Regulation of Integrin β3 Protein Secretion on Implantation Embryo of Mouse (Mus musculus L.) Induced by Oil Atsiri of Purple Nutsedge Tubers (Cyperus rotundus L.)

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WORD COUNT 1755 TIME SUBMITTED 10-MAR-2021 11:23AM
PAPER ID 69796924

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1. INTRODUCTION

This study aims to determine the role of the β3 integrin protein as a receptor for matrix proteins, and an important role in the interaction between tropectoderm and uterine epithelium. Some components that play a role in the process of embryo implantation include cytokines, adhesion molecules [1,2] and proteolytic enzymes [3]. Integrin and fibronectin, trophinin, tastin and blystin are adhesion molecules expressed both in blastosis and endometrium of the uterus and are believed to play a role in the process of implantation [2,4].

These molecules are functional proteins that activate signals in cells during embryo implantation. Functional proteins that regulate certain biological processes are known as regulatory proteins [5,6]. Regulatory proteins secreted during implantation can be classified as signaling proteins [7,8]. Signaling protein in the process of implantation is a protein that plays a role in the process of cell adhesion through activation of signals in cells [7]. The protein is secreted in small amounts, with varying amounts depending on differences in size and relative solubility in water [8].

The integrin protein has a role in influencing and mediating adhesion, migration, invasion and cellular signaling. The αvβ3 and α4β1 integrin proteins are markers of the uterus. Avβ3 integrin shows high expression in embryo attachment, so αvβ3 integrin is associated with infertility problems [9]. In the endometrium, cytotropoblasts participate in regulating the expression of integrins. The expression of β3 integrins in the glandular and luminal epithelium will increase regulation because it coincides with the implantation window of endometrial receptivity. In addition, tropectoderm blastosis also expresses several integrins such as α3, α5, β1, β3, β4 and β5, thought to involve binding of blastocysts on the endothelial surface [10].

On the other hand, some plants including the puzzle grass tubers are as antifertility, especially as blastocytotoxic [11]. In the research results of chloroform extract and methanol tubers of puzzles of grass, are known to have a cytotoxic effect on HeLa cells and SiHa cells (cervical cancer cells) [12]. Kilani et al. [13] have conducted research by testing the tubers of puzzles on leukemia cells (L1210). The results showed a cytotoxic effect by inducing apoptosis. In this connection, the extract of the puzzle grass tuber indicates endometrial dysfunction. This condition can be used as a clinical marker in the assessment of endometrial receptivity for infertile women.

2. MATERIALS AND METHODS

Production of Purple Nutsedge Tubers Essential Oil

The initial process in this research is to identify and determine the plants that will be used based on observing the morphological characteristics of plants such as flowers, leaves, stems, roots and tubers. The tubers are washed clean, then dried at room temperature for about one week, after that chopped into small size. A total of 10 kg of simplicia purple nutsedge tubers distilled Stahl with distilled water 2/3 the contents of the flask and heated at a temperature of 100°C for 3 hours until a mixture of oil and water is obtained (until no essential oil drops are formed). Then the oil mixture is evaporated in vacuo at low temperatures to obtain essential oils of tubers. After that the essential oil is separated, the essential oil which is still mixed with a little water is removed by adding $MgSO_4$ $7H_2O$ until saturated and then separated. The essential oil obtained is used as a sample for the next process.

Determination of Doses of Purple Nutsedge Tubers Essential Oil

According to Sa'roni and Wahjoedi (2002) [14] about "the effect of infusion of rhizome of turf grass on the estrous cycle and uterine weight in white rats", the treatments given are:

Control group with 1 ml/100 g body weight (A)
Dosage group 11.25 mg/100 g body weight (B)
Dosing group 112.5 mg/100 g body weight (C)
Dosing group 337.5 mg/100 g body weight (D)

The dosage was obtained from the 11.25 mg purple sedge tubers extract and was given to the white rat weight tested animal; 100 g (2.5 x body weight of mice), then converted to body weight of mice randomly grouped into 4 groups, so that the dose of the essential oil of tubers used in this study are:

1. Group C was given 0.4 ml of aquabides as a control
2. Group T1 (treatment 1) is given a dose of 4.5 mg/40 g body weight in 0.4 ml of aquabides
3. Group T2 (treatment 2) is given a dose of 45 mg/40 g body weight in 0.4 ml of aquabides
4. Group T3 (treatment 3) was given a dose of 135 mg/40 gr body weight in 0.4 ml of aquabides

Provision of Purple Nutsedge Tubers Essential Oil in Experimental Animals

Mice maintained in laboratory conditions that are controlled by feeding and drinking are given *ad libitum*. The study was conducted in a series of stages. The first step is mating by combining one female mouse with one male mouse. Mated female mice are characterized by the formation of a vaginal plug. The day the vaginal plug was found was assumed to be the first day of pregnancy. In this study the control animal estrous cycle was determined by vaginal smears. The second stage is the maintenance of mice that have been mated. Mice that have been mated or that have been determined to have estrous phases are separated and maintained in a solitary cage. Then each treatment group was given a fraction of the essential oil of the tuber according to the dose of the treatment by means of being fed (orally) using a syringe whose edges were blended and given a small rubber pipe. The scraping is done once a day for 6 days, to determine the effect of the purple sedge tubers essential oil fraction on experimental animals.

Statistical Analysis

Data were analyzed using the data normality test (Kosmogorov-Smirnov test), while the homogeneity of the data tested using the Levene test. Criteria for testing decisions is if the Sig/p-value is highter than alpha = 0.05 then it is said that the data is normally distributed. While the test decision criteria in the Levene test are homogeneous sample data if the Sig or p-value> α = 0.05. The data then proceed by one-way Anova test. If the one way Anova test results in the conclusion Ho is rejected, it means that there are significant differences and the analysis is continued with a multiple comparison test, in this case the Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

The homogenity test of variance based on the Levene test shows that the value is (0.501> 0.05). It means that the research data of Integrin β3 (pg/ml) has the same variant (homogeneous). Then the data proceed by Anova test to find out whether there is an effect of the purple sedge tubers essential oil on the β3 integrin level of experimental animals, which is the value of F = 70,852 with p = 0,000.

Anova statistical test showed that there was an effect of the puzzle essential oil on integrin β3 (p 0.00) in each group. Furthermore, differences were made between groups with the post hoc test as in the following table.

Table 1. Anova test effects of purple nutsedge tubers essential oil on β3 integrin

Group	β3 Integrin levels	p
C	277,46 ± 2,93	
T1	243,42 ± 3,90	0,000
T2	265,83 ± 4,98	
T3	257,13 ± 4,59	

Table 2 shows that there is a significant difference in the level of β3 integrin between the control group and P1, P2 when compared to the control. In this study, it was suspected that a decrease in the uterine β3 integrin level of mice was caused by the effect of the essential oil of the tubers on the luminal epithelial cells and endometrial glands so that the endometrial response was reduced.

It is known that the essential oil of the tuber with its chemical content is cytotoxic and apoptotic to cells such as epithelial cells and endometrial gland cells [15]. The results of this study are in

Table 2. Post hoc test β3 integrin levels after giving purple nutsedge tubers essential oil

Group	T grade			
	C	T1	T2	T3
C	-	0,000	0,000	0,000
T1	0,000	-	0,000	0,000
T2	0,000	0,000	-	0,002
T3	0,000	0,000	0,002	-

line with the opinion of Garrido et al. (2002) [16] that the expression of integrins in the endometrium provides an overview for the assessment of endometrial receptivity. Avβ3 integrins are produced by endometrial glands and luminal epithelium, with the implantation window opening. In the event of interruption/damage, the luteal phase will suppress the integration of αvβ3 integrin during the implantation window. If integrins are found in low conditions this will indicate an effect on endometrial stimulation. Such cases are suspected due to interference / damage to endometrial receptivity.

A number of adhesion molecules, especially integrins, are expressed by the endometrium during the menstrual cycle and pregnancy. Integrins are needed for successful interactions between embryos and endometrium [17]. Reduced uterine receptivity and β3 integrin expression result in endometrial abnormalities. This can be caused by several pathological disorders such as in the case of hydrosalpinges, endometriosis and damage in the luteal phase [18,19].

The αvβ3 integrin is closely related to the morphological maturation of the endometrial glands. Perfect growth of the endometrial gland expresses high levels of αvβ3 integrin [20]. Disturbances in endometrial morphology can affect integrin expression when the implantation window opens. In this study, it is known that due to the administration of essential oils of rooting tubers causes low secretion of β3 integrin during the process of implantation, this is thought to be due to the disruption of the morphology of endometrial cells [21].

Changes in the expression and distribution of integrin receptors in tropoblast cells are thought to cause the development of tropoblast attachment and displacement [22]. It is suspected in this study that giving fraction of essential oil of tubers causes disruption to the attachment and displacement of tropoblasts from blastocyst cells. This is in line with the opinion of Raghunath*et al.* (2009) [11] that some plants, including tubers of puzzles, are as antifertility, especially as blastocytotoxic. Thus it becomes clearer that integrin molecules have various expression patterns during the implantation window in the uterus and on the surface of the blastocyst [2].

4. CONCLUSION

In conclusion, the application of essential oils of purple sedge tubers reduce the levels of β3 integrin of uterine mice during the embryo implantation period.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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