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Effects Leaf Ethanol Extract of *Graptophyllum pictum* L. Griff. to Inhibit Vaginal Atrophy of Menopausal Mouse

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ABSTRACT
Menopausal condition with low estrogen level caused vaginal atrophy that it is a condition where vaginal lining become dryer and thinner and it would be easier to get inflammation. So, it was important to find out the treatment to inhibit it. This research was aimed to determine the effects of *Graptophyllum pictum* (L.) Griff. leaf ethanol extract to inhibit vaginal atrophy of menopausal mice. It used 24 female mice divided into six groups, namely, Group 1, 2 and 3 as control group and group 4,5 and 6 as the treatment groups that were given leaf ethanol extract of *Graptophyllum pictum* L.Griff. with dozes 10 mg/kg, 20 mg/kg, and 30 mg/kg. Menopausal mice were obtained by doing ovariectomy in mice. Six weeks after the treatment, all mice were anesthetized and dissected to take the vagina for slide preparation staining with PAS staining, IHC for GLTU1 and for measuring the glycogen content. The data were the epithelium thickness and glycogen expression, GLTU 1 expression and glycogen level of menopausal mice vagina. All data were analyzed by Anova test, and the result showed that {*Graptophyllum pictum* (L.) Griff} leaf ethanol extract effected significantly on the vaginal epithelium thickness and glycogen expression, GLTU1 expression and glycogen level of ovariectomized mice, so it could inhibit vaginal atrophy. The optimal dozes were 10 mg/kg its leaf extract.

Keywords: *Graptophyllum pictum, Glycogen, Menopausal, Vagina*

1. INTRODUCTION

Women had limited reproductive period because of the limited number of eggs in the ovaries. When the number of egg cells was no longer growing, it was also followed by a decrease in the level of the hormone estrogen produced by the follicle cells that protect the egg. Estrogen was a female reproductive hormone that had a very important role for the reproductive organs and other organs throughout the body. One of the reproductive organs that were affected by decreased levels of estrogen was vagina.[1]

After menopause, the vagina underwent atrophy of its constituent cells, including epithelial cells. Vaginal epithelial cells produced glycogen which was necessary for the growth of lactic acid bacteria (*Lactobacillus* spp.) which was important for controlling pathogenic bacteria or fungi in the vaginal canal. Glycogen also played a role in water retention so that the vaginal condition would not be dry. With low estrogen levels during menopause, there was a decrease in glycogen synthesis and would cause dry conditions in the vagina and the vagina was easily infected to pathogenic bacteria or fungi [2].

To increase the level of estrogen in postmenopausal women, synthetic estrogen hormone therapy could be used orally or by injection, but this hormone therapy had side effects, namely it could induce the development of tumors in the reproductive organs, including the cervix, uterus, or mammary glands.[3]. The research using plant extracts was an alternative treatment to treat vaginal atrophy. One of the plants studied for its effect on the vagina is *Graptophyllum pictum* L. Griff. This plant is a medicinal plant originating from Papua and has been widely studied for the treatment of various health disorders. By using 96% ethanol extract, several compounds could be macerated from this plant, among which the important ones were flavonoids, diterpenes and sterols [4]. These three compounds could bind to the

estrogen receptor and could cause estrogen- signaling effects. Therefore, in this study, the effect of ethanol extract of *Graptophyllum pictum* L.Griff leaves was investigated for the inhibition of vaginal atrophy.

2. MATERIAL AND METHOD

This research used 24 ovariectomized mice. They grouped into 6 groups, namely group 1 (normal control). Group 2 (ovariectomy control), group 3 (hormonal control), group 4,5 and 6 (treatment group with leaf extract 10 mg/kg, 20 mg/kg and 30 mg/kg). All mice in Group 2, 3, 4, 5, and 6 were ovariectomized. For hormonal control it was used estrogen conjugate (Esthero) in 0,08 mg/kg.

Ovariectomy was done through abdomen with one section in the middle of abdomen for taking the two ovaries. For anesthesia it was used ketamine 10 %. Peritoneum incision was closed by cat gut suturing, but skin and muscle by silk suturing. After ovariectomy mice were rested for 14 days for wound healing.

The method used to get leaf ethanol extract was maceration. Fresh leaves were prepared and weighed 2,5 kg then it was air-dried in a room temperature. Dry leaves were crushed by blender to become rough powder. Rough powder was macerated in 2 liters 96 % ethanol in a container and closed and kept for three days. The macerate was separated from rough powder by filtering, then it was evaporated the ethanol content in room temperature (+ 30°C) for two days. The result was gelatinous leaf extract as much as 1.52 g.

The Treatments for mice were done until 6 weeks. At the end of the research all the mice were anesthetized by using chloroform and sacrificed to take vagina to make preparate slide and for measuring glycogen level. Staining used for vaginal slide was Periodic Acid Sciff (PAS) that could stain the glycogen with red color. The expression of glycogen was measured by image J program. Vaginal slides also were stained for GLTU1 (Glucose transporter 1) by immunohistochemistry with anti GLTU1.

Vaginal glycogen level was measured by colorimetric method with Glycogen Colorimetric Assay Kit from Elabscience. The OD value of vaginal glycogen level was count in absorbance 620 nm. From OD data of glycogen level, it was counting the level of glycogen with standard data and blank data. Then all the data were analyzed with ANOVA test and continued with Duncan test.

3. RESULTS

3.1. Thickness of vaginal epithelial cell

The result of this research showed that leaf ethanol extract of *Graptophyllum pictum* L.Griff. increased the thickness of vaginal epithelial cell layer significantly. In hormonal control (G3) that used estrogen conjugate showed the greatest effect in its thickness of epithelial cell layer and its thickness liked normal control G1). Treatment with leaf ethanol extract in 10 mg/kg (G4) of this plant also increased the thickness of epithelial cell layer but its effect was lower than hormonal control. So, it was meant that estrogenic activity of leaf ethanol extract of this plant was lower than estrogen conjugate.

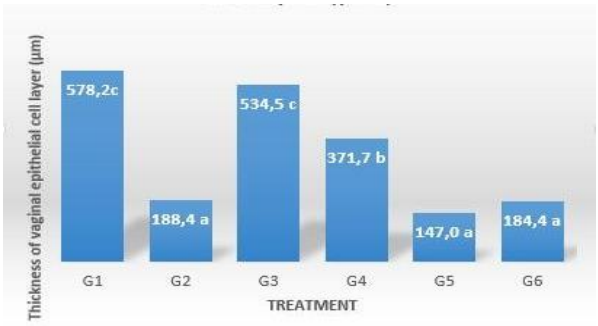

Figure 1. The Thickness of vaginal epithelial cell layer of menopausal mice after treatment with leaf ethanol extract of *Graptophyllum pictum* L. Griff. G1 was normal condition, G2 was ovariectomy condition and G3 was ovariectomy with estrogen treatment, G4, G5 and G6 were treatment that extract in 10 mg/kg, 20 mg.kg and 30 mg/kg. Different letters above bar chart showed significant difference its thickness.

3.2. Glycogen expression and level of vaginal epithelial cell

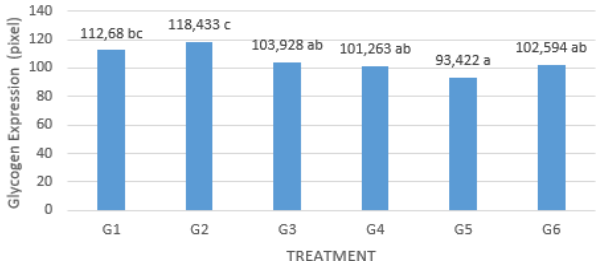

Figure 2. The Glycogen expression of vaginal epithelial cell of menopausal mice after treatment with leaf ethanol extract of *Graptophyllum pictum* L. Griff. G1, G2 and G3 were control group and G4, G5 and G6 were treatment that extract in 10 mg/kg, 20 mg.kg and 30 mg/kg. Different letters above bar chart showed significant difference its glycogen expression.

Glycogen expression was measure by image J. The unit of its measuring was pixel. If the glycogen expression is high, so the pixel was low, but if the glycogen expression was low, so the pixel was high. So, in G2 the value was higher than G3 (hormonal control) and G4, G5 and G6 (treatment with its leaf ethanol extract, that was meant that the glycogen expression in

G2 lower than in hormonal control and treatment with leaf extract.

In this research it was measured the glycogen level of vaginal menopausal mouse with the treatment of leaf ethanol extract of *Graptophyllum pictum* L Griff (Figure 3). And the result showed that the glycogen level in all dozes of leaf extract higher than ovariectomy control (G2). If the effect of its leaf extract on glycogen level compares with hormonal control, it is the same effect. So, it meant that the estrogenic activity of its leaf extract on glycogen level was the same with estrogen conjugate.

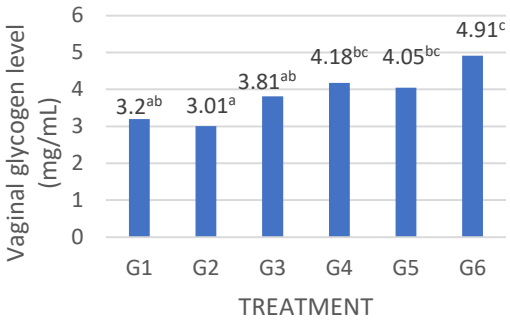

Figure 3. The vaginal glycogen level of menopausal mice after treatment with leaf ethanol extract of *Graptophyllum pictum* L. Griff. G1, G2 and G3 were control group and G4, G5 and G6 were treatment that extract in 10 mg/kg, 20 mg.kg and 30 mg/kg. Different letters above bar chart showed significant difference its glycogen level.

3.3. Vaginal GLTU 1 expression

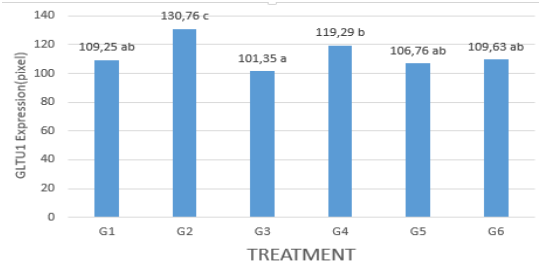

Figure 4. The Vaginal GLTU1 expression of menopausal mice after treatment with leaf ethanol extract of *Graptophyllum pictum* L. Griff. G1, G2 and G3 were control group and G4, G5 and G6 were treatment that extract in 10 mg/kg, 20 mg.kg and 30 mg/kg. Different letters above bar chart showed significant difference its GLTU1 expression.

Expression GLTU in ovariectomy condition showed higher value than hormonal control and its leaf ethanol extract. That was meant that the expression of GLTU1 in Hormonal control and its leaf ethanol extract higher than ovariectomy/menopausal control. Between G4, G5 and G6 there were not different effect significantly. But the GLTU 1 expression in the treatment with its leaf extract lower that with estrogen conjugate (G3), it was meant that the estrogenic effect of *Graptophyllum pictum* L Griff. weaker than estrogen conjugate in inducing GLTU 1 expression.

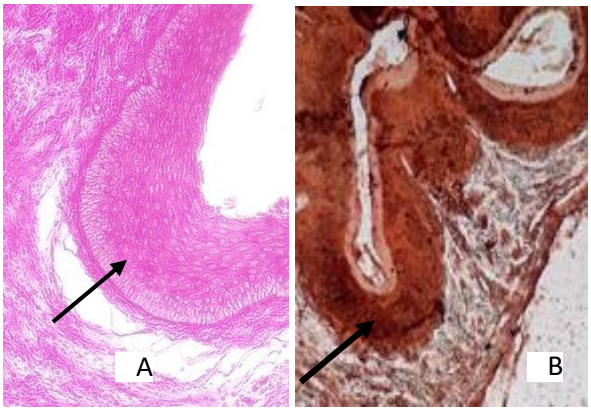

Figure 5. The vaginal cross section of ovariectomized mouse with estrogen conjugate control. A. with PAS staining. B. with Immunohistochemistry staining (Anti GLTU1). The black arrow showed the epithelial cell layer.

4. DISCUSSIONS

The result of maceration using 96 % ethanol showed than the macerate contains especially some active compound such as flavonoid, diterpenoid and steroid [4]. And that all compounds could bind to estrogen receptor α in epithelial cell of vagina and induce cell proliferation in it [5]. The increasing in the number of epithelial cells in vagina increased glycogen level. Glycogen was synthesized from glucose in blood serum in vaginal epithelial cell [6]. Glucose, a major source of energy for all cells, is transported into cells with the help of glucose transporters (GLUTs) [7].

Glycogen synthesis was induced by estrogen signaling [8]. Estrogen through estrogen receptor induce Glucose transporter (GLTU1) synthesis [9]. GLUT1 facilitates the transport of glucose across the plasma membranes of mammalian cells. In the vagina, the basal layers of epithelium were expressed abundantly Glucose Transporter 1 or GLUT1 [10]. Glycogen synthesis was stimulated by glucose uptake and activated by the key enzyme glycogen synthase (GS). Insulin is believed to activate GS mainly through the inhibition of GSK3 [11]. So active compound of *Graptophyllum pictum* L. Griff. could bind to estrogen receptor and induce synthesis of GLTU1. Then GLTU 1 function to transport glucose enter the cell. And for synthesis glycogen (Glycogenesis), glucose underwent some steps, namely glucose phosphorylation (conversion glucose to be glucose-6P), Glucose-1-phosphate formation by phosphoglucomutase enzyme that catalyzed the transfer of the phosphate group from carbon 6 to carbon 1 on the glucose molecule, glucose activation to be uridine

diphosphate glucose (UDPG) and pyrophosphate (PPi), and UDPG with glycogen synthase would form glycogen. Insulin function to inhibit Glycogen synthase kinase (GSK) that inhibit synthesis glycogen. If GSK was inactive, GS could active and function in glycogen synthesis [12].

In ovariectomized or menopausal condition tended to accumulate fat, so it would increase proinflammatory factor such as TNFα [13] and inhibit insulin bound to insulin receptor [14]. If there was not insulin in cell, so there was not glycogen synthesis. Leaf ethanol extract of this plant could decrease proinflammatory factor and increased insulin enter the cell and increased glycogen synthesis.

Leaf ethanol extract of *Graptophyllum pictum* L. Griff. effect in inhibition of vaginal atrophy of menopausal mouse showed in increasing of the thickness of vaginal epithelial cell layers, glycogen level and expression and also in GLTU 1 expression. The optimal dosis was 10 mg/kg.

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