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ORIGINAL RESEARCH

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## EFFECT OF SECANG WOOD (*CEASALPINIA SAPPAN L.*) EXTRACT ON MORPHOLOGY OF SPERMATOZOA, SPERM COUNT, AND REVERSIBLE PROCESS IN MALE RATS

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### ABSTRACT

**Background:** Limited choice of contraception for males is one of the reasons why their participation is low. Secang wood is considered as an alternative contraception that has an influence in the morphology and number of spermatozoa, and reversible in nature.

**Objective:** To examine the effect of extracts of secang wood in the morphology of spermatozoa, sperm count and reversible process in male mice.

**Methods:** This was a quasi experimental study with randomized post test only control group design. There were 32 male rats (*Mus Musculus L*) recruited in this study. Four groups were involved, namely: 1) Ethanol group, 2) Chloroform fraction, 3) Water fraction, and 4) Control group. Each group consisted of 8 mice. There were two treatments in this study: 1) each group was given secang extract with dose 50 mg/25 gram of weight, 2) The observation period was 10 days after the treatment, and 4 mice in each group was dissected to see the morphology and sperm count, while the other 4 mice were allowed to live and maintained until 20 days to see reversible morphology and sperm count

**Results:** The extract of sepang wood at a dose of 50mg/25gram weight could increase the abnormal sperm morphology, and lower sperm count. There was a significant difference between the treatment group and control group with p-value <0.05. The extract was also reversible on the morphology and sperm count.

**Conclusion:** There was a significant effect of secang wood extract on sperm morphology, the number of spermatozoa, and reversible process. This study provides the insight of scientific information about the effect of the sepang extracts on the number and morphology of spermatozoa, and it could be used as a basis for further research in human in the development of natural contraceptive on a reversible man.

**Key words:** Sepang wood, ceasalpinia sappan l, spermatozoa, contraception

## INTRODUCTION

Reasons for low male participation in family planning program (KB) is limited choice of contraception for male, and is more directed to females.<sup>1,2</sup> Until today, the contraception modes for male are just limited to condom, vasectomi, and hormone injections.<sup>2</sup> However, ideally, contraception for men should be able to prevent fertilization, safe, fast performance, no side effects, temporary, and no affect on sex and libido.

In Indonesia, one of the things that is under developed is the use of natural medicinal plant as an alternative anti male fertility. One of them is secang wood (*Caesalpinia sappan*L). This plant empirically and in vitro-proof has ant fertility effect on cell donor of human spermatozoa.<sup>3</sup>

The active compounds of secang wood that can be used as antifertility are flavonoids that can inhibit the enzyme arornatase, namely an enzyme that has a function to catalyze androgen conversion to be estrogen that increases testosterone, so it will inhibit spermatogenesis.<sup>4</sup> Alkaloid can affect the secretion of hormone reproduction, which is needed for the process of spermatogenesis and lower the quality of spermatozoa.<sup>3</sup> Beside flavonoid and alkaloid, another essential compound in the secang wood is aetheric *oil* that can be used as an alternative inferlility, which has function to work on the transportation of sperm, and agglomerate sperm in order to reduce sperm vitality, which consequently affect the sperm not to reach the egg and fertilization can be prevented.<sup>5</sup>

Secang wood is empirically known having many healing properties and is often consumed by community as healthy drink. It also has a powerful antioxidant extracts with antioxidative indexes, which is higher than the commercial antioxidants (BHT and BHA).<sup>6</sup> Thus, the purpose of this study was to examine the effect of

secang wooden extract on sperm morphology, sperm count and reversible process in male rats (*Mus Musculus L*).

## METHODS

### *Design*

This was a Quasy experimental study with randomized post test only control group design.

### *Population and Research Subject*

Thirty two male rats (*Mus Musculus L*) recruited in this study with the inclusion criteria: mature and healthy mice, aged 3 months, 20-30 grams weight. There were four groups in this study: 1) Ethanol group, 2) Chloroform fraction, 3) Water fraction, and 4) Control group. Each group consisted of 8 mice.

### *Intervention*

There were two treatments in this study: 1) each group was given secang extract with dose 50 mg/25 gram of weight, 2) The observation period was 10 days after the treatment, and 4 mice in each group was dissected to see the morphology and sperm count, while the other 4 mice were allowed to live and maintained until 20 days to see reversible morphology and sperm count.

### *Instrument*

The instrument used was an individual mouse cage, maceration tool, Neubauer hemocytometer micro, microscope, watch glass, surgical equipment, pipette test, pipette leukocytes, petri dishes, cover glass, tray stopwatch, books and recording data.

### *Data Analysis*

Data were analyzed using ANOVA test to find out the mean difference between the treatment groups and then continued with Tukey test to compare the mean of the entire treatments in order to know the most influence treatment.

*Ethical Considerations*

This research has been approved by the Health Ministry Polytechnic Semarang with No. 024/KEPK/polytechnic-SMG/EC/2017.

**RESULTS**

*Morphology of Spermatozoa*

The morphological changes in spermatozoa after a given treatment for 10 days shown in table 1 indicated that the means of abnormal sperm morphology in the treatment groups (ethanol extract

13,50; chloroform fraction 11.50; water fraction 6.75) were higher than sperm morphology in the control group (3.75). The highest average of abnormal sperm morphology was found in the group of ethanol extract (13.50) and the lowest was in the control group (3.75). While the reversible morphological changes after 20 days could be seen that the ethanol extract group (6.00), chloroform fraction (5.25), and the control group (3.75) were lower than the water fraction group (3.00).

**Table 1. Morphology of Spermatozoa**

Groups	n	Intervention (X±SD %)	Reversible (X±SD %)
Control	4	3.75±0.957	3.75±0.957
Ethanol extract	4	13.50±2.082	6.00±0.816
Chloroform fraction	4	11.50±1.915	5.25±0.957
Water fraction	4	6.75±2.062	3.00±0.816

**Table 2. Sperm count of male rats**

Groups	n	Intervention (X±SD %)	Reversible (X±SD %)
Control	4	13.25±2.062	11.50±2.246
Ethanol extract	4	8.25±0.957	12.25±1.258
Chloroform fraction	4	7.75±0.957	10.25±1.500
Water fraction	4	8.25±2.500	11.25±3.304

**Table 3. One way Anova of Morphology of Spermatozoa and its reversible**

	Intervention					Reversible				
	Sum of Squares	df	Mean	F	Sig	Sum of Squares	df	Mean	F	Sig
Between Groups	236.250	3	78.750	23.924	.000	22.500	3	7.500	9.474	.002
Within Group	39.500	12	3.292			9.500	12	.792		
Total	275.750	15				32.000	15			

**Table 4. One way Anova of the Number of Spermatozoa and its reversible**

	Intervention					Reversible				
	Sum of Squares	df	Mean	F	Sig	Sum of Squares	df	Mean	F	Sig
Between Groups	80.70	3	26.927	8.730	.002	39.688	3	13.229	2.433	.115
Within Group	37.500	12	3.083			65.250	12	5.438		
Total	117.750	15				104.938	15			

### *Sperm count*

Table 2 showed that changes in the number of spermatozoa after a given treatment for 10 days in the treatment groups (Ethanol extract 8.25; Chloroform fraction 7.75; and Water fraction 8.25) were lower than in the control group (13.25). The lowest number of sperm was found in the group of chloroform fraction. However, the reversible number of spermatozoa showed that the means of sperm count in the treatment groups (Ethanol extract 12.25; Chloroform fraction 10.25; and Water fraction 10.25) were increased than the mean in the control group (11.50). While the results of Shapiro-Wilk test for all variables showed normal distribution, so further analysis was conducted using One Way Anova.

Table 3 showed that morphology of spermatozoa after given the treatment showed p-value = 0.000 and reversible morphology of spermatozoa showed the p-value = 0.002, which was <0.05, indicated that there were significant differences in the mean of morphology of spermatozoa and its reversible.

On the other hand, the number of spermatozoa after given the treatment in Table 4 showed p-value = 0.000, which was < 0.05, indicated that there was a significant difference in the mean of sperm count. But the reversible of spermatozoa count showed the p-value = 0.115. Thus, the p-value was >0.05, which indicated that there was no significant differences in the mean of reversible sperm count.

The Tukey test showed that there were significant differences in the sperm morphology between the control group (0.000) with the other control groups (ethanol group 0.001; chloroform 0.014; and water fraction 0.143). Among the treatment groups, ethanol group (0.001) had the most significant differences compared to the other treatment groups (chloroform 0.14; and water fraction

0.14). While in the number of spermatozoa, it also showed that there were significant differences between the control group (0.008) and the treatment groups (ethanol group 0.977; chloroform 0.008; water fraction 1.000).

The result of Tukey test on the reversible, revealed that there were no significant differences in sperm morphology between group control (0.017) with the other control groups (ethanol group 0.643; chloroform 0.017; and water fraction 0.643). While among the treatment groups, chloroform (0.017) had the most significant differences compared to the other treatment groups (ethanol 0.017; water fraction 0.643).

### **DISCUSSION**

This study found the primary abnormality in the form of a small head, amorphous head, and spiral tail due to a decrease in testosterone levels. Secondary abnormality is seen that headless spermatozoa and tailless, which is in line with the previous study said that these abnormalities were caused by disruption in the process of sperm maturation in epididymis.<sup>7</sup> The chemicals contained in secang wood (*Caesalpinia sappan* L) is an alkaloid which can affect spermatogenesis by pressing the secretion of reproductive hormones (FSH and LH) required for the ongoing spermatogenesis and lower the quality of spermatozoa. FSH serves to spur spermatozoa process, namely the formation of spermatogonia into spermatids.<sup>8</sup> While LH serves to stimulate spermatogenesis process, namely the formation of spermatid cells become spermatozoa.<sup>9</sup>

The decline in the number of spermatozoa after given the extract of sepang wood was because the active substance contained in the sepang wood such as flavanoid compound, as a class of compounds that function as antiandrogenic by inhibiting the enzyme

aromatase, the enzyme that serves to catalyze the immediate conversion of androgens to estrogen that increases the testosterone.<sup>10</sup>

Based on the results of this study, it could be said that the effect of sebang wood on the morphology and sperm count is reversible. It is because after the treatment was stopped, the morphology and sperm count were increased. This is assumed that the active Leydig cells produced testosterone again. These findings were in line with the study conducted by Ermayanti et al<sup>11</sup> revealed that there was an increase in testosterone levels back into the blood plasma of mice after sebang wood treatment was stopped; and it was also reported that an increase in Leydig cell activity in mice by in vitro after the sebang wood treatment stopped.<sup>10</sup>

#### LIMITATIONS OF THE STUDY

Insignificant difference between the time of observation might be due to the time was too short, which only for 20 days. It was possibly required more time for the recovery, since the sperm had already damaged. It was assumed that the restoration of spermatozoa only occurred with spermatozoa that were able to adapt.

#### CONCLUSION

The sebang wood extract significantly affected sperm morphology, the number of spermatozoa, and reversible process. This study provides the insight of scientific information about the effect of the sebang extracts on the number and morphology of spermatozoa and could be used as a basis for further research in human in the development of natural contraceptive on a man who is reversible.

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