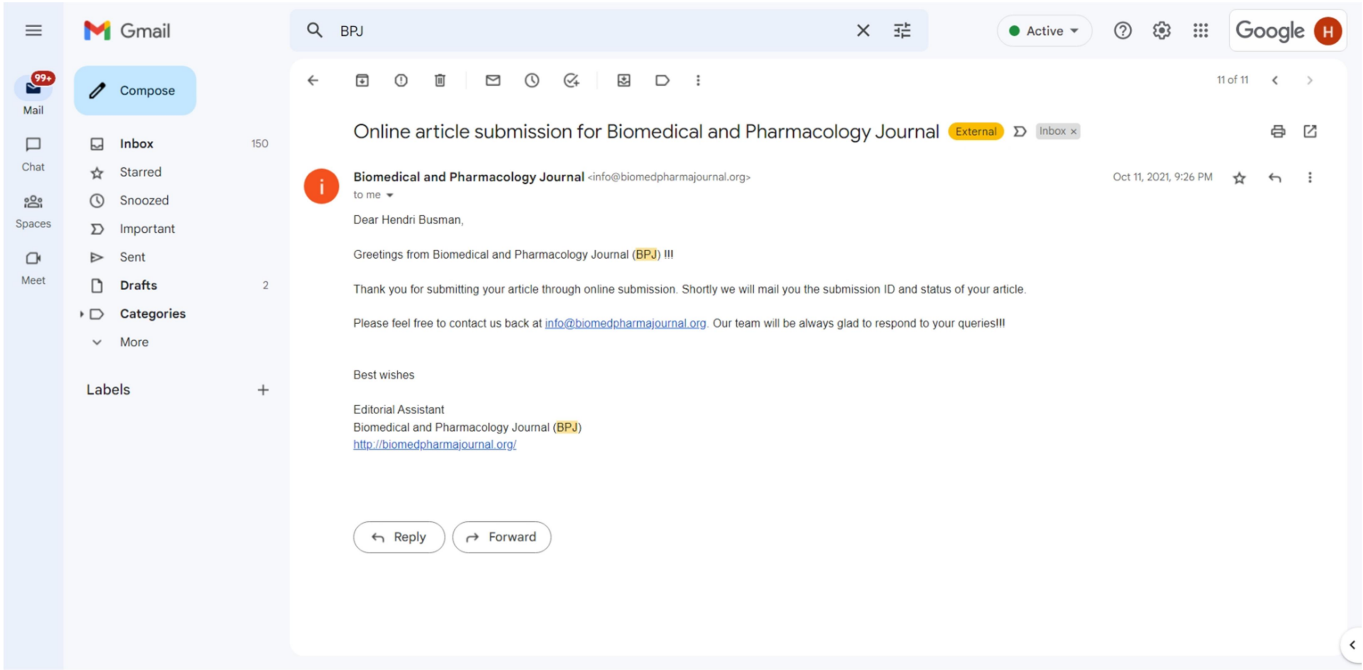

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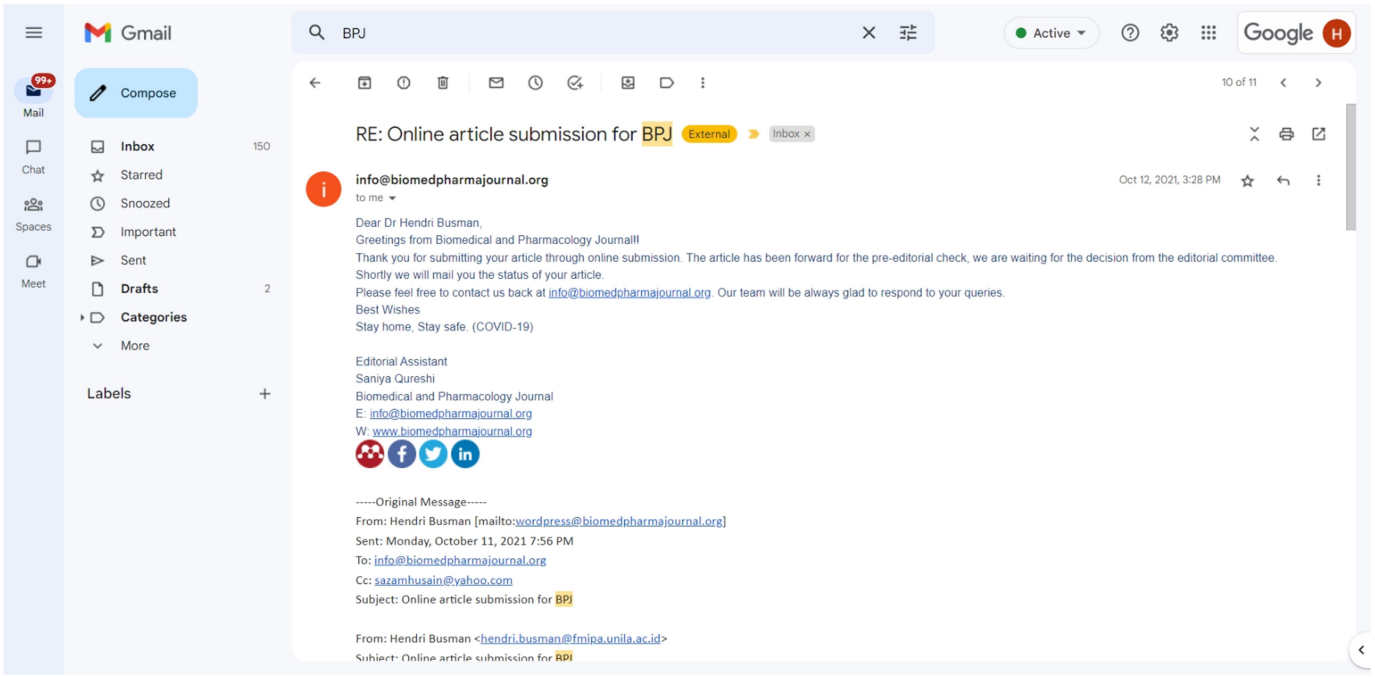

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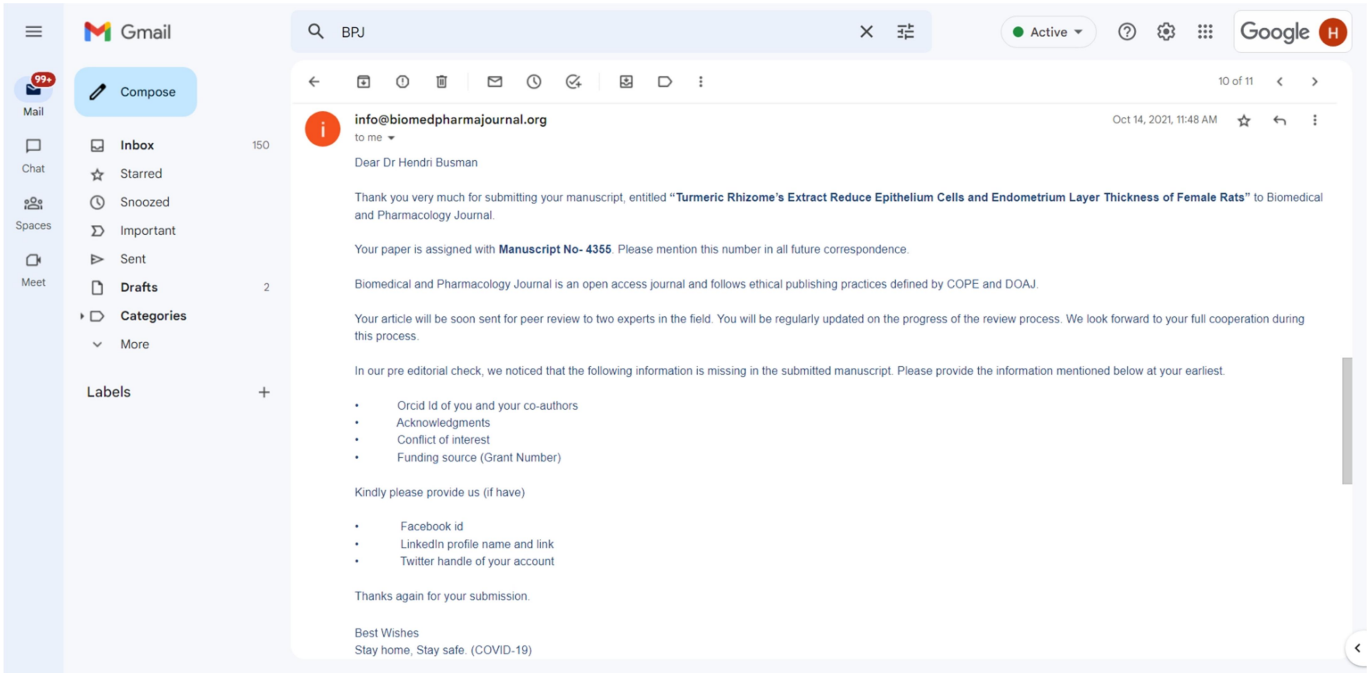

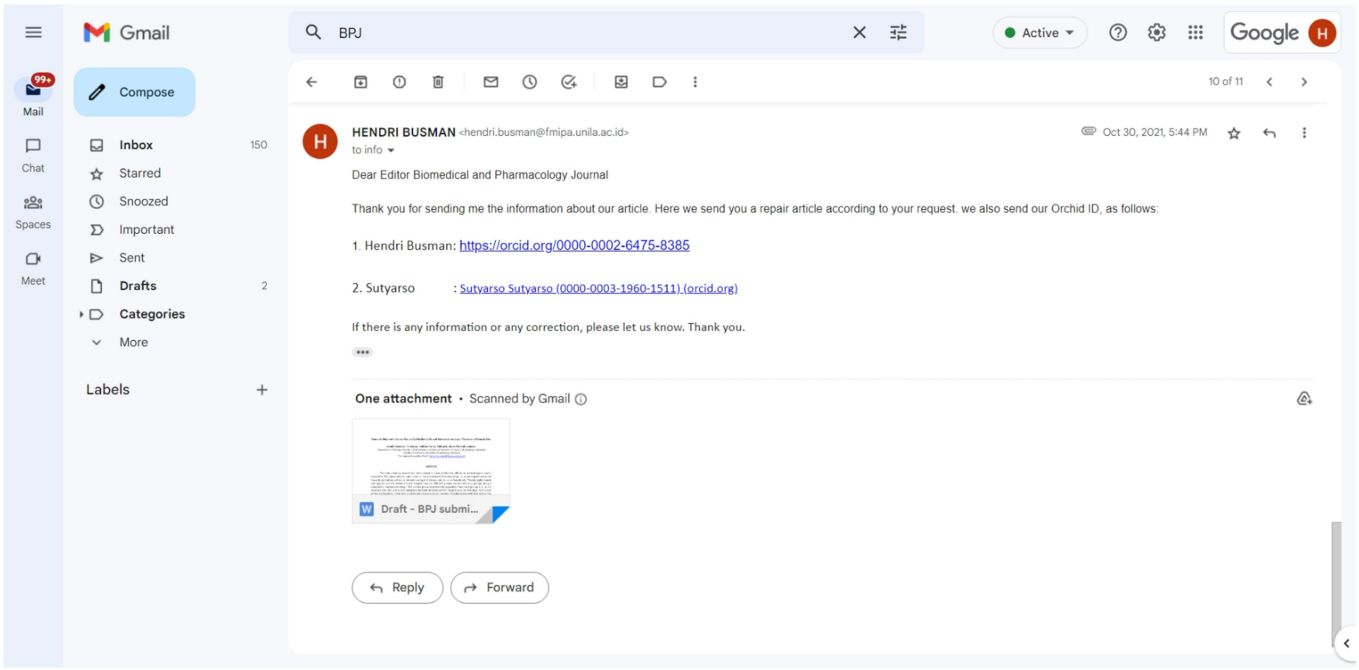

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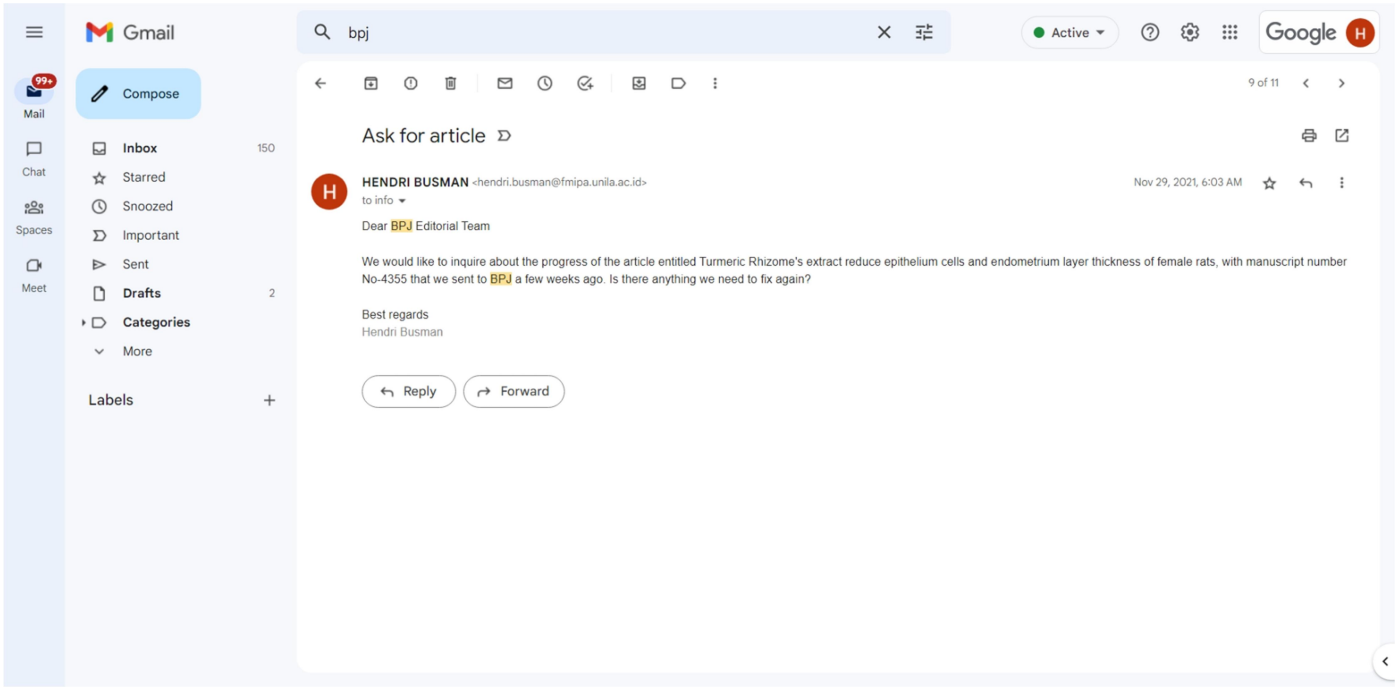

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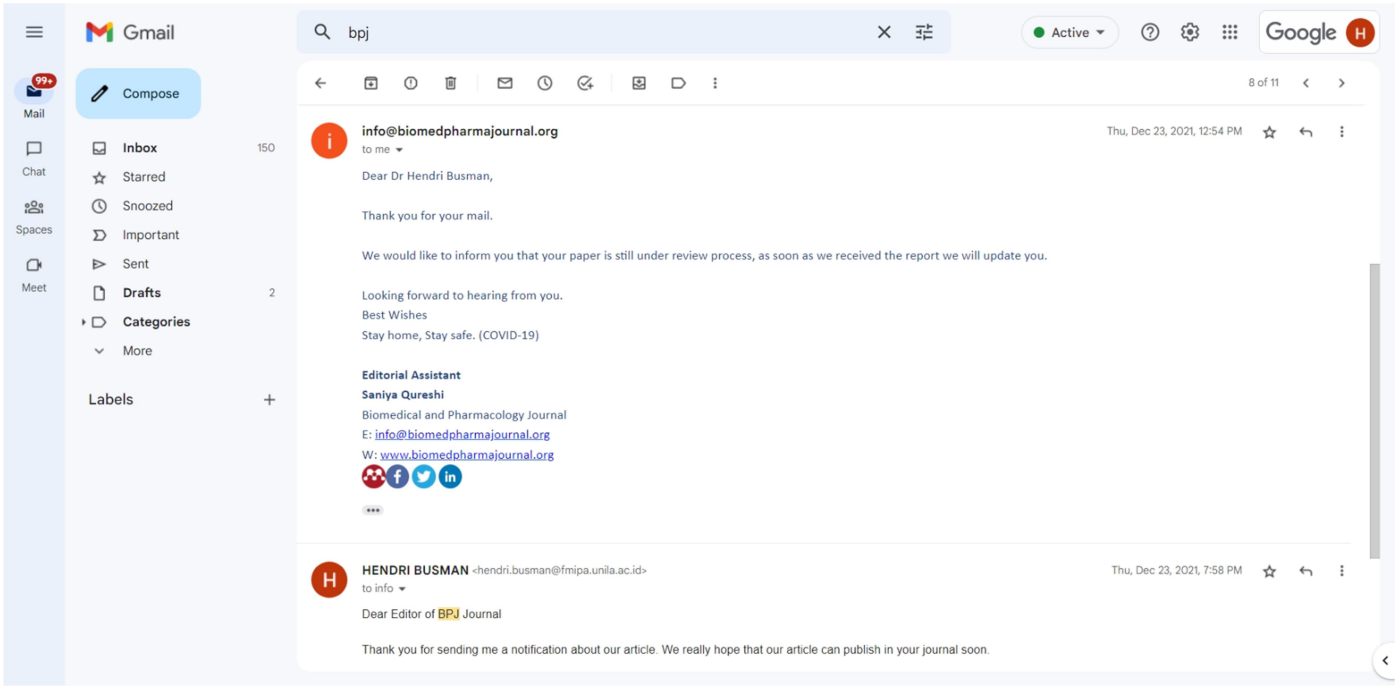

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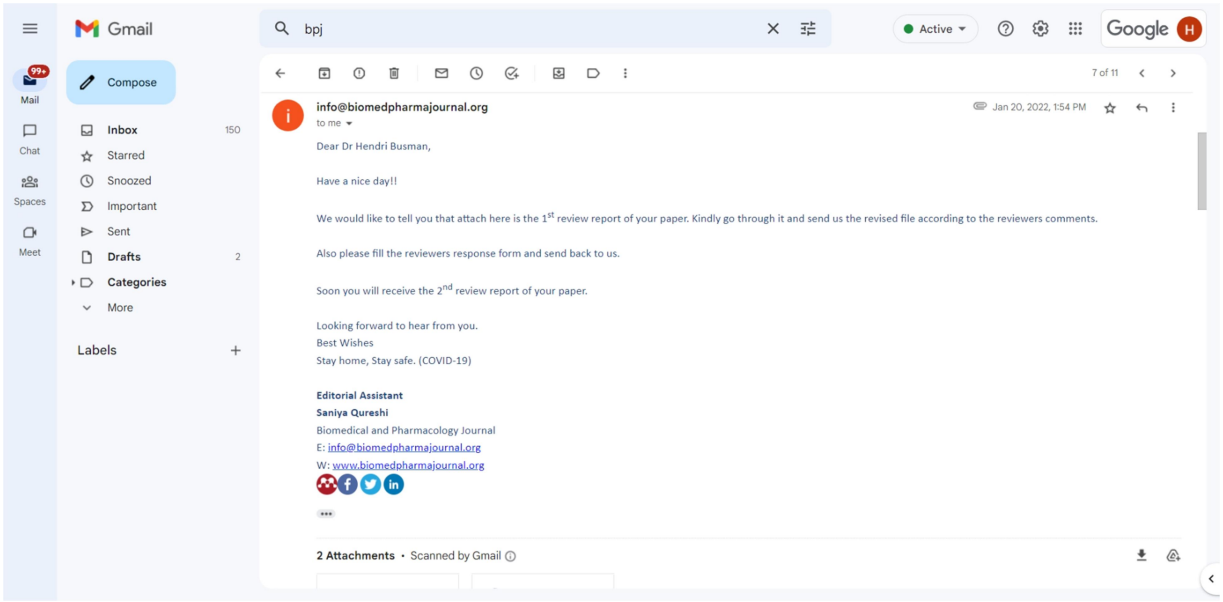

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Ask for the progress about our article

11 messages

HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Thu, Jan 20, 2022 at 8:22 AM
To: info@biomedpharmajournal.org

Dear BPJ editorial team

We want to ask about the progress of our article entitled Turmeric Rhizome's extract reduce epithelium cells and endometrium layer thickness of female rats, with manuscript number No-4355 that we sent to BPJ a few months ago. Is there any progress again?

We hope that our article can publish in your journal. We are looking forward to hearing from you soon.

Best regards
Dr Hendri Busman

info@biomedpharmajournal.org <info@biomedpharmajournal.org> Thu, Jan 20, 2022 at 1:54 PM
To: HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id>

Dear Dr Hendri Busman,

Have a nice day!!

We would like to tell you that attach here is the 1st review report of your paper. Kindly go through it and send us the revised file according to the reviewers comments.

Also please fill the reviewers response form and send back to us.

Soon you will receive the 2nd review report of your paper.

Looking forward to hear from you.

Best Wishes
Stay home, Stay safe. (COVID-19)

Editorial Assistant
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2 attachments

4355-Review Report.doc
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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Sat, Jan 22, 2022 at 9:26 AM
To: info@biomedpharmajournal.org

Dear Editor BPJ Journal

Thank you for sending me the 1st reviewer comment and suggestion. Here I send you a revision article as the reviewer requested. I also send the author response form. If there is any suggestion, please let me know.

We are looking forward to hearing from you soon.

Best Regards
Dr Hendri Busman, M. Biomed
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info@biomedpharmajournal.org <info@biomedpharmajournal.org> Sat, Jan 22, 2022 at 2:26 PM

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REVIEW REPO

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Note: This review report is divided into four sections to evaluate manuscript, kindly explain your observation in a precise manner in each section.

Manuscript Title: **Turmeric Rhizome's Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats**

Section I: Evaluation

1. Scope: Is the work fits better in the scope of the journal?
Comment: Yes.

2. Title: Is the title appropriate, informative, concise, and clear? Does the title clearly and sufficiently reflect the content?
Comment: Yes.

3. Abstract: Is it comprehensive by itself? Is the important and essential information of the article included?
Comment: Yes.

4. References: Are appropriate and adequate references to related works covered sufficiently in the list?
Comment: Yes.

5. Structure and length: Is the overall structure of the article well organized and well balanced? Is the article written with the minimum length necessary for all relevant information?
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6. Logic: Is the article written clearly and correctly? Is it logically consistent?
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7. Figures and tables: Are they essential and clearly presented?
Comment: Yes.

8. Discussion: Are all possible interpretations of the data considered or are there alternative hypotheses that are consistent with the available data?
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9. Conclusion: Are the conclusions of the study supported by appropriate evidence or are the claims exaggerated?
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Section II: Reviewer's remarks to author

Please recommend specific changes below in detail (if any). Review comments may also be listed by page and line number, or marked separately in the manuscript.
Comments:

Turmeric has been widely used in herbal medicine. In this study, the authors have demonstrated the antiestrogenic potential of turmeric rhizome extract on female white rat. They have observed the reduced number of epithelium cells and endometrium's layer thickness in treated rats.

Comments:
1. Check for minor grammatical errors - "The filtrate obtained will be concentrated or thickened using a rotary evaporator at a temperature of 500C for 1 hour."

2. "The number of epithelial endometrium cells was observed using a light microscope with 1.000x magnification in 10 visual fields." - Should this be 1000?

3. In the conclusion, more information is required to emphasize the importance of the present and future study.

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Section III: Overall Paper Rating
Please rate the following fields with the suitable number as: Excellent-5, Very good-4, Good-3, Fair-2, Poor-1 1. Originality of the paper: [4] 2. Relevance: [4] 3. Technical soundness: [3] 4. Significance: [3] 5.Clarity of presentation: [4]

Section IV- Referee's Recommendation

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☐ Accept unconditionally

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Title	Yes	-
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Figures and tables	Yes	-
Discussion	Yes	-
Conclusion	Yes	-
References (Appropriateness)	Accept with minor revisions	Ok, we will do that
Author's reply on Reviewer's Remark	Thank you for sending me some suggestion for revisions our article. We will do the revision that you requested.	

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

Hendri Busman,[*1] Sutyarso,[1] Salman Farisi,[1] Fukrapti,[2] Aulia Rika Fahrumnisa[2]
[1] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia
[2] Faculty of Medicine, University of Lampung, Indonesia
*Corresponding author Email: hendri.busman@fmipa.unila.ac.id

ABSTRACT

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.

INTRODUCTION

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 trophectoderm does not undergo adhesion, and the microfilic portion will assist the application of blastocysts. During adhesion and implantation, the gap between the trophectoderm cell membrane and the surface of the endometrium epithelium will decrease[1].

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 estrogen[1].

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 formed[4]. 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 sites[5].

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 50^0C 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).

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)	*P value*	Mean±SD (µm)	*P 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 α 5%. The mean followed by different letters indicates a significant difference based on the LSD test at 5%.

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 cells[6].

DISCUSSION

This study showed a decrease in the average number of epithelium cells in each 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 research[5], 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 progesterone[7].

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 phase[8].

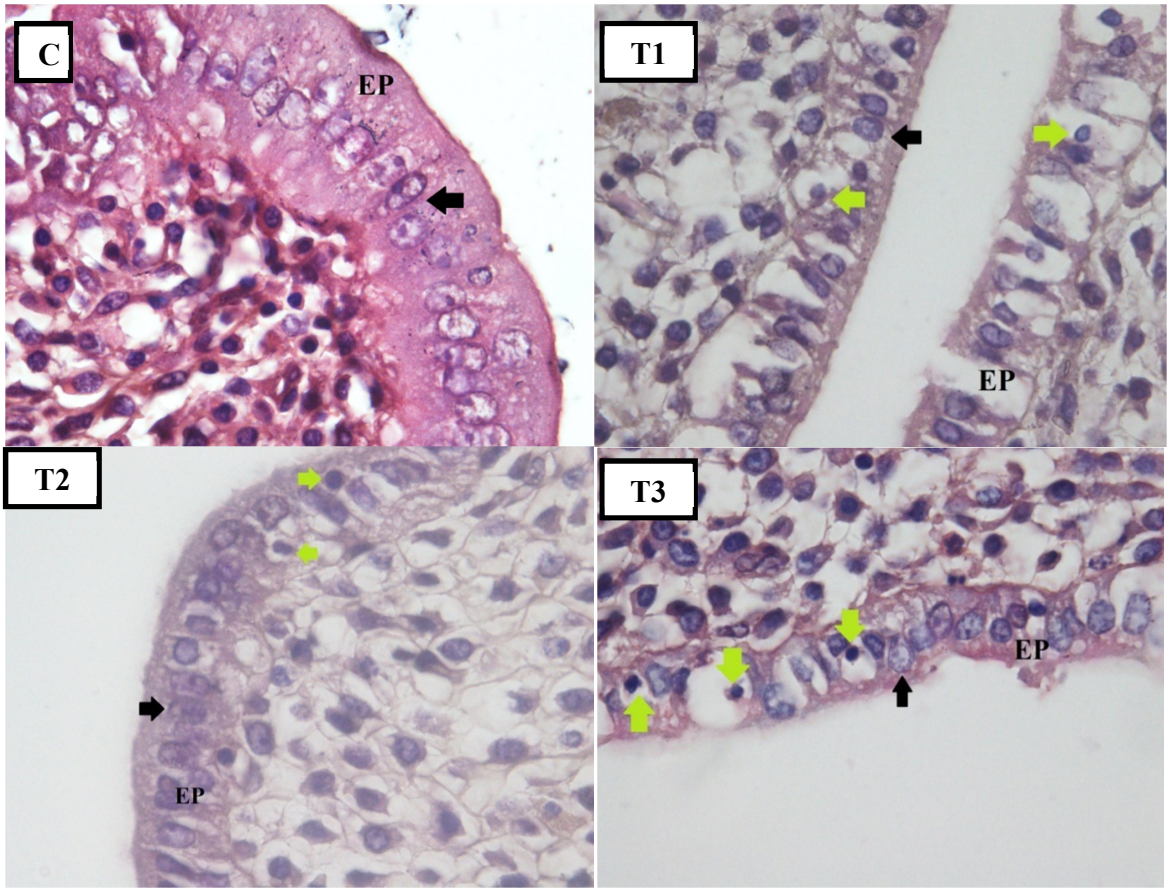

Figure 1. Endometrium epithelium cells with H.E. staining (magnification 100x). Epithelial cells (EP); Nucleus (black arrow); Apoptosis (green arrow); Control Group (C); Treatment 1 (T1); Treatment 2 (T2); Treatment 3 (T3).

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 formed[4].

Estrogen will work if it binds to receptors in endometrium epithelium cells, the receptors ERα, Erβ, 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-B[10]. The receptors are scattered on the surface of the endometrium[11]. This regulation is regulated explicitly by epithelium cells and endometrium stroma under the influence of estrogen[12]. 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 ß3, fibronectin, trophies, tastin, and blystin. The endometrium widely produces integrin ß3 during the menstrual cycle and pregnancy, which functions for adhesion, migration, invasion, and regulation of cellular signals. The expression integrin ß3 illustrates the value of endometrium receptivity[13]. 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 bisdemethoxycurcumin[14]. Curcumin is an ingredient that has the main therapeutic effect of turmeric[15]. Curcumin inhibits the formation of luteal steroid hormones (steroidogenesis) by inhibiting the accumulation of cyclic adenosine 3', 5' monophosphatase (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 pregnenolone[16].

It is known that curcuminoids can bind to the active site of enzymes that play a role in steroidogeneses, such as P450 side-chain cleavage (P450scc), CYP17A1, CYP19A1 (aromatase), and CYP21A2 so that enzymes are inhibited. The P45scc 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 fertility[17].

Prostaglandin E_2 (PGE_2) 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. PGE_2 helps in the process of cumulus expansion, oocyte maturation, ruptured ovarian follicles, and oocyte release in the fallopian tubes[18]. 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 inhibited[19]. 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 ± 80.19 µm. 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 ± 119.50 µm. 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 ± 139.29 µm. 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 ± 68.74 µm.

In this study, the results obtained were lower endometrium thickness in the treatment group compared with the control group. Curcumin has antifertility and antiovulation 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 layer[1]. 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 reproduction[5]. Turmeric extract (*Curcuma longa L.*) has antiovulation, antiimplantation, antiestrogenic effects that inhibit pregnancy. Antiestrogenic activity causes hypothalamic-pituitary inhibitors, which can inhibit ovulation and implantation[20]. Adhesion molecules such as integrin β3 play a role in the process of implantation. If there is interference or damage to the endometrium can inhibit the expression of integrins β3, which can affect the window/implantation window or implantation window[13].

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.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We wish to thank the Pathology and Histology laboratory of the Faculty of Medicine, the University of Lampung, for their cooperation and pathological analyses.

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20. Niazi J, Poonia P, Gupta V, Kaur N. Pharmacotherapeutics of *Curcuma longa* – a potent patent. *International journal of pharma professional's research*, 1(1):24-33 (2018).

To: HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id>

Dear Dr Hendri Busman,

Thank you for your mail and for sending us the revised file and author response form.

Soon we will send you the 2nd review report of your paper.

Looking forward to hearing from you.

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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Tue, Feb 15, 2022 at 10:04 AM
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We want to ask about the progress of our article entitled Turmeric Rhizome's extract reduce epithelium cells and endometrium layer thickness of female rats, with manuscript number No-4355. Is there any comment from the 2nd reviewer again?

We hope that our article can publish in your journal. We are looking forward to hearing from you soon.

Best regards
Dr Hendri Busman
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info@biomedpharmajournal.org <info@biomedpharmajournal.org> Wed, Feb 16, 2022 at 3:30 PM
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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Thu, Feb 17, 2022 at 8:25 AM
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Best Regards
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info@biomedpharmajournal.org <info@biomedpharmajournal.org> Tue, Mar 1, 2022 at 4:47 PM
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We would like to inform you that attach here is the 2nd review report of your paper.

Kindly go through it and send us the revised file as per reviewer comments.

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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Thu, Mar 3, 2022 at 10:09 AM

Biomedical and Pharmacology

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

Section I: Evaluation

1. Scope: Is the work fits better in the scope of the journal?
Comment: Yes

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6. Logic: Is the article written clearly and correctly? Is it logically consistent?
Comment: Yes

7. Figures and tables: Are they essential and clearly presented?
Comment: yes,

8. Discussion: Are all possible interpretations of the data considered or are there alternative hypotheses that are consistent with the available data?
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10. Language: Is the English used in the article readable and good enough to convey the scientific meaning correctly?
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REVIEW REPO

Section II: Reviewer's remarks to author

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Comments:

1. Paper is good

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REVIEW REPO

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Please rate the following fields with the suitable number as: Excellent-5, Very good-4, Good-3, Fair-2, Poor-1 1. Originality of the paper: [4] 2. Relevance: [3] 3. Technical soundness: [2] 4. Significance: [3] 5.Clarity of presentation: [2]

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Please tick the appropriate option:

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Best regards
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Biomedical and Pharmacology

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

Please reply the comments from the reviewer(s) point-to-point on one list but not on your manuscript during the revision.

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Figures and tables	Yes	No Comment
Discussion	Yes	No Comment
Conclusion	Result must be specific	Ok, we will fine it
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Turmeric Rhizome's Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats

Hendri Busman,[*1] Sutyarso,[1] Salman Farisi,[1] Fukrapti,[2] Aulia Rika Fahrumnisa[2]
[1] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia
[2] Faculty of Medicine, University of Lampung, Indonesia
*Corresponding author Email: hendri.busman@fmipa.unila.ac.id

ABSTRACT

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.

INTRODUCTION

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 trophectoderm does not undergo adhesion, and the microfilic portion will assist the application of blastocysts. During adhesion and implantation, the gap between the trophectoderm cell membrane and the surface of the endometrium epithelium will decrease[1].

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 estrogen[1].

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 formed[4]. 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 sites[5].

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 50^0C 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).

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)	*P value*	Mean±SD (µm)	*P 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 α 5%. The mean followed by different letters indicates a significant difference based on the LSD test at 5%.

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 cells[6].

DISCUSSION

This study showed a decrease in the average number of epithelium cells in each 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 research[5], 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 progesterone[7].

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 phase[8].

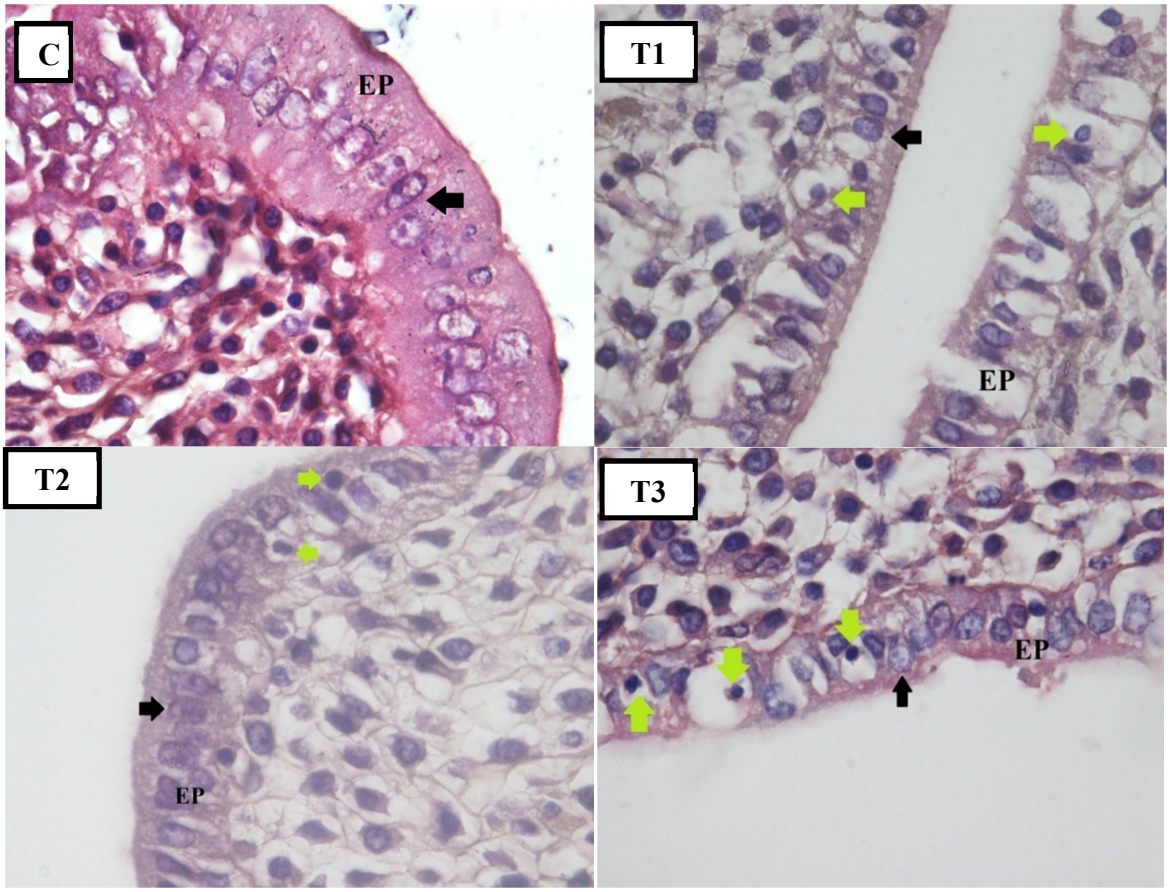

Figure 1. Endometrium epithelium cells with H.E. staining (magnification 1000x). Epithelial cells (EP); Nucleus (black arrow); Apoptosis (green arrow); Control Group (C); Treatment 1 (T1); Treatment 2 (T2); Treatment 3 (T3).

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 formed[4].

Estrogen will work if it binds to receptors in endometrium epithelium cells, the receptors ERα, Erβ, 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-B[10]. The receptors are scattered on the surface of the endometrium[11]. This regulation is regulated explicitly by epithelium cells and endometrium stroma under the influence of estrogen[12]. 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 ß3, fibronectin, trophies, tastin, and blystin. The endometrium widely produces integrin ß3 during the menstrual cycle and pregnancy, which functions for adhesion, migration, invasion, and regulation of cellular signals. The expression integrin ß3 illustrates the value of endometrium receptivity[13]. 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 bisdemethoxycurcumin[14]. Curcumin is an ingredient that has the main therapeutic effect of turmeric[15]. Curcumin inhibits the formation of luteal steroid hormones (steroidogenesis) by inhibiting the accumulation of cyclic adenosine 3', 5' monophosphatase (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 pregnenolone[16].

It is known that curcuminoids can bind to the active site of enzymes that play a role in steroidogeneses, such as P450 side-chain cleavage (P450scc), CYP17A1, CYP19A1 (aromatase), and CYP21A2 so that enzymes are inhibited. The P45scc 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 fertility[17].

Prostaglandin E_2 (PGE_2) 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. PGE_2 helps in the process of cumulus expansion, oocyte maturation, ruptured ovarian follicles, and oocyte release in the fallopian tubes[18]. 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 inhibited[19]. 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 ± 80.19 µm. 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 ± 119.50 µm. 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 ± 139.29 µm. 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 ± 68.74 µm.

In this study, the results obtained were lower endometrium thickness in the treatment group compared with the control group. Curcumin has antifertility and antiovulation 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 layer[1]. 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 reproduction[5]. Turmeric extract (*Curcuma longa L.*) has antiovulation, antiimplantation, antiestrogenic effects that inhibit pregnancy. Antiestrogenic activity causes hypothalamic-pituitary inhibitors, which can inhibit ovulation and implantation[20]. Adhesion molecules such as integrin β3 play a role in the process of implantation. If there is interference or damage to the endometrium can inhibit the expression of integrins β3, which can affect the window/implantation window or implantation window[13].

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.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We wish to thank the Pathology and Histology laboratory of the Faculty of Medicine, the University of Lampung, for their cooperation and pathological analyses.

Funding source

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20. Niazi J, Poonia P, Gupta V, Kaur N. Pharmacotherapeutics of *Curcuma longa* – a potent patent. *International journal of pharma professional's research*, 1(1):24-33 (2018).

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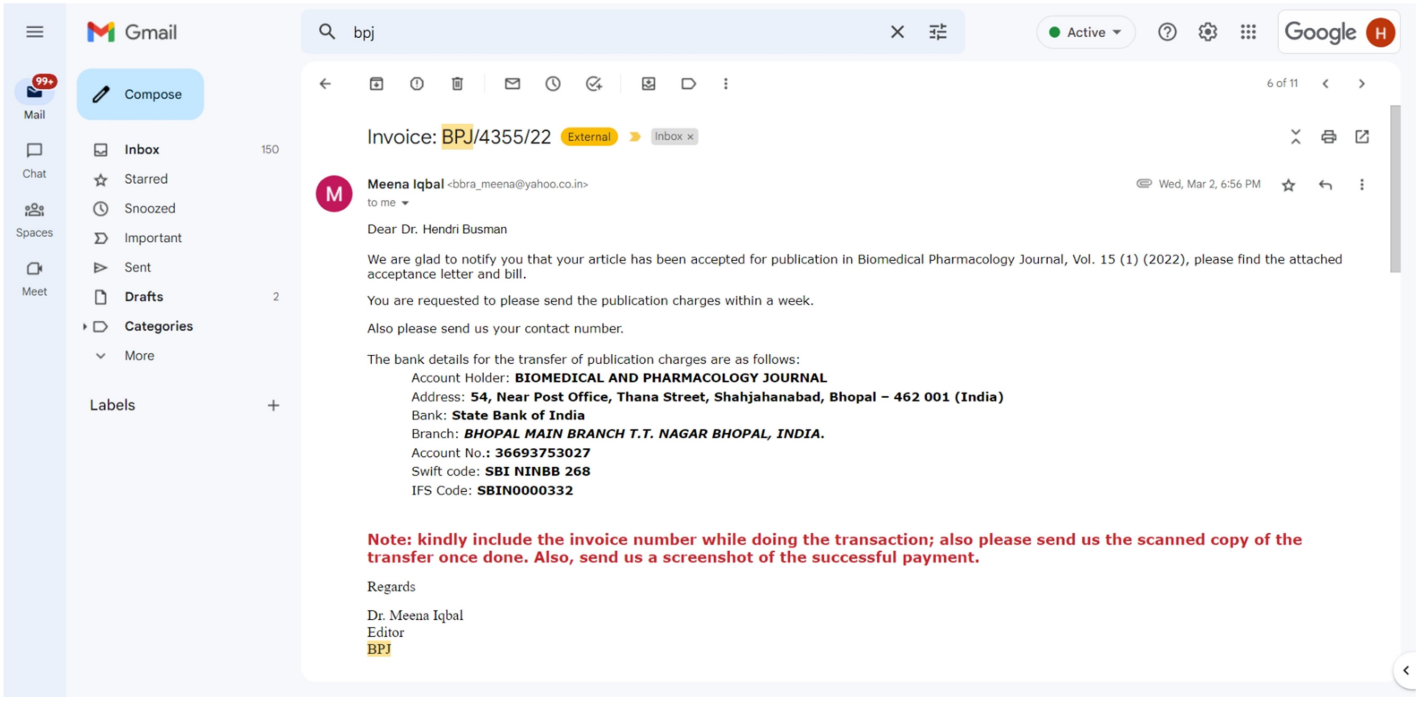

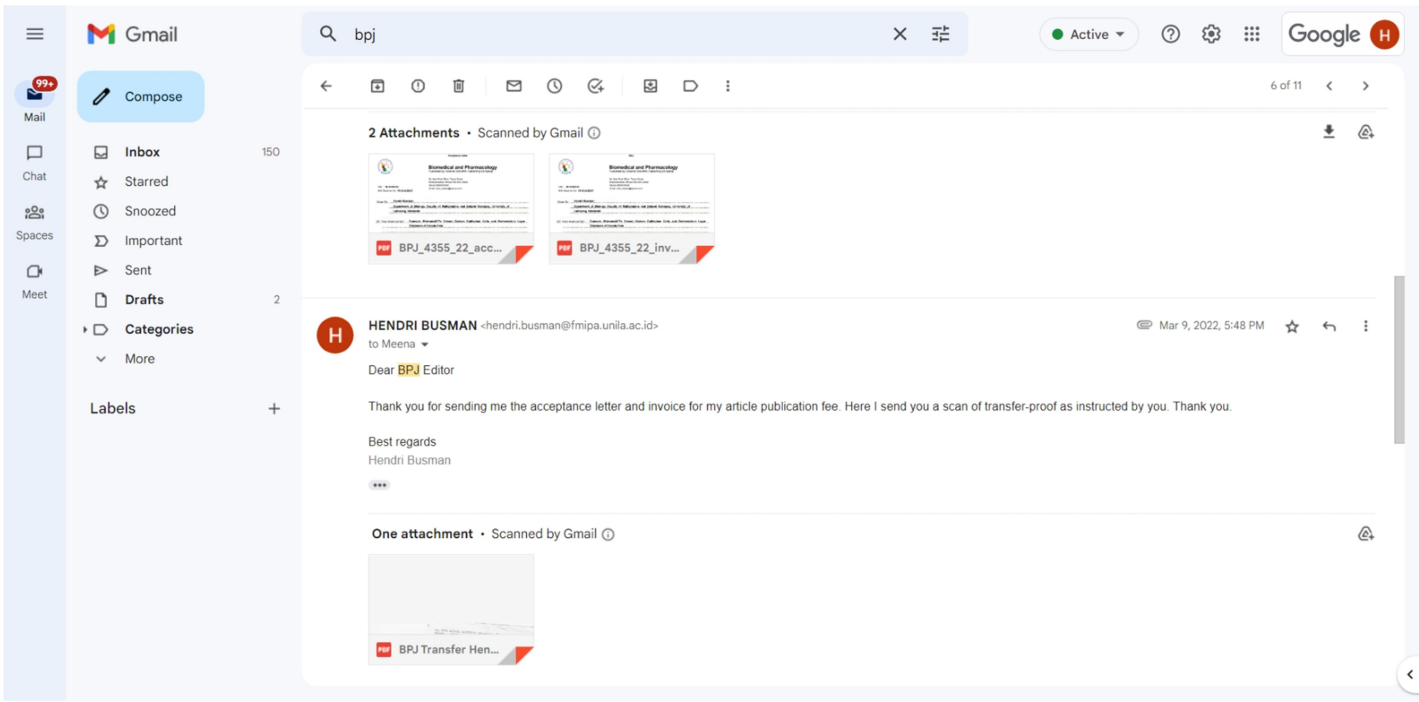

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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Wed, Mar 9, 2022 at 5:48 PM
To: Meena Iqbal <bbra_meena@yahoo.co.in>

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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Mon, Mar 14, 2022 at 8:05 AM
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Meena Iqbal <bbra_meena@yahoo.co.in> Tue, Mar 15, 2022 at 11:17 AM
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Dear Dr. **Hendri Busman**

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia

(A) Your manuscript **Turmeric Rhizomeâ€™s Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats**

has been accepted for publication in **Biomedical and Pharmacology** Vol. **15** No. **01** 20**22**

(B) To expedite the process of publication please send your subscription charges and of your co-authors **Sutyarso, Salman Farisi, Fukrapti,Aulia Rika Fahrumnisa**

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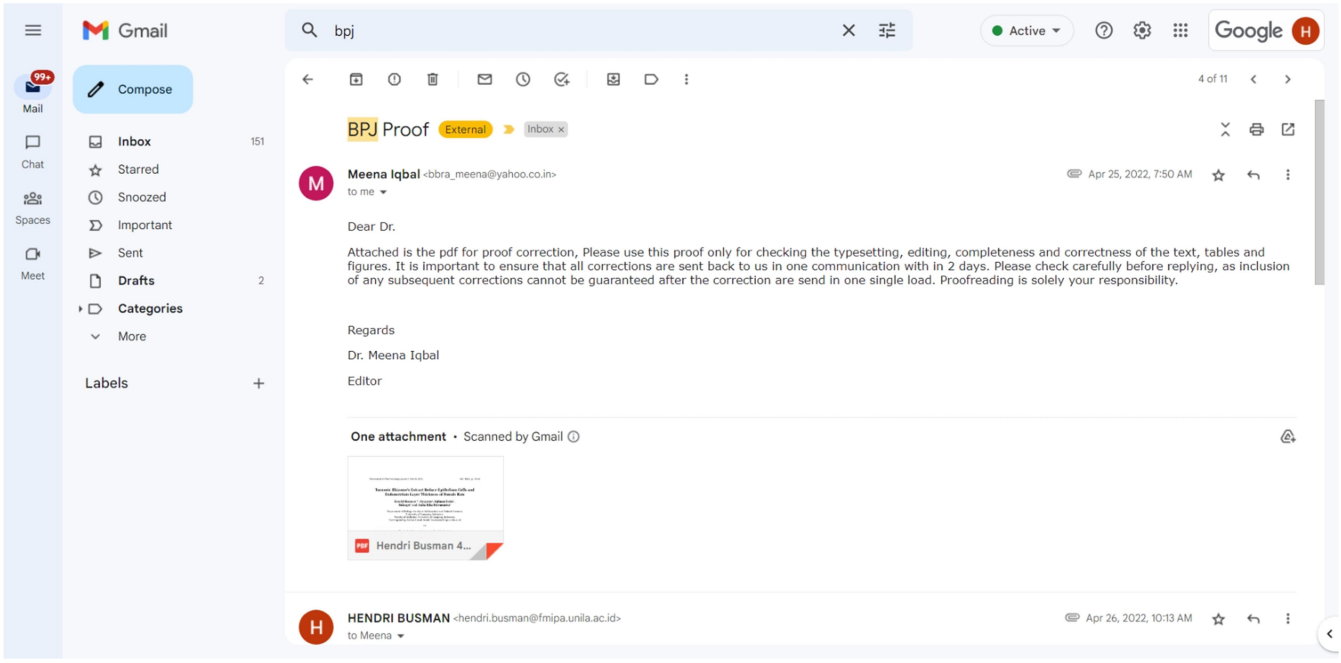
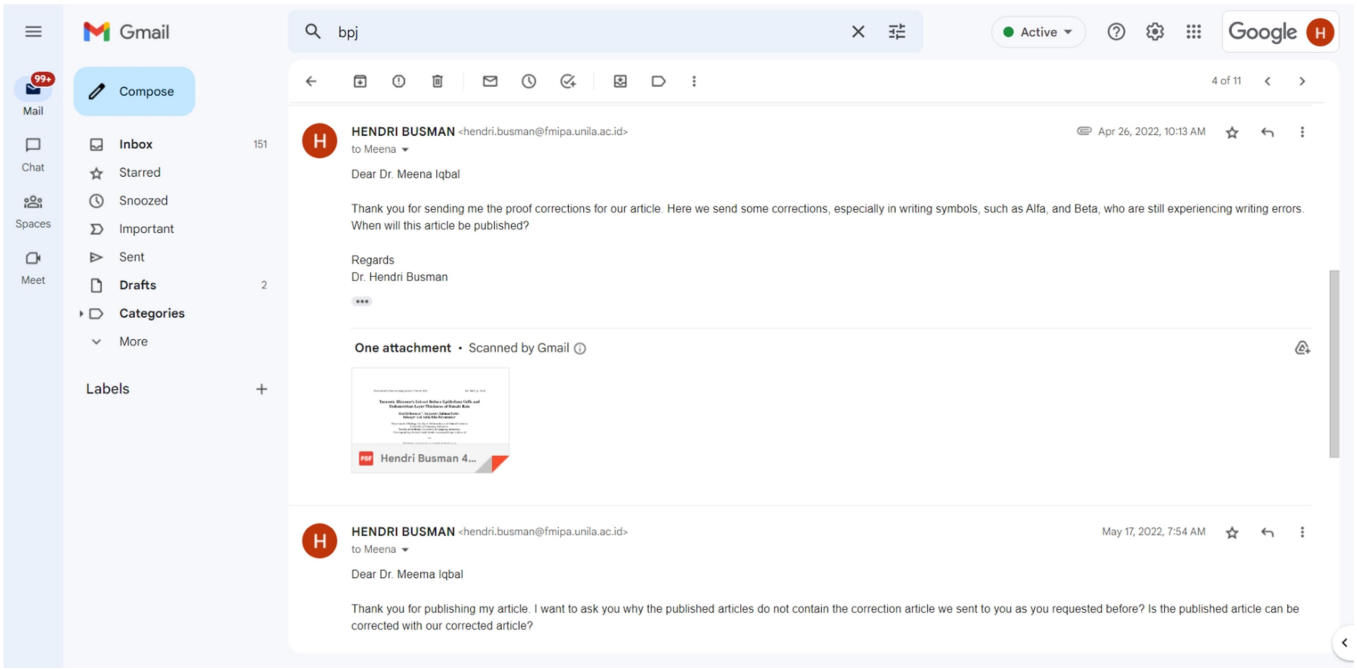
VI. FINAL PROOF

April 25, 2022





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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Tue, May 17, 2022 at 7:54 AM
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To: HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id>

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Sorry for the inconvenience.

Correction of your article will update soon.

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Dr. Meena Iqbal Editor

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HENDRI BUSMAN <hendri.busman@fmipa.unila.ac.id> Wed, May 18, 2022 at 8:43 AM
To: Meena Iqbal <bbra_meena@yahoo.co.in>

Thank you for your response.
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Turmeric Rhizome's Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats

Hendri Busman[1]*, Sutyarso[1], Salman Farisi[1], Fukrapti[2] and Aulia Rika Fahrumnisa[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia.
[2]Faculty of Medicine, University of Lampung, Indonesia.
*Corresponding Author E-mail: hendri.busman@fmipa.unila.ac.id

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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 trophectoderm does not undergo adhesion, and the microfilic portion will assist the application of blastocysts. During adhesion and implantation, the gap between the trophectoderm cell membrane and the surface of the endometrium epithelium will decrease[1].

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 estrogen[1].

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 formed[4]. 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 sites[5].

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 50^0C 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 cells[6].

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)	*P value*	Mean±SD (µm)	*P 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%. nean followed by different letters indicates a significant difference based on the LSD test at 5%.

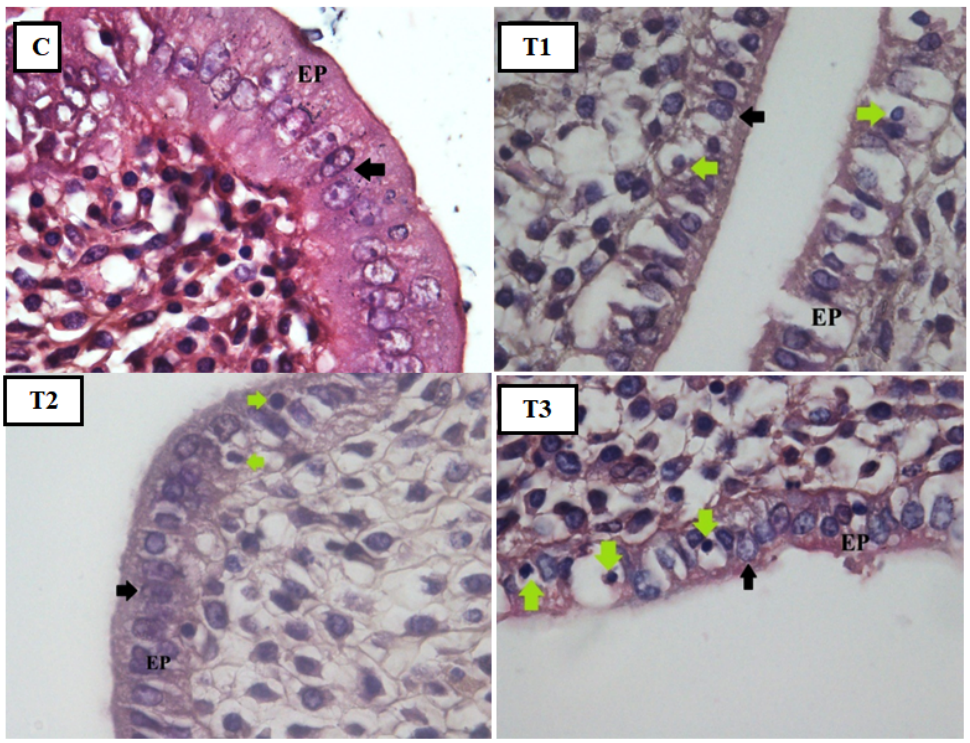

Fig. 1. Endometrium epithelium cells with H.E. staining (magnification 1000x). Epithelial cells (EP); Nucleus (black arrow); Apoptosis (green arrow); Control Group (C); Treatment 1 (T1); Treatment 2 (T2); Treatment 3 (T3)

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 research[5], 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 progesterone[7].

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 phase[8].

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 formed[4].

Estrogen will work if it binds to receptors in endometrium epithelium cells, the receptors ERá, Erâ, G-protein-coupled estrogen receptor 1 (GPER1)[9]. Like estrogen, progesterone works when it binds to progesterone receptors (PR), namely PR-A and PR-B[10]. The receptors are scattered on the surface of the endometrium[11]. This regulation is regulated explicitly by epithelium cells and endometrium stroma under the influence of estrogen[12]. 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 ß3, fibronectin, trophies, tastin, and blystin. The endometrium widely produces integrin ß3 during the menstrual cycle and pregnancy, which functions for adhesion, migration, invasion, and regulation of cellular signals. The expression integrin ß3 illustrates the value of endometrium receptivity[13]. 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 bisdemethoxycurcumin[14]. Curcumin is an ingredient that has the main therapeutic effect of turmeric[15]. Curcumin inhibits the formation of luteal steroid hormones (steroidogenesis) by inhibiting the accumulation of cyclic adenosine 3', 5' monophosphatase (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 pregnenolone[16].

It is known that curcuminoids can bind to the active site of enzymes that play a role in steroidogeneses, such as P450 side-chain cleavage (P450scc), CYP17A1, CYP19A1 (aromatase), and CYP21A2 so that enzymes are inhibited. The P45scc 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 fertility[17].

Prostaglandin E_2 (PGE_2) 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. PGE_2 helps in the process of cumulus expansion, oocyte maturation, ruptured ovarian follicles, and oocyte release in the fallopian tubes[18]. 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 inhibited[19]. 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 ± 80.19 µm. 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 ± 119.50 µm. 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 ± 139.29 µm. 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 ± 68.74 µm.

In this study, the results obtained were lower endometrium thickness in the treatment group compared with the control group. Curcumin has antifertility and antiovulation 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-â, h plays a role in tissue proliferation, making up the endometrium layer[1]. 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 reproduction[5]. Turmeric extract (*Curcuma longa L.*) has antiovulation, antiimplantation, antiestrogenic effects that inhibit pregnancy. Antiestrogenic activity causes hypothalamic-pituitary inhibitors, which can inhibit ovulation and implantation[20]. Adhesion molecules such as integrin â3 a role in the process of implantation. If there is interference or damage to the endometrium can inhibit the expression of integrins â3. ch can affect the window/implantation window or implantation window[13].

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.

ACKNOWLEDGMENTS

We wish to thank the Pathology and Histology laboratory of the Faculty of Medicine, the University of Lampung, for their cooperation and pathological analyses.

Conflict of interest

The authors declare no conflict of interest.

Funding source

This research was fully funded by the authors.

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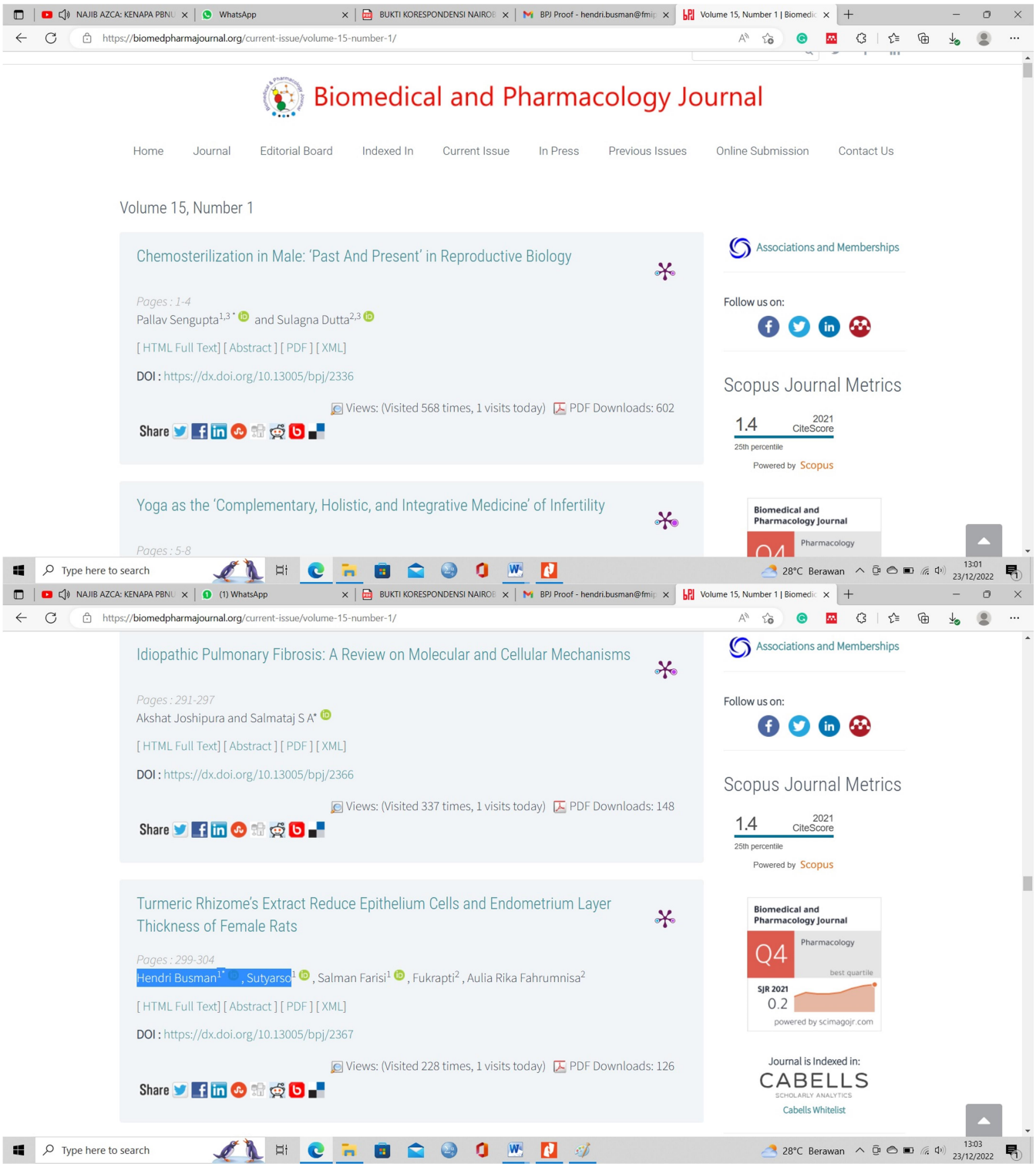

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Turmeric Rhizome's Extract Reduce Epithelium Cells and Endometrium Layer Thickness of Female Rats

Hendri Busman[1*], Sutyarso[1], Salman Farisi[1], Fukrapti[2], Aulia Rika Fahrumnisa[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia

[2]Faculty of Medicine, University of Lampung, Indonesia

Corresponding Author E-mail: hendri.busman@fmipa.unila.ac.id

DOI : https://dx.doi.org/10.13005/bpj/2367

Abstract

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

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