INTRODUCTION

Curcumin (diferuloylmethane) is a polyphenol derived from the Curcuma longa plant, commonly called turmeric with biological activities, including antifertility. Curcumin inhibits COX-2 expression in granulosa cells of ovarian follicles and disrupts vascular endothelial growth factor (VEGF) derived angiogenesis in the endometrium, reducing endometrial receptivity. The purpose of this study was to examine the effects of curcumin on COX-2 and VEGF expression in endometrium of fertile women.

METHODS

A prospective double-blind placebo-controlled clinical trial was conducted in a group of fertile women with regular menstrual cycles, aged between 20-30 y, married, and with children. Subjects were divided into a group receiving daily 800 mg encapsulated curcumin. Curcumin orally for ten days, starting on the third day of the first menstrual day, and a control group. Endometrial biopsy was performed using a microcuret and immunohistochemistry was used to assess VEGF and COX-2 expression. The results were analysed using an independent sample t-test.

RESULTS

In the curcumin-treated group, VEGF expression was significantly lower than the control group (p<0.05), and COX-2 expression was higher but not significantly so (p>0.05).

CONCLUSION

The curcumin causes VEGF expression in endometrium is lower and negatively affects the growth of endometrial stromal cells.

Keywords: Curcumin, COX-2, VEGF, Antifertility

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in VEGF expression in the curcumin group were calculated from epithelial surface, vascular endothelium and glandular. This group was significantly lower at 17.18±12.62 SD per 100 cells compared with the control group at 42.78±32.94 SD per 100 cells (fig. 1, table I; p<0.05). Meanwhile, COX-2/100 cell expression was slightly higher in the curcumin group at 56.68±26.27 SD per 100 cells compared to 44.43±31.82 SD per 100 cells in the control group, but this difference was not significant (fig. 2, table I; p>0.05). In the group that received curcumin, more cells expressed COX-2 on the epithelial surface, stroma and endometrial glands than controls (fig. 2).

### Table 1: VEGF and COX-2 expression calculated from stroma, cytoplasm and endometrial blood vessels in the middle of the menstrual cycle

| Expression          | Curcumin       | Control        | p   |
|---------------------|----------------|----------------|-----|
|                     | n   | X±SD | n   | X±SD |     |
| VEGF (Σ/100 cells)  | 40  | 17.18±12.62 | 40  | 42.78±32.94 | 0.00 |
| COX-2 (Σ/100 cells) | 40  | 56.68±26.27 | 40  | 44.43±31.82 | 0.06 |

Σ = Sum of the immunocytochemistry expression appearance

Fig. 1: Immunocytochemistry of VEGF expression on the surface, endothelium and gland after 10 d of curcumin administration. Endometrial gland cell morphology after Immunocytochemistry staining were characterised by the appearance of purple brown colour from chromogen dianinobenzidine tetrahydrochloride (DAB) in the cytoplasm, and the brown colour in the cytoplasm indicates the presence of VEGF expression. The cell nucleus appears round-oval and the blue/purple colour. Observations using a microscope phase contrast at 40×. VPT = High surface VEGF, VET = Endothelial VEGF High, VKT = High Gland VEGF, VPR = Low surface VEGF, VER = Low Endothelial VEGF, VKR = Low Gland VEGF. The arrows are appearance of Immunocytochemistry of VEGF expression.
DISCUSSION

The effect of curcumin on VEGF expression

The amount of VEGF expression was lower in the curcumin group, indicating that curcumin has an antigonadotropic effect. VEGF targets endothelium to induce proliferation and migration for new blood vessels to grow and supply the endometrium, and works as a vasodilator by stimulating the release of prostacyclin and nitric oxide [24]. Curcumin suppresses VEGF secretion depending on the dose and reduces the growth of endometrial stromal cells, and induces apoptosis in endometrial epithelial cells [13]. Wieser et al. found that in an in vitro model of endometriosis, curcumin can decrease VEGF secretion and cell proliferation and induce apoptosis in normal cells and endometrium [13]. In normal endometrium, VEGF expression can be detected in both glandular and stromal epithelium, and expression in glandular cells increases 3-5 fold from the initial proliferation phase to the final secretion phase [16]. VEGF expression is at its highest in the middle of the secretion phase [17]. Macpherson et al. reported that immunoreactive VEGF significantly increased in the endometrial gland during the normal menstrual cycle [18]. In our study, VEGF levels were very low in the curcumin group, indicating that curcumin decreases VEGF expression in the final follicular phase. The results of this study indicate that there was a decrease in VEGF due to administration of curcumin.
Effect of curcumin on COX-2 expression

LH and FSH directly activate cAMP in granulosa preovulatory cells via the protein kinase A (PKA) pathway to increase the regulation of COX-2 expression [19, 20]. Similarly, LH can stimulate an increase in COX-2 expression [21]. In this study, the increased COX-2 expression in the curcumin group may indicate that the endometrium and granular cells may be different. Granulosa COX-2 expression cells are increase to the level of prostaglandin in ovarium after 24-36 h hCG administration [22]. Purwantii reported that COX-2 expression was highly increased in experimental animals treated with curcumin with LH stimulation compared to animals given curcumin with placebo [8]. An in vitro study conducted by Adiyanti et al. found that administration of curcumin at 100 mg/KgBW yielded a significant difference in increasing VEGF expression compared to a group treated with indomethacin, a non-selective COX inhibitor [9]. Meanwhile, curcumin is a selective COX-2 inhibitor, which stimulates apoptosis and angiogenesis inhibitors [8]. The presence of curcumin inhibitors such as COX-2 inhibitor during the adenylate cyclase and phospholipase pathway will reduce COX-2 expression [8].

Adenylate cyclase catalyses the formation of cAMP from ATP. cAMP stimulates the release of Ca2+ from the endoplasmic reticulum and activates PKA. The released Ca2+ binds to calmodulin and affects the activity of the COX-2 enzyme. Through the phospholipase pathway, active phospholipase will cause degradation of PIP2 (Phatidylinositol bisphosphate) into IP3 (inositol 1,4,5 triphosphat), which stimulates the release of Ca2+ ions that bind to calmodulin, and DAG (Diacylglyceryl), which activates protein kinase c (PKC). PKC and Ca2+ binding with calmodulin will increase COX-2 [21]. Curcumin inhibits COX-2 expression in the follicular granulosa cells in the ovary, with the inhibition taking place prior to CAMP production [23]. Although our study indicated a moderate increase in COX-2 under curcumin treatment, this was not significant, in contrast to our hypothesis.

CONCLUSION

We found that curcumin reduces VEGF expression in the endometrium, but does not affect COX-2 expression. This study indicate that Curcumin is promising in acting as an anti fertility or contraceptive agent. In the future, further research needs to be done, involving multicentres and large sample size with the aim of fertility regulation and anti fertility treatment. This will strengthen the scientific basis for its use as fertility regulating drugs.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

There are no conflicts of interest to declare

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