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Oral Administration of Cogongrass (*Imperata cylindrica L*) Root Ethanol- Extract causes Mouse Epididymal Sperm Abnormality

(PEMBERIAN EKSTRAK ETANOL AKAR ALANG-ALANG (*IMPERATA CYLINDRICA L*) SECARA ORAL MENYEBABKAN ABNORMALITAS SPERMA EPIDIDYMIS MENCIT**)**

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ABSTRACT

Sperm morphology is an important parameter to be observed in the male fertility. Some of the bioactive compounds of cogongrass root such as alkaloid and terpenoid, affect male fertility by interference the spermatogenesis. The objective of the study was to observe the effect of cogongrass root ethanol extract on mouse sperm morphology. This study was carried out by oral administration of two different doses i.e 90 and 115 mg/kg body weight of cogongrass root ethanol extract into 8-10 weeks old DDY strain mice for 14 days to evaluated the acute effect due to the administration of cogongrass root ethanol extract on mouse sperm morphology. The results showed that treatment with cogongrass root ethanol extract significantly increased sperm abnormalities followed a dose depending pattern ($p<0.05$). Interestingly, the administration of cogongrass root extract did not affect sperm head morphology but tailless, folded and bent sperm increased linearly with the administration dose of cogongrass root ethanol extract. In conclusion, cogongrass root ethanol extract causes secondary sperm abnormalitties on mouse sperm.

Key words: Cogongrass root extract; spermatogenesis; sperm morphology

ABSTRAK

Morfologi sperma merupakan salah satu parameter penting untuk mengamati tingkat fertilitas pada pria. Beberapa senyawa bioaktif akar alang-alang seperti alkaloid dan terpenoid dapat memengaruhi fertilitas pria dengan cara mengganggu spermatogenesis Penelitian ini bertujuan menguji pengaruh ekstrak etanol akar alang-alang terhadap morfologi sperma mencit. Penelitian ini dilakukan dengan memberikan secara oral ekstrak etanol akar alang-alang pada mencit strain DDY umur 8-10 minggu selama 14 hari untuk melihat efek akut pemberian ekstrak etanol akar alang-alang dengan dua tingkat dosis yang berbeda yaitu 90 dan 115 mg/kg BB pada morfolgi sperma mencit. Nilai abnormalitas sperma mengalami peningkatan secara signifikan seiring dengan peningkatan dosis ekstrak etanol akar alang-alang yang diberikan ($p<0,05$). Pemberian ekstrak etanol akar alang-alang tidak memberikan pengaruh nyata pada

perubahan abnormalitas kepala sperma mencit tetapi meningkatkan persentase sperma tanpa ekor, sperma dengan ekor melipat dan sperma dengan ekor yang patah. Berdasarkan hasil yang diperoleh, maka dapat disimpulkan bahwa pemberian ekstrak etanol akar alang-alang akan menginduksi kelainan morfologi sekunder pada sperma mencit.

Kata-kata kunci: ekstrak akar alang-alang; spermatogenesis; morfologi sperma

INTRODUCTION

Indonesian have used herb as a traditional medicine to cure some diseases or health problems. The benefits of herb as a traditional medicine are reachable, affordable and effective. Cogongrass (*Imperata cylindrical L*) or *alang-alang* is one of the troublesome weed species in the world. It is a perineal, rhizomatous grass that is variable in appearance and endemic in tropical and subtropical regions throughout the world (Xuan *et al*., 2009). In Asia, cogongrass is used as a traditional medicine for cancer, cold, diarrhea, myalgia, gonorrhea, piles, night sweat (Parkavi *et al*., 2012).

Previous study reported that cogongrass root extract decreased triglyceride and blood glucose absorption in the small intestine, and also had a potential as an anti-hypertension (Ruslin *et al*., 2013). Based on the phytochemical screening, cogongrass contains tannin, saponin, flavonoid, alkaloid, and terpenoid (Krishnaiah *et al*., 2009). Some of the bioactive compounds such as alkaloid, flavonoid, and triterpenoid have several effects on fertility so it can be a candidate for antifertility agent in the future. Hence, there is no study explained about the effect of cogongrass extract on spermatogenesis.

Based on the cogongrass phytochemical compound, the administration of cogongrass extract may induce a disturbance on a spermatogenesis (Margono, 2013), reducing sperm quality (Diantini *et al*., 2008) and changing testicle and reproductive hormone levels (Auta *et al*., 2016) which lead to decreased fertility. The disturbance on spermatogenesis consist of disturbance during mitosis, meiosis, differentiation, maturation and transportation. One of the simple methods to identify the spermatogenesis disturbance is by using a sperm morphology identification. As a preliminary study to evaluated cogongrass root etanol extract on spermatogenesis using sperm morphology assessment was the aim of the study. We expected that the administration of cogongrass root ethanol extract would increase sperm abnormalities. Based on the results, congongrass root may apply as an antifertility agent

RESEARCH METHODS

Preparation of Plant Extract

The cogongrass was obtained from Solo, Central Java, Indonesia. Cogongrass roots were macerated by ethanol 95% for 72 hours, filtrated with a vacuum filter, and concentrated in a vacuum evaporator. The concentrated extracts were suspended with carboxymethylcellulose (CMC) 0.5% and prepared on concentration of 90 mg/kgBW and 115 mg/kg BW.

Animals

The use of animals was approved by Ethics Review Committee Faculty of Medicine Universitas Padjadjaran (1263/UN6.C10/PN/2017). This study was performed in the mouse house in Animal Laboratory Faculty of Medicine and Laboratory of Animal Reproduction and Artificial Insemination Faculty of Husbandry Universitas Padjadjaran. Eighteen male DDY mice strain (Biofarma - Bandung) at the age of 8-12 weeks were housed in a room with a condition of 12/12 h light and dark cycle with good air circulation and *ad libitum* food and drinking water. Mice were given cogongrass root extracts per oral with dose 90 mg/kg (dose 1) and 115 mg/kg BW (dose 2) based on previous study **(Dhianawaty *et al*., 2015)**. The non-treatment group was used as a control. At the termination of the experiment, mice were sacrificed and sperm epididymis were isolated

Sperm Epididymal Isolation

Mice were euthanatized by cervical dislocation. The testicles were isolated and removed from adherent tissues and blood. The left epididymis was isolated and finely minced by anatomical scissors in 1 mL of physiologic saline in a petri dish, then sperms were released from the epididymal tissue at room temperature for 2 minutes.

Epididymal Sperm Morphology

The smear was prepared on clean glass slides after sperm staining with eosin-nigrosin. The slide was air-dried and labeled for subsequent examination. The sperm morphologic was

evaluated by using the light microscope at x1000 magnification with immersion oil. Two hundred sperms in each sample from each mouse were assessed for morphological abnormalities of sperm head and tail then were classified as followed: (1) tailless, (2) amorphous head (3) no-hook head, (4) folded tail and (5) bent tail. The total abnormality was expressed as incidence of 200 sperms/mouse.

Statistical Analysis

All data were analyzed using SPSS software version 16 (SPSS Inc, Chicago, IL, USA). Statistical differences in mean were analyzed using one-way ANOVA followed by Tukey multiple comparison tests. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The abnormalities of sperm morphology will affect male fertility due to the inability of sperms to reach oocyte and/or to penetrate normally. Therefore, sperm abnormality is one of a keys diagnostic tool to assess male fertility. The common sperm abnormalities are the amorphous head, no-hook, tailless, folded tail and bent tail. The Illustration of sperm morphology in this study is presented in Figure 1, while the pattern of sperm morphology after 14 days administration of cogongrass root ethanol extract is presented in Table 1.

The results showed that the administration of cogongrass significantly increased 50 % sperm abnormality in 115mg/kgBW treatment group compared to the control group ($p<0.05$). The sperm abnormal morphology dominated with sperm head abnormal morphology than the tail abnormality. Interestingly, the sperm tail abnormality increased linearly with the administration dose of cogongrass root extract ($p< 0.05$), which is shown in Table 1.

The results of detail sperm head morphology showed that the cogongrass root extract reduced the sperm amorphous head, although statistically insignificant. In the other hand, the tailless, bent tail and folded tail of sperm

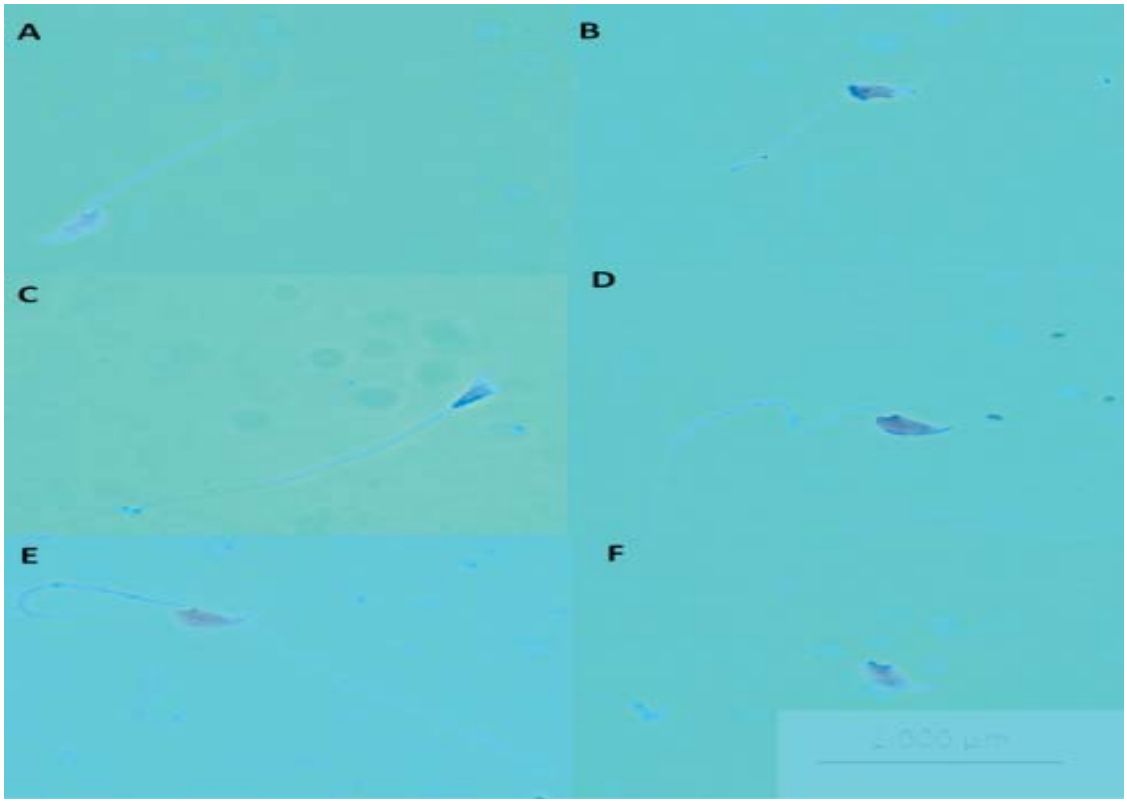

Figure 1. A). The picture shows a normal sperm B). An amorphous sperm. The head appeared thick and the shape is irregular. C) The picture shows a non-hook sperm. The hook is detached from the sperm head. D). The picture shows bent-tail sperm. The tail of the sperm is dent obviously in two site of tail E). Folded sperm. The tail look rounded indicates its abnormality. F).Tailless sperm. Scale bar 1000µm

morphology was significantly increases in the treatment group (Table 2, $p<0.05$).

Head and tail are very important part of sperm morphology. The head contains nucleus densely coiled chromatin fibers, anteriorly surrounded by an acrosome. The acrosome contains proteolytic enzymes that help to destroy the zona pellucida of the egg cell, therefore the sperm to enter easily, while the main function of tail is to allow sperm motility by slithering, snake-like movement (Vasan, 2011; Liu, 2000). Based on our results, insignificantly induced primary sperm morphology defect but significantly induced sperm secondary defect.

The primary defect indicated the disturbance on the testicular, includes the amorphous head, double head, double tail, amorphous head, no hook, no acrosome and double head. Sperm head defects cause sperm failure to penetrate the zona pellucida so that the egg will be difficult to be fertilized (Tourmente *et al*., 2015). Based on the results, the high defect on sperm head morphology indicated the disturbance in the testis. The previous study reported that the administration cogongrass root ethanol extract for 14 days orally significantly reduced the testis weight (Widyastuti *et al.,* 2017), so it's may induced a spermatogenesis disturbance. The results described that of head sperm defect significantly higher compared with tail sperm defect, but overall the administration of cogongrass root ethanol extract reduced the amorphous head and no hook although insignificantly. That is supported with a previous study that the administration of cogongrass root ethanol extract insignificantly increased normal sperm morphology in old mice (Lubis *et al*., 2018). The decreased of sperm head defect may be reflected minimum alteration in sperm chromatin condensation after cogongrass ethanol extract administration (Chemes and Sedo, 2012). However, as long as the reduced sperm head defect mechanism is not fully understood, our results should be interpreted with attention and further studies are necessary to elucidate the mechanism of cogon grass extract action in causing sperm head condensation.

Testicular damage leading a disturbance of testosteron secretion by Leydig cell. One of testosterone function is maintains the epididymis and sperm maturation process. The disturbance of this hormone may induce a secondary sperm

Table 1. Epididymal sperm abnormalities after 14 days administration of cocongrass root ethanol extract

Groups	Total Sperm Abnormal morphology (%)	Part of Sperm	
		Head Abnormality (%)	Tail Abnormality (%)
Group A: Control	38.57 ± 8.70^{a}	33.50 ± 4.26^{a}	5.70 ± 3.09^{a}
Group B: 90mg/kg BW	56.85 ± 3.21^{b}	35.00 ± 5.76^{a}	21.30 ± 3.15^{b}
Group C: 115 mg/kg BW	64.86 ± 3.45^{c}	33.05 ± 4.30^{a}	31.30 ± 4.51^{c}

Note: A different superscripts values in some column indicate statistically significant different ($p < 0.05$).

Table 2. Detail of sperm head and sperm tail abnormality after 14 days administration cocongrass root ethanol extract

Group	Abnormal Epididymal Sperm Head Morphology(%)		Abnormal Epididymal Sperm Tail Morphology(%)		
	Amorphous	No-hook	Tailless	Folded tail	Bent tail
Group A: Control	17.04 ± 2.81^{a}	10.19 ± 2.16^{a}	0.26 ± 0.06^{a}	0.37 ± 0.02^{a}	0.36 ± 0.18^{a}
Group B: 90mg/kg BW	14.92 ± 2.56^{a}	8.93 ± 2.05^{a}	0.92 ± 0.02^{b}	4.98 ± 0.53^{b}	2.21 ± 0.66^{b}
Group C: 115 mg/kg BW	13.92 ± 3.26^{a}	9.90 ± 2.63^{a}	2.13 ± 0.24^{c}	8.13 ± 0.16^{c}	5.05 ± 1.03^{c}

Note: A different superscripts values in some column indicate statistically significant different ($p < 0.05$).

morphology defect. The secondary defect includes the bent tail, folded tail, sperm with a cytoplasm and tail-less. The secondary defect indicates the disturbance during sperm maturation in the epididymis and tail abnormality mainly happen in maturation procces that occur in epididymis. Sperm undergoes a series morphology and physiological changes during maturation such as ultrastructure, metabolism pattern, size, DNA and membrane system (Cornwall, 2009). All of the processes depend on the testosterone level. If the testosterone level decrease, the maturation process will be disrupted. In the tail of the sperm there is mitochondria that supplies ATP for sperm motility. Normal sperm produces sufficient ATP to maintain motility with anaerobic glycolysis and respiration. Changing in mitochondrial integrity and function, lacking of mitochondrial microscopy or altering mitochondrial genome, transcriptase or proteome, as well as reducing mitochondrial membrane potential will result in decreased of sperm motility (Tourment *et al*., 2015; Amaral *et al*., 2013).

The administration effect of cogongrass root extract on sperm abnormality is similar to the administration of other herbs such as *Momordica charantia* L (Astuti *et al*., 2009), *Azadirachta Indica* (Auta *et al*., 2016) and *Semecarpus Anacardium* (Sushma *et al*., 2016), *Cordia dichotoma G Forst* (Sharma *et al*., 2015). The increased of sperm abnormality is induced by the presence of alkaloid, flavonoid, and triterpenoid in cogongrass root ethanol-extract. The previous research found that the administration of alkaloid and triterpenoid had a negative effect on the fertility (Utah *et al*., 2008; Yakubu *et al*., 2012; Meerwal *et al*., 2015).

Alkaloid has an estrogenic effect, which influences the mechanism of testosterone synthesis. The high concentration of testosterone induces the negative feedback mechanism to inhibit luteinizing hormone secretion from anterior pituitary (Ejebe *et al*., 2008). As a result, the synthesis of testosterone in Leydig cell is decreased and spermatogenesis process is disrupted. Terpenoid induces the sperm abnormalities by interfering the reproductive function of the testicle. It will reduce the seminiferous tubular diameter and germinal epithelial cell thickness (Astuti *et al*., 2009). In conclusion, the administration of cogongrass extract increases sperm abnormalities and can induce disruption of male fertility.

CONCLUSION

The administration of cogongrass root ethanol extract increased secondary defect of mouse sperm morphology.

SUGGESTION

A subsequent research should be done *in vitro* and *in vivo* to observe mouse sperm quality, hormonal level and histopathological on testicular after Cogongrass root ethanol extract oral administration.

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