INTRODUCTION

Endometriosis is a pathological disease caused by the uncontrolled proliferation of ectopic tissue outside the endometrial cavity [1]. Giudice (2010) defines endometriosis as an inflammatory condition characterized by tissue lesions such as endometrium outside the uterus and generally associated by pelvic pain and infertility [2]. The endometriosis lesions growth could trigger the concentration of proinflammatory cytokines and growth factors which have a role in adhesion, angiogenesis, and persistence of ectopic endometriosis growth [4-6].

IL-1β is a proinflammatory cytokine that stimulates endometriosis cells producing cytokines and growth factors which have a role in adhesion, growth, invasion, inflammation, and angiogenesis in endometriosis [7,8]. Previous studies showed that IL-1β induced the expression of vascular endothelial growth factor (VEGF) and COX-2, angiogenic factors, in some cancers. As already known, COX-2 and VEGF play an important role in the angiogenesis process in endometriosis [9,10].

At present, the management of endometriosis focused on hormonal therapy and conservative surgery, resulting in approximately 50% of women with endometriosis reduced pain [11], and the recurrence rate after endometriosis is around 11–32% within 1–5 years [12]. Therefore, it is suggested to develop an advanced and effective method as promising management of endometriosis.

ABSTRACT

Objective: The aim of this study is to analyze the effect of octyl gallate and heptyl gallate toward the regulation of interleukin-1β and cyclooxygenase (COX)-2 proinflammatory factor on endometriosis cell culture and analyze its activity toward nuclear factor kappa B (NFkB) target protein through in silico docking technique.

Methods: In vitro study was performed on endometriosis cells cultured treated with two dosages each of heptyl and octyl gallate (51.2 μg/mL and 102.4 μg/mL) for 48 h, then followed by 10 μg/mL lipopolysaccharides (LPS) induction for 24 h. The positive control group was treated by LPS induced and the negative control was treated without LPS. Inflammation regulation was evaluated with enzyme-linked immunosorbent assay technique and in silico docking analyzed using bioinformatics technique.

Results: Molecular docking analysis with gallic acid and their derivatives showed that more stable affinity and stronger binding found on octyl gallate than heptyl gallate and gallic acid at the active site of NFkB.

Conclusions: Based on this study results, octyl gallate and heptyl gallate were proven to be able to reduce COX-2 proinflammatory factor through NFkB pathway as an inflammatory regulator; thus, it has the potential to be developed as a therapy for endometriosis.

Keywords: Heptyl gallate, Octyl gallate, Endometriosis, In silico docking factor kappa B, Cyclooxygenase-2, Interleukin-1β.
with in silico docking techniques to obtain compounds which more potential, stable, and has specific activities in inhibiting NFkB [17,18].

The purpose of this study was to identify the bond strength and potential inhibition of gallic acid derivatives, heptyl gallate, and octyl gallate, toward the protein target, NF-kB through in silico docking technique and the effect toward the regulation of pro-inflammatory cytokines, IL-1β and COX-2, in primary cultures of endometriosis cells.

MATERIALS AND METHODS

Materials
Gallic acid was synthesized by the Chemical Department FKUI, fetal bovine serum (FBS), Fungizone, powder Dulbecco’s modified eagle’s medium F-12 (DMEM F-12) from Gibco/Life Tech USA, penicillin/streptomycin (Sigma-Aldrich), phosphate buffered saline (Merck, IL1β), and COX-2 ELISA Kit (Quantikine R and D and MyBioSource).

In silico docking analysis
In silico docking, the study was performed to analyze docking energy values (ΔG) and amino acids association in the process of interaction between macro NFkB molecules and ligands (octyl gallate and heptyl gallate) using software Marvin Sketch, AutoDock, PyMOL, and LigPlus which are designed for docking.

Isolation and primary culture of endometriosis tissue
The endometriosis tissues patients’ were obtained using laparoscopy procedure, they were put in the transport medium (DMEM F-12 containing 2% penicillin/streptomycin and 2% Fungizone). Then endometriosis cells are obtained by isolating enzymatically cells using Type IV collagenase and culturing it until the cells reached 6×10^5 in complete medium (DMEM F-12 containing 1% penicillin/streptomycin, 1% Fungizone, and 20% FBS). The 2.5×10^4 cells/well were grown in 12 well plates, then treated with heptyl and octyl gallate with two doses (51.2 μg/mL and 102.4 μg/mL) for 48 h, followed by induction of 10 ng/mL lipopolysaccharides (LPS) for 24 h. The positive control group only induced by LPS, and negative control was treated without LPS.

Analysis of levels of cytokines IL-1β and COX-2
Inflammatory regulation was assessed from the level of cytokines, IL-1β and COX-2, in primary cultures of endometriosis cells toward pro-inflammatory cytokines IL-1β in this study showed only induced by LPS, and negative control was treated without LPS. The effect of heptyl gallate and octyl gallate on primary endometriosis cells toward pro-inflammatory cytokines IL-1β in this study showed

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Binding energy score (kcal/mol)</th>
<th>pKi (μM)</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gallic acid</td>
<td>−7.66</td>
<td>2.42</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Heptyl gallate</td>
<td>−7.68</td>
<td>2.37</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Octyl gallate</td>
<td>−7.98</td>
<td>1.41</td>
<td>3</td>
</tr>
</tbody>
</table>

NFkB: Nuclear factor kappa B, pKi: Inhibition constant, HB: Hydrogen bonds

Table 1: Results of in silico docking between ligands and NFkB

Fig. 1: Visualization of nuclear factor kappa residual interactions with compounds (a) Gallic acid, (b) Heptyl gallate, (c) Octyl gallate

RESULTS

Based on the results of in silico docking analysis, the docking energy (binding energy score/ΔG) of gallic acid compounds, heptyl gallate and octyl gallate as ligand against protein NFkB targets respectively −7.66 kcal/mol, −7.68 kcal/mol and −7.98 kcal/mol. ΔG shows the strength of the ligand affinity with the target protein, in which the higher of the negative ΔG value the interaction and conformation between ligand and protein will be more constant and stable [19]. In this study, the octyl gallate showed a stronger and more stable affinity toward NFkB than heptyl gallate and gallic acid.

The amino acids through hydrogen bonds (HB), Tyr285, Lys221, Ser222, Ser226, Ser220, Pro223, and Lys252, performed the bond between ligands with NFkB residues at a distance of <3.31 Å (Fig. 1). HB which could increase the ligand activity was found in the amino acid residues Ala225. The octyl gallate had 3 HB with amino acids Lys221, Ser222, and Ala225, while heptyl had two amino acids, Ser226 and Lys252. The quantity of HB made ligand interactions between NFkB proteins and compounds heptyl gallate and octyl gallate became stronger. Thus, the octyl gallate showed more potent for interaction and inhibitory activity toward NFkB than the heptyl gallate. Another docking indicator is the value of the inhibition constant (pKi), which showed the inhibitor value between the ligand-protein complexes. A low pKi value is a good indicator for the formation of a ligand-protein complex. The result of ΔG, pKi and HB is presented in Table 1.

The effect of heptyl gallate and octyl gallate on primary endometriosis cells toward pro-inflammatory cytokines IL-1β in this study showed
derivatives of gallic acid such as heptyl gallate and octyl gallate had the ability to suppress proliferation and induced apoptosis in some cancer cells [12].

CONCLUSIONS

In the *in silico* docking study showed octyl gallate had a stronger binding and more stable affinity in inhibiting NFkB protein because it had the highest docking energy (ΔG), the lowest pKi, and the highest number of HB compared to heptyl gallate and gallic acid. In addition, we proved that octyl gallate and heptyl gallate have the potential to suppress IL-1β secretion driving to NFκB mRNA expression and COX-2 decrease in endometriosis cells. Octyl gallate and heptyl gallate can be developed as promising agents in the management of endometriosis through their inhibitory effects in the proinflammatory pathway. *In vivo* study is still needed to prove the effects of these two compounds as endometriosis management.

ACKNOWLEDGMENTS

The funding of this research was supported by PUPT 2017 grant. The authors are thankful to Dr. R. Muharam, SpOG (K) for providing the necessary samples for this research and also to Dr. Ade Arsianti for preparing octyl gallate and heptyl gallate.

AUTHORS’ CONTRIBUTIONS

Dr. Arleni has the role of supervising research and directing the making of manuscripts; Mrs. Fajar Sulistya Utami performed the experiment and wrote the manuscript; Dr. Heri helped in supervising data processing analysis, and Mrs. Rahmi Budarti performed endometriosis cells isolation and culture.

CONFLICTS OF INTEREST

We declare there are no conflicts of interest in this research.

REFERENCES

6. McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation...
and nerve fiber interaction in endometriotic pain. Trends Endocrinol Metab 2015;26:1-0.