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Spermatozoa Structure Treated by Plucheaindica Tannin: A Case Study on Male White Mice

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----------ABSTRACT----------
Plucheaindica is one of herbal plants that is potential for antifertility due to tannin content on its leaves. This study aimed at studying the structural change on spermatozoa of male white mice treated by Plucheaindica tannin. The tannin extraction was on the basis of Lowenthal-Procter method. In practice, there were three sorts of treatments, comprising: (A) administering aquadest to control group; (B) giving pure tannin; and (C) administering Plucheaindica tannin. After 49 days of treatment, spermatozoa were observed by using electron microscope. There were some parameters considered to measure the structure of spermatozoa, i.e. size and shapes of head, neck, and tail. The result indicated that the structure of spermatozoa from the three different treatments were all of uniformity. For that reason, pure and Plucheaindica tannins did not yield significant difference on the spermatozoa structure of male white mice.
KEYWORDS: *tannin, antifertility, Plucheaindica*

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I. INTRODUCTION

The presence of contraception remains crucial for human's life. Generally speaking, the use of contraception is closely related to family planning program [1]. Indeed, since 1960s, many life couples have been making use of contraception to avoid pregnancy [2]. In addition to undesired intention to have another pregnancy, contraception is commonly used to set up birth spacing [3]. Contraception is deemed as an effective key to dropping out maternal mortality and morbidity due to unplanned pregnancy [4]. What is more, contraception can be the simplest way to avoid sexually transmitted disease [5], [6]. One of contraception forms is the use of antifertility agent.

Antifertility agent refers to a specific substance with an ability of controlling fertility [7]. It works throughout series of mechanism, such as preventing fertility, thwarting ovulation, mortifying zygote, resisting testosterone, and improving sperm mortality [8]. In common practice, synthetic estrogen and progesterone are used by the majority of people despite their negative impacts to organs and organ systems [9]. In response to such a fact, traditional medication, such as consuming medical herbs, has been popular to most of populations over the world in an attempt to regulate fertility since then [10]. Therefore, exploring medical herbs that contain antifertility agent is in need of actualization [7], [9].

One of medical herbs that has been existent and potential to be a source of antifertility agent is Plucheaindica. P. indicais one of native plants to South East Asia, including Indonesia. This plant is consumed as vegetable and medical herb to all people within the communities in the region [11]. P. indica is consumed for traditional medication by making use of some parts of the plant, such as flower, leaves, stem, and root [12]. Investigation on a number of plants on Pluchea genus exhibits the presence of various bioactive substances, i.e. flavonoid, sterol, triterpene, phenylpropanoid, chalcones, sesquiterpene lactones, and tannin [13], [14]. The existence of bioactive substances is the basic reason why this plant is considerably effective for medication to several illnesses, such as diabetes mellitus, tumor, hypertense, cystitis, wound, brain tonic, kidney stone, hemorrhoid, soreness, backache, vaginal discharge, lumbago, ulcer, tuberculosis, and inflammation [11], [12]

With reference to antifertility potential, some previous researches have studied the significances of P. indicato the fertility of model organism. A study from Muchtaromah, Mukholifah, Nasiroh, Ahmad, & Romaidi (2018) informs that P. indica alongsideasiatica can reduce the number of ovulation, follicles, and estrogen level of mice. In addition, another study from Amalina, Suyatmi, & Suparyanti (2010) also indicates that spermatid cells of mice decrease after being treated using the extract of P. indica leaves. Secondary metabolite substances are meant to become responsible substances for antifertility contained at P. indica; one of which is tannin.

Tannin signifies secondary metabolite that is mostly generated in any plants [17]. It mainly plays role as a defensive system from the threat of herbivores [18]. There are some parts of plants of which tannin concentration is relatively high, to name root, seed, stem, fruit, and leaf [17]. In certain cases, some researches

classified tannin as a substance that was responsible for antifertility in particular kinds of plants, such as calliandra and pineapple peels [19], Dactylocteniumaegyptium[20], and Citrus aurantifolia[21]. There were also some analyses concerned on tannin effect yielded by P. indicaupon the fertility of male mice. However, the analyses only studied tannin effect to spermatozoa concentration [22], testosterone level, [23], and glutamate acid level in mice's cement [24]. Furthermore, a research that studies the effect of secondary metabolite on the sperm structure has never been conducted. For that reason, this current study focused more on the tannin effect yielded by P. indica upon the spermatozoa structure of male white mice.

II. RESEARCH METHODS

Preparation of Model Organism

Model organism used in this research was white mice (wistar mice) with the age of 3-4 months old originated from inbreeding. The mice were taken care from their birth to applying treatments. Specifically, it is the healthy, mature (in term of sex), and male white mice that were selected as the sample with the total of 12.

Extraction of Tannin Fraction

The process of extraction was through maceration-percolation procedure with addition of ethanol solvent, Soxhlet isolation, extraction fractionation, and componential segregation in the fraction. For tannin extraction, Lowenthal-Procter method was used.

Treatments to the Mice

After having the mice adapt to the given environment (cage) placed in laboratory for about two weeks, treatments were administered to the targeted groups of experiment. There were three treatments designed for the groups: (A) giving aquadest to the control group; (B) administering pure tannin; and (C) giving P. indica tannin. The treatments run in three times a day, for 49 days in total, with the dose in each treatment signifying 0.8 ml.

Observation on Spermatozoa Structure

After 49 days of treatments, spermatozoa were observed using electron microscope. The parameters for observation included size and shapes of head, neck, and tail.

III. RESULTS

Data that serve observational result of spermatozoa structure of male white mice treated by using P. indica tannin are presented in the following Table 1.

Treatments	**Sizes of Spermatozoa**	**Forms**		
		Head	Neck	Tail
Control	82 µm	hook-shaped	oval	tapered-long
Pure Tannin	82 µm	hook-shaped	oval	tapered-long
P. indicaTannin	82 µm	hook-shaped	oval	tapered-long

Table 1. Data about size and shapes of head, neck, and tail of spermatozoa found in male white mice treated using P. indica tannin

According to Table 1, it is shown that the shapes of head, neck, and tail on male white mice were totally the same in all groups with different treatments. The illustration of spermatozoa structure found on male white mice after aquadest treatment is shown in Figure 1. In addition, the illustrations of spermatozoa structure on male white mice after P. indica and pure tannin treatments are respectively shown in Figure 2 and 3.

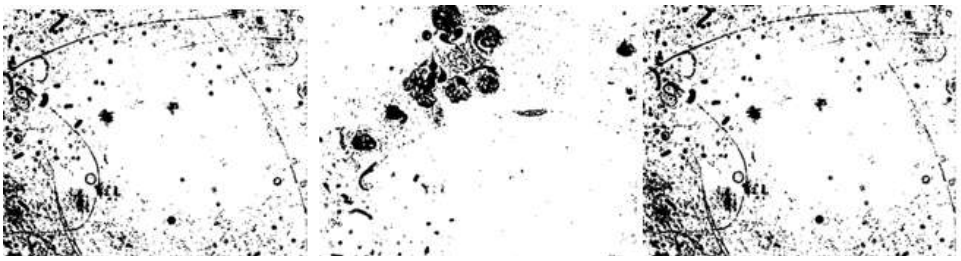

Figure 1. The spermatozoa structure after aquadest treatment (control group) with the repetition of 1,2,3

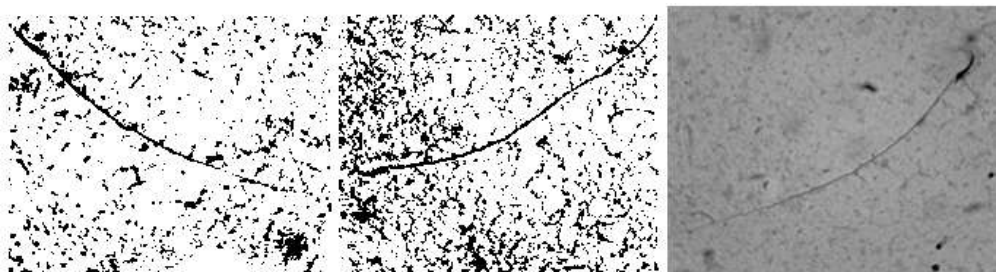

Figure 2. The Spermatozoa structure after P. indica treatment with the repetition of 1,2,3

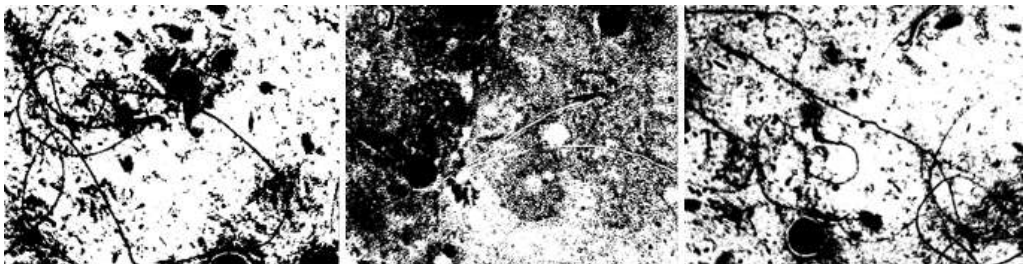

Figure 1. The spermatozoa structure after pure tannin treatment with the repetition of 1,2,3

The spermatozoa structure of male white mice without any tannin treatment and with tannin treatments typified similarities in terms of head, neck, and tail shapes. The abovementioned Figure 1, 2, and 3 have shown that the spermatozoa structure found in male white mice from vas deferent were characterized with hook-shaped head, oval neck, and tapered-long tail. It implied that both tannins, pure and P. indica-made, did not affect the spermatozoa structure.

The sperm under observation was actually limited to one taken out from vas deferent. Vas deferent constitutes a part of male reproduction system which is responsible for transporting sperm from epididymis to ejaculatory ducts [25]. In epididymis, sperm undergoes maturation and is through a process of morphogenesis alongside the formation of head, neck, and tail to form the perfect shapes [26]. The degree of perfection in sperm's size and structure is considered as one of underlying factors that determines the mobility of the sperm in female reproduction tract [27]. Sperm's morphology is also another consideration to define the success of fertility [28]

This finding corroborates an assumption that the mechanism of antifertility on P. indica is much more likely as a result of its effectiveness in reducing the number of spermatogenic cells than in affecting spermatozoa structure. Tannin extracted from P. indicahas been deemed effective to affect the degree of fertility in mammals, both female [15] and male [16]. Referring to male fertility, tannin has been proved effective to decrease spermatozoa concentration [22]. The decrease of spermatozoa concentration, further, is generated by tannin activity to efficiently obstruct cell division [29].

P. indicatannin is also potential to result in damage on the structure of plasm membrane on mitochondria [30]. The corruption of mitochondria would result in obstruction during the process of cell metabolism. This occurrence would decrease the number of ATPs yielded by cells [31]. For that reason, in addition to the obstruction of spermatogenic process, the level of sperm's mortality is also getting higher due to the lack of energy.

In addition to the lack of energy, the higher probability of mortality is also a result of P. indicatannin intervention to the level of glutamate acid in cement. Previous studies have informed that tannin from this plant is effective to reduce the level of glutamate acid contained by cement on male white mice [24]. Glutamate acid is needed in order to preserve the quality of spermatozoa, especially from the damage on cell membrane due to lipid peroxide. The acid would contribute to being antioxidant to protect spermatozoa from free radical generated from the process of oxygen consumption performed by spermatozoa.

IV. CONCLUSION

Hook-shaped head, oval neck, and tapered-long tail have typified the structure of spermatozoa found in male white mice treated by aquadest, pure tannin and P. indica tannin. The structure was similar to the one in common condition. Therefore, to wrap up, adding pure or P. indica tannin will not result in significant difference on the spermatozoa structure of male white mice.

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