

## THE POTENCY OF PULUTAN (*Urena lobata* L.) LEAVES DECOCTION AS ANTIFERTILITY BASED ON ITS EFFECT ON UTERINE DEVELOPMENT OF BALB C MICE (*Mus musculus*)

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### ABSTRACT

The purpose of this research was to determine the effect of decoction of pulutan leaves simplicia (*Urena lobata* L.) on the development of the uterus of Balb C mice (*Mus musculus*). Female mice aged 10-12 weeks were given decoction of pulutan leaves simplicia with concentration of 5%, 7.5%, 10%, 12.5%, 15%, and control. The administration of pulutan leaves was performed by gavage for 11 consecutive days. On day 12, mice were dissected, then, the uterus was taken for histological preparations using paraffin method. Data such as uterine diameter and myometrium and epimetrium thickness were analyzed using one way analysis of variance followed by least significant different. The results showed that decoction of pulutan leaves simplicia decrease the uterine diameter and epimetrium and myometrium thickness compared to control. The decrease of uterine diameter and uterine myometrium and epimetrium thickness was started at the concentration of 7.5%. Decoction of pulutan leaves simplicia could reduce uterine diameter and thickness of uterine myometrium and epimetrium, thus, it might decrease the uterine function as the site of implantation. The decline in the function of the uterus might decrease the number of born children, thus decoction of pulutan leaves simplicia is potential as antifertility agent.

Key words: epimetrium thickness, mice, myometrium thickness, pulutan, uterine diameter

### ABSTRAK

Tujuan dari penelitian ini adalah mengetahui pengaruh rebusan simplisia daun pulutan (*Urena lobata* L.) terhadap perkembangan uterus mencit (*Mus musculus*) Balb C. Mencit betina umur 10-12 minggu diberi rebusan simplisia daun pulutan dengan konsentrasi 5%; 7,5%; 10%; 12,5%; 15%; dan kontrol. Pemberian dilakukan secara gavage selama 11 hari. Setelah perlakuan pada hari ke 12, mencit dibedah, uterus diambil untuk dibuat sediaan histologi dengan metode parafin. Data berupa diameter uterus dan ketebalan epimetrium+miometrium dianalisis dengan analisis varian tunggal dilanjutkan dengan uji beda nyata terkecil. Hasil penelitian menunjukkan bahwa rebusan simplisia daun pulutan menyebabkan diameter uterus dan ketebalan epimetrium-endometrium menjadi lebih rendah dan berbeda dengan kontrol. Konsentrasi yang mulai menurunkan diameter uterus dan ketebalan epimetrium+miometrium adalah konsentrasi 7,5%. Rebusan simplisia daun pulutan dapat menurunkan diameter uterus dan ketebalan epimetrium+miometrium sehingga dimungkinkan dapat menurunkan fungsi uterus sebagai tempat implantasi. Penurunan fungsi uterus dapat menurunkan jumlah anak yang dilahirkan, dengan demikian rebusan simplisia daun pulutan berpotensi sebagai bahan anti fertilitas.

Kata kunci: ketebalan epimetrium, mencit, ketebalan miometrium, pulutan, diameter uterus

### INTRODUCTION

As the one of natural material, pulutan (*Urena lobata* L.) can be utilized as alternative for medical material. Society of Central Kalimantan used *Urena lobata* and *Bauhinia tomentosa* as an agent for men contraception and cancer treatment as well. Pulutan leave is a source of antioxidant since it has flavonoid content (Singh and Singh, 2014). In accordance with study of Fagbohun *et al.* (2012), pulutan leaves acknowledged as containing alkaloid, tannin, terpenoid, flavonoid, saponin, steroid, and phlobatanin, as well as containing mangiferin and stigmaterol. A study suggested that mangiferin dan stigmaterol had antifertility properties (Rinku *et al.*, 2008). Furthermore, Sosa and Carmelo (2010) stated that *Urena sinuate* L. which was a subspecies of *Urena lobata* L., containing steroid (stigmaterol,  $\beta$ -sitosterol), xantone (mangiferin), flavonoid, sugar, and vitamin.

Pulutan as a natural agent containing alkaloid, tannin, saponin, terpenoid, stigmaterol, mangiferin, and flavonoid had been suggested as an antifertility agent. Studies on the potency of pulutan as antifertility agent were infrequent. One of them was conducted by

Rahman (1992) which suggested that 10% infusion of *Urena lobata* and *Bauhinia tomentosa* root at a dose of 0.25 mL, 0.5 mL, and 1 mL could in hibit spermatogenesis process in mice. In vitro studies resulted that the administration of pulutan leaves tent to increase the abnormal spermatozoa in mice (*Mus musculus*) Balb C. Another study of Dhanapal *et al.* (2012) reported that root of *Urena lobata* at dose of 600 mg/kg weight decrease spermatozoa motility in Wistar mice. In accordance with Handayani and Tenzer (2014), the administration of pulutan leaves simplicia decrease the sperm quality in mice. Regarding to those studies, it could be assumed that the administration of pulutan leaves simplicia has a potency to decrease spermatozoa quality hence it was potential for antifertility agent.

The available contraception methods were not yet easily acceptable in mostly society. Beside of the inability to prevent pregnancy at rate of 100%, contraception methods might produce side effects. Recently, it had been studying contraception methods of hormonal and non-hormonal and the feasibility of contraception via vaccine. Therefore, further studies are essential to find out the alternative material from nature

which had minimal adverse effects. One of the suggestions for antifertility potential agent was the pulutan leaves.

### MATERIALS AND METHODS

This research was an experimental study conducted in randomized group trial. The concentrations of pulutan leaves simplicia administration were 5%, 7.5%, 10%, 12.5%, 15%, and control, each sample was examined 4 times. The observed variables were uterine developments including diameter of uterus and the thickness of myometrium+epimetrium.

The animals used in this experiment were mice strain of Balb C, with body weight of 25-29 grams, and aged of 10-12 weeks. The administration of pulutan leaves simplicia was carried out using a tool of gavage for 11 consecutive days (two times of estrus cycle) started from metestrus phase. At the 12<sup>th</sup> day, all mice were euthanized by neck dislocation, dissected to collect the uterine, and then, followed by histological preparation using paraffin method and HE staining. The data obtained were analyzed using analysis of variance (ANOVA) and followed by least significant difference (LSD) test.

### RESULTS AND DISCUSSION

The average of the uterine diameter and epimetrium+myometrium thickness receiving the pulutan leaves for 11 days were shown in Table 1. Based on the analysis, the uterine diameter of mice

receiving the pulutan leaves was narrower than that of control (Figure 1). The concentration of pulutan leaves administration that initiated a positive effect on uterine diameter was at concentration of 7.5% (Figure 2). The diameter of uterine decreased from its size from 78.083  $\mu\text{m}$  (control) to 58.480  $\mu\text{m}$ . Similarly, the thickness of miometrium+epimetrium started to differ from of the control occurred at concentration of 7.5% as well (Figure 2). The thickness of miometrium+epimetrium decreased from 21.498  $\mu\text{m}$  at control group to 19.438  $\mu\text{m}$ .

Uterine is an organ prepared to receive embryo implantation. The preparation is carried out by increasing the cells proliferation; hence the epimetrium, myometrium, and endometrium layers of uterine would be thickened. As a result of the administration of pulutan leaves, the uterine diameter decreased to be narrower than of the control, hence it suggested that it would alter the implantation process and reduce the number of implantation. If the number of implantation reduced, the number of offspring would be reduced as well.

The potency of pulutan leaves as antifertility agent was potential due to its chemical compounds content. Pulutan leaves as natural agent containing alkaloid, tannin, saponin, terpenoid, stigmasterol, maniferin, and flavonoid. Herdiningrat (2002) stated that there were two mechanism principles of the antifertility materials, such as by destructing the cells (cytotoxic and cytostatic) and by altering the hormonal function (hormonal effect). Further more, Ali *et al.* (2013) observed that methanol extraction of pulutan leaves had cytotoxic activity.



**Figure 1.** Uterine condition of the control. A= Uterine diameter, B= Myometrium+epimetrium thickness



**Figure 2.** Uterine condition of treated mice with the decoction of pulutan leaves simplicia at concentration of 7.5%. A= Uterus diameter, B= Myometrium+perimetrium thickness

**Table 1.** Diameter of uterine and thickness of myometrium+epimetrium of the mice (*Mus musculus*) Balb C treated with the decoction of pulutan leaves simplicia for 11 days

Concentration (%)	Uterine diameter ( $\mu\text{m}$ )	Myometrium+epimetrium thickness ( $\mu\text{m}$ )
0	78.083 <sup>d</sup>	21.498 <sup>d</sup>
5	70.023 <sup>d</sup>	25.333 <sup>cd</sup>
7.5	58.460 <sup>c</sup>	19.438 <sup>bc</sup>
10	48.248 <sup>b</sup>	16.335 <sup>b</sup>
12.5	41.535 <sup>ba</sup>	11.918 <sup>a</sup>
15	35.375 <sup>a</sup>	10.485 <sup>a</sup>

<sup>a, b, ba, bc, c, cd, d</sup> Different superscripts within the same column indicates significant correlation ( $P < 0.05$ )

The narrowing of uterine diameter in the treated mice was possibly an impact of tannin and alkaloid contained in pulutan leaves. Durian bark extract have been observed to have cytotoxic properties such as tannin and alkaloid (De Pandua, 1978 as cited in Nurliani and Santoso, 2010) as well as saponin, triterpenoid, flavonoid (Rusmiati, 2010). Nurliani *et al.* (2005) found that the cytotoxic and cytostatic properties from saponin, flavonoid, and tannin in durian bark extract result in a decreased number of spermatogenic cells. Moreover, methanol extract of pulutan had properties of antiproliferation on breast cancer cells (Pieme *et al.*, 2012). The tannin and alkaloid in pulutan leaves was also suspected to have cytotoxic properties hence it was destructive toward cells layering uterine wall. Due to the numerous cells destruction of uterine wall, it resulted in a narrowing diameter of the uterine of treated mice than of the control, as well as the result on the myometrium+epimetrium of the treated mice that were thinner than of the control. The narrowing of uterine wall might lead to a reduced ability to receive embryo implantation. The lesser the uterine ability to receive implantation, the lesser the probability of female mice to do the reproduction. In result, the fertility of female mice was decreased.

It suggests that tannin might also result in shrinking of cell membrane hence it might alter the transportation of nutrients through membrane. As the result, it altered the cell metabolism to generate energy. It was suggested that tannin in pulutan leaves had the identical mechanism. If the energy production was depleted, then the cell metabolism would be reduced, it might result an alteration in cell activity that required energy. The cell activity that required energy was such as cell division. If there was alteration in the cell division, then the number of cells would be depleted, as well as depletion of the cells that layered uterine wall, it resulted in narrowed diameter of uterus and reduced thickness of myometrium+epimetrium. This process was possible to decrease the ability of uterine to receive implantation. If there was decreased number of implantation, then there was decreased number of breeds, as the indicator of fertility.

Alkaloid in pulutan leaves might change the activity of ATP-ase enzyme. In accordance with Ashfanani *et al.* (2010), alkaloid in *Curcuma zedoaria* might alter ATP-ase enzyme activity in the cell membrane at middle part of spermatozoa tail. The ATP-ase enzyme had a role to maintain internal homeostasis of sodium and potassium ion. The change on ATP-ase enzyme activity would lead to alteration in homeostasis of sodium and potassium that resulted in alteration of the membrane permeability. Cell membrane permeability alteration might impede the nutrients transportation for cell metabolism producing energy, thus alteration in energy producing occurred. Division of cell was an activity requiring energy hence depletion of energy would affect the cell division activity. It might result in a decreased diameter of uterine and a decreased thickness of myometrium+epimetrium.

The content of tannin, saponin, and flavonoid in pulutan leaves was suggested to affect the similar mechanism. As an anticancer agent, flavonoid in pulutan leaves might inhibit the cell division. When the number of cells layering the epimetrium, myometrium, and endometrium of mice uterine became lesser, then it resulted in a decreased diameter of uterus. In the same attitude, it leads to a decreased the thickness of myometrium+epimetrium. These two mechanisms would result in alteration on uterine function to receive implantation which would cause a decreased number of implantation. The decreased implantation was the indicator of the decreased fertility rate in mice.

## CONCLUSION

The decoction of pulutan leaves simplicia able to decrease the diameter of uterine of mice or the thickness of myometrium+epimetrium of the mice.

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