Conference Paper

The Effect of Mahkota Dewa (*Phaleria macrocarpa*) Pulp Extract by Peroral Administration Toward The Percentage of Capacitation and Acrosome Reaction in Rat (*Rattus norvegicus*)

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Abstract

This research was conducted to find outthe influence of mahkota dewa (*Phaleria macrocarpa*) pulp extract against the percentage of capacitation and acrosome reaction of male rat (*Rattus norvegicus*). The experimental animals used are 20 male rats with the average body weight of 200g and were divided into four different groups. Po group was the control group and given only CMCNa 0.5%. P1, P2,and P3 was given mahkota dewa pulp extract with a dosage of 7.5 mg/200gr BW/ day, 15mg/200gr BW/day,and 30 mg/200gr BW/day respectively through peroral administration for 14 days. The research used Completely Randoized Design (CRD) and the data was then analyzed by one way ANOVA (Analysis of Variance) followed by Duncan test with the significance of 0.5. The results show that the mahkota dewa pulp extract shows significant impact on capacitation and acrosome reaction of rat. P2 group shows the highest percentage capacitation significance the a mean of 30.00 ± 4.24 and 5.60 ± 0.89 for acrosome reaction percentage.

Keywords: Phaleria macrocarpa pulp extrtact; capasitation; acrosome reaction; sperm potention.

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Received: 03 October 2017
Accepted: 10 October 2017
Published: 29 November 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the VMIC Conference Committee.

1. Introduction

Indonesia's meat consumption development per capita fluctuates and tends to rise from 1993 to 2014 from 0.704kg/capita/year to 2.36kg/capita/year in 2014 [1]. Unfortunately, so far Indonesia has yet to supply all the needs of meat. To supply the rising meat demands a large increase of cattle population is needed. Therefore technology

innovation development is direly in need to optimize and empower local resources. To encourage beef cattle business the farm biotechnology development including IVF (In Vitro Fertilization) needs to be acknowledged [2-3].

In Vitro Fertilization (IVF) is embryo reproduction technology in a media outside the body [4]. In Vitro Fertilization can be defined as the process of fertilization by extracting eggs, retrieving a sperm sample, and the manually combining an egg and sperm in a laboratory dish with culture media [5-6]. Generally, fertilization involves two important processes which are capacitation and acrosome reaction [7]. Those two processes are mutually sustainable. Without capacitation process, spermatozoa is unable to fertilized [8]. One of the main barrier in the application of in vitro fertilization technology is in vitro spermatozoa capacitation [9-10]. Capacitation is meant to dismissed decapacitation factor (protects the stability of the spermatozoa plasma membrane) that is found in semen plasma so that capacitation and acrosome reaction can occur [10].

Spermatozoa needs Reactive Species Oxygen (ROS) in low concentration to induce capacitation and acrosome reaction [11]. If free radical production increases or antioxidant production decreases, it will cause oxidative stress [12]. Oxidative stress is considered one of the factors which causes infertility by giving negative effect to spermatozoa quality such as increasing motility loss, membrane damage, decreasing normal morphology, viability and capacitation ability of spermatozoa.

To prevent those things above additional external antioxidant is needed. Herbal substance which is potential as exogen antioxidant is mahkota dewa fruit which fluorishis in Indonesia. Mahkota dewa can be utilized as antioxidant to reduce the effect caused by free radicals [13].

Keeping those things in mind, this experiment is done to determine the effect of mahkota dewa *(Phaleria macrocarpa)* pulp extract by peroral administration toward the percentage of capacitation and acrosome reaction in rat *(Rattus norvegicus)*.

2. Materials and methods

2.1. Treatment of Experimental Animals

Rat aged 2-3 months with an approximate weight of 200 grams is adapted for 7 days and given treatment for 14 days. Experimental animals are grouped consists one group of negative control and 3 treatment groups. Each group consists of five rats chosen randomly. Control group (Po) consists of five rats given 1 ml of CMCNa 0.5%. P1 group was given 1 ml of mahkota dewa pulp extract 7.5 mg/200 gr BW. P2 group was given

Table 1: Experimental result of capacitation and acrosome reaction of rat (*Rattus norvegicus*) spermatozoa.

Treatment	Capacitation Percentage ($\overline{X}\pm$ SD, %)	Acrosome Reaction Percentage ($\overline{X}\pm$ SD, %)
Po	$19.20^{a}\pm 4.39$	$2.00^{a}\pm 2.00$
P1	$24.80^{b}\pm 3.35$	$3.60^{ab}\pm 1.67$
P2	$30.00^{b}\pm 4.24$	$5.60^{b}\pm 0.89$
P3	$28.00^{b}\pm 4.00$	$4.00^{ab}\pm 1.41$

1 ml of mahkota dewa pulp extract 15 mg/200 gr BW. P3 group was given 1 ml of mahkota dewa pulp extract 30 mg/200 gr BW.

2.2. Capacitation and Acrosome Reaction Examination

Semen was fixated with formaldehyde 4% then washed by adding PBS 3ml and centrifuged 1500 rpm for 10 minutes. Supernatant was then thrown away and added in 0.3ml FITC con A (Sigma) with concentration of 10µg/ml in PBS *dulbecos*. Staining is done 25 minute at room temperature then washed twice by centrifuged 1500 rpm for 10 minutes. Supernatant was thrown away and the sediment was stroked to flow labs slide (specimen), and was added griserol 90% drops. Then the specimen was observed with epifluorescent microscope (Nikon Japan) with B excitation (490 rpm excitation with 525 nm emission) to determine fluorescence of FITC spermatozoa results [14].

3. Results

Experimental result of capacitation and acrosome reaction of rat (*Rattus norvegicus*) which was given with mahkota dewa pulp (*Phaleria macrocarpa*) after stained with FITC (Fluorescent Isotiocianat) and was observed with epifluorescent microscope 400x magnification as shown in Tabel 1 and Figure 1-2.

Different superscripts in one column shows significant difference ($p<0.05$). Po group was given 1 ml of CMCNa 0.5%. P1 group was given 1 ml of mahkota dewa pulp extract 7.5 mg/200 gr BW. P2 group was given 1 ml of mahkota dewa pulp extract 15 mg/200 gr BW. P3 group was given 1 ml of mahkota dewa pulp extract 30 mg/200 gr BW.

Ability of spermatozoa to initiate fertilization must be supported by spermatozoa membrane that has optimum integrity and fluidity. Optimum integrity and fluidity is essential for the capacitation process and acrosome reaction. The presence of free radicals in the tissue that produces spermatozoa can be marked by the rising of the production of ROS that causes membrane damage and alter membrane stability and

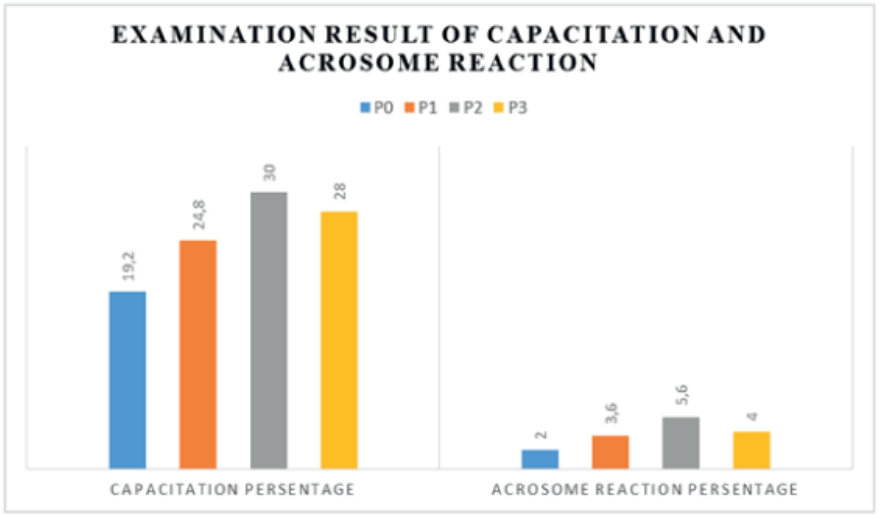

Figure 1: Experimental result of capacitation and acrosome reaction of rat *Rattus norvegicus* spermatozoa in bar diagram.

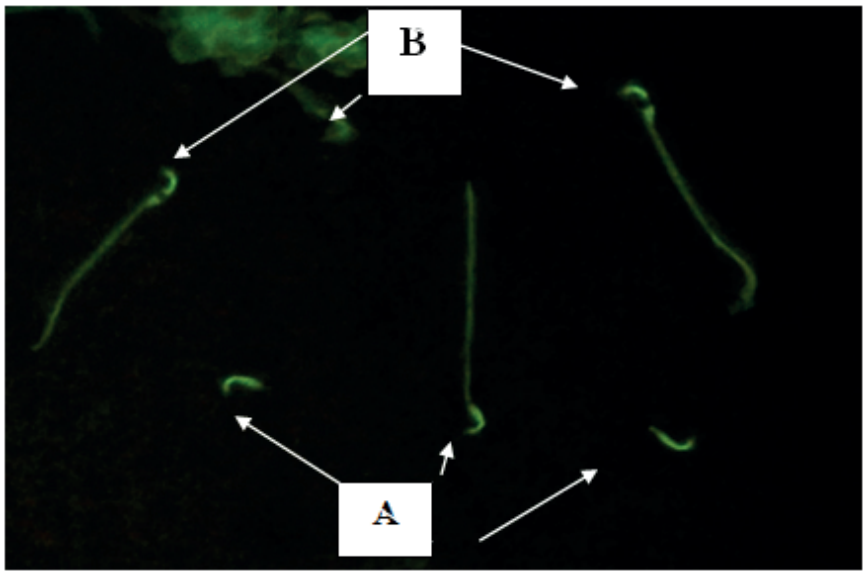

Figure 2: Rat (*Rattus norvegicus*) spermatozoa colored with FITC (Fluorescent Isotiocianat). A. Capacitation (there are acrosome intact and the top of the head of spermatozoa is fluorescent). B. Acrosome reaction (there are no acrosome intact and the aquatorial part of the spermatozoa head is fluorescent).

function [15] and ultimately reducing fertility. In normal conditions, spermatozoa produces ROS in small quantities for sperm regulations this is because spermatozoa needs Reactive Oxygen Species (ROS) in a small concentration to induce capacitation process and acrosome reaction [11].

Spermatozoa is easily damaged by induction of oxidative stress because its sell membrane contains a lot of polyunsaturated fatty acids [16]. This statement is strengthened by [17] dalam [18] Spermatozoa is really sensitive to oxidation because it consists of polyunsaturated fatty acids (PUFA) in high concentration so ROS can cause

the decrease of spermatozoa quality. Sperm parameter changes like motility, viability, capacitation and acrosome reaction can lower the fusion ability of spermatozoa and ovum which causes decrease in fertility.

Po group shows lowest average result of acrosome reaction, it was assumed that the low average result of acrosome reaction was caused by spermatozoa membrane which mostly consists of unsaturated fatty acid that has been damaged by excessive free radicals, considering the free radicals characteristic which easily oxidize unsaturated fatty acid while there is no antioxidant protecting it. The plasma membrane of spermatozoa consist a massive amount of phospholipid and unsaturated fatty acid where unsaturated fatty acid is very vulnerable to free radicals strike mainly hydroxyl radical, so that ROS easily penetrated into plasma membrane. Hydroxyl radical would initiate chained reaction called lipid peroxidation [19]. The result of this chain was the broken chain of fatty acid became toxic substance to the spermatozoa cell [19-20]. Furthermore, there will be a damage of cell membrane which caused membrane fluidity and membrane integrity increased so it will interrupted the process of capacitation and acrosome reaction.

P1 and P2 group shows the increasing of capacitation and acrosome reaction percentage. It is suitable with the function of flavonoid substance as antioxidant. Flavonoid substance has proven to have many advantages which are as antioxidant, anticarcinogenic, antimicrobial, antimutagenic, anti inflammation and reduce the risk of cardiovascular disease [21-22]. As one of antioxidant, flavonoid group acted through free radicals scavenger directly [23-26]. At first, flavonoid was oxidized by radical substance, then it changed to become stable because it reacted with other radical flavonoid so that formed fenoxyl radical which less reactive then free radical reactivity could be muted. Meanwhile, [27] said that acrosome reaction only occurred in spermatozoa which has undamaged membrane. This was because antioxidant in the mahkota dewa pulp extract (Flavonoid) could protect the plasma membrane of spermatozoa from the damaged that caused by free radical oxidation (ROS) so that the membrane integrity stay preserved acrosome reaction can occur. According to [28] that the existence of antioxidant in flavonoid substance of mahkota dewa (*Phaleria macrocarpa*) pulp extract could give protection to spermatozoa's plasma membrane of the rat. The statement was reinforced with the research by [29] who stated that flavonoid can protect plasma membrane of spermatozoa from excessive ROS.

P3 group shows the decreasing of capacitation and acrosome reaction average percentage. This was assumed that excessive administration of mahkota dewa (*Phaleria*

macrocarpa) pulp extract caused toxicity to spermatozoa so that caused the morphology damage of the spermatozoa mainly the cell membrane. Cell membrane damage would cause increasing of membrane fluidity, membrane integrity disturbance and inactivation of the membrane with enzyme and receptor. It would cause the rise of cell damage including spermatozoa cell [14]. If the ATP production in mitochondria was decreased and intracellular ATP was reduced rapidly, it would cause the damage of axonem, decreasing of spermatozoa viability, increasing of midpiece's morphology damage and the loss ability of capacitation and acrosome reaction [30].

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