

ISBN : 978-602-98439-7-2

PROCEEDINGS

International Seminar



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Food Sovereignty and Natural Resources in Archipelago Region

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in Archipelago Region"



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ISBN 978-602984397-2



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ICC-IPB Botani Square
23th -24th Oct-2012



BENEFITS OF KEBAR GRASS (*Biophytum petersianum* KLOTZSCH) ON TESTES QUALITY IN RATS AS ANIMAL MODEL

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ABSTRACT

This research aimed to study the potential extract kebar grass on testes of male rat that exposed to cigarettes smoke. This research used factorial CRD with longer treatment and the time taking. The extract of kebar grass given to rats was 0.0945 mg/g body weight/day. The extraction of kebar grass 0.0945 mg/g body weight/day was used to observe the reproduction function in male rat that exposed to cigarettes smoke. Eighteen male rats were divided into 2 treatment groups (20 days and 60 days). Collecting data were done three times, firstly after exposing, secondly after giving extract kebar grass, and finally after terminating given extract kebar grass. The results showed that extract kebar grass 0.0945 mg/g body weight/day increased testes weight, and the concentrations of DNA and RNA testes of male rat exposed with cigarettes smoke.

Keywords : Cigarettes smoke, kebar grass, *Biophytum petersianum* Klotzsch, testes.

INTRODUCTION

Kebar grass *Biophytum petersianum* Klotzsch (Oxalidaceae family) is one of potentially medicinal herbs for reproduction but has not yet widely known in Indonesia. It, an herbaceous plant, on the other hand, is very famous for people in the Arfak mountains area especially in Kebar, Manokwari, West Papua, known as "banondit", which means lots of children. Kebar grass is believed as an herbal associated with the immune systems, such as inflammation, fever, malaria, and injuries (Inngjerdingen *et al.* 2004; 2006; 2008) and fertility (Sawen 2011).

Rachmy (2009) reported that kebar grass contains secondary metabolites of flavonoids, phenolics and alkaloids compounds and derivatives in the form of isoflavones daidzein. Kebar grass contains compounds that are suggested to improve reproductive function such as proteins, carbohydrates, tannins, flavonoids, antioxidants, phosphor, iron, calcium, vitamin E and vitamin A (Unitly and Inara 2011). Vitamin E and

vitamin A are an antioxidant that can neutralize toxic. Vitamin E can inhibit the oxidation reaction by binding vitamin E to vitamin E free radical that functions as an antioxidant again (Pavlovic *et al.* 2005). The presence of active substances such as antioxidants substances, nutrients and amino acids contained in kebar grass, can improve the testes quality.

This research was conducted to determine the effect of extract kebar grass 0.0945mg/g body weight/day on the reproductive performance such as testes weight, DNA testes, and RNA testes of male rats that have been exposed with cigarette smoke.

MATERIALS AND METHODS

This research used a completely randomized design (CRD). Exposing cigarette smoke to male rat *Rattus norvegicus* with a dose of a 10 cigarette/rat/day was done in the smoking chamber. Oxygen valve was opened by the pressure 0.5 atmospheres then cigarettes were attached to pipe which is connected to the pump. Cigarette was burned and the pump was turned on. The smoke would enter into smoking chamber and be inhaled by rats. The length of time was 15 minutes/cigarette/male rat, so total exposed time for 10 cigarettes per male rats was 2.5 hours.

Five kg of kebar grass were dried and milled into a powder. It was added into thirty liters of ethanol and then homogenized for two hours. It was allowed during 24 hours and then filtered. Filtrates were evaporated with a rotavaporat 40°C until condensed and turned to be powdered. The powder was dissolved in 1 ml of CMC (*Carboxil Methyl Celulose*) 1% to produce extract kebar grass. The extract was given to male rat using *sonde* needle. Determination dose of extract kebar grass in rat based on the standard dose that given to humans (Laurenceand Bacharach 1986). In this research the dose calculation of extract kebar grass is 0.0945 mg/g body weight/day.

Eighteen male rats were divided into 2 groups, which consisted of 9 rats in each group. The first group was a-20-days group as labelled 20h group that was exposed to cigarette smoke for 20 days then given extract kebar grass for 20 days and discharged 20 days; and the second group was a-60-days group as labelled 60h group that was exposed to cigarette smoke for 60 days then given extract kebar grass for 60 days and discharged 60 days. Exposing to cigarette smoke was held on the morning then given extract kebar grass by oral. During the study, data were taken three times, firstly after exposing cigarette smoke (T0), secondly after giving extract kebar grass (T1), and finally after stopping given extract kebar grass (T2). At the time of data collection, three male rats in each treatment were necropted, and then examined parameters of testes weight and the concentration of DNA and RNA testes. Analyzed data used Analysis of Variance (ANOVA) following by Duncan test using software SAS

RESULT AND DISCUSSION

Mean of testes weight, the concentration of DNA, and RNA testes after exposing cigarette smoke, giving extract kebar grass and terminating given extract kebar grass for 20h and 60h groups are presented in Table 1. Statistical analysis showed that given extract kebar grass has a significant relation with testes weight ($P > 0.005$), but no interaction to the concentration of both DNA and RNA testes.

Testes Weight

Length time of given extract kebar grass affected significantly on testes weight of both grouped rats ($P < 0.05$). A-60-days group, however, has a heavier testes than a-20-days group. The testes weight of both groups remained stable after terminating given extract kebar grass. This phenomenon explains that extract kebar grass can increase testes weight and keep it off.

Decreasing testes weight in the exposure of cigarette smoke treatment was suspected as a result of disorder function testes caused by toxic substances from cigarette smoke entering through the endocrine glands in the testes. This can cause an interruption in the seminiferous tubules which is a place for synthesis of spermatozoa. Results of this study showed that the given extract kebar grass for 20 days and 60 days can enhance the activity of the testes. Testes are the organ that has function of reproduction through spermatogenesis, and produce androgenic hormones (Mayes 2003; Cox and John 2005). Kebar grass contains protein which made up of amino acids needed for the production and reproduction activities, and also vitamin E. Linder (2006) stated that vitamin E plays a role in normalizing epithelium in the seminiferous tubules, and repairing epithelial cells can affect the increase of testes weight.

Table 1. Mean of testes weight, the concentration of DNA and RNA testes on rats after exposing cigarettes smoke, giving extract kebar grass and terminating given extract kebar grass.

Parameters	Exposure Time	Decision Time			Mean
		T0	T1	T2	
Testes Weight (g)	20h	2.38±0.10 ^c	2.64±0.29 ^b	2.64±0.12 ^b	2.55
	60h	2.05±0.01 ^d	2.96±0.02 ^a	2.93±0.05 ^a	2.65
	Mean	2.21 ^b	2.80 ^a	2.79 ^a	P<0.05
Concentration of DNA Testes (mg/g)	20h	32.22±19.70 ^{bc}	208.47±156.10 ^a	201.07±61.56 ^a	147.25
	60h	10.17±8.57 ^c	220.04±60.19 ^a	182.10±108.51 ^{ab}	137.44
	Mean	21.20 ^b	214.25 ^a	191.59 ^a	tn
Concentration of RNA Testes (mg/g)	20h	75.29±2.78 ^b	104.40±10.87 ^a	90.98±17.39 ^{ab}	90.23
	60h	78.88±5.93 ^b	105.27±12.29 ^a	106.19±8.63 ^a	96.78
	Mean	77.09 ^b	104.84 ^a	98.59 ^a	tn

Note: Data shown are mean values ± standard deviation. Different letters in the same row or the same column indicate significant difference (p < 0.05). T0 = time after exposure cigarette smoke, T1 = time after administration extract kebar grass, T2 = time after stopping administration extract kebar grass, 20h = group that were exposed to cigarette smoke for 20 days then given extract kebar grass for 20 days and discharged 20 days and 60h = group that were exposed to cigarette smoke for 60 days then given extract kebar grass for 60 days and discharged 60 days.

DNA Testes

Length time of given extract kebar grass treatment to the concentration of DNA testes of both grouped rats increased significantly (P < 0.05). A-60-days group, however, has a higher concentration of DNA testes than a-20-days group. The concentration of DNA testes of both groups remained stable after terminating given extract kebar grass.

This shows that the extract kebar grass can repair cells so that the concentration of DNA testes increased, and after terminating given extract kebar grass still remained in the same condition. According to Rastogi (2007), the amount of DNA per cell in each species is constant. The lose of DNA per organ describes decrease of efficiency of functional organ, while, the increase of DNA content describes the increase of cells number present in the testicular. The concentration of DNA testes in all the given extract kebar grass treatment increases highly because the concentration of DNA/unit dry weight of testicular also enhances. Increasing concentration of DNA testes in treated group given of extract kebar grass showed that mitotic activity increased in that treatment.

RNA Testes

Length period of given extract kebar grass treatment to the concentration of RNA testes of both grouped rats has no significantly effect ($P < 0.05$). The concentration of RNA testes of both groups remained stable after terminating given extract kebar grass. This shows that the extract kebar grass can repair cells so that the concentration of RNA testes increased, and after terminating given extract kebar grass still remained in the same condition.

The content of RNA describes synthesis activity in testes cells and in this study showed a significant correlation with testes weight. RNA testes levels in group given extract kebar grass increased along with the increased of weight testes.

Synthesis activity appears to be lower in the treatment of given cigarette smoke and then rises up in the given extract kebar grass. Kebar grass contains flavonoids and vitamin E which play role as an antioxidant to bind free radicals from cigarette smoke and to repair cells in tissues of testes, therefore tissues of testes can produce spermatozoa. Increasing cell activity is described by increasing concentrations of RNA (Rastogi 2007).

CONCLUSION

It can be concluded that the treatment of extracting kebar grass 0.0945 mg/g body weight/day for both a-20-days and a-60-days rat group could increase the testes weigh, the concentrations of DNA, and RNA testes.

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