

## Effect of *Hylocereus Polyrhizus* Rind Extract Toward Interleukin-1 $\beta$ , Vascular Endothelial Growth Factor Expression, Endometriosis Implant Area

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### ABSTRACT

Retrograde menstruation lead to I Kappa B Kinase (IKK) fosforilation in peritoneum macrophage and cause secretion of proinflammatory cytokine interleukin1 $\beta$  then stimulate endometriosis cell to produce Vascular Endothelial Growth Factor which lead to increasing of endometriosis lesion seen as endometriosis implant area. Cytokine secretion was inhibited through prevention of NF- $\kappa$ B activation by dragon red fruit rind extract (*Hylocereus polyrhizus*). The aim of this reserach is to know the effect of dragon red fuit rind extract with 0,25; 0,5; and 1 mg/g bodyweight dosage toward IL-1 $\beta$ , VEGF expression and implant area in endometriosis mice model. The design of this experiment was randomized post test only control group design. Endometriosis mice model were made in 14 days and split into two group, positive control group and treatment group after two week negative control group and postive control group were given Na-CMC 0,5% solution consecutively, and treatment group were given dragon red fruit extract with different dosage. Signification number for IL-1 $\beta$  is  $p < 0,05$ , signification number for VEGF is  $p < 0,05$ , and implant area signification number is  $p < 0,05$ . Administration of dragon red fruit rind extract can decrease IL-1 $\beta$ , VEGF, and implant area.

**Keywords:** *Hylocereus polyrhizus*, endometriosis, IL-1 $\beta$ , VEGF, implant area.

### INTRODUCTION

The theory to explain mechanism of endometriosis still cannot be accepted by all community. The general theory that accepted is implantation from retrograde menstruation from endometrium<sup>2,4</sup>.

Surgical therapy has some side effects like infection, the damage of internal organ, new adhesion and hemorrhage. Nowadays the innovation is searching for alternative drugs to suppress the development of endometriosis without disturbing the ovulation with minimum side effect. Based on research on effectivity test of the anti-inflammatory property of red dragon fruit peel in male Webster Strain mice, red dragon fruit peel extract has anti-inflammation effect on dosage 0.25, 0.5 and 1 mg/g bodyweight given during 14 days, and this is becoming the reference of this research<sup>6</sup>.

Menstruation blood from patient with endometriosis on peritoneum will stimulate phosphorylation I kappa B kinase (IKK) in peritoneum macrophage and next it will secrete more cytokine pro-inflammation like Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL-1, IL-2, IL-6, IL-8, IL-10), and Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES)<sup>1,5,14</sup>. IL-1 $\beta$  is cytokine pro-inflammation that will stimulate endometriosis cell to produce some cytokine and growth factor that plays important role on adhesion, growth,

invasion, inflammation and angiogenesis on endometriosis tissue. The formation of new blood vessel known as angiogenesis has big role on endometriosis progressivity through vascular endothelial growth factor (VEGF)<sup>12,26</sup>. Cosin<sup>12</sup> stated that the increasing of VEGF on endometrium cell and peritoneal fluid is more significant compares with normal female. Stimulation of VEGF is increasing progressivity of endometrium lesion signed with implant area become larger. Secretion of IL-1 $\beta$  and VEGF by endometrium cell can be inhibited by preventing activation of Nuclear Factor-kappa Beta (NF- $\kappa$ B). Red dragon fruit (*Hylocereus polyrhizus*)<sup>31</sup> peel extract known as its ability to inhibit NF- $\kappa$ B activation besides curcumin.

### MATERIAL AND METHOD

This study has been received approval ethical clearance letter of animal subjects from Faculty Veterinary Medicine, Airlangga University with number 708.

The experimental design used in this research is true experimental with randomized posttest only control group design. Characteristic of inclusion in this research is mice (*Mus musculus*) model of endometriosis, aged 2-3 months old, weighed 20-25 gram, soft haired, and there is no stretch mark.

Material used in this research are peritoneum fluid and lesion of endometriosis from mice model of endometriosis,

Hylocereus polyrhizus peel extract that the fruit got from dragon fruit garden Rembangan Jember district,

then injected with cyclosporine A Sandimmum (Novartis Malaysia) in the first day. In the first day of treatment,

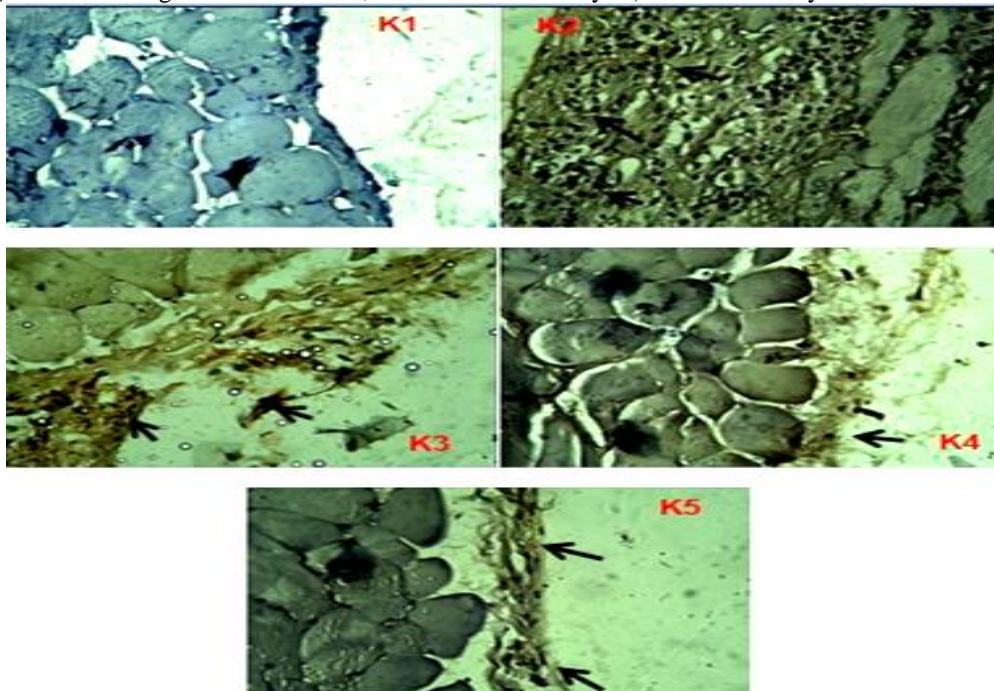


Figure 1: Differences in VEGF expression among treatment groups. The expression of VEGF in K2 group is dominant than another groups. (Nikon Eclipse e-100 Microscope, 400x Magnification, Optilab Viewer 2.2, Image Processing Software Image raster 3.0).<sup>9</sup>

Comparison of the Influence of Red Dragon Fruit Peel Extract on Progressivity of Endometriosis.

Table 1: Mean and Standard Deviation Interleukin-1 $\beta$  Concentration.

	$\bar{x} \pm SD$ Interleukin-1 $\beta$ Concentration				
	K1	K2	K3	K4	K5
IL-1 $\beta$	88,65 $\pm$ 22,46 <sup>a</sup>	182,15 $\pm$ 54,57 <sup>c</sup>	117,7 $\pm$ 43,6 <sup>b</sup>	100,75 $\pm$ 19,49 <sup>b</sup>	111,71 $\pm$ 4,86 <sup>b</sup>

Table 2: Mean and Standard Deviation Expression VEGF.

	$\bar{x} \pm SD$ Expression of VEGF				
	K1	K2	K3	K4	K5
VEGF	0,84 $\pm$ 0,456 <sup>a</sup>	6,96 $\pm$ 0,96 <sup>c</sup>	3,84 $\pm$ 1,53 <sup>b</sup>	2,52 $\pm$ 0,228 <sup>b</sup>	2,12 $\pm$ 1,188 <sup>b</sup>

Table 3: Mean and Standard Deviation Implant Area of Endometriosis.

	$\bar{x} \pm SD$ Implant Area of Endometriosis				
	K1	K2	K3	K4	K5
Implant Area	0 $\pm$ 0 <sup>a</sup>	202,15 $\pm$ 73,79 <sup>c</sup>	62,72 $\pm$ 18,76 <sup>b</sup>	58,37 $\pm$ 19,18 <sup>b</sup>	44,62 $\pm$ 8,27 <sup>b</sup>

Note: Different alphabets in the same line indicates significant differences.

cyclosporine A Sandimmun (Novartis Malaysia), water fluid injection, biopsy of endometrium gained from benign uterine tumor surgery ethinyl estradiol, Enzyme-linked immunosorbent assay kit for IL-1 $\beta$  (Bioassay Technology Laboratory), polyclonal antibody vascular endothelial growth factor Bioass Inc. The research will be conducted in Laboratory of Experimental Animal, and Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga.

Before the research was started, the mice were adapted for 7 days in animal cage. Mice were fed with chicken feed and drinking water ad libitum. The mice were divided into positive control (K2) and treatment group (K3, K4, K5)

group K2, K3, K4 and K5 were also injected with endometrium tissue intraperitoneal. The endometrium tissue was taking with spuit 3 ml and every mouse will be injected intraperitoneal 0.1 ml by using spuit 1 ml and 16G needle so that the endometrium tissue can deliver into the body. The injection of ethinyl estradiol were intramuscularly in the first day and fifth day. Termination of six mice from K2 done in the fourteenth day to prove that the mice were already become model of endometriosis. Mice were inducing with Hylocereus polyrhizus peel extract with various dosage during 14 days and in the day 29 all group of mice will be

terminated to collect the fluid of peritoneum and lesion of endometriosis.

differences in the distribution of VEGF among the negative control group, positive control group, 0.25

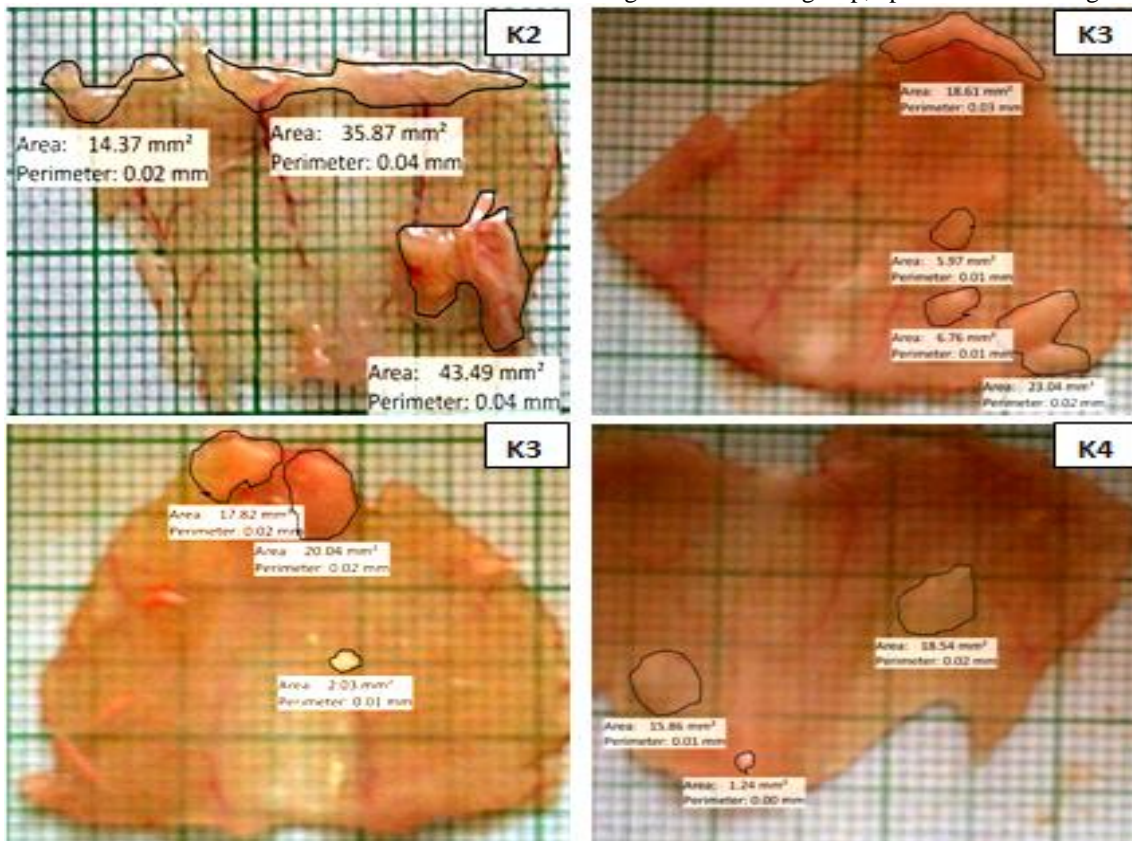


Figure 2: The difference of endometriosis implant area.

Methanolic extract of *Hylocereus polyrhizus* peel was obtained by means of maceration method soaked in 96 % ethanol. Concentration of interleukin-1 $\beta$  was measured with Enzyme-linked immunosorbent assay kit method by using Enzyme-linked immunosorbent assay kit (Bioassay Technology Laboratory). Preparation to examine expression of VEGF was colored by using immunohistochemistry method, while the implant area of endometriosis from peritoneum was measured by using Motic image. Data were analyzed statistically by using Mann Whitney in SPSS (Statistical Programs for Social Scientific) version 22.0

**RESULT**

There were differences in the distribution of IL-1 $\beta$  level among the negative control group, positive control group, 0.25 mg/gram bodyweight dose treatment group, 0.5 mg/g bodyweight treatment group, and 1 mg/g bodyweight treatment group. The differences were showed by the decline in IL-1 $\beta$  level in relation to the increased dose of red dragon fruit peel extract provided as therapy. The expression of VEGF in every sample were evaluating semi-quantitative based on Remmele method that have been modified. Immuno Reactive Score (IRS) is the result of multiplication percentage of positive immune-reactive cell and color intensity of immune-reactive cell. Table 5.3 indicated the average and standard deviation expression of VEGF model endometriosis mice among control group and treatment group. The table indicated that there were

mg/gram bodyweight dose treatment group, 0.5 mg/g bodyweight treatment group, and 1 mg/g bodyweight treatment group.

The implant area of endometriosis in superficial of peritoneum was measured by using motic image and calculating white or brown cyst, measured in millimeter using millimeter block paper. The millimeter block was transferred into motic images. There were differences in the distribution of the implant area of endometriosis among the negative control group, positive control group, 0.25 mg/gram bodyweight dose treatment group, 0.5 mg/gram bodyweight treatment group, and 1 mg/gram bodyweight treatment group. The differences were pointed out by reduced implant area of endometriosis in response to the increased dose of red dragon fruit peel extract provided as therapy.

**DISCUSSION**

Cytotoxic test of red dragon fruit peel extract known that the potency of cytotoxic effect from red dragon fruit peel can inhibit target protein I $\kappa$ B, which is IKK<sup>23</sup>. Inhibition of activation NF- $\kappa$ B indirectly is decreasing cytokine concentration pro-inflammation like TNF- $\alpha$  and IL-1 $\beta$  which is pro-inflammation cytokine and cascade activator to activate another cytokine. Research from Elvina, 2016 to know flavonoid concentration, total of phenol and antioxidant activity from dried red dragon fruit peel, the result of the research showed, flavonoid from red dragon fruit peel is 11,38/100 g, total of the phenols is 11,49/100

g, the activity of antioxidant is 9,5 g/100 g. Research done by Batra and sharma<sup>10</sup> stated flavonoid can capture cell cycle that correlate with the ability to inhibit Cyclin Dependent Kinase (CDK)<sup>11,30</sup>. Flavonoid also can inhibit Mitogen Activated Protein Kinase (MAPK) activity as possibility mechanism of anti-neurodegenerative<sup>24,25</sup> and protection from autoimmune diseases, allergic, and cardiovascular diseases<sup>29,31</sup>. Process to produce interleukin 1 $\beta$  by macrophage beside activate NF- $\kappa$ B pathway also activate Mitogen-Activated Protein Kinase (MAPK) pathway.

There were significant differences in vascular endothelial growth factor expression with greater dose of red dragon fruit peel ethanol extract given by using IRS in groups K1, K2, K3, K4, and K5. Figure 2 showed that the greater dosage of red dragon fruit peel extract that given, the lower vascular endothelial growth factor expressed on endometrium.

This research also supported research done by Hendarto<sup>15</sup>, about effect of Curcumin on vascular endothelial growth factor expression in mice model endometriosis that showed as anti-angiogenesis which is suppressing vascular endothelial growth factor as main factor of angiogenesis. Curcumin can inhibit activity of transcription factor NF- $\kappa$ B that have been proved before by Jobin and Gururaj<sup>34,35</sup>. Mechanism of red dragon fruit peel extract on suppressing endometriosis as anti-inflammation or anti-angiogenesis effect from suppressing vascular endothelial growth factor<sup>33</sup>. The decreasing of vascular endothelial growth factor in this research caused by the decrease activity of NF- $\kappa$ B from red dragon fruit peel extract.

In this research the implant area of endometriosis is red lesion with the most hyperemia on peritoneum. Inflammation area was measure by using millimeter scale then using motic images. Table 5.3 it could be inferred positive control group is 202.15, and K3 with dosage 0.25 mg/gram bodyweight is 62.72, K4 with dosage 0.5 mg/gram BW is 58.37, and K5 with dosage 1 mg/gram bodyweight showed the lowest mean which is 44.62. The difference showed by decreasing implant area of endometriosis along with increasing dosage of red dragon fruit peel extract. The increasing of inflammation is possibly caused by healing immunity of mice that given red dragon fruit peel extract and make clearance in implant area of endometriosis.

As well as research done by Hendarto<sup>14</sup> about effect of Curcumin on implant area as anti-inflammation, it will suppress cellular transformation, proliferation, angiogenesis, invasion from NF- $\kappa$ B<sup>1</sup>. The significant different as the result sign by the differs of endometriosis implant area of given and not given the red dragon peel extract.

Endometriosis implant area on K5 is lower than K2, this caused by the effect of red dragon fruit peel extract that successfully suppress the endometriosis. Mechanism of red dragon fruit peel extract is the anti-angiogenesis effect by suppressing the VEGF<sup>23</sup>.

## CONCLUSION

The concentration of IL-1 $\beta$ , expression of VEGF, implant area in endometriosis model mice treated with ethanol extract of red dragon fruit peel (*Hylocereus polyrhizus*) in various doses are lower compared to the negative control groups and positive control groups.

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