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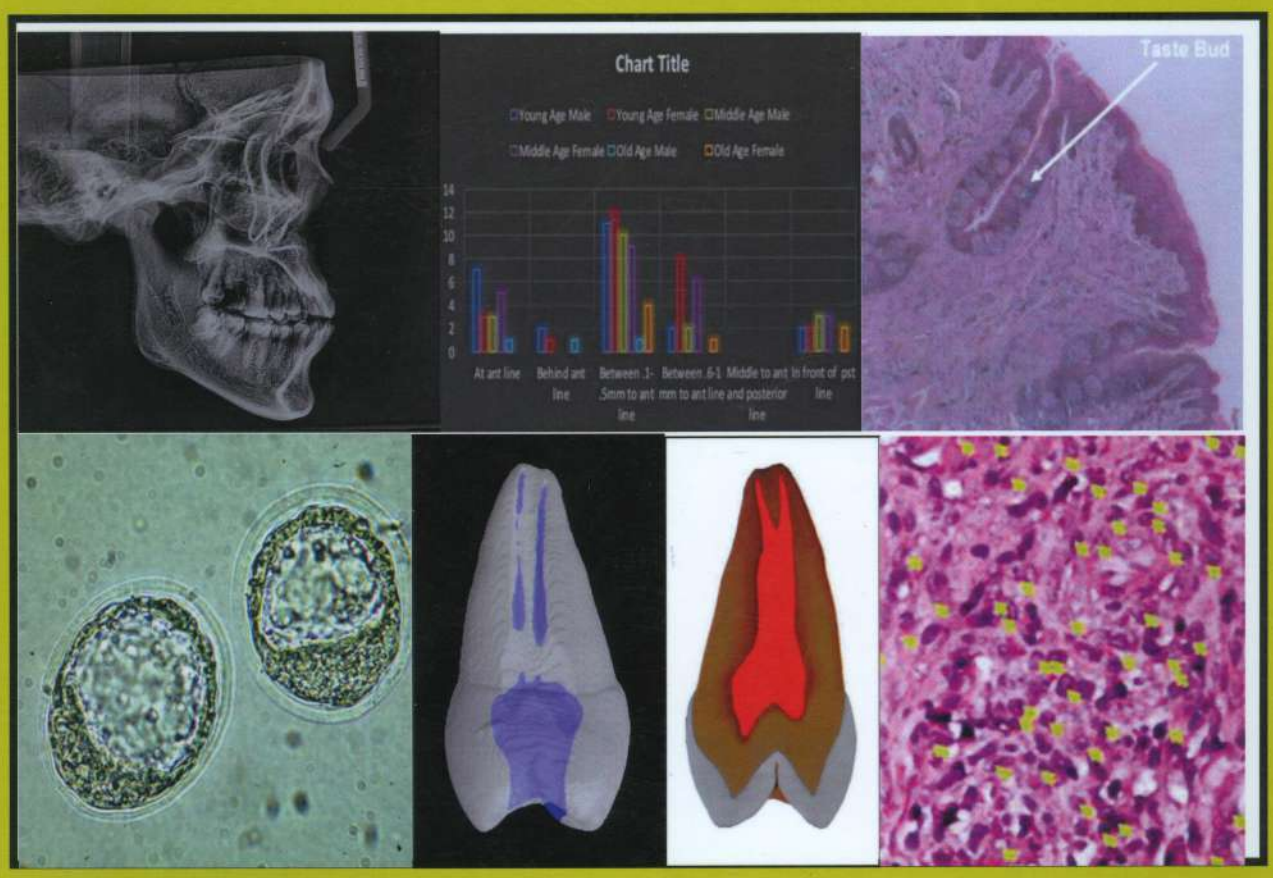

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Hylocereus Polyrhizus Peel Ethanol Extract- the Potential Effect to Tumor Necrosis Factor-A, Macrophage, and Matrix Metalloproteinase-9 in Endometriosis Mice

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Abstract

On endometriosis, macrophage and TNF-α was found in higher concentration. Tumor Necrosis Factor-α activates NF-κB pathway and increase the expression of MMP-9's gen. NF-κB pathway can be blocked by Hylocereus polyrhizus peel.

The objective of this study was to know the effect of Hylocereus polyrhizus peel ethanol extract at dose 0,25; 0,5 mg/gram BW/day; and 1 mg/gram on TNF-α concentration, number of macrophage, and MMP-9 expression on mouse model of endometriosis.

This study was a laboratory experimental research. Thirty female mice were used as samples and divided into 5 groups: 1 positive control, 1 negative control, and 3 treatment groups. Positive control and treatment groups were induced as model of endometriosis for 14 days. The next 14 days, Na-CMC 0,5% was given to both control groups, while Hylocereus polyrhizus peel ethanol extract dose 0,25; 0,5 mg/gram BW/day; and 1 mg/gram were given to treatment groups orally. Peritoneum fluid and endometriosis lesion were examined.

There were significantly differences on TNF-α concentration (p=0.021), number of macrophage (p=0.00), and MMP-9 expression (p=0.002) among control groups and treatment groups. TNF-α concentrations were higher on treatment groups.

Hylocereus polyrhizus peel ethanol extract can be used as alternative therapy for attenuating the alteration of macrophage and MMP-9 in endometriosis disease.

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Keywords: Hylocereus polyrhizus peel ethanol extract, endometriosis, TNF-α, macrophage, MMP-9.

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Introduction

Endometriosis is defined as the presence of endometriosis-like tissue outside the uterus, which induces a chronic inflammatory reaction.[1] The prevalence of endometriosis in the population is difficult to determine but estimates between 2 to 10% within the female population while 50% in infertile women. Most women with endometriosis experience painful symptoms thus infertility.[2] Management of endometriosis can be divided into empirical treatments and surgery. However, the recurrence rate after treatment is higher, about 35% in mild endometriosis and 74% in severe endometriosis.[3] The high recurrence rate due to endometriosis is a progressive disease and needed long-time treatment.[4]

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Materials and methods

This study has been received approval ethical clearance letter of animal subjects from Faculty of Veterinary Medicine Universitas Airlangga with number 710-KE. This study was a laboratory experimental research. Thirty female mice (*Mus musculus*), aged 2-3 months, weighing 20-25 gram, were used as samples. This study was conducted in Faculty of Veterinary Medicine Universitas Airlangga from June to July 2017.

After adaptation for a week, female mice then divided into 5 groups, which are: positive

control group (K1), negative control group (K2), treatment group with *Hylocereus polyrhizus* peel ethanol extract dose 0,25 mg/gram BW/day (K3), treatment group with *Hylocereus polyrhizus* peel ethanol extract dose 0,5 mg/gram BW/day (K4), and treatment group with *Hylocereus polyrhizus* peel ethanol extract dose 1 mg/gram/day (K5). Positive control and treatment groups were induced as model of endometriosis by this following steps: 1) injection of cyclosporin A (0,2 ml/mice) intramuscular on day one to make mice in immunodefficiency state. Cyclosporin A was purchased from Sandimmun (North Ryde, Australia); 2) injection of *ethinyl estradiol* 20.000 IU (0,1 ml/mice) on day one and five; 3) injection of implant tissue (0,1 ml/mice) in the peritoneal cavity on day one. Implant tissue is derived from the myometrium and endometrium of gynecologic benign tumor patient who underwent surgery procedure and didn't use hormonal contraception at least for last 3 months before surgery. The animal model was observed for 14 days to be the mice model of endometriosis.

Starting from 15th day, Na-CMC 0,5% (Clorogreen Gemilang™, Bandung, Indonesia) was given to both control groups as placebo. *Hylocereus polyrhizus* peel ethanol extract dose 0,25 mg/gram BW/day was given to group K3, *Hylocereus polyrhizus* peel ethanol extract dose 0,5 mg/gram BW/day was given to group K4, and *Hylocereus polyrhizus* peel ethanol extract dose 1 mg/gram/day was given to group K5. These placebo and extract was administered orally (0,2 ml/25 mg/gram BW/day) for 14 days with an oral gavage.

At the end of experiment, mice were anesthetized with ketamin (Ketamin Hydrochloride Pfizer®, New Jersey, USA) and acepromazine (Castran®,Venray, Holland). Peritoneal fluid samples were obtained by peritoneum puncture to examine TNF-α concentration using Enzyme Linked Immunosorbent Assay kit (Elabscience™, Wuhan, China), then endometriosis lesions in peritoneum cavity were collected. Formalin-fixed, paraffin-embedded tissue sections from endometriosis lesion were tested by immunohistochemistry to see MMP-9 expressions (Bioss Antibody Incorporation™, Massachussets, USA). The other tissue sections from endometriosis lesion were tested by Hematoxillin Eosin (HE) staining (DAKO™, California,USA) to count macrophages on the lesions. The number of macrophages from 5 field-view then categorized by Klopfleisch scoring system. This score criterias are: 0 if there was no macrophage infiltration, 1 if there were less than 10 macrophage infiltration, 2 if there were 11-50 macrophage infiltration, 3 if there were 51-100 macrophage infiltration, and 4 if there were more than 100 macrophage infiltration.[13]

Matrix Metalloproteinase-9 expressions were analyzed using modified Remmele and Stegnes semiquantitative scoring system (1986). This Immuno Reactive Score (IRS) is a result from multiplication between immunoreactive cells procentage (A) and colour intensity score on immunoreactive cells (B). Datas were collected from 5 field-view. The immunoreactive cells score citerias (A) are: 0 if there was no positive cell, 1 if procentage of positive cells was less than 10%, 2 if procentage of positive cells was 11-50%, 3 if procentage of positive cells was 51-80%, and 4 if procentage of positive cells was more than 80%. While colour intensity score (B) criterias are: 0 if there was no colour reaction, 1 if the intensity of colour is low, 2 if the intensity of colour is average, and 3 if the intensity of colour is high.[14]

Shapiro Wilk test was used to know the normality of data and Levene test was used to know the homogenity of data. If distribution and homogenity of data were normal (p>0.05), One Way Anova test was conducted, followed with posthoc Bonferroni, but, if the data distribution or homogenity wasn't normal, Kruskall-Wallis test followed with Mann Whitney test, were conducted. Analysis was performed with IBM SPSS Statistics versions 24.00 (New York, USA).

Results

Result showed that there were *mean* differences among K1, K2, K3, K4, and K5 groups, as seen on table 1.

	$\bar{x}$ ± SD TNF-α Concentration				
	K1	K2	K3	K4	K5
TNF-α	18.60±0.31	19.63±0.26	20.05±1.23	20.49±1.84	19.55±0.75

Table 1. TNF-α Concentration on Mice Model Endometriosis AmongzControl Groups and Treatment Groups.

The level of TNF-α concentration was higher in the endometriosis model groups (K2, K3, K4, and K5) compared to the negative control group (K1). All doses of *Hylocereus polyrhizus* peel ethanol extract also have higher level of TNF-α concentration compared to K1.

Statistical analysis was conducted by One Way Anova test with p=0.021, that means there was significantly differences among groups. Posthoc Bonferroni test showed that there were significantly differences between K1 and K4.

Result showed that there were *mean* differences among K1, K2, K3, K4, and K5 groups, as seen on table 2. The numbers of macrophage were decreased on treatment groups. This reduction was linear with the increasing of Hylocereus polyrhizus peel ethanol extract doses. Statistical analysis was conducted by Kruskall Wallis test with p=0.000, that means there was at least 1 significantly differences between 2 groups. Mann Whitney test showed that there were significantly differences between: K1 and K2, K1 and K3, K1 and K4, K2 and K3, K2 and K4, K2 and K5, and K3 and K5.

	$\bar{x}$ ± SD Number of Macrophage				
	K1	K2	K3	K4	K5
Macrophage	0,20±0,45	4±0	3,20±0,45	3±0	1,80±1,10

Table 2. Number of Macrophage on Mice Model Endometriosis Among Control Groups and Treatment Groups.

	$\bar{x}$ ± SD MMP-9				
	K1	K2	K3	K4	K5
MMP-9	1.12±0.867	6.16±0.829	3.64±0.817	2.28±0.743	1.56±1.633

Table 3. Matrix Metalloproteinase-9 on Mice Model Endometriosis Among Control Groups and Treatment Groups.

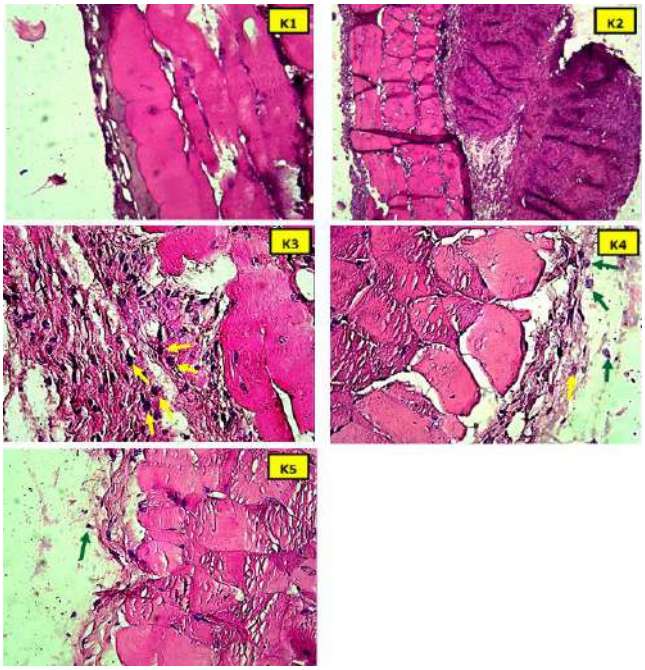

Figure 1. The macrophage inflitration. Massive macrophage infiltration in positive control group (K2) compared with another groups (Hematoxillin Eosin, magnification 400x, Nikon H600L microscope from Nikon Instrumen Inc™, New York, USA).

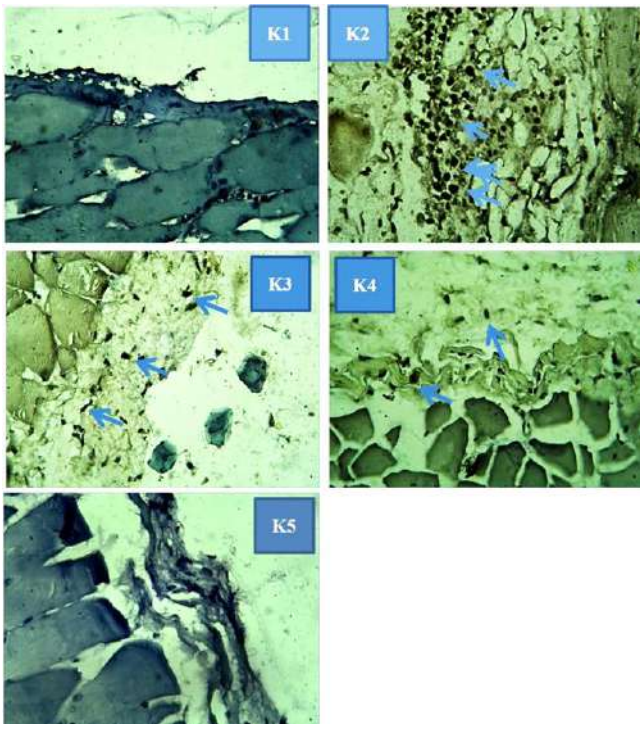

Figure 2. The MMP-9 expression (blue arrow). MMP-9 expression in positive control group (K2) was the strongest among another groups (immunohitochemistry, magnification 1000x, Nikon H600L microscope from Nikon Instrumen Inc™, New York, USA).

Result showed that there were *mean* differences among K1, K2, K3, K4, and K5 groups, as seen on table 3, figure 1, 2.

The MMP-9 expressions were decreased on treatment groups. This reduction was linear with the increasing of *Hylocereus polyrhizus* peel ethanol extract's doses. Statistical analysis was conducted by Kruskall Wallis test with p=0.002, that means there was at least 1 significantly differences between 2 groups. Mann Whitney test showed that there were significantly differences between: K1 and K2, K1 and K3, K2 and K3, K2 and K4, K2 and K5, and K3 and K4.

Discussion

The result of this study showed that *Hylocereus polyrhizus* peel ethanol extract was able to reduce the number of macrophage and MMP-9 expression on mice model endometriosis.

But, the extract wasn't able to reduce TNF-α concentration.[10] *Hylocereus polyrhizus* peel ethanol extract which contain betalain, were given to mice model endometriosis to inhibit NF-κB pathway, which is activated by macrophage and endometriosis cells when these cells bind to their receptors.[15,16]

By blocking NF-κB pathway, it was supposed that the genes expression of TNF-α, MMP-9, and MCP-1 reduced. This study result presented that the secretion of TNF-α by macrophage wasn't reduced. This may because the production of TNF-α by macrophage isn't only through NF-κB pathway[16,17]. There is possibility that TNF-α can be produced through MAPK (mitogen-activated protein kinase) pathway.[19,20] So that, although the extract inhibited NF-κB pathway, the production of TNF-α was still high through MAPK pathway.

This study also presented that MMP-9 and number of macrophage were significantly greater in the positive control group compared to other groups. These increased levels of MMP-9 in mice model endometriosis were significantly reduced by 0,5 mg/gram BW/day and 1 mg/gram administration of *Hylocereus polyrhizus* peel ethanol extract to those comparable to the negative control group. Administration of *Hylocereus polyrhizus* peel ethanol extract dose 1 mg/gram also can reduce the number of macrophage to those comparable to the negative control group.

Conclusions

The conclusion of this study was *Hylocereus polyrhizus* peel ethanol extract can be used as alternative therapy for attenuating the alteration of macrophage and MMP-9 in endometriosis disease.

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Declaration of Interest

The authors declare no conflict of interest.

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