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THE POTENTIAL EFFECT OF RED DRAGON FRUIT (*Hylocereus polyrhizus)* PEEL ETHANOL EXTRACT ON ENDOMETRIOSIS PROGRESSIVITY IN ENDOMETRIOSIS MICE

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ABSTRACT

The aim of this study was to know the effect of red dragon fruit (*Hylocereus polyrhizus*) peel ethanol extract at dose of 0.25, 0.5, and 1 mg/g bw on endometriosis progressivity, signed by granuloma, on mouse model of endometriosis. Twenty five 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, placebo was given to both control groups, while red dragon fruit peel ethanol extract at dose of 0.25, 0.5, and 1 mg/g bw were given to mice in treatment groups as much as 0.2 mL/25 g bw/day orally. Endometriotic lesion was examined. Result showed that there were significantly differences on granuloma among control groups and treatment groups. The conclusion was red dragon fruit peel ethanol extract could inhibit endometriosis progressivity, signed by granuloma, on mouse model of endometriosis.

Key words: endometriosis progressivity, granuloma, red dragon fruit peel ethanol extract

ABSTRAK

Penelitian ini bertujuan mempelajari pengaruh dari ekstrak etanol kulit buah naga merah (Hylocereus polyrhizus) dosis 0,25; 0,5; dan 1 mg/g bb terhadap progresivitas endometriosis yang ditandai dengan granuloma pada hewan coba mencit model endometriosis. Sampel penelitian adalah 25 ekor mencit betina, yang dikelompokkan dalam 5 kelompok: 1 kelompok kontrol positif, 1 kelompok kontrol negatif, dan 3 kelompok perlakuan. Kelompok kontrol positif dan kelompok perlakuan dijadikan mencit model endometriosis selama 14 hari. Selanjutnya, selama 14 hari berikutnya, kelompok kontrol negatif dan kelompok kontrol positif diberi plasebo, sedangkan kelompok perlakuan diterapi ekstrak etanol kulit buah naga merah dosis 0,25; 0,5; dan 1 mg/g bb mencit sebanyak 0,2 ml/25 g bb/hari. Lesi endometriosis kemudian diperiksa. Hasil penelitian menunjukkan terdapat perbedaan secara bermakna pada granuloma antara kelompok kontrol dan kelompok perlakuan. Kesimpulan penelitian ini adalah ekstrak etanol kulit buah naga merah mampu menghambat progresivitas endometriosis, yang ditandai dengan granuloma pada hewan coba mencit model endometriosis.

Kata kunci: progresivitas endometriosis, granuloma, ekstrak etanol kulit buah naga merah

INTRODUCTION

Infertility is the inability of a sexually active, non-contraception couple to achieve pregnancy in one year. Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Schmidt, 2006). The prevalence of infertility in whole world is increasing, reaching 10-15% among all reproductive-aged couples (Deka and Sarma, 2010). Infertility can be caused by many factors; one of them is endometriosis (Hendarto, 2015).

Endometriosis is the presence of endometriosis-like tissue outside the uterus, which induces a chronic inflammatory reaction (ESHRE, 2013). This chronic inflammatory process make endometriosis as a progressive disease that can destroy structures at peritoneum cavity so that reproductive system, functions, and processes can be disturbed (Schenken and Guzick, 1997). The progressivity of endometriosis on peritoneum cavity can be seen by seeing peritoneum level of destruction or abdominal adhesion. This destruction is signed by inflammation, granulomas, abscess/necrosis, and fibrosis (Klopfleisch, 2013). Granuloma or granulomatous inflammation is a distinctive form of chronic inflammation produced in response to various infectious, autoimmune, toxic, allergic, and neoplastic conditions. Granuloma is defined by the presence of mononuclear leucocytes, particularly macrophages that respond to various chemical mediators of cell injury. Granuloma can occurs in all age groups and within all tissue sites (Shah *et al.*, 2017).

To prevent endometriosis progressivity, there are 2 common managements that can be done, empirical treatments and surgery. However, the recurrence rate after treatment is still high, reaching about 35% in mild endometriosis and 74% in severe endometriosis (Speroff and Fritz, 2005). The high recurrence rate due to endometriosis is a progressive disease that needed long-time treatment (Annas *et al.*, 2014).

As an alternative therapy to overcome chronic inflammation condition, endometriosis patients may consume traditional drugs that have anti-inflammatory effects. One of plants that has been known for its anti-inflammatory effect and was able to inhibit carcinoma mammae cells (MCF-7) in vitro is red dragon fruit (*Hylocereus polyrhizus*) (Sarasmita and Laksmiani, 2015). Red dragon fruit is a plant that is included in Cactaceae family dan Hylocereanea subfamily (Wybraniec *et al.*, 2007; Nurliyana *et al.*, 2010). Ethanolic extract of red dragon fruit peel contains betalain that is able to inhibit transcription factor NF-κB so that inflammatory genes expression, such as TNF-α and IL-1β, will not be released (Sarasmita and Laksmiani, 2015).

Recently, the information regarding the potential effect of ethanolic extract of red dragon fruit peel on endometriosis progressivity is not available. Therefore, this study was conducted to know the potential effect of ethanolic extract of red dragon fruit peel at dose of 0.25; 0.5; and 1 mg/g bw to endometriosis progressivity, signed by granuloma, on mouse model of endometriosis.

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. Twenty five female mice (*Mus musculus*), aged 2-3 months and weighed 20-25 g, were used as samples.

Female mice divided into 5 groups after adaptation for a week. These groups consist of positive control group (K1), negative control group (K2), treatment group with ethanolic extract of red dragon fruit peel at dose of 0,25 mg/g bw/day (K3), treatment group with ethanolic extract of red dragon fruit peel at dose of 0.5 mg/g bw/day (K4), and treatment group with ethanolic extract of red dragon fruit peel at dose of 1 mg/g bw/day (K5). The mice in positive control and treatment groups were induced endometriosis by this following steps: 1) injection of cyclosporin A (0.2 mL/mice) intramuscularly on day one to make mice in immunodeficiency state; 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 was derived from the myometrium and endometrium of gynecologic benign tumor patients who underwent surgery procedure and did not 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 day 15, 0.5% Na-carboxymethyl cellulose (Na-CMC) was given to both control groups as placebo, while ethanolic extract of red dragon fruit peel was given to 3 treatment groups (K3, K4, and K5) at dose of 0.25, 0.5, and 1 mg/g bw/day, respectively. These placebo and extract was administered orally (0.2 mL/25 g bw/day) for 14 days with an oral gavage.

At the end of experiment, mice were anesthetized with ketamine and acepromazine, and then the endometriosis lesions were collected. Formalin-fixed and paraffin-embedded tissue sections from endometriosis lesion were stained with hematoxillin eosin (HE) to evaluate granulomas. Granulomas from 2 endometriosis lesions then were counted and evaluated based on Klopfleisch scoring. The score criteria are: 0= no granuloma mass, 2= any granuloma mass and/or 11-50 macrophage infiltration observed, 4= granuloma mass with abscess observed, 6= granuloma mass with abscess and muscle necrosis observed and 8= granuloma mass with abscess, muscle necrosis, and fibrosis observed (Klopfleisch, 2013).

Shapiro-Wilk test was used to know the normality of data and Levene test was used to know the homogeneity of data. If distribution and homogeneity of data were normal (P>0.05), one way analysis of variance (ANOVA) test was conducted, followed with posthoc Bonferroni. But, if the data distribution or homogeneity was not normal, Kruskall-Wallis test followed with Mann-Whitney test, were conducted.

Table 1. Granulomas on mice model endometriosis among control group and treatment groups using Mann-Whitney test

	K1	K2	K3	K4	K5
K1	-	0,008*	0,032*	0,310	1,000
K2	0,008*	-	0,016*	0,008*	0,008*
K3	0,032*	0,016*	-	0,151	0,032*
K4	0,310	0,008*	0,151	-	0,310
K5	1,000	0,008*	0,032*	0,310	-

*P= 0.001. K1= Positive control group, K2= Negative control group, K3= Treatment group with ethanolic extract of red dragon fruit peel at dose of 0,25 mg/g bw/day, K4= Treatment group with ethanolic extract of red dragon fruit peel at dose of 0.5 mg/g bw/day, K5= Treatment group with ethanolic extract of red dragon fruit peel at dose of 1 mg/g bw/day

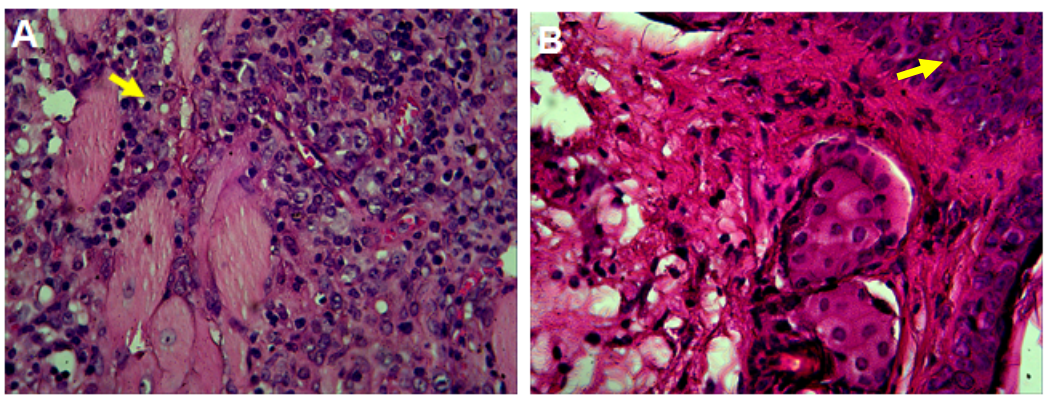

Figure 1. Chronic inflammation signed by fibrous formation and granuloma mass on peritoneum cavity of endometriosis mice. A= Fibrous formation (arrow) among muscles, B= Granuloma mass (arrow) and massive infiltration of inflammatory cells on muscles (HE, 400x)

RESULTS AND DISCUSSION

Result showed that there were chronic inflammation signed by fibrous formation and granuloma mass on peritoneum cavity of endometriosis mice (Figure 1). The granuloma mass was more pronounced in positive control group (K2) than other groups (Figure 2). There were mean differences among K1, K2, K3, K4, and K5 groups, as seen on Table 1. The endometriosis progressivity, signed by granulomas formation, was decreased on treatment groups. This reduction was linear with the increasing dose of ethanolic extract of red dragon fruit peel.

Shapiro-Wilk test showed that data in group 1, 2, 4, and 5 were not normally distributed ($P<0.05$). Levene test also showed that data were not homogenous, therefore one way ANOVA test could not be conducted. Statistical analysis was then conducted by Kruskall-Wallis test with P= 0.001, 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 K5 (Table 1).

The result of this study showed that ethanolic extract of red dragon fruit peel was capable of reducing endometriosis progressivity, signed by less granulomas formation, on mice model endometriosis. Ethanolic extract of red dragon fruit peel at dose of 0.5 and 1 mg/g bw had no significant differences with negative control group, so it could be said that ethanolic extract of red dragon fruit peel on dose 0.5 and 1 mg/g bw could reduce granulomas on endometriosis mice reaching levels to those comparable to healthy mice.

Granuloma is defined by the presence of mononuclear leucocytes, specifically macrophages

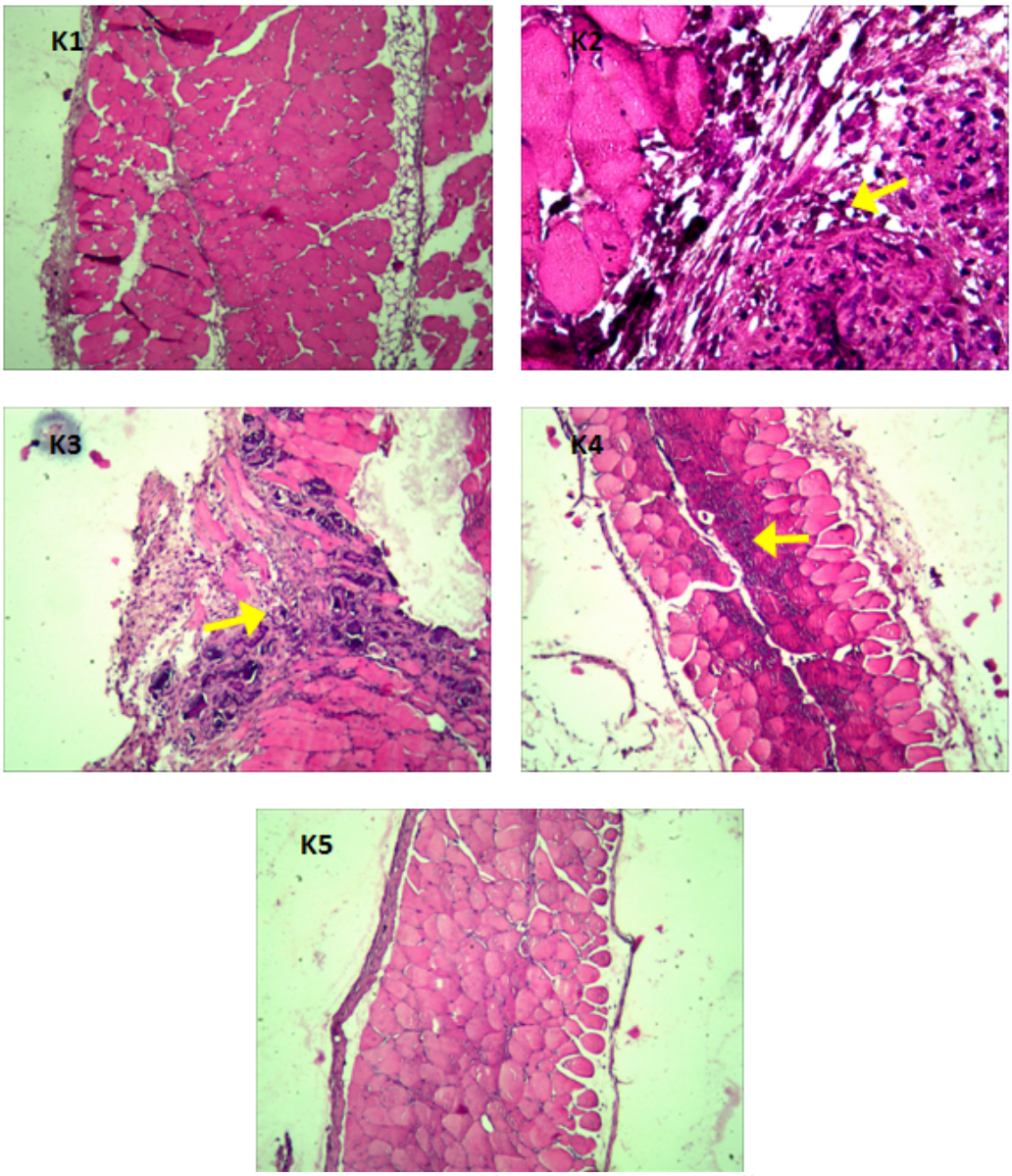

Figure 2. The granuloma mass (arrow) on peritoneum cavity of endometriosis mice in different groups. K1= Positive control group, K= Negative control group, K3= Treatment group with ethanolic extract of red dragon fruit peel at dose of 0,25 mg/g bw/day, K4= Treatment group with ethanolic extract of red dragon fruit peel at dose of 0.5 mg/g bw/day, K5= Treatment group with ethanolic extract of red dragon fruit peel of 1 mg/g bw/day (HE, 400x)

(Shah *et al.*, 2017). On endometriosis, peritoneal macrophages are first cells that are activated. These macrophages play role as secretors for pro-inflammatory cytokines which create ideal micro environment for endometriosis cells to progress (Sourial *et al.*, 2014). One of these pro-inflammatory cytokines is tumor necrosis factor-α. Tumor necrosis factor-α plays its role through NF-κB pathway. It can stimulate endometriosis cells to express monocyte chemotactic protein 1 (MCP-1) which roles as chemo-attractor to attract more macrophages to the lesions. So we can find a large number of macrophages that form granulomatous inflammation on endometriosis lesions and peritoneal fluid (Grund *et al.*, 2008; Gonzalez-Ramos *et al.*, 2012; Zheng *et al.*, 2012).

Ethanolic extract of red dragon fruit peel 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 (Martinez *et al.*, 2014). By blocking NF-κB pathway, it was supposed that the genes expression of TNF-α and MCP-1 reduced, so that macrophage migration to endometriosis lesion would also be decreased and there would be less granuloma formation.

CONCLUSION

Ethanolic extract of red dragon fruit peel could inhibit endometriosis progressivity, signed by less granuloma formation, on mouse model of endometriosis.

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REFERENCES

Annas, J.Y., Hendarto, and H. Widjiati. 2014. Khasiat berbagai dosis suplementasi kurkumin pada progresivitas endometriosis di hewan coba mencit. **Majalah Obstetri dan Ginekologi.** 22(3):118-125.

Deka, P.K. and S. Sarma. 2010. Psychological aspects of infertility. **Br. J. Med. Pract**. 3(3):a336.

ESHRE. European Society of Human Reproduction and Embriology. 2013. **Management of Women with Endometriosis: Guideline of the European Society of Human Reproduction and Embriology.** ESHRE Endometriosis Guideline Development Group, Barcelona.

Gonzalez-Ramos, R., S. Defrere, and L. Devoto. 2012. Nuclear factor-kappaB: A main regulator of inflammation and cell survival in endometriosis pathophysiology. **Fertil. Steril.** 98(3):520-528.

Grund, E.M., D. Kagan, C.A. Tran, A. Zeitvogel, A. Starzinski-Powit, S. Nataraja, and S.S. Palmer. 2008. Tumor necrosis factor-α regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase, p38, and nuclear factor-κb in human endometriotic epithelial cells. **Mol. Pharmacol.** 73(5):1394-1404.

Hendarto, H. 2015. **Endometriosis dari Aspek Teori Sampai Penangangan Klinis.** Airlangga University Press, Surabaya.

Klopfleisch, R. 2013. Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology - a systematic review. **BMC Vet. Re.** Doi.org/10.1186/1746-6148-9-123.

Martinez, R.M., D.T. Longhi-Balbinot, A.C. Zarpelon, L. Staurengo-Ferrari, M.M. Baracat, S.R. Georgetti, R.C. Sassonia, W.A. Verri, and R. Casagrande. 2014. Anti-inflammatory activity of betalain-rich dye of Beta vulgaris: Effect on edema, leucocyte recruitment, superoxide anion, and cytokine production. **Arch. Pharm. Res.** 38:494-504.

Nurliyana, S.Z., I.M.K. Suleiman, M.R. Aisyah, and K.R. Rahim. 2010. Antioxydant study of pulp and peels of dragon fruits: A Comparative Study. **Int. Food Res.** 17:367-375.

Sarasmita, M.A. and N.P.L. Laksmiani. 2015. Uji sitotoksisitas ekstrak etanol limah kulit buah naga merah (*Hylocereus polyrhizus*) pada sel kanker payudara secara invitro dan in silico. **Jurnal Farmasi Udayana.** 4(2):91-97.

Schenken, R.S. and D.S. Guzick. 1997. Revised endometriosis classification american fertility society. **Fertil. Steril.** 67(5):815-816.

Schmidt, L. 2006. Infertility and assisted reproduction in Denmark epidemiology and psychosicial consequences. **Dan. Med. Bull.** 53(4):390-417.

Shah, K.K., B.S. Prit, and M.P. Alexander. 2017. Histopathologic review of granulomatous inflammation. **J. Clin. Tuberc. Other Mycobac. Dis.** 7:1-12.

Sourial, S., N. Tempest, and D.K. Hapangama. 2014. Theories on the pathogenesis of endometriosis. **Int. J. Reprod. Med.** Dx.doi.org/10.1155/2014/179515.

Speroff, L. and M.A. Fritz. 2005. Endometriosis. In **Clinical Gynecologic Endocrinology and Infertility.** Speroff, L. and M.A. Fritz (Eds.). 7th ed. Lippincott William & Wilkins, Philadelphia.

Wybraniec, S., B. Nowak-Wydra, and K. Mitka. 2007. Minor betalain in fruit of hylocereus species. **J. Phytochem.** 68:251-259.

Zheng, Y., X. Liu, and S.W. Guo. 2012. Therapeutic potential of andrographolide for treating endometriosis. **Hum. Reprod.** 27(5):1300-1313.