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Extract of Water Clover (*Marsilea crenata C.Presl*) Leaves Reduced Expression of MMP-1 in Skin of Ovariectomized Rattus norvegicus

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Abstract: ***Science has found a way to prevent skin aging in menopause woman. One of the therapies is estrogen (17β-estradiol) replacement therapy. Skin aging in menopause woman is related with reduced ER-α and ER-β expression and negatively correlated with MMP-1 expression. Syntethic estrogen has several negative effects like breast and ovarian cancer. Phytoestrogen has gain attention due fewer side effects and cheaper. Water clover (Marsilea crenata C.Presl) is easily found in Indonesia which has phytoestrogen properties (isoflavon). Based on our review there was no study about effect of extract of water clover (Marsilea crenata C.Presl) leaves on MMP-1 expression in skin of ovariectomized Rattus norvegicus. This research revealed the effect of extract of water clover (Marsilea crenata C.Presl) leaves on MMP-1 expression in skin of ovariectomized Rattus norvegicus. Thirty-six female rats (Sprague Dawley) with 10-12 weeks old and 250-300 gr weight were randomly chosen and adapted for 7 days. Rats were ovariectomized and healed for 7 days then divided into 2 groups. The plasebo group (control) was rats without intervention and treated grup (treatment) was rats with daily oral 50 mg/kg weight extract of water clove for 28 days. After 48 hours from the last exposure, the rats were sacrificed and MMP1 expression in skin was determined by immunohistochemistry. Independent T-test analysis found significant difference in percentage of fibroblast that expressed MMP1 (p =0.000). The control group expressed MMP1 for 35.099 % and the treatment group expressed MMP1 for 17.463 %. There was decreasing of MMP1 expression for 17.636 % between control and treatment group. This study showed that extract of water clover (Marsilea crenata C.Presl) leaves reduced expression of MMP1 in fibroblast of skin ovariectomized Rattus norvegicus significantly.***

Keywords: water clover leaves, aging, MMP-1, menopause

1. Introduction

Medical science has found a way to prevent aging especially for skin aging in menopause woman. One of the therapies for skin aging is hormone replacement therapy (HRT) especially estrogen (17β-estradiol). Skin aging in menopause woman is related with reduced Estrogen Receptor α and β (ER-α and ER-β) expression and negatively correlated with Matrix Metalloproteinase-1(MMP-1) expression [1]. Increasing MMP-1 expression in menopause woman will degrade collagen in skin and resulting in aging skin. Estrogen replacement can improve quality of skin by increasing thickness and elasticity. This is done by increasing component of extracellular matrix like collagen through reducing MMP-1 expression due to increasing ER-α and ER-β) expression [1].

The usage of estrogen as HRT in menopause women for skin aging has several adverse effects like risk of breast cancer, endometrium cancer, ovarium cancer, and venous thromboembolism [1]. Estrogen as HRT is also contraindication for menopause women with heart diseases, hepatic dysfunction, and lupus erythematosus [2]. The cost is also the issue. Due to its negative side effects, replacement for estrogen by substance likes phytoestrogen is warranted. Phytoestrogen mimics estrogen but fewer side effects and cheaper. Phytoestrogen as 17β –estradiol replacement due to evidence that phytoestrogen is relatively safe [3] and long exposure not related to breast and endometrial cancer [4].

Phytoestrogen has many sources in nature especially in Indonesia. Water clover *(Marsilea crenata C.Presl*) is native and easily to find in Indonesia [5, 6]. Moreover Water clover easily to propagate without high cost production [5, 7]. Water clover *(Marsilea crenata C.Presl*) leaves contain isoflavon as phytoestrogen in form mainly genistein and deidzein [5, 8]. Extract of water clover *(Marsilea crenata C.Presl*) leaves as phytoestrogen application in menopause women is rare. However there was no study about effect of extract of water clover *(Marsilea crenata C.Presl*) leaves on MMP-1 expression in skin of ovariectomized Rattus norvegicus. This research revealed the effect of extract of water clover *(Marsilea crenata C.Presl*) leaves on MMP-1 expression in skin of ovariectomized Rattus norvegicus.

2. Material and Methods

2.1 Water Clover Extract

Water clover leaves were obtained from Cibodas Botanical Garden, Cianjur, West Java, Indonesia. Extraction was performed in Pasundan University, West java, Indonesia (Laboratory of Food and Chemstry) and Phytochemical analysis was conducted in Institute Technology Bandung (Laboratory of Organic Chemistry and Natural Resources), West Java, Indonesia. In brief, water clover leaves were dried in cabinet dryer, and then homogenized then filtered through mesh no 40. Maceration for 24 hours at 28^0C by using 80% methanol and filtered through Whattman Paper

no. 4. The filtrate was evaporated at 45^{0}C in Rotary Evaporator.

Figure 1: (*Marsilea crenata C.Presl*)

2.2 Animals

Thirty-six female rats (Sprague Dawley) were obtained from Animal Lab Unit, Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University. The study passed ethic guideline through Ethical Committee no: 941/UN14.2.9/PD/2019. Female rats age 10-12 weeks, weight 250-300 gram were randomly chosen and adapted for 7 days. Rats were ovariectomized and healed for 7 days then divided into 2 groups. The plasebo group (control) was rats without intervention and treated grup (treatment) was rats with daily oral 50 mg/kg weight extract of water clove for 28 days. After 48 hours from the last exposure, the rats were euthanized.

2.3 MMP1 Examination

Biopsy of skin was performed after rat was euthanized. The skin was immersed into formalin buffer for 24 hours. The skin was dehydrated, cleared and embedded into paraffin block. The skin was cut for 5 µm by using microtome then placed on poly-lysine object glass. Antigen retrieval was performed by citrate buffer pH 6 boiled with 700-watt microwave for 20 minutes followed 140-watt for 10 minutes. After washing 5 minutes 2 times in PBS, followed by peroxidase blocking for 15 minutes, then incubated with labeled polymer-HRP for 30 minutes, and then DAB mixture for 10 minutes. Then counterstain with Meyer Hematoxilin then mounting with Entellan and cover glass. Photomicrograph was using 400X magnifications by microscope (CX-41, Olympus, Japan) and OptilabPro camera (Miconos, Indonesia). Photograph of skin was obtained and analyzed to count the percentage of fibroblast cell which expressed MMP1. Each sample was examined for 3 fields.

2.4 Statistic

Analysis was conducted with independent T-Test by using SPSS 16.0 software.

3. Results

Independent T-test analysis found significant difference in percentage of fibroblast that expressed MMP1 ($p < 0.05$) (Figure 2). The control group expressed MMP1 for 35.099% and the treatment group expressed MMP1 for 17.463%. There was decrease of MMP1 expression for 17.636% between control and treatment group. This study showed that water clover methanol extracts reduced expression of MMP1 in fibroblast of skin of ovariectomized Rattus norvegicus ($p=0.000$). Figure 3 and 4 showed percentage of fibroblast which expressed MMP1 in Control and Treatment group respectively.

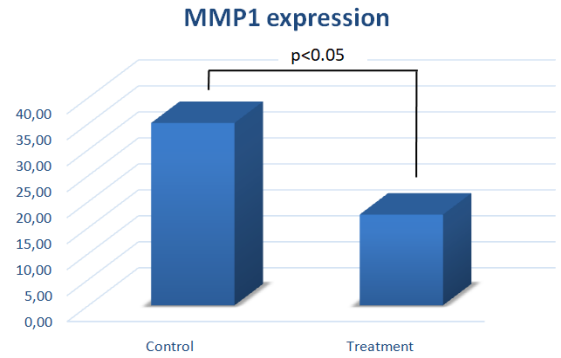

Figure 2: Expression of MMP1 between Control and Treatment Group

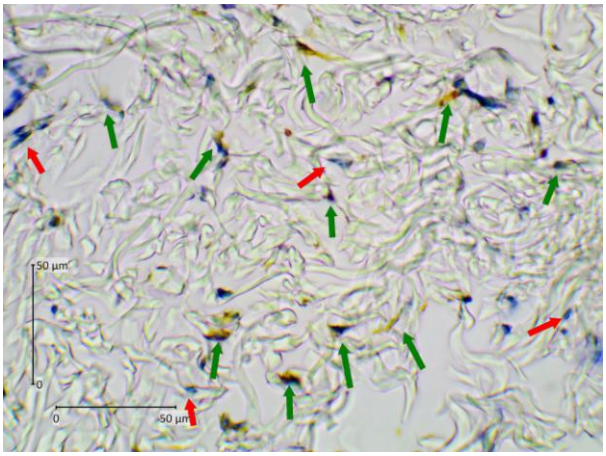

Figure 3: Expression of MMP1 in Control Group. Fibroblast which expressed MMP1 (green arrow) was more abundant than fibroblast which not expressed MMP1 (red arrow) (400x magnification)

4. Discussion

This research revealed phytoestrogen properties of extract of water clover *(Marsilea crenata C.Presl*) which is rich with isoflavon genistein. Phtoestrogen can improve quality of skin by increasing collagen through reducing MMP-1 expression due to increasing ER-α and ER-β expression [1-4]. This study showed that expression of MMP1 in fibroblast significantly reduced by 17.636% between control and treatment group (35.099% vs 17.463%, p= 0.000). Expression of MMP1 was decreased in fibroblast probably through phytoestrogen properties of genistein which was increasing expression of ER-α and ER-β.

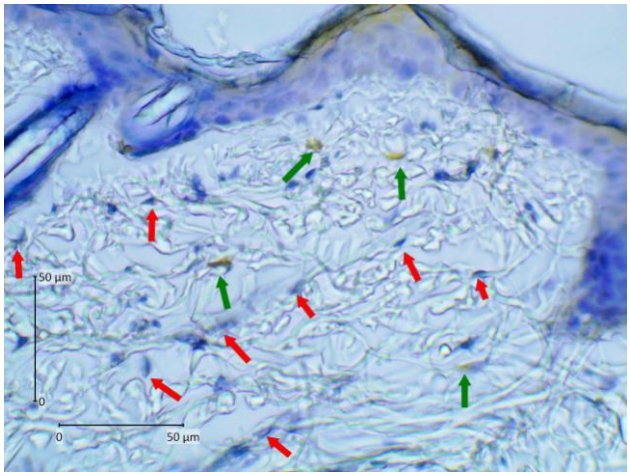

Figure 4: Expression of MMP1 in Treatment Group. Less Fibroblast that expressed MMP1 (green arrow) compared to Control Group (Figure 3). Fibroblast which was not expressed MMP1 was marked by red arrow (400X magnification)

5. Conclusion

Water clover (*Marsilea crenata C.Presl)* leaves extracts effectively reduced expression of MMP1 in skin of ovariectomized Rattus norvegicus through its phytoestrogen mechanism.

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