The Potency of Antifertility Effect of Stem Bark Extract of Mangrove (*Avicennia Marina*) on Male White Rats (*Rattus Novergicus*)

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Abstract: The issue of Indonesian population density is one of must be faced problems. Stem bark of mangrove *Avicennia marina* is one of many plants can be used as contraception. Recently reported, in many countries this *Avicennia marina* used as antifertility nevertheless there are no focus research of the topic proven. The object of this study is to determine the effect of stem bark extract of mangrove *Avicennia marina* on number, motility and viability of spermatozoa of male white rats and also to assess the effect of certain treatment group of some fractions compared to control CMC-Na. This study is experimentally designed in laboratorium. Stem bark of *Avicennia marina* was extracted by soxhlet extraction with methanol solvent, while animal testing were classified to 4 group (each group for 6 rats randomly). The subject were treated along 34 days everyday, the group 1: 1% CMC-Na as a control; Group 2: low dose methanol suspension extract of 1.25%; Group 3: medium dose methanol suspension extract of 2.5%; and Group 4: high dose methanol suspension extract of 5%.Motility test was conducted by putting a drop of sperm on improved neubauer counting chamber and observed by Olympus BX53F microscope (400x magnifying). Then the data analyzed by ANOVA (p value 95%) prior to post hoc test by Duncan (SPSS 20).Motility index of each group 5%, 2.5%, 1.25% and controlwere35%, 47%, 67% and 79% respectively. While, for viability index in group 5%, 2.5%,1.25% and control were 62%, 76%, 73% and 88% respectively.The treatment of stem bark extract of mangrove *Avicennia marina* in male white rats had significantly related to motility and viability index of male rats sperm ($p < 0.005$). This results means that stem bark extract of mangrove *Avicennia marina* showed antifertility effect in male rats.

1 INTRODUCTION

The problem of Indonesian population density is one of many problems that still appearing and not solved. The raising of population number in every year is still alarming because not balanced with the increasing of prosperity. Based on population census in 2010, the number of population raised to 237,641,326 compared to 2000 that only 206,264,597 (BPS,2012). The other problem is Indonesian population growth rate increase 1.38% from 2010-2015 (BPS, 2017).

The growth of population does not only impact to the citizen prosperity in agricultural field but also in job opportunities, education, health, and residences. Therefore, government concerns to promote family planning program to help a person managing birth distance with many methods of contraception offered by government for women and men (satriyasa,*et all,*2017).

Family planning program held by government does not work optimally because the participation of men in the program still in low level compared to women. This situation occurred because there are still no safe and comfort family planning tools for men. Based on the data, the participation of Indonesian men joining family planning program still in 5.5% comparing to other countries like Pakistan of 10.9%; Nepal of 18% and Bangladesh reached to level of 19% (Askrening, 2017). Therefore, it needs to be discovered the men contraception that safe for long term use (Wiryawan*et al*, 2017).

The use of herbal remedies as traditional medicines in Indonesia has been developing since a few decades. Many kinds of plants can be used as herbal remedies to make contraception effect. WHO has formed a group of study to maintain the regulation of men fertility with explorating materials

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or compounds as antifertility agent that safe, effective, and acceptable (Febrianti, 2016).

One of plant that predicted as contraception is stem bark of mangrove *Avicennia marina*. In many contries, stem bark of this kind of mangrove is empirically used as antifertility agent, but there are no study reported focus on this. *Avicennia marina* has many kind of secondary metabolites such as terpenoid,steroid, naphthalene, flavonoid, glucoside iridoid, phenyl propanoid glycoside and diterpenoid glucoside (Esau *et all*., 2015). Flavonoid is one of compounds that have antifertility effect. The preview research showed that flavonoid contained in black tea had antifertility effect on male rats (Delfita, 2014).Therefore, the authors conducted this research to determine the potencial of antifertility effect of *Avicennia marina*on male white rats (*Rattusnovergicus*) especially to count the number, motility, and viability of spermatozoa of male white rats. Also to assess the effect of certain treatment group of some fractions compared to control CMC-Na. Then, this study hopefully can be reference of the next researcher in developing safe and comfort contraception.

2 LITERATURE REVIEW

*Avicennia marina*is known as white mangrove (avicenniaceae). Forming shrubs or trees with a height of 10 meters, and 14 meters in the tropics, it is living in areas with high salinity and coastal protection. And it has been reported that it can tolerate extreme weather and harsh winds (Nayak*et all* , 2014).

Avicennia marina is commonly used as an antioxidant, antitumor, anti-inflammatory, antimicrobial, antiaging, renal anticholine, antiartheroscelerosis, and antituberculin. But it is mentioned that *Avicennia marina* extract is more effective as an antibacterial compared to antifungal. This is related to the chemical content in the form of alkaloids, flavonoids, tannins and glycosides (Danata, 2014). In addition to above compounds there are also other bioactive compounds such as similar components, fatty acids, heterocyclic oxygen, proanthocyanidins, quinones, stilbenes, terpenoids and triterpenoid saponins (behbahani*et all* , 2012).

White rats are rodents and are often used as experimental animals or used for research because rats are animals that represent the class of mammals, which humans are also from the mammal group so that homogenity, organ completeness, nutritional requirements, biochemical metabolism, reproductive system, respiration, blood circulation, genes and excretions resembling humans. Male white rats generally have a weight of 450 - 520 g and females 250 - 300 g. Daily need for food is 5-10 g / 100 g body weight and drink 10 ml / 100 g body weight. Normal cholesterol levels range between 40-130 mg / dl and blood glucose levels 50-135 mg / dl (Wolfensohn& Lloyd, 2013). In contrast to other mammals, white rats have longer spermatozoa around 150-100 nm. The morphology of the rat sperm head is shaped like the same hook as most rodents (Wuwungan*et all,* 2017).

Contraception is a tool or a way that is conducted to inhibit the normal process of ovulation, fertilization or implantation. The decision to use contraception is influenced by the partner, health and method of contraception. (Novita *et all* , 2016)

The method of contraception in men that is currently available is limited to condoms, vasectomy and hormone injections, but the contraceptives above cause many side effects and do not completely prevent pregnancy so that it is less accepted by the public, therefore it is necessary to discover and develop new contraceptive alternatives that prevent fertilization, safely, effective, minimal side effects, and does not reduce the potential for sex and libido (Priastini, 2014).

Antifertility contraception is one of family planning program effort to prevent conception after marriage. Compounds such as alkaloids, flavonoids, terpenoids, steroids, saponins and tannins are found in peel extracts of durian which is proven to have activity as male antifertility (setyowati*et all,* 2015).

Herbal antifertility for men needs to be prioritized because it has several ways to suppress fertilization by suppressing spermatogenesis and decreasing sperm motility so that it fails to enter the cervix temporarily (Satriyasa, 2017).

3 METHODS

3.1 Design of Study

This is an experimental design research with one factor (difference level of stem bark extract of mangrove) completely randomized design and aimed to determine the antifertility potency of stem bark extract of *Avicennia marina* on male white rats.

3.2 Instruments and Materials

3.2.1 Instruments

Instruments used in this study were blender, siever (mesh 40), electric scale (OHAUS), microscope Olympus BX53F, Neubauer counting chamber, rotary evaporator, glasswares, soxhlet instruments, spuit injection, hematocryte pipette, and animal feed equipments.

3.2.2 Materials

Materials used in this study were stem bark of *Avicennia marina*, methanol, 1% CMC-Na suspension, aquadest, alcohol 70%, dye, giemsa, 0.9% saline solution, chloroform, and animal feed.

3.3 Sample Preparation

Stem bark of mangrove *Avicennia marina* were collected from Langkema, West Kabaena, Bombana regency, South East Sulawesi. The bark used must come from a tree with a trunk diameter of about 10-20 cm.

The stem bark were separated from the impurities then washed and dried. Samples were dried premises n aerated for 2-4 days and powdered.

The sampleextracted by the soxhletation method with methanol as a solvent. The soxhletation tool was installed, then the 250- gram of dried sample was wrapped in filter paper, tied with yarn, put into a round bottom flask on the soxhlet, with a solvent volume of 1000 mL (1: 4). Soxhletation carried out at a temperature of 70°C until the cycle droplets were no longer colored. The liquid extract obtained then concentrated using a rotary evaporator at 40°C until viscous methanol extract was obtained.

3.4 Treatment of Subjects

The experimental animals used in this study were Wistar strain male rats that were 8 weeks old and weighed between 150-200 g and placed in a cage. The animals were adapted in the experimental cage one week prior to be treated. The state of the cage is maintained at a temperature of 28-32°C and a dark-light cycle of 12 hours each. Experimental animals were fed standard diet pellets and ad libitum drinking water (Sornalakshmi, TresinaSoris, Paulpriya, PackiaLincy, & Mohan, nd; Suresha et al., 2012). The ethical clearance approval was issued by Commission on ethical clearancefor preclinical research Integrated Research and Testing Laboratory.

3.5 Antifertility Test

For animal testing, the test was divided into 4 groups with each group consisting of 6 rats randomly selected. Rats were weighed and given identification on the tail. Then the treatment was given every day for 34 days with the following procedures:

Group 1 : 1% CMC-Na Control

Group 2 : low dose of methanol extract 1.25% suspension

Group 3 : medium dose of methanol extract 2.5% suspension

Group 4 : high dose of methanol extracts 5% suspension

Furthermore, on day 35 animal surgery and retrieval of sperm in the cauda epididymis obtained by sucking the sperm from the cauda epididmis with 0.5 hematocryte pipette up to the mark then diluted with normal saline (dilution 200 times). This suspension is used to see the number, motility and viability of spermatozoa.

Observation of motility was conducted by dripping sperm preparations in the counting chamber, then observed under an *Olympus BX53F* microscope with magnification 400 times. Motility index of spermatozoa in this study was counted by calculating the percentage of progressive categories which straight forward and fast in 100 spermatozoa. The dyes were used to observe spermatozoa morphological at the same magnification. Viability index of sperm was observed by observing suspension droplets added by one drop of giemsa added with alcohol on glass objects and observed under a microscope, live sperm were stated to be counted from 100 sperm in percent.

3.6 Data Analysis

The analysis of this study used SPSS 20 software. The method used was the analysis of variation (ANOVA) with 95% confidence level and continued with post hoc using the Duncan test.

4 RESULT AND DISCUSSION

4.1 Result

NO	Sample	Σ				Σ	Motility %
		0	1	2	3		
1	5 %	45	20	16	19	100	35 %
2	2.5 %	24	29	30	17	100	76 %
3	1.5 %	15	18	48	19	100	73 %
4	CMC-Na	8	13	53	26	100	88 %

NO	Sample	Σ colored	Uncolored	Percentage %
1	5 %	Σ 32	68	62 %
2	2.5 %	Σ 24	76	76 %
3	1.5 %	Σ 22	73	73 %
4	CMC-Na	Σ 12	88	88 %

NO	Treatment	Sperm (million/ mL)	Motiliy (%)	Viability (%)
1	5 %	1,400	35	62 %
2	2.5 %	2,140	47	76 %
3	1.5 %	1,440	67	73 %
4	CMC-Na	1,900	79	88 %

5 DISCUSSION

Based on ANOVA and post hoc table with Duncan test, it was shown that the administration of stem bark extract (*Avicennia marina*) caused a decrease in the number of spermatozoa motility and viability in the male white rat epididymis (*Rattusnovergicus*). The number of rat spermatozoa (*Rattusnovergicus*) in the treatment of 5% stem bark extract of *Avicennia marina* was significant ($p \leq 0.5$) compared to the control.On the other hand, the 1.25 % and 2.5% stem bark extract of *Avicennia marina* were not significantly different. The average number of white rat spermatozoa in the control was 1,900 million/mL; 1.25% was 1,400 million/ml; 2.5% was 2,140 million/ml and 5% was 1,440 million/ml.

The spermatozoa motility of subjects in the treatment of 5% sample was significantly different ($p \leq 0.5$) with control and other groups of treatment. The mean motility of rat spermatozoa in control was 79%; 1.25% was 67%; 2.5% was 47% and 5% was 35%.

Viability index of rat spermatozoa in the treatment of 5% sample was significantly different ($p \leq 0.5$) with control and other groups, nevertheless the viability index for group treatment between 1.25% and 2.5% sample were significantly different. The average viability of white rat spermatozoa in control, group 2, 3, and 4 were 88%, 73%, 76% and 62% respectively. The average number, motility and viability index of spermatozoa in the control and each treatment can be seen in table 3.

Based on the results of the study it is known that the administration of sample1ml/100g BW adult male rats orally causes a decrease in the number of spermatozoa in cauda epidedimis, motility and viability of spermatozoa. The higher dose of sample was given, the lower amount of motility and viability of rat spermatozoa *(Rattusnovergicus).*

In this study it was known that the administration of sampleaffected the number of spermatozoa in cauda epidedimis. The number of spermatozoa in the group 3 (5% of sample suspension) treatment was significantly different from other groups ($p \leq 0.05\%$). The decrease in the number of spermatozoa was probably caused by a decrease in the number of *leydingcells* by ROS thereby reducing intratestheticteston levels so that it affected spermatogenesis. In other words, ROS causes a decrease in the number of hormone-producing leyding cells that play a role in spermatogenesis, so that spermatogenesis is disturbed/stopped, as a result the number of spermatozoa is reduced. This result is in line with the research of Nayantara et al.,(2008: 3) which showed a decrease in the number of spermatozoa due to increased ROS in rats *(Rattusnovergicus)* treated with monosodium glutamate. The decrease in the number of spermatozoa is also caused by the death of spermatozoa cells due to ROS, especially hydroxyl radicals causing lipid peroxidation.

Based on the results above, it is known that the administration of stem bark extracts of *Avicennia marina* 1ml/100g BW in adult male rats orally causes a decrease in spermatozoa motility. Motility index of spermatozoa between treatments were significantly difference ($p \leq 0.05\%$). Observed spermatozoa motility index were divided into four categories: fast moving, slow moving, localized moving and stationary moving spermatozoa. In this study, the stem bark extract of *Avicennia marina* decreased the spermatozoa motility of rats. This is because mammalian sperm are rich in unsaturated

fatty acids in the plasma membrane so that they are very vulnerable to ROS attack (Tre mallen, 2008: 244).

Spermatozoa motility is produced by a long tail. Tail movements are carried out by energy produced by mitochondria which are concentrated in the middle of the sperm (Shewood, 2004: 520). In this experiment, observation on living and dead sperm cells was carried out by an indication of Eiosinnigrosin. Live sperm cells will be bright white (transparent) while the dead ones will be red. Died sperm cells occurred due to the direct contact with air that lead to sperm oxidation. Long term storage of sperm cells causes a decrease in sperm motility due to remaining cell metabolism namely lactic acid which causes acid condition of the medium and can be toxic to spermatozoa which ultimately causes sperm death (Sugiarti et al., 2004). To assess the viability index of spermatozoa, Eiosinnigrosin staining was used. According to WHO standards, this staining technique provides valid result with a review of the motility obtained data. This technique of staining eiosinnigrosin is a simple technique. In this case the eosin dye will be absorbed by the dead spermatozoa so that they will turn red or pink due to increased cell wall permeability when the spermatozoa die. While nigrosin will color the background of spermatozoa (Septiyani, 2012).

The result also showed that the suspension of samples affected to the viability index of spermatozoa. The higher dose of the extract was given, the lower viability of rat spermatozoa was obtained. This is caused by flavonoids/ antioxidants that contain excessive doses of sample cause an increase in antioxidants in the body. It is also caused by the ability of flavonoids/antioxidants to form ROS, both of which will damage the spermatozoa plasma membrane.

6 CONCLUSION

From the results above, can be concluded that stem bark extract of mangrove *Avicennia marina* had potential activity as antifertility on male rats proven by decreasing of epidedimis weight, spermatozoa number, motility and viability of rats. The best dose as antifertility is 5%.

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