Effect of Pulutan (*Urena lobata* l) Leaves Decoction on the Hormone Testosterone Levels of Balb C Mice (*Mus musculus*)

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Abstract. The side effects of using natural ingredients as antifertility are minor compared to antifertility made from synthetic chemicals. This study aimed to examine the impact of pulutan (*Urena lobata* L) leaf decoction on the fertility of male Balb C mice (*Mus musculus*), reviewed for their effect on testosterone hormone levels in mice. Male mice aged 10-12 weeks were given five pulutan leaves, 5%, 7.5%, 10%, 12.5%, 15%, and controls. Gavage is provided for 36 days. The sample used to measure testosterone levels is the blood and sperm count of mice. Data were analyzed by single ANOVA followed by an LSD test. The results showed that pulutan leaf decoction at the treatment concentration tended to cause the testosterone hormone concentration to be lower than the control. So, it is expected that the decoction of pulutan leaves will potentially be male antifertility.

1 Introduction

One type of plant that has still not revealed much potential is pulutan (*Urena lobata*, L.). As one of the plants that have not been widely publicized, pulutan can be used as an alternative treatment material. As done by the people of Central Kalimantan, who use *Urena lobata* mixed with *Bauhinia tomentosa* as a male birth control drug and cancer drug. Pulutan leaves contain alkaloids, tannins, terpenoids, flavonoids, steroid saponins, and phlorotannins [1]. One of the subspecies of *Urena Lobata* L is *Urena sinuate* L; both contain steroids (stigmasterol, β-sitosterol), Pantone (mangiferin), flavonoids, sugar, and vitamins [2].

Based on the content contained in Pulutan, namely alkaloids, tannins, saponins, terpenoids, stigmasterol, mangiferin, and flavonoids, pulutan is thought to have potential as an antifertility ingredient. Research on the potential of pulses as an antifertility ingredient includes the results of Rahman (1992), who stated that a 10% infusion of the roots of *Urena lobata* and *Bauhinia tomentose* at doses of 0.25 ml, 0.5 ml, and 1 ml could inhibit spermatogenesis in mice [3]. The origins of Urena lobata at a dose of 600 mg/kg bw reduced the motility of spermatozoa of Wistar rats [4]. The decoction of pulutan leaf simplicia can reduce sperm motility in mice, and the decoction of pulutan leaves also reduces the number of sperm in mice [5]. Furthermore, the decoction of pulutan leaves could reduce the diameter of the uterus and the depth of myometrium + epimetrium. Based on the above studies, the

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decoction of pulutan leaf Simplicia can reduce the quality of reproductive organs, so it can potentially be an antifertility material that can reduce the fertility of mice [6].

The quality of spermatozoa dramatically determines the success of fertilization so that it will evaluate individual fertility. Spermatozoon must have a regular shape and be able to move progressively (straight forward) to fertilize the egg. The quality of spermatozoa includes motility, amount, morphology, and viability. One factor that can affect the quality of spermatozoa is the hormone testosterone because spermatogenesis is influenced by the hormone testosterone [7].

Male contraceptive methods include condoms, vasectomy, and interrupted intercourse [8]. The community has not entirely accepted the technique; besides not being able to prevent a pregnancy with 100% success, this method can cause adverse side effects. Currently, contraception is being developed using hormonal and non-hormonal methods and studying the possibility of contraception using vaccines.

Therefore, there is a need for studies to find alternatives by utilizing natural materials that have minimal effects. One of the suspected antifertility potentials is pulutan leaves.

2 Experimental

This research is an experimental study with a randomized block design (RBD). The concentrations of pulutan leaf simplicia used were 5%, 7.5%, 10%, 12.5% , and 15%, as well as controls, and each was repeated four times.

The experimental animals used were male Balb C strain mice weighing 25-29 grams and aged 10-12 weeks. Treatment was administered orally by gavage, and pulutan leaf decoction was administered in 36 days. After treatment, neck dislocation was conducted, and the mice were dissected. The mice's blood was taken to determine testosterone levels. The test used is the ELISA test. And its cauda epididimis was taken. After that, sperm suspension was made to count the amount of its spermatozoa. The amount of spermatozoa were counted using hemocytometer type improved Neubauer.

The data obtained were then analyzed using a single ANOVA with a significance level of 5% to determine whether there was an effect of pulutan leaves if F count> F table, or if there were significant differences between the control group and the treatment group then proceed with the LSD test with a significance level of 5 %.

3 Results and Discussion

Based on the testosterone levels test results, it was found that administering pulutan leaves Simplicia decoction tends to reduce testosterone hormone levels in treated mice. However, it is not statistically significantly different from the control. As shown in the table below, the testosterone levels of the mice treated were all lower than those of the control group.

Table 1. Testosterone levels from mice that were given pulutan leaves Simplicia decoction for 36 days

Treatment (%)	Testosterone Hormone Levels (ng / mL)	Quantity/10-5ml
P1(control)	19.930 ± 0.981^{a}	203.38^{cd}
5	16.877 ± 1.889^{a}	230.67^{d}
7.5	17.632 ± 1.794^{a}	179.25^{bc}
10	14.800 ± 1.119^{a}	159.50^{b}
12.5	15.875 ± 2.203^{a}	113.63^{a}
15	15.959 ± 1.234^{a}	84.00^{a}

Description: Numbers followed by different letters in the same column state that there are influences manifest due to treatment

Based on the Table 1 above, it can be seen that decoction of pulutan leaf leaves given for 36 days tends to reduce testosterone hormone levels, although not significantly different statistically. Pulutan leaves simplicia decoction at all concentrations, causing the testosterone hormone concentration to be lower than the control. The sperm count from treated mice was also less and significantly different from control mice for concentrations of 10 %, 12.5%, and 15%.

The potential of pulutan leaves as an antifertilizing agent is possible because of the chemicals they contain. *Urena sinuate* L and *Urena lobata* L contain steroids (stigmasterol, β-sitosterol), Pantone (mangiferin), flavonoids, sugars, and vitamins [2]. The results of LCMS from pulutan leaves contained quercetin, myricetin, cubebin, Gossypetin, quercetin, hypocretin, and all flavonoids. Decreased testosterone levels from treated mice may be due to flavonoids in pulutan leaves. Isoflavones and flavanones can inhibit cholesterol synthesis and increase LDL receptor expression, lowering blood cholesterol levels [9]. Blood cholesterol levels were significantly reduced due to administering flavonoids in ginger as much as 30 grams/kg per day for 15 days [10]. If the essential ingredients of testosterone are reduced, the testosterone produced will also decrease. This can be seen in mice treated with testosterone levels lower than control.

The essential ingredient for testosterone formation is cholesterol. Cholesterol from blood in Leydig cells will be synthesized into testosterone via paths Δ5 and Δ4. Furthermore, testosterone will be circulated to the target cell through blood vessels and lymph vessels. Some exocrine will be taken to Sertoli cells, and with the help of 5α reductase, testosterone will be converted to dihydrotestosterone, which is more potent testosterone. Furthermore, testosterone and dihydrotestosterone will bind to Sertoli cells' androgen receptor (AR). In addition to being attached to the androgen receptor, testosterone will be bound to the Androgen Binding Protein (ABP) produced by Sertoli cells. It will be taken out of the seminiferous tubule and to the epididymal duct, the vas deferens, which will be needed for the duct function [7].

The decrease in testosterone is also thought to be due to alkaloids in pulutan. Reproductive hormone secretion (testosterone) can be suppressed by alkaloids, disrupting spermatogenesis [11]. Androgen hormones regulate spermatogenesis; AR strongly influences this role, as the luteinizing hormone (LH) concentration and follicle-stimulating hormone (FSH). If spermatogenesis is disturbed, it will cause low male fertility [12]. Based on the research results, pulutan is suspected to inhibit spermatogenesis, as seen from the lower number of spermatozoa in treated mice than controls. Testosterone in Sertoli cells has three functions: maintaining the integrity of the blood-testicular barrier between the basal and adluminal compartments, thus ensuring spermatogenesis is not affected by external influences. Secondly, it is necessary for Sertoli-spermatid Adhesion; thirdly, it is essential to release mature sperm during spermiation. The spermatogenic stage also depends on the hormone testosterone. In addition, the promoter of the AR gene is also sensitive to androgens (testosterone and dihydrotestosterone). Androgens also regulate AR expression and Sertoli cell cycle activity [7]. Spermatogenesis and male fertility depend on the presence of testosterone produced by Leydig cells in the testes. Without testosterone or androgen receptors, spermatogenesis does not continue and only reaches the meiosis stage. The main target of testosterone is Sertoli cells, and its function is in germinal cell development. Sertoli cells can be translated directly as gene expression (classical pathways), or testosterone can activate kinases that can regulate the processes needed to maintain spermatogenesis (non-classical pathways) [13].

Testosterone has a vital role in the reproductive system. These roles include the spermatogenic stage, the formation of AR, regulation of the Sertoli cell cycle, maintenance of the function of the reproductive tract, spermination, and keeping the blood testicular barrier [7]. Based on the research, the pulutan of leaf decoction is potentially an antifertility

agent in males by reducing testosterone production. There are two working principles of antifertility, namely by damaging cells (cytotoxic or cytostatic effects) and by disrupting their hormonal functions (hormonal effects) [14]. Simplicia decoction of pulutan leaves tended to reduce testosterone in treated mice, while the number of spermatozoa decreased significantly in treated mice.

Simplicia decoction of pulutan leaves is thought to work in 2 ways: affecting hormonal function and causing many spermatozoa to be damaged. This can be seen from the testosterone levels of treated mice, which tend to be lower than those of the control group. The number of spermatozoa in treated mice was significantly lower than in controls, possibly due to reduced testosterone levels and the cytotoxic properties of pulutan leaves.

Decreasing testosterone can affect the fertility of mice treated, reducing fertility. Decreased testosterone levels can reduce libido. Male Kejobong deer with higher testosterone concentration than Bligon male deer have better libido and sperm quality [15]. Based on this, a decrease in testosterone levels in treated mice might cause a reduction in libido and the quality of sperm in mice treated. As a result, pregnant female mice are also less than control. Female mice that were mated with pregnant male dick were 75%, whereas, in mice bred with male treatment, 15%, there were no pregnant female mice. Based on these results, the pulutan leaf simplicia decoction can reduce the fertility of male mice.

4 Conclusion

Giving decoction of Pulutan (*Urena lobata* L) leaves tends to reduce testosterone hormone levels and the number of spermatozoa, so decoction of Pulutan leaves has the potential to cause male antifertility.

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